2011



Phytochemical Society of North America 50th Anniversary Meeting

December10-15, 2011 Fairmont Orchid, Hawai'i

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Welcome and ALOHA PSNA50 Conference Delegates, Accompanying Persons, Sponsors and Exhibitors!

We are very pleased to have the 50th anniversary meeting of the Phytochemical Society of North America hosted on the Kohala Coast of the island of Hawaii, the bridge between the west and the east. In true Hawaiian tradition, we welcome each of you (ALOHA and E KOMO MAI). This is a very special time in the society's history, and we hope that you will enjoy the quite remarkable science, the opportunity to meet with scientific colleagues, as well as the wonderful richness and splendor of Hawaii itself.

PSNA 50 represents the culmination of two years intense planning, and brings together a remarkable group and diversity of scientific experts and young scientists alike in each of the main areas of phytochemical research. The program of oral contributions is outstanding, as are the poster sessions. We are very grateful to all of the conference contributors, to our scientific committee and to the local hosts in enabling this wonderful gathering.

We also wish to gratefully acknowledge the wonderfully generous support by our various sponsors to whom we give a big MAHALO! (THANK YOU!). Without their support, this meeting would not be possible.

The meeting begins with reflections about the Society from Professor Eric Conn, one of the pioneers in PSNA, this being followed by key plenary contributions from HRH Princess Chulabhorn Mahidol (on Thailand's phytochemical diversity and promise), Mansukh C. Wani (co-discoverer of taxol and camptothecin), Koji Nakanishi (seminal work on ginkgolides), and Daneel Ferreira (on proanthocyanidin/polyphenols). Together, they provide wonderful examples of the importance of our remarkable phytochemical treasures, much of which has only been discovered in the last 50 years.

We are saddened with the passing of Professor Meinhart H. Zenk, but honored by the wonderful slate of speakers in sessions dedicated to his quite remarkable scientific legacy. He lives on in very many different ways.

The scientific program, thanks to all of you, has wonderful contributions in areas spanning: chemoprevention, natural products drug discovery, medicinal plants, biosynthesis, metabolism/metabolomics, natural products in agriculture, new characterization methods, dietary supplements, phytochemistry/phytomedicine, transcriptomics/metabolomics, as well as the emerging areas of biofuels/bioproducts. There is something for everyone!

PSNA 50 also has special sessions dedicated to our young scientists, at the undergraduate, graduate and postdoctoral levels. We would ask each of you to participate in these activities as well, and to provide your support for their endeavors.

Welcome to Hawaii. Welcome to PSNA 50. (MAHALO! ALOLHA AND MALAMA PONO!) (THANK YOU, WELCOME AND 'TAKE CARE' (BE SAFE) DURING THE CONFERENCE)

Norman G. Lewis Conference Chair PSNA 50 Cecilia Mcintosh President PSNA 50 John Pezzuto Local Host PSNA 50

Scientific Committee

Norman G. Lewis, Chair, Washington State University Ikuro Abe, University of Tokyo Gynheung An, Kyung Hee University Yoshinori Asakawa, Tokushima Bunri University K. Hüsnü Can Baser, Anadolu University Rudolf Bauer, University of Graz Jörg Bohlmann, University of British Columbia G. Paul Bolwell, Royal Holloway, University of London Vanderlan Da Silva Bolzani, Universidade Estadual Paulista, Instituto de Química Wanchai De-Eknamkul, Chulalongkorn University Daneel Ferreira, University of Mississippi Mary Garson, University of Queensland Jonathan Gershenzon, Max Planck Institute for Chemical Ecology De-an Guo, Shanghai Institute of Materia Medica Mahabir P. Gupta, Massuo Jorge Kato, University of São Paulo Ikhlas A. Khan, University of Mississippi Toni M. Kutchan, Donald Danforth Plant Science Center Rachel Mata, Universidad Nacional Autónoma de Mexico Susan McCormick, United States Dept. of Agriculture Cecilia A. McIntosh, East Tennessee State University Birger Lindberg Møller, University of Copenhagen W. David Nes, Texas Tech University Hermann M. Niemeyer, Universidad de Chile M. Soledade C. Pedras, University of Saskatchewan John M. Pezzuto, University of Hawaii at Hilo Richard Robins, Université de Nantes Michel Rohmer, Université de Strasbourg Rosario Rojas. Kazuki Saito, RIKEN and Chiba University, Joachim Stöckigt, Zhejiang University Dieter Strack, Leibniz-Institute of Plant Biochemistry Hermann Stuppner, University of Innsbruck Toshiaki Umezawa, Kyoto University Robert Verpoorte, Leiden University

Organizing Committee

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Committee Chair Director, Institute of Biological Chemistry Washington State University

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David R. Gang, Ph.D. PSNA Past-President Associate Professor and Fellow Institute of Biological Chemistry Washington State University

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Mark A. Bernards, Ph.D. PSNA Past-President Professor & Chair Department of Biology The University of Western Ontario

Laurence B. Davin, Ph.D. Institute of Biological Chemistry Washington State University

Meeting Program

SATURDAY 10 DECEMBER

| 03:00–05:00 pm | Executive Committee Meeting | |
|----------------|------------------------------------|--------------------------------|
| | | Plaza III |
| 02:30–08:30 pm | Registration and Information Table | |
| | | Paniolo Hospitality |
| 02:30–06:00 pm | Poster Setup | |
| | | Salon III & Ballroom Courtyard |
| 06:00–08:30 pm | Welcome Reception | |
| | | Turtle Point |

| 07:30-03:30 | Registration and Information Table | |
|-----------------|--|-------------|
| | Grande Ball | room Foyer |
| Opening Remark | s: | Salon I, II |
| 08:00–08:10 | Hawaiian Protocol John M. Pezzuto and Norman G. Lewis | |
| 08:10–08:15 | Welcome Address: William "Billy" Kenoi, Mayor of Hawaii | |
| 08:15–08:35 | Eric E. Conn (University of California at Davis) <i>PSNA: Some reflections</i> | OR1 |
| Session 1: PSNA | A Plenary Session – A Celebration | Salon I, II |
| Chair: Norman C | G. Lewis | |
| 08:40–09:20 | Chulabhorn Mahidol (Chulabhorn Graduate Institute) Natural products with biological activities from Thai bioresources | S1.1 |
| 09:20–10:00 | Koji Nakanishi (Columbia University) Ginkgolides - Structures, reactions and bioactivities | S1.2 |
| 10:00–10:30 | Break Ballroom Courtyard & Grande Ball | room Foyer |
| 10:30–11:10 | Mansukh C. Wani (RTI International) From yew to me to you: A personal history of the discovery and development of plant-derived anticancer agent, taxol | S1.3 |
| 11:10–11:50 | Daneel Ferreira (University of Mississippi) Half a century of proanthocyanidin/polyphenol research | S1.4 |
| 11:50–12:00 pm | Photos | |
| 12:00-01:00 | Lunch Ballroom Courtyard & Grande Ball | room Foyer |
| 12:00–01:00 | Finding a Graduate Program Workshop Plaza Ballroom Chair: Daniel Owens Panelists: Aruna Kilaru, Norman G. Lewis, Cecilia A. McIntosh, and John Romeo | 1 |
| 01:00–06:00 | Concurrent Session 2: The Legacy of Meinhart H. Zenk | S2 |
| | Salon I, I | I |
| | Concurrent Session 3: Natural Products in Agriculture Promenade Ballroom | S3 |
| | Concurrent Session 4: Chemoprevention | S4 |
| | Plaza Ballroom | 1 |
| 07:00–10:00 | Poster Session I and Student/Postdoctoral Fellow Competition – Rec Salon III & Ballroon | - |
| | | |

| Concurrent Session | on 2: The Legacy of Meinhart H. Zenk | Salon, I, II | | |
|---------------------------|---|--------------|--|--|
| Chair: Nick Amrhein | | | | |
| 01:00–01:30 | Norman G. Lewis (Washington State University) The road from Munich: Meinhart H. Zenk's legacy | S2.1 | | |
| 01:30–02:00 | Birger Lindberg Møller (University of Copenhagen) Functioning dependent metabolons: The "oxime bomb" in cyanogenic glucoside metabolism | S2.2 | | |
| 02:00–02:30 | Fumihiko Sato (Kyoto University) The great footprints of the late Prof. Meinhart Zenk in isoquinoline alkaloi biosynthesis studies | S2.3 d | | |
| 02:30–03:00 | Kazuki Saito (Chiba University and RIKEN) Biosynthetic study of quinolizidine alkaloid – History and recent advancement | S2.4 | | |
| 03:00–03:30 | Joachim Stöckigt (Zhejiang University) Alkaloid synthesis beyond the Rauvolfia alkaloidal network | S2.5 | | |
| 03:30-04:00 | Break Ballroom Courtyard & Grande Bal | llroom Foyer | | |
| 04:00–04:30 | Wanchai De-Eknambul (Chulalongkorn University) My research experience with Prof. Meinhart Zenk, the pioneer of benzylisoquinoline alkaloid biosynthesis | S2.6 | | |
| 04:30–05:00 | Vincenzo De Luca (Brock University) Catharanthus roseus <i>expresses a leaf epidermis specific amyrin synthas</i> <i>involved in triterpene biosynthesis</i> | S2.7 e | | |
| 05:00–05:30 | Kazufumi Yazaki (Kyoto University) Membrane transport of alkaloid in plants | S2.8 | | |
| 05:30–06:00 | Jan Frederik Stevens (Oregon State University) Xanthohumol from hops (Humulus lupulus): Pharmacokinetics and effect on biomarkers of metabolic syndrome in rats | S2.9 s | | |
| 07:00–10:00 | Poster Session I – Student/Postdoctoral Fellow Competition – Recepsion Salon III & Ballroo | | | |

| Concurrent Sess | ion 3: Natural Products in Agriculture | Promenade Ballroom | |
|------------------------|--|---------------------------------------|--|
| Chair: Murray B. Isman | | | |
| 01:00–01:30 | Stephen O. Duke (United States Department of Agriculture) <i>Phytochemicals and genes for their synthesis in pest manageme</i> | S3.1 | |
| 01:30–02:00 | Suzanne R. Abrams (Plant Biotechnology Institute, NRC) Improved crop productivity through manipulation of phytohormor | S3.2 ne signaling | |
| 02:00–02:30 | Robert Stipanovic (United States Department of Agriculture) Biosynthesis of gossypol in cotton: From farnesyl diphosphate to (–)-gossypol | S3.3 o (+)- and | |
| 02:30–03:00 | M. Soledade C. Pedras (University of Saskatchewan) Natural products in agriculture: The arms race between crucifer fungal pathogens | S3.4 s and their | |
| 03:00–03:30 | Murray B. Isman (University of British Columbia) Insecticides based on plant natural products: Long on promise, s products | S3.5 short on | |
| 03:30–04:00 | Break Ballroom Courtyard & G | irande Ballroom Foyer | |
| 04:00–04:15 | Russell L. Rouseff (University of Florida) <i>Sulfur volatiles in</i> Allium canadense <i>and</i> A. tuberosum | O3.1 | |
| 04:15–04:30 | Efraim Lewinsohn (Newe Ya'ar Research Center) <i>L-Methionine catabolism into sulfur aroma volatiles in melon frui</i> | O3.2 ts | |
| 04:30–04:45 | Mitchell L. Wise (United States Department of Agriculture) Plant defense activators as elicitors of oat avenanthramide biosy | O3.3 /nthesis | |
| 04:45–05:00 | Charles L. Cantrell (United States Department of Agriculture) Identification of the mosquito biting deterrent constituents from the folk remedy plant, Jatrophas curcas | O3.4 he Indian | |
| 05:00–05:15 | Anthony D. Wright (University of Hawaii at Hilo) Correlation between tea leaf age and chemical content and shad | O3.5 de levels | |
| 05:15–05:30 | Roslyn Gleadow (Monash University) Impact of climate on allocation of N to cyanogenic glucosides | O3.6 | |
| 07:00–10:00 | Poster Session I – Student/Postdoctoral Fellow Competition Salon III | • – Reception & Ballroom Courtyard | |

| Concurrent Sessi | Concurrent Session 4: Chemoprevention Plaza Ballroom | | | |
|-------------------|---|----------------|--|--|
| Chair: Nanjoo Suh | | | | |
| 01:00–01:30 | Gary D. Stoner (Medical College of Wisconsin) Genetic and epigenetic mechanisms of colon cancer chemoprevention humans by whole berries and berry constituents | S4.1 n in | | |
| 01:30–02:00 | Michael B. Sporn (Dartmouth Medical School) Natural triterpenoids as scaffolds for new drug synthesis | S4.2 | | |
| 02:00–02:30 | Paul Talalay (Johns Hopkins University School of Medicine) <i>The clinical promise of sulforaphane</i> | S4.3 | | |
| 02:30–03:00 | Chung Shu Yang (Rutgers University) Cancer prevention by <i>δ</i> - and γ-tocopherols | S4.4 | | |
| 03:00–03:30 | Kathryn Ann Gold (University of Texas MD Anderson Cancer Center Lung cancer prevention: Reverse migration strategy |) S4.5 | | |
| 03:30-04:00 | Break Ballroom Courtyard & Grande | Ballroom Foyer | | |
| 04:00–04:30 | Arthur Neish Young Investigator Award Lecture Albena T. Dinkova-Kostova (University of Dundee) A novel role for sulfhydryl-reactive activators of transcription factor NF HSF1-dependent upregulation of Hsp70 | S4.6 RF2: | | |
| 04:30–05:00 | Karen Liby (Dartmouth Medical School) Synthetic triterpenoids and chemoprevention: Biological activities and molecular targets | S4.7 | | |
| 05:00–05:30 | Young-Joon Surh (Seoul National University) Cancer chemoprevention with anti-inflammatory phytochemicals | S4.8 | | |
| 05:30–06:00 | John M. Pezzuto (University of Hawaii at Hilo) The phenomena of resveratrol | S4.9 | | |
| 07:00–10:00 | Poster Session I and Student/Postdoctoral Fellow Competition – | Reception | | |
| | Salon III & Bali | room Courtyard | | |

MONDAY 12 DECEMBER

| 07:30–03:30 | Registration and Information Table | |
|-------------|---|----------|
| | Grande Ballroo | m Foyer |
| 08:00–10:00 | Concurrent Session 2: The Legacy of Meinhart H. Zenk | S2 |
| | Salon I, II | |
| | Concurrent Session 4: Chemoprevention | S4 |
| | Plaza Ballroom | |
| 10:00–10:30 | Break | |
| | Ballroom Courtyard & Grande Ballroo | m Foyer |
| 10:30–12:30 | Concurrent Session 2: The Legacy of Meinhart H. Zenk | S2 |
| | Salon I, II | |
| | Concurrent Session 4: Chemoprevention | S4 |
| | Plaza Ballroom | |
| 12:30–01:30 | Lunch | |
| | Ballroom Courtyard & Grande Ballroo | m Foyer |
| 12:30-01:30 | Grantsmanship Workshop Plaza Ballroom | |
| | Chair: Daniel Owens Panelists: Richard A. Dixon, David R. Gang, Toni M. Kutchan, Norman G. Lewis, And Cecilia A. Mcintosh | |
| 01:30-04:00 | Concurrent Session 2: The Legacy of Meinhart H. Zenk | S2 |
| | Salon I, II | |
| | Concurrent Session 4: Chemoprevention | S4 |
| | Plaza Ballroom | |
| 07:00-10:00 | Poster Session II and Student/Postdoctoral Fellow Competition – Recept | otion |
| | Salon III & Ballroom C | ourtyard |

MONDAY 12 DECEMBER

| Concurrent Ses | sion 2: The Legacy of Meinhart H. Zenk | Salon, I, II | | |
|-------------------------|--|--------------|--|--|
| Chair: Vincenzo De Luca | | | | |
| 08:00–08:30 | Bernd Schneider (Max Plank Institute for Chemical Ecology) A blue Anigozanthos root culture as an initial of phenylphenalenone research | S2.10 h | | |
| 08:30–09:00 | Eckhard Leistner (Universität Bonn) Fungal origin of ergot alkaloids in dicotyledonous plants | S2.11 | | |
| 09:00–09:30 | Michael Spiteller (Technical University of Dortmund) <i>Highlights on morphine research with Prof. Meinhart H. Zenk</i> | S2.12 | | |
| 09:30–10:00 | Daneel Ferreira (University of Mississippi) Absolute configuration of secondary metabolites via electronic circular dichroism | S2.13 | | |
| 10:00–10:30 | Break Ballroom Courtyard & Grande Ballro | oom Foyer | | |
| 10:30–11:00 | Yoshinori Asakawa (Tokushima Bunri University) <i>"Why do liverworts biosynthesize marchantins, pungent and bitter substance</i> | S2.14 | | |
| 11:00–11:30 | Michael Müller (University of Freiburg) Regio- and stereoselective intermolecular oxidative phenol coupling in filamentous fungi | S2.15 | | |
| 11:30–12:00 | Brian E. Ellis (University of British Columbia) The MYB75 transcription factor plays a central role in regulating carbon flux into cell wall-related metabolic pathways in Arabidopsis thaliana | S2.16 | | |
| 12:00–12:30 | David R. Gang (Washington State University) Modern tools for ancient medicines: Investigating the biosynthesis of bioactive compounds in important medicinal plants | S2.17 | | |
| 12:30–01:30 | Lunch Ballroom Courtyard & Grande Ballro | oom Foyer | | |
| 12:30–01:30 | Grantsmanship Workshop. Chair: Daniel Owens Plaza Ballroom Panelists: Richard A. Dixon, David R. Gang, Toni M. Kutchan, Norman G. Lewis, and Cecilia A. McIntosh | | | |
| Chair: Wanchai | De-Eknambul | | | |
| 01:30–02:00 | Alfons Gierl (Technical University of Munich) Comparative analysis of benzoxazinoid biosynthesis in monocots and dicots: Independent recruitment of stabilization and bio-activation functions | S2.18 | | |
| 02:00–02:30 | Erwin Grill (Technical University of Munich) <i>Heavy metals; xenobiotics; and more</i> | S2.19 | | |
| 02:30–03:00 | Reinhard Jetter (University of British Columbia) Phenolics in the surface waxes of Secale cereale: Formation and accumulation of cuticular alkylresorcinols | S2.20 | | |
| 03:00–03:30 | Robert L. Last (Michigan State University) Hairy genomics: Studies of secretory glandular trichomes in tomato and relatives | S2.21 | | |
| 03:30–04:00 | Toni M. Kutchan (Donald Danforth Plant Science Center) Post-genomic elucidation of plant natural product pathways | S2.22 | | |
| 07:00–10:00 | Poster Session II and Student/Postdoctoral Fellow Competition – Rece | ption | | |
| | Salon III & Ballroom | Courtyard | | |

MONDAY 12 DECEMBER

| Concurrent Session 4: Chemoprevention Plaza Ballroor | | | |
|--|--|---|-----------------|
| Chair: John M. Po | ezzuto | | |
| 08:00–08:30 | Thomas Kensler (University of Pittsburgh <i>KEAP1-NRF2 signaling as a target for car products</i> | | S4.10 |
| 08:30–09:00 | Clarissa Gerhauser (German Cancer Re Cancer chemoprevention by targeting the future challenges | | S4.11 and |
| 09:00–09:30 | Ah-Ng Tony Kong (Rutgers University) Dietary cancer chemopreventive compour blocking carcinogenesis initiation versus to | | S4.12 s in |
| 09:30–10:00 | Nancy H. Colburn (Center for Cancer Re Dietary prevention of colon carcinogenesis biomarkers | | tute) S4.13 |
| 10:00–10:30 | Break | Ballroom Courtyard & Grande | Ballroom Foyer |
| Chair: Nanjoo Su | h | | |
| 10:30–11:00 | Allan H. Conney (Rutgers University) Studies for cancer prevention: A path from | n tea to caffeine to exercise | S4.14 |
| 11:00–11:30 | Hasan Mukhtar (University of Wisconsin- Cancer chemoprevention: Mission Accom- not so in humans | | S4.15 why |
| 11:30–12:00 | Scott M. Lippman (University of Texas M Natural-agent mechanisms and personaliz | | S4.16 ention |
| 12:00–12:30 | Closing Remarks John M. Pezzuto and Nanjoo Suh | | |
| 12:30-01:30 | Lunch | Ballroom Courtyard & Grande | Ballroom Foyer |
| 12:30–01:30 | Grantsmanship Workshop Chair: Daniel Owens Panelists: Richard A. Dixon, David R. (Norman G. Lewis, and Cecilia A. McInt | | oom |
| Chair: Andre S. E | Bachmann | | |
| 01:30–02:00 | Ning-Sun Yang (Academia Sinica, Agricu Center) <i>Anti-cancer effect of scallion extract again</i> | | h O4.1 |
| 02:00–02:15 | Suzanna M. Zick (University of Michigan) Ginger supplementation and the expression appearing colorectal mucosa of patients a Results from a pilot randomized, controlled | on of NF-kB in the normal- t high risk of colorectal cance | 04.2 er: |
| 02:15–02:30 | Jessica Citronberg (Emory University Ro Ginger supplementation and the expression colorectal mucosa of sporadic colorectal a pilot randomized, controlled trial | on of bax in the normal-appea | aring |

| 02:30–02:45 | Chong-Zhi Wang (University of Chicago) Potential role of ginseng for cancer chemoprevention | 04.4 |
|-------------|---|-------|
| 02:45–03:00 | Ramesh C. Gupta (University of Louisville) Blueberry diet and blueberry bioactives inhibit lung cancer and enhance the activity of paclitaxel | O4.5 |
| 03:00–03:15 | Richard B. van Breemen (University of Illinois) Antioxidant effects of lycopene in men with prostate cancer or benign prostate hyperplasia: A randomized controlled trial | O4.6 |
| 03:15–03:30 | Anait S. Levenson (University of Mississippi Medical Center) Resveratrol and its analogues as potential epigenetic agents for chemoprevention and therapy in prostate cancer | 04.7 |
| 03:30–03:45 | Aimee L. Eggler (Purdue University) <i>Elucidation of structural/functional changes upon modification of Keap1</i> <i>C151, a primary target of botanical chemopreventive agents</i> | O4.8 |
| 03:45–04:00 | Gail B. Mahady (University of Illinois at Chicago) Extracts of collard greens down-regulate the expression of HER2/NEU protein and mRNA in MCF-7 and SK-BR3 breast cancer cells | O4.9 |
| 04:00–04:15 | Eun-Jung Park (University of Hawaii at Hilo) Suppression of cyclooxygenase-2 and inducible nitric oxide synthase expression by 4-[(2-O-acetyl-α-L-rhamnosyloxy)benzyl] isothiocyanate in LPS-stimulated raw 264.7 cells | O4.10 |
| 07:00–10:00 | Poster Session II and Student/Postdoctoral Fellow Competition – Rece Salon III & Ballroom | • |

| 07:30–03:30 | Registration and Informa | tion Table | | |
|-------------|------------------------------|------------------------------|---------------------------|---------|
| | | | Grande Ballroor | • |
| 08:00–10:00 | Concurrent Session 5: | Biochemistry/Enzymology | | S5 |
| | | | Salon, I, II | 0.0 |
| | Concurrent Session 6: | Dietary Supplements | Plaza Ballroom | S6 |
| 10:00–10:30 | Break | | | |
| | | Ballroom Co | urtyard & Grande Ballroor | n Foyer |
| 10:30–12:30 | Concurrent Session 5: | Biochemistry/Enzymology | | S5 |
| | | | Salon, I, II | |
| | Concurrent Session 6: | Dietary Supplements | | S6 |
| 10.00.01.00 | | | Plaza Ballroom | |
| 12:30–01:30 | Lunch | Pallroom Co | urtyard & Grande Ballroor | n Eovor |
| 01:30-03:30 | Concurrent Session 5: | Biochemistry/Enzymology | aliyalu & Glanue Balliool | S5 |
| 01.30-03.30 | Concurrent Session 5. | Biochemistry/Enzymology | Salon, I, II | 35 |
| | Concurrent Session 7: | Botanicals/Medicinals | Calon, I, II | S7 |
| | | | Plaza Ballroom | 01 |
| | Concurrent Session 8: | New Characterization Meth | ods | S8 |
| | | | Promenade Ballroom | |
| 03:30-04:00 | Break | | | |
| | | Ballroom Co | urtyard & Grande Ballroor | n Foyer |
| 04:00-06:00 | Concurrent Session 5: | Biochemistry/Enzymology | | S5 |
| | | | Salon, I, II | |
| | Concurrent Session 7: | Botanicals/Medicinals | | S7 |
| | | | Plaza Ballroom | |
| | Concurrent Session 9: | Natural Products Drug Disc | • | S9 |
| | | | Promenade Ballroom | |

| Concurrent Sessi | on 5: Biosynthesis/Enzymology | Salon, I, II |
|--------------------|--|---------------|
| Chair: Birger Line | dberg Møller | |
| 08:00–08:30 | Michel Rohmer (Université de Strasbourg) Biosynthesis of isoprene units via the MEP pathway: Electron and proto transfers in the formation of IPP and DMAPP | S5.1 n |
| 08:30–09:00 | W. David Nes (Texas Tech University) Unearthing the biosynthetic diversity in the sterol metabolome | S5.2 |
| 09:00–09:30 | Wanchai De-Eknamkul (Chulalongkorn University) <i>Biosynthesis of bioactive acyclic diterpenoids and phytosterols in</i> Crotor stellatopilosus | S5.3 า |
| 09:30–09:45 | James Kirby (University of California, Berkeley) Diterpene biosynthesis in Ricinus communis – Modification of casbene li cytochrome p450 monooxygenase | O5.1 by a |
| 09:45–10:00 | Jim G. Tokuhisa (Virginia Polytechnic Institute and State University) <i>A family of squalene synthases in potato</i> | O5.2 |
| 10:00–10:30 | Break Ballroom Courtyard & Grande Ba | allroom Foyer |
| Chair: Patrick S. | Covello | |
| 10:30–11:00 | Michel Legrand (Université de Strasbourg) Polyketide synthases and tetraketide α-pyrone reductases of Arabidops thaliana are involved in sporopollenin biosynthesis | S5.4 is |
| 11:00–11:30 | Ikuro Abe (University of Tokyo) Engineering plant polyketide synthases | S5.5 |
| 11:30–12:00 | Nicholas Smirnoff (University of Exeter) The role of ascorbate in the acclimation of leaves to high light | S5.6 |
| 12:00–12:15 | Hiroyuki Morita (University of Tokyo) Crystal structure analysis of the type III polyketide synthase that produce curcuminoid | O5.3 es |
| 12:15–12:30 | Mark A. Bernards (University of Western Ontario) Extracellular glycosidases of Pythium irregulare | O5.4 |
| 12:30–01:30 | Lunch Ballroom Courtyard & Grande Ba | allroom Foyer |
| Chair: Michel Ro | hmer | |
| 01:30–02:00 | Vincenzo De Luca (Brock University) Catharanthus roseus as a non-model model system for MIA biosynthesi | S5.7 is |
| 02:00–02:30 | Kye-Won Kim (Washington State University) Stereoselective lignan biosynthesis engendered by dirigent proteins in Arabidopsis thaliana | S5.8 |
| 02:30–02:45 | Chang-Jun Liu (Brookhaven National Laboratory) Deciphering molecular mechanisms of lignin precursor transport | O5.5 |
| 02:45–03:00 | Takeshi Katayama (Kagawa University) <i>Biosynthesis and stereochemistry of 9,9'-deoxyneolignans in</i> Saururus chinensis | O5.6 |

| 03:00–03:15 | James R. Ketudat Cairns (Suranaree U A stress-induced rice enzyme that equilib | , | O5.7 |
|------------------|---|---|---------|
| 03:15–03:30 | Xiaoqiang Wang (Samuel Roberts Noble Structural biology study of plant natural p | , | O5.8 |
| 03:30–04:00 | Break | Ballroom Courtyard & Grande Ballroon | n Foyer |
| Chair: Ikuro Abe | | | |
| 04:00–04:30 | Birger Lindberg Møller (University of Co Cyanogenic glucosides and the P450s in in plants and insects | | S5.9 |
| 04:30–05:00 | Patrick S. Covello (National Research C The biosynthesis of cyclic peptides in the | | S5.10 |
| 05:00–05:15 | Joseph M. Jez (Washington University) Molecular sensors in plant thiol metabolis | sm | O5.9 |
| 05:15–05:30 | Anna Berim (Washington State Universit Elucidating the network to polymethoxyla (Ocimum basilicum) glandular trichomes | | O5.10 |
| 05:30–05:45 | Nancy Terrier (Institut National de la Red The first step of proanthocyanidin galloyla glucosyltransferases | | O5.11 |
| 05:45–06:00 | Nicole A. Horenstein (University of Flori <i>Studies on the biosynthesis of deoxynojir</i> amyloliquefaciens | | O5.12 |

| Concurrent Sessi | on 6: Dietary Supplements | Plaza Ballroom |
|-------------------------|--|----------------|
| Chair: John T. Ar | nason | |
| 08:00–08:30 | Michael Heinrich (Southern Cross University) Dietary supplements and natural health products: Phytochemistry and metabolomics as tools for evidence-based decisions about their use | S6.1 |
| 08:30–09:00 | Wei Jia (University of North Carolina at Greensboro) <i>Metabolomic evaluation of several anticancer agents of plant origin</i> | S6.2 |
| 09:00–09:30 | Rachel Mata (Universidad Nacional Autónoma de Mexico) Copalchi and other selected antidiabetic plants from Mexico | S6.3 |
| 09:30–09:45 | Kamal D. Moudgil (University of Maryland School of Medicine) Huo-luo-xiao-ling dan (HLXL) protects against experimental arthritis by modulating antigen-induced cellular and humoral responses | O6.1 |
| 09:45–10:00 | Cedric B. Baker (Mercer University) New models in translational phytotherapy: dietary phytochemicals in functional nutrition management and pharmaconutrition | O6.2 |
| 10:00–10:30 | Break Ballroom Courtyard & Grande | Ballroom Foyer |
| Chair: John T. Ar | nason | |
| 10:30–11:00 | Susan Murch (University of British Columbia, Kelowna) <i>Traditional Oceanic crops for improved nutrition, nutraceuticals</i> <i>and natural health products</i> | S6.4 |
| 11:00–11:30 | Stefan Gafner (Tom's of Maine) Glycyrrhiza uralensis: <i>Unusual chemistry and unusual application</i> | S6.5 |
| 11:30–12:00 | Kim Colson (Bruker BioSpin) <i>Multilab method validation of blueberry leaf extract by NMR:</i> <i>Qualitative and quantitative</i> | S6.6 |
| 12:00–12:30 | Dale G. Nagle (University of Mississippi) Toxicological mechanisms of botanical dietary supplements | O6.3 |
| 12:30–01:30 | Lunch Ballroom Courtyard & Grande | Ballroom Foyer |
| Concurrent Sessi | on 7: Botanicals/Medicinals | Plaza Ballroom |
| Chair: Rachel Ma | ta | |
| 01:30–01:45 | Agnes M. Rimando (United States Department of Agriculture) <i>Pterostilbene increases PPARα gene expression, activates AMPK,</i> <i>and suppresses expression of genes involved in hepatic lipid</i> <i>metabolism and gluconeogenesis</i> | 07.1 |
| 01:45–02:00 | Edwin D. Lephart (Brigham Young University) The isoflavonoid, equol improves severe and moderate BPH | 07.2 |

| | symptoms in mid-aged Caucasian men: Clinical evidence | |
|-------------|--|------|
| 02:00–02:15 | Pal Pacher (National Institute on Alcohol Abuse and Alcoholism) Nonpsychoactive constituents from Cannabis sativa (marijuana): Therapeutic potential in inflammatory disorders and diabetes | 07.3 |
| 02:15–02:30 | Lucia M. X. Lopes (São Paulo State University) Sesquiterpenes from Holostylis reniformis | 07.4 |

| 02:30–02:45 | Slavko Komarnytsky (Rutgers University) <i>Triptolide ameliorates insulin resistance in obese di</i> | | 07.5 |
|-----------------|--|-----------------------------|-------|
| 02:45–03:00 | Dovi Kelman (University of Hawaii at Hilo) Antioxidant activity of Hawaiian macro-algae | (| 07.6 |
| 03:00–03:15 | Varima Wongpanich (Khon Kaen University) | (| 07.7 |
| | Medicinal plants of high potency and their application medicine | ons in traditional | |
| 03:15–03:30 | Jaspreet Kaur Sihra (University of Surrey) Novel triterpenoid derivatives from Eucomis bicolor Hyacinthoideae) | | 07.8 |
| 03:30–04:00 | Break Ballroom | Courtyard & Grande Ballroom | Foyer |
| Chair: Agnes M. | Rimando | | |
| 04:00–04:15 | Colin Charles Duke (University of Sydney) Discovery of Kangaroo Island medicinal plants using | | 07.9 |
| 04:15–04:30 | Upma Bagai (Panjab University) Antiplasmodial efficacy of ethanolic leaves extract of | | 07.10 |
| 04:30–04:45 | Chinedu Athanasius Eze (University of Nigeria) Assessment of the wound healing activity of methan extracts of Garcinia kola seed in albino rats | | 07.11 |
| 04:45–05:00 | Mamdouh M. Abou-Zaid (Natural Resources Cana Bioproducts from the Canadian forest | ada) (| 07.12 |
| 05:00–05:15 | Gary Loake (University of Edinburgh) Cultured cambial meristematic cells as a source of | | 07.13 |
| 05:15–05:30 | Mark Perry (The University of Western Ontario) Balancing research and sustainability | (| 07.14 |

| Concurrent Session | on 8: New Characterization Methods | Promenade Ballroom |
|---------------------------|---|----------------------|
| Chair: Daneel Fe | rreira | |
| 01:30–02:00 | Margaret Y. Gruber (Agriculture and Agri-Food Canada) Unravelling mysteries in the regulation of plant secondary metab and non-glandular trichomes: New mechanisms and crop applied | |
| 02:00–02:15 | Teagen D. Quilichini (University of British Columbia) The Arabidopsis ABCG26 transporter: a tool for investigating the nature of sporopollenin | O8.2 |
| 02:15–02:30 | Takamitsu Yoshida (Nagoya City University) Quantitative analysis of natural products by qNMR | O8.3 |
| 02:30–02:45 | Claudia S. Maier (Oregon State University) Plant phenolics analysis using electrospray ion mobility time-of-f mass spectrometry | O8.4 light |
| 02:45–03:00 | Pei Chen (United States Department of Agriculture) Mass spectroscopic fingerprinting and chemometric analysis for quality assessment of botanicals and foodstuffs | O8.5 |
| 03:00–03:15 | Sarya Aziz (McGill University) Structural characterization of phenolic lipids obtained by transesterification of 3,4-dihydroxyphenylacetic acid and krill oil | O8.6 |
| 03:30–04:00 | Break Ballroom Courtyard & G | rande Ballroom Foyer |
| Concurrent Session | on 9: Natural Product Drug Discovery | Promenade Ballroom |
| Chair: Jan Freder | rik Stevens | |
| 04:00–04:15 | Tsutomu Hatano (Okayama University) Antibacterial effects of hydrolyzable tannins and related artificial | O9.1 tannins |
| 04:15–04:30 | Catherine P. Tunbridge (University of Surrey) COX-2 specific inhibition from natural product (E)-hinokiresinol a a facile synthesis of 3-vinylphenylindanes | O9.2 |
| 04:30–04:45 | Prasat Kittakoop (Chulabhorn Research Institute) <i>Bioactive compounds from Thai marine-derived fungi</i> | O9.3 |
| 04:45–05:00 | James Hamuel Doughari (Cape Peninsula University of Techno Multi-drug resistance, verotoxin production and efficacy of crude bark extracts of Curtisia dentata among Escherichia coli (non-Or Acinetobacter spp. isolates obtained from water and wastewater | stem 157) and |
| 05:00–05:15 | Martine Cao (University of Liege) 17-O-Acetyl, 10-hydroxycorynantheol, a selective antiplasmodial alkaloid isolated from Strychnos usambarensis leaves | O9.5 |
| 05:15–05:30 | Amani S. Awaad (King Saud University) Evaluation of the antimicrobial activity, sub-chronic toxicity and wound healing effect of Cunninghamella species and some of its isolated compounds | O9.6 |
| 05:30–05:45 | Arif ullah Khan (Kohat University of Science and Technology) Antihypertensive effect of Gentiana floribunda is mediated throug Ca ⁺⁺ antagonistic pathway | O9.7 gh |

WEDNESDAY 14 DECEMBER

| 07:30-03:30 | Information Table | | |
|-------------|--|----------------------------|--------------|
| | | Grande Ballrool | m Foyer |
| 08:00–10:00 | Concurrent Session 9: Natural Products Drug Discover | y Plaza Ballroom | S9 |
| | Concurrent Session 10: Metabolism/Metabolomics | Salon, I, II | S10 |
| 10:00–10:30 | Break Ballroom Courtyard | l & Grande Ballroor | n Foyer |
| 10:30–12:30 | Concurrent Session 9: Natural Products Drug Discover | y Plaza Ballroom | S9 |
| | Concurrent Session 10: Metabolism/Metabolomics | Salon, I, II | S10 |
| 12:30–01:30 | Lunch Ballroom Courtyard | l & Grande Ballroon | n Foyer |
| | Funding for International Collaboration Workshop | | |
| | Chair: Wendy Boss | Plaza Ballroom | |
| 01:30–03:30 | Concurrent Session 10: Metabolism/Metabolomics | Salon, I, II | S10 |
| | Concurrent Session 11: Phytochemistry/Phytomedicine | Plaza Ballroom | S11 |
| 03:30–04:00 | Break Ballroom Courtyard | l & Grande Ballroor | n Foyer |
| 04:00-5:30 | Concurrent Session 10: Metabolism/Metabolomics | Salon, I, II | S10 |
| | Concurrent Session 11: Phytochemistry/Phytomedicine | Plaza Ballroom | S11 |
| 05:00–06:00 | Business Meeting | Plaza Ballroom | |
| 07:00–10:00 | Banquet 50th Anniversary Awards Celebration | | , I, II, III |
| | | | |

WEDNESDAY 14 DECEMBER

| Concurrent Session 9: Natural Products Drug Discovery Plaza Ballroom | | | |
|--|--|----------------|--|
| Chair: Charles L. | Cantrell | | |
| 08:00–08:30 | Andre S. Bachmann (University of Hawaii at Hilo) Syrbactins: New structural class of proteasome inhibitors produced by plant pathogens | O9.8 | |
| 08:30–09:00 | Ning-Sun Yang (Academia Sinica, Agricultural Biotechnology Research Center) <i>Categorization of medicinal plants with immuno-regulatory activities by</i> <i>cytokine expression in mouse bone-marrow derived dendritic cells</i> | O9.9 | |
| 09:00–09:30 | Dulcie Mulholland (University of Surrey) Cembranolides from Croton gratossimus (Euphorbiaceae) | O9.10 | |
| 09:30–09:45 | Yu-Dong Zhou (University of Mississippi) Natural product-based inhibitors of hypoxia-inducible factor-1 (HIF-1) as chemical probes for cellular signaling | O9.11 | |
| 09:45–10:00 | Muriel Cuendet (University of Geneva) Rapid and rational identification of bioactive natural products | O9.12 | |
| 10:00–10:30 | Break Ballroom Courtyard & Grande | Ballroom Foyer | |
| Chair: Dulcie Mu | Iholland | | |
| 10:30–11:00 | Athar Ata (University of Winnipeg) Naturally occurring enzyme inhibitors and their pharmaceutical applications | O9.13 | |
| 11:00–11:15 | Yoshiyasu Fukuyama (Tokushima Bunri University) <i>Neurotrophic seco-prezizaane type sesquiterpenoids from</i> Illicum jiadifengpi | O9.14 | |
| 11:15–11:30 | Mark O'Neil-Johnson (Sequoia Sciences, Inc.) Steering clear of the drug discovery black-hole | O9.15 | |
| 11:30–11:45 | James D. McChesney (Ironstone Separations, Inc.) Enhancing normal phase chromatography for natural products researchers | O9.16 | |
| 11:45–12:00 | Lie-Fen Shyur (Academia Sinica, Agricultural Biotechnology Research Center) <i>Phytoagent deoxyelephantopin cotreatment with cisplatin</i> <i>significantly reduces nephrotoxicity-induced by cisplatin in B16</i> <i>melanoma-bearing mice</i> | O9.17 | |
| 12:00–12:15 | Stephen J. Polyak (University of Hawaii Cancer Center) <i>Antiviral properties of silymarin and purified flavonolignans</i> | O9.18 | |
| 12:30–01:30 | Lunch Ballroom Courtyard & Grande | Ballroom Foyer | |
| 12:30–01:30 | Funding for International Collaboration Workshop Chair: Wendy Boss | | |

Plaza Ballroom

| WEDNESDAY 14 DECEMBER | | | |
|-----------------------|--|---|-------|
| Concurrent Sessi | on 10: Metabolism/Metabolomics | Salon, | I, II |
| Chair: Kasuki Sa | ito | | |
| 08:00–08:30 | Michael H. Beale (Rothamsted Research Realising the potential of plant metabolom | | 10.1 |
| 08:30–09:00 | Bernd Schneider (Max Planck Institute for Spectroscopic metabolite profiling of laser | | 10.2 |
| 09:00–09:30 | Lloyd W. Sumner (Samuel Roberts Noble Exploiting metabolic diversity through integed discovery and elucidation of saponin biosy truncatula | grated metabolomics for the | 10.3 |
| 09:30–09:45 | Jane L. Ward (Rothamsted Research) Unexpected hemiterpenoids in Arabidopsi fingerprinting, give new insights into C/N r | s, revealed by metabolomic | 10.1 |
| 09:45–10:00 | Steven C. Halls (Monsanto Company) <i>Measuring and comparing the magnitudes</i> | | 10.2 |
| 10:00–10:30 | Break | Ballroom Courtyard & Grande Ballroom Fo | oyer |
| Chair: Lloyd Sun | nner | | |
| 10:30–11:00 | Arthur Neish Young Investigator Award Mi Kwon (Korea University) Effects of exogenously applied brassinost yellow poplar | | 10.4 |
| 11:00–11:30 | Kasuki Saito (Chiba University and RIKE Plant metabolomics for phytochemical gen | | 10.5 |
| 11:30–12:00 | Mark Lange (Washington State University Developing mint as an experimental mode and manipulating terpenoid essential oil but | l system for understanding | 10.6 |
| 12:00–12:15 | Jason Q.D. Goodger (University of Melbo Embedded secretory cavities: Natural pro- | / | 10.3 |
| 12:15–12:30 | Dorothea Tholl (Virginia Polytechnic Insti <i>Exploring the organization and function of</i> <i>specialized metabolism in</i> Arabidopsis roc | below ground terpene | 10.4 |
| 12:30–01:30 | Lunch | Ballroom Courtyard & Grande Ballroom Fo | oyer |
| 12:30-01:30 | Funding for International Collaboration | | |

Chair: Wendy Boss

Promenade Ballroom

Chair: Mark Lange

| 01:30–02:00 | Arthur Neish Young Investigator Award Lecture Adrian D. Hegeman (University of Minnesota-Twin Cities) Improving the quantity and quality of LC- and GC-MS data for plant metabolomics | S10.7 |
|-------------|--|-------|
| 02:00–02:30 | Jörg Bohlmann (University of British Columbia) Chemical defence of conifers and bioenergy applications | S10.8 |

| 02:30-03:00 | Mami Yamazaki (Chiba University) Elucidation of biosynthetic pathway of camp | otothecin by metabolomics | S10.9 |
|-------------------|--|-----------------------------|-------------------|
| 03:00–03:15 | Daniel G. Vassão (Max Planck Institute for Tracing glucosinolate metabolism and deto: | | O10.5 s |
| 03:15–03:30 | Marcos Soto-Hernandez (Leiden Universit Phenolic acids in Catharanthus roseus anal approach of metabolomics | | O10.6 |
| 03:30–04:00 | Break E | Ballroom Courtyard & Grande | Ballroom Foyer |
| Chair: Michael H. | Beale | | |
| 04:00–04:30 | Kazufumi Yazaki (Kyoto University) Flavonoid-specific prenyltransferases, a me family responsible for polyphenol diversity | embrane-bound enzyme | S10.10 |
| 04:30–04:45 | Sangeeta Dhaubhadel (Agriculture and Ag Soybean 14-3-3 proteins: Are they involved isoflavonoid biosynthesis in soybean? | | O10.7 |
| 04:45–05:00 | Edmund M.K. Lui (University of Western C The study of North American ginseng metal | | O10.8 |
| 05:00–05:15 | Jun Yin (Shenyang Pharmaceutical University The metabolism of lignans from Fructus sch | | O10.9 |
| 05:15–05:30 | De-Yu Xie (North Carolina State University) Self-pollinated Artemisia annua plants form understand artemisinin biosynthesis | | O10.10 |
| 05:00–06:00 | Business Meeting | | |
| | | | Plaza Ballroom |
| 07:00–10:00 | Banquet 50th Anniversary Awards Celeb | ration | Salon, I, II, III |

WEDNESDAY 14 DECEMBER

| Concurrent Session 11: Phytochemistry/Phytomedicine | | | Plaza Ballroom |
|---|---|-----------------------------|-------------------|
| Chair: Daneel Fe | rreira | | |
| 01:30–02:00 | Yoshinori Asakawa (Tokushima Bunri U Liverworts-potential source of medicinal of | | S11.1 |
| 02:00–02:30 | Vanderlan da S. Bolzani (Universidade l Tracing secondary metabolites on Brazilia it usefully for find new biologically active of | an biodiversity: How to do | S11.2 |
| 02:30–03:00 | Massuo J. Kato (University of São Paulo Biflavonoid biosynthesis |)) | S11.3 |
| 03:00–03:15 | Johannes Westendorf (University Clinic Composition and chemical stability of iride citrifolia L. (noni) | | 011.1 |
| 03:15–03:30 | Jun Wu (South China Sea Institute of Oc Limonoids from mangrove plants of the X bioactivities | | 011.2 |
| 03:30–04:00 | Break | Ballroom Courtyard & Grande | Ballroom Foyer |
| Chair: Vanderlan | da S. Bolzani | | |
| 04:00–04:30 | De-an Guo (Shanghai Institute of Materia Modernization of traditional chinese medi opportunities | | S11.4 |
| 04:30–04:45 | Phila Raharivelomanana (Université de Multidisciplinary assessment of Wikstroer (Thymelaeacea) of Eastern Polynesia | | 011.3 |
| 04:45–05:00 | Norberto Peporine Lopes (Universidade The oxidative in vitro metabolism of lapace biomimetic models | | O11.4 |
| 05:00–06:00 | Business Meeting | | Plaza Ballroom |
| 07:00–10:00 | Banquet 50th Anniversary Awards Cele | ebration | Salon, I, II, III |

THURSDAY 15 DECEMBER

| 07:30-03:30 | Registration and Informati | on Table | | |
|-------------|-------------------------------|---------------------------------|---------------------|-------------|
| | | | Grande Ballroo | om Foyer |
| 08:00-10:00 | Concurrent Session 12: | Transcriptomics | | S12 |
| | | | Salon, I, II | |
| | Concurrent Session 13: | Biofuels and Bioproducts | | S13 |
| | | | Plaza Ballroom | |
| 10:00–10:30 | Break | | | |
| | | Ballroom Courtya | rd & Grande Ballroo | om Foyer |
| 10:30–12:30 | Concurrent Session 12: | Transcriptomics | | S12 |
| | | | Salon, I, II | |
| | Concurrent Session 13: | Biofuels and Bioproducts | | S13 |
| | | | Plaza Ballroom | |
| 12:30-01:30 | Lunch (on your own) | | | |
| 01:30-03:00 | Concurrent Session 12: | Transcriptomics | | S12 |
| | | | Salon, I, II | |
| | Concurrent Session 13: | Biofuels and Bioproducts | | S13 |
| | | | Plaza Ballroom | |
| 03:15–03:30 | Closing | | | |
| | | | S | alon, I, II |
| | | | | |

THURSDAY 15 DECEMBER Concurrent Session 12: Transcriptomics Salon, I, II Chair: Toni M. Kutchan 08:00-08:30 Gregory D. May (National Center for Genome Resources) S12.1 Complex metabolic pathway gene discovery via transcriptome analysis C. Robin Buell (Michigan State University) 08:30-09:00 S12.2 Genomics approaches for biochemical pathway discovery in medicinal plant species Ming-Che Shih (Academia Sinica, Agricultural Biotechnology Research 09:00-09:30 S12.3 Center) De novo assembly of expressed transcripts and construction of a transcriptome database of Phalaenopsis aphrodite 09:30-10:00 **Joaquim Vogt Margues** (Washington State University) S12.4 Transcriptome profiling of Podophyllum hexandrum tissues for genes in podophyllotoxin biosynthesis 10:00-10:30 Break Ballroom Courtyard & Grande Ballroom Foyer Chair: Cecilia A. McIntosh 10:30-11:00 Arthur Neish Young Investigator Award Lecture S12.5 Aruna Kilaru (East Tennessee State University) Insights into storage oil biosynthesis: Comparative transcriptomics of seed and non-seed tissues 11:00-11:30 Peter J. Facchini (University of Calgary) S12.6 Functional genomics using non-model plants and synthetic biosystems for gene discovery in specialized metabolism 11:30-11:45 **Ruifeng He** (Washington State University) 012.1 Transcriptomic and proteomic analysis of reed (Phragmites australis) rhizomes 11:45-12:00 Philipp Zerbe (University of British Columbia) 012.2 Discovery of diterpene biosynthetic pathways using targeted transcriptome analysis and functional characterization of genes and enzymes for metabolic engineering 12:00-12:15 Soheil Mahmoud (University of British Columbia, Okanagan) 012.3 Investigating essential oil metabolism in Lavandula by transcript profiling 12:30-01:30 Lunch (on your own) Chair: Peter J. Facchini 01:30-01:45 Priti Krishna (University of Western Ontario) 012.4 Transcriptome and metabolite analysis of polyunsaturated fatty acid-rich sea buckthorn (Hippophae rhamnoides) seed 01:45-02:00 Padmaja Nagabhyru (University of Kentucky) O12.5 Metabolite and gene expression studies in endophyte infected and

uninfected tall fescue under water deficit stress

| 02:00–02:15 | Jim Mattsson (Simon Fraser University) A genomics approach to gene discovery related to biosynthesis of thujone in western redcedar (Thuja plicata) | O12.6 |
|-------------|---|--------------|
| 02:15–02:30 | Nobuaki Suzuki (Osaka University) <i>EST analysis of trans-rubber producing plant,</i> Eucommia ulmoides <i>Oliver</i> <i>and identification of candidate genes in trans-1,4-polyisoprene production</i> | O12.7 |
| 02:30–02:45 | Wen Zheng (Zhejiang University) Comparative proteomic study reveals the biosynthesis of coumarins in leaves of Clematis terniflora upon UV radiation | O12.8 |
| 03:15–03:30 | Closing | Salon, I, II |

THURSDAY 15 DECEMBER

| Concurrent Session 13: Biofuels and Bioproducts | | | | |
|---|--|----------------|--|--|
| Chair: Laurence B. Davin | | | | |
| 08:00–08:30 | Katrina Cornish (Ohio State University) <i>Biopolymers, bioproducts and biofuels in alternate rubber-producing</i> <i>species</i> | S13.1 | | |
| 08:30–09:00 | Debra Mohnen (University of Georgia) Advances in understanding plant cell wall pectin synthesis and structur and impact on the biofuel industry | S13.2 re | | |
| 09:00–09:30 | Simone Ferrari (Sapienza Università di Roma) Pectin modification improves utilization of plant biomasses to biofuel conversion | S13.3 | | |
| 09:30–10:00 | Michael E. Himmel (National Renewable Energy Laboratory) Engineering improved cellulases for biofuels production | S13.4 | | |
| 10:00–10:30 | Break Ballroom Courtyard & Grande B | Ballroom Foyer | | |
| Chair: Katrina Cornish | | | | |
| 10:30–11:00 | Alessandra Devoto (Royal Holloway, University of London) Engineering plant cell walls for second generation biofuel production | S13.5 | | |
| 11:00–11:30 | Simo Sarkanen (University of Minnesota) Common themes in lignin biosynthesis and lignin biodegradation | S13.6 | | |
| 11:30–12:00 | Art J. Ragauskas (Georgia Institute of Technology) Lignin: The new paradigm in biofuels | S13.7 | | |
| 12:00–12:15 | David Dalton (Reed College) Biopolymer production in transgenic poplar | O13.1 | | |
| 12:15–12:30 | Hong Yang (Washington State University) NAC domain transcription factors and secondary wall formation control in poplar (Populus trichocarpa) | 013.2 | | |
| 12:30-01:30 | Lunch (on your own) | | | |
| Chair: Simo Sarkanen | | | | |
| 01:30–02:00 | Richard A. Dixon (Samuel Roberts Noble Foundation) <i>Metabolic</i> versus <i>transcriptional control targets for lignin modification</i> | S13.8 | | |
| 02:00–02:30 | Toshiaki Umezawa (Kyoto University) Characterization of lignin and related compounds of Erianthus ravenna | S13.9 ae | | |
| 02:30–03:00 | Norman G. Lewis (Washington State University) Northwest Advanced Renewable Alliance (NARA) and the quest for biofuels/petrochemical substitutes | S13.10 | | |
| 03:00–03:15 | Samuel Lopez-Nieves (University of New Mexico) Interactions between the CO ₂ concentrating mechanism and lipid production of two species of algae: Chlamydomonas reinhardtii and Nannochloropsis salina | O13.3 | | |
| 03:15–03:30 | Closing | Salon, I, II | | |
| | | | | |

Poster Session and Student/Postdoctoral Fellow Competition

Salon III & Ballroom Courtyard

Odd numbers, Sunday December 11 and even numbers, Monday December 12.

Natural Products in Agriculture

- NPA 1 **Monique Lacroix** (INRS-Institut Armand-Frappier, Canada) Umu applied for screening herb and plant extracts or pure phytochemicals for antimutagenic activity
- NPA 2 **Heinui Philippe** (Université de la Polynésie Française, French Polynesia) Insecticidal activity of Derris malaccensis from French Polynesia
- NPA 3 **Robert Hollingsworth** (U.S. Pacific Basin Agricultural Research Center, USA) Repellence of essential oils to Frankliniella occidentalis as affected by type of oil and polymer release
- NPA 4 Christopher French (Agriculture & Agri-Food Canada, Canada) Inhibition of potato virus X infectivity by extracts of green tea
- NPA 5 **Julie Ann Luiz Adrian** (University of Hawaii at Hilo, USA) Analysis of guava as a forage - Organic constituents
- NPA 6 **Munkhtsetseg Tsednee** (Agricultural Biotechnology Research Center, Taiwan) Novel phytosiderophores in barley
- NPA 7 **Hanhong Bae** (Yeungnam University, South Korea) Trichoderma *produces antifungal metabolites that inhibit mycelial growth of* Phytophthora *spp.*
- NPA 8 **Patricia Ríos-Chávez** (Universidad Michoacana de San Nicolás de Hidalgo, Mexico) *Effect of* Heliopsis longipes *extracts on* Mycosphaerella fijiensis *Morelet*
- NPA 9 **Rhodesia M. Celoy** (University of Arizona, USA) (+)-Pisatin biosynthesis: From (–)-enantiomeric intermediates to a (+)-derivative
- NPA 10 **Glenn W. Turner** (Washington State University, USA) Improving peppermint essential oil yield and composition through metabolic engineering

Chemoprevention

- C 1 **Suzanna M. Zick** (University of Michigan Medical School, USA) Regulation of ginger root extract on colonic inflammatory signaling in human with normal and high risk of colon cancer
- C 2 **Ryuta Inagaki** (Gifu University, Japan) Synthesis of naturally-occurring furonaphtoquinones and cytotoxicity against HL-60
- C 3 **Tamara P. Kondratyuk** (University of Hawaii at Hilo, USA) Resveratrol derivative (E)-4-(3,5-dimethoxystyryl)aniline is a novel inhibitor of cancer cell invasion
- C 4 **Isela Alvarez-Gonzalez** (Escuela Nacional de Ciencias Biologicas, Mexico) Evaluation of blueberry juice on the precarcinogenic lesions induced by azoxymethane in mouse
- C5 **Laura Marler** (University of Hawaii at Hilo, USA) Thiazole and thiadiazole derivatives of resveratrol as inducers of quinone reductase 1

- C 6 **Tamara P. Kondratyuk** (University of Hawaii at Hilo, USA) Suppression of 12-O-tetradecanoyl-phorbol-13-acetate-induced ornithine decarboxylase activity by resveratrol derivatives
- C 7 **Eun-Jung Park** (University of Hawaii at Hilo) Inhibitory effect of a callophycin A derivative on iNOS expression in lipopolysaccharidestimulated RAW 264.7 cells
- C 8 Hyung Sik Kim (Pusan National University, South Korea) Psammaplin A induces autophagy cell death in doxorubicin-resistant human breast cancer MCF-7/Adr cells
- C 9 **Eun-Jung Park** (University of Hawaii at Hilo, USA) Inhibition of lipopolysaccharide-induced cyclooxygenase-2 and inducible nitric oxide synthase by epimuqubilin a in RAW 264.7 cells

Biochemistry/Enzymology

- B 1 **Trevor H. Yeats** (Cornell University, USA) An extracellular acyltransferase catalyzes cutin polymerization
- B 2 **Keiske Asada** (Nagoya City University, Japan) Molecular cloning and characterization of an iridoid 1-O-glucosyltransferase involved in secologanin biosynthesis
- B 3 **Deborah Hayford** (East Tennessee State University, USA) Heterologous expression and characterization of recombinant putative glucosyltransferase clone 3 from grapefruit (Citrus paradisi)
- B 4 **Fabio C. Chaves** (University of São Paulo, Brazil) Peperomins, chromenones, fungal endophytes and more from Peperomia glabella var. nervulosa
- B 5 **Trinh-Don Nguyen** (University of Calgary, Canada) Probing chemical evolution and diversification in the sunflower family (Asteraceae)
- B 6 **Anye Wamucho** (East Tennessee State University, USA) Heterologous expression in yeast and biochemical characterization of recombinant putative glucosyl-transferase 9 from Citrus paradisi
- B 7 **I. Josefina Flores-Sanchez** (Washington State University, USA) Inhibition of hydroxycinnamoyl-CoA thioesterases from ginger plants (Zingiber officinale)
- B 8 **Alexander W. Chassy** (University of California at Davis, USA) *Tracking the biosynthesis of* ¹³C-labeled grape phenolics in situ
- B 9 **Mark A. Willis** (Washington State University, USA) Two classes of enzymes involved in the biosynthesis of curcuminoids and other diarylheptanoids in ginger and turmeric
- B 10 **Yoshihiko Shimokawa** (The University of Tokyo, Japan) A structure-based mechanism for benzalacetone synthase from Rheum palmatum
- B 11 **Taiji Nomura** (Toyama Prefectural University, Japan) Molecular characterization of tuliposide a-converting enzyme in tulip
- B 12 **Natsajee Nualkaew** (Khon-Kaen University, Thailand) Cloning, characterization and site-directed mutagenesis of Garcinia mangostana benzophenone synthase
- B 13 **Joseph Lynch** (Washington State University, USA) Recombinant expression and characterization of an Arabidopsis thaliana FAD synthetase

- B 14 **Thaniya Wunnakup** (Chulalongkorn University, Thailand) Cloning and characterization of aromatic prenyltransferase genes from Thai medicinal plants
- B 15 **Juraithip Wungsintaweekul** (Prince of Songkla University, Thailand) Methyl jasmonate and yeast extract stimulate mitragynine production in shoot culture of Mitragyna speciosa
- B 16 Shingo Kawai (Shizuoka University, Japan) Diarylheptanoids, myricanol, biosynthesis in Myrica rubra: Incorporation experiments of phydroxycinnamic acid derivatives
- B 17 **Worapan Sitthithaworn** (Chulalongkorn University, Thailand) Expression of 1-deoxy-D-xylulose 5-phosphate synthase, 2C-methyl-D-erythritol 4-phosphate synthase and geranylgeranyl diphosphate synthase, key enzymes of plaunotol biosynthesis in Croton stellatopilosus
- B 18 **Rod Mitchell** (University of Calgary, Canada) Identification and characterization of diterpene synthases in the salvinorin a biosynthetic pathway
- B 19 **Tossaton Charoonratana** (Prince of Songkla University, Thailand) *Mitragynine biosynthesis: Metabolite profiling and mRNA expression of the early steps gene in* Mitragyna speciosa
- B 20 **Camilla Knudsen** (University of Copenhagen, Denmark) Specialized roles for the two UDP-glucosyltransferases UGT85K2 and UGT85K3 in hydroxynitrile glucoside metabolism in Lotus japonicus
- B 21 **Sarah E. Brewer** (Washington State University, USA) Differential expression patterns of arogenate dehydratase genes (ADT1-ADT6) in Arabidopsis thaliana
- B 22 **Tina Frisch** (University of Copenhagen, Denmark) Functional evolution of P450s: The biosynthesis of alliarinoside in Alliaria petiolata
- B 23 **Daniel K. Owens** (East Tennessee State University, Biological Sciences, USA) Biochemical analysis of a putative limonoid glucosyltransferase from Citrus paradisi
- B 24 **Kwang-Hoon Kong** (Chung-Ang University, South Korea) Catalytic site of plant glutathione S-transferase, a herbicide detoxification enzyme

Botanicals/Medicinals

- BOT 1 **Petras Rimantas Venskutonis** (Kaunas University of Technology, Lithuania) Phytochemical composition and antioxidant properties of some herbs grown in Lithuania
- BOT 2 **Petras Rimantas Venskutonis** (Kaunas University of Technology, Lithuania) Phytochemical characterisation of highbush blueberry (Vaccinium × covilleanum) and European cranberry bush (Viburnum opulus) accessions grown in Lithuania
- BOT 3 **Jaspreet Kaur Sihra** (University of Surrey, United Kingdom) Novel triterpenoid derivatives from Eucomis bicolor (Hyacinthaceae: Hyacinthoideae)
- BOT 4 **Amy Keller** (Colorado State University, USA) *Fuzhuan tea: Novel phytochemicals and initial investigations of a fermented preparation of* Camellia sinensis
- BOT 5 **Rocky Graziose** (Rutgers University, USA) Antiparasitic compounds from Cornus florida with activities against Plasmodium falciparum and Leishmania tarentolae

- BOT 6 **Choong Je Ma** (Kangwon National University, South Korea) Neuroprotective compounds isolated from the methanolic extract of Lonicera japonica
- BOT 7 **Chieu Anh Ta** (University of Ottawa, Canada) Inhibition of quorum sensing and biofilm formation by tropical plants
- BOT 8 **Brendan Walshe-Roussel** (University of Ottawa, Canada) Ethnopharmacology of anti-inflammatory botanicals used by the Q'eqchi' Maya of Belize
- BOT 9 **James Doughari Hamuel** (Cape Peninsula University of Technology, South Africa) Antioxidant, antimicrobial and antiverotoxic potentials of extracts of *Curtisia dentata*
- BOT 10 **Olayinka Ayobami Aiyegoro** (North-West University, South Africa) The antioxidant properties, cytotoxicity and monoamine oxidase inhibition abilities of the crude dichloromethane extract of *Tarchonanthus camphoratus* leaves
- BOT 11 Arnason John T. (University of Montreal, Canada) Anti-diabetic potentials of ethanol and water extracts of 17 plants used by the Eeyou Istchee Cree first nations of Northern Quebec
- BOT 12 **Chang-Gue Son** (Daejeon University, South Korea) Myelophil, an extract mix of Astragali Radix and Salviae Radix, ameliorates restrain-induced stress in mice model
- BOT 13 **Mun-Chual Rho** (Korea Research Institute of Bioscience and Biotechnology, South Korea) Phenolic compounds isolated from Psolalea coryifolia inhibit IL-6 induced STAT3 activation
- BOT 14 **Scott Baggett** (Bionovo, USA) Isolation of flavonoid di-C-glycosides from Nelumbo nucifero and their structural determination
- BOT 15 Lee Shu-Chen (National Taiwan University, Taiwan) Alleviating medicine usage and improving pulmonary function with supplements of vegetable and fruit concentrate, fish oil, and probiotics in asthmatic school children
- BOT 16 **Scott Baggett** (Bionovo, USA) Comparing a phenomenex Luna HPLC-DAD method versus a Phenomenex Kinetex UPLC-DAD method for raw material quality control for Menerba
- BOT 17 **Chih-Chun Wen** (China Medical University, Taiwan) *Herbal extracts of* Cibotium barometz, Gentiana scabra, Dioscorea batatas, Cassia tora, *and* Taxillus chinensis *inhibit SARS-CoV replication*
- BOT 18 **Niladri Maity** (Jadavpur University, India) Anti-wrinkle potential of standardized flower extract of Calendula officinalis
- BOT 19 **Worrawat Promden** (Chulalongkorn University, Thailand) *Mushroom tyrosinase inhibition and antioxidant properties of* Dalbergia parviflora
- BOT 20 **Mao Jiang** (University of Western Ontario, Canada) American ginseng acutely regulates contractile function of rat heart
- BOT 21 **Ning-Sun Yang** (Academia Sinica, Agricultural Biotechnology Research Center, Taiwan) The anti-inflammatory effect of specific medicinal plant extracts in DSS-induced colitis model
- BOT 22 Chatchai Chaotham (Chulalongkorn University, Thailand) Antioxidant effect of plaunotol in human renal cells: HK-2
- BOT 23 **Ji Su Kim** (Gyeongsang National University, South Korea) Insecticidal activity of Korean medicinal plant extracts against Myzus persicae
- BOT 24 **Despina Harbilas** (Université de Montréal, Canada) AD03: An alternative treatment to improving hyperglycemia and hyperinsulinemia in a dietinduced obese model

- BOT 25 **Rak-Hun Jeong** (Kyung Hee University, South Korea) Inhibition effect of flavonolignans and lignan glycosides from the aerial parts of Oryza sativa on NO production and tyrosinase activity
- BOT 26 **Mariano Lusakibanza Manzo** (University of Liege, Belgium) In vitro *antiplasmodial and cytotoxic activity of physalin B, epoxyphysalin B from* Physalis angulata
- BOT 27 **Sisay Girmay** (University of Hawaii at Hilo, USA) *Cytotoxic constituents of* Stemphylium solani, *a fungal endophyte of* Morinda citrifolia (*noni*)
- BOT 28 **Chike Godwin Azike** (University of Western Ontario, Canada) Anti-inflammatory effect of North American (NA) ginseng
- BOT 29 Araceli Pérez-Vásquez (Universidad Nacional Autónoma de México, Mexico) Chemical composition of the infusions from the stembark and leaves of Exostema caribaeum
- BOT 30 **Orawan Monthakantirat** (Khon Kaen University, Thailand) Four novel flavonoids from Dalbergia parviflora with the potential to estrogenic and antiestrogenic activities
- BOT 31 **Viridiana Morales-Sánchez** (Universidad Nacional Autónoma de México, Mexico) Development of quality control parameters for the medicinal orchids Cyrtopodium macrobulbon and Scaphyglottis fasciculata
- BOT 32 **Luiz Fernando Pereira** (Pontifical Catholic University of Parana, Brazil) Chorioallantoic membrane (CAM) of chick embryo assay to aqueous extract of Pteridium aquilinum evaluation
- BOT 33 **Pimpimon Tansakul** (Prince of Songkla University, Thailand) Corosolic acid production from Lagerstroemia speciosa callus
- BOT 34 **Choong Je Ma** (Kangwon National University, South Korea) Neuroprotective effect of seed of Lotus plumule in the mouse hippocampal HT22 cell line
- BOT 35 **Varima Wongpanich** (Khon Kaen University, Thailand) Marine natural products as ingredients in traditional medicine
- BOT 36 **Monique Lacroix** (INRS-Institut Armand-Frappier, Canada) Antimicrobial and potential cancer preventive substances from marine algae and cyanobacteria collected in Hawaii and the Caribbean
- BOT 37 **Kanichiro Ishiuchi** (Washington State University, USA) Candidate huperzine A and other Lycopodium alkaloids in cultured cells of Huperzia species
- BOT 38 **Valdir F. Veiga Junior** (Universidade Federal do Amazonas, Brazil) Hypolipidemic activity of triterpenes from Burseraceae oleoresins
- BOT 39 **Hyun-Mee Oh** (Korea Research Institute Bioscience and Biotechnology, South Korea) Cell adhesion inhibitory activities of stilbene derivatives isolated from Rheum undulatum
- BOT 40 **Changmu Kim** (National Institute Biological Resources, South Korea) Sargassumol, a novel antioxidant from the brown alga Sargassum micracanthum
- BOT 41 **JinA Ko** (Korea Research Institute of Bioscience and Biotechnology, South Korea) *In vitro* inhibitory activity of *Ecklonia cava* against porcine epidemic diarrhea coronavirus infection
- BOT 42 **Gail B. Mahady** (University of Illinois, USA) Reduction of OVA-induced lung inflammation in mice treated with Ayurvedic herbs using 2-D and 3-D imaging
- BOT 43 **Hui-Ting Chang** (National Taiwan University, Taiwan) Effect of Abies kawakamii *leaf extracts on life span extension in* Drosophila melanogaster

- BOT 44 **Young Bae Ryu** (Korea Research Institute of Bioscience and Biotechnology, South Korea) *Tanshinones from* Salvia miltiorrhiza *displaying potent 3CL*^{pro} *inhibition*
- BOT 45 **Young Min Kim** (Korea Research Institute Bioscience and Biotechnology, South Korea) Characteristic of viral neuraminidases inhibitory homoisoflavonoids from Caesalpinia sappan

New Characterization Methods

- NCM 1 **Marya Aziz** (McGill University, Canada) Recovery of endogenous phenolic compounds from potato tuber using conventional and highpressure extraction methods
- NCM 2 **Ian Castro-Gamboa** (São Paulo State University, Brazil) How to separate the wheat from the chaff? Exploring Brazilian biodiversity using NMR dereplication techniques
- NCM 3 **Ivette Guzman** (North Carolina State University, USA) Simultaneous extraction and quantification of carotenoids and tocopherols in Brassica species
- NCM 4 **Kimberly L. Colson** (Bruker BioSpin, USA) Detection of adulterated natural product extracts containing sildenafil (Viagra) derivatives

Natural Product Drug Discovery

- NPD 1 Senthilkumar Ravichandran (Bharathiar University, India) Hepatoprotective activity of Rhodiola imbricata acetone extract against Paracetamol induced hepatopathy in Wistar rats
- NPD 2 **Onyekachi Ogbonnaya Iroanya** (University of Lagos, Nigeria) Nephro-hepatoprotective and antioxidant properties of a triherbal formulation
- NPD 3 **Dovi Kelman** (University of Hawaii at Hilo, USA) The antioxidative and antimicrobial roles of associated fungi of the lichen Usnea australis from Hawaii
- NPD 4 **Dovi Kelman** (University of Hawaii at Hilo, USA) Antioxidant activity of Hawaiian lichens
- NPD 5 **Emmanuel Olofu Ogbadoyi** (Federal University of Technology, Nigeria) Evaluation of therapeutic effects of Nigerian medicinal plants in experimental African trypanosomiasis
- NPD 6 **Catherine P. Tunbridge** (University of Surrey, United Kingdom) COX-2 specific inhibitors from Ledebouria ovatifolia and Ledebouria socialis (Hyacinthaceae:Hyacinthoideae)
- NPD 7 **Jun-Ran Kim** (Seoul National University, South Korea) *Microtitre plate-based antibacterial assay towards* Asarum heterotropoides *active principles against human intestinal bacteria*
- NPD 8 **Young Ho Kim** (Chungnam National University, South Korea) Ginsenosides from heat-processed Korean ginseng roots, leaves and flower buds
- NPD 9 **Cristina Theodulo**z (Universidad de Talca, Chile) New diterpene and heterocycles hybrid compounds: Synthesis and gastroprotective mechanisms of action using human cell cultures
- NPD 10 **Marco A Botelho** (University Potiguar, Brazil) A nanostructured gel of Lippia sidoides essential oil

- NPD 11 **Omonike Oluyemisi Ogbole** (University of Ibadan, Nigeria) Evaluation of anti-poliovirus activity of medicinal plants selected from Nigerian ethnomedicine
- NPD 12 **Chih-Chun Wen** (Agricultural Biotechnology Research Center, Academia Sinica, Taiwan) Novel quinolone CMQ induces apoptosis and mitotic catastrophe in prostate cancer cells via reactive oxygen species- and mitochondria-dependent pathways
- NPD 13 **Tonibelle N. Gatbonton-Schwager** (Case Western Reserve University, USA) A mechanistic analysis of bryonolic acid transcriptional control: Perturbation of inflammatory and antioxidant genes in vitro and in vivo
- NPD 14 **Ajudhia Nath Kalia** (ISF College of Pharmacy, India) Antidiabetic active fractions from Momordica balsamina fruit pulp
- NPD 15 **Narinthorn Khositsuntiwong** (Chiang Mai University, Thailand) Melanin production enhancement of human tyrosinase plasmid (pAH7/Tyr) by TAT and an entrapment in elastic cationic niosomes
- NPD 16 **Mauro Cafundo Morais** (São Paulo State University UNESP, Brazil) Synthesis of alkaloids from Pterogyne nitens and its antitumoral activity in nude mice BALB/C
- NPD 17 **Sang Kook Lee** (Seoul National University, South Korea) *WNT/β-catenin signaling mediates the antitumor activity of magnolol in colorectal cancer cells*
- NPD 18 **Jin-Kyu Kim** (Hallym University, South Korea) Constituents of Chamaecyparis obtusa with inhibitory activity on aldose reductase and sorbitol accumulation
- NPD 19 Adnan Jathlan Al-Rehaily (King Saud University, Central Kingdom of Saudi Arabia) Resorcinol and flavonoids compounds from Ononis natrix
- NPD 20 **Patricio Rojas-Silva** (Rutgers, The State University of New Jersey, USA) Isolation of sesquiterpene lactones from roots of Cichorium intybus with leishmanicidal activity
- NPD 21 **Ji-Hae Park** (Kyung Hee University, South Korea) Metabolites from the silkworm (Bombyx mori) droppings promote the activity of HO-1 and SIRT1
- NPD 22 **Che-Yi Lin** (Medical and Pharmaceutical Industry Technology and Development Center, Taiwan)

A botanic lead of attention deficit hyperactivity disorder

- NPD 23 **Mariano Walter Pertino** (Universidad de Talca, Chile) 1,2,3-Triazole-substituted oleanolic acid derivatives: Synthesis and antiproliferative activity
- NPD 24 **Mariano Walter Pertino** (Universidad de Talca, Chile) New dimeric diterpenes: Synthesis and antiproliferative activity
- NPD 25 **Jin Hee Kim** (Daegu Hanny University, South Korea) Haginin A, a PTP1B inhibitor from the branch of Lespedeza cyrtobotrya, stimulates glucose uptake through AMPK activation in skeletal muscle cells
- NPD 26 Ha-Na Youn (Konkuk University, South Korea) Antiviral activities of the aqueous extract from peat moss against influenza virus in vitro and in vivo
- NPD 27 Young Eun Jeon (Hallym University, South Korea) Ecklonia cava extract prevents aggregation of β-amyloid and reduces β-amyloid mediated neuronal death
- NPD 28 **Xing Fu Yin** (Hallym University, South Korea) Nelumbo nucifera *rhizome extract ameliorates the scopolamine-induced reductions of cell proliferation, neuroblast differentiation and BDNF levels*

- NPD 29 **Dianqing Sun** (University of Hawaii at Hilo, USA) Antimicrobial evaluation of a focused naringenin and resveratrol chemical library
- NPD 30 **Pervin Basaran** (Suleyman Demirel University, Turkey) Production and identification of biologically active compounds of fungi Aspergillus spp.
- NPD 31 **Dan Bi Choi** (Hallym University, South Korea) Neuroprotective effects of ethyl acetate extract of Codonopsis lanceolata on ischemic damage in gerbil
- NPD 32 **Ui Joung Youn** (University of Hawaii at Hilo, USA) Biologically active constitutes from the flower of Vernonia cinerea
- NPD 33 **Young Woo Kim** (Daegu Haany University, South Korea) Inhibition of SREBP-1C-dependent hepatic steatosis by sauchinone, an AMPK-activating lignan in Saururus chinensis
- NPD 34 Leng Chee Chang (University of Hawaii at Hilo, USA) Evaluation of smoking cessation agents from natural products
- NPD 35 Leng Chee Chang (University of Hawaii at Hilo, USA) Cytotoxic dihydrobenzofurans from Mitrephora wangii
- NPD 36 **Veronique Guillemard** (IRD, Natural Product and COREUS Research Units, New Caledonia) Uncovered therapeutic potential of marine red algae from New Caledonia
- NPD 37 **Christoph Eibl** (University of Hawaii at Hilo, USA) Development of potential CNS therapeutics derived from the alkaloid cytisine
- NPD 38 **Jun Lee** (Korea Institute of Oriental Medicine, South Korea) Anti-proliferative dineolignans from Saururus chinensis against human cancer cell lines
- NPD 39 **Young Woo Kim** (Daegu Haany University, South Korea) Inhibition of LXRα-mediated hepatic steatosis by liquiritigenin, a licorice flavonoid, in associated with NRF2 activation
- NPD 40 **Mi Jeong Jo** (Daegu Haany University, South Korea) Resveratrol prevents mitochondrial dysfunction through PARP-LKB1 activation
- NPD 41 **Sang Chan Kim** (Daegu Haany University, South Korea) Anti-inflammatory effects of Isatidis radix and its active component, tryptanthrin in lipopolysaccharide-activated RAW264.7 macrophage cells
- NPD 42 **Sang Mi Park** (Daegu Haany University) Red ginseng extract prevents CCI₄-induced liver fibrosis

Metabolism/Metabolomics

- M 1 **Carolina Cieniak** (University of Ottawa, Canada) *Traditional Cree anti-diabetic medicine: Advanced metabolomic assessment in experimental models of diabetes and Alzheimer's disease*
- M 2 **Kim Jin-Hee** (Seoul National University, South Korea) Analysis of Ginkgo biloba levopimaradiene synthase (LPS) promoter in Arabidopsis thaliana
- M 3 **Siau Sie Voo** (Washington State University, USA) Unraveling the biosynthetic capacity of monoterpenes in specialized epithelial cells of grapefruit peel
- M 4 **Dong Sik Yang** (The Samuel Roberts Noble Foundation, USA) Metabolite profiling of triterpene saponins in Medicago truncatula core collection

- M 5 **Guillaume Beaudoin** (University of Calgary, Canada) Virus-induced gene silencing of cytochromes P450 putatively involved in alkaloid biosynthesis in opium poppy
- M 6 **Purnima Ashok** (KLE University's College of Pharmacy, India) Bioavailability studies of vitamin C in Amla and its combinations
- M 7 **Sunghyouk Park** (Inha University, South Korea) Combined genomic-metabolomic approach for the differentiation of geographical origins of natural products
- M 8 **Claudia L. Cardenas** (Washington State University, USA) Arabidopsis *fiber–reduced* (snd1/nst1) *mutants: Nanoindentation probing of mechanical properties in distinct cell wall types*
- M 9 **Min-Kyoung Kang** (Seoul National University, South Korea) *Promoter analysis of multi copy* Ginkgo biloba 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate reductase (IDS) gene
- M 10 **Mi Kwon** (Korea University, South Korea) Functional characterization of Medicago truncatula MYB transcription factors: MtMYB2 and MtMYB70
- M 11 **Thais Guaratini** (University of São Paulo, Ribeirão Preto, Brazil) *Grandisin* in vitro *metabolism using biomimetical models*
- M 12 **Kiva Ferraro** (University of Calgary, Canada) Pisum sativum *is a novel bioinformatics platform to study proanthocyanidin biosynthesis*
- M 13 Alan David Budgeon (Washington State University, USA) Metabolomic consequences in arogenate dehydratase and NAC transcription factor modified Arabidopsis thaliana
- M 14 **Zhaoyang Wei** (Washington State University, USA) Characterization of Arabidopsis thaliana serine hydroxymethyltransferases from mitochondria and the cytosol
- M 15 **Nanna Bjarnholt** (University of Copenhagen, Denmark) Merger of primary and secondary metabolism: endogenous turnover pathway of cyanogenic glucosides enables sorghum to channel nitrogen from defense compounds into primary metabolism
- M 16 **Ara Kirakosyan** (The University of Michigan, USA) *Bioavailability of tart cherry anthocyanins*
- M 17 **Stephen C. Grace** (University of Arkansas at Little Rock, USA) Metabolomic analysis of antioxidant phytonutrients in the high pigment (hp-1, hp-2) photomorphogenic mutants of tomato
- M 18 **Milen Ivanov Georgiev** (Leiden University, The Netherlands) Metabolomics and pharmacognosy of mulleins (Verbascum)

Phytomedicine/Phytochemistry

- P 1 **Hou-Wen Lin** (Second Military Medicinal University, China) Bioactive secondary metabolites from the Xisha marine sponges
- P 2 **João M. Batista Jr.** (São Paulo State University UNESP, Brazil) Absolute configuration determination of complex chiral natural product molecules using vibrational circular dichroism (VCD): a case study
- P 3 **Minoru Ueda** (Tohoku University, Japan) Bioorganic studies on jasmonate glucoside, a putative trigger for ion channel activation in plant

- P 4 **Dorota A. Nawrot** (University of Surrey, United Kingdom) Chemical constituents of east European forest species
- P 5 **Guillermo Schmeda-Hirschmann** (Universidad de Talca, Chile) *Resin diterpenes from* Austrocedrus chilensis
- P 6 **Haribalan Perumalsamy** (Seoul National University, South Korea) Larvicidal activity of compounds identified in Pongamia pinnata seed against Aedes aegypti and Culex pipiens pallens
- P 7 **Bohyoung Lee** (Kangwon National University, South Korea) Quantitative analysis of compounds in fermented Insampaedok-san and its neuroprotective activity in HT22 cells
- P 8 **Choong Je Ma** (Kangwon National University, South Korea) Quantitative analysis of the eight major compounds in the Samsoeum using a HPLC coupled with DAD and ESI-MS
- P 9 Young-Soo Bae (Kangwon National University, South Korea) Polyphenolic secondary metabolites from Juglans mandshurica
- P 10 **Masayuki Ninomiya** (Gifu University, Japan) Chalcone glycosides from Brassica rapa and their synthetic analogues inhibit LPS-induced NO production
- P 11 **Swapan Pramanick** (University of Hawaii at Hilo, USA) *Phytochemical study of the native Hawaiian plant,* Metrosideros polymorpha
- P 12 **Guillermo Schmeda-Hirschmann** (Universidad de Talca, Chile) Direct identification of phenolic constituents in Fabiana imbricata infusions by HPLC-DAD and HPLC-MSN
- P 13 **Benjamin R. Clark** (University of Hawaii at Hilo, USA) *Pigment analysis of spathes in* Anthurium *species*
- P 14 **Kwang-Jin Lee** (University of Hawaii at Hilo, USA) *Quantitative analysis of curcumin and related compounds in* Curcuma longa *by HPLC and LC-MS*
- P 15 **Atsuyoshi Nishina** (Yonezawa Women's Junior College, Japan) Effect of Gyojya-na extracts on UVB-induced apoptosis in HaCat cell
- P 16 **Se-Yeong Park** (Kangwon National University, South Korea) A tetrasaccharide isolated from the fruits of Taxus cuspidata
- P 17 **Heiko Rischer** (VTT Technical Research Centre, Finland.) Differential lipid profiles of microalgae co-cultivated with marine bacteria
- P 18 **Morio Yoshimura** (Matsuyama University, Japan) Diarylheptanoid sulfates and related compounds from Myrica rubra bark
- P 19 **Caroline Cristina Fernandes-Silva** (University of São Paulo, Brazil) Chemical characterization of three samples of Brazilian propolis by NMR and GC/MS
- P 20 **Dorota A Nawrot** (University of Surrey, United Kingdom) Coumarins from the Malagasy Cedrelopsis rakotozafyi (*Ptaeroxylaceae*)
- P 21 **Takashi Yoshida** (Matsuyama University, Japan) Phenolic compounds from sunflower (Helianthus annuus) seeds
- P 22 An Keun Kim (Sookmyung Women's University, South Korea) Luteolin enhances tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of SK-BR3 human breast cancer

- P 23 **Dianqing Sun** (University of Hawaii at Hilo, USA) Syntheses of macrocyclic engelhardione analogs as potential antituberculosis agents
- P 24 **Bernhard W.G. Vogler** (University of Alabama in Huntsville, USA) Isolation, characterization, and bioactivities of prenylated isoflavonoids from Rhynchosia edulis
- P 25 **Jong Hwan Kwak** (Sungkyunkwan University, South Korea) Phenolic glycosides from the stem of Stewartia pseudocamellia
- P 26 **Juan Diego Hernández** (Universidad Michoacana de San Nicolás de Hidalgo, México) Lignan derivatives from polar extracts from stems and dried exudate of five species of burseraceae
- P 27 **Valdir F. Veiga Junior** (Universidade Federal do Amazonas, Brazil) *A flavonoid glycoside from* Eperua glabriflora
- P 28 **Mamoru Koketsu** (Gifu University, Japan) Isolation of secondary metabolites from microalgae
- P 29 **Meri Emili Pinto Ferreira** (São Paulo State Univeristy "Júlio de Mesquita Filho", Brazil) A cyclopeptide isolated from Jatropha ribifolia and its solid phase synthesis
- P 30 **Rosa del Rio** (Universidad Michoacana de San Nicolás de Hidalgo, México) *A thymol derivative from* Ageratina glabrata
- P 31 **Hamzeh Amiri** (Lorestan University, Iran) Essential oil composition and secretory structures of Grammosciadium scabridium
- P 32 **Sung-Joon Lee** (Korea University, South Korea) *T-FBS-02 is a liver X receptor-α ligand and stimulates reverse cholesterol transport without inducing hepatic lipogenesis*
- P 33 **Osman Elhady Mosa** (El Neelain University, Sudan) Preliminary phytochemical evaluation and seed proximate analysis of Surib (Sesbania leptocarpa)-Sudan
- P 34 **Debora Esposito** (Rutgers University, USA) Brassinosteroid decreases hyperglycemia in a diet-induced obesity mouse model
- P 35 **William N. Setzer** (University of Alabama in Huntsville, USA) Caffeine attenuates the cytotoxic activities of intercalating aromatic alkaloids
- P 36 **Debora Esposito** (Rutgers University, USA) Wound healing activity of phytoecdysteroids
- P 37 **Jose Angel Marañon** (Tradichem Biotech., Spain) Polyphenol synergism augments therapeutic potential of resveratrol: Updating of the French paradox
- P 38 **Kamal D. Moudgil** (University of Maryland School of Medicine, USA) Celastrus aculeatus *suppresses autoimmune arthritis in rats by inhibiting pathogenic T-cell and antibody responses*
- P 39 **Chang-Gue Son** (Daejeon Oriental Hospital of Daejeon University, South Korea) *Comparison of anti-hepatofibrotic effect:* Artemisia capillaries *vs.* Artemisia iwayomogi
- P 40 **Marco Antonio Botelho** (Federal Institute of Science and Technology, Brazil) Nanotechnology in phytomedicines
- P 41 **Misagh Alipour** (Northern Ontario School of Medicine, Canada) Synergistic effects between an aqueous extract of Panax quinquefolius and tobramycin against Pseudomonas aeruginosa in a rat model of lung infection

- P 42 **Chinedu Athanasius Eze** (University of Nigeria, Nigeria) The anti-ulcer activities of the oil extract of Balanites aegytiaca seed in guinea pigs
- P 43 **Bohyoung Lee** (Kangwon National University, South Korea) The alteration of components in the fermented Hwangryunhaedok-tang and its neuroprotective activity
- P 44 **Ning-Sun Yang** (Academia Sinica, Agricultural Biotechnology Research Center, Taiwan) In vitro and in vivo antitumor effects of deoxyelephantopin on human breast cancer cells
- P 45 **Chih-Chun Wen** (China Medical University, Taiwan) Adjuvant effect of specific microtubule-depolymirizing agents on dendritic cell-based cancer vaccines
- P 46 **Jeong Yong Moon** (Jeju National University, South Korea) Antioxidant activity of a HPLC-MS/MS fingerprinted ethyl acetate fraction derived from Cattley guava leaf and its potential activity towards H₂O₂ induced DNA damage in HEPG2 cells
- P 47 **Kazumi Yagasaki** (Tokyo University of Agriculture and Technology, Japan) Anti-diabetic effect of piceatannol, a stilbenoid, in vitro and in vivo
- P 48 **Pradeep Kamboj** (ISF College of Pharmacy, India) Anti-urolithiatic screening of aerial parts of Erythrina stricta
- P 49 **Nidia C. Yoshida** (University of São Paulo, Brazil) Occurrence of lignoids in Schilleria and Macrostachys clades of Piper
- P 50 **Li-Yeh Chuang** (I-Shou University, Taiwan) The antibacterial and antioxidant activities from leaves and sticks of Illicium verum
- P 51 **Jinwoong Kim** (Seoul National University, South Korea) Hypolipidemic effect of Dioscorea opposita on diet-induced obesity in mice
- P 52 **Minh Hien Hoang** (Korea University, South Korea) *Cy-FBS-01, is a PAN-agonist for PPARS*
- P 53 **Eun Young Kim** (Sookmyung Women's University, South Korea) Luteolin sensitizes SK-Hep1 human hepatocellular carcinoma cells to trail-induced apoptosis through upregulation of death receptors
- P 54 **Noor Rabihah binti Aid** (Sirim Berhad, Industrial Biotechnology Research Centre, Malaysia) Phytochemical screening and aphrodisiac study of Mallotus muticus and Rhizophora mucronata extracts
- P 55 **Sarifah binti Rejab** (Sirim Berhad, Industrial Biotechnology Research Centre, Malaysia) *Phytochemical screening of mangrove extracts for aphrodisiac potentials*

Transcriptome Profiling

- T 1 **Thu-Thuy T. Dang** (University of Calgary, Canada) Functional genomics to elucidate new enzymes in benzylisoquinoline alkaloid biosynthesis
- T 2 **Siriluk Sintupachee** (Chulalongkorn University, Thailand) *Transcription profile of cytochrome P450-hydroxylases potentially involved in plaunotol biosynthesis in* Croton stellatopilosus
- T 3 **Scott Cameron Farrow** (University of Calgary, Canada) Integration of transcript and metabolite profiling in cell cultures of 18 plant species from four families that produce benzylisoquinoline alkaloids
- T 4 **Kim K. Hixson** (Washington State University, USA) Advanced proteome analysis of arogenate dehydratase knockout mutants in Arabidopsis thaliana

Biofuels and Bioproducts

- BIO 1 **Fumihiko Sato** (Kyoto University, Japan) Microbial production of plant isoquinoline alkaloids
- BIO 2 **Jianfeng Xu** (Arkansas State University, USA) Application of hydroxyproline-O-glycosylation for enhanced plant-based production
- BIO 3 **Vaishali Sharma** (Lawrence Berkeley National Labs, Berkeley, USA) Tools for developing glycosyl-transferase assays and methods for xylan profiling in plants
- BIO 4 **Sivakumar Ganapathy** (Arkansas State University, USA) Lipid, monosaccharides and vitamin e chemistry of Stichococcus bacillaris strain siva2011: Source for biodiesel and value-added products
- BIO 5 **Danielle Julie Carrier** (University of Arkansas, USA) The extraction of high value phytochemicals in the context of a biorefinery
- BIO 6 **Mahmoud Gargouri** (Washington State University, USA) Engineering of non-mevalonate pathway for the enhancement of oil content in microalgae Chlamydomonas reinhardtii
- BIO 7 **Ji Young Jung** (Gyeongsang National University, South Korea) Optimization of potassium hydroxide pretreatment from steam-exploded soybean hull using response surface methodology
- BIO 8 **Selim Kermasha** (McGill University, Canada) Biosynthesis of novel natural nutraceuticals and antioxidants: A biotechnological approach
- BIO 9 **Jae-Kyung Yang** (Gyeongsang National University, South Korea) Combinations of steam explosion and chemical pretreatment for fermentation sugar production from Miscanthus sinensis

Invited Speakers



As the founding President of the Chulabhorn Research Institute, Her Royal Highness **Princess Chulabhorn** has inspired the development of a scientific research institute that has earned acclaim worldwide both for the outstanding quality and also for the excellence of its training programs. H.R.H. Princess Chulabhorn has wide ranging interests in science in general and in chemistry area in particular, with special interests in the chemistry and synthesis of natural products and in Thai medicinal plant research. She is also interested in the toxicology and the cancer risk from exposure to carcinogenic compounds present in the air pollution in vulnerable populations. This research has been identified by WHO as a priority area for concern in the Asia Pacific region and is the focus of the Institute's regional training role as a WHO collaborating center. In Thailand HRH Princess Chulabhorn's dedication to scientific research through

her inspired leadership has earned the respect of all Thais who have universally accorded her the title of "The Scientist Princess". H.R.H. Princess Chulabhorn has received many international recognitions for her scientific accomplishments. In addition, she has also been visiting professor at Universities in Germany, Japan and USA, and has received numerous honorary doctoral degrees from universities in the USA, United Kingdom, Japan, Russia and elsewhere. In organic chemistry, she had received many awards and the recent awards included: The Nagoya Special Award Gold Medal in recognition of her vision and for her dedication and efforts in promoting science in general and organic chemistry in particular (2006); the Albert Hoffmann Centennial Gold Medal from the University of Zurich for her outstanding contribution to the Chemistry of Natural Products (2007); the Windaus Medal from the German Chemical Society and the Georg-August-University Göttingen for her outstanding work in Organic Chemistry (2009); the N.D. Zelinksky Award from the Zelinksky Institute of Organic Chemistry (2011) and the "Distinguished Women in Chemistry and Chemical Engineering" from IUPAC and ACS (2011).



Eric Conn received a B.A. in Chemistry at the University of Colorado in 1944. My mentor there, Dr. Reuben Gustavson, was determined that I not see military service in World War 2 and arranged for me to work at Oak Ridge. My research involved isolating fission products from the uranium pile that formed minute amounts of plutonium used in the first bomb over Japan, over the objection of many of the scientists working at that site. After the war, when everyone was returning to graduate school, I was accepted at the University of Chicago and worked with Professor Birgit Vennesland doing plant biochemistry. My project was to isolate TPN, now known as NADP. Initially, I had to kill some rats for their livers as a source of TPN; (that experience quickly turned me into a plant biochemist.) Larger amounts of TPN were soon required and that involved working up 50 pounds of hog liver obtained from a west side slaughter house. I

also needed spent brewer's bottom yeast for larger amounts of Zwischenferment; that also came from a brewery on the west side. All of this resulted in a rather unimpressive thesis, but after 2 years of such work, I received my Ph. D in 1948. Vennesland asked me to stay on for 2 years to work with her students while she was on medical leave. She arranged for me to lecture in the biochemistry-biology sequence in the famed Robert Maynard Hutchins College at Chicago. That gave me teaching experience, a significant factor in receiving my first appointment as a Lecturer at Berkeley in 1952, By 1959 I'd received tenure at Berkeley, and my good friend and colleague, Paul Stumpf and I, moved to Davis which the Regents had decided to make into a full campus of the University. The rest is history: I received research grants from the US Public Health Service and the National Science Foundation and with the help of wonderful students and post-docs established a body of work on natural products in plants. If someone were to ask me what my most important paper was, I would say the discovery and isolation of the enzyme, phenylalanine ammonia lyase, coauthored with Jane Koukol in1961 and affectionately known as PAL.

PSNA Plenary Session – A Celebration



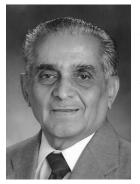
Daneel Ferreira, Chair of the Department of Pharmacognosy, University of Mississippi, graduated from the University of Pretoria in 1963 and completed the D.Sc. (1973) program of the Chemistry Department, UOFS, Bloemfontein. In 1969 he was appointed as Technical Assistant, progressed to the rank of Professor of Organic Chemistry in 1985, and served as chairperson of the Chemistry Department (1994-1997). He spent 1977 at Imperial College under the supervision of Sir Derek Barton, and was awarded the DIC for his work on the synthesis of aminoglycoside antibiotics. In 1990 he was invited to establish a Research Unit for Polyphenol- and Synthetic Chemistry at UOFS by the Foundation for Research Development. He joined the NCNPR at the University of Mississippi in 1999 as Visiting Scholar/Principal Scientist, and was appointed in his current position in 2004. His research focuses on the chemistry of proanthocyanidins and absolute

configuration studies of natural products based on chiroptical methods. He is co-author of about 320 peer-reviewed papers, serves on the Editorial Board of *Phytochemistry*, and is Associate Editor for *Journal of Natural Products*. He is a recipient of the Gold Medal of the S.A. Chemical Institute, the Havenga Prize of the S.A. Academy for Arts and Sciences, the Centenary Medal and an Honorary Doctorate of Free State University, and served as President of the PSNA in 2003.



Dr. Koji Nakanishi was born in Hong Kong (1925) and raised in Lyon, London and Alexandria before going to Japan in 1937. His father worked for Yokohama Species Bank (now Bank of Tokyo). Attended Yamate Primary School (Ashiya), Konan Middle/High School (Kobe), and Nagoya University in 1947. During graduate years while working with Prof. Fujio Egami, he joined Harvard University, 1950-1952, Prof. Louis F. Fieser, as a GARIOA student (Fulbright predecessor). He received his Ph.D. (1953, Nagoya University) Prof. Yoshimasa Hirata. He was professor at Tokyo Kyoiku University (now Tsukuba University) 1958-1963, at Tohoku University 1963-1969, before joining Columbia University. He became Centennial Professor of Chemistry in 1980, retired in 2007, but continues research. He was a founding member and Director of Research at International Centre of Insect Physiology and Ecology, Kenya, 1969-1977, Director of Suntory Institute for Bioorganic Research, Osaka, 1979-1991, and Director of the

Chemistry unit at Biosphere 2 (Columbia University), Arizona in 2001 until its termination in 2003. His research is in natural products chemistry and spectroscopy (initially IR, now in chiroptical spectroscopy). He works on the mechanism of vision and bioorganic studies of ginkgolides. He has published 800 papers, authored /coauthored/edited 11 books and co-edited a 9 volume series on natural products. He published an autobiography (1991, "A Wandering Natural Products Chemist-Am. Chem. Soc.). Since 1996, a Nakanishi Prize is awarded in alternate years by the Chem. Soc. Japan and Am. Chem. Soc.



Mansukh C. Wani (Ph.D., Chemistry, Indiana University) is Principal Scientist Emeritus at the Research Triangle Institute (RTI), Research Triangle Park, NC. Dr. Wani's main areas of research include the isolation and characterization of biologically active natural products and synthesis of anticancer and antifertility agents. In the area of natural products research at RTI, he has been involved in the isolation, purification, and characterization of a wide variety of antineoplastic agents including camptothecin and taxol. He has published extensively with over 200 publications to his credit. As of today, there have been several thousand citations to his work on taxol and camptothecin in internationally recognized scientific journals. Dr. Wani is the recipient of many awards, including the Bruce F. Cain Memorial Award given by the American Association for Cancer Research, the National Cancer Institute Award of Recognition, the 2000 Charles F. Kettering

Prize of the General Motors Cancer Research Foundation, and the 2003 Distinguished Alumni Award from Indiana University. In April 2003, the American Chemical Society designated the discovery of camptothecin and Taxol at Research Triangle Institute a National Historic Chemical Landmark.



Ikuro Abe is Professor of Natural Products Chemistry at The University of Tokyo. He obtained his Ph.D. in 1989 from The University of Tokyo under the direction of Professor Yutaka Ebizuka, where he studied chemistry and biochemistry of natural products biosynthesis. After two years postdoctoral research with Professor Guy Ourisson at the CNRS Institut de Chimie des Substances Naturelles, and mostly with Professor Michel Rohmer at the Ecole Nationale Supérieure de Chimie de Mulhouse (1989-1991), he went to the USA to work with Professor Glenn D. Prestwich at the State University of New York at Stony Brook (1991-1996) and then at the University of Utah (1996-1998) as a Research Assistant Professor. In 1998, he returned to Japan at University of Shizuoka, School of Pharmaceutical Sciences (1998-2009), and served as an investigator of PRESTO, Japan Science and Technology Agency (2005-2009). In 2009, he moved back to the University of Tokyo, Graduate School of

Pharmaceutical Sciences as Professor of Natural Products Chemistry. His research interests focus on exploring and engineering the natural products biosynthesis.



Suzanne R. Abrams is currently the Research Director for the Plant Biotechnology Institute of the National Research Council Canada in Saskatoon, with responsibility for the Institute's research programs as well as the platform technologies. Sue obtained her B.Sc. at Carleton University, her Ph.D. at Dalhousie University in synthetic organic chemistry, and had a postdoctoral fellowship at the University of Alberta. She began her career with the NRC in Saskatoon in 1977 and for more than thirty years worked on synthesis of natural products including lipids and plant hormones, particularly on abscisic acid, and plant hormone profiling as a technology for plant functional genomics research programs, publishing over 150 papers in refereed journals.



Yoshinori Asakawa first studied biology at the Tokushima University, and then went to graduate school at the Hiroshima University in 1964 and studied organic chemistry there. He has been actively involved in bryophyte research since the early 1970s, when he was a post-doc with Professor Guy Ourisson at the Institut de Chimie, Université Louis Pasteur, Strasbourg, France. In 1976, he moved to the Faculty of Pharmaceutical Sciences of Tokushima-Bunri University (TBU) as an Associate Professor, and was promoted to Full Professor in 1981.He has studied not only bryophyte constituents and their biosynthesis, but also bioactive secondary metabolites of pteridophytes, inedible mushrooms and aromatic and medicinal plants, as well as biotransformation of secondary metabolites by fungi and mammals, and oxidation reactions of organic peracids. He has authored more than 550 original papers, 24 reviews and 27 books and monographs. For

his outstanding research, Prof. Asakawa was awarded the first Hedwig Medal from the International association of Bryologists, the Phytochemistry Prize and Certification from Elsevier, International Symposium on Essential Oils Award from ISEO, Jack-Cannon Gold Medal from Malaysian Natural Product Society, Japanese Society of Pharmacognosy Award, and Tokushima News Paper Award. He was the editor of Phytomedicine and Spectroscopy, and is on the editorial boards of numerous scientific journals which include Phytochemistry, Phytochemistry Letters, Planta Medica, Flavor and Fragrance Journal, Fitoterapia, Natural Product Communication, Natural Product Research, Malaysian Journal of Sciences, Current Chemical Biology, Scientia Pharmaceutica, Journal of Traditional Complementary Medicine, among others. He served twice as Dean of TBU and is Director of Institute of Pharmacognosy (1986-present) and currently leads Phytochemistry as the President of the Phytochemical Society of Asia since 2007.



Mike Beale is a Research Leader at Rothamsted Research, Harpenden, UK and also holds honorary appointments at the Universities of Nottingham and Bristol. He is a biological chemist with a long career in both synthetic and biosynthetic chemistry of plant natural products. His early work was focussed on the biosynthesis of diterpenes particularly the gibberellin plant hormones. His research group currently specialises in high throughput plant metabolomics, using combined NMR and MS technologies, as a means of discovery of metabolite biomarkers and biosynthetic genes involved in plant stress biology. However, he maintains an interest in the plant terpenoids, and has his group has recently been active in the identification of sesquiterpene synthase genes and also in the production of hemiterpenes in *Arabidopsis*.



Jörg Bohlmann is a Professor and Distinguished University Scholar in the Michael Smith Laboratories at the University of British Columbia, Vancouver, Canada (www.michaelsmith.ubc.ca/faculty/bohlmann/). He received his PhD from the Technical University Braunschweig, Germany (1995), was a Feodor Lynen Fellow of the Alexander von Humboldt Foundation at Washington State University, USA (1995-1998) and research associate at the Max Planck Institute for Chemical Ecology, Germany (1998-2000). He holds academic appointments in the Departments of Botany and Forest Sciences, and is an associate of the UBC Wine Research Centre. His research deals with the molecular biochemistry and biology of terpenoids, genomics of conifers, grapevines, and various medicinal plants, and plant defense against insects. His research is funded by the Natural Sciences and Engineering Research Council of Canada (NSERC),

and other sources. He has been the project leader of five large-scale, Genome Canada funded genomics projects (Treenomix I and II, www.treenomix.ca/; Tria I and II, http://thetriaproject.ca/; SMarTForests). He is co-PI on two other Genome Canada projects on Grape and Wine Genomics and Synthetic Biology of Natural Products. He has received several national and international awards and distinctions including a Feodor Lynen Fellow of the Alexander von Humboldt Foundation, the C.D. Nelson Award of the Canadian Society of Plant Physiologists, the Charles A. McDowell Award for Excellence in Research awarded by UBC, the E.W.R. Steacie Memorial Fellowship of the Natural Sciences and Engineering Research Council of Canada; he is an elected Fellow of the American Association for the Advancement of Science.



Vanderlan da S. Bolzani is Full Professor at São Paulo State University (UNESP) and currently is Vice-Director of the UNESP Innovation Agency (AUIN). With over 186 research publications, five patents, four book chapters, she is also a Fellow of the Royal Society of Chemistry (UK). Recently, she was elected a member of the Deliberative Advisory Board of the Brazilian National Research Council (CNPq), and has received several awards, the most recent being the Simão Mathias Medal, the highest honor given to a Brazilian Chemist (Brazilian Society of Chemistry – SBQ) and Distinguished Woman in Science Chemistry and Chemical Engineering, conceived by ACS. Her Ph.D. degree was

in organic chemistry under guidance of Otto R. Gottlieb, University of São Paulo, and did a post-doctorate at Virginia Polytechnic Institute (VPISU-USA), under the supervision of David G. I. Kingston. In 1990, she was awarded a fellowship from DAAD for a short training at the University of Hanover. Since 2003, she is a member of the Biota-FAPESP Program Coordination. Her field of interest is plant science, where she has been involved in the isolation, bioactivity and function of secondary metabolites and peptides from plants. Recently she has been involved on metabolomics of medicinal plants and sugar cane. She also has strong involvement in human resource training of the master and Ph.D. student and post-doctoral level. She has strong collaboration with Pharmaceutical and Cosmetic Industries looking for new drugs from plant species. Dr. Bolzani was President of the Brazilian Chemical Society from 2008-2010, and is currently Counselor. She serves as member of several Editorial Boards of Scientific Journals.



C. Robin Buell is a Professor of Plant Biology at Michigan State University. Her current research activities are centered on genomic aspects of plant biology and plant pathogens. The research primarily involves projects focused on high throughput sequencing, functional genomics, comparative genomics, and bioinformatics. She has been involved with the generation of genomic resources for medicinal plants and her group performed the bioinformatics analyses of 14 medicinal plant species as a member of the NIH-funded Medicinal Plant Consortium (http://medicinalplantgenomics.msu.edu). This project has generated robust representations of the transcriptomes for 14 plant species of pharmaceutical interest including *Panax*, *Camptotheca*, *Atropa*, *Gingko*, and *Digitalis*. In collaboration with other members of the Medicinal Plant Consortium, we have assembled the transcriptome for all 14 species and provided functional

annotation to facilitate data interpretation by biologists. We have also developed a computational pipeline to quantitate expression abundances for each transcriptome in a range of tissues and treatments to facilitate correlation of expression levels with metabolite levels. These transcriptome resources, when coupled with metabolome data, permit identification of key genes involved in metabolites of pharmaceutical interest.



Dr. Colburn received her BS in Chemistry from Swarthmore College and PhD in Biochemistry from the McArdle Laboratory for Cancer Research, University of Wisconsin. Following faculty positions at the Universities of Delaware and Michigan, she was appointed Chief, Cell Biology Section, Laboratory of Viral Carcinogenesis at the NCI in 1979, and in 2003, she was appointed Chief, Laboratory of Cancer Prevention, Center for Cancer Research. Dr. Colburn was among the first to demonstrate that transcription factors AP-1 and NF κ B were drivers of tumorigenesis in multistage carcinogenesis that could be targeted for cancer prevention. Her laboratory discovered the tumor suppressor Pdcd4 and identified Pdcd4 as an inhibitor of translation initiation whose stabilization could be targeted to prevent cancer. The Colburn laboratory now engages in intervention studies to prevent colon cancer and discover biomarkers in mice and humans. Former Colburn laboratory trainees now serve as distinguished

Professors or Institute Directors at Medical Schools and as Directors in pharmaceutical or biotechnology companies. A member of distinguished editorial and scientific advisory boards, Dr. Colburn has served as organizer of major international symposia on molecular targets for cancer prevention.



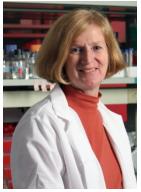
Kim Colson, Ph.D., has over thirty years of experience in NMR spectroscopy. Her use of NMR spectroscopy began in the area of natural products structure elucidation and molecular dynamics. After working in the pharmaceutical industry, including Bristol-Myer Squibb and Burroughs Wellcome, for 9 years she joined Bruker BioSpin in Billerica Massachusetts. Since joining Bruker she has held various roles including applications scientist, product manager and now business development manager.

Dr. Colson's role as business development manager is in the research and development department. Her mission is to identify and establish applications for NMR spectroscopy in non-traditional areas. This includes the development of new methods, hardware and software to implement NMR into these new disciplines. She leads a team of scientists and engineers in the US and Europe to develop the new tools. Her team is currently heavily focused on developing tools for quality control of dietary supplements.



Allan H. Conney, Ph.D., received his BS degree in pharmacy and his Ph.D. degree in oncology at the University of Wisconsin-Madison. He is the State of New Jersey Professor of Pharmacology and Garbe Professor of Cancer and Leukemia Research in the Department of Chemical Biology of the Ernest Mario School of Pharmacy at Rutgers University. Dr. Conney is also Director of the Susan Lehman Cullman Laboratory for Cancer Research. He held positions at the NIH for 3 years and in the pharmaceutical industry for 27 years prior to joining Rutgers University in 1987. He has contributed to our understanding of factors influencing the metabolism and action of drugs, carcinogens, environmental chemicals and steroid hormones; regulation and biological significance of multiple cytochromes P-450; drug interactions; induced synthesis of microsomal enzymes; carcinogenesis and mutagenesis by polycyclic aromatic hydrocarbons; cancer chemotherapy by differentiating agents; and cancer

prevention by dietary chemicals, drugs and exercise. Dr. Conney is a member of the National Academy of Sciences (U.S.A.).



Katrina Cornish, an Ohio research Scholar, holds an Endowed Chair in Bioemergent Materials at The Ohio State University, emphasizing alternative rubber production and exploitation of opportunity feedstocks from agriculture and food processing wastes for value-added composite products and biofuels. Her inventions at USDA were licensed by Yulex Corporation and form the foundation of the US domestic rubber industry. Prof. Cornish led the USDA's development of domestic natural rubber and rubber latex sources for >15 years. Post-USDA, as Senior Vice President, Research & Development at Yulex, Prof. Cornish oversaw the company's ongoing research, development, production, validation and regulatory programs for the commercialization of Guayule latex for safe medical devices and specialty consumer products. She has >150 papers and patents of which >120 are related to rubber biosynthesis and production, including co- and by-products. Amongst her numerous awards, Prof. Cornish

received the AAIC Outstanding Researcher of the Year, the Good Housekeeping Award for Women in Government (2004), was elected Fellow of the American Association for the Advancement of Science (2002), won a presidential award from the American Chemical Society (2002), was honored as the USDA's Outstanding Senior Scientist of the Year (1998), and was recognized by the Agricultural University of Antonio Narro, Mexico for her research (1997). Also, her guayule latex received the Connect 2005 Most Innovative New Product Award, Medical Devices & Diagnostics. Dr. Cornish received a first class honors degree in biological sciences and a Ph.D. in plant biology from the University of Birmingham, England.



Pat Covello is curious about how plants make chemicals. In his undergraduate, graduate and postdoctoral training in Canada and the U.K., he studied chemistry and plant physiology, biochemistry and molecular biology. As a postdoctoral fellow in Michael Gray's lab, he was a co-discoverer of RNA editing in plant mitochondria and published one of the first examples of evolutionary gene transfer from mitochondrion to nucleus. As Senior Research Officer at the National Research Council of Canada's Plant Biotechnology Institute and Adjunct Professor at the University of Saskatchewan, Dr. Covello made important contributions to the understanding of plant enzymes which modify fatty acids. In the last decade, he has focused on functional genomics approaches to understanding plant natural product biosynthesis in a number of different pathways (isoprenoids, alkaloids, cyclic peptides). Dr. Covello has made a major

contribution to the elucidation of the biosynthesis of the antimalarial compound artemisinin. The results of this work are expected to support the development of a microbial production system for commercial scale of antimalarial drugs for the developing world, on a not-for-profit basis.



Wanchai De-Eknamkul is a faculty member at Chulalongkorn University, Faculty of Pharmaceutical Sciences, Department of Pharmacognosy and Pharmaceutical Botany. He received his Ph.D. (Plant Biochemistry) in 1987, under the supervision of Prof. Brian E. Ellis (University of Guelph). During 1989-1990, he received a research fellowship from the Alexander von Humboldt Foundation, Bonn, to do post-doc. with Prof. Dr. Meinhart H. Zenk at the University of Munich. His main research interest has been on the biosynthetic studies of pharmaceutically-important natural products, and biotechnological applications of biosynthetic genes and enzymes for yield improvement of useful products. He has been working on the biosynthesis of the anti-peptic plaunotol in *Croton stellatopilosus* and yield improvement of the anti-malarial artemisinin in *Artemisia annua*. He is a project

leader of the Natural Product Biotechnology Network granted by Thailand's National Science and Technology Development Agency. Recently, with the support of the International Center of Science and High Technology of UNIDO in Trieste, Italy, he established a new drug discovery center at Chulalongkorn University, Bangkok. In addition to research, he has contributed to development of Thailand's National Research University Project, a new initiative of the Office of the Higher Education Commission. He received a best research award in 1999 from the Thailand Research Fund (TRF), the Innovation Ambassador Award 2008 from the National Innovation Agency and the Invention Award 2009 from the National Research Council of Thailand. He has published more than 50 international research articles and patents from his work.



Alessandra Devoto, Senior Lecturer, School of Biological Sciences, Royal Holloway University of London (RHUL), UK. She completed her M.Sc. in Biology in 1991 and her Ph.D. in Plant Molecular Biology, in 1996 at the University of Rome "La Sapienza", Italy. She carried out her post-doctoral studies at The Sainsbury laboratory, John Innes Centre, and at The University of East Anglia, Norwich, UK. She became Senior Lecturer at RHUL, UK in 2006. Her research interests are on plant hormone signalling and in particular on the role of jasmonates on the cell cycle and on the production of secondary products. She also uses transcriptomics, proteomics and computational biology to model and infer signalling networks. In collaboration with Professor Paul Bolwell, at Royal Holloway, she studies how to improve the composition of cell wall polymers to improve processing of biomass.

She has several publications in relevant fields. She is an alumna of the JSPS (Japan Society for the Promotion of Science), acting as a Gatsby Mentor, and as an advisor on the GarNET (Genomic *Arabidopsis* Resource Network) as well as being Vice Chair on EU LIFE Panel.



Albena Dinkova-Kostova obtained her PhD in Biochemistry in 1996 from Washington State University under the mentorship of Professor Norman Lewis. Her doctoral work was directed towards purification and characterization of enzymes of the phenylpropanoid pathway. During that time she became intrigued by the fact that plant phenylpropanoids, some of which (podophyllotoxin and the semi-synthetic etoposide/tenoposide) are used in cancer chemotherapy; others (nordihydroguaiaretic acid) are potent antioxidants, are also inducers of anticarcinogenic enzymes, as shown by Professor Paul Talalay at Johns Hopkins. She joined his laboratory where she became interested in chemoprevention by chemical and dietary induction of cytoprotective proteins. While remaining parttime faculty member of the Dept. of Pharmacology and Molecular Sciences at

Johns Hopkins, since 2007 Albena holds a position of Lecturer and Research Councils UK Academic Fellow at the Medical Research Institute, Univ. Dundee, Scotland. Her work on the mechanism of induction of cytoprotective proteins (through the Keap1/Nrf2/ARE pathway) and the chemistry of inducers has been published in many journals, including *Biochemistry*, *PNAS*, *Chem. Biol., J. Biol. Chem., J. Med. Chem., Chem. Res. Toxicol., Cancer Prev. Res.* and *Methods Enzymol..* It has been highlighted in commentaries and attracted more than 2000 citations; one paper was featured on the American Chemical Society Publications website as being in the top 1% of the most-cited papers during the past 10 years.



Richard A. Dixon is Distinguished Professor and Samuel Roberts Noble Research Chair, Senior Vice President, and Director of the Plant Biology Division at the Samuel Roberts Noble Foundation. He holds Adjunct Professorships at Rice University, the University of Texas at Austin, and the University of Oklahoma. He received his Bachelor's and Doctoral degrees in Biochemistry and Botany from Oxford University (UK), and postdoctoral training in Plant Biochemistry at Cambridge University (UK). He was awarded the Doctor of Science degree for his research achievements by Oxford University in 2004. His research interests center on molecular biology and metabolic engineering of plant natural product pathways. He has published over 400 papers on these and related topics in international journals, and has been named by the Institute for Scientific Information as one of the 10 most cited authors in the plant and animal

sciences. Professor Dixon is Co-Editor-in-Chief of the journal BioEnergy Research, and a member of the Editorial Boards of four other international journals. He is Fellow of the American Association for the Advancement of Science, and was elected to membership of the US National Academy of Sciences in 2007.



Stephen Duke directs a USDA, ARS research group of twelve scientists at the National Center for Natural Product Research (Univ. Mississippi) involved in discovery of pest management products from natural sources. His own research is primarily involved in discovery of natural phytotoxins and determination of their molecular target sites, as well as the role of some of these compounds in allelopathy and other chemical ecology aspects of plants. He has published over 350 peer reviewed papers and book chapters, edited six books, and co-written one book. He is President of the International Allelopathy Society, Vice Chair of the Agrochemicals Division of the American Chemical Society, and Past President of the International Weed Science Society and the Weed Science Society of America. Editorial responsibilities include Executive Board of *Pest Management Science* and Editorial Boards of several other journals. His awards

include Fellow of AAAS, International Award for Research in Agrochemicals of ACS, and honorary doctorate from the University of the Basque Country.



Brian Ellis received his undergraduate education at the University of New Brunswick in biology and chemistry (1965), and his Ph.D. in plant biochemistry from the University of British Columbia (1969). After postdoctoral training with Meinhart Zenk in West Germany, and with Stewart Brown at Trent University in Ontario, he accepted a faculty position at the University of Guelph in 1973 in the Department of Chemistry and Biochemistry. In 1989, he became Head of the Department of Plant Science at the University of British Columbia, a position that he held until 1998. He is now a Professor in the UBC Michael Smith Laboratories, where he also served as Associate Director from 2001 to 2008. Nationally, Dr. Ellis has served as a member of the 'Committee on Research Grants' at the Natural Sciences and Engineering Research Council of Canada (NSERC), as 'Group Chair for Life Sciences' at NSERC, and as a member of the 'Science and Industry Advisory Committee' of Genome Canada. In 2000/01, he

served as co-Chair of the Expert Panel on *The Future of Food Biotechnology* for the Royal Society of Canada. His research projects focus on mapping the signaling systems that plants use to sense and respond to environmental changes, and on learning how plant cells control the structure and chemistry of their cell walls. In 2009, he was awarded the Gold Medal of the Canadian Society of Plant Physiologists for outstanding research contributions to the field.



Peter Facchini is Professor of Plant Biochemistry in the Department of Biological Sciences at the University of Calgary, and he holds the Canada Research Chair in Plant Metabolic Processes Biotechnology. He obtained his Ph.D. from the University of Toronto in 1991, and conducted postdoctoral research at the University of Kentucky and the Université de Montréal prior to his current appointment in 1995. His research is focused on the basic and applied biochemistry of bioactive metabolites in medicinal plants. In particular, he has been working for almost two decades toward a comprehensive understanding of the biochemistry, molecular and cell biology of pharmaceutical alkaloid metabolism in opium poppy, and he is regarded as an international authority in the field. His research team has made numerous important contributions including the isolation of several key genes involved in the biosynthesis of

morphine, codeine and other compounds, the establishment of an extensive collection of genomics resources for opium poppy and related plants, and the identification of the specific cell types that participate in alkaloid biosynthesis. He presently leads the Genome Canada-funded PhytoMetaSyn Project, a consortium of Canadian researchers targeting the commercial production of high-value plant metabolites in microbial fermentation systems. He has published over 100 research papers and scholarly articles, and is work is frequently featured in the public media. In 2003, he received the C.D. Nelson Award from the Canadian Society of Plant Physiologists as the outstanding young plant biologist in Canada.



Simone Ferrari is Assistant Professor in Plant Physiology at the University of Rome "La Sapienza" since 2007. He obtained his major degree in Biological Sciences in 1997, and a Ph.D. in Plant Sciences in 2002 at the Università of Roma "La Sapienza". He has been a Research Fellow in Molecular Biology at the Massachusetts General Hospital (Boston, USA) from 1999 to 2003, and Assistant Professor in Plant Pathology at the University of Padua (Italy) from 2004 to 2007. His research activity aims at the role of plant cell walls in development and interactions with microbial pathogens. His early work dealt with the role of pectin-degrading enzymes and their inhibitors in determining the outcome of fungal infections. He has also studied the signalling pathways activated by pectin fragments in the regulation of defence responses. and the

impact of pectin modifications on growth, hormone sensitivity and resistance to pathogens. Recently he investigated the role of pectin methylation in determining cell wall accessibility to hydrolytic enzymes, with the aim of improving biomass conversion into biofuels and other products.



Stefan Gafner, Director of Analytical Chemistry at Tom's of Maine. He obtained his B.Sc. in Pharmacy at the University of Bern in 1992, and his Ph.D. (Phytochemistry) under the direction of Prof. K. Hostettmann at the University of Lausanne in 1997. He completed his post-doctoral studies with Prof. J. M. Pezzuto at the University of Illinois at Chicago in 1999 and in the same year joined Tom's of Maine. His research interests are focused on quality control of botanical raw materials and finished products and on plant metabolites with anti-inflammatory properties. He has authored/co-authored over 30 peer-reviewed publications. In 2007, he co-chaired the organization of the 48th Annual meeting of the American Society of Pharmacognosy in Portland, Maine.



David R. Gang is a member of the Institute of Biological Chemistry at WSU. He earned his BS and BA degrees in Botany/Plant Molecular Biology and Germany Literature at Brigham Young University and his PhD at WSU. He did post-doctoral work at the University of Michigan. His first faculty position was in the Department of Plant Sciences and the BIO5 Institute at The University of Arizona. He has earned several awards, including the Benson Presidential Scholarship at BYU; the Loyal H. Davis Graduate Student Fellowship at WSU; the Sokol Post-Doctoral Fellowship in the Sciences from the Rackham Graduate School, UM; the Arthur Neish Young Investigator Award, PSNA; and the Young Investigator Award in Plant Genome Research from the United States NSF. Dr.

Gang's research involves an interdisciplinary approach, seeking to elucidate the biosynthetic pathways that produce novel and important metabolites in plants, to uncover the mechanisms responsible for the evolution of these pathways in the plant kingdom, to understand the function of a given natural product/metabolite in the biology and physiology of a given plant species, and to develop the tools needed to analyze metabolites in complex biological systems. He is also very interested in identifying mechanisms responsible for controlling the development of plant cells and tissues that have evolved to produce high levels of such metabolites, such as glandular trichomes and storage rhizomes. The most productive approach in this area is now a multidisciplinary approach—which utilizes the best tools from the fields of chemistry, biochemistry, molecular biology, genomics, proteomics and metabolomics.



Clarissa Gerhauser heads the Cancer Chemoprevention and Epigenomics Group at the German Cancer Research Center (DKFZ) in Heidelberg, Germany. She has worked as a research associate and research assistant professor in the area of cancer chemoprevention with J.M. Pezzuto at The University of Illinois at Chicago from 1993 to 1996, funded through a Feodor-Lynen Fellowship awarded by the Alexander-von-Humboldt-Foundation, Germany. In 2003, she was awarded with the EACR Young Cancer Researcher Award Highly Commended and the Phoenix Pharmacy Scientific Research Price for "Pharmaceutical Biology" for the identification of the hops compound xanthohumol as a novel cancer chemopreventive agent. Her research interest is the identification and proof of efficacy of novel plant- or diet-derived compounds and synthetic analogs with potential chemopreventive activity and the elucidation of underlying

molecular mechanisms, with a current strong focus on epigenetic mechanisms. She has authored more than 80 research articles, reviews and book chapters on cancer chemopreventive agents, holds four patents, and is regularly invited for lectures both nationally and internationally. She is an editorial and advisory board member of "Planta Medica" and "Phytomedicine" and has guest edited special issues on "xanthohumol" for "Molecular Nutrition and Food Research" and on "Nutrition, Chemoprevention and Natural Products" for "Planta Medica". Also, she has co-edited a comprehensive reference book on "Chemoprevention of Cancer and DNA damage by dietary factors" (Wiley Press, 2009). Her research is funded by grants from the German Brewers Association, the German Research Council, the German Federal Ministry of Education and Research, and the European Union. She studied Pharmacy at the University of Würzburg, Germany, and obtained a Ph.D. (*summa cum laude*) in Pharmaceutical Biology at the University of Munich.



Alfons Gierl obtained his Dr. rer. nat in Biology (1982) from the Ludwig-Maximilians-University in Munich. His PhD study was done at Max-Planck-Institute for Biochemistry, Martinsried in the lab of Wolfram Zillig. After a postdoctoral year in the same lab, he joined Heinz Saedler's Department of Molecular Plant Genetics at the Max-Planck-Institute for Plant Breeding in Cologne. The studies of plant transposable elements resulted in the determination of the complete structure and the elucidation of the transposition mechanism of McClintock's *En/Spm* element. The detailed characterization of transposase function in transgenic tobacco and the introduction of *En/Spm* into Arabidopsis provided the basis for using this transposon for gene tagging and reverse genetic systems. Since 1993, he became full Professor at the Technische Universität München and Director of the Department of Genetics. Gene tagging with maize transposons led into the analysis of DIMBOA biosynthesis, a plant secondary metabolic pathway that confers broad pest resistance to cereals. By now, Alfons

Gierl and his team have identified and functionally characterised the complete set of eight genes that encode benzoxazinoid biosynthesis in maize and other grasses. An evolutionary highlight was the discovery of Bx1 gene function in DIMBOA biosynthesis. BX1 catalyzes the branch point reaction from primary metabolism, has evolved from tryptophan synthase, and its structure was determined. The Bx1 homologue *Igl* encodes an enzyme with identical function, however, this maize gene is involved in the production of volatile signals that are emitted after herbivore damage. Evolution of secondary metabolism is a main focus of the lab.



Kathryn A. Gold is an Assistant Professor of Thoracic/Head and Neck Medical Oncology at the University of Texas MD Anderson Cancer Center. Her research focuses on cancer prevention and the use of targeted therapies for lung cancer and head and neck cancer. She is a member of the American Society of Clinical Oncology and the American Association for Cancer Research, and she has received research funding from the American Society of Clinical Oncology. She holds a B.S. in Chemistry from Duke University and an M.D. from Washington University in St. Louis. She completed her internal medicine residency at the Hospital of the University of Pennsylvania and her medical oncology fellowship

at the University of Texas MD Anderson Cancer Center.



Erwin Grill studied Biology in Munich, Germany, and then joined the group of Prof. M.H. Zenk for postgraduate studies. In 1987, he received his Ph.D. for the discovery and characterization of phytochelatins in plants. He worked as a postdoctoral fellow in the group of Prof. Somerville at the Plant Research Laboratory, MSU, and as a group leader of Prof. Amrhein at the Swiss Federal Institute in Zurich. Since 1996, he is Head of Botany at the Technische Universität München. His research has a long-standing interest in elucidating the molecular mechanisms of abiotic stress responses in plants. The research focuses on the detoxification of heavy metals and xenobiotics as well as responses to water deficit and abscisic acid signaling in plants. Erwin Grill is member of the German National Academy of Sciences, Leopoldina, and of the Bavarian Academy of Sciences.



De-an-Guo, received Ph.D. of Pharmacognosy in Beijing Medical University in 1990. He worked as a postdoctoral in Texas Tech University, USA from 1993 to 1996. Currently in the Shanghai Institute of Materia Medica as a chair professor and director of National Engineering Lab for TCM Standardization. Principal social concurrent posts include Expert Committee Member of Chinese Pharmacopeia (2005 and 2010 Editions); Editor in chief of Chinese Pharmacopoeia Vol. 1 (2010 English Edition); Expert Committee Member of States Pharmacopeia, Associate of of United editors Journal Ethnopharmacology and editorial board members of 10 international journals such as Planta Medica, Phytomedicines, Fitorerapia, Phytochemistry Letters, etc. Received the First grade Natural Science Awards from Ministry of Education in 2005 and 2007 respectively. He has published over 400 papers, among which

260 papers were published in SCI cited journals with over 2800 citations. His Hirsh Index of published papers is 27. He has supervised over 45 Ph.D. students.



Adrian D. Hegeman is an Assistant Professor in the Microbial and Plant Genomics Institute at the University of Minnesota Twin Cities with appointments in the Departments of Horticultural Science and Plant Biology. He received his B.A in Biochemistry from Oberlin College in 1992, and completed his Ph.D. in Biochemistry from the University of Wisconsin-Madison in the laboratory of Perry A. Frey in 2001 studying the structure and function of the enzyme of dTDP-glucose 4,6-dehydratase. He then continued his post-doctoral studies at the University of Wisconsin in the laboratory of Michael R. Sussman using mass spectrometry-based proteomics and metabolomics (2002-2007). During his postdoc Adrian co-authored a textbook with his graduate advisor (Frey) titled: *Enzymatic Reaction Mechanisms* (Oxford, 2007). He has held his appointment at the University of Minnesota since 2007 studying plant metabolomics and the use of stable isotopes and mass spectrometry for methodological innovations. Other projects include: exploring the botanical origins of propolis collected by

honey bees, examining the mechanistic enzymology of plant hormone (auxin) biosynthesis, measuring bioactive constituents of kava ('awa; *Piper methysticum*), and prospecting for antimicrobial and antioxidant compounds in native and naturalized prairie plants to use as preservatives in cosmetics.



Professor **Michael Heinrich** is a pharmacognosist, biologist and anthropologist specializing in medicinal and food plant research, especially bioactive natural products as well as food and medicinal plant usage (ethnopharmacology), especially in Mexico and the Mediterranean. He is currently Director of Southern Cross Plant Science Centre at Southern Cross University and leads a team working across a range of research and commercial platforms including metabolomics / phytochemistry, phytopharmacology, ethnobiology, DNA technologies, genomics, plant improvement and bioinformatics. Michael has edited and co-authored five books on medicinal and food plant research and published well over 180 refereed scientific papers and a large number of international conference papers. He is Editor in Chief of the new journal Frontiers in Ethnopharmacology (www.frontiersin.org, together with Prof. Ding-Feng Su, Shanghai), Review Editor of the Journal of Ethnopharmacology and

Subject Editor of Phytochemistry Letters and the Journal of Pharmacy and Pharmacology. For many years he has worked on bioactive secondary metabolites from plants with a particular focus on antiinflammatory compounds using the transcription factor NF-kappaB as a key lead. Some more recent examples of projects include: Metabolomic research on medicinal and food plants, the use of herbal medicines in migrant communities in London, history of medicinal plant use in Europe and food and medicinal plant usage in the Mediterranean. Recent research projects centre on the value chain between producers and consumers of herbal medicines and food supplements.



Michael E. Himmel, Principal Scientist and Principal Group Manager in the Biosciences Center at the National Renewable Energy Laboratory. He has 33 years of progressive experience in conducting, supervising, and planning research in protein biochemistry, recombinant technology, enzyme engineering, new microorganism discovery, and the physic-chemistry of macromolecules. In 2004, he was co-recipient of the R&D 100 "Advanced Cellulase System for Biomass Conversion" Award for his role working with industry; and in 2010, he was given the C.D. Scott Award for career long contributions to the biomass conversion field. He has served as PI for the

DOE EERE Office of the Biomass Program since 1992, wherein his responsibilities have included managing research designed to improve cellulase performance, reduce biomass pretreatment costs, and improve yields of fermentable sugars. He has developed new facilities at NREL for biomass conversion research, including a Cellulase Biochemistry Laboratory, a Biomass Surface Characterization Laboratory, a Protein Crystallography Laboratory, and a new Computational Science Team. During the past three decades, Dr. Himmel has published over 450 articles, meeting abstracts, books, and patents. In 2008, he edited a new book for Blackwell Publishers entitled "Biomass Recalcitrance" which is a top selling book in science and has been translated into Chinese. He also works closely with the biomass conversion industry today; as demonstrated by the numerous CRADAs underway in his laboratory.



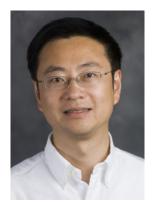
Murray B. Isman is a Professor of Entomology and Toxicology at the University of British Columbia, Vancouver, and Dean of the Faculty of Land and Food Systems. He has performed extensive research for over 30 years in the areas of insect toxicology/behavior, and insect-plant chemical interactions, with particular emphasis on the discovery and development of botanical insecticides and antifeedants. He has authored over 170 refereed publications, including 25 book chapters, and coedited two books. He serves on the editorial boards of five scientific journals and has provided peer reviews for over 80 different journals. He has held visiting professorships in Brazil and China, and been appointed a visiting scientist in Germany and Korea. He is currently an international advisor for African Dryland Alliance for Pesticidal Plant Technologies (ADAPPT), an EU-funded research network. Dr. Isman is a former President of the International Society of Chemical

Ecology, the Phytochemical Society of North America and the Entomological Society of British Columbia. Earlier this year he received the Gold Medal for outstanding achievement from the Entomological Society of Canada. His current teaching includes contributions to courses in entomology and environmental toxicology, and coordination of his Faculty's first year course. He has supervised 24 graduate students, 12 postdoctoral fellows and 14 visiting scholars. Dr. Isman received his doctorate in Entomology from the University of California at Davis in 1981 and holds BSc and MSc degrees in Zoology from UBC.



Reinhard Jetter received his BSc in chemistry (University of Munich), which he finished with a diploma thesis on organic synthesis and the physical chemistry of antiaromatic compounds. He obtained his Ph.D. (1993) at the University of Kaiserslautern, with studies on waxes of various poppy species under guidance of Markus Riederer and in close collaboration with Meinhart H. Zenk. Next, he moved to the Institute of Biological Chemistry at Washington State University, where he worked as a Postdoctoral Fellow with Rod Croteau (1994-96). His work there focused on cloning and characterization of enzymes involved in biosynthesis of conifer resin diterpenoids. He then worked as a Research Associate and independent group leader in the Biology Department, University of Würzburg (1996-2003), where he began investigations into polyketides/ triterpenoids found in plant skins. In 2003, he joined the University of British

Columbia, where he is now Professor and Canada Research Chair in Plant Natural Products Chemistry. He is leading a group of chemists and biologists focusing on the various aspects of plant surfaces, using a wide range of techniques. His projects are highly interdisciplinary, ranging from molecular genetics over enzyme mechanism and chemical product identification to physiology of water transport and chemical ecology of plant-insect-interactions.



Wei Jia is Professor at Department of Nutrition, the University of North Carolina at Greensboro (UNCG) and Co-Director of The UNCG Center for Research Excellence in Bioactive Food Components, located at the North Carolina Research Campus in Kannapolis. He also serves as Director of the Metabolomics Core Lab at the David H Murdock Research Institute, Kannapolis, North Carolina.

Dr. Jia's M.S. and Ph.D. were completed at the University of Missouri-Columbia. He has worked for years on biochemical profiling and evaluation of botanical preparations (traditional Chinese medicine, TCM) in various pharmacological models. His current research focuses on mass spectrometry (MS)-based metabolomics profiling technologies to investigate metabolic phenotypes and metabolic transformation in cancer and metabolic disorders, and pharmacokinetic and metabolomic characterization of TCMs. He utilizes global

metabolic and chemical profiling approaches, as a way to scientifically bridge different pharmaceutical and nutritional concepts and methodologies. A top-down or 'from whole to parts' strategy is taken in his research to capture the holistic and dynamic variations of biological systems in response to environmental stimuli or drug intervention, and to elucidate the underlying mechanisms of disease onset and pathological development.



Massuo J. Kato graduated in chemistry at State University of São Paulo, Araraquara (1981), followed by Ms Sciences (1984), and Ph.D. in Organic Chemistry (1989) at University of São Paulo working on the chemistry of Myristicaceae species (1981-1989). After two years of fellowship at National Institute of Amazonian Research (INPA, Manaus), he worked at Rhone-Poulenc bioprospecting antitumorals and antibiotic compounds from plants. He obtained a position at the Chemistry Institute in 1991 and then had a sabbatical period at Institute of Biological Chemistry, Washington State University at Pullman (1992-1993) working with Dr. Norman Lewis. He is currently a member of the editorial boards of Phytochemistry, Phytochemical Analysis, The Open Bioactive Compounds Journal, Encyclopedia of Life Support System-Unesco, and Global Journal of Biochemistry. His current interests are related to natural product chemistry, bioactivity, biosynthesis and evolution of secondary compounds.



After graduating from Hamilton College, Thomas Kensler received his Ph.D. in toxicology from M.I.T. Following postdoctoral fellowships at the McArdle Laboratory for Cancer Research, University of Wisconsin and at the National Cancer Institute in Bethesda, Maryland, he joined the faculty of the Johns Hopkins Bloomberg School of Public Health in 1980. In 1992, he was promoted to the rank of Professor. From 2000 to 2006, he served as Director of the Division of Toxicology. He has recently joined the Faculty at the University of Pittsburgh and holds appointments in the Department of Pharmacology & Chemical Biology and the Department of Environmental and Occupational Health. He also holds several Visiting Professorships in China. His research interests are in environmental carcinogenesis and cancer prevention. He is a past chairman of the NIH Chemo/Dietary Study Section and serves as the Cancer Prevention Editor for the journal Carcinogenesis. He has received several honors including the 2007 AACR-American Cancer Society Award for Research Excellence in Cancer Epidemiology and Prevention, the 2009 Society of Toxicology Translational Impact Award, and the 2010 Friendship Award from Jiangsu Province, People's Republic of China.



Kye-Won Kim received her BSc (Microbiology) from Kyung-Pook National University in 1992, and her MSc from the same institution in 1995 under the supervision of Professor Jong-Guk Kim. Her research was a part of a *Bacillus subtilis* genome sequencing project. After that, her interests gravitated towards plants with her PhD studies being undertaken in the Division of Biochemistry, National Institute of Agricultural Science and Technology (NIAST), Rural Development Administration (RDA). Graduating in 2001, her PhD thesis involved studying capsaicinoid biosynthesis–related genes and metabolic engineering of their genes in pepper (*Capsicum annuum* L.) in order to modify capsaicinoid contents. In 2001, she joined Dr. Norman G Lewis' laboratory at the Institute of

Biological Chemistry at Washington State University as a Research Associate. Her research studies focused initially on the underlying reasons for distinct enantioselectivities of pinoresinol/lariciresinol reductases (PLRs), as well as on a laccase gene expression project. Dr. Kim's work then included the study of dirigent proteins, with the exciting discovery of a second form of dirigent proteins in *Arabidopsis* able to facilitate stereoselective coupling of coniferyl alcohol to afford (-) pinoresinol. Prior to that, our lab had discovered corresponding (+) pinoresinol-forming dirigent proteins. Dr. Kim was the recipient of the Arthur Neish young investigator awards in 2010.



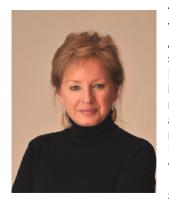
Aruna Kilaru is Assistant Professor, Department of Biological Sciences at East Tennessee State University, USA. She completed her B.Sc. in Biology and Chemistry at Andhra University, India in 1991, and her M.Sc. in Biotechnology at Maharaja Sayajirao University, India in 1993. She earned her Ph.D. in Environmental and Evolutionary Biology, from the University of Louisiana at Lafayette, USA in 2005, under the guidance of Prof. Karl Hasenstein. Dr. Kilaru pursued post-doctoral studies with Prof. Kent Chapman at University (2009-11). Her research interests are in plant biochemistry and physiology, with particular emphasis on lipid synthesis and signaling. Specifically, she continues to unravel

the metabolic pathway of N-acylethanolamines, a class of bioactive lipids, in plants. Dr. Kilaru also has ongoing research to understand the regulation of triacylglycerol biosynthesis in non-seed tissues. Her work was published in high impact journals including PNAS, Plant Cell, JBC, Plant Journal, among others. As a graduate student and postdoctoral fellow, Aruna received various honors and travel awards to present her research. Dr. Kilaru is an active member of the American Society of Plant Biologists, Sigma Xi, and Association for Women in Science, in addition to the Phytochemical Society of North America.



Ah-Ng Tony Kong is Professor II (Distinguished), Glaxo Chair Professor of Pharmaceutics and Director of the Graduate Program in Pharmaceutical Sciences at Rutgers, The State University of New Jersey. He is also Associate Director for the Center for Cancer Prevention Research and Co-Leader of the Carcinogenesis and Cancer Prevention program at the Cancer Institute of New Jersey. He received his B.S. in Pharmacy (1983) from the University of Alberta, Canada and his Ph.D. in Pharmacokinetics and Pharmacodynamics (1989) from the State University of New York at Buffalo. He received post-doctoral training in molecular genetics and cellular signaling from 1989-1991 at the National Institutes of Health (NIH). He was on the faculty of Thomas Jefferson University Medical School and the University of Illinois at Chicago before joining Rutgers in early 2001. He

continues to serve on the NIH Study Section since 1999 and has funding support from the NIH since 1993. He has trained more than 30 post-doctoral fellows, visiting professors, Ph.D. and M.S. students. He has published more than 160 original research, review articles and book-chapters. He is an Editor of *Pharmaceutical Research*, and member of editorial advisory boards of *Carcinogenesis, Molecular Carcinogenesis, Biopharmaceutics and Drug Disposition* and *Cancer Prevention Research (AACR)*. His research areas are in dietary phytochemicals (signaling and gene expression, nutrigenomics, cancer chemoprevention), animal tumor models of prostate, colon and skin, epigenetics, oxidative/redox/ inflammatory stress response and Nrf2-mediated nuclear transactivation & signaling, pharmacokinetics and pharmacodynamics of phytochemicals.



Toni M. Kutchan is the Oliver M. Langenberg Distinguished Investigator and Vice President for Research at the Donald Danforth Plant Science Center and Adjunct Professor of Biology at Washington University in St. Louis. Previously, she spent twenty years carrying out research in Germany, holding positions as Professor and Department Head at the Leibniz Institute for Plant Biochemistry in Halle, Germany, as well as Managing Director of that institute. Her primary research interests are the biosynthesis of plant medicinal compounds such as alkaloids and metabolic engineering of medicinal plants. She has been a member of the Board of Scientific Advisors of the Schering Research Foundation, a member of the Central Selection Committee of the Alexander von Humboldt Foundation, is on the editorial board of *The Journal of Biological Chemistry* and *Phytochemistry* and has served as Coordinator of the German Science Foundation Priority Program "Evolution of Metabolic Diversity." She is

currently a Scientific Advisory Committee Member of the William L. Brown Center for Plant Genetic Resources of the Missouri Botanical Garden. She has published numerous articles reporting her research, is a frequent international lecturer and is a member of the Berlin-Brandenberg Academy of Sciences, a Fellow of the Academy of Science St. Louis, and a member of the German National Academy of Science Leopoldina. She holds a B.S. in Chemistry from the Illinois Institute of Technology, a Ph.D. in Biochemistry from St. Louis University and the *Dr. Habil.* from the University of Munich.



Mi Kwon, Research Professor, College of Life Science and Biotechnology at Korea University, South Korea. She completed her B.S. in Forest Product Technology in 1991, her M.S in wood anatomy in 1993, at Seoul National University, and her Ph.D. (Plant Physiology Program) under supervision of Prof. Norman G. Lewis at Washington State University in 2000, and post-doctoral studies with Prof. Nicki Engeseth at University of Illinois Urbana-Champaign (2001-2002), and Prof. Sunghwa Choe (2002-2006) at Seoul National University. Her research interests are in tree growth and development, with special emphasis on molecular control of secondary cell wall biosynthesis during xylem formation in woody plants. She served as an Adjunct Research Institute from 2009. She is a recipient of the Future Scientist & Best Article Award (2007) from the Botanical Society of Korea.



Bernd Markus Lange is an Associate Professor at the Institute of Biological Chemistry and is currently co-directing the M.J. Murdock Metabolomics Laboratory at Washington State University. He received his Bachelor's and Master's degrees in Chemistry from the University of Bonn and his Doctoral degree in Botany from the University of Munich. Upon graduation, Dr. Lange held postdoctoral positions with Lutz Heide at the University of Tübingen and Rodney Croteau at Washington State University. Subsequently, he led research groups in the biotechnology industry (Novartis Agricultural Research Institute Inc., Torrey Mesa Research Institute of Syngenta and Diversa Inc.), before taking up his current faculty position in 2004. Dr. Lange's research interests center on using integrative approaches to characterize the regulation of biochemical pathways with particular emphasis on terpenoid biosynthesis in specialized tissues and cell types.



Robert L. Last is Barnard Rosenberg Professor of Biochemistry at Michigan State University, where his laboratory uses genomics approaches to study plant biochemistry and physiology. Prior to joining MSU in 2004, he last served as a Program Officer at the National Science Foundation. From 1998 to 2002, he was a founding Science Director at Cereon Genomics LLC, where his group established genomics platforms for phenomics using Arabidopsis and model cellular systems. He began his independent career at the Boyce Thompson Institute at Cornell University in 1989 where he rose through the ranks to Full Scientist, studying amino acid biosynthesis and plant stress adaptation mechanisms. He was a postdoc with Gerald Fink at Whitehead Institute at MIT, received his PhD in Biological Sciences from Carnegie-Mellon University and a BA in Chemistry from Ohio Wesleyan University. He is a Fellow of the AAAS, the American Society of Plant Biologists and a Monsanto Fellow. He was a recipient of a NSF Presidential

Young Investigator Award. He has served the broader community in a variety of ways including on Editorial Boards of Plant Physiology and Current Opinions in Plant Biology, as Editor in Chief of The *Arabidopsis* Book. He has been involved on various advisory boards including three years as Founding Chair of the iPlant Cyberinfrastructure Collaborative Board of Directors.



Michel Legrand is Director of research at IBMP (Institut de Biologie Moléculaire des Plantes) of CNRS, Strasbourg, France. One of his major research interests concerns the role of secondary metabolism in plant defense and development. In the past, he has studied the regulation of the phenylpropanoid pathway upon infection by pathogens and investigated particularly the implication of *O*-methyltransferases in the induced synthesis of phenolic compounds. Different classes of *O*-methyltransferases (COMTs and CCoAOMTs) were shown to be implicated in the biosynthesis of lignin monomers by producing and analyzing a range of antisense plants whose lignin quantity and/or quality was modified. A new acyltransferase involved in lignin synthesis (HCT, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase) was characterized and shown to catalyze the synthesis of the shikimate and quinate esters of *p*-coumaric acid,

which are the substrates of cyp450 3-hydroxylase. More recently, characterization of *Arabidopsis thaliana* genes and corresponding enzymes involved in pollen cell wall formation has demonstrated the role of fatty acid derivatives as precursors of sporopollenin building units.



Eckhard Leistner studied Biology and Chemistry at the Ludwig-Maximilian-University in Munich, Germany. He received his PhD in botany under the supervision of Professor Meinhart Zenk in 1968. He subsequently joined Professor Ian Spenser at the Department of Chemistry in Hamilton, Ontario, Canada from 1969 through 1971 as a research associate and got his habilitation at the Ruhr-University in Bochum, Germany in 1973. He was appointed Associate Professor at the Department of Pharmaceutical Biology of the Westfälische Wilhelms-University in Münster, Germany, where he stayed from 1975 to 1983. He then became Full Professor and Head of the Department of Pharmaceutical Biology of the Rheinische Friedrich Wilhelms-University in Bonn, Germany. He served his university in different functions including a chairmanship of the Department of Pharmacy. He was visiting Professor in the School of

Pharmacy at Purdue University (1979) and in the Department of Chemistry at the University of Washington (1992). His current research is focused on the mode of action of ginkgotoxin in the human body which helped to recommend a restricted use of Ginkgo medications by German authorities. His research is also concerned with the role of plant associated microorganisms. His research was funded by various institutions including the Deutsche Forschungs-gemeinschaft. Approximately 60 PhD students graduated under his supervision, some holding chairs in Pharmaceutical Biology and Pharmaceutical Biotechnology at German universities. The Deutsche Pharmazeutische Gesellschaft conferred the Carl Mannich medal upon him in 2007.

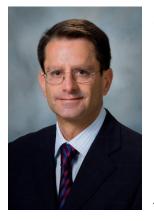


Norman G. Lewis, Regents Professor and Arthur M. Katie Eisig-Tode Distinguished Professor, is Director, Institute of Biological Chemistry, at Washington State University, USA. He completed his B.Sc. (Honors) in Chemistry at the University of Strathclyde in 1973, his Ph.D. (Chemistry) at the University of British Columbia in 1977, and post-doctoral studies with Prof. (Sir) A.R. Battersby at Cambridge University (1978-80). His research interests are in plant biochemistry, with particular emphasis on plants phenols (lignins, lignans allyl/propenyl phenols) and plant cell walls, with over 220 refereed/invited papers. Studies have also involved space flight experiments (Space Shuttle and MIR). Of particular interest is the work on the proteins that control phenoxy radical coupling in plants, the dirigent proteins, which were discovered in his lab. He has held various offices, including Phytochemical Society of North

America (President), American Society of Gravitational Biology (President), and American Society of Plant Biology (Public Affairs Committee). He serves on various editorial boards and advisory boards, with a main responsibility as Phytochemistry Regional Editor. Prof. Lewis is an Elected Fellow of the Royal Society of Edinburgh, Scotland's National Academy of Science and Letters.



Karen T. Liby is a Research Assistant Professor at Dartmouth Medical School. Her research focus is developing new drugs for the prevention and treatment of cancer. Her studies have identified the importance of HO-1 and the Nrf2/ARE/Keap1 cytoprotective network to explain many of the biological activities of synthetic oleanane triterpenoids. She has also studied various drugs, alone and in combination, for prevention and treatment of experimental lung cancer, estrogen receptor-negative breast cancer, and pancreatic cancer. She earned a B.S. in biology and history from Hillsdale College and a Ph.D. in cellular and molecular biology from the University of Cincinnati and was awarded the Wilson S. Stone Memorial Award from the M.D. Anderson Cancer Center and a Sidney Kimmel Foundation for Cancer Research Scholar Award.



Scott M. Lippman, MD, holds the Charles A. LeMaistre Distinguished Chair in Thoracic Oncology, is professor of Cancer Medicine and Cancer Prevention, and is chair of the Department of Thoracic/Head and Neck Medical Oncology at The University of Texas M.D. Anderson Cancer Center in Houston, Texas. He graduated from Johns Hopkins University School of Medicine and is board certified in internal medicine, hematology, and medical oncology. Dr. Lippman's major fields of research are translational/molecular studies of cancer risk, carcinogenesis, and molecular-targeted drug development. He has a long-standing record of National Cancer Institute funding in these research areas, including current funding as principal investigator of a P01 and a SPORE. Dr. Lippman's record of service includes the FDA Oncologic Drugs Advisory Committee (ODAC) and the editorial boards of several top-tier peer-reviewed journals, including *Cancer Research, JNCI,* and *Journal of Clinical Oncology* (*JCO*). He currently is chair of the ASCO Nominations Committee, AACR

Cancer Prevention Committee, and NIH study section CDP; member of the NIH Clinical Trials/Translational Research Advisory Committee [CTAC]); and Co-Director of the ECCO/AACR/EORTC Flims Clinical Methods Workshop. He also is a member of the Association of American Physicians (AAP). He received the ASCO-ACS Award in 2007 and AACR Cancer Research and Prevention Foundation Award in 2005. Dr. Lippman is editor-in-chief of the AACR journal *Cancer Prevention Research*. He has authored over 300 publications including in the high-impact journals the *NEJM, JAMA, PNAS, Nature Medicine, Cancer Cell, Cancer Research, JNCI*, and *JCO*.



Vincenzo De Luca, is a Professor in the Department of Biological Sciences at Brock University in St. Catharines, ON, Canada, where he has been a Tier 1 Canada Research Chair in Plant Biotechnology since August, 2001. Previously he held positions as Principal Scientist with Novartis/Syngenta (1998-2001), as Professor of Biological Sciences with the University of Montreal (1989-98) and as Associate Research Officer with the National Research Council of Canada (1984-89). His research interests have focused on combining modern tools of genomics and metabolomics with biochemical characterization to elucidate secondary metabolism pathways in plants. His lab has made contributions to our understanding of monoterpenoid indole alkaloid (MIA) biosynthesis and on the production of anticancer dimeric indole alkaloids in the Madagascar periwinkle (*Catharanthus roseus*). His group has shown that some MIAs appear to

accumulate in the leaf surface and that a primary reason the lack of dimer production is the spatial separation of the 2 precursors in the plant. These and other fundamental studies are helping to understand how particular plant cells are programmed for manufacturing natural products. The results of this research are being used to manipulate the level and quality of the metabolites being produced. In this manner, the products of photosynthesis can be directed to produce commercially useful pharmaceuticals (Examples: vinblastine, taxol and morphine), aromas, nutraceuticals, flavours, colors and pesticides.



Rachel Mata received her M Sc. and Ph.D. degrees from Purdue University in 1976 and 1979, respectively. After postdoctoral appointments at the Institute of Chemistry of the National Autonomous University of Mexico, she moved to the School of Chemistry of the same University at the end of 1984 where she is currently the head of the Pharmacy Department. Her research projects are intended to isolate and characterize bioactive compounds from selected medicinal plants and fungi from Mexico in order to discover new leads for the development of new drugs and pesticide agents friendlier to the environment. This work has crystallized in more than 170 refereed papers. She has held various offices, including Phytochemical Society of North America and served on various editorial boards and advisory boards.



Gregory D. May is President of the National Center for Genome Resources, Santa Fe, New Mexico, USA. He completed his BS in Biology at Southeast Missouri State University in 1987, his PhD in Plant Physiology in the Department of Biochemistry and Biophysics at Texas A&M University in 1992, and his post-doctoral studies with Dr. Charles Arntzen at the Institute of Biosciences & Technology, Houston Texas, USA in 1993. His research interests are in genome biology with a focus on the effects of genotypic and epigenetic variation on gene expression and phenotype. Emphasis in the more than 90 refereed publications is in the application of next-generation DNA sequencing technologies to characterize complex eukaryotic genomes and the discovery of novel genes. He serves as editor for *The Plant Genome* and *Frontiers Genetics*.



Debra Mohnen received her Ph.D. in plant biology from the University of Illinois with research conducted at the Friedrich Miescher Institute in Basel, Switzerland. She held postdoctoral research associate positions at the USDA's Richard Russell Research Center and at the Complex Carbohydrate Research Center (CCRC) in Athens, GA where she won an NIH National Research Service Award for her postdoctoral research. She was appointed to the CCRC faculty in September 1990 and is currently Professor in the Department of Biochemistry and Molecular Biology and also adjunct faculty member in the Department of Plant Biology and member of the Plant Center at the University of Georgia (UGA). She served on the Committee on the Status of Women in Plant Physiology of the American Society of Plant Physiologists, invited faculty sponsor for the UGA Association for Women in Science (AWIS), past member-

at-large in the Cellulose and Renewable Materials Division of the American Chemical Society, and is currently a member of the Council for Chemical and Biochemical Sciences, Chemical Sciences, Geosciences, and Biosciences Division in the Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy. As Co-PI on the NSF-funded "Plant Cell Wall Biosynthesis Research Network" Dr. Mohnen established, and continues to run, the originally NSF-funded service "CarboSource Services" that provides rare substrates for plant wall polysaccharide synthesis to the research community. Dr. Mohnen was awarded the 2008 Bruce Stone Award by the Plant Polysaccharide Workshop for contributions and promising research in the area of Pectin Biosynthesis. She was Chair of the 2009 Plant Cell Walls Gordon Research Conference. Dr. Mohnen is Activity Lead in Plant Cell Wall Biosynthesis Research and Focus Area Lead in Plant Biomass in the DOE-funded BioEnergy Science Center (BESC). She serves on scientific advisory boards for multiple bioenergy centers and is a joint Editor-in-Chief for the journal Biotechnology for Biofuels. Her research centers on the biosynthesis, function and structure of plant cell wall polysaccharides and is supported by funding from the USDA, NSF and DOE. Her emphasis is on pectin biosynthesis and pectin function in plants and on human health, and on the improvement of plant cell wall structure so as to improve the efficiency of conversion of plant wall biomass to biofuels.



Birger Lindberg Møller obtained his MSc, PhD and DSc from the University of Copenhagen in 1972, 1975, and 1984, respectively. BLM was a Fulbright Fellow at Eric Conn's laboratory, UC Davis, in 1975-77 working with cyanogenic glucosides. From 1977-1984 he was Senior Scientist and Niels Bohr Fellow at the Department of Physiology, Carlsberg Laboratory studying photosynthesis. In 1984 he was appointed Research Professor and in 1989 Professor at the Royal Veterinary and Agricultural University, Copenhagen, now part of the University of Copenhagen. In the period 1998-2008 he served as Head of Center for Molecular Plant Physiology (PlaCe) founded by the Danish National Research Foundation. In 2008, he was appointed Director of the research centre "Pro-Active Plants" supported by the Villum Foundation and in 2010, Director of the Center for Synthetic Biology funded by the Danish Ministry of Science. In 2011 he became Director of the section for "Plant Pathway Discovery" in the Novo

Nordisk Foundation Center for BioSustainability. One of his main research interests is the synthesis, turnover and storage of cyanogenic glucosides, and their role in plant insect and plant microbe interactions. Birger Lindberg Møller has been the main advisor of 35 PhD students. He is an elected member of the Royal Danish Academy of Science and Letters and of the Danish Academy of Natural Sciences. He is member of the International Human Rights Network of Academic and Scholarly Societies, Washington. In 2007, Birger Lindberg Møller was awarded the Villum Kann Rasmussen Research Prize, the largest Danish research award (350.000 Euro).



Hasan Mukhtar is Helfaer Professor of Cancer Research, Director and Vice Chair for Research, Department of Dermatology, School of Medicine and Public Health, University of Wisconsin, Madison, Wisconsin, USA. He is also the Coleader of Cancer Chemoprevention Program, Paul P Carbone Comprehensive Cancer Center, University of Wisconsin. He is an author of 450 original peer reviewed publications, many in very high impact journals and is an author of 68 book chapters and two books. He serves as an Editorial Board member /Associate Editor of 32 scientific journals that include Experimental Dermatology, Photochemistry and Photobiology, Clinical Cancer Research, Carcinogenesis,

International Journal of Cancer, Apoptosis Molecular Nutrition and Food Research, Nutrition & Cancer, and Cancer Research. He serves on grant review committees of National Institutes of Health, Department of Veterans Affairs, Department of Defense and many private organizations. Dr Mukhtar has deep passion for research in cancer chemoprevention with focus on skin, lung and prostate cancers. His other interests include defining adverse effects of ultraviolet radiation on skin, skin cancer mechanisms and the role of botanical antioxidants to prevent such damages. Dr Mukhtar obtained PhD in biochemistry in 1971 from Kanpur University, India.



Michael Müller studied Chemistry at the University of Bonn and obtained his diploma in 1991 working with Prof Dr W. Steglich on natural product synthesis. He then moved with his mentor to Munich, where he obtained his PhD in Organic Chemistry in 1995 from the Ludwig-Maximilians-University. He spent 1996 to 1997 at the University of Washington as a Research Assistant, working with Prof. Dr. H. G. Floss on biosynthesis of antibiotics. In September 1997, he started his independent scientific work as the Head of the Bioorganic Chemistry Group at the Institute of Biotechnology, Research Centre Jülich. He finished his Habilitation in Organic Chemistry and Bioorganic Chemistry in October 2002. In 2004, he was appointed Full Professor of Pharmaceutical and Medicinal Chemistry at the University of Freiburg. His research interests are in the study of chemoenzymatic synthesis, natural product chemistry and asymmetric synthesis.



Susan Murch received her PhD from the University of Guelph, Guelph, Canada in 2000 specializing in plant biochemistry and biotechnology. The highlight of her thesis was the discovery of the human hormone melatonin in leaves and flowers of many popular medicinal plants. Susan joined the Chemistry faculty at the University of British Columbia, Okanagan Campus in 2005, was awarded a Canada Research Chair in Natural Products Chemistry (2005-2010) and was President of the Natural Health Products Research Objectives with practical applications of phytochemistry. Current research programs in Dr. Murch's lab include: efforts to bring the underutilized traditional crop breadfruit (*Artocarpus altilis*) to world markets, medicinal activity of the traditional North American medicinal plant 'osha' (*Ligusticum* species), phytochemistry and metabolomics of cranberry species, and the presence and persistence of the naturally occurring

neurotoxin β -methylamino-L-alanine. She has published more than 75 peer reviewed journal articles, 15 book chapters and co-edited a volume entitled "The Journey of a Cell to a Whole Plant".



W. David Nes, born in Bethesda, Maryland and educated at Gettysburg College, is the Paul Whitfield Horn Professor and Chair of Biochemistry Division at Texas Tech University. He received a M.S. at Drexel University in 1977 with his father, William R. Nes, with whom he published a series of research papers and a book on sterols and a Ph.D. at the University of Maryland in 1979 where he studied plant biochemistry. In 1980, he began his career at the ARS-USDA Western Regional Research Center in Albany, CA and after relocation to the Russell B. Research Center in Athens, GA was promoted to Lead Scientist in 1988 and during these early years was appointed adjunct Research Professor status at the University of Georgia (Natural Products Center) and Auburn University (Chemistry Department) and a DAAD fellowship from Germany. He joined the Chemistry and Biochemistry faculty at Texas Tech University, Lubbock, Texas in 1993 and in the last quarter century has mentored over 60 graduate students, post-doctoral fellows and Visiting Scientists. From 2003 to 2005, he was Visiting Scientist and Program Director at the National Science Foundation, Molecular and Cellular Biosciences Division and Visiting Professor in 2008 at the Max

Planck Institute for Chemical Ecology, Jena, Germany. His research, funded by the NSF, NIH, Welch Foundation and Industry, is broadly in natural products chemistry with a major focus on enzyme mechanisms, biosynthesis and function of phytosterols which has resulted in 165 publications and 9 books.



M. Soledade C. Pedras is currently the Tier 1 Canada Research Chair in Bioorganic and Agricultural Chemistry in the Department of Chemistry of the University of Saskatchewan, Canada, where she is Professor of Chemistry since July 1994. Her research interests and studies involve the discovery of chemical and biochemical mediators of the interaction between plants (crucifers) and fungal pathogens, and application of this knowledge to understand disease resistance and control plant pathogens. Her projects include biosynthetic pathways of phytoalexins, phytoanticipins, elicitors and phytotoxins, isolation of detoxifying enzymes from plant pathogens and design and synthesis of paldoxins and other inhibitors of metabolic processes specific to fungi.



John Michael Pezzuto received his B.S. degree in chemistry from Rutgers University (1973), and Ph.D. in biochemistry from the University of Medicine and Dentistry of New Jersey (1977). He then performed two years of postdoctoral work in the Department of Chemistry at Massachusetts Institute of Technology, where he was the recipient of a postdoctoral fellowship from the National Cancer Institute. Following a one-year stay at the University of Virginia, as Instructor of Chemistry, he accepted a faculty position at the University of Illinois at Chicago (1980). He was promoted to the rank of Associate Professor in 1984, and to full professor in both the College of Pharmacy (1991-2002) and the College of Medicine (1994-2002) at the University of Illinois at Chicago. He was named Distinguished University Professor in 2002. He held various administrative

positions prior to moving to Purdue University in 2002, where he served as Professor of Medicinal Chemistry and Molecular Pharmacology, and Dean of the College of Pharmacy, Nursing and Health Sciences (2002-2006). He is currently Professor and Founding Dean of the College of Pharmacy at the University of Hawaii at Hilo (2006-present), and holds adjunct professorial positions at the University of Illinois at Chicago, Purdue University, and the Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii at Manoa. He is an author of over 500 publications, co-inventor of several patents, the editor of three books, member of several editorial boards, and editor-in-chief of *Pharmaceutical Biology*. He resides in Hilo, Hawaii, with his wife and three children.



Arthur Ragauskas held the first Fulbright Chair in Alternative Energy and is a Fellow of the International Academy of Wood Science and TAPPI. His research program at Georgia Institute of Technology is seeking to understand and exploit innovative sustainable bioresources. This multifaceted program is targeted to develop new and improved applications for nature's premiere renewable biopolymers for biomaterials, biofuels, biopower, and bio-based chemicals. His Fulbright sponsored activities at Chalmers University of Technology, Sweden were focused on the forest biorefinery and new biofuel conversion technologies for lignocellulosics. Currently, he is a program leader for the GA Tech team leader for Biological Energy Science Center (BESC) research efforts. Dr. Ragauskas has been an invited visiting professor at Universidade da Beira Interior, Portugal; Chalmers University of Technology, Sweden; Royal Institute of Technology/ STFi, Stockholm, Sweden; and South China University of Technology, China.



Michel Rohmer completed a PhD thesis with Professsor Guy Ourisson at the Université Louis Pasteur of Strasbourg (1975), working on the chemistry and biochemistry of prokaryotic triterpenoids. Meanwhile, he was "assistant" and later "maître-assistant" in Pharmacognosy at the Faculty of Pharmacy (1974-1979). After post-doctoral work with Professor Carl Djerassi at Stanford University (1978-1979) on sterols from marine organisms, he was promoted as Professor of organic and bio-organic chemistry, first at the Université de Haute Alsace (Ecole Nationale Supérieure de Chimie) in Mulhouse (1979-1994) and later in 1994 at the Faculty of Chemistry of the University of Strasbourg. He is Member of the "Institut Universitaire de France" (1997), the Deutsche Akademie der Naturforscher Leopoldina (2000) and the "French Académie des Sciences"

(2003)". Michel Rohmer is a specialist of the chemistry and biochemistry of isoprenoids from microorganisms and higher plants and published over 215 papers. Main discoveries include the biohopanoids, a series of pentacyclic bacterial triterpenoids, precursors of a ubiquitous family of molecular fossils, and the methylerythritol phosphate pathway, a novel metabolic route towards the isoprene units in bacteria and plants plastids. Awards: Vaillant Prize, Académie des Sciences (1984); French-British Prize, the Royal Society/Académie des Sciences (1993); Humboldt–Gay-Lussac Prize, Alexander von Humboldt Foundation (1997); Nakanishi Prize, Chemical Society of Japan/American Chemical Society (2008); Schroepfer Medal Award, American Oil Chemists Society (2008); Albert Hofmann Award, University of Zürich, Switzerland (2008).



Kazuki Saito graduated from the Faculty of Pharmaceutical Sciences, the University of Tokyo, Japan in 1977 and then obtained his Ph D for bio-organic chemistry/biochemistry from the University of Tokyo in 1982. After staying in Keio University in Japan and Ghent University in Belgium (Prof. Marc Van Montagu's laboratory), he became a faculty member at the Graduate School of Pharmaceutical Sciences, Chiba University, Japan. There he has been appointed as a Full Professor since 1995, until now. Since April, 2005, he has been additionally appointed as a group director at RIKEN Plant Science Center to direct Metabolomic Function Research Group. He also holds the post of Deputy Director of RIKEN Plant Center. In 2010, he was awarded The Prize for Science and Technology (Research Category) by the Minister of Education, Culture, Sports, Science and Technology, Japan. He was also selected as one

of ASPB TOP AUTHORS in 2010. In 2011, he received The Award for Distinguished Research from Japanese Society for Plant Cell and Molecular Biology. His research interests are metabolome-based functional genomics, biochemistry, molecular biology and biotechnology of primary and secondary metabolism in plants. In particular, he is engaging in the biosynthetic studies of sulfur compounds, flavonoids, terpenoids and alkaloids by means of metabolomics.



Simo Sarkanen is a Professor in the Department of Bioproducts and Biosystems Engineering at the University of Minnesota. He received his B.A. degree (Natural Sciences, Chemistry) from Cambridge University (King's College) in England, and his Ph.D. (Bio-organic Chemistry) from the University of Washington in 1976. He subsequently entered the realm of lignin chemistry and biochemistry as a postdoctoral research associate (Chemical Engineering) with Prof. J. L. McCarthy at the University of Washington. His interests range from the development of lignin-based polymeric materials to the mechanism of lignin biosynthesis and the enzymology of lignin biodegradation. The first lignin-based thermoplastics were formulated in his research group. He is particularly curious about how the assembly of lignin macromolecules is controlled in muro, and how the powerful noncovalent interactions in lignin biodegradation.



Fumihiko Sato received his Ph.D. from Graduate School of Agriculture of Kyoto University (1981). He was appointed Assistant Professor at Kyoto University in 1979, and carried out postdoctoral research at University of Muenster as an Alexander von Humboldt fellow (1983-1984). He was promoted as Professor in Faculty of Agriculture in 1995 and moved to his current position in 1999. He has authored and co-authored *ca.* 200 publications. Molecular and cellular biological studies on totipotency in plant cells have been carried out in his laboratory using in vitro cultured cells and transgenic plants. Especially, functional differentiations, e.g. molecular mechanism of photoautotrophism and molecular biology of secondary metabolism, such as biosynthesis of isoquinoline alkaloids in plant cells, have been investigated to understand the totipotency in plant cells. Developments of novel genetic engineering techniques such as differential RNAi, metabolic engineering and synthetic biology of secondary metabolism have been also investigated.



Bernd Schneider is the head of the Biosynthesis/ NMR group of the Max Planck Institute for Chemical Ecology Jena, Germany, since 1997. Between 1981 and 1996, he was a researcher at the Institute for Plant Biochemistry in Halle, Germany. In 1991 he received a research fellowship of the Alexander von Humboldt-Foundation to work with M.H. Zenk at the University of Munich. In 2008/2009, he was a guest professor at Université "Jules Verne", Amiens, France. Major research interests are in the area of natural product chemistry and NMR spectroscopic applications in chemical ecology with a focus on the biosynthesis of plant secondary metabolites. He has published approximately 200 original papers and review articles. He holds a diploma and doctoral degree in chemistry from the University of Halle/S. and Dr. habil. from the Universities of Halle and Jena.



Ming-Che Shih is the director and distinguished research fellow of the Agricultural Biotechnology Research Center (ABRC), Academia Sinica, Taiwan. He was a faculty member of the Department of Biology, University of Iowa between 1988 and 2009 and the director of Carver Center for Comparative Genomics at the University of Iowa between 2005 and 2007. His lab has used a powerful selection scheme to isolate mutants that are defective in hypoxia induction of the alcohol dehydrogenase gene in Arabidopsis. His lab has taken full advantage of these mutants to gain an understanding of the hypoxia signaling mechanism. They showed that hypoxia triggers several signaling pathways and that one of these pathways is mediated by ethylene. More recently, he has used a functional genomic approach to reconstruct transcriptional regulatory pathways associated with hypoxia in both Arabidopsis and rice. Since joining ABRC in 2008, he has extended his research into functional genomics for biomass conversion and orchid biotechnology.

constructed transcriptomes for several fungal and orchid species by high throughput sequencing techniques, namely Illumina Solexa and Roche 454 Titanium. His lab has developed an efficient workflow for assembly of sequence data, annotation and functional analysis. The entire workflow minimizes time and labor for informatics process and maximizes output in both novel gene discovery and sequence information. This workflow can easily be adapted to most research subjects on demand.



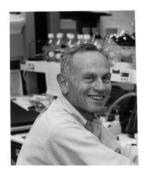
Nicholas Smirnoff is Professor of Plant Biochemistry in the College of Life and Environmental Sciences, University of Exeter, Devon, UK. He studied at Bristol University (BSc in Botany, 1978) and St. Andrews University (PhD in plant physiological ecology, 1981) followed by postdoctoral research on nitrogen metabolism and drought responses with George R. Stewart at Birkbeck College, London (1982-5). He was instrumental in the elucidation of the biosynthetic pathway of ascorbic acid in plants and has ongoing interests in the control of ascorbate synthesis and its biological functions. Other research interests include the roles of reactive oxygen species and antioxidants in stress responses and development. He is part of a consortium that is using

systems biology approaches to deciphering transcriptional responses of Arabidopsis to abiotic and biotic stress. He is also working on the biosynthesis of alkanes by cyanobacteria and plants. He is an editor of "Annals of Botany" and an advisory board member of "Journal of Experimental Botany".



Michael Spiteller was born in Herrsching/Ammersee in Bavaria on 24th April, 1954. After studying chemistry at the Georg-August-University in Göttingen, he changed to geosciences and was appointed to a professorship in 1985 at the Faculty of Forestry in Göttingen. In the same year, he got a position as group leader at Bayer Crop Science AG. In 1989, Michael Spiteller was offered a professorship for organic sediment analysis at the University of Heidelberg and four years later he moved to the University of Kassel on appointment to the chair in Ecological Chemistry and Ecotoxicology. In 1999, he was appointed to the chair in Environmental and Analytical Chemistry at Dortmund University of Technology, and since then he is the head of the Institute of Environmental Research (INFU) of the Faculty of Chemistry(see homepage: http://www.infu.tu-

dortmund.de). His basic field of research is concerned with the detection of natural and anthropogenic substances in soil, water and plants, the development of new analytical methods for the detection of trace contaminants in the environment as well as the detection and identification of unknown metabolites in plants and fungi. Michael Spiteller is co-editor and member of the editorial board of various scientific journals, honorary member of the Serbian Chemical Society and the Institute of Organic Chemistry and Phytochemistry at the Bulgarian Academy of Science in Sofia as well as honorary doctor of the Universities of Novi Sad in Serbia and Sofia in Bulgaria.



Michael B. Sporn received his M.D. degree at the University of Rochester, and then started a 35-year career at the National Institutes of Health, where he became the Chief of the Laboratory of Chemoprevention in the National Cancer Institute in 1978. In the 1980s, his laboratory in Bethesda played a key role in the original discovery of the multifunctional cytokine known transforming growth factor-beta (TGF-beta). In 1995, he moved to Dartmouth Medical School, where he has held an endowed chair as Professor of Pharmacology and Medicine. He has been a strong advocate for prevention of cancer for many years, and much of his own research has dealt with the development of new drugs to be used as chemopreventive agents. These drugs have included synthetic retinoids and rexinoids (analogs of Vitamin A), synthetic deltanoids (analogs of Vitamin D), as

well as selective estrogen response modulators (SERMs). Most recently he has been focusing on the use of new synthetic triterpenoids as agents for preventing breast and lung cancer and for suppressing inflammation and oxidative stress.



Jan Frederik ('Fred') Stevens received his M.Sc. in Pharmacy (1988), pharmacy license (1990), and Ph.D. in Medicinal Chemistry (1995) from Groningen University, The Netherlands. He received postdoctoral training at Oregon State University (1995-1999), the Free University of Amsterdam (1999-2000), and the Leibniz Institute for Plant Biochemistry, Halle/Saale, Germany (2000-2002). In 2002, he joined the faculty at Oregon State University (OSU), Corvallis, where he is now an Associate Professor of Medicinal Chemistry in the College of Pharmacy. He is affiliated with the Linus Pauling Institute at OSU as one of 12 Principal Investigators. He has authored or co-authored 70 articles in peer-review journals, 15 of which were published in Phytochemistry. His research is aimed at determining the role and function of vitamin C and dietary

phytochemicals in human health and disease. His research group relies heavily on mass spectrometry as a tool for studying small organic molecules in complex biological matrices such as blood plasma, cell lysates, tissue homogenates and plant extracts. Mass spectrometry-based metabolomics is a new direction in his laboratory for discovery of biological effects of vitamins and phytochemicals. One of his research projects is focused on xanthohumol from hops (*Humulus lupulus*), which has gained interest in recent years as a dietary supplement and food additive. Dr. Stevens and his OSU colleagues have published 20 papers on the chemistry and biological activities of xanthohumol and related prenylated flavonoids. In preparation for clinical studies with xanthohumol, Dr. Stevens currently studies its pharmacokinetics and its effects relevant to metabolic syndrome.



Robert D. Stipanovic has conducted natural product research for over 35 years focusing on compounds occurring in plants in the Malvaceae family, primarily *Gossypium* (cotton). He is employed at the, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, Texas, USA. His work on compounds produced by the cotton plant has been focused on those that protect the plant from herbivorous insects and pathogens. However, for many years he has been intrigued with the possibility of utilizing cottonseed as a feed for non-ruminant animals such as chicken and fish, which more efficiently convert food intake into body mass. Cottonseed contains ~22.5% of a good quality protein. Average annual world production of cottonseed is ~43MMT, which would provide almost 10MMT of protein. Utilization of cottonseed as feed

for chickens would be a boon for many developing countries where cotton is already grown. Unfortunately, commercial cottonseed cannot be fed to non-ruminants because it contains the toxin gossypol which occurs naturally as a mixture of enantiomers, and (+)-gossypol is significantly less toxic to chickens than the (-)-gossypol. Cottonseed from commercial plants contain an approximate 3:2 ratio of (+)-gossypol to (–)-gossypol. In collaborative work, ARS scientists in College Station have developed plants that produce seed with >95% (+)-gossypol. The subject of the talk will be the biosynthesis of gossypol from farnesyl diphosphate to hemigossypol and the recent discovery that a dirigent protein controls the free radical coupling of hemigossypol to give (+)-gossypol.

PSNA 50 Invited Speakers



Joachim Stöckigt is presently Professor at Zhejiang University (China), where he continues his research at the College of Pharmaceutical Sciences after his retirement from Mainz University. He interests in natural products biosynthesis includes phytochemistry, enzymology, molecular and structural biology as well as chemo-enzymatic syntheses. As Full Professor he spent twenty years for research in the field of Pharmaceutical Biology at the Institute of Pharmacy at Mainz, after working together with M. H. Zenk at Bochum and Munich University for about two decades. He was a member of the Founder Committee of the Munich Gen Center and received the Tate & Lyle Award of the Phytochemical Society of Europe. He holds a Diploma in Chemistry from University of Kiel, a Ph. D. in Organic Chemistry from University of Münster, the *Dr. Habil.* from University of Bochum and the *Dr. Habil.* from University of Munich.



Gary Stoner is Professor of Medicine at the Medical College of Wisconsin (MCW) and Director of the Molecular Carcinogenesis and Chemoprevention Program in the MCW Cancer Center. He became involved in chemoprevention research in the early 1980's, initially investigating the chemopreventive potential of the naturally-occurring compounds, ellagic acid and phenethyl isothiocyanate in the rodent lung and esophagus. As an extension of research with ellagic acid, his laboratory developed a "food-based" approach to the prevention of esophageal and colorectal cancer in rodents and in humans using freeze-dried black raspberries (BRBs). His laboratory has shown that BRBs inhibit esophageal and colon cancer in rodents and humans by modulating cell signaling pathways associated with proliferation, apoptosis, inflammation and angiogenesis, and by reactivating tumor suppressor genes that have been silenced by methylation. His research is documented in more than 300 peer-reviewed publications and book chapters, and he has edited several books. He

has served on several grant and contract review committees, most recently as Chair of the NIH Chemo/Dietary Prevention Study Section. He has received numerous awards including the NIH MERIT award, and the Distinguished Alumni Award and Honorary Doctorate from Montana State University. He is also a Fellow in the American Association for the Advancement of Science.



Lloyd W. Sumner acquired his Ph.D. in analytical chemistry in 1993 from Oklahoma State University. He then joined Texas A&M University as the Director of the Mass Spectrometry Applications Laboratory. He moved to the Noble Foundation in 1999 and has risen to the rank of Professor within the Plant Biology Division. Dr. Sumner's research program focuses on the development and application of large-scale biochemical profiling (ie metabolomics, proteomics and transcriptomics) for novel gene discovery and understanding of plant natural products biosynthesis. Dr. Sumner has published over 90 peer reviewed articles and book chapters. His research is currently supported by the Samuel Roberts Noble Foundation, NSF 2010, NSF MCB, NSF MRI, NSF-JST Metabolomics, and the Oklahoma Commission for the Advancement of Science and Technology. Dr. Sumner is currently a Fellow of The American Association for the Advancement of Science, current Treasurer and past President of the

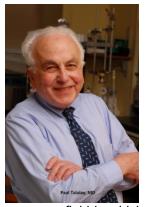
Metabolomics Society, Co-founding Member of the International Advisory Committee for Plant Metabolomics, Adjunct Professor at Oklahoma State University Dept. of Biochemistry and Molecular Biology, and a Distinguished Alumni of Cameron University. Dr. Sumner serves as a Managing Editor for Plant Physiology. He is also an Editorial Board member for the journal Metabolomics and the newly formed journal Frontiers in Plant Biotechnology

PSNA 50 Invited Speakers



Young-Joon Surh is a Professor of Biochemistry and Molecular Oncology, College of Pharmacy, Seoul National University, South Korea. He graduated from Seoul National University with B.S. (Pharmacy) and M.S. (Biochemistry). Professor Surh earned his Ph.D. degree at the McArdle Laboratory for Cancer Research, University of Wisconsin-Madison and completed his postdoctoral training at the Massachusetts Institute of Technology (MIT). In 1992, he was appointed as a tenure-track Assistant Professor at Yale University School of Medicine. Since relocating to Seoul National University in 1996, Dr. Surh has been investigating the molecular mechanisms of cancer prevention with antiinflammatory and antioxidative phytochemicals, with emphasis on intracellular signaling molecules as prime targets. Professor Surh has served as a member

of the editorial board member of more than 30 international journals, including *Carcinogenesis*, *International Journal of Cancer*, *Molecular Carcinogenesis*, *Cancer Letters*, *Mutation Research*, *Life Sciences*, *Molecular and Cellular Biochemistry*, *Free Radical Research*, *Food and Chemical Toxicology*, *Biofactors*, *Genes and Nutrition*, *Molecular Nutrition and Food Research*, *Journal of Clinical Biochemistry and Nutrition*, etc. He is also editor of the following books: *Oxidative Stress*, *Inflammation and Health* (CRC Press), *Molecular Targets and Therapeutic Use of Curcumin* (Springer-Verlag), and *Dietary Modulation of Cell Signaling Pathways* (CRC Press). Professor Surh has published more than 200 papers in peer-reviewed international journals and more than 80 invited editorials, reviews and book chapters. He received numerous awards including McCormic Science Institute Award from American Society for Nutrition and the E.C. Miller and J.A. Miller Distinguished Scholar Lecture Award.



Paul Talalay, M.D., is the John Jacob Abel Distinguished Service Professor of Pharmacology and Molecular Sciences at Johns Hopkins University School of Medicine. He received the S.B. Degree in Biophysics from M.I.T. and the M.D. Degree from Yale. Following surgical training at the Massachusetts General Hospital, he moved to the University of Chicago, where he became a Professor of Biochemistry, Professor of Medicine, and Professor in the Ben May Laboratory for Cancer Research. He was appointed in 1963 as Director of the Department of Pharmacology at the Johns Hopkins School of Medicine. In 1975, he relinquished this position to devote himself full time to cancer prevention research. Dr. Talalay has devoted his entire career to the cancer problem. His early work involved the elucidation of the enzymatic basis of the metabolism of steroid hormones and their control of hormone-dependent malignancies. For the last 30 years, he has devised strategies for chemoprotection against the risk of

cancer, a field in which he is recognized as a pioneer. His efforts have focused on achieving protection by raising the enzymes concerned with the detoxication of carcinogens. Analysis of the chemistry and the molecular biology of boosting enzymes of detoxication led him and his colleagues to devise simple cell culture methods for detecting chemical and especially dietary phytochemicals that raise these enzymes. This work made possible the isolation of sulforaphane as the most potent inducer of protective enzymes in broccoli. These findings led to the organization of the *Brassica Chemoprotection Laboratory* (1993) and the *Lewis B. & Dorothy Cullman Cancer Chemoprotection Center* (2003) at the Johns Hopkins Medical Institutions. The principle of protection against cancer and other chronic diseases by boosting endogenous cellular mechanisms has now been validated in animal models and by clinical studies. Dr. Talalay's honors, in addition to his appointment as a University Distinguished Service Professor, include: one of the first life-time Professorships of the American Cancer Society; Membership in the National Academy of Sciences; in the American Academy of Arts and Sciences; and in the American Philosophical Society. Among many honors, he has received an honorary D.Sc. Degree from Acadia University, and the Linus Pauling Institute Prize for Health Research.

PSNA 50 Invited Speakers



Toshiaki Umezawa received his Ph.D. from the Graduate School of Agriculture of Kyoto University (1987). The thesis title was "Mechanisms for chemical reactions involved in lignin biodegradation by *Phanerochaete chrysosporium*", which was the work supervised by Professor Takayoshi Higuchi. He has been working mainly on the area of wood chemistry and biochemistry since 1982, and more recently, focusing on biosynthesis of lignans and norlignans and metabolic engineering of lignification, with publications of over 140 research articles since 1982. 1982-1993: Instructor in Wood Research Institute, Kyoto University, 1989-1990: Postdoctoral fellow in Dept. of Forestry and Biochemistry, Virginia Polytechnic Institute, Kyoto University, 1993-2005: Associate Professor in Wood Research Institute, Kyoto University, 2005-present: Professor in Research Institute for Sustainable Humanosphere, Kyoto University, 2006-present: Research fellow in Institute of Sustainability Science, Kyoto

University. In 1992, he was awarded the Japan Wood Research Society Prize for 1991 for the work entitled "Mechanisms for chemical reactions involved in lignin biodegradation". He was elected as a fellow of International Academy of Wood Science in 2000.



Mami Yamazaki, Associate Professor, Department of Molecular Biology and Biotechnology, Graduate School of Pharmaceutical Sciences, at Chiba University, Japan. She graduated and completed her Ph.D. (Pharmaceutical Sciences) at Chiba University. She spent a year as a visiting researcher with Prof. M. Van Montagu at Gent University Belgium in 1996. Her research interests are in the basis for the evolution of chemical diversities in plants. She has conducted molecular biological and comparative studies using chemo types, cell types and related species that are differ in secondary metabolisms such as anthocyanins or alkaloids.



Chung S. Yang is a Professor II in the Department of Chemical Biology and the Director of the Center for Cancer Prevention Research in the Ernest Mario School of Pharmacy at Rutgers University. He holds the title of the John L. Colaizzi Endowed Chair in Pharmacy. He is also the Leader of the Carcinogenesis and Chemoprevention Program of the Cancer Institute of New Jersey. Dr. Yang received his B.S. Degree from National Taiwan University and his Ph.D. Degree from Cornell University, Ithaca, New York. Before joining Rutgers University in 1987, he was a professor at UMDNJ- New Jersey Medical School. Dr. Yang's major research interests are in the molecular mechanisms of carcinogenesis and the prevention of cancer by dietary factors and

pharmaceutical agents. In the 1980's, he initiated the large scale Joint US-China Nutritional Intervention Trial on Esophageal/Gastric Cardia Cancer. In the 1990's, he studied the molecular mechanism of human esophageal carcinogenesis and created animal models for esophageal adenocarcinoma and ulcerative colitis-associated colon carcinoma. In the past 20 years, Dr. Yang has conducted extensive research on the cancer preventive activities of tea and tea polyphenols. In recent years, he is also studying cancer prevention by different forms of tocopherols and developing models for colon and prostate cancers induced by the dietary carcinogen, PhIP, in CYP 1A-humanized mice. Dr. Yang has been serving on many study sections and grant review committees as well as editor, associate editor and member of editorial boards of several journals.



Kazufumi Yazaki received his Ph.D. from Graduate School of Pharmaceutical Sciences of Kyoto University under the supervision of Prof. M. Tabata (1988). He was appointed as Assistant Professor at Faculty of Pharmaceutical Sciences of Okayama University in 1986. As an Alexander von Humboldt fellow (1989 to 1991), he carried out postdoctoral research in Germany at University of Bonn, working in the group of Dr. L. Heide in the laboratory of Prof. E. Leistner, and successively at the laboratory of Prof. K. Hahlbrock in Max-Planck-Institute Cologne. He moved to Kyoto University as Assistant Professor in Faculty of Pharmaceutical Sciences in 1992, and promoted as Associate Professor in Agricultural Faculty of Kyoto University in 1996. He got promoted to Professor in the former Wood Research Institute of Kyoto University in 2002, and is currently

Professor of Research Institute for Sustainable Humanosphere due to the reorganization of Research Institutes in Kyoto University. The current research topics of the Yazaki Lab include (i) identification and characterization of plant genes for prenylation of aromatic compounds, (ii) biosynthesis and emission of plant volatile terpenoids, (iii) metabolic engineering of plant natural products in heterologous hosts, and (iv) membrane transport of natural products in plant cells. Recently, membrane transport of small molecules involved in symbiotic nitrogen fixation in legume plants has been also studied. He has authored and co-authored more than 150 publications, and also serves on editor of three international journals, e.g., Plant Cell Physiology.

Oral Session Abstracts

OR1

PSNA: SOME REFLECTIONS

<u>Eric E. Conn</u> (Section of Molecular and Cellular Biology, University of California at Davis, Davis, CA 95616-8535, USA; eeconn@sbcglobal.net)

A sabbatical leave in England during the summer of 1960 provided an opportunity to attend a meeting of the Plant Phenolics Group of Europe with Tony Swain and Jeffrey Harborne at Royal Holloway College on the west side of London. This was the first meeting I'd attended that was devoted to this specific group of compounds. I decided that a similar organization in the States would meet a specific need for workers in the field. To my surprise and delight, I found that efforts were already under way, details of which can be found in Dr. Stewart Brown's 1992 history of the PSNA in *Recent Advances in Phytochemistry* (Volume 26). Today we can reflect upon the 50th anniversary of this society and the exciting contributions that ii continues to make.

S1.1 NATURAL PRODUCTS WITH BIOLOGICAL ACTIVITIES FROM THAI BIORESOURCES

Chulabhorn Mahidol (Chulabhorn Graduate Institute, Bangkok, 10210 Thailand; somsak@cri.or.th)

Research on natural products is essential for the discovery of lead compounds because of the incredible diversity of chemical structures that are produced by animals, plants, marine, and micro-organisms. It is considered that because of the diversities of the structures as well as the biological activities of their constituents, terrestrial plants, marine and micro-organisms offer a unique and renewable resource for the discovery of potential new drugs and biological entities.

Our interest in research of bioactive compounds from Thai medicinal plants and other sources has been long standing. In this presentation, we will focus on the bioactive compounds derived from various Thai plants, as well as from marine animals and microorganisms. The presentation will include the structure elucidation, chemistry, biological activity and, in some unique cases, the biosynthesis of the compounds isolated from these bioresources.

S1.2

GINKGOLIDES - STRUCTURES, REACTIONS AND BIOACTIVITIES

Koji Nakanishi (Columbia University, Chemistry, New York, NY 10027, USA; kn5@columbia.edu)

The origin of the *Ginkgo biloba* tree,¹ the "living fossil", dates back 250 million years ago, the dinosaur era, and it is said that the leaf fossils are similar to current leaves. The ginkgo extract had been used in China since the 11th century for curative purposes. The isolation, structure determination, and reaction studies of the cage structured diterpenoid ginkgolides from *Ginkgo biloba* will be outlined,² followed by recent bioorganic findings. It is the sole terrestrial natural product with a *tert*-Bu group, the presence of which led to the discovery of the intramolecular nuclear Overhauser effect (NOE) in structural studies.

The unique cage structured ginkgolides consisting of five 5-membered rings, i.e., three lactones, one ether and a five/five spirocyclic ring system, lead to many chemical reactions and properties, which will be outlined. The ginkgolides interact with a number of receptors R, the glycine, GABA and platelet activating receptors. Electrophysiological measurements indicated that impairment of the brain hippocampal ("memory") region by oligomeric amyloid-beta is reversed by ginkgolides, an aspect that rationalizes its use against dementia. The biodistribution of tritiated ginkgolide B into the rat tail after 5 hours showed that it has receptors in a variety of organs, the brain, heart, lung, liver, kidney, adrenal gland, and spleen. Ginkgolides that penetrated the brain blood barrier of rat and rabbit showed two receptors in the hippocampus ("center of memory"). In attempts to capture these two receptors, biotinylated ginkgolides with a benzophenone photoaffinity label have been synthesized. Experiments aimed towards characterization of these two receptors will be outlined.

1. Ginkgo biloba, van Beek, T.A., Ed., Harwood Academic Publishers, 2000, Netherlands.

2. Nakanishi, K., Bioorg. & Med. Chem., 2005, **13**, 4987-5000.

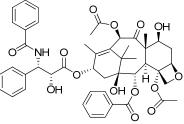
S1.3

FROM YEW TO ME TO YOU: A PERSONAL HISTORY OF THE DISCOVERY AND DEVELOPMENT OF PLANT-DERIVED ANTICANCER AGENT, TAXOL

Mansukh C. Wani (RTI International, Organic and Medicinal Chemistry, Research Triangle Park, NC 27709, USA; mcw@rti.org)

Taxol, a secondary metabolite obtained from the wood bark of *Taxus brevifolia*, is found in the Pacific Northwest coastal region of the United States. It was isolated by the bioassay-guided fractionation of the crude plant material. The structure of Taxol was established by single crystal x-ray analysis. Taxol has a unique mechanism of antitumor activity. It inhibits cancer cell growth via stabilization of microtubules.

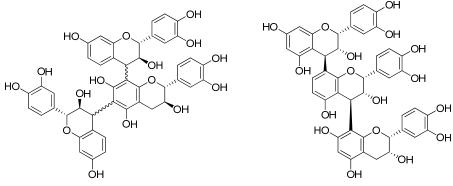
Currently, Taxol is approved for clinical use in the USA by the FDA for the treatment of refractory ovarian, breast, and non-small cell lung cancers and Kaposi's Sarcoma. This presentation will describe the 30-year efforts which transformed this compound from an interesting plant secondary metabolite to a life-saving chemotherapeutic agent.



S1.4 HALF A CENTURY OF PROANTHOCYANIDIN/POLYPHENOL RESEARCH.

<u>Daneel Ferreira</u>, Christina M. Coleman (Research Institute of Pharmaceutical Sciences and Department of Pharmacognosy, University of Mississippi, University, MS 38677, USA; dferreir@olemiss.edu)

The 5-deoxyproanthocyanidin pools of plants are extremely complex due to variation in oxygenation pattern and a variety of regio-/stereochemical and conformational phenomena. These structural complexities also confound the isolation and structure elucidation processes, especially NMR protocols where ¹H and ¹³C spin systems are often broadened and/or multiplied due to restricted rotation about the interflavanyl bond(s). Our research to understand the intricate structural, configurational, conformational, and chemical behavior began in the 1970s when we designed a synthesis protocol aimed at defining the linkage mode(s) and the absolute configuration of the constituent flavanyl moieties. Some key issues that emanated from our own and other studies, e.g., control of the regio- and stereochemistry of the interflavanyl bond formation process, the development of an electronic circular dichroism method to define the absolute configuration at C-4 of the chain extension unit and corroboration of the results via. theoretically calculated ECD spectra, and the chemical manipulation of some crucial bonds in the proanthocyanidin architecture will be discussed.



S2.1

THE ROAD FROM MUNICH: MEINHART H. ZENK'S LEGACY

Norman G. Lewis (Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340 USA; lewisn@wsu.edu)

Professor Meinhart Zenk is remembered as a true visionary and pioneer in many areas of plant metabolism, largely involving remarkable excursions into phenolic (including phenylpropanoid) metabolism and alkaloid biosynthesis, as well as phytoremediation.

We retrace his remarkable and fascinating scientific journey, taking into account that all scientific contributions must be judged from the time when they were made and the technologies available at the time. Driven by a passion for excellence and perfection, as well as an insatiable thirst for knowledge, his scientific legacy and persona have left an indelible mark. From a personal perspective, some of the pivotal steps and peaks scaled are retraced, and the legacy of the "Zenk School" is highlighted.

S2.2

FUNCTIONING DEPENDENT METABOLONS: THE "OXIME BOMB" IN CYANOGENIC GLUCOSIDE METABOLISM

<u>Birger Lindberg Møller</u> (University of Copenhagen, Faculty of Life Science, Plant Biochemistry Laboratory, Department of Plant Biology and Biotechnology, Frederiksberg C, 1871, Denmark; blm@life.ku.dk)

Cyanogenic glucosides are classical phytoanticipins. Following tissue disruption they release toxic hydrogen cyanide to provide protection of the plant from generalist herbivorous insects. However, numerous fungi are not deterred by hydrogen cyanide. Sorghum contains the cyanogenic glucoside dhurrin. Its synthesis from tyrosine is catalyzed by CYP79A1, CYP71E1 and UGT85B1 with (E)- and (Z)p-hydroxyphenylacetaldoxime as intermediates. Experimental evidence indicate that the isomerization of the (E) to (Z) oxime is catalyzed by CYP71E1. CYP71E1 is highly labile and sensitive to oxygen. The reactive oxygen species generated at the site of infection as a plant defense response to fungal infection may thus inactivate CYP71E1. As a result, the dhurrin metabolon would dissociate resulting in the formation of an oxime as the final product. Oximes are toxic to fungal pathogens. A cyanogenic glucoside producing plant would thus be able to preferentially combat fungal infection if the metabolon catalyzing cvanogenic glucoside formation dissociated in a functioning dependent manner. This "moonlighting" function and reactivity of the oxime produced may give rise to the formation of a diverse array of conjugation products activating an innate immune response. Experimental studies of oxygen formation as a result of metabolon dissociation will be presented. Detonation of an "oxime bomb" to combat fungal infection would supplement the ability of cyanogenic glucoside containing plants to detonate a "cyanide bomb" when attacked by chewing insects. Other P450 based metabolons may serve similar functioning dependent properties.

S2.3

THE GREAT FOOTPRINTS OF THE LATE PROF. MEINHART ZENK IN ISOQUINOLINE ALKALOID BIOSYNTHESIS STUDIES

<u>Fumihiko Sato</u> (Graduate School of Biostudies, Kyoto University, Department of Plant Gene and Totipotency, Kyoto, 606-8502, Japan; fsato@lif.kyoto-u.ac.jp)

Higher plants are rich sources of medicinal compounds. Many medicinal plants, however, are still harvested in the wild due to technical difficulties of cultivation, as well as for economic reasons. Due to the increased demands for quantities of these materials, production of metabolites by cell cultures has been intensively investigated. Prof. Zenk established many cell cultures of medicinal plants with high secondary metabolite productivity. This allowed him to investigate the respective biosynthetic pathways, and, most notably, isoquinoline alkaloid (IQA) pathways. One of his major contributions in IQA biosynthesis was the revision of the early steps of the reticuline biosynthetic pathway. His intensive biochemical studies further identified many biosynthetic enzymes. There is not enough space to describe

them all. I simply note that he clarified the roles of many cytochrome P450s in alkaloid biosynthesis. His achievement is not limited to biosynthesis; Prof. Zenk also proposed the idea of transport and the regulation of gene expression through jasmonate, a key regulator in elicitor responses. These achievements clearly paved the way for the current developments of molecular biology, metabolic engineering, and synthetic biology in secondary metabolism, especially in IQA biosynthesis. I also note that Prof. Zenk's interests were not restricted to plant metabolism. He identified morphine biosynthesis in human cells, and opened the door of plant natural product studies in human cells. The footprints of the late Prof. Zenk in IQA studies are, indeed, great, and we shall all miss him.

S2.4

BIOSYNTHETIC STUDY OF QUINOLIZIDINE ALKALOID - HISTORY AND RECENT ADVANCEMENT

<u>Kazuki Saito</u>,^{1,2} Somnuk Bunsupa,¹ Mami Yamazaki^{1,3} (¹Chiba University, Graduate School of Pharmaceutical Sciences, Chiba, Japan 263-8522, ²RIKEN, Plant Science Center, Yokohama, Japan 230-0045, ³Japan Science and Technology, CREST, Kawaguchi, 332-0012, Japan; ksaito@faculty.chiba-u.jp)

Quinolizidine alkaloid (QA) forms a major group of plant alkaloids that contain several hundred structurally related compounds distributed mostly in Leguminosae. QAs are biosynthesized through cyclization of a cadaverine unit, which is produced through action of lysine decarboxylase. The molecular mechanism underlying QA biosynthesis is poorly understood; only very few studies have been aimed at charactering the enzymes and regulatory mechanism for QA biosynthesis. We have cloned lysine/ornithine decarboxylase (L/ODC) in the first step of their biosynthesis from alkaloid-containing cultivars of *Lupinus angustifolius* by using PCR-select-subtraction and 5'/3'-RACE techniques. We also characterized L/ODC genes from *Sophora flavescens* and *Echinosophora koreensis* which produce QAs. These three purified recombinant L/ODCs showed decarboxylase activity toward two substrates, L-lysine and L-ornithine, with similar kinetic properties. The heterologous expression of L/ODC in *Arabidopsis* and tobacco resulted in the enhanced accumulation of cadaverine and tobacco alkaloids derived from lysine, indicating the actual function of this enzyme for formation of cadaverine and subsequent production of alkaloids. This is the first report on an L/ODC involved in QAs biosynthesis from plants.

S2.5

ALKALOID SYNTHESIS BEYOND THE RAUVOLFIA ALKALOIDAL NETWORK

<u>Joachim Stöckigt</u> (Zhejiang University, Institute of Materia Medica, College of Pharmaceutical Sciences, Hangzhou, China)

The biosynthetic network of monoterpenoid indole alkaloids of the Indian and Chinese medicinal plant *Rauvolfia* has been elaborated in great detail during many years. Broad biosynthetic knowledge accumulated on the participating enzymes, which included their detection, their biochemical characterization, search for the cDNAs and their heterologous expression.

Crystallization of major enzymes and three-dimensional X-ray analyses of substrate, product and cofactor complexes allow now the development of new synthetic and chemo-enzymatic strategies in order to generate novel and unusual alkaloids.

S2.6

MY RESEARCH EXPERIENCE WITH PROF. MEINHART ZENK, THE PIONEER OF BENZYL-ISOQUINOLINE ALKALOID BIOSYNTHESIS

<u>Wanchai De-Eknamkul</u> (Chulalongkorn University, Dept. of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Bangkok, 10330, Thailand; dwanchai@chula.ac.th)

It all started when I first met Prof. Zenk at his all-time favorite meeting: "The First Princess Chulabhorn International Congress on Natural Products", held in Bangkok, Thailand in December 1987. Later, I had an opportunity to undertake my post-doc studies (1989-1990) in his laboratory in Munich. With the aim of

elucidating the biosynthetic pathway of morphine, by focusing on the step of (*S*)-reticuline to (*R*)-reticuline conversion, I experienced both hard times and good times in working with him. It was a difficult task that the conversion step possibly involved different enzymatic reactions. However, Prof. Zenk impressively guided me to the right direction until finding the target enzyme eventually. I was proud to be a part of his research group and his success in clarifying one of the world's most important biosynthetic pathways of natural products. While working with Prof. Zenk, I admired his abilities to predict and solve problems and his expertise in the field of biosynthesis of natural products. It is, thus, not surprising that he was also the first to resolve many other biosynthetic pathways of pharmaceutically-important benzylisoquinoline alkaloids, such as berberine, sanguinarine, protopine, papaverine, etc. From his remarkable contributions to the field of biosynthesis, Prof. Zenk is truly "The Pioneer of Benzylisoquinoline Alkaloid Biosynthesis".

S2.7

CATHARANTHUS ROSEUS EXPRESSES A LEAF EPIDERMIS SPECIFIC AMYRIN SYNTHASE INVOLVED IN TRITERPENE BIOSYNTHESIS

Fang Yu,¹ Antje Thamm,¹ Darwin Reed,² Patrick Covello,² <u>Vincenzo De Luca</u>¹ (¹Brock University, Department of Biological Sciences, St Catharines, ON L2S 3A1, Canada, ² National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, SK S7N 0W9, Canada; vdeluca@brocku.ca)

The plant kingdom produces many thousands of biologically active triterpenes that are derived from (3S)oxidosqualene to generate over 80 different carbon skeletons. Different oxidosqualene cyclase (OSC) enzymes carry out the carbocation rearrangements responsible for this biological diversity. For example, *Catharanthus roseus* accumulates 2.5% of their leaf dry weights as the α -amyrin-derived ursane-type triterpene, ursolic acid, on the leaf surface. Sequencing of a leaf epidermis enriched cDNA library generated most of the mevalonic acid pathway as well as a new OSC gene with high amino acid sequence identities to amyrin synthases (*Cr*AS) from other species. Functional expression of *Cr*AS in *Saccharomyces cerevisiae* resulted in the production of α -amyrin and β -amyrin in an approximate 8 to 2 ratio. Transcription analysis showed that *Cr*AS is predominantly expressed in the leaf epidermis of young *Catharanthus* leaves. These results strongly suggest that expression of triterpene biosynthesis genes is highly regulated during plant growth and development, that triterpenes appear to be produced in the specialized epidermis of young leaves, and that synthesis is closely associated with secretion on the leaf surface where they fulfill particular biological roles.

S2.8

MEMBRANE TRANSPORT OF ALKALOID IN PLANTS

Kazufumi Yazaki (Kyoto University, Research Institute for Sustainable Humanosphere, Uji, 611-0011, Japan; yazaki@rish.kyoto-u.ac.jp)

Plant alkaloids comprise an important group of secondary metabolites due to their strong and divergent biological activities. Most alkaloids have aromatic ring structures containing a nitrogen atom, which provides the basic property. Alkaloids often preferentially accumulate in a particular organ of the plant body, e.g., berberine in the inner bark of *Phellodendron amurense*, caffeine in the seeds of *Coffea* spp. In the 1980s, intensive studies on the accumulation mechanism of alkaloids in plant cells were done, mostly using plant cell cultures. In this field, Dr. Zenk first pointed out that the vacuolar transport of alkaloids was stereo-specific, and suggested the involvement of a specific transporter molecule for vacuolar sequestration of alkaloids, not simply by an ion-trap mechanism.

Many years later, we identified a couple of transporter proteins responsible for the membrane transport of alkaloids. Cjmdr1, an ATP-binding cassette transporter localized at the plasma membrane of *Coptis japonica* cells, can transport berberine, while this alkaloid is transported by proton antiport mechanism at tonoplast. To honor the pioneering works by Dr. Zenk, recent progresses in alkaloid transport will be discussed.

S2.9

XANTHOHUMOL FROM HOPS (*HUMULUS LUPULUS*): PHARMACOKINETICS AND EFFECTS ON BIOMARKERS OF METABOLIC SYNDROME IN RATS

LeeCole Legette,^{1,2} Lian Ma,¹ Ralph L. Reed^{1,,2} Cristobal L. Miranda,^{1,2} J. Mark Christensen,¹ Rosita R. Proteau,¹ Jan Frederik Stevens^{1,2} (¹Oregon State University, Pharmaceutical Sciences, Corvallis, OR 97331, USA, ²Oregon State University, Linus Pauling Institute, Corvallis, OR 97331, USA; fred.stevens@oregonstate.edu)

Xanthohumol (XN) is the principal prenylated flavonoid of the hop plant, *Humulus lupulus*. It has received much attention due to its cancer chemopreventive, antiangiogenic, anti-inflammatory, antihyperlipidemic, and antihyperglycemic effects *in vitro* and *in vivo*. XN has been marketed as a dietary supplement. To determine whether biologically relevant concentrations of XN can be attained after oral administration, we conducted a pharmacokinetic study in male jugular vein-cannulated Sprague-Dawley rats. Rats received either an intravenous injection or oral gavage of XN at three dose levels. Plasma samples, treated with and without glucuronidase, were analyzed for XN and its metabolites (isoxanthohumol, 8-prenylnaringenin, and 6-prenylnaringenin) using LC-MS/MS. The combined bioavailability of conjugated and free xanthohumol was approximately 0.33, 0.13 and 0.11 for the low, medium and high dose of XN, respectively. The half-life of XN at the tested oral doses was in the range 18-30 hours. We also studied the chronic effects of dietary XN in a rat model of metabolic syndrome (Zucker fa/fa rats) by daily administration of XN per os at the three dose levels. The highest dose resulted in lower plasma glucose levels and decreased weight gain. This suggests that XN may be a viable therapeutic agent for the treatment of metabolic disorders and obesity.

S2.10

A BLUE ANIGOZANTHOS ROOT CULTURE AS AN INITIAL OF PHENYLPHENALENONE RESEARCH

<u>Bernd Schneider</u> (Max Plank Institute for Chemical Ecology, Beutenberg Campus, Hans Knöll Str. 8, Jena, 07745, Germany; schneider@ice.mpg.de)

Meinhart Zenk established and maintained in his Munich laboratory a collection of up to 1000 plant cell and organ cultures, which were used for "chasing the enzymes" [Phytochemistry 30, 3861-3863 (1991)] and genes of secondary metabolite biosynthesis.

Among these cultures, a unique blue-colored root culture attracted my special interest when I visited his lab as a Humboldt research fellow in the early 1990's. On request, Meinhart Zenk allocated this particular culture to me with the comment that it probably accumulates so-called phenylphenalenones, which may be worthwhile to be investigated.

This was the starting point of a fruitful research area, first about phytochemistry and structure elucidation, than occurrence in the plant kingdom and tissue-specific distribution, biosynthetic experiments including

¹³C labelling and characterization of recombinant biosynthetic enzymes, some synthetic work, and finally bioactivity studies and ecological functions. Some highlights of our phenylphenalenone research will be presented.



Root culture of Anigozanthos preissii (Haemodoraceae)

S2.11 FUNGAL ORIGIN OF ERGOT ALKALOIDS IN DICOTYLEDONOUS PLANTS

<u>Eckhard Leistner</u>,¹ Ulrike Steiner² (Universität Bonn, Institut für Pharmazeutische Biologie, Bonn, 53115, Germany, ²Universität Bonn, Institut für Nutzpflanzenwissenschaften und Resourcenschutz (INRES)-Phytomedizin, Bonn, 53115, Germany; eleistner@uni-bonn.de)

Convolvulaceous plants such as *Ipomoea asarifolia* and *Turbina corymbosa* are esteemed in southern Mexico as one of the principal hallucinogens for use in divinations as well as magico-religious rituals. The physiologically active principles are ergot alkaloids which are also known to be biosynthesized by clavicipitaceous fungi. The disjunct occurrence of ergot alkaloids in higher plants and fungi seemed to contradict the principle of chemotaxonomy that identical or at least similar natural products have a common evolutionary history and, thus, occur in taxonomically related plants or microorganisms.

Recently we have shown, however, that *I. asarifolia* and *T. corymbosa* are not the producer of ergot alkaloids but that the plants are colonized by the first ergot alkaloid producing clavicipitaceous fungi described that are apparently mutualistic symbionts of dicotyledonous plants. The fungi belong to a newly established genus which we named *Periglandula* with reference to the close association of the fungi with secretory glands of its plant hosts. The secretory glands are likely to be mediators of a metabolic dialogue between plant and fungus. Ergot alkaloids participate in this dialogue.

S2.12 HIGHLIGHTS ON MORPHINE RESEARCH WITH PROF. MEINHART H. ZENK

<u>Michael Spiteller</u> (Technical University of Dortmund, Institute of Environmental Research (INFU) of the Faculty of Chemistry, Chair of Environmental Chemistry and Analytical Chemistry, Dortmund, 44227, Germany; spiteller@infu.tu-dortmund.de)

Over a decade Meinhart H. Zenk made significant contributions concerning the biosynthesis of morphine in plants and mammals. His work was characterized by sharpness of thought and experimental elegance, which constantly fascinated his pupils and colleagues. It was his particular talent to ornament the scientific discussion with convincing arguments presented with vast graciousness. We had the opportunity and honour to accompany Meinhart's life's work for a small distance. It all started on the 11th of June 2007, when he asked me to record a high resolution mass spectrum of papaverine. This initial measurement resulted in an intensive cooperation over the next three years concerning the biochemical precursors of morphine in plants and animals. The highlights of this cooperation are presented on the occasion of the Meinhart H. Zenk Memorial and emphasized with original citations from more than 300 emails, from which it is possible to follow the ups and downs and finally the successful publication of the results. Meinhart has left us with an important task: the significance of the result that mammals are able to carry out de novo synthesis of morphine from simple biochemical precursors for pain therapy will certainly occupy the fellow workers of M. H. Zenk in the years to come.

Grobe, M. et al.(2010)PNAS USA,107(18),8147-8152. Han, X. et al.(2010)Phytochemistry,71,1305-1312.

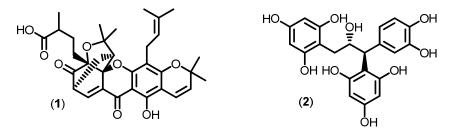
S2.13

ABSOLUTE CONFIGURATION OF SECONDARY METABOLITES via ELECTRONIC CIRCULAR DICHROISM

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Chiroptical methodology represents a powerful tool towards definition of the absolute configuration of stereogenic centers in secondary metabolites, irrespective of the physical state of the compound. The utility of the CD method has been strengthened with the introduction of TDDFT calculations that permit the theoretical calculation of electronic CD spectra to greatly enhance our ability to interpret ECD data in terms of absolute configuration. We will cover the configurational assignment of a significant array of

natural products ranging from the caged xanthone, (–)-morellic acid (1), to conformationally labile polyphenols of the 1,1,3-triarylpropan-2-ol-type (2).



S2.14 "WHY DO LIVERWORTS BIOSYNTHESIZE MARCHANTINS, PUNGENT AND BITTER SUBSTANCES?"

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The most characteristic chemical phenomenon of liverworts is that most sesqui- and diterpenoids are enantiomers of those found in higher plants. It is very noteworthy that different species of the same genus like Frullania tamarisci and F. dilatata, each produces different sesquiterpene enantiomers. Liverworts are also rich sources of various types of bis-bibenzyls, such as marchantins, riccardins, plagiochins, and isoplagiochins, which are structurally similar to bis-bibenzylisoguinoline alkaloids such as tubocurarine, and bibenzyl cannabinoids as well as its related bibenzyls. Prof. Meinhart Zenk's question was that why do liverworts produce potent pungent, bitter or muscle relaxing products when the author presented his lecture in Munich. The author predicted that they might be defense substances against insects and mammals. He had been quite interested in biosynthesis of marchantin-type bis-bibenzyls. Asakawa and Matsuda [1] proposed that cyclic bis-bibenzyls might be biosynthesized from bibenzyls that correspond chemically to dihydrostilbene. Zenk and Asakawa et al. [2] proved their hypothesis by feeding experiments of radioactive and ¹³C labeled phenylalanine and dihydro-p-coumaric acid. The A- and Brings of the marchantin molecule are derived from the benzene ring of L-phenylalanine via trans-cinnamic acid and p-coumaric acid. Enzymatically hydrogenated dihydrocoumaric acid from coumaric acid condenses with three molecules of malonyl-Co A to form prelunularic acid which is aromatized to yield lunularic acid and possibly lunularin which is followed by condensation of lunularin or lunularic acid to form marchantin A. This is the first experimental result of biosynthesis of macrocyclic bis-bibenzyls obtained from liverworts. Since this work, more than 70 cyclic- or acyclic bis-bibenzyls and their monomer have been isolated from liverworts and their total synthesis and bioactivity reported.

- 1. Asakawa, Y., and Matsuda, R. (1982) *Phytochemistry* **21**, 2143-2144
- Friederich, S., Maier, U. H., Deus-Neumann, B., Yoshinori, A., and H. Zenk, M. (1999) *Phytochemistry* 50, 589. Friederich, S., Rueffer, M., Asakawa, Y., and Zenk, M. H. (1999) *Phytochemistry* 52, 1195.

S2.15

REGIO- AND STEREOSELECTIVE INTERMOLECULAR OXIDATIVE PHENOL COUPLING IN FILAMENTOUS FUNGI

Christian Gil Girol,¹ Wolfgang Hüttel,¹ Silke Foegen,¹ Katja M. Fisch,² Jörn Piel,² Thorsten Heinekamp,³ Axel Brakhage,³ <u>Michael Müller</u>¹ (Institute of Pharmaceutical Sciences, University of Freiburg, Freiburg, 79104, Germany, ²Institute of Organic Chemistry and Biochemistry, Universität Bonn, Bonn, 53121, Germany, ³Department of Molecular and Applied Microbiology; Leibniz-Institute for Natural Product Research and Infection Biology (HKI), Jena, 07745, Germany; michael.mueller@pharmazie.uni-freiburg.de)

More than 50 years ago, Barton and Cohen correlated the structures of many phenol-coupled natural products of higher plants to oxidative phenol coupling. In 1997 Lewis et al. reported that in some plant families, lignan formation, via oxidative phenol coupling, is a two-enzyme-process. The late Meinhart Zenk showed that P450 enzymes catalyze regio- and stereoselective intramolecular oxidative phenol coupling in the biosynthesis of several alkaloids in plants and mammals.

Our aim is directed toward characterizing the enzymes that are responsible for the regio- and stereoselective oxidative phenol coupling in fungi. For that reason we have chosen the filamentous fungi *Penicillium citreo-viride* and *Aspergillus niger*, producers of the dimeric metabolites vioxanthin and kotanin, respectively. In order to develop an assay system we synthesized the dimeric target molecules as well as the monomeric precursors semi-vioxanthin and demethylsiderin, the latter ones specifically ¹³C-labeled. Feeding studies revealed the polyketide origin and the regio- and atropselective dehydrodimerization of demethylsiderin. Progress towards the identification of the enzymes involved in these transformations will be discussed.

S2.16

THE MYB75 TRANSCRIPTION FACTOR PLAYS A CENTRAL ROLE IN REGULATING CARBON FLUX INTO CELL WALL-RELATED METABOLIC PATHWAYS IN *ARABIDOPSIS THALIANA*

Apurva Bhargava, Carl Douglas, <u>Brian Ellis</u> (University of British Columbia, Vancouver, BC V6T 1Z4, Canada; bee@msl.ubc.ca)

Formation of plant secondary cell walls, which represent a major metabolic sink for photosynthate, typically requires coordinated synthesis of large amounts of both carbohydrate and phenylpropanoid polymers. The broad outline of the transcriptional network that regulates the channeling of carbon into these products has begun to be revealed but the interactions that define its architecture remain largely unknown. We found that the *Arabidopsis* R2R3-class MYB transcription factor, MYB75, which is known to physically interact with other transcriptional regulators such as TT8 and bHLH012, also interacts with the KNOX-class transcription factor, KNAT7. Analysis of gain-of-function and loss-of-function mutants allows us to place MYB75 within a regulatory circuit that represses the activity of key players in secondary cell wall synthesis, both in the *Arabidopsis* inflorescence stem and in the developing seed coat, possibly as part of a mechanism to fine tune commitment to secondary cell wall biosynthesis according to available metabolic resources.

S2.17

MODERN TOOLS FOR ANCIENT MEDICINES: INVESTIGATING THE BIOSYNTHESIS OF BIOACTIVE COMPOUNDS IN IMPORTANT MEDICINAL PLANTS

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The number and types of specialized compounds produced by any plant is very unique, producing considerable variation and resulting in distinct chemotypes. Many medicinal herbs possess chemotypes with distinctive biochemical profiles in specific tissues, such as rhizomes or trichomes. Using a biochemical genomics-based approach we have identified several classes of enzymes that play important

roles in controlling the diversity of the terpenoids, such as turmerones and other sesquiterpenoids, and phenylpropanoid-derived compounds, such as gingerols and curcuminoids, in several medicinal plants. Specialized metabolism appears to be organized at times and in certain species (if not all) in a modular fashion: groups of metabolites that are biosynthetically linked can accumulate in a concerted manner in biosynthetic modules. In many cases, a large collection of related metabolites (e.g., sesquiterpenoids or gingerols/curcuminoids) accumulate that do not all belong to the same module. Indeed, subgroups of compounds accumulate in separate modules. This suggests that multiple enzymes must be involved in production of these compounds, which has been verified in our recent efforts. Identification of such enzymes and characterization of their individual functions are important steps in understanding how such complex arrays of metabolites can evolve in specific plant lineages. Evaluating such information is an important step in understanding how the great diversity of plants has evolved.

S2.18

COMPARATIVE ANALYSIS OF BENZOXAZINOID BIOSYNTHESIS IN MONOCOTS AND DICOTS: INDEPENDENT RECRUITMENT OF STABILIZATION AND BIO-ACTIVATION FUNCTIONS.

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Stabilization by glucosylation and bio-activation by hydrolysis is essential for phytoanticipin function in plant defense. Benzoxazinoids represent protective and allelopathic phytoanticipins that are found in a multitude of species of the family Poaceae and occur sporadically in a single species of phylogenetically unrelated dicots. We have isolated and functionally characterized the benzoxazinoid-specific UDP-glucosyltransferase and β -glucosidase from the dicot *Consolida orientalis* (larkspur). A phylogenetic comparison of these enzymes with their counterparts in the grasses indicates convergent evolution by repeated recruitment of these functions during evolution. Protein modeling supports the phylogenetic analysis. The data indicate a great evolutionary flexibility in recruitment of these essential functions of secondary plant metabolism.

S2.19

HEAVY METALS; XENOBIOTICS; AND MORE

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Plants are constantly challenged by a variety of noxious compounds, including heavy metal ions, microbial toxins and agrochemicals. The tripeptide glutathione (GSH) plays a central role in the detoxification of such compounds in plants. GSH alleviates metal ion-induced oxidative reactions. In addition, GSH is a precursor of phytochelatins (PCs), which bind and sequester heavy metals. A third role of GSH is to form conjugates with xenobiotics, which are subsequently catabolized and sequestered to the vacuole by the action of ATP-driven ABC-type transporters. PCs provide a basal form of metal-tolerance and -homeostasis even in some fungi and *Caenorhabditis elegans*. PCs are generated by the action of a specific dipeptidyl-transferase, PC synthase (PCS), which catalyses the repeated transfer of glutamylcysteinyl units of GSH onto GSH or PC. PCs bind the heavy-metal ions more avidly than the monothiol GSH. Interestingly, PCS also catalyses the turnover of GS-conjugates to glutamylcysteinyl-conjugate formation of herbicides has been identified as a catabolic pathway characteristic for plants. Our studies with PCS-knockout lines of *Arabidopsis* reveal that PCS provide the cytosolic activity for glutamylcysteinyl-conjugate formation. Thus, PCS appear to fulfill at least two functions in plants; PC biosynthesis for heavy metal detoxification and the turnover of GS-conjugates.

S2.20

PHENOLICS IN THE SURFACE WAXES OF SECALE CEREALE: FORMATION AND ACCUMULATION OF CUTICULAR ALKYLRESORCINOLS

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Alkylresorcinols are phenolic lipids found in diverse taxa of higher plants and at particularly high levels in grass species. They have strong anti-bacterial/anti-fungal activities and are often deposited at or near the surfaces of plants, making it plausible that they serve as a first line of defense against pathogens. However, direct evidence showing that surface alkylresorcinols are biosynthesized for a protective function at the surface is still insufficient. Earlier work in our lab had shown that in *Secale cereale* leaves very-long-chain alkylresorcinols (C19 to C27) accumulate mainly in the cuticular wax mixtures, near the tissue surface. Additionally, alkylresorcinol accumulation was found to be synchronized with production of other wax components during leaf ontogenesis. A homology-based approach has now been used to clone several genes potentially encoding CHS-like type III polyketide synthases. Heterologous expression of these genes in yeast showed that one of them indeed encodes an alkyl resorcinol synthases. A second alkylresorcinol synthase was cloned from *Brachypodium distachyon*, a closely related genetic model system. The rye alkylresorcinol synthase gene was strongly expressed in green leaves, only weakly in etiolated leaves, and not in roots. Leaf expression levels were found correlated with alkylresorcinol accumulation rates. All results together indicate that alkylresorcinols are indeed biosynthesized specifically for a defensive function associated with the wax lining the surface of grass leaves.

S2.21

HAIRY GENOMICS: STUDIES OF SECRETORY GLANDULAR TRICHOMES IN TOMATO AND RELATIVES

<u>Robert L Last</u>,¹ A. Daniel Jones,¹ Eran Pichersky,² Cornelius S. Barry,¹ Jeongwoon Kim,¹ Anthony Schilmiller,¹ Kiyoon Kang,¹ Eliana Gonzales-Vigil¹ (¹Michigan State University, Biochemistry and Molecular Biology, Chemistry, Horticulture and Plant Biology, East Lansing, MI 48824-1319, USA; ²University of Michigan, Molecular, Cellular and Developmental Biology, Ann Arbor, MI 48109-104, USA; lastr@msu.edu)

Secreting Glandular Trichomes (SGTs) are epidermal protuberances that produce a wide variety of specialized metabolites in many plant species. For example, the tastes and smells of Mediterranean cooking herbs such as basil and oregano are from essential oils produced and stored in SGTs. Medicinal compounds such as the anti-malaria artemisinin and anti-emetic cannabinoids are also found in SGTs. These structures contribute to defense against biotic stress agents such as herbivores and pathogens.

The collaborative Solanum Trichome Project (www.trichome.msu.edu) is taking a combined chemistry, biochemistry, genomics and genetics approach to study the biosynthetic pathways in the SGTs of tomato and its close relatives. Targets include simple and modified terpenes, acylsugars and methylated flavonoids. Results of these studies will be described, including evidence that there is great diversity in the metabolites produced by Solanum SGTs, presumably in response to selective pressure imposed by biotic stress agents over evolutionary time.

S2.22

POST-GENOMIC ELUCIDATION OF PLANT NATURAL PRODUCT PATHWAYS

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The study of the biosynthesis of plant alkaloids at the enzyme and gene level has greatly advanced in A number of genes are available from the monoterpenoid indole-, recent vears. tetrahydrobenzylisoquinoline-, and structurally related to both of the previous classes, the terpenoidisoquinoline alkaloid biosynthetic pathways. To date, however, only partial understanding of the formation of medicinal natural products at the enzyme and gene levels has been attained. The explosive increase in understanding of biology over the past two decades has been enabled by work on model genetic organisms. The study of selected species-specific medicinal natural products, however, requires investigation of those plant species that harbor all or most components of the focal biosynthetic pathway. Detailed genetic and biochemical information on these highly specialized species is often missing. Having comprehensive medicinal plant transcriptomes would greatly advance research on medicinal plant species. We now seek to generate and use transcriptome data to understand the complete formation, storage and regulation of plant-derived medicinal compounds at the enzyme and gene level. Results will be presented from efforts to date to produce deep transcriptome datasets from members of the Papaveraceae and to interrogate the datasets for candidate alkaloid biosynthetic genes.

S3.1

PHYTOCHEMICALS AND GENES FOR THEIR SYNTHESIS IN PEST MANAGEMENT

<u>Stephen O. Duke</u>, Franck E. Dayan, Charles L. Cantrell, Agnes M. Rimando, David E. Wedge, Zhiqiang Pan, Scott R. Baerson, Kumudini M. Meepagala (Agricultural Research Service, United States Department of Agriculture, Natural Products Utilization Research Unit, University, MS 38677, USA; sduke@olemiss.edu)

Natural products and their derivatives represent almost 20% of the pest management products being sold worldwide. Phytochemical-derived compounds are strongly represented among insecticides, with less representation in other pesticides. This presentation will briefly review those compounds that have been successful and will provide examples of phytochemicals with promising activity as herbicides, insecticides, insect repellents, molluscicides, and fungicides. Genetically engineering synthesis of natural pesticides into crops will be discussed with focus on the example of sorgoleone, a natural herbicide from the genus *Sorghum*.

S3.2

IMPROVED CROP PRODUCTIVITY THROUGH MANIPULATION OF PHYTOHORMONE SIGNALING

<u>Suzanne R. Abrams</u>, Ken M. Nelson, L. Irina Zaharia (National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, SK Canada S7N 0W9; sue.abrams@nrc-cnrc.gc.ca)

Manipulation of plant hormone metabolism and of hormone signaling pathways are powerful strategies to improve productivity of agricultural crops, for enhancing a plant's tolerance to environmental stresses (drought, heat, cold stress), improving seedling vigour, modifying plant architecture, etc. Towards these ends, an in depth understanding of hormone-induced complex signaling networks in controlling specific developmental pathways or physiological responses in plants is critical. This presentation will focus on recent studies on stress and development in crop plants, integrating profiles of the metabolites of the hormones auxin, cytokinin, gibberellin and abscisic acid, with genomic and physiological data. Application of metabolism resistant plant hormone analogs for agricultural applications and to probe hormone induced gene expression will also be described.

S3.3

BIOSYNTHESIS OF GOSSYPOL IN COTTON: FROM FARNESYL DIPHOSPHATE TO (+)- AND (-)- GOSSYPOL

Tanya Wagner, Jinggao Liu, Lorraine Puckhaber, Alois Bell, <u>Robert Stipanovic</u> (U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, TX 77845, USA; bob.stipanovic@ars.usda.gov)

Gossypol is a dimeric sesquiterpene that occurs in some members of the Malvaceae family. It occurs as an enantiomeric mixture in the foliage, seeds and roots of the cotton plant (*Gossypium*). It provides protection from insect and animal herbivory. Early studies demonstrated that gossypol is the product of cyclization of *E*,*E*-farnesyl diphosphate to (+)- δ -cadinene, which is converted to 8-hydroxy-(+)- δ -cadinene. Proposed intermediates beyond 8-hydroxy-(+)- δ -cadinene include desoxyhemigossypol and hemigossypol. At the time of its first discovery, hemigossypol was proposed to be the immediate precursor of gossypol; it was subsequently shown that hemigossypol is converted into gossypol by peroxidase, and 30 years later that a peroxidase in concert with a flower petal dirigent protein provides a 56% enantiomeric excess of (+)-gossypol. To complete the last step in the gossypol biosynthesis, a temporal study has now identified hemigossypol in developing cottonseed.

S3.4

NATURAL PRODUCTS IN AGRICULTURE: THE ARMS RACE BETWEEN CRUCIFERS AND THEIR FUNGAL PATHOGENS

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Plants and their microbial invaders are involved in an arms race that continues to cause sustainability issues for agriculture. The use of fungicides and pesticides to prevent crop losses causes pollution and health hazards that make these agricultural practices unacceptable. To decrease the enormous social disparities of our world, food availability and safety is critical. Therefore, continuing efforts to develop methodologies that allow agriculture practices to be sustainable are critical to improve our existence. To devise sustainable methods to prevent and deter cruciferous pathogens, their molecular interaction with crucifers, both cultivated and wild species, is under intense investigation. Cruciferous plants (e.g. canola, mustard, cauliflower, broccoli, turnip, thale cress) produce complex blends of secondary metabolites with diverse ecological roles, which include self-protection against microbial pathogens, pests and other sorts of stress, whereas their fungal pathogens produce phytotoxic metabolites and macromolecules that facilitate plant invasion. Although many of the natural products involved in crucifer defense reactions are detoxified by fungal pathogens, these fungal detoxifications can be stopped. That is, inhibitors (paldoxins) of these transformations could protect plants by boosting their natural chemical defenses and prevent pathogen growth. The fundamental aspects and challenges of this strategy to treat plant fungal diseases will be presented.

S3.5

INSECTICIDES BASED ON PLANT NATURAL PRODUCTS: LONG ON PROMISE, SHORT ON PRODUCTS

<u>Murray B. Isman</u> (University of British Columbia, Faculty of Land and Food Systems, Vancouver, BC Canada V6T1Z4; murray.isman@ubc.ca)

The spectacular success of the synthetic pyrethroid insecticides in agricultural, structural pest control and public health for over three decades beginning in the mid-1970s, and the more recent success of the neonicotinoid insecticides (since the mid-1990s) has long provided the impetus for the discovery of novel plant natural products with insecticidal properties. Although a number of plant natural products with truly insecticidal actions have been discovered, these are rare in nature and their commercial potential even more rarely realized. The only commercially successful botanical insecticides developed in the past two decades have been those based on neem seed extracts, containing the remarkable triterpenoid, azadirachtin, and those based on certain plant essential oils, typically comprised largely of more ubiquitous monoterpenoids and sesquiterpenoids. Prolonged attempts to synthesize or otherwise produce azadirachtin on a viable commercial scale have proven unsuccessful. Azadirachtin (and natural analogs) is the primary active insecticidal constituents of neem, and commercial neem insecticides are based on semi-refined extracts enriched in azadirachtin. Certain plant essential oils widely used in the flavouring and fragrance industries has been formulated for insect control, but in this case efficacy is seldom correlated with one or more putative active constituents. In contrast, efficacy appears to depend on internal 'synergy' among both active and putatively inactive constituents. Evidence for this phenomenon of synergy in insects and mites will be presented.

03.1

SULFUR VOLATILES IN ALLIUM CANADENSE AND A. TUBEROSUM

Russell L. Rouseff, John M. Smoot, Rajinder S. Mann, William S. Castle, Lukasz L. Stelinski (University of Florida, IFAS, Citrus Research and Education Center, Lake Alfred, FL 33850, USA; rrouseff@ufl.edu)

Headspace volatiles from wild onion (*A. canadense* L.) and garlic chives (*A. tuberosum* Rottl.) were examined using GC-MS and GC-PFPD (pulsed flame photometric detector, sulfur mode). Although both

species contain numerous sulfur volatiles, only crushed garlic chive leaves and oil repelled the Asian citrus psyllid, *Diaphorina citri* Kuwayama, which is the insect vector for the fatal citrus greening or huanglongbing disease.¹ Since sulfur volatiles have been shown to repel this insect², the purpose of this study was to examine the sulfur volatiles in both species to help determine which might have biological activity. Twelve sulfur volatiles were identified in garlic chive and 18 sulfur peaks were observed in the wild onion. Eight volatiles were common to both. Identified volatiles consisted primarily of thiols and sulfides with various methyl, allyl, and propyl substitutions. Low temperature and gradient temperature injections were performed to determine if thermal artifacts were formed.

- 1. Mann, R. S.; Rouseff, R. L.; Smoot, J. M.; Castle, W. S.; Stelinski, L. L., Sulfur volatiles from *Allium* spp. affect Asian citrus psyllid, *Bulletin of Entomological Research* **2011**,101, 89-97.
- 2. Onagbola, E. O.; Rouseff, R.; Smoot, J.; Stelinski, L. L., Guava leaf volatiles and dimethyl disulphide inhibit response of *Diaphorina citri* Kuwayama. *J. Appl. Entomol.* **2011**.

03.2

L-METHIONINE CATABOLISM INTO SULFUR AROMA VOLATILES IN MELON FRUIT

Itay Gonda,^{1,2} Einat Bar,¹ Noga Sikron,² Vitaly Portnoy,¹ Ya'akov Tadmor,¹ Arthur A Schaffer,¹ Nurit Katzir,¹ Yosef Burger,¹ Aaron Fait,² <u>Efraim Lewinsohn</u>¹ (¹Institute of Plant Sciences, Newe Ya'ar Research Center, Agricultural Research Organization, Vegetable Crops, Ramat Yishay, Israel 30095, ²Ben-Gurion University of the Negev, Jacob Blaustein Insts. for Desert Research, Midreshet Ben-Gurion, Dept. of Dryland Biotechnology, Sedeh Boker, Israel 84990; twefraim@agri.gov.il)

Volatiles derived from essential amino acids have a strong impact on melon and other fruit aromas. Different plants utilize different biochemical routes to produce such volatiles. We have previously shown that amino acid aminotransferases are key factors in the production of aromatic- and branched-chainamino-acid-derived aroma volatiles in developing melon fruit (Gonda *et al.*, 2010, J. Exp. Bot. 61: 1111-1123). We present evidence that in fruit melon slices, ${}^{13}C_5$ - L-methionine was incorporated into sulfur aroma volatiles following two different labeling patterns. We also present results indicating that melon fruit possess L-methionine aminotransferase as well as L-methionine gamma lyase enzymatic activities. Data mining from transcriptomic databases yielded two sequences that putatively code for such proteins. The sequences are currently being tested for their biochemical role by functional expression in *E. coli*. Further, metabolic flux analysis will be carried out to generate information on the active pathways in the fruit. Integrating this with existing genomic, transcriptomic, enzymatic and metabolomic data, will contribute to deciphering the metabolic pathways of the conversion of essential amino acids into aroma volatiles in melon fruits.

03.3

PLANT DEFENSE ACTIVATORS AS ELICITORS OF OAT AVENANTHRAMIDE BIOSYNTHESIS

Mitchell L. Wise (USDA, ARS, Cereal Crops Research Unit, Madison, WI 53726, USA; mlwise@wisc.edu)

Oats produce a group of phenolic secondary metabolites termed "avenanthramides". Among food crops these metabolites are unique to oat. In addition to their biological role as phytoalexins, the avenanthramides are potent antioxidants in vitro and have potential as nutraceuticals. In cellular assays and animal models they demonstrate potent anti-inflammatory activity through inhibition of nuclear factor kappa beta. Although produced constitutively in the oat grain, the levels of avenanthramides tend to be highly variable and the levels are strongly influenced by environment, genotype and genotype × environment interactions. Recent work in my laboratory has shown that avenanthramide levels in vegetative tissue, and to some extent in the grain, can be enhanced by treatment with plant defense activators such as acibenzolar-*S*-methyl (benzothiadiazoles, BTH) and isonicotinic acid (INA). Treatment of the plants with BTH or INA produced a strong up-regulation of avenanthramide biosynthesis within 48 hours. This response tends to be fairly long lasting (days to weeks). The dynamics of avenanthramide biosynthesis in various tissues of oat plants will be described in detail. Genotypic variation in avenanthramide production will also be described.

IDENTIFICATION OF THE MOSQUITO BITING DETERRENT CONSTITUENTS FROM THE INDIAN FOLK REMEDY PLANT, JATROPHA CURCAS

<u>Charles L. Cantrell</u>,¹ Abbas Ali,² Stephen Duke,¹ Ikhlas Khan² (¹United States Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, University, MS 38677, USA, ²University of Mississippi, National Center for Natural Products Research, University, MS 38677, USA; clcantr1@olemiss.edu)

An investigation of the Indian folk remedy plant, Jatropha curcas, was performed to identify the constituents responsible for the mosquito biting deterrent activity of the oil. J. curcas seed oil is burned in oil lamps in India and parts of Africa to repel biting insects, primarily mosquitoes. The seed oil was thoroughly analyzed by ¹H NMR, ¹³C NMR, HPLC-RI, and GC-FID to identify the constituents in the oil. Identified constituents, both free fatty acids and triglycerides, were evaluated for activity in Aedes aegypti biting deterrent assays. Furthermore, an oil condensation trap was used to demonstrate that free fatty acids or triglycerides are partially volatilized during the combustion process. These compounds were found to be responsible for the biting deterrency of the burned oil. Specifically, oleic, palmitic, linoleic, and stearic acids were all active at 25 nmol/cm² above that of solvent control in A. aegypti biting deterrent assays. Oleic, palmitic, and linoleic acids were all more active than stearic acid in the same bioassay. Evaluation of the triglycerides containing each of these fatty acids revealed that tripalmitin, tristearin, trilinolein, and triolein all demonstrated significant activity above a solvent control at 10 µg/cm², whereas tripalmitin was the most active. Due to literature reports suggesting larvicidal activity of the oil, J. curcas seed oil and its free fatty acid constituents were also evaluated against 1-day old A. aegypti larvae up to 500 ppm. Oleic acid was the only fatty acid having larvicidal activity against 1-day old AP nutrients. aegypti larvae with an LC₅₀ of 47.9 ppm.

O3.5

CORRELATION BETWEEN TEA LEAF AGE AND CHEMICAL CONTENT AND SHADE LEVELS

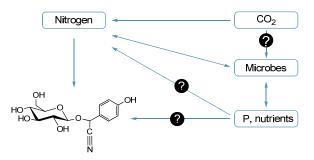
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This study investigated tea leaf samples from a Hawaiian tea plantation and the relationship between tea leaf age, the relative concentrations of three naturally occurring compounds commonly found in them; *L*-theanine, caffeine and epigallocatechin gallate (EGCG), all of which are reported to have positive effects on human health, shade levels and FRAP determined antioxidant activity. An HPLC method was developed that utilized a reversed phase C-18 stationary phase and a mobile phase composed of water and acetonitrile to quantify the relative amounts of the three natural products. The outcome of the analyses showed that the concentration of *L*-theanine and caffeine decreased as leaf age increased moving from bud to first and then second leaf, while EGCG concentration increased in moving from the bud to first, second and lower leaves. The influence of shade on the relative concentrations of these three compounds in tea leaves was also was investigated and shown to have a positive correlation with EGCG levels. Antioxidant activity, as determined using the FRAP assay system, was found to correlate positively with increasing EGCG levels. This is the first investigation of its type and also of tea samples from Hawaii. The presented findings show that certain chemical components of tea can potentially be used as markers for the age, quality and authenticity of various teas now and into the future.

IMPACTS OF CLIMATE CHANGE ON ALLOCATION OF N TO CYANOGENIC GLYCOSIDES

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The allocation of resources to bioactive products is likely to change as a result of climate change, affecting plant nutritive value. Cyanogenic glucosides, which break down to release toxic HCN, are synthesized by about 5% of all plants as well as many insects. In humans, epidemics of the neurological disorder Konzo are more common during periods of drought. Similarly, animals grazing on forage sorghum can die if plants are young, highly fertilized or water stressed. Toxicity of cassava and other C_3 plants (e.g. clover) is higher in plants grown at elevated CO_2 whereas drought effected-toxicity in sorghum (C_4) is moderated by higher concentrations of atmospheric CO_2 . We created an EMS mutagenized population of sorghum and identified individuals with reduced, enhanced or zero dhurrin. Allocation of N to different metabolites in different genotypes is compared with growth using a high through-put phenomics in order to help develop predictive models.



S4.1

GENETIC AND EPIGENETIC MECHANISMS OF COLON CANCER CHEMOPREVENTION IN HUMANS BY WHOLE BERRIES AND BERRY CONSTITUENTS

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Our laboratories have been evaluating the ability of freeze-dried berries to prevent gastrointestinal tract cancers in animals and in humans. Most studies have used black raspberries (BRBs), due to their high antioxidant potential and their high content of anthocyanins and fiber. In rodent studies, the consumption of BRB powder, at concentrations of 2.5, 5 and 10% (w/w) of a synthetic diet, results in a 40-70% inhibition of carcinogen-induced cancer in the rat esophagus and colon. Mechanistically, BRBs exhibit a broad range of chemopreventive effects on a cellular level including inhibition of cell proliferation, inflammation, angiogenesis, and stimulation of apoptosis, cell adhesion, and differentiation, and they protectively modulate the expression levels of genes associated with all of these cell functions. Based upon these preclinical observations, we have conducted a series of pilot clinical trials of BRBs in patients at high risk for cancer. The oral administration of BRB powder (45g/day) to Barrett's esophagus patients for 6 months led to a reduction in parameters of oxidative stress, but minimal effects on the lesion itself. Oral administration of strawberry powder (60g/day) to 37 Chinese patients with esophageal dysplasia led to histologic regression of 80% of the dysplastic lesions and reduced levels of iNOS, COX-2, and phospho-NF-kB-p65 proteins. Treatment of 20 colorectal cancer patients with BRB powder for about 3 weeks led to a reduction in cell proliferation and demethylation of suppressor genes in the Wnt signaling pathway in colorectal tumors. Finally, a trial in 14 patients with familial adenomatous polyposis showed that daily treatment with rectal BRB suppositories for 9 months caused a 36% regression of rectal polyps. These preliminary trials indicate that berries have significant promise for chemoprevention of esophageal and colon cancer in humans.

S4.2 NATURAL TRITERPENOIDS AS SCAFFOLDS FOR NEW DRUG SYNTHESIS

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There are many triterpenoids that exist in nature that have interesting biological activities. However, most of these triterpenoids are not potent enough to be practically used in clinical medicine as preventive or therapeutic agents. Thus, it is necessary to enhance activity by chemical modification of the naturally occurring triterpenoid scaffold. We will discuss the application of this principle, as exemplified by the synthetic oleanane triterpenoids that have been made in the Department of Chemistry at Dartmouth College by Professor Gordon Gribble and his colleagues, and then tested for biological activity in my own laboratory in the Department of Pharmacology at Dartmouth Medical School. Many of these agents have profound anti-inflammatory activity, by virtue of their ability to modulate the synthesis of enzymes involved in the inflammatory process. They also have similar anti-oxidative effects, again by inducing the synthesis of enzymes that destroy reactive oxygen species. One such derivative of oleanolic acid, namely CDDO-Methyl ester (bardoxolone methyl), is now in Phase III clinical trial for treatment of advanced kidney disease in diabetic patients.

S4.3

THE CLINICAL PROMISE OF SULFORAPHANE (SF)

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Isolation of sulforaphane (SF) from broccoli as the principal transcriptional inducer of a network of cytoprotective genes provided a rationale for the health benefits of high plant consumption (especially crucifers). It established that aerobic cells contain elaborate gene networks for defense against damage

by oxidative stress, electrophiles, inflammation, and radiation. Upregulation of these genes, mediated by the Keap1-Nrf2-ARE signaling pathway, protects cells against these damaging processes which lead to neoplastic and other chronic diseases. Broccoli sprouts deliver standardized doses of glucoraphanin (the glucosinolate precursor of SF) which undergoes hydrolysis to its active metabolite SF by plant myrosinase, or by microflora of the gastrointestinal tract. Microbial conversion efficiency varies enormously among individuals (2-40%), raising important health implications. SF and all other inducers are thiol reagents that target the cysteine-rich intracellular sensor Keap1, and upregulate a wide variety of genes. But SF also affects a broader range of functions that include induction of heat shock proteins, modulation of NF κ B-dependent inflammatory pathways, and suppression of histone deacetylase. The magnitudes of these responses probably depend on inducer levels, cell type and prevailing stress conditions. Insight into the multiple cytoprotective effects of sulforaphane, and the ease with which this dietary component can be delivered to humans has generated considerable interest in translating these findings into the clinic. Many clinical trials currently in progress are targeting malignancies (breast, prostate, bladder, skin), asthma, COPD, and radiation damage.

S4.4

CANCER PREVENTION BY δ - AND γ -TOCOPHEROLS

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Epidemiological studies have shown an inverse correlation between vitamin E intake and cancer risk. However, recent large-scale human trials with high doses of α -tocopherol (α -T) have produced disappointing results. This points out the need for a better understanding of the biological activities of different forms of tocopherols. Using a tocopherol mixture that is rich in γ -T (γ -TmT, containing 57% γ -T, 24% δ -T and 13% α -T), we have demonstrated the inhibition of cancer formation and growth in animal models for colon, lung, mammary gland and prostate cancers. δ -T was found to be more active than γ -T in the inhibition of cancer cell growth in culture and xenograft tumors as well as in AOM-induced colon carcinogenesis in rats, whereas α -T was ineffective. The blood and tissue levels of δ -T were low and those of α -T were high, but the levels of side-chain degradation metabolites of δ -T were high, suggesting that metabolites of δ -T contribute to the inhibitory activity. The inhibitory activity was associated with the quenching of reactive oxygen and nitrogen species as well as anti-inflammatory activities. We suggest the use of γ -TmT, δ -T or γ -T, rather than pure α -T, for cancer prevention (Supported by NIH grants CA141756, CA122474 & CA133021 and the John Colaizzi Chair Endowment Fund).

S4.5

LUNG CANCER PREVENTION: REVERSE MIGRATION STRATEGY

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Despite extensive research, there are no known effective chemoprevention agents for lung cancer. Clinical trials in the past, using agents without a clear target in an unselected population, have shown interventions to be ineffective or even harmful. We propose a new approach to drug development in the chemoprevention setting: Reverse migration; that is, drawing on our experience in the treatment of advanced cancer to bring agents, biomarkers, and study designs into the prevention setting. Our institution has experience with biomarker-driven clinical trials, as in the recently reported Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial, and we now propose to bring this trial design into the prevention setting.

S4.6

ARTHUR NEISH YOUNG INVESTIGATOR AWARD LECTURE A NOVEL ROLE FOR SULFHYDRYL-REACTIVE ACTIVATORS OF TRANSCRIPTION FACTOR NRF2: HSF1-DEPENDENT UPREGULATION OF Hsp70

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Transcription factors NRF2 and HSF1 provide broad defence mechanisms by regulating the expression of several hundred genes that encode proteins with versatile cytoprotective functions. The identification of small-molecule inducers of these pathways is actively being pursued as a strategy to prevent or delay disease onset and to extend healthy lifespan. Such compounds, many of which are abundant in plants, and their synthetic analogues protect against the otherwise detrimental consequences of the toxic, neoplastic, and pro-inflammatory effects of xenobiotics and endogenous substances in experimental models of carcinogenesis, cardiovascular disease, and neurodegeneration. We found that structurally distinct small-molecule NRF2 activators, all of which react with sulfhydryl groups, but differ in potency by 15,000-fold, upregulate Hsp70, a prototypic HSF1-target gene, thus implicating the heat shock response as a target for NRF2 activators. Hsp70 upregulation requires functional HSF1, but is NRF2-independent. In addition, a sulfoxythiocarbamate inducer conjugates to the negative regulator of HSF1, Hsp90. The differential concentration-dependence of the two responses suggests that activation of NRF2 precedes that of HSF1. These findings support the future development of potent "dual" activators of this type as mechanism-based comprehensive cytoprotective agents.

S4.7

SYNTHETIC TRITERPENOIDS AND CHEMOPREVENTION: BIOLOGICAL ACTIVITIES AND MOLECULAR TARGETS

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Synthetic oleanane triterpenoids are multifunctional agents with potent anti-inflammatory, antiproliferative, and pro-apoptotic activities *in vitro*. Although they are not conventional cytotoxic molecules, the triterpenoids can prevent and treat cancer in a variety of experimental animal models. Both CDDOmethyl ester (CDDO-Me) and CDDO-ethyl amide (CDDO-EA) significantly decrease the number, size, and histopathology of lung adenocarcinomas in A/J mice challenged with vinyl carbamate. CDDO-Me also delays the development of estrogen receptor negative mammary tumors induced by overexpression of the ERbB2 oncoprotein or by deletion of the BRCA1 tumor suppressor gene. Both CDDO-Me and CDDO-EA also extend survival in a transgenic mouse model of pancreatic cancer, driven by mutations in Kras and p53. In addition to their profound effects on the redox status of cells, the triterpenoids directly interact with regulatory proteins containing reactive cysteines. Keap1, IKK, STAT3, PTEN, and mTOR are all validated molecular targets of the triterpenoids, and all of these proteins and their associated downstream signaling pathways are highly relevant targets for the prevention of cancer.

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S4.8

CANCER CHEMOPREVENTION WITH ANTI-INFLAMMATORY PHYTOCHEMICALS

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A new horizon in cancer chemoprevention research is the recent discovery of molecular links between inflammation and cancer. Modulation of cellular signaling involved in chronic inflammatory response by anti-inflammatory agents hence provides a rational and pragmatic strategy in molecular target-based chemoprevention and cytoprotection. Many substances derived from herbs and spices have been found to activate this particular redox-sensitive transcription factor, thereby potentiating cellular antioxidant or detoxification capacity. It is noteworthy that there is a good correlation between anti-inflammatory activity of some chemopreventive/cytoprotective agents and their ability to induce antioxidant gene expression. The current research in my laboratory concerns evaluation of chemopreventive and cytoprotective effects of some edible antioxidative and anti-inflammatory phytochemicals and elucidation of their underlying molecular mechanisms. Our research program has attempted to unravel common events mediated by transcription factors, such as NF- κ B, STAT3 and Nrf2, and their regulators, involved in the cellular signaling network for molecular target-based chemoprevention with selected dietary and medicinal phytochemicals.

S4.9

THE PHENOMENA OF RESVERATROL

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Cancer chemoprevention entails the ingestion of dietary or pharmaceutical agents that can prevent, delay or reverse the process of carcinogenesis. We have been actively engaged in the systematic discovery and characterization of natural product chemopreventive agents. The typical approach involves identifying active crude substances, such as extracts derived from terrestrial plants or marine organisms, utilizing *in vitro* bioassay systems, followed by isolation of pure active components. As part of this project, an extract obtained from a nonedible Peruvian legume, *Cassia quinquangulata* Rich. (Leguminosae), was evaluated and found to be active as an inhibitor of cyclooxygenase. The active component was identified as resveratrol. A surprisingly broad spectrum activity was observed, indicative of potential to inhibit carcinogenesis at the stages of initiation, promotion and progression. This discovery has led to many additional research efforts. There are now around 4000 papers concerning various aspects of resveratrol action, some of which have generated controversy. The molecule is unusually promiscuous and specific mechanisms remain elusive. Rapid and near complete metabolism add to the conundrum. Recently, we have exploited the broad spectrum of activities mediated by this simple stilbene to design derivatives with much greater potency and specificity. An overview of the field and a personal perspective will be presented. (Supported by NCI program project P01 CA48112).

S4.10 KEAP1-NRF2 SIGNALING AS A TARGET FOR CANCER PREVENTION BY NATURAL PRODUCTS

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Health reflects the ability of an organism to adapt to stress. Stresses - metabolic, proteotoxic, mitotic, oxidative and DNA-damage stresses - not only contribute to the etiology of cancer and other chronic degenerative diseases but are also hallmarks of the cancer phenotype. Activation of the Kelch-like ECH-associated protein 1 (KEAP1)-NF-E2-related factor 2 (NRF2)-signaling pathway is an adaptive response to environmental and endogenous stresses and serves to render animals resistant to chemical carcinogenesis, other forms of toxicity, and inflammation whilst disruption of the pathway exacerbates these outcomes. Protection against these stresses is manifest in multiple ways: (i) prevention of

macromolecular damage through induction of electrophile detoxication and antioxidative enzymes, as well as dampening of inflammatory processes, (ii) induction of macromolecular damage repair/removal systems including the proteasome, DNA repair and autophagy, and (iii) activation of tissue repair/regeneration pathways. These cytoprotective effects of Nrf2 reflect responses mediated by direct activation of downstream effector genes and through cross-talk with other signaling networks contributing to cellular plasticity. The Keap1-Nrf2 pathway can also be induced by thiol-reactive small molecules including dithiolethiones, isothiocyanates and triterpenoids that demonstrate protective efficacy in preclinical chemoprevention models and in clinical trials.

S4.11

CANCER CHEMOPREVENTION BY TARGETING THE EPIGENOME - STATE OF THE ART AND FUTURE CHALLENGES

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The term epigenetics refers to modifications in gene expression caused by heritable, but potentially reversible, changes in chromatin structure. Major epigenetic mechanisms include DNA methylation, histone acetylation and methylation, and non-coding (micro) RNAs. Given the fact that epigenetic modifications occur early in carcinogenesis, they have been identified as promising new targets for prevention strategies. Recent years have provided a wealth of information on the potential impact of chemopreventive agents on epigenetic mechanisms (reviewed in Huang, Plass, Gerhauser, Curr Drug Targets 2010). Food components targeting the epigenome include micronutrients (folate, selenium, retinoic acid, vitamin E), butyrate, polyphenols (from green tea, apples, coffee, and other dietary sources), genistein and soy isoflavones, curcumin, ellagitannin, indol-3-carbinol, lycopene from tomatoes, and sulfur-containing compounds from Allium and cruciferous vegetables. Their effects on the epigenome have potential impact on multiple mechanisms relevant for cancer prevention, including detoxification, cell cycle progression, signal transduction, apoptosis induction, and others. In vivo studies that demonstrate the functional relevance of epigenetic mechanisms for health promoting efficacy of natural products are still limited. Future projects will identify best strategies for chemopreventive intervention with micronutrients and dietary food components, taking into account the importance of epigenetic mechanisms for gene regulation.

S4.12

DIETARY CANCER CHEMOPREVENTIVE PHYTOCHEMICALS: SIGNALING AND EPIGENETICS IN BLOCKING CARCINOGENESIS INITIATION VERSUS TUMOR PROGRESSION

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Diverse dietary phytochemicals can prevent diseases including cancer. These dietary "antioxidants", can trap RONS, also trigger cellular signaling events including "proteins thiol modifications" leading to expression of cellular defense genes or other cellular effects. We study dietary phenolic antioxidants, isothiocyanates, tocopherols, *w*-3 fatty acids and herbal medicines, which are effective against various many animal carcinogenesis models. These compounds could modulate kinases, activate Nrf2 signaling, and induce cellular defense genes HO-1, GST, NQO1, and GCS. Integrating results from Nrf2-/- mice with microarray bioinformatics, other genes including apoptosis, cell adhesion, cell growth, kinases, electron transport, transcription factors, and ubiquitination, are also Nrf2-mediated, leading to the overall cellular protective effects against oxidative/carcinogenic damages. These Nrf2-/- mice are more prone to carcinogen-induced skin, colon and other cancers. Incidentally, in the prostate TRAMP tumors, as cancer progresses, a shut-down of Nrf2 via CpG methylation of the promoter region, attenuating Nrf2-mediated genes, which were reversed by dietary PEITC, curcumin and tocopherols. Hence, daily intake of these phytochemicals would induce Nrf2-mediated anti-oxidative stress genes either directly or indirectly through epigenetic pathways, reduce inflammation, induction of apoptosis/autophagy of initiated tumor cells during early lesions. (Supported by NIH grants).

S4.13 DIETARY PREVENTION OF COLON CARCINOGENESIS AND DISCOVERY OF PREDICTIVE BIOMARKERS

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Our group has established multiple collaborations to prevent carcinogenesis by dietary intervention and to discover predictive molecular indicators. We have identified potential molecular targets and biomarkers of efficacious response to dry bean-based diets in intervention studies to prevent colon carcinogenesis in humans and mice. These studies are based on the observation that high dry bean intake decreased advanced colorectal adenoma recurrence in humans and protected against chemically induced colon carcinogenesis in obese mice. Both ethanol extract (containing flavonols and other soluble compounds) and residue (containing fiber) as well as whole cooked beans were active in attenuating carcinogenesis in the Ob/Ob mice. IL-6 has been identified as a potential biomarker and molecular target. Serum IL-6 concentrations were elevated in participants of the Polyp Prevention Trial (PPT) who developed high risk or advanced adenomas and were lower in participants consuming a flavonol-rich diet to which dry beans primarily contribute. Similarly, serum IL-6 concentrations were elevated in obese, carcinogen-induced mice with pre-neoplastic lesions and were lower in mice fed the dry bean-rich diets. RNA concentrations of IL-6 in colon tissue were elevated in mice receiving the carcinogen and were attenuated in mice fed the dry bean-rich diets (Mentor-Marcel et al., 2009). In a short-term human feeding (LIFE) study, we identified from fecal colonocyte microarray analysis sets of 3 genes that could be used as potential indicators of risk or exposure to dietary dry beans. The LIFE study, which paralleled the diets found efficacious in the PPT demonstrated that a legume-enriched low-glycemic index diet produces improvements in biomarkers of insulin resistance and inflammation as well as improvements in serum lipid profiles and plasma leptin. Indicators of insulin resistance and inflammation also improved in the 4-year Polyp Prevention Trial. Current studies are identifying serum metabolites as indicators in the PPT and LIFE studies.

S4.14

STUDIES FOR CANCER PREVENTION: A PATH FROM TEA TO CAFFEINE TO EXERCISE

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Oral administration of green or black tea to SKH-1 mice inhibited UVB-induced skin carcinogenesis, but the decaffeinated teas were inactive and caffeine had a strong inhibitory effect. Mechanistic studies indicated that caffeine inhibited UVB-induced carcinogenesis by increasing UVB-induced p53 and by inhibiting UVB-induced increase in the ATR/Chk1 pathway thereby enhancing UVB-induced apoptosis. Caffeine had no effect on apoptosis in normal epidermis. Since caffeine administration increased locomotor activity and decreased tissue fat, we evaluated the effect of voluntary exercise (running wheel in the cage) on UVB-induced apoptosis. Exercise, like the effect of caffeine, also enhanced UVB-induced apoptosis, decreased tissue fat and inhibited UVB-induced carcinogenesis. Caffeine or running wheel exercise-related inhibition of UVB-induced carcinogenesis was associated with increased apoptosis in the tumors but not in areas away from tumors. A combination of caffeine and exercise had a much stronger stimulatory effect on UVB-induced apoptosis and a stronger inhibitory effect on UVB-induced carcinogenesis than either treatment alone. Since both regimens decreased tissue fat, we evaluated the effect of removal of the parametrial fat pads on UVB-induced apoptosis and found that UVB-induced apoptosis was enhanced, suggesting that tissue fat may secrete anti-apoptotic factors. These results suggest that tissue fat may enhance carcinogenesis by inhibiting apoptosis in DNA-damaged precancer cells and in cancer cells.

S4.15 CANCER CHEMOPREVENTION: MISSION ACCOMPLISHED IN RODENT MODELS BUT WHY NOT SO IN HUMANS

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I define cancer chemoprevention as slowing the process of carcinogenesis. Based on our experience with hundreds of chemopreventive agents in animal models, the process of carcinogenesis could be slowed by many agents in every spontaneous, induced and transgenic model. Thus, we can say that the mission of cancer chemoprevention has been successfully accomplished in rodent models. However, the picture of cancer chemoprevention in humans is not that glowing. The results of prospective randomized human trials of chemopreventive agents have in many cases been less impressive or have conflicted with the results of animal and observational studies. First of all, understandably any successful animal protocol for cancer chemoprevention in rodent models has never been duplicated in human settings. For reasons unclear and unfortunately any human large scale cancer chomoprevention trial done to date before initiation has never been first validated in a reliable rodent model. In addition, the timing of the intervention during multistep carcinogenesis, the complexity of dietary interactions, baseline levels in a given individual or population, duration of the study and the dose-response effects further complicates translation of animal data to human settings. It is also possible that because of our lifestyle of consuming many chemopreventive agents present in fruits and vegetables and other sources, we have already reaped some benefits of cancer chemoprevention. Thus, we might have to settle for only modest delays in cancer occurrence as a result of intervention. Some thoughts will be presented for further slowing the process of carcinogenesis in the human population.

S4.16

NATURAL-AGENTS MECHANISMS AND PERSONALIZING MARKERS FOR CANCER PREVENTION

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Mechanism-based, personalized approaches with natural agents are on the march in cancer prevention. The natural agents discussed here represent recent advances in molecular targeting and predictive markers for natural-agent cancer prevention. Promising preclinical/clinical results with green tea and its polyphenolic flavonoid constituent EGCG (epigallocatechin gallate) include randomized trials in the head and neck and prostate. Elegant new mechanistic data indicate that EGCG binds to the peptidyl prolyl cis/trans isomerase Pin1, which is required for EGCG effects on cell growth, c-Jun activation, and NF-kB and AP-1-mediated transcription regulation. EGCG analogs that target Pin1 more specifically could be developed, then tested in phase 0 trials, such as that recently conducted with an indole-3 carbinol compound (derived from cruciferous vegetables; primarily inhibits Akt), which was the first phase 0 trial for chemoprevention. Promising preclinical/early clinical results of myoinositol include inhibiting phospho-Akt and regulating PI3K expression signatures in the lungs of smokers. With promising preclinical/early clinical activity through effects on AMPK and IGF1R signaling, metformin (derived from French lilac) recently was found to have germline predictive markers. Selenium did not prevent prostate cancer in SELECT or high-grade PIN patients, but pharmacogenetics suggest genotypes that predict selenium benefit. Developing standard personalized chemoprevention, with either natural or other agents, is becoming clearer with studies of molecular targets and predictive markers. The recent mechanistic study of EGCG is a model of molecular-targeted research in this field.

O4.1 ANTI-CANCER EFFECT OF SCALLION EXTRACT AGAINST COLON TUMOR

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Colorectal cancer is a common malignancy and a leading cause of cancer death worldwide. Diet is known to play an important role in the etiology of colon cancer and, recently, dietary chemoprevention has received increasing attention for the prevention and alternative treatment of colon cancers. Scallion is used as a spice/vegetable worldwide, and as a traditional Chinese medicine in treating a variety of diseases. The possible beneficial effects of scallion on mouse colon cancer were evaluated in this study. The in vivo anti-tumor effects of scallion extracts were assessed in a subcutaneously inoculated CT-26 colon tumor model in BALB/c mice. Tumor tissues were subjected to western blotting and immunohistochemistry for analysis of key inflammatory markers, and ELISA for analysis of cytokines. A specific preparation of scallion extracts, orally fed to test mice at 50 mg/kg b.w./day, resulted in a significant suppression of tumor growth and enhanced the survival rate of test mice. Dosage of specific scallion extract, when translated into human application, is equivalent to 10 g fresh weight/70 kg b.w./day. This amount of scallion is highly acceptable as a dietary uptake for both Asian and Western food cultures. At the molecular level, scallion extracts inhibited the key inflammatory markers COX-2 and iNOS, and suppressed the expression of various cellular markers known to be involved in tumor apoptosis (apoptosis index), proliferation (cyclin D1 and c-Myc), angiogenesis (VEGF and HIF-1 α), and tumor invasion (MMP-9 and ICAM-1), when compared with vehicle control-treated mice. Our findings may warrant further investigation on the use of common scallion as a chemopreventive dietary agent to lower the risk of colon cancers.

04.2

GINGER SUPPLEMENTATION AND THE EXPRESSION OF NF-KB IN THE NORMAL-APPEARING COLORECTAL MUCOSA OF PATIENTS AT HIGH RISK OF COLORECTAL CANCER: RESULTS FROM A PILOT RANDOMIZED, CONTROLLED TRIAL

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Background: Ginger has been proposed as a promising candidate for colorectal cancer (CRC) prevention. To assess the potential of ginger for modulating inflammation in normal human colorectal mucosa, we measured the expression of NF-kB, a transcription factor involved in the control of inflammation, in normal-appearing colon mucosa of patients at high risk for CRC in a pilot, randomized, double-blinded, placebo-controlled, clinical trial.

Methods: A total of 20 patients were treated with either 2.0 g (eight 250 mg capsules) of encapsulated ginger (standardized to 5%-gingerols) or placebo for 28 days. Overall expression and distributions of NF-kB in colorectal crypts in biopsies of normal-appearing colon mucosa were detected and measured using automated immunohistochemistry and quantitative image analysis. For quality control, 10% of slides were randomly selected for repeat measurements, which yielded an intra-rater reliability of 0.96.

Results: In the ginger group relative to the placebo group, NF-kB expression decreased 25.8% (p = 0.08) along the full length of the crypts, with a 29.4% (p = 0.08) decrease in the upper 40% (differentiation zone) of the crypts, and 23.7% (p = 0.08) in the lower 60% (proliferative zone) of the crypts relative to the placebo group.

Conclusions: These results suggest that ginger may reduce inflammation in the colorectal epithelium.

GINGER SUPPLEMENTATION AND THE EXPRESSION OF BAX IN THE NORMAL-APPEARING COLORECTAL MUCOSA OF SPORADIC COLORECTAL ADENOMA PATIENTS: RESULTS FROM A PILOT RANDOMIZED, CONTROLLED TRIAL

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Background: Ginger has been proposed as a promising candidate for colorectal cancer (CRC) prevention. To assess the potential of ginger for modulating apoptosis in the normal human colorectal mucosa, we measured the expression of bax, an apoptosis promoter, in the normal-appearing colon mucosa of patients at high risk for CRC in a pilot, randomized, double-blinded, placebo-controlled, clinical trial.

Methods: A total of 20 patients were treated with either 2.0 g (eight 250 mg capsules) of encapsulated ginger (standardized to 5%-gingerols) or placebo once daily for 28 days. Overall expression and distributions of bax in colorectal crypts in biopsies of normal-appearing colon mucosa were detected and measured using automated immunohistochemistry and quantitative image analysis. For quality control, 10% of slides were randomly selected for repeat measurements, which yielded an intra-rater reliability of 0.98.

Results: In the ginger group, relative to the placebo group, bax expression decreased 15.6% (p = 0.81) along the full length of the crypts, 6.6% (p = 0.38) in the upper 40% (differentiation zone) of the crypts, and 21.7% (p = 0.77) in the lower 60% (proliferative zone) of the crypts; however, there was a 19% increase (p = 0.80) in the proportion of the expression of bax in the upper 40% relative to the whole crypt. Conclusions: These preliminary results suggest that ginger may reduce apoptosis in the colorectal epithelium overall while shifting more of its expression from the proliferative zone of the crypt to the luminal, or differentiation zone.

04.4

POTENTIAL ROLE OF GINSENG FOR CANCER CHEMOPREVENTION

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The clinical management of cancer invariably involves diverse conventional modalities, including surgery, radiation, and chemotherapy. However, the complexity of human cancer requires some alternative management to improve the therapeutic efficacy of conventional treatment and/or the guality of life of cancer patients. Medicinal botanicals have recently gained more attention for cancer management. Numerous effective anticancer drugs have been developed from botanicals, and identifying new herbal sources to develop ideal chemoprevention remains an essential step in advancing the treatment of colorectal cancer. In this study, potential roles of ginseng herbs, especially American ginseng and notoginseng, in cancer chemoprevention are evaluated. The major pharmacologically active constituents of ginsengs are ginsenosides, which can be mainly classified into protopanaxadiol and protopanaxatriol groups. The recognized active anticancer compounds from American ginseng and notoginseng are ginsenosides Rg3, Rh2, and protopanaxadiol. The structure-activity relationship between their chemical structures and pharmacological activities is discussed. Sugar molecules within a ginsenoside have a high impact on cancer cells. Anticancer activities increase with the decrease of sugar number. In addition, we observed that various steaming temperatures and time treatments of the ginseng herbs can change their ginsenoside profiles and enhance their anticancer activities. This heat treatment process may increase the efficacy of ginseng in cancer chemoprevention. (This work was supported in part by the NIH/NCCAM grants AT003255, AT004418 and AT005362).

BLUEBERRY DIET AND BLUEBERRY BIOACTIVES INHIBIT LUNG CANCER AND ENHANCE THE ACTIVITY OF PACLITAXEL

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Unlike significant progress made in the prognosis of certain cancers, the prognosis for lung cancer remains grim. We describe efficacy of blueberry powder and bioactives for their anti-lung cancer activity, and for their chemosensitizing effects. Nude mice were provided AIN-93M diet or diet supplemented with blueberry powder (7.5%, w/w) and then inoculated with highly aggressive human lung cancer H1299 cells. This intervention showed significant inhibition of the tumor burden progressively. To determine if blueberry anthocyanidins (anthos) were the main bioactives, H1299 cells were treated in culture with blueberry anthos individually and their equimolar mixture. These compounds showed a time- and dose-dependent inhibition of the cell growth, with much greater effect observed with the antho mixture. Nude mice carrying H1299 cell xenograft when treated i. p. with either delphinidin (1.5 mg/mouse) or a native mixture of blueberry anthos (0.5 mg/mouse) resulted in 60-65% inhibition of the tumor burden. Further, nude mice upon i. p. treatment with suboptimal doses of the chemotherapeutic drug paclitaxel and blueberry anthos elicited significant tumor inhibition only in the combination groups. Analyses of H1299 cells from the various treatments by Western blot revealed that the synergistic effects observed were due to the attack of anthos either on overlapping and/or distinct targets associated with cell proliferation, apoptosis, inflammation and metastasis.

04.6

ANTIOXIDANT EFFECTS OF LYCOPENE IN MEN WITH PROSTATE CANCER OR BENIGN PROSTATE HYPERPLASIA: A RANDOMIZED CONTROLLED TRIAL

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Consumption of tomato products is associated with a decreased risk of prostate cancer, and lycopene, the red carotenoid in the tomato, is a potent antioxidant that might contribute to this chemoprevention activity. A double-blind, randomized, placebo-controlled trial of 120 men, 90% African American veterans, with either prostate cancer or benign prostate hyperplasia was carried out to investigate whether oral administration of lycopene increases lycopene levels in blood and prostate tissue and lowers markers of oxidative stress. Urology patients were randomly assigned to receive either 30 mg/d of lycopene as a tomato oleoresin or placebo for 21-days prior to prostate biopsy for possible diagnosis of prostate cancer. For the men receiving lycopene, the mean lycopene concentration in plasma increased 2-fold compared to placebo and 2-fold in prostate tissue. There was a trend in the reduction the DNA oxidation product 8-oxo-deoxyguanosine in men diagnosed with benign prostate hyperplasia but not in men diagnosed with cancer. Lipid peroxidation measured as malondialdehyde in plasma was negatively correlated with lycopene levels.

RESVERATROL AND ITS ANALOGUES AS POTENTIAL EPIGENETIC AGENTS FOR CHEMOPREVENTION AND THERAPY IN PROSTATE CANCER

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Epigenetic silencing of tumor suppressor gene(s) is a contributing factor to the pathogenesis of prostate cancer (PCa). Reversal of epigenetic silencing is therefore a potentially desirable modality of targeted chemoprevention and therapy for PCa. We report on the epigenetic HDAC inhibitory activity of Resveratrol (Res) through inhibition of metastasis-associated protein 1 (MTA1), which plays a critical role in PCa progression. Resveratrol down-regulates the MTA1 protein by promoting its degradation and dissociation from the NuRD (nucleosome remodeling and deacetylation) repressor complex, which results in reverse deacetylation of tumor suppressor p53. Further, silencing MTA1 by genetic approaches (shRNA) in combination with Res treatment significantly enhances p53 acetylation and subsequent apoptosis in PCa cells. In the present study, we compared and contrasted the anti-proliferative and MTA1-regulation of Res and its natural (PTER, PIC and 3M-Res) and synthetic (2Ac-Res, 3Ac-Res and DMSA) analogues in a panel of PCa cells: LNCaP, Du145 and PC3M. Results indicate that selected Res analogues have higher biopotency in inhibiting MTA1 and androgen receptor (AR) therefore promising greater efficacy as potential "epigenetic" chemopreventive and therapeutic agents in PCa.

04.8

ELUCIDATION OF STRUCTURAL/FUNCTIONAL CHANGES UPON MODIFICATION OF KEAP1 C151, A PRIMARY TARGET OF BOTANICAL CHEMOPREVENTIVE AGENTS

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Many botanical agents, including sulforaphane from Cruciferae species, activate the transcription factor Nrf2. Numerous cytoprotective genes are upregulated by Nrf2, comprising a promising therapeutic strategy for the prevention of cancer and other diseases. At basal conditions, Nrf2 is repressed by the cysteine-rich Keap1 protein, which targets Nrf2 for ubiquitination by a Cul3-mediated ubiquitination complex and subsequent degradation. Modification of Keap1 cysteines, in particular C151, by sulforaphane and other compounds leads to Nrf2 activation, by downregulating Nrf2 ubiquitination and degradation. We find C151 to be highly modified by sulforaphane and other promising chemopreventive botanical compounds. We and others have shown by co-immunoprecipitation experiments that modification of C151 disrupts the Keap1-Cul3 interaction. We sought to understand the structural and functional changes that occur upon modification of Keap1 C151. Interestingly, we find that when Keap1 C151 is modified, in addition to a decrease in the Keap1-Cul3 affinity, there is a conformational change in the Keap1-Cul3 complex that does form. We propose that this conformational change reduces the ability of the Keap1-Cul3 complex to target Nrf2 lysines for ubiquitination.

EXTRACTS OF COLLARD GREENS DOWN-REGULATE THE EXPRESSION OF HER2/NEU PROTEIN AND mRNA IN MCF-7 AND SK-BR3 BREAST CANCER CELLS

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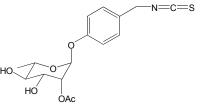
Cruciferous vegetables such as broccoli, cabbage, and brussel sprouts have been shown in animal studies, as well as clinical trials, to have chemopreventative effects. We have been investigating plants with potential chemopreventive activity from the Lumbee Tribe of North Carolina, including Brassica oleracea var. acephala L., or 'collard greens', a vegetable common in the Lumbee diet. Our preliminary data show that MeOH extracts of collards enhanced secreted human placental alkaline phosphatase (SEAP) reporter activity. However, the ethyl acetate liquid fraction (EtOAc, 20 µg/ml) reduced SEAP expression and expression of endogenous pS2, an estrogen dependent gene, when administered with 10 nM estradiol. Additionally, both crude MeOH extract and the EtOAc fraction inhibited the activity of HER-2 tyrosine kinase completely at 20 µg/ml. The inhibition of HER2/erbb2 protein expression was corroborated by In-Cell Western analysis in SK-BR3 cells. The EtOAc partition inhibited the expression of HER2 protein by 30% at 37 µg/ml in SKBR3 cells, and became significantly cytotoxic at higher concentrations. These results indicate that collard greens may potentially convey chemoprotection when consumed regularly. In both MCF-7 and SK-BR3 breast cancer cells, the MeOH extract and the EtOAc partition at 20 µg/ml down-regulated the expression of the mRNA for the HER-2 receptor. These data suggest that collard greens have chemopreventative effects and may be developed as new anti-HER-2 agents.

O4.10

SUPPRESSION OF CYCLOOXYGENASE-2 AND INDUCIBLE NITRIC OXIDE SYNTHASE EXPRESSION BY 4-[(2'-O-ACETYL-α-L-RHAMNOSYLOXY)BENZYL] ISOTHIOCYANATE IN LPS-STIMULATED RAW 264.7 CELLS

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4-[(2'-O-Acetyl- α -L-rhamnosyloxy)benzyl]isothiocyanate (RBITC) from *Moringa oleifera* Lamarck suppressed the expression of COX-2 and iNOS at both the protein and mRNA levels through the inhibition of phosphorylation of the extracellular-signal-regulated kinase and the stress-activated protein kinase, as well as ubiquitin-dependent degradation of inhibitor $\kappa B\alpha$ ($l\kappa B\alpha$). In accordance with $l\kappa B\alpha$ degradation, nuclear accumulation of NF- κB , and subsequent binding of NF- κB to the NF- κB *cis*-acting element, was attenuated by treatment with RBITC. These data suggest RBITC should be included in the dietary armamentarium of isothiocyanates potentially capable of mediating anti-inflammatory or cancer chemopreventive activity.



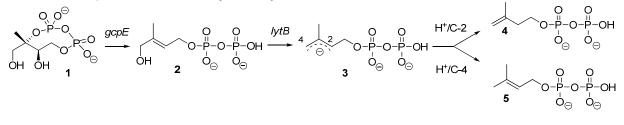
4-[(2'-O-acetyl-α-L-rhamnosyloxy)benzyl]isothiocyanate (RBITC)

S5.1

BIOSYNTHESIS OF ISOPRENE UNITS VIA THE MEP PATHWAY: ELECTRON AND PROTON TRANSFERS IN THE FORMATION OF IPP AND DMAPP

<u>Michel Rohmer</u>, Lionel Charon, Catherine Grosdemange-Billiard, Myriam Seemann, Jeanne Toulouse, and Denis Tritsch (Université de Strasbourg/CNRS, Institut de Chimie, 67070 Strasbourg Cedex, France)

Methylerythritol cyclodiphosphate (MEcPP, 1) reductase (GcpE) and hydroxymethylbutenyl diphosphate (HMBPP, 2) reductase (LytB), the last two enzymes of the mevalonate-independent MEP pathway, catalyze respectively the reductions of MEcPP into HMBPP and HMBPP into isopentenyl diphosphate 4 and dimethylallyl diphosphate 5. These reactions involve the transfer of two electrons provided by the [4Fe-4S] cluster of the enzymes and the protonation of an allylic anion intermediate 3. Experiments performed with the isolated enzymes suggest that the electron source for the Fe/S cluster is in bacteria NADPH/flavodoxin/flavodoxin reductase for both enzymes and in plants photosynthesis via ferredoxin in the light or NADPH/ferredoxin reductase/ferredoxin in the dark for GcpEpE. Incubation of [1-²H]glucose into the terpenoids of the bacterium *Zymomonas mobilis* was in accordance with the role of the flavine of flavodoxine as proton donor in the LytB catalyzed reduction.



S5.2 UNEARTHING THE BIOSYNTHETIC DIVERSITY IN THE STEROL METABOLOME

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Eukaryotes exhibit diversity in the composition of membrane sterols. This is largely due to the sterol side chain construction of various combinations of alkyl groups, which can be synthesized by a family of structurally similar enzymes known as sterol C24-methyltransferases (24-SMTs). Discovery trails to early 24-alkyl sterols involving geochemistry, phylogenetic distribution studies and comparative genomic and mechanistic analyses of 24-SMTs reveal remnants of ancient phytosteranes that date back to the Precambrian eon, phyla-specific differences in the length of the side chain (C8 to C11) and configuration of the C24-alkyl(idene) group (alpha/beta- and cis-trans-orientations) and evolutionary accommodation in substrate differences to form single versus multiple products. Notably, fitness requirements determined in a range of organisms show the importance of targeted 24-alkyl sterol homeostasis (type and amount of compound) in growth and/or reproduction and therefore implicates a mating of 24-alkyl sterol structure to function in systems biology. This talk covers the secrets of sterol side chain complexity unearthed in the topics outlined above.

S5.3

BIOSYNTHESIS OF BIOACTIVE ACYCLIC DITERPENOIDS AND PHYTOSTEROLS IN CROTON STELLATOPILOSUS

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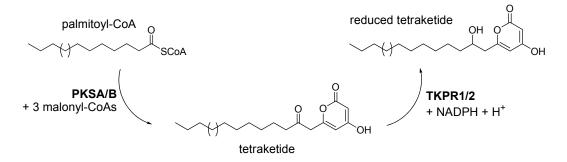
Croton stellatopilosus, a Thai medicinal plant containing an antipeptic plaunotol, has been used in forms of whole plants, callus and cell suspension cultures as models for biosynthetic studies of acyclic diterpenoids and phytosterols. Plaunotol is a simple linear acyclic diterpene alcohol derived from 4 isoprene units whereas the phytosterols, β -sitosterol and stigmasterol, are from the same monomer of 6 units. Various techniques, including feeding experiments, enzymology, subcellular compartmentalization molecular cloning and metabolic profiling, have been used to clarify the nature of the biosynthesis of both isoprenoid groups. Interesting results have been obtained with respect to their origin of isoprene units, degree of metabolite exchange between the mevalonate pathway and the deoxyxylulose pathway and the transcription profiles of their biosynthesis.

S5.4

POLYKETIDE SYNTHASES AND TETRAKETIDE-PYRONE REDUCTASES OF ARABIDOPSIS THALIANA ARE INVOLVED IN SPOROPOLLENIN BIOSYNTHESIS

Benjamin Lallemand,¹ Etienne Grienenberger,^{1,2} Sung Soo Kim,² Thierry Heitz,¹ Carl J. Douglas,² <u>Michel</u> <u>Legrand</u>¹ (¹IBMP du CNRS, Université de Strasbourg, Strasbourg, 67000, France, ²University of British Columbia, Department of Botany, Vancouver, BC V6T 1Z4, Canada; michel.legrand@ibmpcnrs.unistra.fr)

Fatty acyl-CoA esters synthesized by ACYL-COA SYNTHETASE (ACOS5) are condensed with malonyl-CoA by POLYKETIDE SYNTHASE A (PKSA) and PKSB and then reduced by TETRAKETIDE α -PYRONE REDUCTASE 1 (TKPR1) and TKPR2 to yield α -pyrone polyketides required for pollen development and sporopollenin biosynthesis. Genes encoding the enzymes are present in all plants surveyed to date, thus suggesting they participate in an ancient conserved biochemical pathway.



S5.5

ENGINEERING PLANT POLYKETIDE SYNTHASES

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The superfamily of type III polyketide synthases (PKSs) produce a variety of plant secondary metabolites with remarkable structural diversity and biological activities. In the last 10 years, there have been significant advances in understanding the structures and functions of the enzymes. Because of the remarkable catalytic potential and the substrate promiscuity, the structurally simple type III PKSs can be an excellent platform for engineering to design and develop supra-natural enzyme with novel catalytic functions. In this presentation, our recent progress of engineering of plant type III PKS enzymes by structure-based and precursor-directed approach will be discussed.

S5.6

THE ROLE OF ASCORBATE IN THE ACCLIMATION OF LEAVES TO HIGH LIGHT

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Arabidopsis thaliana leaves accumulate ascorbate (vitamin C) and anthocyanins over a similar time course when transferred from low to high light conditions. Ascorbate and anthocyanin deficient mutants are more susceptible to photodamage in high light (HL). Ascorbate deficient mutants (vtc1 and vtc2) have severely decreased anthocyanin accumulation in HL. However, flavonol glycosides, also produced from the flavonoid biosynthesis pathway, show much smaller HL-induced increases and are unaffected in vtc mutants. HL induces increased transcript levels of flavonoid biosynthesis enzymes and the transcription factors that control anthocyanin synthesis (e.g. PAP1 and 2). Induction of these transcripts is decreased in vtc mutants suggesting that ascorbate status influences the signal perception or transduction processes upstream of PAP1 and 2. HL-induced ascorbate biosynthesis appears to be controlled to a large extent at the first committed step of the biosynthesis pathway catalysed by GDP-L-galactose phosphorylase (VTC2 and VTC5). Both VTC2 and anthocyanin gene expression may be controlled via photoreceptors such as cryptochrome and the HY5 transcription factor or via poorly understood chloroplast/photosynthesis-derived signals. Investigations of these signal transduction pathways are underway to provide information on how ascorbate accumulation is controlled and how ascorbate in turn influences anthocyanin accumulation. The roles of ascorbate both as a photoprotectant in its own right and as a proposed redox buffer that modulates the acclimation of leaf metabolism to light intensity will be discussed.

S5.7

CATHARANTHUS ROSEUS AS A NON-MODEL MODEL SYSTEM FOR MIA BIOSYNTHESIS

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Approximately 20% of plant species contain alkaloids with a broad range of physiological properties that protect them from various types of herbivores & pathogens. Within this abundant group of nitrogen containing secondary metabolites the monoterpenoid indole alkaloids (MIAs) make up a large and most diverse class of compounds that are characteristically found within the Apocynaceae, the Loganiaceae, & the Rubiaceae plant families. Their complexity MIA chemistry is matched by their remarkably diverse effects on living organisms that has led to their use as drugs for preventing malaria (quinine) & for treating neurological disorders (reserpine), cancer (camptothecin, vinblastine and vincristine) & as vasodilators (yohimbine) in human beings. Since the MIAs of *Catharanthus roseus* are among the best characterize with respect to their chemistry, biochemistry, & molecular biology, our laboratory has continued to develop this model non-model system by broadening the scope of our genomic research & pathway discovery to a number of other medicinally important MIA producing plant species that accumulate distinct classes of these compounds. The approach involves directed metabolic profiling of each plant species to

identify the best MIA biosynthesis tissues. Selected tissues are then used to harvest mRNA enriched in MIA biosynthesis & transcripts are processed for large scale sequencing. The data produced from several selected species can then be used to assemble genes with putative functions for incorporation into bioinformatic databases in order to perform comparative genomics & for identification of interesting MIA pathway candidate genes. Progress made will be described.

S5.8

STEREOSELECTIVE LIGNAN BIOSYNTHESIS ENGENDERED BY DIRIGENT PROTEINS IN ARABIDOPSIS THALIANA

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Regiospecific and/or stereoselective coupling reactions are often key steps in natural product biosynthesis. Our discovery of a (+)-pinoresinol forming dirigent protein (DP) in Forsythia intermedia, and the observation of (-)-lariciresinol accumulation in Arabidopsis thaliana root tissue, motivated us to search for the corresponding (-)-pinoresinol forming DP in this plant species. Using a GUS-reporter gene strategy and in silico analysis, we provisionally identified AtDP6 as a possible candidate for this physiological role in the DP multi-gene family in Arabidopsis. AtDP6 (At4g23690) was then established to engender stereoselective lignan biosynthesis both in vitro using heterologous proteins from both insect and tomato cell culture systems, as well as in vivo using reverse genetics. Up- and down-regulated AtDP6 mutants also clearly showed alteration of their enantiomeric compositions and contents of both pinoresinol and lariciresinol. Another homolog, AtDP5 (At1q64160) closest to AtDP6, was also established to be a (-)pinoresinol forming DP based on our in vitro, analyses with recombinant protein. Additionally, in order to begin to understand how such different stereoselectivities are controlled, sequence analyses, protein structure modeling and site-directed mutagenesis approaches were employed to identify putative substrate binding sites and amino acid residue(s) or domains which not only bind incoming substrates, but also differentially orientate them in such a way as to engender distinct (+)- and (-)-pinoresinol forming DP stereoselectivities.

S5.9

CYANOGENIC GLUCOSIDES AND THE P450S INVOLVED IN THEIR FORMATION IN PLANTS AND INSECTS

<u>Birger Lindberg Møller</u> (University of Copenhagen, Faculty of Life Science, Plant Biochemistry Laboratory, Department of Plant Biology and Biotechnology, Frederiksberg, C 1871, Denmark; blm@life.ku.dk)

For more than 420 million years, plants, insects and their predators have co-evolved based on a chemical arms race including deployment of refined chemical defense systems by each player. Cyanogenic glucosides are produced by numerous plants (e.g. sorghum, barley, cassava, clover, flax, almonds) whereas some specialized insects as part of this arms race are able to sequester cyanogenic glucosides from their food plant as well as to carry out de novo biosynthesis. The classes of intermediates involved in de novo synthesis are identical in plants and insects whereas the enzymes involved have been derived by convergent evolution. The genes encoding the biosynthetic enzymes in plants are clustered on the genome and in different higher plant lineages appear to have been repeatedly and independently recruited from members of similar gene families. Following tissue disruption, the cyanogenic glucosides are hydrolyzed and release toxic hydrogen cyanide to protect the plant or insect from generalist herbivorous insects or predators. Cyanogenic glucosides serve numerous additional metabolic functions in addition to defense. They may function as storage reservoirs of nitrogen and sugar and as guenchers of reactive oxygen species. Forage sorghum contains the cyanogenic glucoside dhurrin and following adverse growth conditions, the amounts of HCN released may be toxic to grazing livestock. In collaboration with Australian researchers, biochemical screens and TILLING approaches have been used to identify a single amino acid change in the CYP79A1 enzyme in sorghum that resulted in an inactive enzyme and acyanogenic plants. Acyanogenic mutants have also been obtained in Lotus japonicus. In

synthetic biology approaches, we have shown that P450 catalyzed hydroxylations may be linked to photosystem I and driven by light.

C.K.Blomstedt et al. (2011) Plant Biotech J., in press; doi: 10.1111/j.1467-7652.2011.00646.x

S5.10 THE BIOSYNTHESIS OF CYCLIC PEPTIDES IN THE CARYOPHYLLACEAE

<u>Patrick S. Covello</u>, Janet A. Condie, Goska Nowak, Carla J.S. Barber, Sheila Chiwocha, Darwin W. Reed, John Balsevich (National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, SK S7N 0W9, Canada; patrick.covello@nrc-cnrc.gc.ca)

Plants within the Caryophyllaceae produce a wide range of cyclic peptides (CPs) consisting of 5-9 proteinogenic amino acids. Many of these CPs have interesting bioactivity in mammalian systems. Despite this, there is very little information on the *in planta* function and biosynthesis of CPs in the Caryophyllaceae. A *Saponaria vaccaria* expressed sequence tag collection was investigated for information about CP biosynthesis. This revealed genes that appear to encode CP precursors which are cyclized to mature CPs. Expression of CP precursor genes in transgenic roots resulted in the production of cyclic peptides. Recent efforts to understand the enzymes involved in CP biosynthesis will be discussed.

05.1

DITERPENE BIOSYNTHESIS IN *RICINUS COMMUNIS* – MODIFICATION OF CASBENE BY A CYTOCHROME P450 MONOOXYGENASE

<u>James Kirby</u>, Minobu Nishimoto, Genevieve Park, Jeffrey L. Fortman, Jay D. Keasling (University of California, Berkeley, California Institute of Quantitative Biosciences (QB3), Berkeley, CA 94720, USA; jimkirby@berkeley.edu)

Many Euphorbiaceae plants are known to synthesize diterpenes of medicinal interest, such as the latent HIV-1 activator prostratin (12-deoxyphorbol 13-acetate), the analgesic resiniferatoxin, and the anticancer drug candidate ingenol 3-angelate. We have previously reported that casbene (1) is the key diterpene olefin produced by several members of this family, including castor bean (*R. communis*). Many of the functionalized diterpene products found in these plants could in theory be synthesized from casbene but their biosynthetic routes have not been elucidated. In order to investigate steps downstream from casbene synthesis, we undertook an investigation into cytochrome P450 monooxygenase candidates from the Euphorbiaceae. Utilizing a metabolically engineered strain of yeast for heterologous expression, we have found a P450 from *R. communis* that efficiently oxidizes casbene. This work has provided insights into heterologous production of oxidized diterpenes and improved our understanding of diterpene biosynthesis in the Euphorbiaceae.

05.2

A FAMILY OF SQUALENE SYNTHASES IN POTATO

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Squalene synthase (EC 2.5.1.2.1; SQS) catalyzes the condensation of farnesyl diphosphate to form squalene and is located at a critical juncture in isoprenoid metabolism. In plants, SQS activity contributes to the formation of phytosterols, brassinosteroids, cholesterol, and in potato plants, steroidal glycoalkaloids (SGAs). Unlike most eukaryotes, higher plants have more than one gene coding for SQS. *S. chacoense* accumulates transcript for at least three genes encoding SQS homologs. The pattern of transcript accumulation in the plant differs for each gene. The predicted polypeptides have 74 to 83% identity and have differences in the active sites of the enzyme. Current research focuses on characterizing SQS activity by heterologous expression and generating hairy root cultures transformed

with specific antisense gene constructs to test the function of the three genes in sterol metabolism. Each of the three genes contained an intron in the 3'-UTR. We are generating gene constructs with premature stop codons with and without the intron to test the role of the intron in nonsense-mediated decay of mRNA.

05.3

CRYSTAL STRUCTURE ANALYSIS OF THE TYPE III POLYKETIDE SYNTHASE THAT PRODUCES CURCUMINOID

<u>Hiroyuki Morita</u>, Ikuro Abe (Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, 113-0033 Japan, and JST, CREST, Tokyo, 102-0075, Japan; hmorita@mol.f.u-tokyo.ac.jp)

Curcuminoid synthase (CUS) from *Oryza sativa* is a plant-specific type III polyketide synthase (PKS) that catalyzes the remarkable one-pot formation of the C_6 - C_7 - C_6 diarylheptanoid scaffold of bisdemethoxycurcumin, by the condensation of two molecules of 4-coumaroyl-CoA and one molecule of malonyl-CoA. The crystal structure of *O. sativa* CUS was solved at 2.5 Å resolution, which revealed a unique, downward expanding active-site architecture, previously unidentified in the known type III PKSs. The large active-site cavity is long enough to accommodate the two C_6 - C_3 coumaroyl units and one molecule, which forms hydrogen bond networks with Ser351-Asn142-H₂O-Tyr207-Glu202, neighboring the catalytic Cys174 at the active-site center. These observations suggest that CUS employs unique catalytic machinery for the one-pot formation of the C_6 - C_7 - C_6 scaffold. Thus, CUS utilizes the nucleophilic water to terminate the initial polyketide chain elongation at the diketide stage. Thioester bond cleavage of the enzyme-bound intermediate generates 4-coumaroyldiketide acid, which is then kept within the downward expanding pocket for subsequent decarboxylative condensation with the second 4-coumaroyl-CoA starter, to produce bisdemethoxycurcumin.

05.4 EXTRACELLULAR GLYCOSIDASES OF PYTHIUM IRREGULARE

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The ginseng (*Panax quinqufolius* L.) pathogen *Pythium irregulare* (Buis) is able to selectively metabolize the 20(*S*)-protopanaxadiol ginsenosides Rb1, Rb2, Rc, Rd, and gypenoside XVII *in vitro* via extracellular glycosidases, leading to the formation and partial assimilation of ginsenoside F2. To determine whether there is a correlation between the activity of ginsenoside metabolizing β -glucosidases and the pathogenicity of *P. irregulare* towards ginseng, the production of ginsenoside-specific glycosidases and pathogenicity of various isolates of *P. irregulare* were determined. For this, 10 isolates of *P. irregulare* were selected on the basis of their genetic variability and the host plant they were isolated from (including ginseng), and obtained from the Canadian Collection of Fungal Cultures. These isolates were cultured *in vitro*, in the presence of ginsenosides and the level of ginsenoside-specific glycosidase activity in their extracellular proteins was measured. Meanwhile ginseng seedlings were inoculated with the same suite of *P. irregulare* isolates and scored for disease symptoms to estimate the relative pathogenicity of each isolate towards ginseng plants. When combined this data shows a positive correlation between glycosidase activity in *P. irregulare* and the pathogenicity of this organism towards ginseng.

05.5

DECIPHERING MOLECULAR MECHANISMS OF LIGNIN PRECURSOR TRANSPORT

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Lignin is the second most abundant terrestrial biopolymer after cellulose. It reinforces and waterproofs cell walls of vascular plants, therefore, is essential for the viability of plants. However, the presence of

lignin in cell wall impedes the efficient utilization of cellulosic fibers in agricultural and industrial processes. Lignin precursors, the monolignols, are synthesized within the cytoplasm of the cell. Thereafter, these monomeric precursors are translocated into the cell wall, where they are polymerized and integrated into the wall matrix. While the biosynthesis of monolignols is relatively well understood, our knowledge on transport of these monomers is sketchy. To explore the molecular mechanisms underlying monolignol transport, we used isolated plasma and vacuolar membrane vesicles prepared from *Arabidopsis*, together with applying different transporter inhibitors in the assays, to examine the uptake of monolignols and their derivatives. We found that the transport of lignin precursors across plasmalemma and sequestration of them into vacuoles are ATP-dependent primary-transport processes. Therefore, transport across cell membrane likely involves ATP-binding cassette (ABC) transporters. By gene expression correlation analysis, we then identified a set of ABC transporter genes that their expressions potentially link to lignin biosynthesis. By *in vitro* biochemical analysis using a dedicated transporter expression system and genetic disturbance of gene expression, we identified one specific ABC transporter acting as exporter of monolignols and responsible for the tissue-specific lignin deposition. The detailed analysis will be presented and discussed.

05.6

BIOSYNTHESIS AND STEREOCHEMISTRY OF 9,9'-DEOXYNEOLIGNANS IN SAURURUS CHINENSIS

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9,9'-Deoxyneolignans were isolated mainly from Myristicaceae, Lauraceae, and Piperaceae plants. However, their biosynthesis is unknown. The present report describes stereochemistry and biosynthesis of D7'-4,7-dihydroxy-3,3'-dimethoxy-8-O-4'-neolignans, whose erythro and threo isomers are named as machilins C and D, respectively, in Saururus chinensis (Saururaceae). (1) The neolignans were prepared as racemic standards by dehydrogenation of isoeugenol with horseradish peroxidase and hydrogen peroxide. The diastereomeric ratio (erythro:threo) was 16:84. (2) Machilin D (threo) was isolated from the underground parts of the plant with an enantiomeric ratio [(+):(-)] of 2:98. Machilin C (erythro) was not isolated. (3) To determine the enantiomeric ratio of the preferred diastereomer (machilin D), its terminal double bond was saturated by catalytic reduction with 10% palladium carbon and chiral HPLC of the resulting dihydromachilin D was done. (4) Dehydrogenation of isoeugenol with a soluble enzyme preparation from the plant in the presence of hydrogen peroxide gave machilins C and D in the ratio of 14:86, respectively. This machilin D was racemic. (5) Dehydrogenation of isoeugenol with an insoluble enzyme preparation from the plant in the presence of hydrogen peroxide afforded the neolignans in the ratio of 16:84, respectively. The enantiomeric ratio of the resulting machilin D (as dihydro form) was (+):(-) = 2:3 (20% e.e.). Stereoselective formation of (-)-machilin D by an enzyme activity in S. chinensis was suggested.

05.7

A STRESS-INDUCED RICE ENZYME THAT EQUILIBRATES GLUCOSYL CONJUGATES

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Os9BGlu31, a rice (*Oryza sativa* L.) member of a monocot-specific subcluster of glycoside hydrolase family 1 (GH1) was found to transfer glucose to carboxylic acids and alcohols, rather than hydrolyze 4nitrophenyl β -D-glucopyranoside (4NPGlc). The enzyme could also transfer β -D-fucoside and β -Dxyloside, but not other sugars from 4NP glycosides. Among natural glucose conjugates, 1-*O*- β -D-feruloylglucose, 1-*O*- β -D-4-hydroxybenzoyl-glucose, 1-*O*- β -D-4-coumaroyl-glucose, 1-*O*- β -D-vanillyl-glucose, 1-*O*- β -D-sinapoyl-glucose, phlorizin, apigenin 7-*O*-glucoside and GA4 glucosyl ester could also act as donors. Of natural substrates available, the 4-coumaric acid ($k_{cat}/K_m = 33 \text{ s}^{-1} \text{ mM}^{-1}$) and ferulic acid ($k_{cat}/K_m = 25 \text{ s}^{-1} \text{ mM}^{-1}$) showed the highest efficiency as acceptors, while a wide range of molecules could act as acceptors with lesser efficiency. The OsBGlu31 gene was most highly expressed in the first day of germination and upon ethephon, abscisic acid, jasmonate, auxin and drought treatments of seedlings, suggesting a role in germination and stress response.

05.8 STRUCTURAL BIOLOGY STUDY OF PLANT NATURAL PRODUCT BIOSYNTHESIS

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Plants synthesize a larger number of natural products which play important roles in plant defense against microorganisms and herbivores and also have significant health benefits for animals and humans. The biosynthesis of plant natural products is very complex with many different types of enzymes involved in a variety of different chemical reactions. We are working on three types of enzymes involved in plant natural product biosynthesis, including glycosyltransferases for glycosylation, reductases involved in reduction reactions, and cytochrome P450s involved in hydroxylation and dehydration. We have determined crystal structures of several uridine diphosphate glycosyltransferases, NADPH-dependent reductases, and cytochrome P450s. These structures provide essential insights into their structure-function relationships and catalytic mechanisms in their complex biosynthetic processes. Structure-based mutagenesis and the further functional studies were carried out to explore the roles of key residues for catalysis and substrate specificity, and to further decipher the mechanisms. These studies also provide a basis to manipulate enzyme activity and substrate specificity and the biosynthetic processes for enhancing plant disease resistance or producing more or new health-promoting chemicals.

05.9 MOLECULAR SENSORS IN PLANT THIOL METABOLISM

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Sulfur is essential for plant growth and development, and the molecular systems for maintaining sulfur state and thiol metabolism are tightly controlled. From a biochemical perspective, the regulation of plant thiol metabolism highlights nature's ability to engineer pathways that respond to multiple inputs and cellular demands under a range of conditions. Recent work on the cysteine and glutathione biosynthesis pathways in plants reveals that macromolecular changes in protein structure play a pivotal role in changing enzyme activities in response to cellular signals. For example, formation of the cysteine regulatory complex by the biosynthetic enzymes serine acetyltransferase and *O*-acetylserine sulfhydrylase is critical for modulating activity of the pathway. Likewise, the rate-limiting enzyme in glutathione biosynthesis, glutamate-cysteine ligase, uses a thiol-based structural switch to sense cellular redox state and to modulate enzyme activity. Ultimately, biochemical regulation of plant metabolic pathways through sensing changes in cellular state may be more general than previously believed.

05.10

ELUCIDATING THE NETWORK TO POLYMETHOXYLATED FLAVONES IN SWEET BASIL (OCIMUM BASILICUM L.) GLANDULAR TRICHOMES

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Sweet basil (*Ocimum basilicum* L.) accumulates significant amounts of polymethoxylated flavones in its leaves. The metabolic network involved in production of this array of extensively modified compounds has not been elucidated. Analysis of an EST database from the peltate glandular trichomes of four different

basil lines that accumulate not only different volatile metabolite bouquets, but also different flavone profiles, afforded a number of candidate flavone *O*-methyltransferase (FOMT) genes. Recombinant FOMTs display distinct substrate preferences and product specificities that can account for most detected 7-/6-/4'-methylated, 8-unsubstituted flavones. Apparent K_M values in the low micromolar range and specific gene expression profiles support the involvement of specific FOMTs in the biosynthesis of specific flavones in the different sweet basil lines. Structure homology modeling suggested the involvement of several amino acid residues in defining the proteins' stringent regiospecificities. The roles of these individual residues were confirmed by site-directed mutagenesis. A parallel study of flavone A-ring hydroxylases allowed us to delineate the network from apigenin to salvigenin, gardenin B and nevadensin, the major polymethoxylated flavones that accumulate in sweet basil.

05.11

THE FIRST STEP OF PROANTHOCYANIDIN GALLOYLATION INVOLVES GLUCOSYL-TRANSFERASES

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Grape proanthocyanidins (PA) play a major role in organoleptic properties of wine. They are accumulated mainly in skin and seed during the early stages of berry development. Despite the recent progress in understanding PA biosynthesis, the mechanisms involved in PA galloylation are still not elucidated in plants.

Two Myb transcription factors controlling the PA pathway in grapevine have recently been identified and ectopically over-expressed in an homologous system. In addition to already known PA genes, three genes coding for glucosyltransferases were significantly induced in hairy roots over-expressing those Myb factors (Terrier *et al.*, Plant Physiology, 2009). The three glucosyltransferases display high sequence similarities with other plant glucosyltransferases able to catalyze formation of glucose esters. Studies of the *in vitro* properties of these 3 enzymes were performed through production of recombinant proteins and they are able to catalyze the formation of 1-O-acyl-Glc esters of phenolic acids but are not active on flavonoids and stilbenes. The transcripts are expressed in the early stages of grape berry development, mainly in skins and seeds. The results presented here suggest that these enzymes could be involved in PA galloylation.

05.12

STUDIES ON THE BIOSYNTHESIS OF DEOXYNOJIRIMYCIN IN BACILLUS AMYLOLIQUEFACIENS

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Deoxynojirimycin (DNJ) is an analog of glucose containing a nitrogen atom in place of the endocyclic ring oxygen of the pyranosyl ring. This natural product, and related azasugars have been long known as inhibitors of glycosidase and glycosyltransfer enzymes, and more recently, have become of keen interest for use as molecular chaperones. Though azasugars have been widely popular and have defined the paradigm for molecular features of many glycosidase inhibitors, the biosynthetic pathway had remained unreported for many years. We report here our progress on the identification of genes involved in deoxynojirimycin biosynthesis in *Bacillus amyloliquefaciens*, and studies on their function. Initially guided by genomic annotation, blast analyses and chemical logic, we identified a cluster of three genes, gabT1, yktC1 and gutB1 that were likely candidates for the pathway. Knockout of gabT1 resulted in abolition of DNJ production based on kinetic and mass spectrometric analyses. Chemical complementation with a putative biosynthetic intermediate, downstream of the mutation site, restored DNJ production. Further, transformation of *E. coli* with these three genes resulted in production of a key biosynthetic intermediate, mannojirimycin. Further aspects of work including functional studies of pathway enzymes and the prospects for identification of the remainder of the pathway will be discussed.

S6.1

DIETARY SUPPLEMENTS AND NATURAL HEALTH PRODUCTS: PHYTOCHEMISTRY AND METABOLOMICS AS TOOLS FOR EVIDENCE-BASED DECISIONS ABOUT THEIR USE

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Different (and mostly limited) levels of evidence are available for widely used health products. Using examples from our own metabolomic research and from our critical reviews of the scientific evidence, I discuss new opportunities for an evidence-based assessment of such products.

Euterpe oleracea Mart. (açai) with reportedly high levels of polyphenols (anti-oxidants) has become a poster child of the power of the internet. *In vitro* and *in vivo* evidence on its "effectiveness" is very limited and mostly inconclusive (Heinrich et al. 2011). *Garcinia mangostana* L. (mangosteen) yields "liquid botanical supplements", but evidence for their health benefits of is still lacking. Central to the species biological activity are xanthones. A serious weakness is the lack of clinical data (Obolskiy et al. 2009). *Serenoa repens* (Bartrum) Sm. (saw palmetto) is used in the treatment of Benign Prostatic Hyperplasia. Overall, the clinical evidence is much better, but very often the composition of the extracts used is not known and is variable. A metabolomic analysis (Booker, Suter and Heinrich, unpublished) identified oleic acid and caproic acid ethyl ester as potential marker compounds.

A more rigorous systematic strategy which integrates phytochemical and pharmacological is needed in order to assess the health claims of such products. With the rapid rise of internet-driven marketing, we also need strategies to prioritise products that need to be assessed.

Heinrich, M., T. Dhanji, I. Casselman (2011) Phytochem. Lett. 4:10 - 21. Obolskiy, D., I. Pischel, N. Siriwatanametanon, M. Heinrich (2009) Phytother. Res. 27:1047-1065.

S6.2

METABOLOMIC EVALUATION OF SEVERAL ANTICANCER AGENTS OF PLANT ORIGIN

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Emerging evidence indicates that altered cellular metabolism is the defining characteristic of nearly all cancers regardless of cellular or tissue origin. Therefore, a view of cancer as primarily a metabolic disease suggests that metabolomic technology can be applied in cancer research for the investigation of pharmacological effects as well as mechanism of action. We recently evaluated the cancer-preventive effect of several plant-derived compounds, guercetin, resveratrol, salvianolic acid B (Sal-B, isolated from Salvia miltiorrhiza Bge, Danshen), and breviscapine (a flavonoid isolated from Herba Erigerontis), which were previously reported to have inhibitory effect against the malignant transformation of precancerous lesion. A precancerous colorectal lesion model with azoxymethane (AOM) treated male wistar rats was investigated to evaluate dietary resveratrol and guercetin treatment, alone or in combination. Additionally, a 7,12-dimethylbenz(a)anthracene (DMBA)-induced oral carcinogenesis model in hamster was used to evaluate the Sal-B and breviscapine treatments. The global metabolic variations in sera and tissues of model groups and treatment groups were characterized by gas chromatography time of flight mass spectrometry (GC-TOFMS). The dynamic changes of metabolic profiles indicate that these natural compounds were able to attenuate chemical-induced metabolic perturbation, which is consistent with the findings of significantly decreased lesion/ carcinoma incidences in the treatment groups. Differentially expressed metabolites involved glycolytic intermediates, amino acids, TCA intermediates, fatty acids and nucleosides.

S6.3

COPALCHI AND OTHER SELECTED ANTIDIABETIC PLANTS FROM MEXICO

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There is currently no cure for diabetes, but the condition can be managed so that patients can live a relatively normal life. Oral medications are available to lower blood glucose in type-II diabetics but most of these products possess side effects after prolonged use. In consequence, the search for new therapeutic agents for treating type-II DM, including plants used in folk medicine has increased notably in recent years. Mexico is rich in medicinal plants highly prized by the population for the treatment of diabetes and, according to a recent review there are about 306 species from 235 genera and 93 families used as hypoglycemic agents. Copalchi, *Hintonia latiflora* (Rubiaceae), *Ligusticum porteri* (Apiaceae), and *Brickellia cavanillesii* (Asteraceae) are some of these. A concise overview on these species will be presented including phytochemistry aspects, quality control methods, as well as preclinical safety and efficacy parameters, stemming from our own work.

S6.4

TRADITIONAL OCEANIC CROPS FOR IMPROVED NUTRITION, NUTRACEUTICALS AND NATURAL HEALTH PRODUCTS

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Breadfruit, *Artocarpus altilis* (Parkinson) Fosberg, has been a staple food and traditional crop in the Pacific for more than 3,000 years and is widely cultivated in the Caribbean and other tropical regions. In 1787 Captain W. Bligh sailed the Bounty to Tahiti to collect breadfruit trees for food in the Caribbean. A single breadfruit tree produces 150-200 kg of fruit per year and the fruit can be eaten fresh or processed into fermented products or flours. There are hundreds of cultivars of breadfruit selected by indigenous peoples across Oceania that have been collected and curated at the Breadfruit Institute germplasm bank of the National Tropical Botanical Garden, Hawaii. Individual varieties vary in specific vitamins, nutrients, proteins, salt tolerance, and medicinal phytochemicals. The process of bringing breadfruit to modern markets required the negotiation of international agreements for equitable benefit sharing, development of mass-propagation technologies for large scale production of disease free plants, and studies to identify nutrient-rich varieties, develop new products and create opportunities for not-for-profit distribution.

S6.5

GLYCYRRHIZA URALENSIS: UNUSUAL CHEMISTRY AND UNUSUAL APPLICATIONS

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The phytochemical investigation of a supercritical fluid extract of *Glycyrrhiza uralensis* led to the isolation of 20 known isoflavonoids and coumarins, as well as glycycarpan, a new pterocarpan. The presence of two isoflavan-quinones, licoriquinone A and licoriquinone B, in a fraction subjected to gel filtration on Sephadex LH-20 is believed to be due to metal-catalyzed oxidative degradation of licoricidin (1) and licorisoflavan A (2). The licorice extract as well as 1 and 2 were able to reduce volatile sulfur compounds (VSCs) production by *Porphyromonas gingivalis, Prevotella intermedia*, and *Solobacterium moorei* as well as in a human saliva model. Although the extract and isolates did not inhibit the proteolytic activity of bacteria, they blocked the conversion of cysteine into VSCs by *P. intermedia*. Compounds 1 and 2 also showed potent antibacterial activities, causing a marked growth inhibition of the cariogenic species

Streptococcus mutans and Streptococcus sobrinus at 10 μ g/mL and the periodontopathogenic species *P. gingivalis* (at 5 μ g/mL) and *P. intermedia* (at 5 μ g/mL for **1** and 2.5 μ g/mL for **2**). Only **1** moderately inhibited growth of *Fusobacterium nucleatum* at the highest concentration tested (10 μ g/mL).

S6.6 MULTILAB METHOD VALIDATION OF BLUEBERRY LEAF EXTRACT BY NMR: QUALITATIVE AND QUANTITATIVE

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The ability to quickly assay natural product extracts for new compounds or higher levels of known compounds or confirm the natural source or origin can enhance the selection of natural products for ensuring efficacy, safety, origin, and quality. Similarly, the ability to perform these assays reproducibly on different instruments and at multiple locations frees users from time consuming sample validation on multiple instruments independently. NMR is well documented as an analytical technique which provides structurally definitive and quantitative data simultaneously in a single NMR experiment. NMR's high reproducibility across platforms allows qualitative assessment (chemometric modeling) of highly complex samples such as botanical extracts, which enables data comparison at different sites. Concepts and results of a 12 site multi-site reproducibility study are presented, which aims to identify and quantify compounds directly from the spectra of raw blueberry leaf extract and qualitatively identify the natural source of the raw plant material.

06.1

HUO-LUO-XIAO-LING DAN (HLXL) PROTECTS AGAINST EXPERIMENTAL ARTHRITIS BY MODULATING ANTIGEN-INDUCED CELLULAR AND HUMORAL RESPONSES

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial inflammation and joint damage. Proinflammatory cytokines, antibodies, matrix-degrading enzymes and nitric oxide mediate the pathogenic events in arthritis. Accordingly, we examined whether an herbal formula, HLXL, which has long been used in traditional Chinese medicine for the treatment of arthritic conditions, targets these mediators of inflammation leading to attenuation of arthritis. We tested HLXL in the rat adjuvant-induced arthritis model of human RA. We demonstrated that oral administration of HLXL (2.3g/kg) to Lewis rats after the onset of arthritis significantly reduced the severity of arthritis compared with the water-fed controls. Interestingly, HLXL-fed rats revealed a lower concentration of the proinflammatory cytokine IL-10 in recall response to antigen than controls. HLXL feeding also suppressed the serum levels of antigen-specific antibodies as well as nitric oxide. These results provide a strong rationale for further testing and validation of the use of HLXL in patients with RA.

NEW MODELS IN TRANSLATIONAL PHYTOTHERAPY: DIETARY PHYTOCHEMICALS IN FUNCTIONAL NUTRITION MANAGEMENT AND PHARMACONUTRITION

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The new fields of Functional Nutrition Management (FNM) and Pharmaconutrition (PN) deal with the transdisciplinary integration of the multifarious dietary phytochemicals in functional foods, medical foods, and botanical dietary supplements. Total evidence review from epidemiology, *in vitro* tests, and clinical trials supports health benefits from multicomponent dietary phytochemical mixtures, whole foods, and dietary patterns, but overall appears to be lacking in isolated individual dietary phytochemicals. The need for a new model that addresses this disparity in health benefits leads us to propose a novel way to develop therapies based on a dietary phytochemical model that integrates chemical ecology with traditional medical systems from a functional nutrition and pharmaconutrition perspective with medicinal foods and food components. A case study of the Zingiberaceae plant family in this integrative context will explore ginger (*Zingiber officinale*), tumeric (*Curcuma longa*), galangal (*Alpinia galangal*), and Thai finger root/krachai (*Boesenbergia pandurata*), and their respective phytochemical profiles (e.g., gingerols, shogaols, diarylheptanoids/curcuminoids, chalcones, and other phenolic compounds/flavonoids) with known functional and pharmacological bioactivity.

06.3

TOXICOLOGICAL MECHANISMS OF BOTANICAL DIETARY SUPPLEMENTS

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Reports indicate that a variety of botanical dietary supplement (BDS) products are associated with hepatotoxicity or other forms of idiosyncratic toxicity. Extracts from more than 350 species of plants and other organisms used in traditional Chinese, Indian, African, and Western herbal medicine were evaluated for their ability to disrupt mitochondrial function in cultured cells. Extracts from 15 different plant species suppressed mitochondrial respiration. Extracts from other species potently uncoupled oxidative phosphorylation. Several plant species that have been associated with severe hepatic or cardiac toxicity contain compounds that have been reported to interfere with mitochondrial function. While the pharmaceutical industry has recently come to recognize the importance of implementing measures to assess the potential mitochondrial toxicity of new drug leads, no such methods have been widely used to evaluate the botanical constituents found in herbal medicines. These results indicate that certain plant extracts, including some that were previously thought to be safe, contain components that induce mitochondrial dysfunction and may be responsible for potential BDS-induced liver or heart toxicity.

PTEROSTILBENE INCREASES PPARα GENE EXPRESSION, ACTIVATES AMPK, AND SUPPRESSES EXPRESSION OF GENES INVOLVED IN HEPATIC LIPID METABOLISM AND GLUCONEOGENESIS

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Pterostilbene (a dimethylether analog of resveratrol) at 10, 20 and 50 μ M dose-dependently increased PPAR α gene expression in H4IIE rat hepatoma cells, consistent with earlier studies that showed it is a PPAR α agonist, and provided greater increases in PPAR α gene expression than 100 and 200 μ M fenofibrate. Expectedly, pterostilbene increased gene expression of fatty acyl-CoA oxidase and carnitine palmitoyltransferase-1, enzymes involved in fatty acid catabolism. Phosphorylation of 5'-adenosine monophosphate-activated protein kinase (AMPK), known to regulate fatty acid metabolism, was increased by pterostilbene dose-dependently (at 10, 20 and 50 μ M); 50 μ M pterostilbene activated AMPK to a greater extent than AICAR (0.5 mM) or metformin (2 mM). AMPK is also known to repress transcription of phosphoenol pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), enzymes involved in hepatic gluconeogenesis. In this study, dexamethasone was used to induce gluconeogenesis, and insulin as positive control to suppress gluconeogenesis. Similar to insulin, pterostilbene dose-dependently decreased gene expression of PEPCK and G6Pase. Additionally, pterostilbene decreased glucose production in H4IIE cells. This is the first study to show pterostilbene activates AMPK, regulates FA metabolism, and suppresses gluconeogenesis, providing mechanistic support for its use in management of dislipidemia and type 2 diabetes.

07.2

THE ISOFLAVONOID, EQUOL IMPROVES SEVERE AND MODERATE BPH SYMPTOMS IN MID-AGED CAUCASIAN MEN: CLINICAL EVIDENCE

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Equol, the major metabolite of the isoflavone daidzein, specifically binds and blocks the hormonal action of 5α -dihydrotestosterone (DHT) *in vitro* and *in vivo* (Reprod Biol Endo, 2011, 13; 9:4). Equol can at the same time bind estrogen receptor beta that has important implications for prostate disorders. The objective of this clinical study was to provide proof of principle that equol at a low effective dose improves health symptoms of benign prostatic hyperplasia (BPH). A proprietary non-racemic equol mixture (6 mg) was taken twice per day for 4 weeks by 18 healthy males between 49 and 60 years old that have BPH according to the International Prostate Symptoms Score (IPSS) form before treatment started (baseline). Institutional Board Review approval, informed consent, medical histories and qualification produces were followed. At baseline (0 weeks), at 2 weeks and then at 4 weeks all subjects completed the IPSS forms indexing 7 BPH parameters; total scores and self-perceived quality of life changes. Data was analyzed by repeated ANOVA. In brief, the overall IPSS scores in both groups significantly improved from baseline levels at 0 weeks compared to 2 or 4 weeks with equol treatment. In the severely symptomatic group (n = 8): each IPSS indicator and the quality of life significantly improved by 4 weeks with equol treatment. These results demonstrate great promise for equol at a low effective dose to improve BPH symptoms in men. Funding: HoHo

NONPSYCHOACTIVE CONSTITUENTS FROM *CANNABIS SATIVA* (MARIJUANA): THERAPEUTIC POTENTIAL IN INFLAMMATORY DISORDERS AND DIABETES

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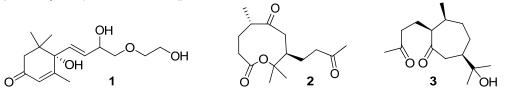
Medical marijuana plant (*Cannabis sativa*) has been used for centuries for various therapeutic indications in human medicine. Previously it was thought that its only active constituent is the δ -9tetrahydrocannabinol (THC). THC is a nonselective agonist of both cannabinoid receptors 1 and 2 (CB1, CB2). While CB2 activation in immune cells results in potent anti-inflammatory effects, CB1 stimulation in the central nervous system (CNS) decreases pain, but leads to undesirable psychoactive consequences, thus limiting the therapeutic potential of THC and marijuana. However, numerous recent studies, including those from our laboratories have demonstrated that several previously considered inactive constituents of *C. sativa*, such as cannabidiol or δ -9-tetrahydrocannabivarin (THCV), which do not exert psychoactive effects via CB1 activation, may exert very potent anti-inflammatory and antioxidant properties, and attenuate the disease progression in preclinical models of diabetes/diabetic complications, ischemic-reperfusion injury, inflammation, and cancer, just to mention a few. Examples of the powerful anti-inflammatory and tissue protective effects of these natural plant derived constituents from preclinical disease models will be presented, and their tremendous therapeutic potential will be discussed.

07.4

SESQUITERPENES FROM HOLOSTYLIS RENIFORMIS

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The hexane extract of *Holostylis reniformis* roots yield oily fractions which were analyzed by GC/MS and ¹H and ¹³C NMR spectroscopy. Palmitic acid, guaiol, 1-*epi*-cubenol, 10-*epi*-eudesmol, γ -eudesmol, bulnesol, elemol, and β -eudesmol were the main constituents in the oil. In addition, the new megastigmane (1) and nine-membered lactone (2), together with the 4,5-*seco*-guaiane (3), with an unusual carbon skeleton, were isolated from root extracts and their structures were determined by spectroscopic analyses.(FAPESP, CNPq).

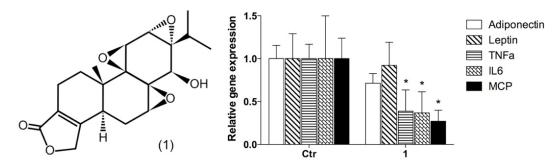


07.5 TRIPTOLIDE AMELIORATES INSULIN RESISTANCE IN OBESE DIABETIC MICE

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Diabetes arises because of failed insulin action, however the role of inflammation in insulin resistance remains unclear. To determine if targeting systemic inflammation ameliorates diabetes, we orally administered 50 μ g/kg triptolide every 3 d for 9 wk to high fat diet-induced obese C57BL/6J mice. Triptolide (1) is a diterpene triepoxide isolated from a traditional Chinese medicinal plant (*Tripterygium wilfordii*) with anti-inflammatory and immunosuppressive properties. Mice treated with triptolide exhibited

higher glucose disposal by oral glucose tolerance test with no significant changes in body weight, lean body mass, or fat mass. We next determined changes in macrophage recruitment and cytokine signature of adipose tissue in these animals. Visceral fat from triptolide-treated mice showed decreased expression of inflammatory markers $TNF\alpha$, IL-6, and monocyte chemoattractant protein (MCP). These results show the importance of adipose tissue inflammation and gene regulation in systemic glucose metabolism and insulin sensitivity.



07.6 ANTIOXIDANT ACTIVITY OF HAWAIIAN MACRO-ALGAE

Ellen Kromkowski Posner,¹ Karla J. McDermid,¹ Anthony D. Wright,² <u>Dovi Kelman</u>² (¹University of Hawaii at Hilo, Department of Marine Science, Hilo, HI 96720, USA, ²University of Hawaii at Hilo, College of Pharmacy, Department of Pharmaceutical Sciences, Hilo, HI 96720, USA; dkelman@hawaii.edu)

Marine macro-algae are known to contain a wide variety of bioactive compounds, many of which have commercial applications in pharmaceutical, medical, cosmetic, nutraceutical, food and agricultural industries. Natural antioxidants, found in many macro-algae, are important bioactive compounds that play an important role against various diseases and ageing processes through protection of cells from oxidative damage. In this respect, relatively little is known about the bioactivity of Hawaiian macro-algae that could be a potential natural source of such antioxidants. The antioxidant activity of organic extracts of 27 species of Hawaiian macro-algae from 25 different genera was determined. The activity was determined by employing the FRAP (Ferric Reducing Antioxidant Power) assay. Of all of the algae tested, the extract of *Turbinaria ornata* was found to be the most active. Bioassay-guided fractionation of this show, for the first time, that numerous Hawaiian macro-algae exhibit antioxidant activity, a property that could lead to an application in one of many useful healthcare or related products.

07.7 MEDICINAL PLANTS OF HIGH POTENCY AND THEIR APPLICATIONS IN TRADITIONAL MEDICINE

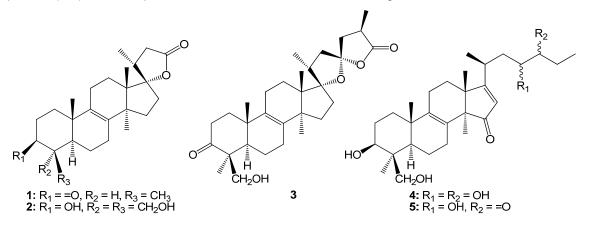
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Ethnopharmacological information has a role to play as one of the approaches to drug discovery and a number of medicinal plants are well recognized to provide active principles for modern medicine. However, many failed along the empirical pipeline of development because of their toxicity. In this work, we review 12 medicinal plants recognized in Thai Traditional Medicine as potent medicinal ingredients, 10 of which are mentioned possibly as fatal. Five medicines are Euphorbiaceous products, the others are derived from Anacardiaceae, Cannabaceae, Convolvulaceae, Lauraceae, Papaveraceae, Solanaceae, and Strychnaceae. Their indications are not only strictlydescribed, but applications also require some special traditional technique prior to incorporating to the formulated preparations which will be discussed herein. Other aspects of concern with respect to attitudes towards modern and traditional medicines will be described in this presentation.

07.8 NOVEL TRITERPENOID DERIVATIVES FROM *EUCOMIS BICOLOR* (HYACINTHACEAE: HYACINTHOIDEAE)

<u>Jaspreet Kaur Sihra</u>,¹ Dulcie A. Mulholland,¹ Moses K. Langat,¹ Neil R. Crouch,² Jean-Marc Nuzillard,³ Alfred E. Thumser⁴ (¹University of Surrey, Chemical Sciences, Faculty of Health and Medical Sciences, Guildford, GU2 7HX, United Kingdom, ²South African National Biodiversity Institute, Ethnobotany Unit, Durban, 4007, South Africa, ³University of Reims, Pharmacognosy Laboratory, Reims, 51687, France, ⁴University of Surrey, Biological Sciences, Faculty of Health and Medical Sciences, Guildford, GU2 7XH, United Kingdom; j.sihra@surrey.ac.uk)

The phytochemical investigation of the dichloromethane extract of *Eucomis bicolor* has yielded five novel compounds (1-5). The triterpenoid derivatives have been screened against several tumour cells.



07.9 DISCOVERY OF KANGAROO ISLAND MEDICINAL PLANTS USING HONEYBEES

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Kangaroo Island with its unique endemic flora is a potential source of medicinal plants. With very limited introduced flora, the island is well suited to employ honeybees (*Apis mellifera*) to source out the medicinal plants. Honeybees collect resins or exudates from plants to produce bee glue (propolis) to seal and disinfect their hives. Propolis has been well recognized for its medicinal property since ancient civilization, and still retains current worldwide popularity as a traditional medicinal product. As a sanctuary for Ligurian honeybees (*Apis mellifera ligustica*), Kangaroo Island has strict quarantine regulations with well established beekeeping practice including propolis production.

Methods were established to use honeybees to identify the plant sources of novel bioactive natural products. Single plant source propolis, resin/exudate from the plants and propolis carried on bee legs were matched, mainly through comparison of ¹H-NMR spectra analytical profiles. Our survey of distribution of source plants has identified chemotypes and their abundance on the island.

Our studies showed that the composition of propolis from Kangaroo Island is novel, containing many new chemical entities as the main constituents. In particular, novel prenylated cinnamates and prenylated tetrahydroxystilbenes were identified and biologically evaluated.

ANTIPLASMODIAL EFFICACY OF ETHANOLIC LEAVES EXTRACT OF XANTHIUM STRUMARIUM

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Traditional medicines have always focused on the power of natural products to treat and cure diseases. Xanthium has been selected on basis of their ethnopharmacological use. The leaves of Xanthium strumarium were collected from (voucher no.17920) Mandi district of Himachal Pradesh, India. Ethanol leaf extract of X. strumarium (ELEXS) was prepared by soxhlet extraction and contained alkaloids, saponins, phenolic compounds and steroids. ELEXS has been found to inhibit Plasmodium berghei schizont maturation in dose dependent manner with IC₅₀ of 5µg/ml. Acute toxicity of ELEXS was determined by limit test of Lorke and extract exihibited LD₅₀ of 1.5g/kg concentration in mice. Extract(500mg/kg) exhibited dose dependent chemosuppressive effect with maximum chemosuppression of 88.6% (p<.001) and 85% in chloroquine (5mg/Kg) in comparison to infected controls. Repository activity of ELEXS was checked using pyrimethamine (1.2mg/kg) as positive control and untreated P. berghei infected mice as negative controls. Extract(350mg/kg) exhibited 90.4% and positive control 90% chemo suppression. 150 mg/kg ELEXS exhibited 91.1% curative activity with significant p value(<.001).Mean survival period was 29.8 days(150mg/kg ELEXS)and 30days for Chloroquine treated group, whereas, all the mice of infected group died within one week of infection. ELEXS can be classified as an active antiplasmodial extract. It should be investigated for isolation of its active components and their role as chemotherapeutic agents against malaria.

07.11

ASSESSMENT OF THE WOUND HEALING ACTIVITY OF METHANOLIC AND *n*-HEXANE EXTRACTS OF *GARCINIA KOLA* SEED IN ALBINO RATS

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High rates of drug resistance in bacteria from wound isolates makes availability and provision of multiple choice/alternative drug sources at reduced costs imperative for effective management of wounds of any type. Medicinal plants are believed to be an important source of the new chemical substances with potential therapeutics. The assessment of wound healing activity of methanolic and *n*-hexane extracts of *Garcinia kola* seeds was studied using full skin thickness excision model on dorsum of albino rats of both sexes. 5% and 10% of both extracts were prepared using white soft paraffin (base) as a vehicle. The ointment was topically and aseptically applied on the wound. Group A animals were treated with Cicatrin[®] powder; (positive control), while groups B, C, D, and E received 10%, 5% methanol and 10%, 5% *n*-hexane extracts of *Garcinia kola* seed respectively. Group F rats were treated with White soft paraffin (negative control). The wound healing effect of the extracts was assessed using the wound appearance, mean rate/ percentage of wound contraction as well as histopathology. The parameters obtained from the ointment treatment groups were compared with those of Cicatrin[®] powder (standard) and Base treated (negative controls) group animals respectively.

The results showed that the healing rate was faster in group C than in other animal groups. However, the healing rate in group C treatment was comparable to group A animals on post-surgery days 18 and 21. The group D animals showed the least rate of healing throughout the study period. The surfaces of the wound in all groups were found to be wet and inflamed on day 3 post surgery. On day 9 post surgery, the wound surfaces of the animals in groups A, B, C and E were all dry while those of groups D and F were oily and fairly wet. The oily wound surface in group D animals lasted up to day 15 post surgery. However, no suppuration was noticed in all the groups during the period of experiment. Histopathology shows complete epitheliazation which was recorded in groups A, C and E on day 21 post surgery. The sequence of wound healing has shown that both extracts have wound healing activity. The 5% concentration of methanolic extract has better wound healing activity compared with concentrations of 10% methanolic and 5% and 10% *n*-hexane extract treated groups. Therefore, the 5% methanolic extract concentration may favorably be recommended as a topical ointment for incisional wound.

BIOPRODUCTS FROM THE CANADIAN FOREST

<u>Mamdouh M. Abou-Zaid</u>, Domenic A. Lombardo, Linda Buchan, Melanie Coppens, Scott Hopkin (Natural Resources Canada, Canadian Forest Services, Great Lakes Forestry Centre, Sault Ste. Marie, ON P6A 2E5, Canada; mabouzai@nrcan.gc.ca)

Canada's boreal forest is a "natural synthetic manufacturing factory" continuously working on the production of enormous numbers of complex organic substances playing a variety of roles and possessing diverse bioactivities. Each species has a unique phytochemical profile with characteristic classes of substances, a phenomenon known as phytochemical diversity and redundancy. Their biosyntheses, the metabolic sequences leading to the production of the various classes of natural products, are thoroughly interconnected.

Canadian forest flora provides for a diverse and rich source of bioactive natural product compounds. Among the compounds of interest are those of the phenolic, polyphenol, and flavonoids classes of compounds. Extracts of indigenous plant species have been screened for antioxidant (maple syrup), and antifungal and anticancer activities. Those plants whose whole foliage extracts exhibited preliminary activity were subjected to a bioassay-guided fractionation and isolation of the active constituents from the plant extracts.

This presentation describes our laboratory's on-going research interests involving the screening, fractionation, isolation, purification and structural elucidation of the antioxidant/antifungal-active compounds from *Acer*, *Pinus*, *Taxus*, *Chimaphila*, and *Abies* species, and considers their role in health promotion and disease prevention.

07.13

CULTURED CAMBIAL MERISTEMATIC CELLS AS A SOURCE OF PLANT NATURAL PRODUCTS

Eun-Kyong Lee,² Young-Woo Jin,² Joong Hyun Park,² Young Mi Yoo,² Sun Mi Hong,² Rabia Amir,¹ Zejun Yan,¹ Eunjung Kwon,¹ Alistair Elfick,³ Simon Thomlinson,⁴ Florian Halbritter,⁴ Thomas Waibel,¹ Byung-Wook Yun,¹ <u>Gary Loake</u>¹ (¹University of Edinburgh, Institute of Molecular Plant Sciences, Edinburgh, EH9 3JR, United Kingdom, ²Unhwa Corp, 874-1, 2Ga, Wooah-Dong, Dukjin-gu, Jeonju, South Korea, ³University of Edinburgh, School of Engineering, Edinburgh, EH9 3JL, United Kingdom, ⁴University of Edinburgh, Institute of Stem Cell Research, Edinburgh, EH9 3JH, United Kingdom; gloake@ed.ac.uk)

A plethora of important, chemically diverse, natural products are derived from plants. In principle, plant cell culture offers an attractive production platform for some natural products but often is not a commercially viable strategy because of difficulties associated with culturing dedifferentiated plant cells (DDCs) on an industrial scale. To address this problem, we have isolated and cultured innately undifferentiated cambial meristematic cells (CMCs). Utilizing a combination of deep sequencing technologies, we identified marker genes and transcriptional programs consistent with a stem cell identity. This notion was further supported by CMC morphology, hypersensitivity to γ -irradiation and radiomimetic drugs and the ability of these cells to differentiate at high frequency. CMCs derived from *Taxus cuspidata*, source of the key anticancer drug, paclitaxel, circumvented obstacles routinely associated with the commercial growth of DDCs. These cells may therefore provide a cost-effective and environmentally friendly platform for sustainable production of a variety of important plant natural products.

07.14

BALANCING RESEARCH AND SUSTAINABILITY

<u>Mark Perry</u> (University of Western Ontario, Faculties of Law and Science, London, Ontario N6A 5K7, Canada; mperry@uwo.ca)

There is tension between the need to find new compounds and precursors to give relief from the conditions and diseases that plague humanity, with the need to ensure that sustainability of botanical sources is not compromised. An additional aspect is the desire to reward those that provide clues and knowledge on the efficacy of botanicals (traditional knowledge) that can lead to the mass production of

useful compounds. This tension can be seen in the international arena with the contrasting approaches of the Agreement on Trade Related Aspects of Intellectual Property, which the vast majority (153) of nations have accepted, and the Convention on Biological Diversity, with some 193 national parties (with the notable exception of the United States of America). In 2000, the World Intellectual Property Organisation established an intergovernmental committee to provide resolution for the need for international instruments to protect the exploitation of traditional knowledge and genetic resources, and although much progress has been made, a common agreement for such instruments has yet to be met.

This paper looks at the background and outcomes of these tensions over the last decade and proposes steps that could lead to the resolution of the two basic needs, namely advancement of science and the protection of rights.

UNRAVELLING MYSTERIES IN THE REGULATION OF PLANT SECONDARY METABOLITES AND NON-GLANDULAR TRICHOMES: NEW MECHANISMS AND CROP APPLICATIONS

<u>Margaret Yvonne Gruber</u>,¹ Xiang Li,³ Peng Gao,² Dejun Cui,¹ Min Yu,¹ Ali Taheri,¹ Nagabushana Nayidu,¹ Ushan Alahakoon,^{1,4} Jennifer Holowachuk,¹ Sharon Regan,⁶ Abdelali Hannoufa,⁷ Zakir Hossein,⁷ Isobel Parkin,¹ S. Wei,⁵ P. Bonham-Smith,⁴ B. Yu² (¹Saskatoon Research Centre, Agriculture and Agri-Food Canada, Saskatoon, SK S7N0X2, Canada, ²Plant Biotechnology Institute, National Research Council, Saskatoon, SK S7N0W9, Canada, ³Jinlin University, College of Plant Sciences, Changchun, Jinlin 130062, China, ⁴University of Saskatchewan, Dept. of Biology, Saskatoon, SK S7N0W9, Canada, ⁵Anhui Agricultural University, School of Tea and Food Science, Hefei, Anhui 20036, China, ⁶Queen's University, Dept. of Biology, Kingston, ON K7L3N6, Canada, ⁷Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food, London, ON N5V4T3, Canada; Margie.Gruber@agr.gc.ca)

Anthocyanins, proanthocyanidin, lignin, and carotenoids are important secondary metabolites which protect plants against stress and provide nutritional and health benefits to humans and livestock. Plants with modified lignin show improved cellulose (energy) availability, and trichomes (plant hairs) discourage insects and animals from foraging on plants. Some of the mystery surrounding gene regulation for these plant defence strategies is being unravelled by the new SK population of *Arabidopsis* enhancer lines and yellow/brown-seeded and wild *Brassica systems*. The presentation will highlight new tools and new regulatory mechanisms taken from the analysis of a collection of new *Arabidopsis* mutant lines and *Brassica* lines with modified flavonoid/phenolic, carotenoid, and trichome pathways and will highlight several useful applications for crop plants.

08.2

THE ARABIDOPSIS ABCG26 TRANSPORTER: A TOOL FOR INVESTIGATING THE NATURE OF SPOROPOLLENIN

<u>Teagen D. Quilichini</u>, A. Lacey Samuels, Carl J. Douglas (University of British Columbia, Botany, Vancouver, BC V6T 1Z4, Canada; tdquilic@interchange.ubc.ca)

Sporopollenin, a tough biopolymer in spore and pollen walls, protects these structures from environmental stresses. From the data available, sporopollenin is a polymer of fatty acids and oxygenated aromatic compounds. Analyses of Arabidopsis male sterile mutants defective in pollen wall formation have revealed genes required for sporopollenin biosynthesis and/or deposition, including MS2, ACOS5, PKS-A/PKS-B. TKPR1 and ABCG26. Based on genetic and biochemical analysis of these genes and the corresponding enzymes, it has been proposed that an aliphatic polyketide sporopollenin monomer is synthesized in the tapetum. ABCG26, an ABC transport protein, is thought to function in sporopollenin export from tapetum cells. However, the substrate transported by ABCG26 is unknown. In the abcg26 mutant, sporopollenin precursors are predicted to accumulate in the tapetum. Through the analysis of abcg26 by two-photon microscopy, lipidic and autofluorescent compounds in tapetum cells can be visualized. No differences between wild type and *abcg26* tapetum lipids were observed. Conversely, abcg26 exhibits autofluorescence in tapetum vacuoles, not observed in wild type. Transmission electron microscopy supports these findings, with enlarged, debris-filled vacuoles in the tapetum of abcg26 mutants. Identification of the autofluorescent components accumulating in abcg26 tapetum cells by biochemical methods will provide an opportunity to examine the composition of sporopollenin in planta. Using live-cell imaging and biochemical methods, we are also investigating the nature of sporopollenin precursors that accumulate in double mutants of *abcg26* and *acos5*, *pks-a/pks-b*, and *tkpr1*.

QUANTITATIVE ANALYSIS OF NATURAL PRODUCTS BY qNMR

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qNMR is a powerful tool for quantitative determination of natural products since (1) it requires no standard compounds to prepare calibration lines, and (2) SI-traceable determination of the organic molecules can be attained by using a certified reference material as an internal standard. We applied qNMR to determine the purities of protoberberine alkaloid reagents obtained in the market. The purity of the reagent estimated by ¹H-NMR was, in general, lower than that claimed by the manufacturer, leading to over-estimation of the alkaloid contents of Coptis Rhizome when determined by HPLC. The present quantitative NMR method was also applicable to direct determination of protoberberine alkaloid contents in *Coptidis rhizoma*.

We also successfully applied the method to evaluate carthamin contents in commercial Carthamus red colourants without using carthamin as a standard compound.

08.4

PLANT PHENOLICS ANALYSIS USING ELECTROSPRAY ION MOBILITY TIME-OF-FLIGHT MASS SPECTROMETRY

Fereshteh Zandkarimi¹, Sasidhar Nirudodhi¹, Jan F Stevens², <u>Claudia S. Maier</u>¹ (¹Oregon State University, Department of Chemistry, Corvallis, OR 97331, USA, ²Oregon State University, Department of Pharmaceutical Sciences and Linus Pauling Institute, Corvallis, OR 97331, USA; claudia.maier@oregonstate.edu)

We report on the characterization of plant phenolics using an electrospray hybrid quadrupole time-of-flight mass spectrometer equipped with a traveling wave ion mobility separation device. There is increasing evidence that polyphenols have health benefits. Considering the increasing interest in plant phenolics as nutraceuticals, comprehensive profiling methods for plant extracts are highly needed. We highlight the application of advanced mass spectrometric fragmentation techniques in combination with ion mobility separation and also its current limitations for the characterization of plant extracts and plant phenolic libraries. We discuss MS and ion mobility data for the characterization of phenolics isolated from hops with focus on the prenylated flavonoids, xanthohumol (XN) and its isobaric and isomeric flavanone, isoxanthohumol (IX), and the two isobaric and structurally related flavanones, 6-prenylnaringenin (6PN) and 8-prenylnaringenin (8PN). We observed a slight difference for the arrival time distributions (ATD) for XN and IX (0.1msec), but identical ATDs were observed for the two prenylnaringenins. We also show the application of ion-mobility separations and time-aligned parallel fragmentation for the characterization of proanthocyanidins from hops and grapes. These analyses provide snapshots on the complexity of proanthocyanidin polymer mixtures but are highly informative. Once established these images may guide extraction processes of plant materials and serve as quality control fingerprints.

08.5

MASS SPECTROSCOPIC FINGERPRINTING AND CHEMOMETRIC ANALYSIS FOR QUALITY AS-SESSMENT OF BOTANICALS AND FOODSTUFFS

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The chromatographic fingerprint technology was accepted by the WHO as a strategy for identification and quality evaluation of herbal medicines in 1991. However, the reproducibility of chromatograms is not consistent and it often takes 60 minutes or longer to generate a single chromatographic fingerprint. Flow-

injection mass spectrometry (FIMS), on the other hand, can generate a mass spectrometric fingerprint in 1 minute or less. FIMS can be used to detect differences in chemical composition arising from genetic, environmental, and other factors.

Principal component analysis (PCA) and other chemometric tools can also be used to process MS fingerprints. The results are score plots that are easily interpreted visually and statistically, and can pinpoint exactly what components are responsible for the chemical difference found between different sample sets. Differentiation between *Panax quinquefolius*, *P. ginseng*, and *P. notoginseng* species, between *Scutellaria lateriflora* and the germander species, and between organic and conventionally grown grapefruits have been successfully demonstrated. The results prove that MS fingerprinting is an easy, fast, and powerful tool for quality assessment of botanicals and foodstuffs.

08.6

STRUCTURAL CHARACTERIZATION OF PHENOLIC LIPIDS OBTAINED BY TRANSESTERIFICATION OF 3,4-DIHYDROXYPHENYLACETIC ACID AND KRILL OIL

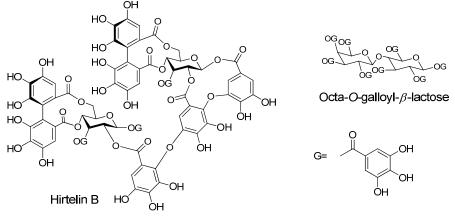
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The biosynthesis of novel biomolecules, phenolic lipids, rich in ω -3 polyunsaturated fatty acids (PUFAs) and phospholipids, by the enzymatic transesterification in solvent-free medium of krill oil with 3,4-dihydroxyphenylacetic acid (DHPA), using Novozym 435 was investigated. The krill oil components and its esterified phenolic lipids were separated by HPLC, using a Zorbax column (3.5 m, packed with stable bond C₁₈-reversed-phase) as well as a solvent gradient of acetonitrile and isopropanol. The detection was performed simultaneously by ultraviolet/diode array detector (UV/DAD) at 215 and 280 nm as well as with an evaporative light scattering detector (ELSD). The experimental results indicated that ELSD was shown to be a more appropriate tool for the analysis of the krill oil components and its esterified phenolic lipids, obtained by a transesterification of phospholipids and DHPA. Liquid chromatography/mass spectrometry-electrospray ionisation/atmospheric-pressure chemical ionization (LC/MS-ESI/APCI) successfully characterized the molecular structures of the synthesized phenolic lipids.

ANTIBACTERIAL EFFECTS OF HYDROLYZABLE TANNINS AND RELATED ARTIFICIAL TANNINS

Airi Doi,¹ Mohamed A. A. Orabi,² Shoko Taniguchi,³ Takuya Ogawa,³ Yasuo Takeuchi,³ Teruo Kuroda,³ Tomofusa Tsuchiya,⁴ Hideyuki Ito,³ <u>Tsutomu Hatano</u>^{1,3} (¹Okayama University, Faculty of Pharmaceutical Sciences, Okayama, 700-8530, Japan, ²Al-Azhar University, Faculty of Pharmacy, Assiut, 71524, Egypt, ³Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, 700-8530, Japan, ⁴Ritsumeikan University, College of Pharmaceutical Sciences, Kusatsu, 525-8577, Japan; hatano@pharm.okayama-u.ac.jp)

Hydrolyzable tannins isolated from *Tamarix nilotica* and *T. tetrandra*, such as hirtellin B, showed *in vitro* antimicrobial effects on methicillin-resistant *Staphylococcus aureus* (MRSA). Artificial tannins produced on the galloylation of oligosaccharides, such as octa-O-galloyl-beta-lactose, also showed antibacterial effects on MRSA. The minimum inhibitory concentrations (MICs) of these compounds were largely different from each other depending on their structures. Inhibition of pathogenic pigment production in *Pseudomonas aeruginosa* was observed for almost all of the *Tamarix* tannins and artificial tannins.

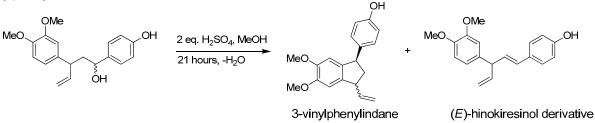


09.2

COX-2 SPECIFIC INHIBITION FROM NATURAL PRODUCT (E)-HINOKIRESINOL AND A FACILE SYNTHESIS OF 3-VINYLPHENYLINDANES

<u>Catherine P. Tunbridge</u>,¹ Neil R. Crouch,³ Alfred E. Thumser,² Dulcie A. Mulholland¹ (¹University of Surrey, Chemical Sciences, Guildford, GU2 7XH, United Kingdom, ²University of Surrey, Biological Sciences, Guildford, GU2 7XH, United Kingdom, ³South African National Biodiversity Institute, Ethnobotany Unit, Durban, 4007, South Africa; c.tunbridge@surrey.ac.uk)

The total synthesis of natural product (*E*)-hinokiresinol had an overall yield of 20% from starting materials 4-hydroxyacetophenone and 4-hydroxybenzaldehyde. COX-1 and COX-2 assays confirmed that (*E*)-hinokiresinol had anti-inflammatory activity, with it showing selectivity towards COX-2 at 10μ M. During the synthesis of derivatives, an intermolecular cyclisation occured resulting in a facile route to 3-vinylphenylindanes.



BIOACTIVE COMPOUNDS FROM THAI MARINE-DERIVED FUNGI

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Marine-derived fungi are isolated from tissues of Thai marine invertebrates, and are rich sources of bioactive compounds. In our experience, these fungi could grow rapidly in salt containing media (sea water), but most of them hardly grow in media without salt supplements, and sometimes, those that do grow in non-saline media change their morphology when cultured under such conditions. We define these fungi as "marine-derived fungi" rather than marine fungi which require seawater for their growth. We have chemically explored biologically active compounds from marine-derived fungi. Results of this research will be presented.

09.4

MULTI-DRUG RESISTANCE, VEROTOXIN PRODUCTION AND EFFICACY OF CRUDE STEM BARK EXTRACTS OF *CURTISIA DENTATA* AMONG *ESCHERICHIA COLI* (NON-O157) AND *ACINETOBACTER* SPP. ISOLATES OBTAINED FROM WATER AND WASTEWATER SAMPLES

<u>James Hamuel Doughari</u>, Patrick Alois Ndakidemi, Izanne Susan Human, Spinney Benade (Faculty of Applied Sciences, Cape Peninsula University of Technology, P.O. Box 652, Cape Town 8000, South Africa; jameshamuel@yahoo.com)

Drug resistant diarrhea and nosocomial infections caused by verotoxic Escherichia coli and some Acinetobacter spp. has posed serious therapeutic challenges especially in developing countries. The aim of this work was to investigate multi-drug resistance, verotoxin-production and susceptibility of E. coli and Acinetobacter spp. isolated from some water samples to crude stem bark extracts of Curtisia dentata. Culture of 62 water samples on Brilliance E. coli/coliform selective medium (BECSM, Oxoid), Eosin Methylin Blue (EMB) agar, or Baumann's enrichment medium (BEM) and Leeds Acinetobacter Medium (LAM) yielded 69 isolates of E. coli and 41 isolates of Acinetobacter spp. with 26 (53.06%) of the E. coli and 6 (14.63%) of the A. haemolyticus isolates producing verotoxins, and no A. lwoffii isolate produced the toxins. Multi-drug resistance index (MDRI) values of isolates ranged between 7-33.00% for both isolates with 12 (17.39%) of the E. coli and 10 (24.39%) of the Acinetobacter spp. resistant to 3 or more classes of the antibiotics. C. dentata stem bark extracts demonstrated low MIC values of 150-300 µg/ml for E. coli and 150-2000 µg/ml for Acinetobacter spp. The plant also contained saponins, tannins, glycosides, anthraquinones, flavonoids, steroids and phenols. The presence of verotoxic multidrug resistant E. coli and Acinetobacter spp. in the environments investigated calls for further surveillance of more water bodies and other environments. Proactive control measures need to be in place to curtail possible contamination of food and drinking water sources. Purification of C. dentata phytoconstituents, toxicological as well as *in vivo* studies for their antimicrobial potentials against pathogenic bacteria. should be carried out with a view to utilizing the plant in developing novel antibiotic substances.

09.5

17-O-ACETYL, 10-HYDROXYCORYNANTHEOL, A SELECTIVE ANTIPLASMODIAL ALKALOID ISOLATED FROM STRYCHNOS USAMBARENSIS LEAVES

<u>Martine Cao</u>,¹ Raymond Muganga,^{1,2} Monique Tits,¹ Luc Angenot,¹ Michel Frederich¹ (¹University of Liege, Pharmacy, Laboratory of Pharmacognosy, CIRM, Liege, 4000, Belgium, ²National University of Rwanda, Pharmacy, Faculty of Medecine, Butare, 117, Rwanda; Martine.Cao@ulg.ac.be)

In the course of our investigations on *Strychnos usambarensis* leaves in order to isolate isostrychnopentamine, the main alkaloid responsible for the antiplasmodial activity of the plant, a new tertiary indolic alkaloid, has been isolated: 17-O-acetyl, 10-hydroxycorynantheol I. Its structure was determined by means of spectroscopic and spectrometric methods such as UV, IR, CD, NMR and ESI-MS. It is one of the most active monomeric indole alkaloids known to date showing an *in vitro* activity against *Plasmodium falciparum* close to 5 μ M and high selectivity.

EVALUATION OF THE ANTIMICROBIAL ACTIVITY, SUB-CHRONIC TOXICITY AND WOUND HEALING EFFECT OF *CUNNINGHAMELLA SPECIES* AND SOME OF ITS ISOLATED COMPOUNDS

<u>Amani S. Awaad</u>,¹ Derek J. Maitland,² G. A. Soliman,³ Nabilah A. Al-Jaber, Mounerah R. Al-Outhman, M. E. Zain,³ Tahani A. Alhamed (¹King Saud University, Chemistry Department, Faculty of Science (Girls Section), Riyahd, 11495, Saudi Arabia, ²Bradford University, Chemical and Forensic Sciences Department, School of Life Science, Bradford, BD7 1DP, United Kingdom, ³ Al-Kharj University, Medical Laboratory Sciences Department, Faculty of Applied Medical Sciences, Al-Kharj, Saudi Arabia; alazab@ksu.edu.sa)

The alcohol extracts of Cunninghamella blakesleeana, Cunninghamella elegans and Cunninghamella homothallica, as well as their successive extracts and isolated compounds, were evaluated for their antimicrobial activity against a number of microbes. The tested extracts showed significant antimicrobial activity. Total alcohol and successive extracts of C. elegans showed the highest activity against Staphylococcus aureus. Three fatty acids were isolated and identified as palmitic acid, oleic acid and stearic acid; they showed variable activities against Staphylococcus auras. Five compounds: 2-(6-amino-9H-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol (adenosine), uridine, pyrimidine-2,4-dione uracil, gallic acid, and 3-(methoxycarbonyl)but-3-enoic acid were isolated from the extract of Cunninghamella elegans and identified. The results of the antibacterial activity of the isolated compounds revealed that the activity was attributed to uridine as the most active one (MIC=20 μ g mL⁻¹) followed by uracil, gallic acid and 3-(methoxycarbonyl) but-3-enoic acid (MIC=150,130 and 210 µg mL⁻¹, respectively). The total methanol extracts of C. blakesleeana, C. elegans, and C. homothallica did not induce any signs of toxicity or mortalities in mice when administered orally at doses up to 5000 mg kg⁻¹. In a sub-chronic experiment, oral administration of the methanolic extracts of three fungi to rats in a dose of 200 mg kg⁻¹ for 35 days did not produce any significant change in their liver and kidney functions. The topical application of methanol extracts of C. elegans and uridine wound healing process was at a concentration of 5 mg mL⁻

09.7

ANTIHYPERTENSIVE EFFECT OF GENTIANA FLORIBUNDA IS MEDIATED THROUGH Ca⁺⁺ ANTAGONISTIC PATHWAY

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The crude extract of *Gentiana floribunda* (Gf.Cr) caused a dose-dependent (3.0-300 mg/kg) fall in arterial blood pressure (BP) of rats under anesthesia. In rat aortic ring preparations denuded of endothelium, Gf.Cr (1.0-10 mg/MI) relaxed high K⁺ (80 mM) and phenylephrine (PE, 1 μ M)-induced contractions and shifted Ca⁺⁺ dose-response curves to the right, similar to that caused by verapamil. It also suppressed PE (1 μ M) control peak responses at 0.3-1.0 mg/mL, obtained in Ca⁺⁺-free medium, like verapamil. Pre-treatment of tissues with Gf.Cr produced rightward non-parallel shift of PE-curves with decline of the maximum contractile response. The vasodilator effect of Gf.Cr was endothelial-independent, as it was not blocked by *N*-nitro-*L*-arginine methyl ester hydrochloride (0.1 mM), atropine (1 μ M) or indomethacin (1 μ M) in endothelium-intact aortic tissues. These data indicate that BP-lowering action of *Gentiana floribunda* occurred via Ca⁺⁺ antagonism (inhibition of Ca⁺⁺ ingress and release from intracellular stores) which provides a pharmacological basis to justify its effectiveness in hypertension.

SYRBACTINS: NEW STRUCTURAL CLASS OF PROTEASOME INHIBITORS PRODUCED BY PLANT PATHOGENS

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The syrbactin natural products belong to a new class of irreversible proteasome inhibitors which include syringolins and glidobactins. These small molecules are derived from the plant pathogen *Pseudomonas syringae* pv. *syringae* (Pss) and an unknown species of the order Burkholderiales, respectively. They are structurally distinct from other, well-established proteasome inhibitors (e.g., bortezomib/velcade), and bind the eukaryotic 20S proteasome by a novel mechanism (Nature, 2008, 452: 755-8). Despite the irreversible binding mode, these molecules show surprising selectivity for covalent proteasome inhibition, highlighting their potential as promising lead structures for drug discovery. Syrbactins exhibit strong antitumor activity *in vitro* against neuroblastoma, multiple myeloma, and ovarian cancer cells (Biochem Pharmacol, 2010, 80: 170-8) and *in vivo* against neuroblastoma-tumor bearing mice. The chemical synthesis of these natural products has been accomplished (PNAS, 2009, 106: 6507-6512; Org Lett, 2010, 12: 2402-5) and novel syrbactin-inspired analogues have been synthesized with improved proteasomal inhibitory activity and elevated anti-tumor potency. Proteasome inhibition is a promising strategy for targeted anticancer therapy and syrbactins are a new class of inhibitors which provide a structural platform for the development of novel, proteasome inhibitor-based drug therapeutics.

09.9

CATEGORIZATION OF MEDICINAL PLANTS WITH IMMUNO-REGULATORY ACTIVITIES BY CYTOKINE EXPRESSION IN MOUSE BONE-MARROW DERIVED DENDRITIC CELLS

<u>Ning-Sun Yang</u>,^{1,2,3} Wen-Chi Wei,^{1,4} Sheng-Yen Lin,^{1,5} Yung-Tsung Chen,^{1,2} Chia-Chi Wu¹ (¹Academia Sinica, Agricultural Biotechnology Research Center, Taipei, 115, Taiwan, ²National Taiwan University, Institute of Biotechnology, Taipei, 106, Taiwan, ³National Central University, Department of Life Science, Taoyuan County, 32001, Taiwan, ⁴National Sun Yat-Sen University, Department of Marine Biotechnology and Resources, Kaohsiung, 804, Taiwan, ⁵National Defense Medical Center, Graduate Institute of Life Science, Taipei, 114, Taiwan; nsyang@gate.sinica.edu.tw)

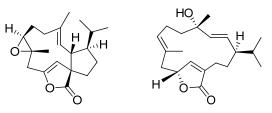
Traditional medicinal plants (TMP) are increasingly recognized for use in public health care throughout the world. Numerous TMPs are reputed to confer various medicinal efficacies or effectiveness including anti-inflammation and immuno-modulatory activities. However, systematic investigation and concrete demonstration of TMPs on defined, specific and heath care-applicable immuno-regulatory activities is limited. Dendritic cells (DCs), a key type of professional antigen presenting cells (APC) are key mediators in human's immune systems. Cytokine stimulation by DCs is known to play a crucial role in DC-mediated immuno-regulatory activities in various immune responses. Therefore, DCs are considered by many as a viable pharmacological platform or target for evaluating TMP's immuno-regulatory activities. The objective of this study was to evaluate the regulatory activities of specific TMPs on DC maturation and activities, especially on cytokine expression. Our preliminary results show that a number of the ethanol and ethyl acetate extracts of test TMPs can exhibit an inhibitory activity on LPS-induced expression of TNF- α , IL-6 and IL-12 in mouse bone marrow derived dendritic cells (BMDCs). In addition, these test herbal extracts can be categorized into several functional groups based on their capacities to regulate cytokine. We therefore hypothesize that these key cytokines may be usefully employed as a guide or index for grouping, classifying, monitoring and manipulating the molecular and immunological specificities of different anti-inflammatory herbal extracts in key immune cell systems., These findings and future studies many have potential pharmacological application to the development of TMPs.

O9.10

CEMBRANOLIDES FROM CROTON GRATISSIMUS (EUPHORBIACEAE)

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The phytochemical study of the leaves and stem bark of *Croton gratissimis* has yielded a range of novel cembranolide diterpenoids such as 1-2. Compounds 1 and 2 were screened against the PEO1 and PEO1TaxR ovarian cancer cell lines and found to have lower potency than paclitaxel (IC_{50} values of 132 and 125 nM respectively against PEO1, cf paclitaxel 2.3 nM).



09.11

NATURAL PRODUCT-BASED INHIBITORS OF HYPOXIA-INDUCIBLE FACTOR-1 (HIF-1) AS CHEMICAL PROBES FOR CELLULAR SIGNALING

<u>Yu-Dong Zhou</u>, Fakhri Mahdi, Jun Li, Lin Du, Sandipan Datta, Dale G. Nagle (University of Mississippi, School of Pharmacy, Department of Pharmacognosy, University, MS 38677-1848, USA; ydzhou@olemiss.edu)

The transcription factor hypoxia-inducible factor-1 (HIF-1) regulates oxygen homeostasis. Decreases in oxygen tension (hypoxia) activate HIF-1, which then increases the expression of genes that promote cellular adaptation and survival under hypoxic conditions. Employing a combined approach that incorporates natural product chemistry with bioassays, we have discovered chemically diverse natural products that suppressed HIF-1 activation. Compounds (rotenone, skimmiarepins, etc.) that disrupt mitochondrial respiration selectively inhibited HIF-1 activation by hypoxia. Further mechanistic studies revealed that mitochondrial respiration inhibitors trigger a cellular stress response that stalls protein translation. In addition, mitochondrial respiration and fission. Cellular signaling pathways that regulate mitochondrial morphology and protein translation may constitute part of a signaling network that transmits acute exposure-associated cellular effects exerted by natural product-based mitochondrial respiration inhibitors.

O9.12

RAPID AND RATIONAL IDENTIFICATION OF BIOACTIVE NATURAL PRODUCTS

<u>Muriel Cuendet</u>, Andreas Nievergelt, Claudia Simoes Pires, Nadine Bohni, Jean-Luc Wolfender (University of Geneva, School of Pharmaceutical Sciences, Geneva, 1211, Switzerland; muriel.cuendet@unige.ch)

As established by ample precedent, nature provides broad chemical diversity and compounds isolated from plants are essential in therapy. Successful hit discovery of candidates relies on rational screening strategies involving *in vitro* assays, compound isolation, and identification. These steps are often time consuming and require large amounts of plant material or other biological sources. Advances in analytical methods and bioassay development helped to push forward the research in this area. The development of high resolution methods related to HPLC for both chemical and biological profiling has significantly increased the efficiency of classical bioactivity-guided fractionation procedures. A comprehensive investigation becomes feasible starting with only tenth of milligram of crude extract. Enriched extracts can be fractionated in a single step by semi-preparative HPLC, and the activity of microfractions is evaluated,

enabling a rapid localisation of the corresponding bioactive LC-peaks. Test samples are then analyzed by UHPLC-TOF-MS to get first information on their chemical composition. Compound identification and their approximate concentration in the original sample are feasible in the low microgram range by offline coupling with microflow-NMR equipped with automated sample injection. This discovery platform will be illustrated by a practical example of phytochemical investigation performed at the microgram level. This represents a key advantage for rapid localisation of the biological activity and subsequent identification of the compounds of interest.

09.13 NATURALLY OCCURRING ENZYME INHIBITORS AND THEIR PHARMACEUTICAL APPLICATIONS

<u>Athar Ata</u> (University of Winnipeg, Department of Chemistry, Winnipeg, MB R3B 2G3, Canada; a.ata@uwinnipeg.ca)

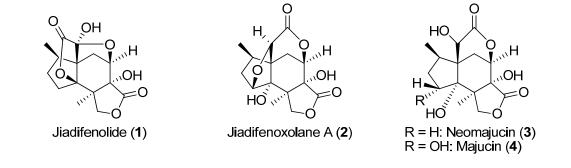
Enzymes are essential to human life, mediating biochemical processes including metabolism, cellular signal transduction, cell cycling, and development. Malfunction in these biochemical systems often leads to diseases that can be caused either by the dysfunction, overexpression, or hyperactivation of the enzymes involved. An understanding of diseases at the molecular level has provided several enzyme inhibitors in clinics. These enzyme inhibitors are used to treat several human health related issues. For instance, glutathione S-transferase inhibitors have applications in overcoming the drug resistance problems in cancer and parasitic chemotherapy. Fatty acid synthase inhibitors are used to discover antimalarial, anti-parasitic, anti-TB and anti-fungal compounds. We are involved in discovering new natural products exhibiting inhibitory activities against glutathione S-transferase (GST), acetylcholinesterase (AChE), and α -glucosidase. In this presentation, structures of potent enzyme inhibitors and their structure-activity relationships will be discussed.

09.14

NEUROTROPHIC SECO-PREZIZAANE-TTYPE SESQUITERPENOIDS FROM ILLICIUM JIADIFENGPI

<u>Yoshiyasu Fukuyama</u>, Miwa Kubo (Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Tokushima, 770-8514, Japan; fukuyama@ph.bunri-u.ac.jp)

As part of our continuing studies on neurotrophic compounds in *Illicium* species, we investigated the chemical constituents of the pericarps of *I. jiadifengpi*, resulting in the isolation of two *seco*-prezizaane-type sesquiterpenoids 1 and 2 named jiadifenolide and jiadifenoxolane A. The Dess-Martin oxidation of the known sesquiterpene, neomajucin (3), gave rise to 1 in a straightforward fashion. This means that the absolute configuration of 1 can be assigned to be the same as that of 3. Jiadifenolide (1) was found not only to significantly enhance neurite outgrowth in the primary cell cultures of rat cortical neurons at concentrations ranging from 0.01 to 10 μ mol L⁻¹, but also to have a potential to specifically promote differentiation of multipotent neural stem cell line MEB5 cells into neurons at 10 μ mol L⁻¹.



O9.15

STEERING CLEAR OF THE DRUG DISCOVERY BLACK-HOLE

<u>Mark O'Neil-Johnson</u>,¹ Gary R. Eldridge,¹ Courtney Starks,¹ Russell Williams,¹ Werner Maas² (¹Sequoia Sciences, Inc., Saint Louis, MO 63114, USA, ²Bruker Instruments, Inc., Billerica, MA 01821, USA; moj@sequoiasciences.com)

There is not one single scientific innovation that you can point to and say, "this has revolutionized drug discovery." The billions of dollars that have been invested in proteomics, genomics, metabolomics, etc. has not created the proportionate output to dollar input. Mergers and acquisitions have done nothing to invigorate the discovery process. In fact, financial data has indicated that this aggressive strategy for business growth has resulted in \$1 trillion reduction of valuation over the past decade. Is this the path towards innovation as an industry? As natural product chemists, the question that we need to ask is, "what about chemical diversity?"

Sequoia Sciences identifies novel chemistry from its library of structurally diverse small molecules isolated from plants. Sequoia built this analytical process such that rapid isolation and structure elucidation of active compounds could be accomplished. Using the sensitive Bruker TCI 1.7mm MicroCryoProbe, structure elucidation of active compounds is completed on samples of limited mass.

The scientific strategy that Sequoia employs to rapidly uncover the chemical diversity contained in plant natural products will be outlined. This presentation will outline Sequoia's unique process that is used to create a library of compounds. The MicroCryoProbe has now extended the high-throughput process to include NMR data acquisition. Sequoia's inclusion of the MicroCryoProbe compliments its current platform technologies for high-throughput natural products research for drug discovery allowing it to uncover the chemical diversity contained in natural products.

O9.16

ENHANCING NORMAL PHASE CHROMATOGRAPHY FOR NATURAL PRODUCTS RESEARCHERS

<u>James D. McChesney</u>, Douglas L. Rodenburg (Ironstone Separations, Inc., Etta, MS 38627-9519, USA; jdmcchesney@yahoo.com)

Ironstone Separations has developed a propriety technology for the fabrication of high efficiency preparative chromatographic columns applicable to both normal and reversed phase columns, called C4 technology. Importantly, we have also perfected a technology which regenerates the efficiency of normal phase columns such that they can also be reused for hundreds of separations without adsorbent replacement. Since high quality normal phase adsorbent costs about \$5,000 per kilogram, these technologies provide cost savings to users of many thousands of dollars over the lifetime of a preparative normal phase chromatographic column. Thus the advantages of normal phase preparative chromatography, increased capacity per column run and ease of purified compound recovery, are captured without the disadvantage of frequent adsorbent replacement. Ironstone fabricates and markets this technology to the synthetic chemistry and natural products research and development communities. Due to their significant economic advantages, Ironstone's technologies are especially beneficial to academic natural product researchers.

09.17

PHYTOAGENT DEOXYELEPHANTOPIN COTREATMENT WITH CISPLATIN SIGNIFICANTLY REDUCES NEPHROTOXICITY-INDUCED BY CISPLATIN IN B16 MELANOMA-BEARING MICE

<u>Lie-Fen Shyur</u>, Wen-Wan Chao, Ya-Wen Cheng, Ching-Yi Chiou (Academia Sinica, Agricultural Biotechnology Research Center, Taipei, 115, Taiwan; lfshyur@ccvax.sinica.edu.tw)

This study investigated the *in vitro* and *in vivo* efficacy of deoxyelephantopin (DET), a major sesquiterpene lactone constituent of *Elephantopus scaber* L. (Asteraceae), against B16 melanoma. Isobologram analysis revealed the synergism of DET and chemotherapeutic drug cisplatin (CP) against B16 cell proliferation. A series of drug treatment protocols were thus designed in this study, i.e., DET or CP treatment alone, DET co-treated with CP (DET+CP), and sequential treatment with CP then DET (CP-

DET). A stable B16 melanoma cell clone carrying COX-2 promoter driven-luciferase reporter gene was established to monitor the lung metastasis of melanoma in syngeneic mice. Our results showed that Pre-DET10 and CP-2 have a similar profound effect on inhibiting lung metastasis of B16 melanoma and increase of median survival rate in tested mice. CP treatment, however, resulted in renal damage and haematological toxicity in mice that was not detected in DET or DET and CP cotreatment groups. The metabolomic results obtained by UPLC-QTOF MS showed that CP-induced nephrotoxicity in mice kidney can be reflected in the levels of specific metabolites involved in primary metabolism or urea cycle in animals. Mechanistic study indicated that DET could induce cell cycle arrest at G2/M phase, as well as apoptosis in B16 melanoma cells. Our findings may prove useful for the future application of combinational DET and CP treatment against metastatic melanoma.

09.18 ANTIVIRAL PROPERTIES OF SILYMARIN AND PURIFIED FLAVONOLIGNANS

<u>Stephen J. Polyak</u>,¹ Jessica Wagoner,¹ Jan McClure,¹ Erica Lovelace,¹ Tyler Graf,² Nicholas Oberlies² (¹University of Washington, Laboratory Medicine, Seattle, WA 98104, USA, ²University of North Carolina at Greensboro, Chemistry and Biochemistry, Greensboro, NC 27412, USA; polyak@uw.edu)

Silymarin, an extract of milk thistle seeds [Silybum marianum (L.) Gaertn. (Asteraceae)], prevents liver injury and disease progression in animal models. To maximize the clinical value of this natural product, we are pursuing the molecular mechanisms by which silvmarin protects the liver. We have previously shown that silymarin and silymarin-derived purified flavonolignans block hepatitis C virus (HCV) infection in liver cells and have immunomodulatory and anti-inflammatory effects on T cells. We now report that SIL suppresses human immunodeficiency virus (HIV) infection in peripheral blood mononuclear cells (PBMC) in vitro, the results of which have been validated in 5 different donor PBMC preparations. Furthermore, SIL inhibits 2 replication-competent viruses and 4 pseudoviruses in TZM-BI cells. Thus, SIL inhibits Clade A, B, and C HIVs. Cumulatively, the data show that silymarin-derived compounds have antiviral effects against HCV and HIV in their natural cellular contexts: liver and T cells. We hypothesize that these antiviral effects against divergent viruses in vastly different cell types arise through the interaction of silymarin-derived compounds will cellular biomolecules that regulate virus infection. Current work is focused on identifying the cellular targets of silymarin flavonolignans using biochemical, genetic, and systems biology approaches. These studies may lead to novel cell-targeted antiviral therapies, identification of biomarkers of silymarin treatment and efficacy, and refinements in silymarin-based treatments for liver disease in HCV and HCV/HIV infected patients. This research is partially supported by NCCAM and the University of Washington Virology Division Pilot Award.

S10.1 REALISING THE POTENTIAL OF PLANT METABOLOMICS

<u>Michael H. Beale</u>, Jane L. Ward (Rothamsted Research, Plant Sciences, Harpenden, AL5 2JQ, United Kingdom; mike.beale@rothamsted.ac.uk)

Plant metabolomics technology has now developed to an extent where large-scale screening programmes are possible. A key feature of untargeted metabolite profiling is that both primary and secondary metabolites are observed in the same spectra. This enables the study of how plants switch metabolic flow from primary to secondary metabolism in response to stress. We describe a sample preparation protocol, streamlined for robotic operation, that is used to generate NMR-MS fingerprint data from many thousands of samples. The data, because they originate from multiparallel analysis of single solvent extracts, can be interpreted together to identify discriminatory metabolites. Furthermore, structural information on those metabolites can be extracted from the fingerprints, in a way that is analogous to the classical structure determination of isolated, pure natural products. Use of this technology for the screening of recombinant inbred lines of Arabidopsis revealed that metabolic variation could be ascribed to relatively few areas of the genome. The metabolome is dynamic and studies must take into account the natural diurnal rhythms, tissue specificity and developmental programming. The presentation will describe examples of natural product biomarkers detected in Arabidopsis, either locally or systemically in response to microbe infection. A very striking example of the power of the technique has emerged from recent work on nutrient deprived Arabidopsis plants, where novel hemiterpenoids, directly associated with leaf N depletion, are induced by metabolic re-programming that is co-ordinated with synthesis of root phenylpropanoids.

S10.2

SPECTROSCOPIC METABOLITE PROFILING OF LASER-MICRODISSECTED PLANT CELLS

<u>Bernd Schneider</u> (Max Planck Institute for Chemical Ecology, Biosynthesis/NMR, Jena, 07745, Germany; schneider@ice.mpg.de)

The analysis of single plant cells and special plant cell populations is of considerable interest in natural product chemistry, chemical ecology and other disciplines of plant science. Laser-microdissection has become an established method for harvesting cells from plant tissue for RNA isolation and amplification. Identification of metabolites from single cells and microscopic tissue pieces so far has been reported by mass spectrometry. However, miniaturization of NMR and the enhanced sensitivity of cryogenically cooled probes enable NMR-based metabolic profiling of secondary metabolites specifically accumulating in special plant cells. ¹H NMR, 2D COSY and HSQC spectra were recorded at 500 MHz from extracts obtained from a limited number of cells and the metabolites were identified in the mixture. Using ¹H NMR, the relative proportions of metabolites in the samples were determined by integration and/or quantified by means of added standards. The method and results from plant species accumulating metabolites in specialized cells will be discussed.

S10.3

EXPLOITING METABOLIC DIVERSITY THROUGH INTEGRATED METABOLOMICS FOR THE DISCOVERY AND ELUCIDATION OF SAPONIN BIOSYNTHETIC GENES IN *MEDICAGO TRUNCATULA*

Dong Sik Yang, John H Snyder, David V. Huhman, Vered Tzin, Stacy Allen, Yuhong Tang, and <u>Lloyd W.</u> <u>Sumner</u> (Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK 73401, USA; Iwsumner@noble.org)

Triterpene saponins are a class of structurally diverse plant natural products with a wide range of demonstrated bioactivities including allelopathic, antifungal, antibacterial, anti-insect, anticancer, and antinutritive activities. The antinutritive properties of triterpene saponins in legume forages such as alfalfa and soybean are of particular and substantial economic importance. However, the genes and proteins

responsible for the biosynthesis of legume saponins are mostly unknown. Thus, we are using cuttingedge metabolomics, correlated gene expression profiling, and eventually genome wide association mapping to identify, prioritize and characterize gene candidates related to triterpene saponin biosynthesis in *M. truncatula*. This presentation will provide a specific example whereby large-scale metabolite profiling (*i.e.* metabolomics) was used to survey a diverse collection of *M. truncatula* germplasm for the identification of hyper- (high) and hypo- (low) saponin accumulating lines. Comparative gene expression analyses were then performed on the hyper and hypo saponin accumulating lines, and correlation analyses performed to identify and prioritize genes candidates likely involved in triterpene saponin biosynthesis and regulation. This presentation will focus on a specific cytochrome P450 that was characterized as a multi-functional oxidase in saponin biosynthesis.

S10.4

Arthur Neish Young Investigator Award Lecture EFFECTS OF EXOGENOUSLY APPLIED BRASSINOSTEROID IN SECONDARY XYLEM OF YELLOW POPLAR

Hyunjung Jin,² Wook Kim,² <u>Mi Kwon</u>^{1,2} (¹Institute of Life Science and Natural Resources, Korea University, Seoul, Korea 136-701, ²Biotechnology, Korea University, Seoul, 136-701, Korea; mikwon@korea.ac.kr)

Brassinosteroids (BRs) are group of plant steroidal hormones with various physiological roles including control of cell elongation and cell division, tracheary element differentiation, and resistance aginst biotic and abiotic stresses in plants. Recently, roles of BRs in the vascular cambium development were proposed based on the analysis of Arabidopsis thaliana BR mutants as well as suspension cells of Zinnia elegans. However, the effects of BRs in the vascular development have not been demonstrated in the woody species which possesses a well-developed vascular system. Thus, this study was designed to uncover the roles of brassinosteroids during secondary xylem formation in woody species. When 24-epibrassinolide (BL) had been applied to the vascular cambium of the vertical stem of two-year-old yellow poplar, the growth promotion of tracheary elements were clearly visualized. Statistical analysis of cell length and cell diameter indicate that the length of both types of cells, fiber and vessels, was significantly increased upon BL application. Since anatomical and histochemical analysis implied changes in the cell wall structure and chemistry, an expression analysis was performed for the genes involved in cell wall biosynthesis at the transcriptional level. In the case of lignin, almost all lignin biosynthetic genes were significantly down-regulated in the stem where BR was applied exogenously, as compared to the control stem. On the other hand, cellulose synthase (CesA) was significantly up-regulated, indicating an active involvement of BR in secondary xylem formation in woody plants.

S10.5 PLANT METABOLOMICS FOR PHYTOCHEMICAL GENOMICS

<u>Kazuki Saito</u> (RIKEN, Plant Science Center, Yokohama, 230-0045, Japan, and Chiba University, Graduate School of Pharmaceutical Sciences, Chiba, 263-8522, Japan; ksaito@faculty.chiba-u.jp)

Metabolomics plays a major role in plant functional genomics and biotechnology. We have established an excellent analytical platform of plant metabolomes based on the combination of multiple mass spectrometers. An integrated analysis of metabolome and transcriptome data led to the prediction of gene-to-metabolite relations with a model plant, *Arabidopsis thaliana*. Holistic identification of genes involved in biosynthesis and modification of flavonoids in *Arabidopsis* has been carried out by combination of a transcriptome co-expression network. Metabolomics developed in *Arabidopsis* is further applicable to crops to decipher their gene functions and to improve their traits by biotechnology. An excellent coverage of chemical diversity of our analytical platform was suitably applied to the assessment of objective substantial equivalence of genetically-modified tomatoes over-expressing the taste-modifying protein miraculin. Application to a study in rice leading to the prediction of the rice agronomical and food traits was made by regression analysis of the metabolome for the World Rice Core Collection (WRC). Metabolome QTL analysis has been also performed to figure out the overview of metabolic genomics in

rice. In this presentation, the crucial roles of metabolomics in plant functional genomics and crop biotechnology will be discussed.

S10.6

DEVELOPING MINT AS AN EXPERIMENTAL MODEL SYSTEM FOR UNDERSTANDING AND MANIPULATING TERPENOID ESSENTIAL OIL BIOSYNTHESIS

<u>Mark Lange</u> (Washington State University, Institute of Biological Chemistry, Pullman, WA USA 99164-6340; lange-m@wsu.edu)

Mints have been used and valued as aromatic herbs and sources of essential oils for thousands of years. The biosynthesis of the terpenoid essential oils in mint is confined to modified epidermal hairs called glandular trichomes. These structures contain highly specialized, non-photosynthetic, cells - termed secretory cells - that are solely responsible for the synthesis of essential oils. An EST sequencing effort with isolated secretory cells, which began in 1997, provided sequences of cDNAs with potential roles in terpenoid essential oil biosynthesis. By 2004 all of the structural genes with direct involvement in mint essential oil biosynthesis had been cloned and characterized, thus demonstrating the utility of working with specialized plant cell types for gene discovery. A metabolic engineering effort aimed at manipulating essential oil yield and composition commenced in 1998 and has continued to generate a large number of transgenic mint lines with purposefully altered oils. More recently (since 2005), mathematical modeling has provided fascinating novel insights into the regulatory control of this process, which has enabled further metabolic engineering advances. The genome of a diploid mint was sequenced using Illumina technology in 2011, which has opened up new opportunities for using integrative approaches, based on metabolic engineering and/or molecular breeding, for the sustainable agricultural production of high quality essential oils at a competitive cost.

S10.7

Arthur Neish Young Investigator Award Lecture IMPROVING THE QUANTITY AND QUALITY OF LC- AND GC-MS DATA FOR PLANT METABOLOMICS

Paul G. Boswell¹, Will I. Menzel¹, Mikel R. Roe¹, Jerry D. Cohen¹, <u>Adrian D. Hegeman^{1,2,3}</u> (¹University of Minnesota-Twin Cities, Department of Horticultural Science and the Microbial and Plant Genomics Institute, Saint Paul, MN 55108, USA, ²University of Minnesota-Twin Cities, Plant Biology, Saint Paul, MN 55108, USA; hegem007@umn.edu)

Current LC- and GC- mass spectrometry-based metabolomics approaches are capable of providing thousands of chromatographic "features" from typical plant extracts. While accurate mass alone is insufficient to allow annotation of most of those observed peaks, orthogonal information, in the form of chromatographic retention, is usually captured but not under suitably controlled conditions to be useful for metabolite identification. Some success in using retention information in this way has come in the field of GC-MS (Kovats indices etc.), where the theoretical basis for compound retention is relatively simple and instrumentation is more easily standardized. In LC-MS, the higher degree of complexity in analyte retention and operational inconsistencies from run to run and greater differences between instruments have decreased the usefulness of retention information for metabolite identification. Here we describe a new approach that allows us to harness LC retention data for metabolite identification by carefully measuring multi-variable retention properties of compounds on several widely used C18-reversed phase media and by precisely accounting for variation across time and instrument platforms using a new methodology in which we "back-calculate" all significant instrument-related factors controlling retention from the gradient retention times of a small set of standard compounds. In addition, we will briefly discuss our recent efforts in constructing two environmental chambers for stable isotopic labeling of plants using [¹³C]-carbon dioxide for both absolute and relative quantification and for measurement of metabolic flux.

S10.8 CHEMICAL DEFENCE OF CONIFERS AND BIOENERGY APPLICATIONS

<u>Jörg Bohlmann</u> (University of British Columbia, Michael Smith Laboratories, Vancouver, BC V6T1Z4, Canada; bohlmann@msl.ubc.ca)

Conifer trees produce large volumes of terpenoid oleoresin defenses for their protection against insects (e.g. bark beetles and weevils) and insect-associated fungal pathogens (e.g., ophiostomaoid fungi). Using a combination of genomics, transcriptomics, proteomics, and biochemical approaches, we have functionally characterized large gene families of terpenoid synthases (TPS-d family) and cytochrome P450 dependent monoxygenases (CYP720B family) of conifer oleoresin biosynthesis. The TPS-d and CYP720B gene families are critical for the plasticity and diversity of secondary metabolism in conifer defense and the successful evolution of long-lived conifer trees, which often survive for several hundred years in the same location defeating many generations of faster evolving insect pests and pathogens. In parallel, using genome sequencing of fungi and bark beetles, we have discovered new genes that allow fungal pathogens to overcome the toxic defenses of conifer hosts. The gene space of conifer defense against insects revealed genes for the improved production of biofuels and bioproducts.

S10.9 ELUCIDATION OF BIOSYNTHETIC PATHWAY OF CAMPTOTHECIN BY METABOLOMICS

<u>Mami Yamazaki</u>,^{1,2} Takashi Asano,^{1,2} Ko Aoki,^{2,3} Kazuki Saito^{1,4} (¹Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, 263-8522, Japan, ²CREST, JST, Tokyo, 102-0075, Japan, ³Kazusa DNA Institute, Kisarazu, 292-0818, Japan, ⁴RIKEN Plant Science Center, Yokohama, 230-0045, Japan; mamiy@faculty.chiba-u.jp)

Camptothecin (CPT), a monoterpenoid indole alkaloid, is a natural toxin that binds topoisomerase I, and the CPT derivatives are clinically used as anticancer drugs. The plant species producing CPT, such as *Camptotheca acuminata, Nothapodytes foetida* and *Ophiorrhiza pumila* posses CPT-resistant topoisomerase I to survive with CPT produced by themselves. The catalytic reactions and intermediates are still unclear in the late steps in CPT biosynthesis. To find out intermediate compounds and figure out the whole pathway of CPT biosynthesis, we conducted metabolome analysis of genetically modified hairy roots of *Ophiorrhiza pumila*. The CPT production was observed in the hairy roots induced by *Agrobacterium rhizogenes*, however not in the dedifferentiated cell suspension culture. The amount of CPT accumulation was correlated with the gene expression level of both tryptophan decarboxylase (TDC) and secologanin synthase (SLS) in hairy roots in which corresponding genes were knocked down by RNAi technique. The general metabolic change in these tissues was analyzed by using infusion FT-MS, LC/FT-MS and LC/MS. Among the specific mass ion peaks detected in hairy root but not in cell suspension culture, several peaks exhibited positive or negative correlation with the gene expression levels of TDC and SLS in RNAi hairy roots as well as CPT peak. The pathway mining using these data will be discussed.

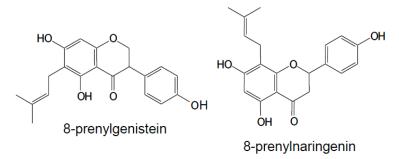
S10.10

FLAVONOID-SPECIFIC PRENYLTRANSFERASES, A MEMBRANE-BOUND ENZYME FAMILY RESPONSIBLE FOR POLYPHENOL DIVERSITY

<u>Kazufumi Yazaki</u>, Kanako Sasaki, Tomoyoshi Akashi, Hirobumi Yamamoto, Shin-ichi Ayabe (Research Institute for Sustainable Humanosphere, Kyoto University, Uji 611-0011, Japan; Department of Applied Biological Sciences, Nihon University, Fujisawa 252-0880, Japan; Faculty of Life Science, Toyo University, Gunma 374-0193, Japan; yazaki@rish.kyoto-u.ac.jp)

Prenylation largely contributes to the diversification of aromatic natural products, such as the flavonoids, coumarins, and phenylpropanoids, via differences in the prenylation position on the aromatic rings, various lengths of prenyl chains, and further modifications of the prenyl moiety, e.g. cyclization and hydroxylation. The entry steps represents the crucial coupling process of the shikimate and the polyketide pathways providing an aromatic moiety and the isoprenoid pathway derived from either mevalonate or

MEP (methyl erythritol phosphate) pathways, which provides the prenyl (isoprenoid) chain. Recently, membrane-bound prenyltransferases have been reported as being responsible for these prenylation reactions. An updated understanding about this enzyme family is discussed.



O10.1 UNEXPECTED HEMITERPENOIDS IN *ARABIDOPSIS*, REVEALED BY METABOLOMIC FINGERPRINTING, GIVE NEW INSIGHTS INTO C/N METABOLIC BALANCING

<u>Jane L. Ward</u>, John M. Baker, Aimee M. LLewellyn, Nathaniel D. Hawkins, Michael H. Beale (Rothamsted Research, National Centre for Plant and Microbial Metabolomics, Harpenden, AL5 2JQ, United Kingdom; jane.ward@rothamsted.ac.uk)

Unlike many other species, the model plant Arabidopsis thaliana contains relatively low levels of terpenoids, consisting mainly of sesquiterpene volatiles produced in flowers. However, metabolomic analysis of polar solvent extracts, using combined NMR-MS, has revealed the presence of two novel (to Arabidopsis) hemiterpenoid glycosides (HTGs) that accumulate, in leaves, to guite high levels (Ward et al PNAS, 2011,108,10762-10767). The structure of the compounds was determined by 2D-NMR and confirmed by synthesis. Using a hydroponic growth system we applied a series of stresses to roots and could follow the formation of these compounds in leaves. The formation of the HTGs was induced specifically in leaves by nitrate deficiency, and some other, but not all, root applied stresses such as oxidative stress. Replacement of growth media nitrate with ammonia failed to suppress the formation of the HTGs indicating that nitrate ion sensing was a key factor in signalling their formation. The formation of the HTGs in leaves was strongly correlated with the induction of phenylpropanoid secondary metabolites (coniferin and scopolin) in roots of the same plants. Feeding of MEP pathway intermediates to detached leaves of control and nitrate deficient plants was used to delineate the pathway to the hemiterpenoids and investigate the regulatory processes behind the induction. The shunts of photosynthetic carbon flow to HTGs will be discussed in terms of safety valve/overflow mechanisms that are involved in the balancing leaf photosynthetic carbon flow against nitrogen availability.

O10.2 MEASURING AND COMPARING THE MAGNITUDES OF METABOLOMIC CHANGE

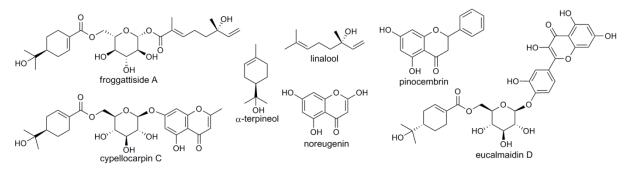
<u>Steven C. Halls</u>, Jay M. Harrison, George G. Harrigan, Angela Hendrickson Culler, Marie A. Coffin (Monsanto Company, St. Louis, MO 63167, USA; steven.c.halls@monsanto.com)

Metabolomic analyses allow examination of numerous biochemical pathways networks and complex interactions. Several statistical tools have been developed to help identify potential metabolic effects related to a particular treatment. When metabolomic analyses are combined with multiple experimental design factors, it becomes challenging to understand the relative magnitude of the overall metabolic effect from each factor because of the complexity of the data. Developing tools such as Principal Variance Component Analysis (PVCA), oPLS-DA Eigen values and Stochastic Drift Ratios allow an overall comparison to be made between the relative magnitudes of change for each experimental factor (genotype, location, treatment, etc.)

EMBEDDED SECRETORY CAVITIES: NATURAL PRODUCT BIOFACTORIES

<u>Jason Q.D. Goodger</u>, Allison M. Heskes, Ian E. Woodrow (University of Melbourne, School of Botany, Parkville, Victoria 3010, Australia; jgoodger@unimelb.edu.au)

Many plants possess specialised extracellular secretory structures, such as glandular trichomes and embedded secretory cavities, which produce a range of high-value natural products. There have been rapid advances in research on the biosynthesis of natural products from trichomes because, unlike embedded cavities, these structures can be readily isolated from leaf surfaces and purified in a functional state. We have recently developed a method to isolate functional embedded cavities from within leaves and make use of this to study unique aspects of the structure, biosynthetic function and metabolome of embedded secretory cavities. In particular, we have shown that *Eucalyptus* secretory cavities house an array of natural products, in addition to the well known terpene essential oils, including chromanones, flavanones, flavonol glycosides and a large number of monoterpenoid glucose esters.



010.4

EXPLORING THE ORGANIZATION AND FUNCTION OF BELOW GROUND TERPENE SPECIALIZED METABOLISM IN ARABIDOPSIS ROOTS

Martha Vaughan,² Qiang Wang,¹ Jung-Hyun Huh,¹ Reza Sohrabi,¹ Jim Tokuhisa,¹ <u>Dorothea Tholl</u>¹ (¹Virginia Tech, Biological Sciences, Blacksburg, VA 24061, USA, ²USDA-ARS, Center for Medical, Agricultural & Veterinary Entomology, Gainesville, FL 32608, USA; tholl@vt.edu)

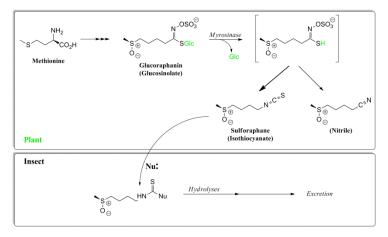
Understanding plant metabolism as a whole requires its analysis in both above ground and below ground tissues. Specifically, how specialized metabolism is maintained in plant roots at cell and subcellular levels is not well understood. We investigate the organization of terpene specialized metabolic pathways and their function in *Arabidopsis* roots. We have found that constitutively expressed terpene biosynthetic enzymes and modules are largely restricted to specific cell types generating layers or gradients of terpene metabolites in the root tissue. As an example, we have identified a novel diterpene olefin (rhizathalene), which is exclusively produced by the terpene synthase TPS08 from MEP pathway precursors in leucoplasts of the root vascular tissue. Diffusion of the diterpene compound from the root stele into the surrounding cell layers has an anti-feeding effect on root herbivores. As a second example, we have characterized two (*E*)- β -farnesene synthases (TPS22, TPS25) with complimenting expression patterns in different root growth zones and surprising subcellular compartmentation in mitochondria. In addition to the constitutive formation of terpenes, we characterize biosynthetic modules involved in pathogen-induced volatile homoterpene formation. An unusual biosynthetic route in the formation of the homoterpene DMNT from the triterpene arabidiol and its potential function will be discussed.

O10.5 TRACING GLUCOSINOLATE METABOLISM AND DETOXIFICATION IN SMALL HERBIVORES

<u>Daniel G. Vassão</u>, Katharina Schramm, Michael Reichelt, Kimberly L. Falk, Jonathan Gershenzon (Max Planck Institute for Chemical Ecology, Biochemistry Department, Jena, TH 07745, Germany; vassao@ice.mpg.de)

The glucosinolates present in Brassicales plants are phytoanticipins that, upon activation, result in the socalled "mustard oil bomb". This "bomb" comprises a number of toxic glucosinolate-hydrolysis products, most prominently their isothiocyanate derivatives, but also nitriles and thiocyanates. While their biosyntheses from amino acids and their activation steps are well-studied, we know only little regarding their mode(s) of action and ecological effects. Even less is understood about the biochemical means employed by some small herbivores to disarm or safely "detonate" this bomb, a necessary strategy to permit successful herbivory and development.

We are now producing and utilizing isotope-labeled glucosinolates to detect, elucidate and quantify these different biochemical strategies within generalist herbivores. More specifically, we are characterizing the metabolic fates of the major *A. thaliana* methionine-derived alkylglucosinolate (4-methylsulfinylbutyl glucosinolate, glucoraphanin) in several insect herbivores, as well as in the mollusk pest *Arion lusitanicus*. We have found these processes to primarily consist of conjugation to amino acids and derivatives. Such results are now allowing us to correlate these metabolic strategies to the apparent herbivory success of these herbivores, i.e. associating their growth and development to their management of intaken toxic glucosinolate products.



O10.6 PHENOLIC ACIDS IN CATHARANTHUS ROSEUS ANALYZED BY A TARGETED APPROACH OF METABOLOMICS

<u>Marcos Soto-Hernandez</u>,^{1,2} Young Hae Choi,¹ Robert Verpoorte¹ (¹Natural Products Laboratory, Institute of Biology, Leiden University, Leiden, 2300RA, The Netherlands, ²Colegio de Postgraduados, Campus Montecillo, Botanica, Texcoco, 56230, México; msoto@colpos.mx)

Phenolic acids containing leaves of intact plants from *Catharanthus roseus* responded to treatment with an elicitor from the oomicete *Pythium aphanidermathum* by shifting their phenol metabolism towards wall-bound phenylpropanoids derivatives: ferulic and *p*-coumaric acids.

The regulation of these metabolic changes, the integration of the phenylpropanoic acids and the signal transduction after elicitation were studied.

SOYBEAN 14-3-3 PROTEINS: ARE THEY INVOLVED IN THE REGULATION OF ISOFLAVONOID BIOSYNTHESIS IN SOYBEAN?

Xuyan Li,¹ <u>Sangeeta Dhaubhadel</u>^{1,2} (¹Southern Crop Protection and Food Research Center, Agriculture and Agri-Food Canada, London, ON N5V 4T3, Canada, ²University of Western Ontario, Department of Biology, London, ON N6A 5B7, Canada; sangeeta.dhaubhadel@agr.gc.ca)

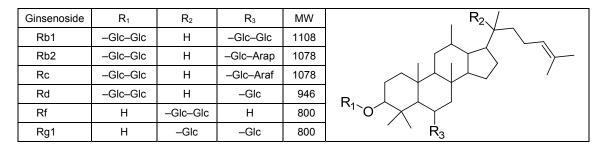
Isoflavonoids are legume specific plant natural compounds that play important roles in nitrogen fixation as well as biotic and abiotic stresses. Many clinical studies have suggested a role for isoflavonoids in human health and nutrition. Therefore, understanding the regulation of isoflavonoid biosynthesis is critical to develop soybean cultivars with altered levels of isoflavonoids. Recently, we have identified an R1 MYB transcription factor, GmMYB176, which regulates CHS8 gene expression and affects isoflavonoid biosynthesis in soybean. Further, we identified the requirement of a 14-3-3 protein binding site within GmMYB176 for its cytoplasmic localization. Plant 14-3-3 proteins have been found to regulate a variety of biological processes such as metabolic, growth and developmental or signaling pathways via interactions with their target proteins. We identified 18 14-3-3 family members in soybean genome, of which 16 are transcribed. Tissue specific expression pattern of soybean 14-3-3 genes in various soybean tissues indicated that all 16 14-3-3s were expressed in embryos during the development suggesting that 14-3-3 proteins may play an important role in seed development. All of the 14-3-3s expressed in soybean were able to interact with GmMYB176 both in in vivo and in vitro condition. The detailed analysis of 14-3-3 binding sites within GmMYB176 identified a critical motif for 14-3-3 protein-GmMYB176 interaction where Ser29 located within the motif is potentially phosphorylated. Our results demonstrate that soybean consists of the largest members of 14-3-3 gene family identified to date and that 14-3-3 regulate the intracellular localization of GmMYB176 and control turnover of GmMYB176 thereby affecting isoflavonoid biosynthesis in soybean. The role of 14-3-3s in isoflavonoid biosynthesis is demonstrated by altering its expression in soybean hairy roots and using virus induced gene silencing in soybean and monitoring the impact on isoflavonoids levels.

010.8

THE STUDY OF NORTH AMERICAN GINSENG METABOLISM IN RATS BY LC-MS/MS

Edmund M.K. Lui,¹ Takeo Sakuma,² Suma Ramagiri,² Tom Moy² Carmai Seto² (¹University of Western Ontario, Physiology and Pharmacology, Ontario Ginseng Innovation and Research Consortium, London, ON N6A 5C1, Canada, ²AB-Sciex, Concord, ON L4K 4V8, Canada; ed.lui@schulich.uwo.ca)

The metabolism of ginseng in Zucker rats was studied using high resolution accurate mass spectrometry (HRMS) techniques coupled with UPLC. Fast and generic single injection HRMS methods allow for simultaneous qualitative and quantitative analysis of complicated ginsenosides in biological samples. Plasma and intestinal content were collected after chronic oral treatment with aqueous and alcoholic extracts. LC-MS and MS/MS technology and software programs have allowed for rapid analysis of complex mixtures of ginsenosides and their metabolites as well as metabolic pathways involved. The data showed that the primary ginsenoside, Rb1, was extensively metabolized in the intestine with the formation of oxidative metabolites and its aglycones. In contrast, the other less abundant ginsenosides, Re, Rd and Rc were detected the plasma but not in the colon. This indicated their intestinal absorption and systemic bioavailability after oral administration (supported by MRI: RE02-049).



O10.9 THE METABOLISM OF LIGNANS FROM FRUCTUS SCHISANDRA

<u>Jun Yin</u>,¹ Feng Yun Cao,^{1,2} Ling Yang² (¹Shenyang Pharmaceutical University, School of Traditional Chinese Medicines, Shenyang, 110016, China, ²Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Laboratory of Pharmaceutical Resource Discovery, Dalian, 116023, China; yinjun826@sina.com)

After metabolites from dibenzocyclooctadiene lignans of Schisandra chinensis incubated with human liver microsomes were isolated by HPLC and their structures were identified, the study combined correlation analysis, chemical inhibition studies, assays with recombinant CYPs and enzyme kinetics indicated that CYP3A4 was the main hepatic isoform. Three metabolites were identified and the results showed little difference among the mentioned species. Deoxyschizandrin undergo hydroxylation in HLMs, and schizandrin was the corresponding metabolite specially mediated by CYP3A4. The high affinity and high turnover make deoxyschizandrin hydroxylation an excellent probe drug for CYP3A4 activity in vitro. The metabolic pathway of schisantherin A validated the inhibitory mechanism against CYP3A4 undergoes demethylenenation in the human liver microsomes. IC₅₀ drift and dynamics test indicate that schisantherin A is inhibitor which shows dependency to NADP⁻ and time, which illustrates that the methylene of schisantherin A produced active intermediate during the catalyzed reaction, thereby presents forceful inhibitory effect against CYP3A4. In mice liver microsomes, schisantherin A, schizandrol B and schizandrin B showed strong inhibition against metablism schizandrin and deoxschizandrin. Our research results indicate that why deoxyschizandrin presented forceful anti-multidrug resistance in vitro but reduced or vanished in vivo, and the extract of Fructus Schisandra containing deoxyschizandrin presents forceful anti-multidrug resistance in vivo.

010.10

SELF-POLLINATED ARTEMISIA ANNUA PLANTS FORM A NEW PLATFORM TO UNDERSTAND ARTEMISININ BIOSYNTHESIS

<u>De-Yu Xie</u>, Fatima Alejos-Gonzalez, Guosheng Qu (North Carolina State University, Plant Biology, Raleigh, NC 27695, USA; dxie@ncsu.edu)

Artemisinin is the currently most effective medicine for human being to fight against malaria disease. However, its low production in *Artemisia annua*, the only natural resource, limits its sufficient supply for the treatment of malarial victims. The past endeavors have made certain great progresses in understanding the biochemical pathway of artemisinin formation; however, little is known in the genetics of its biosynthesis, which leads to difficulties in metabolic engineering of this medicine. In this presentation, we report the development of self-pollinated *A. annua* plants in growth chambers. The F5 progeny plants were developmentally and morphologically uniform and were predicted to be homozygous. LC-MS based metabolic profiling showed that except for roots, seedlings after the removal of roots, leaves, and capitula produced artemisinin. ADS and CYP71AV1 genes, two pathway genes involved in the artemisinic acid formation, were expressed in young leaves and flowers. It was interesting that CYP71AV1 was expressed in roots of seedling although this tissue did not produce artemisinin. Selfpollinated plants form a new genetic resource to understand the biosynthesis of artemisinin.

S11.1 LIVERWORTS-POTENTIAL SOURCE OF MEDICINAL COMPOUNDS

<u>Yoshinori Asakawa</u> (Tokushima Bunri University, Faculty of Pharmaceutical Sciences, Tokushima, 770-8514, Japan; asakawa@ph.bunri-u.ac.jp)

The present paper concerns chemical constituents from liverworts (Marchantiophyta) and their biological activity. The liverworts are taxonomically placed between algae and pteridophytes (ferns) and there are 6000 species. Almost all liverworts possess beautiful cellular oil bodies. Over several hundred new compounds (terpenoids, phenolic compounds and acetogenins) have been isolated from liverworts [1-3]. Many of them show characteristic scents, pungency and bitterness, and some can give allergenic contact dermatitis. Many also have cytotoxic, anti-HIV inhibitory, antimicrobial and antifungal, insect antifeedant and mortality, nematocidal, superoxide anion radical release and NO production inhibitory, neurotrophic, piscicidal, muscle relaxing, antiobesity and vasorelaxant effect and antiinfluenza activities [1-5]. The chemical structures of the active compounds and some biological activities including fragrant and tasty components will be discussed.

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S11.2

TRACING SECONDARY METABOLITES ON BRAZILIAN BIODIVERSITY: HOW TO DO IT USEFULLY TO FIND NEW BIOLOGICALLY ACTIVE COMPOUNDS?

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Natural products represent a vast and complex structural diversity that is not matched by any other sources of small molecules, as well as providing not only a source of raw material, but an inspiration for the discovery of new molecular targets. Brazilian biodiversity holds a tremendous resource of secondary metabolites, which has still hardly been explored. During the past ten years, we have isolated and published *ca.* 834 compounds from Cerrado and Atlantic Forest species that constitute a valuable set of leads, useful for further medicinal chemistry studies. Some casearin diterpenes, piperidine alkaloids and small cyclic peptides have been used as starting materials for synthetic and semi-synthetic derivatives, aiming at SAR studies for optimization of pharmacological properties. [Biota-FAPESP, CNPq, FINEP].

S11.3 BIFLAVONOID BIOSYNTHESIS

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Flavonoids are ubiquitous in the plant kingdom but their dimers, the biflavonoids, are restricted to some Gymnospermae families such as Ginkgoacea and Araucariaceae and a few Angiospermae species. The biosynthesis of these compounds are controversial, but their formation could involve the oxidative coupling of two apigenin or two chalcones moieties possibily mediated by a peroxidase. However, this important biosynthetic step has not been examined in terms of precursor or enzymes involved. Phytochemical studies carried out on *Araucaria angustifolia* leaves demonstrated the presence of

amentoflavone-type biflavonoids and lignans. On the other hand, its callus culture produced only *p*-coumaroyl/feruloyl esters but no flavonoids. Nevertheless, when apigenin is provided to the cell cultures, a methoxylated amentoflavone-type biflavonoid, isoginkgetin is formed. Then, the biosynthesis of biflavonoids in *A. angustifolia* proceeds through a long sequence of steps involving the apigenin production followed by the action of peroxidase and dirigent protein to mediate oxidative coupling forming the 3'-8'' linkage, and methylation reaction by *O*-methyl transferase. These enzymes and the dirigent protein were detected and characterized in cell cultures.

S11.4 MODERNIZATION OF TRADITIONAL CHINESE MEDICINE: CHALLENGES AND OPPORTUNITIES

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Traditional Chinese medicine (TCM) has over 3000 years of history and played important role in the peoples' health and social development in Chinese history. In the past 50 years, Chinese scientists have made great effort to modernize TCM to make it a evidence-based medicine from the experience-based origin. The current review has summarized the recent advances in the modern research on the various respects of TCM including the resources investigation, GAP cultivation, phytochemistry, quality assessment, safety issues, metabolic investigation, pharmacology, systems biology etc. It pointed out that GAP for Chinese herbs is the first and key step in the quality control cycle of traditional Chinese medicine. In addition, the research on TCM active principles and quality control methods were also summarized. Over 12,000 chemical constituents have been isolated from over 600 species of traditional Chinese medicines, among which over 3000 new compounds were discovered, which laid a solid foundation for clarifying the material basis of action and providing reference substances for the quality control of TCM. Systems biology approach is now being actively practiced for the action mechanism studies of TCMs and their active principles, which provided a valuable approach for complex TCM systems. Currently, genomics, proteomics and metabolomics have obtained good application in TCM studies. Finally, the progress of TCM new drug research and development has been outlined. Several famous new drugs from TCM or based on TCM have been successfully marketed. Examples are artemisinin, artemether, huperzine A, etc. Other types of new drugs include Fufang Danshen Dripping Pill. Diao Xinxuekang, etc.

011.1

COMPOSITION AND CHEMICAL STABILITY OF IRIDOIDS OCCURRING IN *MORINDA CITRIFOLIA* L. (NONI)

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Morinda citrifolia L. (noni) is among the most important medicinal plants used by ancient Polynesian people. Pharmacological activities, such as anti-inflammatory, immunostimulting and anti-oxidative properties have been confirmed by modern research. Although the chemistry of the noni plant has been widely investigated, the compounds that are responsible for the pharmacological profile, which is best explained as adaptogenic are not known. Possible candidates for the adaptogenic properties are a group of structurally related iridoids. We investigated a variety of noni fruit samples from different tropical areas with respect to their iridoid content. Local differences in the quantitative composition of iridoids could be observed, however, the qualitative composition was almost the same. Most prominent were the structurally related iridoids deacetyl asperulosidic acid (DAA) and asperulosidic acid (AA). Both compounds are stable under acidic conditions of the stomach (pH 1-2). DAA is stable at room temperature and pH-values between 1-12, whereas AA decomposes to DAA at pH >10. The hydrophilic glycosides do not enter the bloodstream after oral ingestion. DAA and AA are hydrolyzed by β glycosidases, present in the human intestinal wall and in fecal bacteria. The resulting aglycones are highly reactive and have a short half life in biological fluids. Our findings make it unlikely that DAA and AA or their aglycones are responsible for the pharmacological activity of noni, which might nevertheless be due to reaction products formed by the reactive aglycones and biological molecules occurring in the body.

LIMONOIDS FROM MANGROVE PLANTS OF THE XYLOCARPUS GENUS AND THEIR BIOACTIVITIES

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Mangrove plants are a large group of different salt tolerant plants growing in tropical and subtropical intertidal estuarine zones. We will introduce mangrove resources worldwide, and then focus on new limonoids from mangrove plants of the genus *Xylocarpus*. Limonoids, derived from a precursor with a 4,4,8-trimethyl-17-furanylsteroid skeleton, are tetranortriterpenoids with a β -furyl ring moiety. During the last 10 years, we have identified more than 100 new limonoids from *Xylocarpus* plants collected in south China and India. The structures of these compounds were elucidated by NMR spectroscopic method combined with single-crystal X-ray diffraction techniques. The absolute configurations of limonoids with a new carbon skeleton were established by circular dichroism quantum chemical calculations. Antifeedant, insecticidal, and antitumor activities of some limonoids are reported.

011.3

MULTIDISCIPLINARY ASSESSMENT OF WIKSTROEMIA ENDEMIC SPECIES (THYMELAEACEA) OF EASTERN POLYNESIA

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Wikstroemia genus (Thymelaeaceae family), used in traditional medicine in Asia and Pacific areas, is well known to possess various interesting therapeutic properties (such as cytotoxicity, anti-mitotic, anti-inflammatory, anti-oxidant, antiviral, antifungal, antimalarial...) and also to contain many bioactive components.

Three endemic species of *Wikstroemia* are found in French Polynesia: *W. coriacea* (endemic of Eastern Polynesia), *W. raiateensis* (endemic of Raiatea Island) and *W. johnplewsii* (endemic of Hiva Oa island). We report herein a first investigation to establish their chemical composition and pattern. Eleven constituents belonging to different metabolite classes (sesquiterpenoids, lignans, triterpenoids, biflavonoids, coumarin and phenyl derivatives) were identified including the new natural compounds, oleodaphnoic acid, 2-hydroxy-1,5-diphenylpentan-1-one and 3-hydroxy-1,5-diphenylpentan-1-one. Comparison of the chemical profile of these species put in evidence the chemodiversity of these three endemic species which could be also discriminated by some morphological traits and genetic patterns. These findings will be helpful to solve remaining taxonomic confusion among *Wikstroemia* species from French Polynesia.

011.4

THE OXIDATIVE IN VITRO METABOLISM OF LAPACHOL, CHARACTERIZED BY BIOMIMETIC MODELS

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Lapachol, a natural naphthoquinone, has been demonstrated in the past to have a great number of biological properties, such as activity against enterovirus and *Trypanosoma cruzi*. Envisioning future therapeutic applications, twelve potential oxidation metabolites were therefore generated *in vitro* by means of different oxidizing agents with the Jacobsen catalyst or metalloporphyrins. Thereafter, characterized derivatives were compared to lapachol with isolated rat liver microsomes. Acknowledgements: To FAPESP, CAPES and CNPg for the financial support.

S12.1

COMPLEX METABOLIC PATHWAY GENE DISCOVERY VIA TRANSCRIPTOME ANALYSIS

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The evolution of DNA sequencing technologies has changed the ways in which we address complex biological questions. Due to greater data output of these technologies and advances in assembly algorithms, de novo transcript assembly and analysis are now routine for previously uncharacterized plant transcriptomes. Numerous plant species are considered to have medicinal, herbal or health protective value. Most of the underling beneficial compounds have complex chemical structures and biochemical pathways. Many of these biochemical pathways and their genes, however, are poorly characterized. Assembly and analysis of RNA sequencing (transcriptome) data present unique classes of problems. An over-arching philosophy for addressing these problems has been developed to address both experimental design and data analysis. We utilize hybrid assembly and alignment techniques in which we apply different algorithms either in parallel or sequentially to build on the strengths of different underlying algorithms. Our primary assemblers are a combination of ABySS, Velvet, and SSAKE for short read data, and CAP3, Mira, and Phrap for processing longer sequences. Our goal is to generate the needed infrastructure to help establish operative biochemical pathways to their compounds, including that of gaining critical knowledge as to cell/tissue specificities and regulatory processes. For each species reported, a reference sequence has initially been established through deep, Illumina RNA sequencing and assembly of target tissue RNAs. Expression profiling and genetic variant analysis of specific tissues/developmental stages using Illumina RNA-Seq is being performed. These analyses include above and below ground tissues that generally have distinct natural product profiles, together with harvesting of cell types (in a limited number of cases) that harbor compounds of interest. Application of next-generation DNA sequencing technologies to the characterization of plant transcriptomes will be reported. Results of data generation, de novo assembly and analysis will be discussed.

S12.2

GENOMICS APPROACHES FOR BIOCHEMICAL PATHWAY DISCOVERY IN MEDICINAL PLANT SPECIES

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Medicinal plants produce a wide range of compounds of pharmaceutical interest. The great structural diversity and biological activities of plant-derived compounds suggest that additional relevant compounds remain to be discovered. However, the secondary metabolic pathways that produce such compounds are poorly understood. To address this problem, whole transcriptome sequencing using next generation sequencing methods used Medicinal Plant was bv the Consortium (http://medicinalplantgenomics.msu.edu/) to generate the transcriptomes of 14 medicinal plant species for which reference genomes are not available. Our genomics and bioinformatics approach integrates transcript abundance data with functional annotation for these species to identify genes in key biochemical pathways associated with the synthesis, transport and accumulation of target compounds. We have sequenced an array of cDNA libraries and assembled the transcriptome using the de novo short read assembler Velvet/Oases. Pseudo-reference sequences were constructed from unigenes that represent the transcriptome. To estimate the transcript abundance, single end reads were mapped to the pseudo-reference sequence and expression levels were quantified. Functional annotation of the unigenes, coupled with downstream analyses of "expression matrices" for each species in conjunction with metabolomics data, has enabled the identification genes in biochemical pathways related to the medicinal compounds of interest.

S12.3

DE NOVO ASSEMBLY OF EXPRESSED TRANSCRIPTS AND CONSTRUCTION OF A TRANSCRIPTOME DATABASE OF *PHALAENOPSIS APHRODITE*

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Orchids are one of the largest families in Angiosperm with more than 25,000 species, displaying a wide diversity of unique and interesting biological features. Molecular and genetic research studies of orchids are hindered due to limited genomic information available in current databases. Recent advances in high throughput DNA sequencing technology make it economically feasible to generate orchid genomic information. We have designed an effective workflow to fast accumulate genomic information for these non-model organisms in order to facilitate their genome research. We used 454 GS FLX Titanium and Illumina Genome Analyzer IIx platforms to sequence cDNAs prepared from different organs of *Phalaenopsis aphrodite*. After quality trimming and assembly pipeline, 246,242 contigs were obtained, with 43,358 annotated to protein coding genes. Designed into the workflow is to achieve both gene discovery and expression profiling with the same sequencing efforts of Solexa reads. The expressed genes were blasted to database and found their functional identities in Pfam, Gene Ontology and KEGG. All datasets and analysis results were organized in a user-friendly manner in the Orchidstra website (URL: http://orchidstra.abrc.sinica.edu.tw).

S12.4

TRANSCRIPTOME PROFILING OF *PODOPHYLLUM HEXANDRUM* TISSUES FOR GENES IN PODOPHYLLOTOXIN BIOSYNTHESIS

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The roots and rhizomes of *Podophyllum hexandrum* have been used for decades as a source of the aryltetralin lignan podophyllotoxin, precursor to drugs used to treat several types of cancer. Yet in spite of its extensive use in medicine, much of the biosynthetic pathway to podophyllotoxin is mostly speculative. In an effort to further our understanding of its biosynthetic pathway, we have taken advantage of recent developments in gene sequencing technologies, as part of a broader program to study several medicinally important plant species. (To date we have examined 24 important medicinal plant species, see http://uic.edu/pharmacy/MedPlTranscriptome/index.html for transcriptome/metabolome data). Using Illumina sequencing approaches and various gene assembly construction strategies, we have, for example, generated a transcriptome database using different *Podophyllum* tissues and are mining these to identify the candidates responsible for the putative steps in the podophyllotoxin biosynthetic pathway. This approach has been initially successful first in identifying (and confirming assembly protocols for) all potential gene family members in this species for known biochemical steps, as well as for identification of putative unknown upstream enzymes in the phenylpropanoid pathway. Progress in identifying the missing steps (methylenedioxy bridge formation etc) in podophyllotoxin biosynthesis is described. This work is supported by 5 RC2 GM092561-02

S12.5 ARTHUR NEISH YOUNG INVESTIGATOR AWARD INSIGHTS INTO STORAGE OIL BIOSYNTHESIS: COMPARATIVE TRANSCRIPTOMICS OF SEED AND NON-SEED TISSUES

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Storage oils in the form of triacylglycerols (TAGs) in seeds serve as a high-energy carbon resource for post-germinative growth whereas they provide a source of food for seed dispersers when they occur outside the seed such as mesocarp. Using high-throughput deep transcriptional profiling tools, we generated 10 million ESTs for various developing seed and non-seed tissues. I will present insights from analysis of the similarities and differences in lipid gene expression and regulation in developing seed (rapeseed and castor) and non-seed (mesocarp of oil palm and avocado) tissues.

S12.6

FUNCTIONAL GENOMICS USING NON-MODEL PLANTS AND SYNTHETIC BIOSYSTEMS FOR GENE DISCOVERY IN SPECIALIZED METABOLISM

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Among the vast catalogue of plant natural products are ~2500 benzylisoquinoline alkaloids (BIAs) that include codeine and morphine produced in opium poppy. cDNAs corresponding to most of the enzymes involved in morphine biosynthesis and several functioning in other branch pathways have been identified. The first committed step in BIA metabolism, norcoclaurine synthase, is catalyzed by a unique enzyme. All other known enzymes belong to a limited number of families including cytochromes P450, *O*- and *N*-methyltransferases, FAD oxidoreductases, dioxygenases, acyltransferases and three different types of reductases. Tapping into the enzyme variants responsible for the immense diversity of BIAs requires the generation of resources for a variety of plant species and the development of tools that provide a common functional genomics platform. The PhytoMetaSyn Project (www.phytometasyn.com) represents a consortium of Canadian researchers advancing the application of genomics and synthetic biology to the discovery of novel natural product biosynthetic genes and the reconstitution of pathways in microbes. The tools generated are allowing the discovery of novel BIA biosynthetic genes using plug-and-play synthetic biology based on candidate genes in our deep transcriptome databases, and complement our plant functional genomics tools such as virus-induced gene silencing.

O12.1 TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS OF REED (*PHRAGMITES AUSTRALIS*) RHIZOMES

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The common reed (*Phragmites australis*), one of the most widely distributed of all angiosperms, uses its rhizomes (underground stems) to invade new territory, making it one of the most successful weedy species worldwide. To identify candidate genes and proteins involved in rhizome growth, development and metabolism, we employed next-generation sequencing and quantitative proteomics technologies to characterize the reed rhizome transcriptome and proteome. Combining 336,514 Roche 454 Titanium reads and 103,350,802 Illumina paired-end reads in a hybrid assembly yielded 124,450 unique transcripts with an average length of 549 bp, including 31,675 contigs (average length 1,283 bp) and 92,775

singletons, of which 54,202 were annotated. Rhizome-specific and differentially expressed transcripts were identified between rhizome tips (apical meristematic region) and rhizome elongation zones. A total of 1,280 non-redundant proteins were identified by label-free proteomics coupled to GeLC-MS/MS, of which 174 and 77 proteins were up regulated in the rhizome tip and elongation zone tissues, respectively. Identification and characterization of specific genes with potential roles in rhizome differentiation, development and function will be discussed.

012.2

DISCOVERY OF DITERPENE BIOSYNTHETIC PATHWAYS USING TARGETED TRANSCRIPTOME ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF GENES AND ENZYMES FOR METABOLIC ENGINEERING

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Plant diterpenoids are well characterized for their diverse physiological functions and many specialized (i.e., secondary) diterpenoids are also of substantial value as pharmaceuticals and other industrial products. Therefore, plants are perhaps the best known renewable resource for diterpene natural products, including compounds for the fragrance industry (e.g., *cis*-abienol, sclareol), precursors for industrial resins and coatings (e.g., conifer diterpene resin acids), and a plethora of pharmaceuticals with a wide range of applications as anti-cancer drugs (e.g., taxol), anti-inflammatory agents (e.g., marrubiin), or antimicrobials/antifungals (pseudolaric acids). The goal of our research is the discovery of enzymes for the production of a suite of diterpenoid compounds in metabolically engineered microbial host systems. For a set of ten different plant species we characterized the diterpenoid metabolite profiles, followed by tissue-specific 454- and Illumina transcriptome sequencing. Sequence databases were established for the discovery and functional characterization of diterpene biosynthetic pathway genes, targeting new diterpene synthases and cytochrome P450 monooxygenases. We will present the overall strategy for the rapid and successful genomics-based gene discovery of various diterpenoid biosyntheses and highlight specific examples of the functional characterization of enzymes, and details of reactions mechanisms gleaned from structural analyses.

012.3

INVESTIGATING ESSENTIAL OIL METABOLISM IN LAVANDULA BY TRANSCRIPT PROFILING

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Several members of the genus *Lavandula* (lavenders) produce valuable essential oils (EO), which are extensively used in cosmetics, hygiene products and medicines. These oils are predominantly constituted of monoterpenes, the C_{10} class of the isoprenoids. Although numerous genes encoding monoterpene synthase enzymes have been described from a wide range of plants including gymnosperms and angiosperms, regulation of production and secretion of EO constituents is still poorly understood. We are investigating EO metabolism in lavender glandular trichomes, and have generated extensive genomics resources for these plants. We obtained over 22,000 expressed sequence tags (ESTs) from flowers, leaves and glandular trichomes, and assessed their expression pattern in various tissues of three lavender species by microarrays. Our investigation led to the cloning and functional characterization of two EO biosynthetic enzymes. We also identified putative genes that control trafficking of EO constituents in glandular trichomes. Further, our data confirmed that the biosynthesis of EO constituents is regulated at multiple levels, including transcriptional control of the terpene synthase genes.

TRANSCRIPTOME AND METABOLITE ANALYSIS OF POLYUNSATURATED FATTY ACID-RICH SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*) SEED

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Sea buckthorn (*Hippophae rhamnoides* L.) is a hardy, fruit producing, plant known historically for its medicinal and nutraceutical properties. The most recognized product of sea buckthorn is its seed and pulp oil. Sea buckthorn is fast gaining popularity as a source of functional food and nutraceuticals, but currently has few genomic resources; therefore, we explored the fatty acid composition of Canadiangrown cultivars (ssp. mongolica) and the seed transcriptome using 454 sequencing. GC-MS profiling of fatty acids in seeds and pulp of berries indicated that the seed oil contained linoleic and α -linolenic acids at 33-36% and 30-36%, respectively, while the pulp oil contained palmitoleic acid at 32-42%. 454 sequencing of sea buckthorn cDNA collections from mature seeds identified sequences related to fatty acid biosynthesis. A subset of these was examined for transcript expression at four developing stages of the berry. This study provides the first comprehensive genome sequences for sea buckthorn, and demonstrates that the seed oil of Canadian-grown sea buckthorn cultivars contains high levels of linoleic acid and α -linolenic acid in a close to 1:1 ratio, which is beneficial for human health. These data provide the foundation for further studies on sea buckthorn oil, the enzymes involved in its biosynthesis, and the genes involved in the general hardiness of sea buckthorn against environmental conditions.

012.5

METABOLITE AND GENE EXPRESSION STUDIES IN ENDOPHYTE INFECTED AND UNINFECTED TALL FESCUE UNDER WATER DEFICIT STRESS

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Tall fescue plants symbiotic with the endophytic fungus, *Neotyphodium coenophialum* (E+), have better survivability and persistence under stressful conditions, especially under drought stress, than plants lacking the endophyte (E-). To understand more about the grass-endophyte interactions, how endophyte affects the host plant physiology and gene expression especially when the plants are subjected to water deficit stress conditions, we conducted a time course water deficit stress experiment using 3 clone pairs of tall fescue. Upon rewatering, survival and retillering was significantly greater for E+ than E- plants starting from day 2 or 3 of the treatment. We observed higher accumulation of the free sugars like glucose, fructose, trehalose, and amino acid proline in E+ plants at early days of onset of stress compared to E- plants. Loline alkaloids and mannitol, which are fungal metabolites, also increased with water deficit stress. Thus endophyte aids in survival and recovery of plants from drought, and may act in part by inducing rapid accumulation of these compatible solutes, soon after imposition of stress. Illumina mRNA sequencing of these E+ and E- clones shown 125 unigenes were differentially expressed two-fold or more between them. Sequencing of these E+ and E- stressed as well as watered controls tissues, is in progress to see any specific effects of endophyte on plant gene expression especially under water deficit stress.

A GENOMICS APPROACH TO GENE DISCOVERY RELATED TO BIOSYNTHESIS OF THUJONE IN WESTERN REDCEDAR (*THUJA PLICATA*)

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Reforestation with *T. plicata* is severely hampered by extensive ungulate browsing of plantlets. High foliar monoterpenoid content correlates with reduced browsing, providing a target for resistance breeding. The most abundant monoterpenoids in T. plicata foliage are α - and β -thujone, both of which strongly deter ungulate browsing. We found that these compounds are stored in foliar resin glands. Thereafter, we used next generation DNA sequencing technology to compare transcript profiles of foliage with and without glands to identify >600 genes those expression associated with these structures. The differential expression was confirmed for the majority of tested genes by RT-Q-PCR. One of the most differentially expressed genes encodes a putative monoterpene synthase. *In situ* RNA hybridization showed that this gene is expressed in the epithelium of foliar resin glands. Furthermore, *in vitro* enzyme assays showed that the corresponding protein converts geranyl pyrophosphate almost entirely into sabinene, a known precursor of thujone synthesis. We are currently assessing whether candidate genes for the conversion of sabinene into thujones, primarily cytochrome P450s and reductases, are also expressed in gland epithelium.

012.7

EST ANALYSIS OF *TRANS*-RUBBER PRODUCING PLANT, *EUCOMMIA ULMOIDES* OLIVER AND IDENTIFICATION OF CANDIDATE GENES IN *TRANS*-1,4-POLYISOPRENE PRODUCTION

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Eucommia ulmoides Oliver is one of the few woody plants producing abundant *trans*-polyisoprene rubber in leaf, bark and seed coat. Two cDNA libraries derived from the bark and inner tissue in the stem were constructed and total of 27752 expressed sequence tags (ESTs) including 10520 unigenes consisting of 4302 contigs and 6218 singlets were generated. Homologues of major latex protein (MLP) and rubber particle membrane proteins (RPMPs) which function for effective synthesis of high molecular poly-isoprene in latex were isolated and shared high proportions in the ESTs, indicating abundant expression for *trans*-polyisoprene rubber biosynthesis. The six MVA pathway genes in which synthesize isopentenyl diphosphate (IPP), a starting material of poly-isoprene biosynthesis, were isolated and functioned for IPP biosynthesis. Five full length trans-isoprenyl diphosphate synthase were also isolated and two of them functioned to synthesize farnesyl diphosphate which is assumed as an intermediate of rubber biosynthesis.

COMPARATIVE PROTEOMIC STUDY REVEALS THE BIOSYNTHESIS OF COUMARINS IN LEAVES OF *CLEMATIS TERNIFLORA* UPON UV RADIATION

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Phytochemical studies have been carried out to reveal the effects of UV radiation on leaves of *Clematis terniflora* DC. Three coumarins were prominently induced by UV-B radiation and could not be detected in control and UV-A treatment groups. Harmful effects of UV-B radiation were found on the surface of leaves. A comparative proteomic research method has been used to study the different expression levels of proteins under high intensity (120.8 μ w/cm²) UV-A and UV-B radiation. Seventy three differentially expressed proteins were identified by MALDI-TOF/TOF MS. Functions of the successfully identified sixty three proteins that mainly focus on photosynthesis and respiration, transporting, amino acid biosynthesis, secondary metabolism, defence and stress responses, carbohydrate metabolism, energy metabolism and other categories. Results showed that proteins which were involved in the secondary metabolic pathway of phenylpropanoid biosynthesis were significantly stimulated. These proteins are very important in the UV-B induced production of coumarins. This study helps to increase our understanding of the comprehensive functional network *Clematis terniflora* uses to adapt to UV-A and UV-B stress. More importantly, this opens up new areas for the exploration of the changes in plant secondary metabolic pathways under UV radiation.

S13.1 BIOPOLYMERS, BIOPRODUCTS AND BIOFUELS IN ALTERNATE RUBBER-PRODUCING SPECIES.

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Efforts are underway to move sustainable biorefineries, powered with renewable fuels, from a subsidized concept to commercial reality. The production of rubber, resins, bioproducts and bio-fuels from *Parthenium argentatum*, or rubber, inulin and biofuels from *Taraxacum kok-saghyz*, are both attractive to a true biorefinery capable of producing a wide range of phytochemicals. A combination of products and fuels, from single feedstocks, is needed for profitability. However, as both are new industrial crops, scale-up issues are extremely complex, and species-specific chemistry and biochemistry must be understood and capitalized upon. The expansion of acreage must be intimately tied to expansion of processing capacity and to specific markets.

S13.2

ADVANCES IN UNDERSTANDING PLANT CELL WALL PECTIN SYNTHESIS AND STRUCTURE AND IMPACT ON THE BIOFUEL INDUSTRY

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Plant cell walls are comprised of cellulose, so-called matrix polysaccharides (pectin and hemicellulose), cell wall proteins, and in many secondary walls, lignin. Among these, pectin is the most structurally complex polysaccharide and is often considered as mainly associated with primary cell walls, although recent results indicate an important role in secondary walls as well. Studies in our group on pectin structure and synthesis have centered on the potential function of pectin in the recalcitrance of plant biomass to deconstruction for biofuel production and on the role of a family of glycosyltransferases known as GAUTs in pectin synthesis. The results suggest that pectin may be more realistically viewed as a polysaccharide domain within a complex glycoconjugate matrix. The role of GAUTs in the synthesis of pectic domains and implications for engineering plants for enhanced biofuel production will be discussed.

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S13.3

PECTIN MODIFICATION IMPROVES UTILIZATION OF PLANT BIOMASSES TO BIOFUEL CONVERSION

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Cell wall recalcitrance to enzymatic hydrolysis is the main bottleneck for the industrial scale-up of biomass processing and bioconversion to fermentable sugars. We aimed to overcome the difficulties of converting plant biomass into usable products by genetically engineering or selecting plants with altered expression of proteins that help either break down the components of the cell wall or prevent the cell wall polysaccharides from forming crosslinks. Pectin acts as a glue by affecting cell wall stiffening through homogalacturonan (HGA) calcium-mediated cross-links. Saccharification efficiency of dicot and monocot biomass can be improved by reducing the amount of acidic HGA domains through the constitutive expression of a fungal polygalacturonase (PG) or the overexpression of an inhibitor of pectin methylesterase (PMEI). We are also exploring the possibility to improve cell wall degradation without causing growth defects through the controlled expression of pectin-degrading enzymes. Saccharification is also improved in *Arabidopsis* mutants with a lower content of de-methylated stretches of HGA as compared to the wild type, indicating that the level of unesterified HGA is a useful parameter to isolate natural variants with improved saccharification efficiency. Understanding the biochemical and genetic determinants of cell wall degradability will be useful to identify markers for the breeding of new varieties suited for the dual food and bioenergy production

S13.4

ENGINEERING IMPROVED CELLULASES FOR BIOFUELS PRODUCTION

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The *Trichoderma reesei* Families 6 and 7 cellulases (Cel6A and Cel7A) are key industrial enzymes used for the production of biofuels from lignocellulosic biomass. These enzymes are multi-modular, with a Family 1 carbohydrate-binding module linked to a large catalytic domain via a flexible O-glycosylated linker. We have used simulation to elucidate new functions for these three sub-domains in general, and have demonstrated a new route to increase the activity of Cel7A. These findings include new roles for glycosylation, which we have shown can be used to tune the binding affinity. We have also examined the structure of the catalytically-active complex of Cel7A and its non-processive counterpart, Cel7B, engaged on cellulose, which suggests allosteric mechanisms may be involved in chain binding when these cellulases are complexed on cellulose. Our computational results also suggest that product inhibition varies significantly between Cel7A and Cel7B, and we offer a molecular-level explanation for this observation. These results highlight new considerations in protein engineering for processive and non-processive cellulases.

S13.5

ENGINEERING PLANT CELL WALLS FOR SECOND GENERATION BIOFUEL PRODUCTION

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The substrate for second generation biofuels is lignocellulosic material obtained from plant cell walls. Genetic modification of the cell wall has the potential to improve cellulose accessibility and hydrolysis, therefore decreasing the cost and energy input in biofuel production. This study aims to improve understanding of cell wall biosynthesis and organisation to increase cellulose content and extractability by genetic modification and pretreatment with white rot fungus *Phanerochaete chrysosporium*.

Enzymatic saccharification assays have shown differences in soluble sugars released from transgenic tobacco lines down-regulated in both lignin and xylan. Significantly, *TOBACCO PEROXIDASE 60* down-regulated line 1074 shows 30% increase in glucose release as compared to the wildtype. Xylan down-regulation by suppression of *UDP-GLUCURONATE DECARBOXYLASE*, which synthesises the xylan precursor xylose, also caused improvement in saccharification. Treatment of the cell wall modified lines with *P. chrysosporium, a* white rot fungus that naturally hydrolyses and metabolises lignin further improved saccharification after pretreatment.

We also show that lignin biosynthesis pathway is down-regulated at the transcriptional level in lignin modified lines, while the polysaccharide biosynthesis response differs depending on the position of disruption in lignin biosynthesis.

S13.6

COMMON THEMES IN LIGNIN BIOSYNTHESIS AND LIGNIN BIODEGRADATION

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The primary structures of lignins may be replicated through a direct template polymerization mechanism during the final step of lignin biosynthesis. The process is governed by powerful forces arising from electron correlation between precursor aromatic rings and the pre-existing substructures about to undergo replication. Correspondingly, the enzymatic degradation of lignin macromolecules requires two consecutive steps. After enzyme-catalyzed cleavage, dissociation between individual lignin fragments and components may be facilitated by other proteins that can compete with the strong noncovalent interactions remaining between the oligomers and polymeric lignin chains.

S13.7

LIGNIN: THE NEW PARADIGM IN BIOFUELS

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The biological conversion of biomass to biofuels is a multi-faceted technical challenge that has focused on the basic structure of the plant cell wall and its chemical components. Although the native recalcitrance of biomass is attributed to many factors including cellulose crystallinity/degree of polymerization, lignin/hemicellulose structure, lignin-carbohydrate complexes, cell wall structure and accessibility, recent advances in transgenic plants have highlighted the special role that lignin holds on recalcitrance. By down-regulating select monolignol pathways it has been possible to significantly decrease recalcitrance and improve the overall efficiency of cellulosic ethanol by +20%. Research studies in lignin structure for transgenic plants have shown that the observed reduction in recalcitrance for lignin-lite transgenic plants is due to reductions in lignin content and structure. Furthermore, pretreatment a key technology in 2nd and 3rd generation biofuels significantly alters the structure of lignin and facilities biological deconstruction of plant polysaccharides. This presentation will integrate our investigations into the structure of lignin pre and post pretreatment for native and transgenic biomass with a special emphasis on the role of β -O-aryl ethers, condensed and non-condensed lignin structures as determined by 1D and 2D NMR. Finally, lignin to date remains an under-utilized biofuels resource in itself especially from cellulosic ethanol technologies. Understanding its structure after pretreatment has facilitated its utilization as a feedstock resource for heterotrophic oleaginous microorganism leading to the generation of triacylglycerols which opens new avenues for securing biodiesel from lignocellulosics.

S13.8 METABOLIC VERSUS TRANSCRIPTIONAL CONTROL TARGETS FOR LIGNIN MODIFICATION

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Lignocellulosic biomass is recalcitrant to saccharification, and this is, at least in part, due to the presence of the cell wall polymer lignin. Analysis of alfalfa (*Medicago sativa*) plants in which lignin content and composition had been modified through independently down-regulating each of eight enzymes in the lignin pathway revealed that reduction in lignin content progressively increased saccharification efficiency. However, the gains in fermentable sugar production are partially offset by reductions in plant yield in some, but not all, of these transgenic lines. Levels of the stress hormone salicylic acid (SA) inversely mirror lignin levels in a series of transgenic alfalfa plants, and genetic experiments in *Arabidopsis* point to SA production as being responsible for most of the reduced growth phenotypes of lignin down-regulated plants. Targeting transcriptional regulators of the lignin or whole secondary cell wall pathways provides an alternative strategy for lignin down-regulation that potentially avoids metabolic spillover effects. Forward genetic screening of a transposon mutagenized population of barrel medic (*Medicago truncatula*) has led to the identification of NAC and WRKY master switches that work as positive and negative regulators of lignification, respectively. Loss of function of WRKY12 in *Arabidopsis* leads to a 50% increase in stem biomass density. Manipulation of lignin biosynthetic and regulatory genes has potential to deliver bioenergy feedstocks or forages combining increased cell wall density with improved digestibility.

S13.9

CHARACTERIZATION OF LIGNIN AND RELATED COMPOUNDS OF ERIANTHUS RAVENNAE

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Recently, plant biomass such as *Erianthus*, switchgrass, *Miscanthus*, *Jatropha* and oil-producing algae have been receiving a lot more interest as chemical and biofuel feedstocks. Liquid fuels from the plant biomass or lignocellulosic materials offer an attractive alternative to fossil fuels. Lignocellulosic biomass is composed of cellulose, hemicelluloses and lignins. The three components constitute a suprastructure, where lignin encrusts cellulose microfibrils and confers mechanical strength and imperviousness to the cell wall. At the same time, lignins are obstacles in the enzymatic conversion of plant cell wall polysaccharides into biofuels. However, little is known about lignins of energy-producing plants, especially *Erianthus ravennae*. Recently, we established a microscale, high-throughput, and highly reproducible nitrobenzene oxidation method. We have successfully applied the method as well as a number of analytical methods to the analysis of *E. ravennae*, thereby lignins, related compounds, and enzymatic saccharification efficiency of the plant have been characterized.

S13.10

NORTHWEST ADVANCED RENEWABLE ALLIANCE (NARA) AND THE QUEST FOR BIOFUELS/PETROCHEMICAL SUBSTITUTES

Norman G. Lewis (Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA; lewisn@wsu.edu)

Vascular plants have evolved remarkable phytochemical armouries, not only from a lignocellulosic recalcitrance perspective but also through specialized metabolism leading to a variety of bioactive/defense molecules. Many countries now wish to explore the potential of sustainably using these renewable (woody) lignocellulosic materials as "feedstocks" for liquid fuels and petrochemical substitutes at the scale and cost needed.

The Northwest Advanced Renewables Alliance has been created to establish the feasibility of using various woody biomass (Douglas-fir, western hemlock, poplar and red alder) for such applications and uses; this presentation focuses upon the progress made thus far in genetically modifying model plant (*Arabidopsis*) and poplar in efforts to overcome lignocellulosic recalcitrance through gene downregulation/transcription factor modulation/introduction of new biochemical pathways.

O13.1 BIOPOLYMER PRODUCTION IN TRANSGENIC POPLAR

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Poplar is an attractive model species for the production of commercially promising biopolymers such as polyhydroxybutyrate (PHB). A PHB-producing system was developed in poplar based on the introduction of three bacterial transgenes under the control of an ecdysone-inducible promoter. The negative impacts of PHB production on plant health were minimized by targeting of PHB synthesis to the chloroplast and delaying production until application of an inducing chemical - the licensed insecticide Intrepid. PHB concentrations up to 1-2 % (dry weight) were detected in leaves of 18 separate events after 6-8 weeks of application of the inducing chemical. There was no direct toxicity due to the inducing agent, but growth (mass and height) was reduced substantially in plants in which the concentration of PHB exceeded 1%. PHB granules were visualized within chloroplasts using confocal fluorescence microscopy and found to measure 3.6 μ m in length. Ongoing efforts to develop new senescence-associated promoters that might enable enhanced PHB yields will be discussed.

013.2

NAC DOMAIN TRANSCRIPTION FACTORS AND SECONDARY WALL FORMATION CONTROL IN POPLAR (POPULUS TRICHOCARPA)

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The use of woody plants, such as poplar, as bioenergy/bioproduct plantation feedstock is limited, due in part to lignocellulosic recalcitrance that impedes facile fermentation/saccharification into products suitable as scalable petrochemical replacement/intermediate commodity chemicals. To begin to consider new ways to overcome this form of recalcitrance, it was recently discovered that NST/SND and VND subgroups of NAC domain transcription factors can function as "master switches" for fiber and vessel secondary wall formation in the model plant, *Arabidopsis*. This raised the possibility of engineering woody plants more amenable to lignocellulosic processing. Accordingly, genes encoding poplar NAC TFs (PtNAC1, PtNAC6, PtNAC7, PtNAC13 and PtNAC17) were initially studied for their abilities to restore secondary wall formation in the *Arabidopsis* SND1/NST1-RNAi double mutant. Overexpression of all five PtNACs in the double mutant rescued secondary wall formation in the fiber cells and restored lignin contents to near WT levels as well, as determined by comprehensive histochemical and chemical analyses. These findings suggest that all five PtNAC are functional (potentially) redundant TFs involved in poplar secondary wall formation and lignin biosynthesis. In-depth functional analyses using overexpression and silencing of five poplar PtNAC TFs are also currently underway with transgenic poplars.

013.3

INTERACTIONS BETWEEN THE CO₂ CONCENTRATING MECHANISM AND LIPID PRODUCTION OF TWO SPECIES OF ALGAE: CHLAMYDOMONAS REINHARDTII AND NANNOCHLOROPSIS SALINA

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The demand for energy is rapidly depleting available resources and creating a need for renewable energy. Wind and solar power are valuable sources of electricity, but they do not generate energy dense compounds needed for long distance uses such as long-haul trucking and air transportation. Algal lipid production may help to solve these needs without impacting food sources. However, many fundamental

questions remain unanswered in algal biology and this is impairing development of viable algal lipid production systems. We have chosen to examine the basic research question of how algal CO_2 concentrating mechanism (CCM) function affects lipid production. CCM function is often induced at low CO_2 and it increases the efficiency of CO_2 capture, though the allocation of carbon under these conditions is not well understood. We analyzed lipid content and CCM function at low CO_2 (CCM expressed) and high CO_2 (CCM suppressed) to determine if CCM expression increases or decreases lipid storage. Our measurements of online carbon isotope discrimination showed that high CO2 suppressed the CCM in both *C. reinhardtii* and *N. salina*. Our preliminary discrimination data also provided evidence for an unusual HCO_3^- pump- CO_2 leak style CCM in *N. salina* that was first characterized in other species of *Nannochloropsis*. In addition, our preliminary data using a hyperspectral imaging suggest lipid content increases in both species after exposure to high CO_2 . However, the effect is smaller in *C. reinhardtii* and much of the lipid accumulation occurred in the eyespot. **Poster Session Abstracts**

NPA 1

UMU APPLIED FOR SCREENING HERB AND PLANT EXTRACTS OR PURE PHYTOCHEMICALS FOR ANTIMUTAGENIC ACTIVITY

<u>Monique Lacroix</u>, Stéphane Caillet, Stéphane Lessard (INRS-Institut Armand-Frappier, Laval, Quebec H7V1B7, Canada.)

Antimutagenic activities of twelve herb extracts and twenty two plant extracts or pure phytochemicals assessed using a method based on the umu test system for screening natural antimutagens. All herb extracts tested showed antimutagenic properties except for Italian parsley that had mutagenic activity. Sage, mint, vervaine and oregano were the most antimutagenic. With regard to the metabolites, those from most herb extracts showed antimutagenic properties and those from garlic and thyme showed very strong antimutagenic activities, while those from camomile, rosemary and tarragon showed mutagenic activities, and those from celeriac and sage showed very strong mutagenic activities. Among pure compounds, pycnogenol metabolites showed strong antimutagenic activities.

NPA 2

INSECTICIDAL ACTIVITY OF DERRIS MALACCENSIS FROM FRENCH POLYNESIA

<u>Heinui Philippe</u>,¹ Taivini Teai,¹ Maurice Wong,² Christian Moretti,³ Phila Raharivelomanana¹ (¹Université de la Polynésie Française, Laboratoire BIOTEM, Faa'a, 98702, French Polynesia, ²Service du Développement Rural, Papeete, 98713, French Polynesia, ³Institut de Recherche pour le Développement, Papeete, 98713, French Polynesia.)

Derris malaccensis (G. Bentham) D. Prain, a tropical member of the Fabaceae growing in French Polynesia, was investigated to determine concentrations of metabolites (rotenoids and flavonoids) with pesticidal potential. Comparison of chemical composition of the different plant parts confirmed the prevalence in the roots of rotenone, a rotenoid used as a pesticide and in phytopharmacy. The root extract of D. malaccensis exhibited insecticidal activity against major pests in French Polynesia such as aphids (Toxoptera spp.), and the ant species Monomorium destructor and Wasmannia auropunctata (little fire ant). W. auropunctata, in particular, is an invasive ant known for its painful stings and impact on the environment; as a result, it is a great nuisance to humans in agriculture areas. The little fire ant is classified among the 100 worst invasive alien species in the world, and could become the greatest ant species threat in the Pacific area.

NPA 3

REPELLENCE OF ESSENTIAL OILS TO FRANKLINIELLA OCCIDENTALIS AS AFFECTED BY TYPE OF OIL AND POLYMER RELEASE

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Eight essential oils (at 0.125-1.0% V/V in acetone) were separately deposited on leaf disks to evaluate their potential to repel Western flower thrips [Franliniella occidentalis (Pergande] adult females (WFT). The proportion of thrips counted on control leaf disks in choice bioassays was used as the measure of repellence. The most repellent essential oils were incorporated into polymer matrices i.e. methylcellulose or alginate (0.5 or 1% W/V) in order to verify the potential of the polymer to extend repellence over time (24-120 h). At a concentration of 0.5%, Thymus vulgaris and Satureja montana were the most repellent essential oils. For these treatments, no WFT were counted on treated leaf disks 60 minutes after the start of the test. Thymus serpyllum and Origanum compactum also showed repellence values of 0.9 at this concentration. When S. montana and T. serpyllum were incorporated within polymer matrices, close to 100% repellence was achieved even after 48 hours regardless of the concentration of the alginate polymer used (0.5 or 1%). This level of repellence was maintained for 3 days in the presence of T. serpyllum and for 4 days in the presence of S. montana. Results also showed that the alginate based-coating was repellent by itself.

NPA 4

INHIBITION OF *POTATO VIRUS X* INFECTIVITY BY EXTRACTS OF GREEN TEA

<u>Christopher French</u>, Michel Bouthillier (Agriculture & Agri-Food Canada, Pacific Agri-Food Research Center, Summerland, BC VOH 1ZO, Canada.)

Infectivity of *potato virus X* in a bioassay using *Chenopodium quinoa* was strongly inhibited by preincubation of the purified virus with aqueous solutions of five commercial leaf extracts of green tea, *Camellia sinensis*. Similar results were obtained using equivalent concentrations of the compound epigallocatechin-3gallate (EGCg), suggesting that this was the active compound in the extracts. This is the first report of antiviral activity of green tea extracts and EGCg on PVX. Natural products, such as green tea extracts, have the potential for development as low toxicity, antiviral, disinfectants in agricultural applications. NPA 5

ANALYSIS OF GUAVA AS A FORAGE - ORGANIC CONSTITUENTS

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This study was designed to determine the nutrient concentration of guava, Psidium guajava L., tree components. The analysis compared various tree tissues, seasons and locations. Nutritional analyses of guava tree parts suggest that guava tree shoots had the highest protein concentration (16.8% CP), while branches were relatively low in protein concentration (2.9% CP). Guava tree branches were high in fiber (65.9% ADF, 76.5% NDF), while the shoots (27.4% ADF, 37.1% NDF) and bark (27.1% ADF, 33.2% NDF) had the least fiber. Leaves were highest in hemicelluloses (11.3%), while the bark (6.1%), immature fruit (6.1%), breaker stage fruit (5.8%) and ripe fruit (5.6%) had the least. Total digestible nutrients were highest in breaker stage (72.6%) and ripe fruit (72.7%) while branches had the lowest value (52.6%). The breaker stage fruit and ripe fruit were highest in energy values (both at NEL 1.7 Mcal/kg, NEM 1.7 Mcal/kg, NEG 1.1 Mcal/kg), while the branches were lowest (NEL 0.7 Mcal/kg, NEM 0.9 Mcal/kg, NEG 0.4 Mcal/kg).

NPA 6

NOVEL PHYTOSIDEROPHORES IN BARLEY

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Iron (Fe) is one of the most important minerals in all organisms as a catalytic cofactor in vital metabolic pathways. Understanding plant Fe acquisition from the environment is critical to improve not only agricultural productivity but also human nutrition as plants are a major nutritional component of the human diet. Under Fe deficiency, graminaceous plants secrete Fe-chelating compounds called phytosiderophores (PSs) into the rhizosphere to take up Fe. Accurate analysis of PSs in grasses could therefore facilitate the use of these natural Fe-chelating compounds to improve Fe availability in plants. For this purpose, we have developed a rapid and highly sensitive LC-ESI-TOF-MS method for direct and simultaneous determinations of free PSs and their ferric complexes. Using this method, we have identified two more PSs, AVA and HAVA, in addition to previously reported PSs, DMA, MA and epi-HMA, in *Hordeum vulgare*, L.cv. Himalaya roots as well as in root exudates under Fe deficiency. MS results of root exudates and PS-ferric complexes suggest that the two PSs identified could be responsible for Fe acquisition.

NPA 7

TRICHODERMA PRODUCES ANTIFUNGAL METABOLITES THAT INHIBIT MYCELIAL GROWTH OF PHYTOPHTHORA SPP.

Soo Jung Bae, Vivek Bajpai, Kwang-Hyun Baek, <u>Hanhong Bae</u> (Yeungnam University, School of Biotechnology, Gyeongsan, 712-749, South Korea.)

Trichoderma fungal species, that colonize plant roots, are well-known for their potential to control plant pathogens. *Trichoderma* are ubiquitously distributed soil fungi that produce a variety of antibiotic metabolites. We extracted extracellular metabolites from a liquid culture of 130 *Trichoderma* isolates and screened the metabolites for antifungal activity against seven *Phytophthora* species. Several metabolites have the potential to control *Phytophthora* pathogens. The structures of the metabolites will be determined later.

NPA 8

EFFECT OF HELIOPSIS LONGIPES EXTRACTS ON MYCOSPHAERELLA FIJIENSIS MORELET

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Mycosphaerella fijiensis is the most devastating pathogen of plantain and banana worldwide. Since the fungus has developed resistance to several conventional fungicides, it is a necessity to look towards biofungicides. In searching for a biological control agent for this pathogen, we assessed an *in vitro* extract of *Heliopsis longipes*, a plant that contains a high level of affinin. Four extracts, differing in preparation date, were compared. Evaluations were conducted over 15 days by measuring the inhibition radius (mm) and the number of the growing colonies (cm²). Activity appeared to depend on the date of the ethanolic extract. We found that extracts at 20% and 30% were the most inhibitory.

NPA 9 (+)-PISATIN BIOSYNTHESIS: FROM (-)-ENANTIOMERIC INTERMEDIATES TO A (+)-DERIVATIVE

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(+)-Pisatin, the major phytoalexin of pea (Pisum sativum L.), is an isoflavonoid derivative belonging to the pterocarpan family. It was the first chemically identified phytoalexin and subsequent research has demonstrated that most legumes produce pterocarpans with the opposite stereochemistry. Studies have shown that fungal pathogens are often more sensitive to a pterocarpan phytoalexin that has the opposite stereochemistry of its host's phytoalexin. Interestingly, pea produces two pterocarpanoid phytoalexins-minor amounts of (-)-maackiain and large amounts of (+)pisatin. Studies on the biosynthesis of (+)-pisatin have compounds that (-)-enantiomeric shown are intermediates in its synthesis and are in the same pathway with that of (-)-maackiain biosynthesis. However, the step(s) from the (-)-intermediates to a (+)derivative is still unknown. Previous studies have shown 2'-dihydroxy-4', 5'-methylenedioxythat (-)-7, isoflavanone [(-)-sophorol] is an intermediate in (-) maackiain and (+) pisatin biosynthesis. Chemical reduction of (-)-sophorol produces two isomers, cis and trans (-)-7. 2'-dihvdroxy-4'. 5'-methylenedioxyisoflavanol [(-)-DMDI]. However, NMR analysis of the product of (-)-sophorol reduction by sophorol reductase revealed the product to be the cis (-)-DMDI isomer and we propose that cis (-)-DMDI is the branching point for the production of (-)-maackiain and (+)-pisatin. Time course enzyme assays comparing the proteins from elicited and non-elicited pea tissues using cis (-)-DMDI as substrate revealed the early and increasing production of an achiral 7,2'-dihydroxy-4', 5'-methylenedioxyisoflavene (isoflavene) from the elicited pea tissues as compared to the non-elicited pea tissues. The same protein preparation from elicited tissues also converts the isoflavene into unknown products. We propose that the production of the achiral isoflavene intermediate could serve as the step for the change in the configuration that will ultimately produce a (+)-derivative.

NPA 10

IMPROVING PEPPERMINT ESSENTIAL OIL YIELD AND COMPOSITION THROUGH METABOLIC ENGINEERING

<u>Glenn W. Turner,</u>¹ Bernd Markus Lange,¹ Soheil S. Mahmoud,⁴ Mark R. Wildung,¹ Edward M. Davis,¹ Iris Lange,¹ Raymond C. Baker,² Richard A. Boydston,³ Rodney B. Croteau¹ (¹Washington State University, Institute of Biological Chemistry, Pullman, WA 99164-6340, USA, ²Washington State University, Irrigated Agriculture research and Extension Center, Prosser, WA 99350, USA, ³United States Department of Agriculture, Agricultural Research Service, Vegetable and Forage Crops Research Unit, Prosser, WA 99350, USA, ⁴University of British Columbia at Okanagan, Department of Biology, Kelowna, BC V1V 1V7, Canada.) Peppermint oil production in the United States has been declining in recent years. Disease-resistant, high yielding varieties could help reverse this trend, but peppermint is a sterile hybrid, so conventional breeding is not an option. We conducted a project to evaluate the potential of genetic engineering to produce these badly needed peppermint varieties. We achieved increases in oil vield by overexpressing selected genes from the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway. We also analyzed a number of two-gene combinations for their effects on oil yield and oil composition. The most promising results came from coupling expression of an antisense version of (+)-menthofuran synthase with overexpression of the MEP pathway gene 1-deoxy-Dxylulose 5-phosphate reductoisomerase. This line showed an oil yield increase of up to 61% over the yield of wild-type controls with favorable oil composition. In addition, we transformed peppermint with a gene encoding (+)-limonene synthase which accumulated sufficiently to demonstrate its potential as a marker of transgenic oil. Our study illustrates the utility of metabolic engineering for the sustainable agricultural production of high quality essential oils at a competitive cost.

C 1

REGULATION OF GINGER ROOT EXTRACT ON COLONIC INFLAMMATORY SIGNALING IN HUMAN WITH NORMAL AND HIGH RISK OF COLON CANCER

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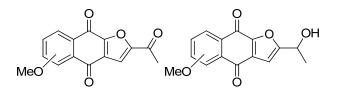
Ginger and other zingiberaceous plants have been reported to contain anti-inflammatory activities with significant colon cancer risk (CRC) preventive potential in vitro. Elevated prostaglandin E2 (PGE2) produced by cyclooxygenase (COX) has been shown as an early event of CRC development. In this study, healthy subjects and subjects with high risk of CRC were given 2.0 g/day ginger root extract or placebo for 28 days. The protein levels of COX-1, the constitutive form of COX and 15-hydroxyprostaglandin dehydrogenase (15 PGDH), the rate limiting enzyme in PGE2 catabolism, were measured in colon biopsies obtained from flexible sigmoidoscopy at baseline and the end of the study. Colonic COX-1 was significantly reduced (by 24%+13, p=0.03) from baseline in ginger group in high CRC risk subjects and there was a trend toward significant decreases in COX-1 (p=0.055) in healthy volunteers. On the other hand, 15 PGDH protein was not altered by the intervention in either healthy or high CRC risk subjects. Therefore ginger has the potential to decrease human colonic PGE2 synthesis without affecting PGE2 catabolism. Further investigation in larger studies with longer ginger intervention is necessary to exam the efficacy of ginger in regulation of the tissue level of prostaglandin synthesis.

C 2

SYNTHESIS OF NATURALLY-OCCURRING FURONAPHTOQUINONES AND CYTOTOXICITY AGAINST HL-60

<u>Ryuta Inagaki</u>, Masayuki Ninomiya, Mamoru Koketsu (Gifu University, Department of Materials Science and Technology, Faculty of Engineering, Gifu, 501-1193, Japan.)

Naturally-occurring furonaphthoquinones are known as anti-cancer compounds. Especially, 2-acetylnaphtho[2,3b]furan-4,9-dinone and 2-(1-hydroxyethyl)-naphtho[2, 3b]furan-4,9-dinone from *Tabebuia cassinoides* have been used as a folk remedy in South America. Their cytotoxicity against KB, P388 and other cell types are known. We synthesized several furonaphthoquinones to examine structure-activity relationships. The furonaphthoquinones so obtained were systematically investigated for the cytotoxicity against HL-60 cells.

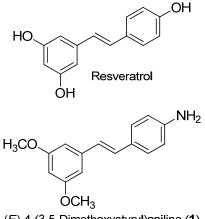


C 3 RESVERATROL DERIVATIVE (*E*)-4-(3,5-DIMETHOXYSTYRYL)ANILINE IS A NOVEL

INHIBITOR OF CANCER CELL INVASION

Tamara P. Kondratyuk,¹ Eun-Jung Park,¹ Tyler Hirokawa,¹ Ethyn Leong,¹ Bin Sun,² Mark Cushman,² John M. Pezzuto¹ (¹College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA, ²Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, and The Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA.)

Resveratrol is a structurally simple stilbene that interacts with numerous targets. Following evaluation of scores of structural analogs, the profile of biological responses mediated by (E)-4-(3,5-dimethoxystyryl)aniline (**1**) was found to be similar to resveratrol, but *in vivo* absorption and metabolic stability were much greater. Based on *in vitro* matrigel tests conducted with MCF7, MDA-MB-231, PC3 or RPMI 8226 human cancer cells, we now report resveratrol and compound **1** are active inhibitors of cell migration and invasion. Additional mechanistic studies are underway.



(E)-4-(3,5-Dimethoxystyryl)aniline (1)

C 4

EVALUATION OF BLUEBERRY JUICE ON THE PRECARCINOGENIC LESIONS INDUCED BY AZOXYMETHANE IN MOUSE

<u>Isela Alvarez-Gonzalez</u>,¹ Luis Fernando Gacia-Melo,¹ Victor Islas-Islas,¹ Eduardo Osiris Madrigal-Santillan,² Eduardo Madrigal-Bujaidar¹ (¹Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biologicas, Mexico City, DF 07738, Mexico, ²Universidad Autonoma del Estado de Hidalgo, Instituto de Ciencias de la Salud, Pachuca, Hidalgo, Mexico.)

The consumption of fruits and vegetables has been suggested to prevent the development of cancer. Blueberry, in particular, has shown activity against heart disease, inflammation, diabetes mellitus, urinary tract infections and neurodegeneration. Therefore, the objective of this project was to evaluate the chemopreventive effect of blueberry juice (BJ) obtained from Vaccinium virgatum in three different doses against the damage caused by azoxymethane (AOM) in mouse colon cells. For the study, we quantified aberrant crypt foci (ACF), which are considered precarcinogenic lesions. In our assay, we found the following results: BJ per se did not alter the weight of the animals, in contrast with AOM, which reduced 20% the weight of the animals at the third week. In regard to precarcinogenic lesions, BJ showed no effect, while AOM induced 104.57 ACF in the colon of the mice. BJ did not show any precarcinogenic damage when administered alone. When we combined the two agents BJ plus AOM, the results showed that the lower and middle doses (0.4 and 1.2 µL/g) significantly decreased the frequency of ACF (70.35 and 88.64%, respectively). However, the high tested dose of BJ (15 µL/g) increased 326.73% the level of ACF. These results establish that the protective effect of BJ corresponds to low doses, while higher ones may act as co-mutagens.

C 5

THIAZOLE AND THIADIAZOLE DERIVATIVES OF RESVERATROL AS INDUCERS OF QUINONE REDUCTASE 1

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Although a number of resveratrol derivatives have been demonstrated to possess potent cancer chemopreventive potential, the stilbene ethylenic bridge is problematic since it is metabolized to the potentially dangerous *trans*-stilbene oxide. To circumvent this problem, a series of derivatives was synthesized with a thiazole or thiadiazole in place of the ethylenic bridge. A number of the resulting derivatives were found to be potent inducers of quinone reductase 1 (QR1), with CD (concentration required to double activity) values as low as 60 nM. Induction of QR1 may indicate activation of the antioxidant responsive element (ARE), which mediates expression of a number of phase II detoxifying enzymes. This activity indicates a reduction in oxidative stress, which could have implications not only for cancer chemoprevention, but for a number of other diseases, including heart disease, diabetes, Alzheimer's, and Parkinson's disease. Structure-activity relationships within the series were analyzed, and a number of assays (e.g., glutathione S-transferase induction, glutathione reductase induction, xanthine oxidase inhibition, total antioxidant capacity) were employed to investigate the potential of these compounds to reduce oxidative stress.

C 6

SUPPRESSION OF 12-0-TETRADECANOYL-PHORBOL-13-ACETATE-INDUCED ORNITHINE DECARBOXYLASE ACTIVITY BY RESVERATROL DERIVATIVES

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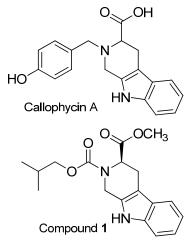
As demonstrated previously, resveratrol (3,4',5trihydroxy-trans-stilbene) inhibits 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase (ODC), the key rate limiting enzyme in mammalian polyamine synthesis. Using human bladder epithelial carcinoma HTB-24 cells in culture, where resveratrol inhibits induction with an IC₅₀ of 8.8 μ M, we now report potential metabolites [(E)-4-(3,5dihydroxystyryl)phenyl sulfate (IC₅₀ 1.2 µM), resveratrol 3,5,4'-trisulfate (IC₅₀ 1.8 µM), resveratrol 3,4'-disulfate (IC₅₀ 1.8 µM), and resveratrol 3,5-disulfate (IC₅₀ 2.3 µM)] demonstrate greater activity. Based on RT-PCR studies, ODC inhibition occurs at the transcriptional level, but this was not due to direct inhibition of protein kinase C (e.g., resveratrol IC₅₀, 79 µM; resveratrol 3,5-disulfate IC₅₀, 49 µM). Additional work is underway to more fully investigate this potentially important observation. [This work was supported by program project P01 CA48112 awarded by the National Cancer Institute. SL acknowledges Indo-US Science and Technology Forum (IUSSTF), New Delhi for a Research Fellowship]

C 7

INHIBITORY EFFECT OF A CALLOPHYCIN A DERIVATIVE ON INOS EXPRESSION IN LIPOPOLYSACCHARIDE-STIMULATED RAW 264.7 CELLS

Eun-Jung Park, Li Shen, Dianqing Sun, Tamara P. Kondratyuk, John M. Pezzuto (College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA.)

Callophycin A, found in the red algae Callophycus oppositifolius, has been reported as a potential antitumor agent. In our studies, this compound showed modest inhibition of LPS-induced iNOS activity with RAW 264.7 cells (<50% at 50 µM). However, after chemical modification of callophycin A, several derivatives exerted more potent inhibitory effects. In particular, compound 1 showed the most potent inhibition (IC₅₀ = 2.8 μ M), and blocked iNOS protein and mRNA expression in a dosedependent manner. Since compound 1 is of potential or value an anti-inflammatory as cancer chemopreventive agent, further mechanistic studies are underway.



C 8

PSAMMAPLIN A INDUCES AUTOPHAGY CELL DEATH IN DOXORUBICIN-RESISTANT HUMAN BREAST CANCER MCF-7/ADR CELLS

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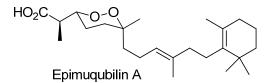
Psammaplin A (PsA) is a natural product isolated from marine sponges, which has been demonstrated to have anticancer activity against several human cancer cell lines through cell cycle arrest and apoptosis. Recently, development of new drugs that are less toxic and more effective against multidrug resistance in cancer patient are needed urgently. Here, we report the anticancer potential of PsA in doxorubicin-resistant human breast cancer MCF-7/Adr cells. PsA significantly inhibited the proliferation of MCF-7/Adr cells in a dose-dependent manner and markedly increased the G2/M phase of cell cycle. PsA significantly decreased SIRT1 enzyme activity, which is more potent than that of nicotinamide, a well known SIRT1 inhibitor. In addition, PsA markedly increased the expression of autophagy-related proteins LC3 and beclin-1 levels. The PsA-induced autophagy cell death was confirmed by acridine orange staining, which is a marker of acidification of autophagic vacuoles.These results suggest that PsA is sufficient to overcome multidrug resistance cancer through SIRT1mediated autophagy in breast cancer MCF-7/Adr cells, thus indicating therapeutic potential for clinical use.

C 9

INHIBITION OF LIPOPOLYSACCHARIDE-INDUCED CYCLOOXYGENASE-2 AND INDUCIBLE NITRIC OXIDE SYNTHASE BY EPIMUQUBILIN A IN RAW 264.7 CELLS

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Epimuqubilin A, from the marine sponge *Latrunculia* sp., suppressed nitric oxide production with LPS-stimulated RAW 264.7 cells ($IC_{50} = 7.6 \mu$ M). Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were suppressed at both the mRNA and protein levels in a dose-dependent manner through blockage of the phosphorylation of inhibitor kinase (IKK β). This resulted in stabilization and inhibition of NF-65 nuclear translocation and DNA binding. This is an unique mechanistic relationship that suggests epimuqubilin A warrants further exploration as a potential therapeutic agent.



Β1

AN EXTRACELLULAR ACYLTRANSFERASE CATALYZES CUTIN POLYMERIZATION

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A waxy cuticle covers the aerial epidermis of plants and provides protection against desiccation and other stresses. It is contiguous with the polysaccharide cell wall and and consists of waxes associated with a polyester matrix of cutin. Previous work has revealed early steps in cutin monomer biosynthesis, but the mechanism of cutin polymerization has remained a mystery. A model system to address this question is provided by tomato (Solanum lycopersicum) fruit, which are typically covered with an exceptional amount of cutin composed primarily of esterified 10.16dihydroxyhexadecanoic acid. We have identified a tomato mutant, cutin deficient 1 (cd1) that has a 90% reduction in polymerized cutin. The CD1 gene was mapped and its identity confirmed by transgenic complementation of the cd1 mutant. Immunolocalization showed the protein to localize within the nascent cuticle. GC-MS analysis of soluble surface lipids in the cd1 mutant showed accumulation of a non-polymerized putative monomeric cutin precursor. Recombinant CD1 showed acyltransferase activity using an analogous substrate as an acvl donor. Taken together, our results indicate that cutin is synthesized via successive transesterification reactions that are catalyzed by CD1. The presence of close orthologs of CD1 in diverse plant genomes suggests that this is a conserved mechanism of extracellular polyester biosynthesis in plants.

B 2

MOLECULAR CLONING AND CHARACTERIZATION OF AN IRIDOID 1-O-GLUCOSYLTRANSFERASE INVOLVED IN SECOLOGANIN BIOSYNTHESIS

<u>Keiske Asada</u>, Mai Nagatoshi, Kazuyoshi Terasaka, Hajime Mizukami (Nagoya City University, Graduate School of Pharmaceutical Science, Nagoya, 467-8603, Japan.)

Catharanthus roseus is a sole source of commercially important anticancer drugs, vincristine and vinblastine, which are derived from strictsidine, a condensation product of tryptamine and secologanin. Despite an important biogenetic role of secologanin linking terpenes and indole alkaloids, the biosynthetic pathways, especially glucosylation step, leading to secologanin are pooly understood. We attempted to isolate and characterize a cDNA encoding a glucosyltransferase which is involved in secologanin biosynthesis.

Homology-based PCR cloning using the highly conserved motif among plant secondary product glucosyltransferases and the EST database search led us to obtain three full-length glucosyltransferase clones from *C.roseus* cultured cell (CrUGT6) and leaves (CrUGT7 and 8). Enzyme assays using the recombinant proteins revealed that CrUGT8 has high and specific glucosylation activity toward 7-deoxyloganetic acid. To our knowledge, this is first report identifying the glucosylation step in the secologanin pathway in *C.roseus*.

В3

HETEROLOGOUS EXPRESSION AND CHARACTERIZATION OF RECOMBINANT PUTATIVE GLUCOSYLTRANSFERASE CLONE 3 FROM GRAPEFRUIT (*CITRUS PARADISI*).

<u>Deborah Hayford</u>, Daniel K. Owens, Cecilia A. Mcintosh (¹East Tennessee State University, Biological Sciences, Johnson City, TN 37614, USA, ²East Tennessee State University, School Of Graduate Studies, Johnson City, TN 37614, USA.)

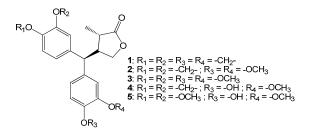
The grapefruit plant, Citrus paradisi, tends to accumulate high levels of flavonoid glycosides such as flavanones and flavones. Flavonoids have a vast array of important functions in plants and also in humans. Most naturally occurring flavonoids exist in glycosylated forms and this suggests that glycosylation is a key part of plant biochemical processes. Glucosyltransferases (GT's) involved in secondary metabolism share a loosely conserved UDP sugar binding motif called a PSPG box. Even though there is some degree of homology within the PSPG box, comparison of overall nucleotide or amino acid sequence of these enzymes tends to be low. The use of amino acid sequences alone cannot be used to predict specific functions and biochemical assays remain the only way to conclusively establish function. In our pursuit to study the structure and function of flavonoid GT's, we have used molecular approaches to identify, clone, express, and functionally characterize the enzymes. In this work, clone PGT3 was obtained through EST mining of a directionally cloned young grapefruit leaf cDNA library. PGT3 has been modified and cloned into E. coli and also into Pichia pastoris. Expression of recombinant PGT3 has been confirmed with the E. coli, however the majority of the protein was found in insoluble inclusion bodies. Expression with Pichia pastoris overcame the challenge of inclusion bodies. Results of expression in Pichia pastoris and purification of PGT3 by immobilized metal affinity chromatography are presented. PGT3 has been tested for GT activity with compounds representing the subclasses of flavonoids as well as some simple phenolics.

Β4

PEPEROMINS, CHROMENONES, FUNGAL ENDOPHYTES AND MORE FROM *PEPEROMIA GLABELLA* VAR. NERVULOSA

<u>Fabio C. Chaves</u>, Lydia F. Yamaguchi, Camila A. Rodrigues, Massuo J. Kato (University of São Paulo, Chemistry Institute, São Paulo, SP 05508-900, Brazil.)

In studying the yet unknown biosynthetic origin of secolignans from *Peperomia glabella* var. nervulosa, three major (1-3) and two minor (4, 5) peperomins were characterized by GC/MS and HRESI following isolation of 1-3. Additionally, various chromenones and other polyketides were identified. In order to further understand the biosynthetic pathway leading to peperomin formation, cell cultures were initiated using leaf and stem explants from adult plants. Unexpectedly, a variety of fungi isolated from these plant tissues was identified based on ITS sequences, and their metabolites in culture were determined.



B 5

PROBING CHEMICAL EVOLUTION AND DIVERSIFICATION IN THE SUNFLOWER FAMILY (ASTERACEAE)

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Asteraceae, the largest and cosmopolitan flowering plant family, is characterized by its contents of sesquiterpene lactone (STL). Thousands of bioactive STLs have been documented, but their biochemistry is poorly understood. Studying STLs in Asteraceae thus provides an insight into the adaptive evolution of enzymes that has led to the family's enormous chemical diversification. Natural variants of closely related enzymes (e.g. terpene synthase and P450) can serve as excellent templates for enzyme evolution studies. We focused on the key P450 catalyzing three-step enzymes oxidations of sesquiterpene backbone at C12. Homologs of Artemisia annua amorphadiene C12 oxidase from three major and the basal subfamilies of Asteraceae were functionally characterized to catalyze the conversion of germacrene A (GA) to germacrene A acid (GAA), substantiating that this activity is highly conserved. Interestingly. GA evolutionary oxidases (i.e., predecessors of amorphadiene oxidase) showed promiscuous activities toward various non-natural substrates while amorphadiene oxidase displayed strict substrate specificity. With GA oxidase, we further identified the lactone-forming enzymes. A P450 clone encoding 8- β -GAA hydroxylase from sunflower was functionally identified. Its closest homolog in lettuce, however, was elucidated to catalyze the 6- α -hydroxylation of GAA, which result in the formation of the simplest STL, costunolide. Evolutionary significance of GA oxidases, 8- β - and 6- α -GAA hydroxylases will be discussed in the context of Asteraceae phylogeny.

B 6

HETEROLOGOUS EXPRESSION IN YEAST AND BIOCHEMICAL CHARACTERIZATION OF RECOMBINANT PUTATIVE GLUCOSYL-TRANSFERASE 9 FROM *CITRUS PARADISI*

<u>Anye Wamucho</u>, Daniel K. Owens, Cecilia A. McIntosh (East Tennessee State University, Biological Sciences, Johnson City, TN 37614, USA, ²East Tennessee State University, School of Graduate Studies, Johnson City, TN 37614, USA.)

The wide diversity of plant secondary products is a result of different modifications they undergo among which is glucosylation. Glucosylation of plant secondary metabolites increases their bioavailability, solubility, stability and also affects their organoleptic properties. Due to low homology between the nucleotide and amino sequences of plant secondary product acid glucosyltransferases (GTs), it is not possible to ascribe function based on sequence only. One approach is to identify and isolate putative GT clones, express them heterologously, and biochemically characterize the proteins. Grapefruit has been shown to accumulate high levels of glucosylated flavonoids, predominantly flavanones, flavones and flavonols, Eleven putative GT clones have been isolated from Citrus paradisi and some have been biochemically characterized. The hypothesis being tested is that PGT9 is a plant secondary product GT. A PGT9 contig was identified from the harvEST database using the plant secondary product glucosyltransferase (PSPG) box as identifier. It was amplified from young grapefruit leaf cDNA using specific primers. It was cloned into E. coli and successfully expressed but was localized to inclusion bodies and could not be tested for GT activity. PGT9 was subsequently cloned into Pichia pastoris using the pPICZA vector with zeocin resistance for selection. Expression of recombinant PGT9 in Pichia pastoris has been confirmed by Western blot analysis using anti myc antibodies. Enrichment of recombinant PGT9 by IMAC has been achieved and fractions with highly enriched PGT9 were pooled, desalted, concentrated, and used to screen for secondary product GT activity using a variety of flavonoid substrates as well as some simple phenolics.

Β7

INHIBITION OF HYDROXYCINNAMOYL-COA THIOESTERASES FROM GINGER PLANTS (ZINGIBER OFFICINALE)

<u>I. Josefina Flores-Sanchez</u>, David R. Gang (Washington State University, Institute of Biological Chemistry, Laboratory for Cellular Metabolism and Engineering, Pullman, WA 99164-6340, USA.)

Ginger (Zingiber officinale Rosc.), a plant from the Zingiberaceae, is widely used in traditional Asian cuisine and herbal medicine. Gingerols and diarylheptanoids, important compounds from this plant, are produced by type III polyketide synthases (PKSs), based on in vitro characterization of recombinant enzymes identified in our large scale transcriptome sequencing project. Previous efforts to detect PKS activities in ginger tissues were marginally successful because of hydrolysis of the hydroxycinnamoyl-CoA substrates in these assays, presumably due to the presence of thioesterases in these tissues. Three inhibitors of thioesterases were tested in efforts to identify these enzymes in leaf and rhizome crude protein extracts: orlistat, a reduced form of lipstatin and peptide 1 and peptide 2 from hydrolysates of soybean β -conglycinin. Significant differences in enzyme activities for feruloyl-CoA thioesterase and p-coumaroyl-CoA thioesterase were detected in rhizome crude extracts (P<0.05) while there were no significant differences for the same enzyme activities present in leaf. However, the inhibition of feruloyl-CoA thioesterase and p-coumaroyl-CoA thioesterase was significantly different in leaf extracts, with inhibitions of 4-fold or 5-fold, respectively; while no significant differences were found for inhibition of both enzyme activities (P<0.05) in the rhizome. The relationship of these thioesterase activities to the biosynthesis of the gingerols and diarylheptanoids in ginger will be discussed.

B 8

TRACKING THE BIOSYNTHESIS OF ¹³C-LABELED GRAPE PHENOLICS IN SITU

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Phenylalanine (Phe) is one of the primary building blocks for phenolic compounds in plants, including hydroxycinnamates and flavonoids. While many of the pathways surrounding phenolic biosynthesis have been established, uncertainty remains in the regulation and characteristics of Phe metabolites *in vivo*. The novel method presented here describes a technique able to probe the biosynthetic regulation as well as the nature of Phe metabolites. A ¹³C-labeled Phe tracer was incorporated into grape berries on the vine (*in situ*), producing similarly ¹³C-labeled phenolics. Following incubation with the tracer, labeled phenolic compounds were monitored over time by LC-DAD-MS. Labeled Phe was quickly metabolized and the allocation of tracer depends primarily on grape berry maturity. As expected, incorporation into immature grapes resulted in the production of labeled hydroxycinnamate esters whereas incorporation following the onset of ripening resulted in significant concentrations of labeled anthocyanins. This methodology presents a novel technique useful to (1) track the regulation of phenolic biosynthesis following environmental or genetic manipulation, (2) understand the nature of phenolics within their *in vivo* environment, and (3) search for novel Phe metabolites. Future studies using this technique will address the catabolism of flavonoids.

В9

TWO CLASSES OF ENZYMES INVOLVED IN THE BIOSYNTHESIS OF CURCUMINOIDS AND OTHER DIARYLHEPTANOIDS IN GINGER AND TURMERIC

Yuying Sang, <u>Mark A. Willis</u>, David R. Gang (Washington State University, Institute of Biological Chemistry, Pullman, WA 99164, USA.)

Ginger and turmeric have been used for centuries for both culinary and medicinal purposes throughout Asia and now much of the rest of the world. The curcuminoids and other compounds of the diarvlheptanoid class found in both of these plants appear to have very important medicinal properties that lead to their application against numerous diseases and ailments. Curcumin in particular has undergone clinical trials for use in treating Alzheimer's disease, diabetes, and various cancers. However, some now believe that tetrahydrocurcumin, which has undergone two double-bond reductions, may be the more bioavailable and relevant diarylheptanoid for human medicinal use. Here we describe the differential production of various diarylheptanoids in ginger and turmeric and two classes of enzymes involved in their production. The first class, type III polyketide synthases, catalyze the formation of the diarylheptanoid backbone. Several different enzymes in two subclasses have now been found to be involved in production of different subgroups of these compounds. Several genes belonging to the second class of enzymes, the doublebond reductase family, have now been cloned from ginger and turmeric. The expression levels of specific genes correlates well with the production of specific diarylheptanoids, which are produced in *in vitro* assays with the corresponding enzymes, suggesting specific roles for specific enzymes in the production of these compounds. We also describe a modeling analysis of these proteins to rationalize their different substrate preferences and catalytic activities.

A STRUCTURE-BASED MECHANISM FOR BENZALACETONE SYNTHASE FROM RHEUM PALMATUM

<u>Yoshihiko Shimokawa</u>, Hiroyuki Morita, Ikuro Abe (Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, Japan.)

Benzalacetone synthase (BAS), a plant-specific type III polyketide synthase (PKS), catalyzes a one-step decarboxylative condensation of malonyl-CoA and 4coumaroyl-CoA to produce the diketide benzalacetone. We solved the crystal structures of both the wild-type and chalcone-producing I207L/L208F mutant of Rheum palmatum BAS at 1.8 Å resolution. In addition, we solved the crystal structure of the wild-type enzyme, in which a monoketide coumarate intermediate is covalently bound to the catalytic cysteine residue, at 1.6 Å resolution. This is the first direct evidence that type III PKS utilizes the cysteine as the nucleophile and as the attachment site for the polyketide intermediate. The crystal structures revealed that BAS utilizes an alternative, novel activesite pocket for locking the aromatic moiety of the coumarate, instead of the chalcone synthase's coumaroyl-binding pocket, which is lost in the active-site of the wild-type enzyme and restored in the I207L/L208F mutant. Furthermore, the crystal structures indicated the presence of a putative nucleophilic water molecule which forms hydrogen bond networks with the Cys-His-Asn catalytic triad. This suggested that BAS employs novel catalytic machinery for the thioester bond cleavage of the enzyme-bound diketide intermediate and the final decarboxylation reaction to produce benzalacetone. These findings provided a structural basis for the functional diversity of the type III PKS enzymes.

B 11

MOLECULAR CHARACTERIZATION OF TULIPOSIDE A-CONVERTING ENZYME IN TULIP

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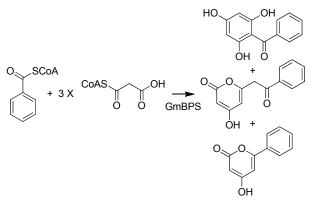
Tuliposides are representative secondary metabolites in tulip (Tulipa gesneriana). Their lactonized aglycones, tulipalins, are considered to function as defense chemicals due to their biological activities. We recently found that tuliposides are converted to tulipalins by tuliposide-converting enzyme (TCE), which has been purified from tulip bulbs. Although all parts of tulip possess TCE activity, specific activities in the crude extracts remarkably differ between bulbs and other tulip tissues. In order to investigate the functional diversity of TCE in each tulip tissue, we purified the TCE from petals as a representative of the tissues other than bulbs. The purified enzyme preferentially accepted tuliposides as substrates, with tuliposide A being the best substrate, and exhibited similar characteristics to the bulb enzyme with respect to substrate specificity, temperature and pH optima, and susceptibility to various inhibitors. However, the specific activities and the molecular masses differed greatly between the petal and bulb enzymes. Degenerate RT-PCR and the subsequent RACE PCRs with petal mRNA resulted in the isolation of novel cDNAs (TgTCEA1 and TgTCEA2) encoding the petal TCE. Functional characterization of the *E. coli*-expressed recombinant enzymes confirmed the involvement of TgTCEAs in the conversion of tuliposide A into tulipalin A. TgTCEAs were transcribed sufficiently in all tulip tissues, but not in bulbs, showing the presence of another TgTCEA homolog expressing specifically in bulbs, as suggested by the distinct enzymatic characters between the petal and bulb enzymes.

B 12

CLONING, CHARACTERIZATION AND SITE-DIRECTED MUTAGENESIS OF GARCINIA MANGOSTANA BENZOPHENONE SYNTHASE

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The biosynthesis of xanthones in Garcinia mangostana L. (Clusiaceae) involves the first reaction step catalyzed by benzophenone synthase (BPS) which is a type III polyketide synthase. The cDNA of G. mangostana BPS (GmBPS), cloned from young fruit pericarp, was found to consist of 1176 bp encoding a protein of 391 amino acids (Mr of 42.7 kDa). The recombinant enzyme 2,4,6-trihydroxybenzophenone produced as the predominant product when using benzovI CoA as starter. It also accepted other starter substrates and 1-3 units of malonyl CoA to form various phloroglucinol-type and polyketide lactone-type compounds. Site-directed mutagenesis of GmBPS led to change in substrate specificity, and increased the ratio of triketide lactone to benzophenone due to the reduce active site cavity by the mutants T135L, and G339S, respectively.



RECOMBINANT EXPRESSION AND CHARACTERIZATION OF AN ARABIDOPSIS THALIANA FAD SYNTHETASE

<u>Joseph Lynch</u>, Matthew Holm, Sanja Roje (Washington State University, Institute of Biological Chemistry, Pullman, WA 99164-6340, USA.)

FMN and FAD are important cofactors for a variety of enzymes involved in a multitude of metabolic processes in all organisms. These cofactors, as well as their inactive precursor riboflavin, are known to be interconverted by a network of enzyme catalyzed reactions. While examples of these enzymes have been characterized in several different organisms, in plants most of those interconverting enzymes have yet to be identified. Our lab has identified an *Arabidopsis* homolog to the known yeast FAD synthetase FAD1, an enzyme which facilitates the ATP-dependent conversion of FMN to FAD, which is non-homologous to other known plant FAD synthetases. Here we describe the recombinant expression, purification, and characterization of this new plant FAD synthetase.

B 14

CLONING AND CHARACTERIZATION OF AROMATIC PRENYLTRANSFERASE GENES FROM THAI MEDICINAL PLANTS

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In plants, prenylations of aromatic compounds play a major role in the diversification of natural product groups, such as flavonoids, xanthones, phenylpropanoids, and coumarins. These prenvlation reactions are catalyzed by aromatic prenyltransferases which transfer various lengths of prenyl groups to different positions of the aromatic rings of the secondary metabolites. In this study, genes encoding flavonoid prenyltransferases from four Thai medicinal plants, namely Artocarpus lakoocha Roxb L., Clitoria ternatea L., Orthosiphon aristatus Mig. and Morus alba L. were amplified from their cDNA preparation using degenerate primers and cloned. The results showed that three different partial genes (alr1, alr2A and alr2B) were obtained from A. lakoocha and one each, ctl, oam and mal, was from C. ternatea., O. aristatus and M. alba., respectively. These partial genes showed their percentage of identity in the range of 51 -63. when compared with that of the known SfN8DT-1 gene. These prenvltransferase genes are being determined for their full-length sequences which will be used for the cloning and functional expression.

B 15

METHYL JASMONATE AND YEAST EXTRACT STIMULATE MITRAGYNINE PRODUCTION IN SHOOT CULTURE OF *MITRAGYNA SPECIOSA* (ROXB.) KORTH.

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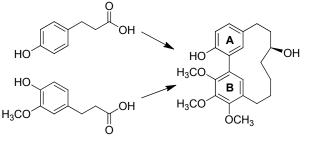
The shoot culture of Mitragyna speciosa was established and maintained in McCown woody plant medium (WPM) supplemented with 2 mg/L thidiazuron (TDZ), 1 mg/L benzyladenine (BA) and 2% (w/v) sucrose. The shoot cultures were elicited at exponential phase (14th day of culture) with methyl jasmonate (MJ) and yeast extract (YE). Mitragynine content was determined by HPLC and transcription profiles of tryptophan decarboxylase (tdc) and strictosidine synthase (sss) were monitored. The results indicated that MJ at 10 μ M, exposed for 24 h and YE at 0.1 mg/L, exposed for 12 h stimulated mitragynine production with a magnitude of 3.4 times and 2 times higher than control. The tdc and sss mRNA expressions were related to the ability of mitragynine production in the elicited shoot culture. The results from this study demonstrated that mitragynine production in the M. speciosa shoot culture was stimulated by MJ and YE. The mechanism of elicitation was up-regulated the tdc and sss gene expressions.

B 16

DIARYLHEPTANOIDS, MYRICANOL, BIOSYNTHESIS IN *MYRICA RUBRA*: INCORPORATION EXPERIMENTS OF *p*-HYDROXYCINNAMIC ACID DERIVATIVES

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To elucidate the hydroxylation and methylation steps in the biosynthesis of myricanol in Myrica rubra, we carried ¹³C-labeled experiments with out feeding p-¹³C-NMR studies hydroxycinnamic acid dervatives. indicated that 3-(4-hydroxyphenyl)propionic acid and 3-(4-hydroxy-3-methoxyphenyl)propionic acid were preferentially incorporated into the A- and B-rings of myricanol, respectively. A biosynthetic pathway originating from 4-coumaric acid and leading to myricanol is discussed.



EXPRESSION OF 1-DEOXY-D-XYLULOSE 5-PHOSPHATE SYNTHASE, 2C-METHYL-D-ERYTHRITOL 4-PHOSPHATE SYNTHASE AND GERANYLGERANYL DIPHOSPHATE SYNTHASE, KEY ENZYMES OF PLAUNOTOL BIOSYNTHESIS IN CROTON STELLATOPILOSUS

Worapan Sitthithaworn,¹ Juraithip Wungsintaweekul,² Tanawan Sirisuntipong,² Tossaton Charoonratana,² Yutaka Ebizuka,³ Wanchai De-Eknamkul⁴ (¹Faculty of Pharmacy, Srinakharinwirot University, Pharmacognosy, ²Facultv Nakonnavok. 26120. Thailand. of Pharmaceutical Sciences, Prince of Songkla University, Pharmacognosy and Pharmaceutical Botany, Songkhla, 90112, Thailand, ³Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, 113-0033, ⁴Faculty of Pharmaceutical Japan, Sciences. Chulalongkorn University, Pharmacognosy, Bangkok 10330, Thailand.)

Plaunotol is an acyclic diterpene alcohol accumulating in the leaves of Croton stellatopilosus. Expression levels of genes encoding key enzymes in the plaunotol biosynthetic pathway, namely 1-deoxy-D-xylulose 5phosphate synthase (dxs), 2C-methyl-D-erythritol 4phosphate synthase (meps) and geranylgeranyl diphosphate synthase (ggpps), were analysed by measuring transcript levels in leaves of different developmental stages. The results showed that dxs. meps, and gapps are all active in young leaves prior to full expansion when plaunotol is synthesised from the deoxy-D-xylulose 5-phosphate precursor in chloroplasts. The dense presence of chloroplasts and oil globules in the palisade cells of these leaves support the view that these genes are involved in plaunotol biosynthesis in chloroplast-containing tissues.

B 18

IDENTIFICATION AND CHARACTERIZATION OF DITERPENE SYNTHASES IN THE SALVINORIN A BIOSYNTHETIC PATHWAY

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Salvinorin A is a hallucinogenic diterpenoid that is found in the glandular trichomes of *Salvia divinorum*. Salvinorin A is the only known hallucinogen to mediate hallucination by binding to and activating the κ -opioid receptor. Derivatives of salvinorin A are candidates for the treatment of hallucination associated disorders such as schizophrenia as a result of this unique receptor binding profile. In the hypothesized pathway of salvinorin A biosynthesis, a class II and a class I diterpene synthase catalyze the conversion of geranylgeranyl pyrophosphate (GGPP) into a novel clerodane diterpene. In order to understand salvinorin A metabolism, S. divinorum young leaf cDNA was subjected to pyrosequencing, and the resulting EST database was used to identify five candidate diterpene synthases by sequence homology to known diterpene synthases. Two of these candidates were found to be similar to type II diterpene synthases and were named copalyl pyrophosphate synthase like (CPPSL) 1 and 2. The remaining 3 candidates were found to be similar to type I diterpene synthases and were named kaurene synthase like (KSL) 1, 2 and 3. CPPSL2, KSL2 and KSL3 are predominantly expressed in the trichomes of S. divinorum, indicating that they may play a role in the salvinorin A pathway. Accordingly, recombinant CPPSL2 enzyme was expressed in Escherichia coli, and the purified enzyme was found to catalyze the conversion of substrate GGPP into a novel product. Structural elucidation of this new compound and additional characterizations of the KSLs are in progress.

B 19

MITRAGYNINE BIOSYNTHESIS: METABOLITE PROFILING AND mRNA EXPRESSION OF THE EARLY STEPS GENE IN *MITRAGYNA SPECIOSA* (ROXB.) KORTH.

Tossaton Charoonratana, Juraithip Wungsintaweekul (Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.) Mitragynine, a terpenoid indole alkaloid, originates from two biosynthetic pathways, the shikimate and the terpenoid pathways. Therefore the genes involved in those pathways are the target in our study. We investigated the mRNA expression level of the 1 month old seedling plant. Tryptophan decarboxylase (tdc), 1deoxy-D-xylulose-5-phosphate synthase (dxs), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (dxr) and strictosidine syntase (sss) of the M. speciosa genes were used to profile genes transcription activities in three organs comprising the whole plant; leaves, roots, and stems. The relative quantitative real-time pcr (gRT-PCR) analysis was used to confirm those upregulated genes. parallel, the metabolites profiles including In secologanin, tryptophan, tryptamine and mitragynine contents were determined using HPLC. For instance, low amounts of tryptophan and tryptamine in M. speciosa might be formed by the concerted action of TDC and SSS, which convert the primary metabolite tryptophan to tryptamine and strictosidine in the presence of excess amount of secologanin. In addition, the upstream DXP genes dxs and meps showed lower mRNA expressions, suggested that those genes had a lesser effect in mitragynine biosynthesis. Adding tryptophan and tryptamine to the culture medium also increases the amount of mitragynine in M. speciosa culture, so tdc and sss play an important role in mitragynine biosynthesis.

SPECIALIZED ROLES FOR THE TWO UDP-GLUCOSYLTRANSFERASES UGT85K2 AND UGT85K3 IN HYDROXYNITRILE GLUCOSIDE METABOLISM IN *LOTUS JAPONICUS*

<u>Camilla Knudsen</u>,¹ Lisbeth Mikkelsen,¹ Fran Robson,² Mohammed Saddik Motawie,¹ Carl Erik Olsen,¹ Birger Lindberg Møller,¹ Adam Takos,¹ Fred Rook¹ (¹University of Copenhagen, Department of Plant Biology and Biotechnology, Frederiksberg, Denmark 1871, ²John Innes Centre, Department of Metabolic Biology, Norwich, NR4 7UH, United Kingdom.)

Cyanogenic glucosides are amino-acid derived plant chemical defense compounds against generalist herbivores. They are α -hydroxynitrile glucosides that are activated by specific β -glucosidases upon tissue disruption. The unstable α -hydroxynitrile will dissociate with the release of hydrogen cyanide. The legume model Lotus japonicus contains the cyanogenic glucosides linamarin and lotaustralin, and the non-cyanogenic yand β -hydroxynitrile glucosides rhodiocyanoside A and D, which are also thought to function as defense compounds. Glucosylation is a key-step in the biosynthesis of hydroxynitrile glucosides as it stabilizes and detoxifies these compounds, and allows for their storage. Both the UDP-glucosyltransferases UGT85K2 and UGT85K3 are able to catalyze the synthesis of linamarin and lotaustralin, but only UGT85K2 showed significant glucosylation activity for the synthesis of rhodiocvanosides in vitro. Mutants in the UGT85K2 obtained bv TILLING. almost aene. lacked rhodiocyanosides and showed severe growth defects. This suggested the toxicity of the rhodiocyanoside aglycones and supports their proposed defense role. The observed specificity of these UGTs further highlights the metabolic flexibility of the hydroxynitrile glucoside based defense pathway in L. japonicus.

B 21

DIFFERENTIAL EXPRESSION PATTERNS OF AROGENATE DEHYDRATASE GENES (ADT1-ADT6) IN ARABIDOPSIS THALIANA

Sarah E. Brewer, Oliver R. A. Corea, Laurence B. Davin, Norman G. Lewis (Washington State University, Institute of Biological Chemistry, Pullman, WA 99164-6340, USA.)

Arogenate dehydratases (ADTs) are enzymes that facilitate the last step in phenylalanine biosynthesis. The six genes encoding these enzymes within *Arabidopsis thaliana*, *ADT1-6*, were recently biochemically characterized, however it is currently unclear whether different ADT isoenzymes act in a functionally redundant manner or whether there are spatial and/or temporal differences in their activity. This is an important area of

investigation because ADT enzymes play a crucial role in synthesizing phenylalanine, which has many important downstream products, including lignin. One possibility is that ADTs may be differentially involved in the synthesis of proteins or the production of lignin and other compounds. To investigate their expression patterns over growth and development, ADT promoter::GUS (Bglucuronidase) fusions were constructed then introduced into Arabidopsis plants. GUS staining was performed on second-generation transformed (T_2) plants at various growth stages (from three-day-old seedlings to mature plants) and various tissues (roots, leaves, stems, flowers, siliques and seeds). The results of this staining established the expression patterns of the six ADTs, including several major differences, providing insight into the possible functions of each enzyme within plants.

B 22

FUNCTIONAL EVOLUTION OF P450S: THE BIOSYNTHESIS OF ALLIARINOSIDE IN ALLIARIA PETIOLATA

<u>Tina Frisch</u>, Mohammed Saddik Motawie, Carl Erik Olsen, Nanna Bjarnholt, Birger Lindberg Møller (University of Copenhagen, Department of Plant Biochemistry and Biotechnology, Frederiksberg, DK-1871, Denmark.)

Nitrile formation in plants involves activity of cytochrome P450s. Hydroxynitrile glucosides are widespread among plants but do generally not occur in glucosinolate (GLS)producing species. Alliaria petiolata (Brassicaceae) is the only species known to produce GLSs as well as a vhydroxynitrile glucoside. Furthermore, A. petiolata has been described to release cyanide, which indicates an unidentified cyanogenic glucoside. Our research on A. petiolata addresses the molecular evolution of P450s. By integrating knowledge of GLS and hydroxynitrile glucoside biosynthesis in other species, we propose a biosynthetic pathway for the γ -hydroxynitrile glucoside, alliarinoside. HomoMet and the corresponding oxime are suggested as shared intermediates in the biosynthesis of alliarinoside and 2-propenyIGLS. The first committed step in the alliarinoside pathway is envisioned to be catalyzed by a P450, which has been recruited to metabolize the oxime. Furthermore, the pathway is suggested to involve enzyme activities common to secondary modification of GLSs. Thus, we argue that biosynthesis of alliarinoside is the first known case of a hydroxynitrile glucoside pathway evolved from the Brassicales-specific GLSs. An intriguing question is if the hydroxynitrile intermediate in the proposed alliarinoside pathway may also be glucosylated into a novel homoMet-derived cyanogenic glucoside. Elucidating the biosynthesis of alliarinoside and other putative hydroxynitrile glucosides in A. petiolata will provide insight into how P450 evolution promotes development of novel natural product pathways.

BIOCHEMICAL ANALYSIS OF A PUTATIVE LIMONOID GLUCOSYLTRANSFERASE FROM *CITRUS PARADISI*

Daniel K. Owens, Cecilia A. McIntosh (East Tennessee State University, Biological Sciences, Johnson City, TN 37614, USA)

Limonoids abundantly accumulate in citrus species, and are of particular interest to the marketability of citrus juices as they convey bitterness. As citrus fruit ages, a natural debittering process occurs which has been attributed to the conversion of bitter limonoid aglycones to the corresponding tasteless limonoid glucosides during maturation. Beyond glucosylation's commercial importance, it is also a significant in planta modification Glucosylation serves a number reaction. of physiologically important roles such as influencing solubility and thereby transport, regulating bioavailability, and stabilizing structure. The enzymes that catalyze glucosylation, glucosyltransferases (GTs), typically function by transferring a UDP activated glucose to the corresponding aglycone. A loosely conserved 44 amino acid residue motif known as the plant secondary product glucosyltransferase (PSPG) box is thought to encompass the GT UDP-glucose binding moiety. In this work, the PSPG box has been used as a marker to identify putative glucosyltransferase genes using a combination of bioinformatics techniques and "fishing" against grapefruit cDNA libraries. One of the identified putative GTs, PGT8, is highly homologous to a limonoid GT from Citrus unshiu (AB033758). PGT8 has been recombinantly expressed in E.coli and strongly enriched using metal affinity chromatography. The recombinant enzyme has been screened for activity with synthesized limonoate A-ring lactone as well as a number of flavonoid substrates. Enzymatic activity with quercetin has been demonstrated with this enzyme.

B 24

CATALYTIC SITE OF PLANT GLUTATHIONE S-TRANSFERASE, A HERBICIDE DETOXIFICATION ENZYME

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Glutathione S-transferase (GST, EC 2.5.1.18) is a family of multifunctional proteins, catalyzing the formation of conjugates between reduced glutathione (GSH) and a wide variety of electrophilic compounds. In plant, this function of GST plays a pivotal role in the detoxification of herbicides, organic pollutants and natural toxins. To gain further insight into herbicide detoxification in plant and GST evolution, we have studied the catalytic mechanism and the relationship between structure and functions of rice GST by the combination of site-directed mutagenesis, X-ray crystallography and in depth kinetic analysis. The substitutions of Tyr8 and Ser13 residue with alanine resulted in approximately 80-90% loss of specific activity. From the pH-log (k_{cat}/K_m^{CDNB}) plot, the pKa values of GSH in enzyme-GSH complex of Y8A and S13A mutants were estimated to be approximately 8.5-8.9, which were about 1.6-2.0 pK units higher than that of the wild-type enzyme. From the 3-dimensional structure of rice GST, we suggested that Ser13 is located in the active site and its side chain is in close proximity to the thiol group of glutathione bound in the enzyme. From these results, we suggest that Ser13 in rice GST is the residue responsible for catalytic activity by lowering the pKa of GSH in enzyme-GSH complex, and by enhancing the nucleophilicity of the thiol of GSH in the active site of plant GST.

PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF SOME HERBS GROWN IN LITHUANIA

Petras Rimantas Venskutonis (Kaunas University of Technology, Department of Food Technology, Kaunas, LT-50254, Lithuania.)

This presentation reviews phytochemical studies of medicinal plants grown in Lithuania. These studies were focused on the antioxidant properties and phytochemical composition of extracts isolated by using different solvents, as well as their fractions and purified compounds. In some cases genotoxicity and cytotoxicity assays were performed. The plants include such less investigated species as Hierochloe odorata, Marrubium vulgare, Chrysanthemum balsamita, Rhaponticum carthamoides, Geranium macrorrhizum, Potentilla fruticosa and some others. It was found that these plants accumulate strong antioxidants as it was measured by using several radical scavenging assays and some other methods. Flavonoids and phenolic acids were the main phytochemicals in the studied plants, some of them were not previously reported as natural compounds or as constituents of the analyzed plants. However, some other structures were also identified. For instance, two very strong antioxidants were found in sweet grass, namely 5,8-dihydroxybenzopyranone (DHBP) and its 8-O-B-D-glucopyranoside. Radical scavenging capacity of these compounds were comparable with rosmarinic acid and other well-known strong natural antioxidants. The reactivity of 5,8-DHBP with peroxidase was similar to the reactivity of quercetin; the extract effectively neutralized the effect of singlet oxygen to erythrocytes, however demonstrated the pro-oxidant character of cytotoxicity. 5,8-DHBP inhibited the contractility of arterial smooth muscles and increased spontaneous basal tone of arteries: it was toxic in high concentrations, while low doses only slightly reduced the contraction ability of small arteries.

BOT 2

PHYTOCHEMICAL CHARACTERISATION OF HIGHBUSH BLUEBERRY (VACCINIUM × COVILLEANUM) AND EUROPEAN CRANBERRY BUSH (VIBURNUM OPULUS) ACCESSIONS GROWN IN LITHUANIA

Petras Rimantas Venskutonis,¹ Vilma Kraujalytë,¹ Elicija Stackevièienë,² Juozas Labokas,² Laima Èesonienë,³ Remigijus Daubaras³ (¹Kaunas University of Technology, Department of Food Technology, Kaunas, LT-50254, Lithuania, ²Nature Research Centre, Institute of Botany, Vilnius, LT-08406, Lithuania, ³Vytautas Magnus University, Kaunas Botanical Garden, Kaunas, LT-46324, Lithuania.)

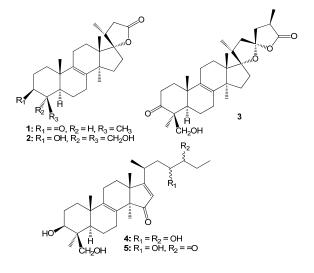
Growing interest in natural food ingredients exhibiting beneficial effects on human health has been an important factor for expanding the studies of less common horticultural plants in recent years. It is well established that berries accumulate high amounts of bioactive compounds. particularly polyphenolic antioxidants; however, some species remain under investigated. This study presents preliminary phytochemical characterization of different anatomical parts of highbush blueberry (Vaccinium × covilleanum) and European cranberry bush (Viburnum opulus), the species domesticated in Lithuania and being investigated in order to assess the possibilities of their commercial cultivation. In total 13 cultivars of highbush blueberry and 7 cultivars of European cranberry were studied. Antioxidant potential of extracts isolated from different plant parts by various solvents was assessed by using in vitro radical scavenging assays and in situ antioxidant capacity determination methods. Phytochemical composition was screened by using LC/MS and GC/MS methods. The variations in the concentration of various constituents such as phenolic acids, flavonoids, proanthocyanidins, glycosides, volatile and unidentified compounds between studied cultivars were determined and assessed by using PCA.

BOT 3

NOVEL TRITERPENOID DERIVATIVES FROM EUCOMIS BICOLOR (HYACINTHACEAE: HYACINTHOIDEAE)

Jaspreet Kaur Sihra,¹ Dulcie A Mulholland,¹ Moses K Langat,¹ Neil R Crouch,² Jean-Marc Nuzillard,³ Alfred E Thumser⁴ (¹University of Surrey, Chemical Sciences, Faculty of Health and Medical Sciences, Guildford, GU2 7HX, United Kingdom, ²South African National Biodiversity Institute, Ethnobotany Unit, Durban, 4007, South Africa, ³University of Reims, Pharmacognosy Laboratory, Reims, 51687, France, ⁴University of Surrey, Biological Sciences, Faculty of Health and Medical Sciences, Guildford, UK GU2 7XH, United Kingdom.)

The phytochemical investigation of the dichloromethane extract of *Eucomis bicolor* has yielded five novel compounds (1-5). The triterpenoid derivatives have been screened against several tumour cells.



FUZHUAN TEA: NOVEL PHYTOCHEMICALS AND INITIAL INVESTIGATIONS OF A FERMENTED PREPARATION OF *CAMELLIA SINENSIS*

<u>Amy Keller</u>, Tiffany Weir, Corey Broeckling, Ajay Kumar, Elizabeth Ryan (Colorado State University, Fort Collins, CO 80523, USA.)

Many fermented foods have been shown to have both unique bioactivity and phytochemicals as a result of the fermentation process. Fuzhuan tea is a traditional preparation of Camellia sinensis L. (Theaceae) from Hunan, China that is fermented with the fungus Eurotium cristatum. This preparation has had ethnobotanical importance in northern China, Mongolia, and Tibet for centuries to counteract negative effects of high fat diets. Preliminary data shows that Fuzhuan tea significantly inhibited cell viability of the human colon cancer cell lines HT-29 as compared to the control at 750 μ g/ml. To explore novel bioactive compounds, metabolomics was performed on Fuzhuan tea extract in comparison with unfermented green tea using UPLC-ToF-MS. Principal Component Analysis shows a unique phytochemical profile for Fuzhuan tea compared to green tea and we have identified candidate fatty acid amide compounds such as linoleamide that, to our knowledge, have not been previously reported in C. sinensis. Linoleamide, a fatty acid amide (FAA) that induces sleep, and several related FAAs act as neurological signaling molecules and are thought to have physiological roles in memory, cardiovascular function, cognition, reproduction, and immune function, providing promising new avenues to explore Fuzhuan tea bioactivity. Tissue histology and liver gene expression analysis performed in ICR mice demonstrated the safety of Fuzhuan tea consumption, and further studies of Fuzhuan tea are underway.

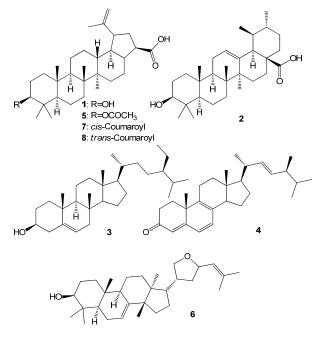
BOT 5

ANTIPARASITIC COMPOUNDS FROM CORNUS FLORIDA L. WITH ACTIVITIES AGAINST PLASMODIUM FALCIPARUM AND LEISHMANIA TARENTOLAE

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Cornus florida, a plant traditionally used in North America for the treatment of malaria, was shown to be active *in vitro* against *Plasmodium falciparum* (D10 strain). Antiplasmodial-guided fractionation of the ethanolic extract of bark afforded 8 compounds: betulinic acid (1), ursolic acid (2), β -sitosterol (3), ergosta-4,6,8,22-tetraene-3-one (4), 3β -O-acetyl betulinic acid (5), 3-epideoxyflindissol (6), 3β -O-*cis*-coumaroyl betulinic acid (7), 3β -O-*trans*-coumaroyl betulinic acid (8), of which 4, 5, 6 and 7 are reported for the first time from this genus and 6 is for the first time here isolated

from a natural product. *In vitro* IC₅₀ values against *Plasmodium falciparum* (D10 strain) (**4**: 61.0 μ M; **6**: 128.0 μ M, **7**: 10.4 μ M, and **8**: 15.3 μ M) are shown for the first time. Compounds were also tested for antileishmanial activity against *Leishmania tarentolae*, with IC₅₀ values reported here for the first time: **4**: 11.5 μ M, **6**: 1.8 μ M, **7**: 8.3 μ M, and **8**: 2.2 μ M). Cytotoxicity toward Chinese Ovarian Hamster cells is discussed for all isolated compounds.



BOT 6

NEUROPROTECTIVE COMPOUNDS ISOLATED FROM THE METHANOLIC EXTRACT OF LONICERA JAPONICA

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The CH₃Cl fraction of the flower of Lonicera japonica (Lauraceae) significantly protected primary cultures of rat cortical cells injured by the excitotoxic amino acid, Lglutamate. loganin (1), secoxyloganin (2), caffeic acid (3) rutin (4), hyperoside (5), quercetin-3-O-glucoside (6), Ionicerin (7), kaempferol-3-O-rutinoside (8), luteolin-7-O-D-glucopyranoside (9), quercetin (10), luteolin (11) were isolated by bioactivity-guided fractionation from the fraction and further separated CH₃CI usina chromatographic techniques. caffeic acid, lonicerin, kaempferol-3-O-rutinoside, quercetin and luteolin had significant neuroprotective activities against glutamateinduced neurotoxicity in primary cultures of rat cortical cells at concentrations ranging from 0.1 μ M to 10.0 μ M.

INHIBITION OF QUORUM SENSING AND BIOFILM FORMATION BY TROPICAL PLANTS

<u>Chieu Anh Ta</u>,¹ Marie Freundorfer,¹ Ammar Saleem,¹ Ana Gargaun,² Muhammad Asim,² Mario Garcia Quesada,³ Marco Otarola Rojas,³ Pablo Sanchez Vindas,³ Luis Poveda,³ Victor Cal,⁴ Tony Durst,² John T. Arnason¹ (¹University of Ottawa, Biology, Ottawa, ON K1N 6N5, Canada, ²University of Ottawa, Chemistry, Ottawa, ON K1N 6N5, Canada, ³Universidad Nacional, Herbario Juvenal Valerio Rodriguez, Heredia, 86-3000, Costa Rica, ⁴Belize Indigenous Training Institute, Punta Gorda, Belize.)

This is a new approach to the discovery of new phytochemicals from tropical plants that can interfere with the formation of bacterial biofilms. Bacteria use a cell-to-cell communication system known as quorum sensing (QS) to coordinate gene expression for the formations of these biofilms. Ethanolic extracts of tropical and traditional anti-infective plants were screened for QS interference and biofilm inhibition. Extracts from the Melastomataceae, Meliceae. Sapindaceae, Lepidobotryaceae, Combretaceae, and Euphorbiaceae showed the highest inhibitory activities. QS inhibition ranges from 7.3 + 0.1 mm to 26.1 + 0.3mm. Inhibition of biofilm growth ranges from 0 to 76.8 + 2.0 %. In particular, one Melastomataceae species (Oxlaju chajom) was most promising with QS inhibition zone of 25.9 + 0.6 mm and biofilm MIC (minimum inhibitory concentration) of 50 μ g/mL. Interestingly, this is the first report of biological activity for this plant and very little is known about its phytochemistry. Bioassayguided fraction of Oxlaju chajom showed that inhibitory activities are in the more polar fractions. Current work is being done on the isolation and identification of the active principles.

BOT 8

ETHNOPHARMACOLOGY OF ANTI-INFLAMMATORY BOTANICALS USED BY THE Q'EQCHI' MAYA OF BELIZE

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Indigenous pharmacopeias recognize the important role of inflammation in disease, and the Q'eqchi' Maya healers of Belize possess a practical understanding of a large number of immunomodulatory botanicals. Ethnobotanical interviews were held with 5 members of the Q'eqchi' Maya Healers Association using a list of 14 inflammatory symptom categories and one hundred and seven plant species were collected from primary and secondary semi-evergreen rainforest in the Maya Mountains of Belize. Ethanolic extracts of fifty-five species were assayed for anti-inflammatory activity in a LPS-stimulated THP-1 monocyte assay. Of these, 76% demonstrated significant anti-inflammatory activity relative to the vehicle control, and three species displayed activity equal to that of the parthenolide positive control. In addition, several sesquiterpene lactones isolated from Neurolaena lobata exhibit potent anti-inflammatory activity. These results demonstrate that plants used by the Q'eqchi' Maya Healers Association for the treatment of inflammatory-related symptoms do indeed possess immunomodulatory properties, and elucidating the active principles of these species can yield compounds with novel bioactivities.

BOT 9

ANTIOXIDANT, ANTIMICROBIAL AND ANTIVEROTOXIC POTENTIALS OF EXTRACTS OF CURTISIA DENTATA

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The potentials of Curtisia dentata as antimicrobial, antioxidant and antiverotoxin against environmental isolates of E. coli and Acinetobacter spp, the presence of phytochemicals and some organic compounds was investigated. concentration The highest of phytochemicals and organic compounds such as anthraquinones, alkaloids, essential oils, glycosides, phenols, steroids, saponins and tannins and the organic compounds quinones, anthocyanins, amines and carboxylic acids were found in the stem bark ethanol extracts compared to other parts of the plant or solvent extracts. Antimicrobial activity as relative inhibition zone diameters (%) ranged between 8-28% (MIC values, 100-2500 mg/ml) against E. coli serotypes, and 10-28% (MIC, 100-850mg/ml) and 6-28% (MIC, 150-2500 mg/ml) against A. Iwoffii and A. haemolvticus respectively. The extracts demonstrated inhibitory action against the expression of both Vtx1 and Vtx2 genes in both E. coli and A. haemolyticus strains with the ethanol extracts demonstrating the highest antiverotoxic activity compared to other solvents. The ethanol root bark extracts consistently showed the highest DPPH radical scavenging activity (62.43%), total phenol content (TPH) (57.62 26 mg GAE/g) and reducing power (RP) (41.32%), followed by those of the stem bark and leaf extracts with the respective values of 54.68%, 37.77 mg GAE/g and 21.83%. Ethanol extracts demonstrated the highest values for both DPPH, TPH and RP followed by dichloromethane, hexane, acetone and distilled water in this order. C. dentata can be used to source novel antimicrobial agents for the treatment of verotoxic bacterial infections.

THE ANTIOXIDANT PROPERTIES, CYTOTOXICITY AND MONOAMINE OXIDASE INHIBITION ABILITIES OF THE CRUDE DICHLOROMETHANE EXTRACT OF *TARCHONANTHUS CAMPHORATUS* L. LEAVES

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Tarchonanthus camphoratus (camphor bush) has been widely used for numerous medicinal purposes. The aim of the present study was to evaluate the antioxidant properties, cytotoxicity and monoamine oxidase inhibition activities of the crude dichloromethane leaf extract of T. camphoratus. The antioxidant activities were assessed using the Thiobarbituric acid-reactive Substances (TBARS) Assay and the Nitroblue Tetrazolium (NBT) assay. The cytotoxicity assays were performed according to the microculture MTT method. From the MTT assay it was determined that at a concentration of 10 mg/ml of crude extract 95% of the neuroblastoma cells were killed. Almost 99% of the cells were viable at a concentration of 0.08 mg/ml extract. The extract also showed the ability to inhibit monoamine oxidase (MAO-A and MAO-B) with the corresponding IC₅₀ values of 1.371 mg/ml and 0.2737 mg/ml respectively. The antioxidant activity and cytotoxic effect of the extracts increased with increase in concentration. This study suggests that the dichloromethane leave extract of Tarchonanthus camphoratus can potentially be used as a readily accessible source of natural antioxidants.

BOT 11

ANTI-DIABETIC POTENTIALS OF ETHANOL AND WATER EXTRACTS OF 17 PLANTS USED BY THE EEYOU ISTCHEE CREE FIRST NATIONS OF NORTHERN QUEBEC

Nan Shang,^{1,2} Lina Musallam,^{1,2} Diane Vallerand,^{1,2} Brendan Walshe-Roussel,^{2,3} <u>Arnason John T</u>.,^{2,3} Pierre S. Haddad^{1,2} (¹Natural Health Products and Metabolic Disease laboratory, Department of Pharmacology, University of Montreal, Montréal, Quebec H3T1J4, Canada, ²Canadian Institutes of Health Research Team in Aboriginal Antidiabetic Medicines and Montreal Diabetes Research Center, Montréal, Quebec H3T1J4, Canada, ³Medicinal Plant and Ethnopharmacology Laboratory, Department of Biology, University of Ottawa, Ottawa, K1N 6N5, Canada.)

Our group (CIHR-TAAM) identified 17 plants used by the Cree to treat symptoms of diabetes and screened their 80% hydrated ethanol extracts (EE), using an *in vitro* bioassay platform. However, traditional preparations are often based on hot water extractions (HWE). We thus compared these two extraction methods on the anti-

diabetic potential of the 17 Cree plants at equal concentrations. Three main bioassays routinely applied in our laboratory were used: 1) stimulation of glucose transport in muscle cells by measuring 3H-2deoxyglucose uptake (C2C12 cell line), and 2) inhibition of hepatic glucose production by measuring inhibition of glucose-6-phosphatase activity (G-6Pase; H4IIE cell line), 3) potentiation of adipogenesis by measuring accumulation of triglycerides (3T3-L1 cell line). Our results show that out of the 17 HWE: A) Eight had less or completely lost the effects on glucose transport, B) Five had lower or lost the effects on G6Pase activity, and C) Eleven had lower or lost their effects on adipogenesis in comparison to their EE counterparts. Interestingly, AD01 is the only plant with almost equal anti-diabetic potential between HWE and EE. So the method of extraction is a significant determinant of the biological activity of a medicinal plant. Some HWE plants have comparable anti-diabetic potentials with EE in the bioassays tested here. As EE better extracts phenolics, higher doses of HWE may be necessary to obtain comparable activity. Alternatively, concentrations of components and hence resulting biological activity could be higher in traditional preparations made by aboriginal healers. Changes in the quality and quantity of extract components related to extract preparation as well as underlying mechanisms of action will require further experimentation. Funded by CIHR and the China Scholarship Council.

BOT 12

MYELOPHIL, AN EXTRACT MIX OF ASTRAGALI RADIX AND SALVIAE RADIX, AMELIORATES RESTRAIN-INDUCED STRESS IN MICE MODEL

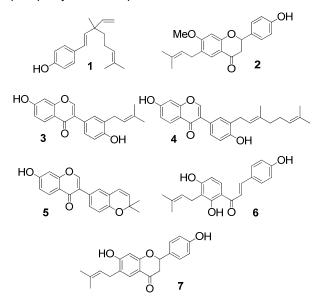
Hyeong-Geug Kim, <u>Chang-Gue Son</u> (Liver and Immunology Research Center, Daejeon Oriental Hospital of Daejeon University, Daejeon, 301-704, South Korea.)

Myelophil is an extract mix of Astragali Radix and Salviae Radix, which has been prescribed for patients with chronic fatigue symptom. This study evaluated the antioxidant effects of Myelophil in restrain-induced stress model. Six-week Balb/c male mice were orally administered Myelophil (0, 100, 200, or 400 mg/kg for 5 days, and then were given to restrain-stress for six hours. Myelophil pre-treatment significantly ameliorated the alteration of serum alanine aminotransferase, aspartate aminotransferase, total ROS, and total antioxidant capacity compared to control group. In addition, Myelophil pre-treatment showed antioxidant effect such as decreasing lipid peroxidation, restoring glutathione depletion in liver tissue compared to control group. Myelolphil pre-treatment lowered tissue levels of pro-inflammatory cytokine, tumor necrotic factor-Yá. Taken together, Myellophil has potent protective effects against restrain-induced stress via antioxidant actions.

PHENOLIC COMPOUNDS ISOLATED FROM PSOLALEA CORYIFOLIA INHIBIT IL-6 INDUCED STAT3 ACTIVATION

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Seven flavonoids (1-7) were isolated from the methanol extracts of the seeds of *Psoralea corylifolia* by bioactivity-guided fractionation using STAT3-dependent luciferase activity. Compounds 1-7 inhibited STAT3 activation by IL-6 in a dose-dependent manner with IC₅₀ values of 4.57±0.45, 3.02±0.53, 2.77±0.02, 0.81±0.15, 1.37±0.45, 2.45±0.13 and 4.89±0.05 μ M, respectively. They also decreased the level of IL-6-induced STAT3 phosphorylation in Hep3B cells.



BOT 14

ISOLATION OF FLAVONOID DI-C-GLYCOSIDES FROM NELUMBO NUCIFERO AND THEIR STRUCTURAL DETERMINATION

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Nelumbo nucifero is one of the raw materials in BioNovo's first in class herbal pharmaceutical drug for the treatment of menopausal hot flashes, Menerba[®]. During our effort to identify marker compounds for each raw material in Menerba[®]: we isolated schaftoside (1) and isoshaftoside (2) from *N. nucifero*. To our knowledge this is the first reported isolation of these compounds from *N. nucifero*. Dictionary of Natural Product and literature searches showed a number of previously flavonoid di-C-glycosides isomers with isolated MW=564. Examples include vicenin 1 and 3, which differs from 1-2, with respect to which sugar is attached at R1 and R2. In addition, a LC-MS trace showed N. nucifero contains 10 peaks with MW=564. To save time and the expense of isolating additional quantities of 1-2 for use in our raw material quality control program, we purchased 1-2 from various sources. However, given the complexity of these compounds and minor structure differences between known isomers we initiated in-depth 1D and 2D NMR studies on the isolated and purchased compounds. We acquired 1D (¹H, ¹³C) and 2D (COSY, HSQC, H2BC, TOCSY, HMBC) NMR spectrum at 400 and 600 MHz. After detail analysis of the NMR data we able to distinguish between the five schaftoside (1) isomers.

BOT 15

ALLEVIATING MEDICINE USAGE AND IMPROVING PULMONARY FUNCTION WITH SUPPLEMENTS OF VEGETABLE AND FRUIT CONCENTRATE, FISH OIL, AND PROBIOTICS IN ASTHMATIC SCHOOL CHILDREN

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We designed a 16-week parallel double-blind randomized placebo-controlled school-based intervention trial to investigate the joint effect of the beneficial dietary components on asthma. A total of 192 asthmatic children aged 10-12 yrs were recruited from elementary schools in metropolitan Taipei. The intervention group received vegetable plus fruit capsules, fish oil capsules and probiotic capsules, while the control group received placebos. Asthma symptoms, pulmonary functions, medicine usage and pediatric asthma quality of life questionnaire score (PAQLQ score) were evaluated at baseline, 8 and 16 weeks. Compared to placebo group, the intervention group had significant improvement in pulmonary parameters such as FVC (178 ml vs. 91 ml) and FEV1 (107 ml vs. 41 ml) and the proportion of children using bronchodilator significantly decreased over the 16 week period. Asthma symptoms and PAQLQ score were not significantly different between the two groups, probably because most children were routinely seen by physicians and medication were adjusted whenever needed. Our study showed that dietary supplements with vegetable and fruit concentrates, fish oil, and probiotics could alleviate bronchodilator usage and increase pulmonary function in asthmatic children.

COMPARING A PHENOMENEX LUNA HPLC-DAD METHOD VERSUS A PHENOMENEX KINETEX UPLC-DAD METHOD FOR RAW MATERIAL QUALITY CONTROL FOR MENERBA

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Menerba^{®;}, a mixture of 21 botanical raw materials, is BioNovo's herbal pharmaceutical for the treatment of menopausal hot flashes. Bionovo and the FDA agreed that a HPLC-DAD fingerprint method be included for quality control of raw materials and drug substance (DS). Aqueous herbal extracts are complex mixtures requiring long gradients to provide sufficient resolution of components for HPLC fingerprinting. Therefore, we required a single, rapid, HPLC-DAD method to compare raw material lots, and DS. Three different HPLC methods were employed during development of the quality control program. The first method used a HP 1100 HPLC and a Phenomenex Luna C18 150 x 4.6. 5 μ m column. This resulted in a method with a very long analysis time (70 min). To reduce the run time and improve resolution we switched to a Phenomenex Kinetex C18 150 x 4.6, 2.7 µm column. Kinetex columns use a core shell silica partial vs. a traditional silica partial. Using the Kinetex column, on a HP 1100 HPLC, injection cycle time was cut to 50 min and resolution of critical compound pairs was improved. Finally, we adapted this method to a Shimadzu UPLC system using a Kinetex C18 150 x 2.1, 1.7 µm column. This combination reduced the cycle time to 25 min and resolution was slightly improved.

In summary, great improvements were made to the HPLC-DAD fingerprinting method by switching to the Kinetics column technology and utilizing a UPLC system.

BOT 17

HERBAL EXTRACTS OF CIBOTIUM BAROMETZ, GENTIANA SCABRA, DIOSCOREA BATATAS, CASSIA TORA, AND TAXILLUS CHINENSIS INHIBIT SARS-COV REPLICATION

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Development of anti-severe acute respiratory syndrome associated coronavirus (SARS-CoV) agents is pivotal to prevent the reemergence of the life-threatening disease, SARS. In this study, more than 200 extracts from Chinese medicinal herbs were evaluated for anti-SARS-CoV activities using a cell-based assay that measured SARS-CoV induced cytopathogenic effect (CPE) *in vitro* on Vero E6 cells. Six herbal extracts, one each from Gentiana scabra, Dioscorea batatas, Cassia tora and Taxillus chinensis (designated as GSH, DBM, CTH and TCH, respectively), and two from Cibotium barometz (designated as CBE and CBM), were found to be potent inhibitors of SARS-CoV at concentrations between 25 and 200 μ g/ml. The concentrations of the six extracts needed to inhibit 50% of Vero E6 cell proliferation (CC₅₀) and 50% of viral replication (EC₅₀) were determined. The resulting selective index values (SI = CC_{50}/EC_{50}) of the most effective extracts CBE, GSH, DBM, CTH and TCH were >59.4, >57.5, >62.1, >59.4, and >92.9, respectively. Among these extracts, CBM and DBM also showed significant inhibition of SARS-CoV 3CL protease activity with IC₅0 values of 39 μ g/ml and 44 μ g/ml, respectively. Our findings suggest that these six herbal extracts may have potential as candidates for future development of anti-SARS therapeutics.

BOT 18

ANTI-WRINKLE POTENTIAL OF STANDARDIZED FLOWER EXTRACT OF CALENDULA OFFICINALIS LINN.

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Traditionally Calendula officinalis L. (Compositae) flower has been claimed for use in inflammation, wound healing, antiseptic, various skin diseases like ulceration, eczema etc. The present study was designed to validate its skin protective activity through inhibition of hvaluronidase, elastase and matrix metalloproteinase-1 (MMP-1). C. officinalis flower was extracted with methanol and fractionated with ethyl acetate, n-butanol and water. Methanol extract and its fractions were tested for enzyme inhibition assay along with standard oleanolic acid. The extract and fractions were standardized through RP-HPLC using syringic acid as biomarker. C. officinalis methanol extract showed significant (cP<0.001) anti-hyaluronidase and antielastase activity with IC_{50} of 6.66±1.54 $\mu g~mL^{-1}$ and $2.70\pm1.73\mu$ g mL⁻¹ respectively and good MMP-1 inhibition (lower fluorescence reading) compared to standard oleanolic acid (35.55±1.60 & 31.57±0.94 µg mL⁻¹). Among all fractions tested, the ethyl acetate fraction showed significant activity. The RP-HPLC analysis revealed that good amount of syringic acid is present in C. officinalis methanol extract (7.3 % w/w), which was higher in the ethyl acetate fraction (13.5 % w/w). C. officinalis showed potent inhibitory activity on hyaluronidase, elastase and MMP-1. Hence, the traditional claim of C. officinalis supports its potential use as an anti-wrinkle agent.

MUSHROOM TYROSINASE INHIBITION AND ANTIOXIDANT PROPERTIES OF DALBERGIA PARVIFLORA ROXB.

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A crude extract of Dalbergia parviflora and its constituents of 35 flavonoids isolated as pure compounds were screened for their inhibitory activity against mushroom tyrosinase. Among the flavonoids tested, only four, namely Khrinone (5), Cajanin (9), (3RS)-3-hydroxy-8-methyoxy vestitol (23) and (6aR,11aR)-3,8-dihydroxy-9-methoxy pterocarpan (33) were shown to have IC_{50} values lower than 100 μ M. These flavonoids were further studied for their inhibition kinetics on the diphenolase activity of the mushroom tyrosinase. The results showed that the inhibition of (5), (9), (23) and (33) were uncompetitive, non-competitive, mixed and competitive inhibitors, respectively. In addition, the D. parviflora extract and the isolates were evaluated for their antioxidant activities: DPPH assay, X/XO assay and ORAC assay. The results revealed that the compounds showed antioxidant activity with, SC₅₀ values of 40-400 µM for DPPH assay, 2.5-250 µM for X/XO assay and 2.8-120 µM Trolox equivalent/10 µM flavonoid for ORAC assay. Based on these findings, it was concluded that D. parviflora heart wood extract is a potential source of natural antioxidants which might be used as anti-browning agents that can inhibit the enzymatic oxidation of phenols by tyrosinase.

BOT 20

AMERICAN GINSENG ACUTELY REGULATES CONTRACTILE FUNCTION OF RAT HEART

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Chronic *Panax ginseng* treatments improve the cardiac performance. However reports of acute administration of *Panax ginseng* on cardiovascular function remain controversial and mechanisms are not clear. In this study, we examined effects of acute American ginseng (*Panax quinquefolius*) administration on rat cardiac contractile functions by using Electrocardiogram (ECG), non-invasive blood pressure measurement and Langendorff isolated-perfused heart measurements. 8-week old male Sprague Dawley rats were gavaged with

water soluble American ginseng at 300 mg/kg body weight. Heart rate and developed pressure were measured at 1hr and 24hr after gavaging. Heart rate was significantly decreased (ECG (6%), non-invasive blood pressure (9-15%) and Langendorff isolated-perfused heart (15-20%)) in water soluble ginseng treated rats comparing with control groups. Markedly decreased developed pressure was observed in ginseng treated Langendorff isolated-perfused hearts but not in noninvasive blood pressure measurements. Direct effect of American ginseng on rat cardiac contractile function was examined by measuring the Langendorff isolated spontaneously beating perfused heart at varying concentrations of water soluble American ginseng. Significantly decreased heart rate and developed force were evidenced after direct ginseng treatments. In our study, we presented the first evidence of depressed cardiac contractile function by acute administration of North American ginseng in rat.

BOT 21

THE ANTI-INFLAMMATORY EFFECT OF SPECIFIC MEDICINAL PLANT EXTRACTS IN DSS-INDUCED COLITIS MODEL

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Approaches for developing evidence-based application of traditional Chinese medicinal (TCM) herbs have received high attention internationally. Recent studies also revealed that induced or chronic inflammation is strongly associated with carcinogenesis. MP, a specific medicinal plant commonly used as TCM in Taiwan, has been shown by our own laboratory and others to confer high anti-inflammatory effects. In this study, we evaluated the effect of the hot water extract of fresh MP (MPHF) in mice with dextran sulphate sodium-induced colitis or colitis-associated colon cancer. We also compared the anti-colitis effects of MP extracts prepared by different extraction methods. MPHF effectively attenuated clinical symptoms of colitis, including loss of body weight, diarrhea and rectal bleeding. It also provided protections against colon-shortening and histopathological changes caused by colon tissue inflammation. MP extracts prepared by different extraction methods showed significantly different levels of anti-colitis activity, and MPHF conferred the best efficacy. In the colitis-associated colon cancer mouse model, mice of the MPHF-treated group showed significantly higher survival rate and better protection on histological manifestations than the cohort mice of nontreated group. In conclusion, we suggest that MP may have good potential for future development into possible adjuvant treatment of colitis and for general protection of gastrointestinal tract.

ANTIOXIDANT EFFECT OF PLAUNOTOL IN HUMAN RENAL CELLS: HK-2

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Renal injury is often found in various oxidative stressrelated pathologies including hypertension, diabetic and toxicity from some medications. Although many ROS (reactive oxygen species) scavengers are proposed for renal protection, most of them are not approved for their safety and efficacy in human. This research work was, therefore, aimed to search for new efficient natural ROS scavengers for the renal protection. Among Thai medicinal plants, Croton stellaopilosus is of particular interest. It contains plaunotol which has been manufactured as an anti-peptic ulcer drug for long time. Its extract has also been shown to have antioxidant activity, and is thus a good source for shedding its novel application. In this study, the antioxidant characteristic profile of plaunotol in human renal cells (proximal tubular renal cells: HK-2) was investigated via flow cytometry analysis. It was found that pretreatment with plaunotol for 6 h significantly decreased the endogenous ROS level. The pretreatment could significantly inhibit the rising of ROS from exogenous ROS treatments such as hydrogen peroxide (H₂O₂) and 2,3-dimethoxy-1,4naphthoquinone (DMNQ). These antioxidant properties might lead to a possibility to develop plaunotol as a safe and effective renopreventive substance for renal damage prevention from oxidative stress.

BOT 23

INSECTICIDAL ACTIVITY OF KOREAN MEDICINAL PLANT EXTRACTS AGAINST *MYZUS PERSICAE*

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Use of natural compounds from plant extracts has been suggested as a viable source of alternative treatments for insect and mite control because many of such compounds have novel modes of action, no or low toxicity to non-target organisms and humans, and are less harmful to the environment. The insecticidal activity of Korean medicinal plant(*Sorbus commixta*, *Akebia quinata* and *Acer tegmentosum*) from ethanol extract, and its fractions using hexane, chloroform, ethyl acetate and water of *Sorbus commixta*, *Akebia quinata* and *Acer tegmentosum* were tested against *Myzus persicae* to examine their effects on mortality. Their composition of volatile substances was determined using GC-MS.

The hexane fraction from *Acer tegmentosum* at a concentration of 1,000 ppm showed 100% *Myzus persicae* mortality after an exposure of 120 min. The results showed that extracts of *Acer tegmentosum* and some of their constituents have potential for development as botanical insecticides.

BOT 24

AD03: AN ALTERNATIVE TREATMENT TO IMPROVING HYPERGLYCEMIA AND HYPERINSULINEMIA IN A DIET-INDUCED OBESE MODEL

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Our team (CIHR-TAAM) conducted ethanobotanical studies in the Cree of Eevou Istchee communities of Northern Quebec and identified 17 plants with antidiabetic potential. Previous in vitro screening studies revealed that one of these plants, AD03, produced strong anti-diabetic effects. Therefore, we hypothesized that AD03 would exert an anti-diabetic effect, in a mouse model of diet-induced obesity and T2D, by lowering glycemia and insulin resistance. We conducted a prevention study where C57BL/6 mice were subjected to high fat (HF) diet for eight weeks to which AD03 was incorporated at 125 and 250 mg/kg. In comparison, in the treatment study, the mice were subjected to HF diet for sixteen weeks. AD03 was introduced in the HF diet for the last eight weeks and tested at 125 and 250 mg/Kg. Results: In the prevention study, AD03 at 250 mg/Kg gradually but significantly decreased whole body and retroperitoneal fat pad weights and improved circulating adipokine levels as compared to HF cognates. No significant effects were observed on glycemia and insulinemia. In the treatment study: AD03 significantly and dose-dependently improved glycemia and insulinemia levels, circulating adipokine levels as well as the G/I index (indicator of insulin resistance) when compared to HF controls. However, AD03 improvement effect on body weight or retroperitoneal fat pad weight was not as pronounced as in the prevention study. In both the prevention and treatment study, no statistical difference was observed in water or food intake. AD03 thus exhibits promising anti-diabetic and slight anti-obesity effects. Mechanisms remain to be elucidated in the liver, muscle and adipose tissue, all targeted by T2D and obesity. Funded by the CIHR.

INHIBITION EFFECT OF FLAVONOLIGNANS AND LIGNAN GLYCOSIDES FROM THE AERIAL PARTS OF ORYZA SATIVA L. ON NO PRODUCTION AND TYROSINASE ACTIVITY

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Rice (Oryza sativa L.) is the principal cereal crop in Asia ingested by the majority of the population. Because there are few reports on constituents or pharmacological activities of the plant, isolation and identification of the bioactive constituents of O. sativa are still required. The aerial parts of Oryza sativa L. were extracted with 80% aqueous MeOH and the concentrated extract was successively partitioned with *n*-hexane. EtOAc. *n*-BuOH. and H₂O. The phytochemical study on O. sativa (leave and stem) led to isolation of four new flavonolignans and two new lignan glycosides along with a known flavonolignan, salcolin B (1). The structures of the isolated compounds were determined on the basis of El-MS, FAB-MS, ¹H and ¹³C-NMR, DEPT, and 2D-NMR (COSY, HSQC, HMBC) experiments. New flavonolignans and lignan glycosides were named as salcolin C (2), salcolinoside A (3), salcolinoside B (4), salcolinoside C (5), oryzanoside A (6), and oryzanoside B (7). The inhibition effect of the isolated compounds on NO production and tyrosinase activity was evaluated. It was suggested that some compounds inhibited NO production in vitro and tyrosinase activity. The alcohol extracts and the compounds could be useful for functionality of cosmetics.

BOT 26

IN VITRO ANTIPLASMODIAL AND CYTOTOXIC ACTIVITY OF PHYSALIN B, EPOXYPHYSALIN B FROM *PHYSALIS ANGULATA* L.

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P. angulata is widely used in popular medicine in many tropical countries to treat various diseases. Crude extracts (aqueous, CH_3OH , EtOH and CH_2CI_2) were prepared by maceration. Physalin B and epoxy-physalin B were obtained by bio-guided fractionation using Si₆₀ liquid chromatography (hexane - ethyl acetate) followed

by RP₁₈ preparative-HPLC in MeOH-H₂O. Crude extracts and pure compounds were then tested in vitro against the 3D7 (chloroquine sensitive) strain of P. falciparum and against the human normal fetal lung fibroblasts WI-38, which allowed determination of selectivity index. Physalin B and epoxy-physalin B were identified by X-ray diffraction. The CH₂Cl₂ and MeOH extracts had a high antiplasmodial activity (IC₅₀: 1.25µg/ml and 1.85µg/ml). Epoxy-physalin B and physalin B gave IC₅₀ values of 0.31 µg/ml and 1.52 μ g/ml. The aqueous extract had moderate activity (IC₅₀: 10.05µg/ml). Epoxy-physalin B and physalin B had a selectivity index of 4.5 and 2.5, respectively. In conclusion, physalin B and epoxy-physalin B could explain the antiplasmodial activity of P. angulata.

BOT 27

CYTOTOXIC CONSTITUENTS OF STEMPHYLIUM SOLANI, A FUNGAL ENDOPHYTE OF MORINDA CITRIFOLIA L. (NONI)

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Morinda citrifolia L. (noni) (Rubiaceae) is a popular medicinal plant indigenous to many pan-tropical regions of the world. The juice from the fermentation of its fruits is claimed to have anticancer properties, but this has remained largely unverified under rigorous pharmacological criteria. The present study investigated the anticancer potential of the metabolites of Stemphylium solani (Ascomycota), an endophytic fungus isolated from the leaves of the noni plant. S. solani was identified during the screening of 30 pure endophyte isolates from the fruits and leaves of noni for cvtotoxic activity. The total ethyl acetate extract of 5-week old malt extract fermentation broth cultures demonstrated IC₅₀ values of 5 and 7 μ g/mL against human lung carcinoma (LU-1) and human prostate carcinoma (LNCaP) cell Bioassay-guided respectively. lines. flash chromatography of the total extract yielded several major cytotoxic fractions. Preliminary purification of one of these fractions using MPLC and reverse phase semipreparative HPLC led to the isolation of two cytotoxic compounds whose structures are being determined by state-of-the-art techniques. Since S. solani is not known to be pathogenic to noni, these cytotoxic constituents may be the product of symbiotic interactions between the fungal endophyte and its host plant. Such cryptic metabolic contributions could conceivably play a role in the purported usefulness of noni in the management of a variety of diseases including cancer.

ANTI-INFLAMMATORY EFFECT OF NORTH AMERICAN (NA) GINSENG

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North American (NA) ginseng extracts exert immunomodulatory effects in vitro. Aqueous (AQ) extract induced immuno-stimulatory effect, while alcoholic (ALC) extract suppressed lipopolysaccharide (LPS)-stimulated macrophage response. The immuno-stimulatory effect of the AQ extract has been attributed to the presence of polysaccharides (PS). The present study focused on the pro-inflammatory and anti-inflammatory effects of ginseng under in vivo and ex vivo conditions. Homocysteine treatment in adult rats induced inflammatory responses in aortic tissues as well as in plasma. Concurrent treatment with both types of ginseng extracts suppressed these inflammatory responses, while ginseng treatment alone in control rats produced no apparent effects. Sub-chronic treatment of rats with both types of extracts was found to have no apparent effect on alveolar macrophage function ex vivo, but they both suppressed LPS-stimulated NO production in culture. This data suggested that systemically acquired ginseng components derived from both extracts possessed anti-inflammatory effect. To further examine the mechanism underlying the anti-inflammatory effect of AQ extract, macrophages (RAW 264.7) were pre-treated with AQ extract or PS fraction for 24 hr prior to LPSstimulation in culture. Results showed that pre-treatment desensitized the responsiveness of macrophages to LPS stimulation; and these effects were absent when AQ or PS ginseng treatment was given concurrently with LPS. It is concluded that AQ and ALC ginseng extracts possess anti-inflammatory effect in vitro and in vivo: and the mode of exposure is an important determinant.

BOT 29

CHEMICAL COMPOSITION OF THE INFUSIONS FROM THE STEMBARK AND LEAVES OF EXOSTEMA CARIBAEUM

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The infusion prepared from the stem-bark of Exostema caribaeum (Jacq.) Roemer & Schultes (Rubiaceae) is widely used for the treatment of malaria and diabetes. Previous chemical studies of the stem-bark revealed the presence of several 4-phenylcoumarins. In the present investigation. we describe the isolation and characterization of two additional 4-phenylcoumarins, namelv 6"-O-acetyl-5-O-β-D-glucopyranosyl-7,3',4'triihydroxy-4-phenylcoumarin and 5-O-B-Dglucopyranosyl-7,3',4'-trihydroxy-4-phenylcoumarin,

from an organic extract of the stem-bark. Chemical analyses of the infusions of the leaves and stem-bark

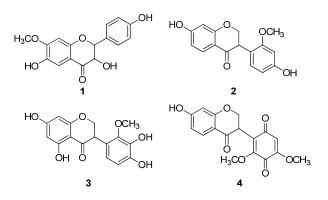
established the presence of 4-phenylcoumarins in both preparations. Thus, as in other Mexican copalchis, 4-phenylcoumarins are the antidiabetic principles of *E. caribaeum*. In addition a suitable HPLC-UV procedure for simultaneous quantification of the major 4-phenylcoumarins in the crude drug of *E. caribaeum* was developed. Altogether, these procedures will be valuable for quality control of the most commercialized medicinal plants in Mexico.

BOT 30

FOUR NOVEL FLAVONOIDS FROM DALBERGIA PARVIFLORA ROXB. WITH THE POTENTIAL TO ESTROGENIC AND ANTIESTROGENIC ACTIVITIES

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The heartwood of Dalbergia parviflora Roxb. (Leguminosae) has been used to normalize the menstruation in Thai traditional medicine. To support its common use, further investigation on its constituents were carried out. A part of its methanol extract (150 g) of the heartwood was subjected to silica gel column chromatography to yield 26 fractions. In this investigation, fraction R was focused for purification by using HPLC. Estrogenic activity and antiestrogenic activity were evaluated by monitoring cell proliferation of estrogen responsive human breast cancer, MCF-7 and T47D cells with various concentrations of isolates. Novel flavavone (1), isoflavanone (2-4), along with 7 known flavonoids, 3',4',6-trihydroxy-7-methoxyflavanone (5), 4',7-dihydroxyflavone (6), 4',7-dihydroxy-8-methoxyisoflavone (7), 4',5,7-trihydroxy-2'-methoxyisoflavone (8) 2',3',7-trihydroxy-4'-methoxyisoflavanone (9). claussequinone (10) and 2'-methoxyisoliguiritigenin (11) were isolated and their structures determined. Further purification and their bioactivity will be presented.



DEVELOPMENT OF QUALITY CONTROL PARAMETERS FOR THE MEDICINAL ORCHIDS CYRTOPODIUM MACROBULBON AND SCAPHYGLOTTIS FASCICULATA

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Cyrtopodium macrobulbon and *Scaphyglottis fasciculata* are employed for treating several diseases in Mexican folk medicine. In the present study, we describe quality control parameters for both species including identity and composition tests. The identity tests comprise chromatographic profiles by HPLC and GC, as well as histological studies using electronic microscopy. In addition, headspace analysis (SPME) of the bulb of both plants using different coated fibers revealed that the principal light volatile compounds were hexanal, eucalyptol, isobornyl formate, 1-nonen-3-ol in the case of *C. macrobulbon* and 3,7-dimethyl-1,6-octadien-3-ol, and 1-nonen-3-ol for *S. fasciculata*. Finally, HPLC method for determining the main active principles of the infusions of this species were developed and validated.

BOT 32

CHORIOALLANTOIC MEMBRANE (CAM) OF CHICK EMBRYO ASSAY TO AQUEOUS EXTRACT OF PTERIDIUM AQUILINUM EVALUATION

Amanda Leitolis, Fernanda Gimenez de Souza, Kelli Freitas, Ricardo Andrukaitis Moledo, Lucas Maracci, Joceline Franco, <u>Luiz Fernando Pereira</u> (Pontifical Catholic University of Parana (PUCPR), Department of Biological Sciences, Curitiba, Paraná 80215-901, Brazil.)

The goal of this study was to evaluate the response of the chorioallantoic membrane to aqueous extract of Pteridium aquilinum (braken fern) and observe its effect on the chick embryo. For the assays (n=8), the eggs were cleaned and a window was opened in the eggshell for access to CAM. All eggs were kept in a humidified incubator at 37°C and gently agitated manually twice a day. The implants with the Pteridium aquilinum extract $(0.1, 0.5, 1, 5 \text{ and } 10 \mu g/ml)$ were performed on gel saline (0.5 ml) previously positioned on the CAM of fertile eggs with six days of incubation. After seven days, the eggs were opened again to the observation on a stereoscopic microscope. The samples were photographed and later, removed for subsequent processing and histological analysis (HE and Masson trichrome). We also evaluated the number of blood vessels and pro- or antiangiogenic response of CAM. The counting was made using a specific program (Image-Pro Plus version 4.5^{TM}). Preliminarily, according to our experimental protocol, aqueous extract of Pteridium aquilinum had no pro- or antiangiogenic effects on CAM. The histological slides showed collagen fibers as possible indicator of inflammatory disorders and/or reparation signals. The majority of chick embryos treated with Braken Fern suffered severe deformities.

BOT 33

COROSOLIC ACID PRODUCTION FROM LAGERSTROEMIA SPECIOSA CALLUS

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Corosolic acid, an ursane-type triterpene acid, is found in Lagerstroemia speciosa leaves (Lythraceae). Because of its pharmacological activity on hyperglycemia, corosolic acid is used as dietary supplement for reducing blood glucose level. In this study, L. speciosa callus was induced from leaves on Murashige and Skoog (MS) medium supplemented with 2 mg/l of 2,4dichlorophenoxy acetic acid (2,4-D) and 0.5 mg/l of kinetin. Under this culture condition, the callus culture produced biomass of 0.67±0.21 g/callus and corosolic acid with yield of 127.55±13.69mg%. The corosolic acid production curve suggested that callus could produce high yield of corosolic acid at the beginning of stationary phase (24 days of culture), which was 166 times higher than by natural leaves.

BOT 34

NEUROPROTECTIVE EFFECT OF SEED OF *LOTUS PLUMULE* IN THE MOUSE HIPPOCAMPAL HT22 CELL LINE

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Glutamate-induced oxidative injury contributes to neuronal degeneration in many central nervous system diseases, such as Alzheimer's disease. The neuroprotective effects of total extract and its fractions (n-hexane, chloroform, ethyl acetate and n-butanol) of seed of L. plumule were investigated in glutamateinduced neurotoxicity in the HT22 cell. The ethyl acetate fraction of seed of L. plumule showed the potent neuroprotective effects by inhibited ROS production in the HT22 cell. Furthermore, the ethyl acetate fraction of seed of *L. plumule* had DPPH radical ($IC_{50} = 90.89$) μ g/ml) and hydrogen peroxide (IC₅₀ = 639.66 μ g/ml) scavenging effect, respectively. These results suggested that the ethyl acetate fraction of seed of L. plumule could show neuroprotective activity by its anti-oxidative activity.

MARINE NATURAL PRODUCTS AS INGREDIENTS IN TRADITIONAL MEDICINE

<u>Varima Wongpanich</u>, Witaya Lowtangkitcharoen (Faculty of Pharmaceutical Sciences, Khon Kaen University, Division of Pharmacognosy and Toxicology, Khon Kaen, 40002, Thailand.)

A survey on official Thai Traditional Formularies has revealed the applications of marine natural products as components in various formulated preparations. More than 40 items are those directly obtained or products modified mainly from animal and mineral origins. A small number is obtained from plant sources. Even though medicinal properties of each item are mentioned, none of the items are used as a single therapeutic material. The indications of formulated preparations that contain these marine-derived components comprise treatment on gastro-intestinal disturbance, reproductive disorders, infections, external and chronic wounds, as well as being feverfew and nourishing. It is also noticed that almost all of the items required pre-incorporation processing prior to compounding. Unlike those of the terrestrials, the ethnopharmacological information of marine natural products is not well documented and has not likely been taken into account for drug discovery and development. As research on marine natural products chemistry has received growing interest in recent decades as a potential new source of drug candidates, this presentation may provide a different angle of connection between chemistry and medicine for sustainable benefit to human life.

BOT 36

ANTIMICROBIAL AND POTENTIAL CANCER PREVENTIVE SUBSTANCES FROM MARINE ALGAE AND CYANOBACTERIA COLLECTED IN HAWAII AND THE CARIBBEAN

Monique Lacroix,¹ Dominic Dussault,¹ Tifanie Vansach,³ Khanh Dang Vu,¹ David Horgen,³ Clarissa Gerhaeuser² (¹INRS, INRS-Institut Armand-Frappier, Laval, Quebec H7V 1B7, Canada, ²German Cancer Research Center, Division Epigenomics and Cancer Risk Factors, Heidelberg, 69120, Germany, ³Hawaii Pacific University, Natural Sciences, Kaneohe, HI 96744, USA.)

We investigated the effects of a small collection of marine-derived extracts and isolates on the reduction of foodborne illness and prevention of cancer. Ten cyanobacterial isolates and nine organic algal extracts from organisms collected in Caribbean and Hawaiian waters were tested against foodborne pathogens using a broth dilution assay. Results showed that several extracts and compounds showed antimicrobial activity against three Gram positive foodborne pathogens at low concentrations (<500 ppm). Results also showed that extracts of *Gracilaria salicornia, Liagora* sp. and *Ulva* sp. demonstrated high potential to suppress carcinogen metabolic activation through induction of NQO1. This research demonstrates the potential of several

cyanobacterial isolates as sources of biologically active substances for the control of foodborne pathogens and for potent inducers of an important enzyme in reducing carcinogen activation.

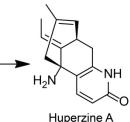
BOT 37

CANDIDATE HUPERZINE A AND OTHER LYCOPODIUM ALKALOIDS IN CULTURED CELLS OF HUPERZIA SPECIES

<u>Kanichiro Ishiuchi</u>, Jeong-Jin Park, David R Gang (Washington State University, Institute of Biological Chemistry, Pullman, WA 99164, USA)

Tissue culture of various *Huperzia* species has been achieved and production of huperzine A (HupA), an anti-Alzheimer's disease drug candidate isolated from the traditional Chinese medicine Qian Ceng Ta (*Huperzia serrata*), has been confirmed in the callus of several species, especially in *H. pinifolia*. The accumulation of various Lycopodium alkaloids, particularly those related to HupA biosynthesis, was also monitored in these tissues using high resolution Q-IMS-TOFMS and ion trap-based MSⁿ analysis.





BOT 38

HYPOLIPIDEMIC ACTIVITY OF TRITERPENES FROM BURSERACEAE OLEORESINS

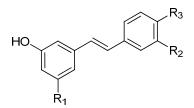
Deborah S. Braz,^{1,2} André Luis Rüdiger,¹ Emerson Silva Lima,² <u>Valdir F. Veiga Junior</u>¹ (¹Universidade Federal do Amazonas, Chemistry Department, Manaus, AM 69079000, Brazil, ²Universidade Feral do Amazonas, School of Pharmaceutical Science, Manaus, AM 69079000, Brazil.)

The phytochemical study of Amazonian Burseraceae oleoresins provided four enriched fractions, named HT, OT, DHT and AT. These fractions had their components identified and these were found to be constituted by isomeric triterpenes that were evaluated for lipase, *a*-amylase and *a*-glycosidase activities. The mixture OT showed higher percentages of lipase inhibition, presenting IC₅₀ (mg/mL) of 3.97 (± 0.41), whereas the TA fraction was the only one with significant *a*-amylase inhibition, showing IC₅₀ (mg/mL) of 23.03 (± 0.5). Given the results for *a*-glucosidase enzymatic inhibition, all compounds tested showed an inhibition greater than 80% and a promise for development of alternative drugs for metabolic syndrome prevention.

CELL ADHESION INHIBITORY ACTIVITIES OF STILBENE DERIVATIVES ISOLATED FROM RHEUM UNDULATUM

<u>Hyun-Mee Oh</u>¹, Seung Woong Lee¹, Mi-Hwa Kim¹, Woo Song Lee¹, Mun-Chual Rho¹ (Eco-Friendly Biomaterial Research Center, Korea Research Inst. Bioscience and Biotechnology, Jeongeup, 580-185, South Korea.)

Six stilbenes were isolated from the methanol extracts of *Rheum undulatum* rhizomes by bioactivity-guided fractionation. Compounds **1-4** inhibited direct binding between sICAM-1 and LFA-1 of THP-1 cells in a dose-dependent manner with IC₅₀ values of 50.1, 25.4, 33.4 and 45.9 μ M, respectively. In addition, the methoxyl group, glycoside, double bond and *trans* configuration of stilbene compounds might be modulatory factors on the binding of LFA-1 and ICAM-1.



- 1. R₁=OH, R₂=H, R₃=OCH₃, Desoxyrhapontigenin
- 2. R₁, R₂=OH, R₃=OCH₃, Rhapontigenin
- **3.** R₁=OH, R₂=H, R₃=OH, *trans*-Resveratrol
- **4.** R₁, R₂, R₄=OH, Piceatannol
- 5. R_1 =OH, R_2 =OGIc, R_3 =OH, Piceatannol-3-O- β -glucopyranoside
- 6. R₁=OGlc, R₂=OH, R₃=OCH₃, Isorhapontin

BOT 40

SARGASSUMOL, A NOVEL ANTIOXIDANT FROM THE BROWN ALGA SARGASSUM MICRACANTHUM

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Free radicals have been implicated in the pathogenesis of various diseases, such as ischemia, arteriosclerosis, diabetes, rheumatoid arthritis, inflammation, and cancerinitiation. There is considerable evidence that antioxidants may help prevent illnesses caused by oxidative stress because they have the capacity to quench free radicals, thereby protecting cells and tissues from oxidative damage. Thus, the demand for alternative antioxidants from natural sources is gradually growing.

The genus *Sargassum* (Sargassaceae), belonging to the large brown algae, are distributed mainly temperate Pacific coast, the Indian Ocean and the Australian coast

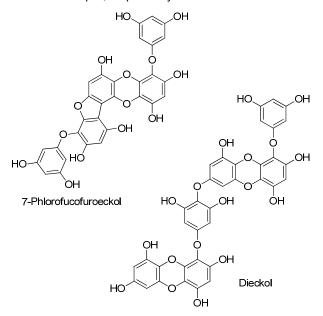
more than 400 species. They are known to produce diverse bioactive principles including plastoquinones, chromanol, cyclopentenone, and polysaccharides. *S. micracanthum* is distributed in Japan and South Korea, mainly south and east coast. The extract of *S. micracanthum* has been known to exhibit antioxidant, anti-viral, and selective vasodilation effects. As part of our ongoing effort to find natural antioxidant, a new free radical scavenger, designated as sargassumol has been isolated from the methanolic extract of *S. micracanthum*

BOT 41

IN VITRO INHIBITORY ACTIVITY OF *ECKLONIA CAVA* AGAINST PORCINE EPIDEMIC DIARRHEA CORONAVIRUS INFECTION

<u>JinA Ko</u>,¹ Hyung-Jun Kwon,¹ Young Bae Ryu,¹ Young Min Kim,¹ Kyoung-Oh Cho,² Woo Song Lee,¹ Su-Jin Park¹ (¹Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, 580-185, South Korea, ²Biotherapy Human Resources Center, College of Veterinary Medicine, Chonnam National University, Gwangju, 500-757, South Korea.)

We evaluated the ability of five polyphenols isolated from the *Ecklonia cava* (EC) to porcine epidemic diarrhea virus (PEDV). We assessed the anti-viral activity of pretreatment, simultaneous treatment, and post treatment. Using the simultaneous treatment assay, we found that EC extracts and fraction and compounds directly blocking viral adsorption to cells. The 50% effective inhibitory concentrations (EC₅₀) of the EC-1 to EC-5 were 12.4-24.5 μ g/mL and compounds **1-5** were 10.8-22.5 μ M, respectively. Moreover, the post treatment assay showed that all extracts and fractions inhibited viral replication with EC₅₀ values of 19.5-28.8 μ g/mL. Also, two compounds (**4** and **5**) showed EC₅₀ values of 12.2 and 14.6 μ M, respectively.



REDUCTION OF OVA-INDUCED LUNG INFLAMMATION IN MICE TREATED WITH AYURVEDIC HERBS USING 2-D AND 3-D IMAGING

Kapil K. Soni, Udeshi Patel, Jim Artwolt, Elizabeth Nunamaker, <u>Gail B. Mahady</u> (University of Illinois at Chicago, Pharmacy Practice and Biological Resource Center, Chicago, IL 60612, USA.)

The medicinal plants Bacopa monnieri (L.) Penn. (Scrophulariaceae). Boswellia serrata Roxb. (Burseraceae) and Ocimum sanctum L. (Lamiaceae) were collected in Vidisha (M.P.), India based on traditional use in Ayurvedia to treat asthma. The plant materials were dried, extracted in methanol and tested in three in vitro assays, leukotriene-C4-synthase, leukotriene-A4-hydroxylase and cyclooxygenase-2. The extracts inhibited all enzymes indicating antiinflammatory and potential anti-asthmatic activities. The extracts were then tested in 24 BALB/c mice (6 arms, 4 animals per arm) sensitized by i.p. injections of ovalbumin (OVA, 50 μ g) weekly for 3 weeks. The animals were then treated by gastric lavage for four days with the herbal extracts (100 mg/kg bw) after intranasal OVA challenge. Animals were subjected to 2-D in vivo imaging and 3-D tomography in a Xenogen IVIS 2000 Imaging System to assess lung inflammation induced by OVA. Animals sensitized and challenged with OVA had significant lung and peritoneal inflammation. Treatment of the mice with dexamethasone reduced OVA-induced inflammation by 50-60%, as compared with the PBS control group. Treatment of the mice with the Ayurvedic herbal extracts also reduced inflammation, with the activities of the extracts being in the order of Ocimum sanctum>Boswellia serrata>Bacopa monnieri.

BOT 43

EFFECT OF ABIES KAWAKAMII LEAF EXTRACTS ON LIFE SPAN EXTENSION IN DROSOPHILA MELANOGASTER

<u>Hui-Ting Chang</u>¹, Jui-Hua Chu², Chau-Ti Ting², Shu Fang³, Li-Sheng Hsu¹, Shang-Tzen Chang¹ (¹National Taiwan University, School of Forestry and Resource Conservation, Taipei, 106, Taiwan, ²National Taiwan University, Department of Life Science, Taipei, 106, Taiwan, ³Academia Sinica, Biodiversity Research Center, Taipei, 115, Taiwan.)

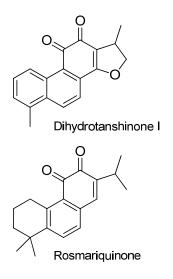
Abies species (Pinaceae) have been used as folk medicines. Abies kawakamii (Hayata) Ito is an evergreen conifer tree in Taiwan with the potential of being a medicinal plant. Antioxidant activity and prolongevity effect in the fruit fly (*Drosophila melanogaster*) lifespan of ethanolic extract and hot water extract from *A. kawakamii* leaf were investigated in this study. Both ethanolic extract and hot water extract exhibited antioxidative activity including scavenging activity of DPPH radicals, reducing power and trolox equivalent antioxidant capacity. Hot water extract showed better antioxidant efficacy than ethanolic extract. Different dosages of hot water extract and ethanolic extracts have various life span extension effects on different sex of *Drosophila melanogaster*. Lifespan extension is longer on fruit flies fed with hot water extract than with ethanol extract, the result was consistent with the antioxidant performance. Prolongevity effect (56.1% and 65.7% for male and female fruit fly respectively) was found in fruit flies fed with hot water extract at a dosage of 0.1 mg/mL dosages. In conclusion, *A. kawakamii* extracts showed a beneficial effect in extension the mean lifespan of fruit fly.

BOT 44

TANSHINONES FROM SALVIA MILTIORRHIZA DISPLAYING POTENT 3CL^{pro} INHIBITION

Ji-Young Park,¹ Young Min Kim,¹ Kyoung-Oh Cho,² Su-Jin Park,¹ Woo Song Lee,¹ <u>Young Bae Ryu¹</u> (¹Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, 580-185, South Korea, ²Biotherapy Human Resources Center, College of Veterinary Medicine, Chonnam National University, Gwangju, 550-757, South Korea.)

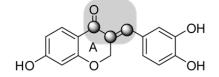
Bioactivity-guided fractionation of the ethanol extract yielded seven tanshinones, identified as tanshinone I (1), tanshinone II A (2), tanshinone II B (3), crytotanshinone (4), dihydrotanshinone I (5), methyl tanshinonate (6), and rosmariquinone (7). The inhibitory activities of these compounds (1-7) against $3CL^{pro}$ from SARS, bovine (KWD3) CoV and porcine epidemic diarrhea virus (PEDV) were evaluated to determine potencies. Analyses using various *in vitro* coronavirus $3CL^{pro}$ assays showed that all seven tanshinones were selective $3CL^{pro}$ inhibitors. Of the tanshienones, rosmariquinone (7) exhibited the most potent inhibitory activity toward SARS-CoV $3CL^{pro}$ (IC₅₀ = 10.2 μ M), whereas dihydrotanshinone I (5) potently inhibited KWD (IC₅₀ = 0.7 μ M) and PEDV (IC₅₀ = 6.1 μ M).



CHARACTERISTIC OF VIRAL NEURAMINIDASES INHIBITORY HOMOISOFLAVONOIDS FROM CAESALPINIA SAPPAN

Young Min Kim, Ji-Young Park, Su-Jin Park, Young Bae Ryu, Woo Song Lee (Eco-Friendly Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology, Jeongeup, 580-185, South Korea.)

In this study, twelve neuraminidase inhibitory compounds **1-12** were isolated from the leaves of *Caesalpinia sappan* on the basis of their biological activities against three types of viral NAs. Of isolated homoisoflavonoids, sappanone A (**2**) showed the most potent NAs inhibitory activities with IC₅₀ values of 0.7 μ M [H1N1], 1.1 μ M [H3N2], and 1.0 μ M [H9N2], respectively, whereas saturated homoisoflavonoids such as **3** did not show significant inhibition. The data revealed that the α , β -unsaturated carbonyl group in the A-ring was key requirement for viral NAs inhibitory activity. In our enzyme kinetic study, all NA inhibitors screened were found to be reversible noncompetitive types.



NCM 1

RECOVERY OF ENDOGENOUS PHENOLIC COMPOUNDS FROM POTATO TUBER USING CONVENTIONAL AND HIGH-PRESSURE EXTRACTION METHODS

<u>Marya Aziz</u>, Sarya Aziz, Selim Kermasha (McGill University, Department of Food Science and Agricultural Chemistry, Ste-Anne de Bellevue, Quebec, H9X 3V9, Canada.)

The recovery of endogenous phenolic compounds (PC) from potato tuber (Solanum tuberosum), usina conventional and high-pressure (HP) extraction methods, was investigated. Mixtures of hexane/ethyl acetate and ethyl acetate/chloroform were used as solvents for the conventional extraction method, whereas ethyl acetate and water/ethanol were investigated with the HP one. The experimental results from the conventional method showed that the hexane/ethyl acetate extraction method was 1.6 times more efficient than that of ethyl acetate/chloroform, resulting in 63.5 mg PC/g extract as compared to 39.4 mg PC/g extract, respectively. To optimize the HP extraction method, selected parameters, including the effect of temperature, number of flushing (cycle) and the potato homogenate/Ottawa sand ratio, were studied. The optimum temperature for the HP extraction by ethyl acetate and water/ethanol HP, was determined to be 50 and 40, respectively. Using the HP method, the results showed that the majority of phenolic compounds (65%) were recovered during the first flush. In addition, the optimum ratio of potato homogenate/Ottawa sand (w/w) was determined to be 5:4 and 5:1 for ethyl acetate and water/ethanol extraction methods, respectively. The overall results the experimental results indicated that the HP extraction with ethyl acetate was 3.5 times more efficient than that with ethanol/water, resulting in 43.0 mg PC/g extract as compared to 12.3 mg PC/g extract.

NCM 2

HOW TO SEPARATE THE WHEAT FROM THE CHAFF? EXPLORING BRAZILIAN BIODIVERSITY USING NMR DEREPLICATION TECHNIQUES.

lan Castro-Gamboa, Fausto Carnevale Neto, Rafael Freire, Alan Cesar Pilon, Patricia Cardoso, Vanderlan Bolzani (Institute of Chemistry, São Paulo State University - UNESP, Organic Chemistry, Araraquara, SP 14800900, Brazil.)

The establishment of new and innovative analytical methods that may shed information towards the composition of complex natural mixtures is critical on bioprospection programs. Our research group NuBBE has incorporated the use of molecular virtual design using NMR aiming to increase the understanding of molecular relationships on dynamic natural matrixes and synergism effects of highly active crude extracts, previously screened using *in vitro* human cell lineages such as HL-60 (leukemia), MDA-MB435 (melanoma),

HCT-8 (colon) and SF-295 (glioblastoma). From several Fabaceae and Asteraceae species, NMR data was acquired, processed and compared with a molecular virtual designed NMR environment containing all known reported metabolites. From the multivariate analysis we detected a recurring array of flavonoids, such as 8methylnaringenin, 4,5,7-trihydroxy-3',6-dimethoxy-8methylflavanone, 3',4',5,6,7-pentahydroxy-8-methyldihvdroflavonol. 4'.5.7-trihvdroxy-3-methoxy-6.8dimethylflavanone. 4',7-dihydroxy-5-methoxy-6methyldihydroflavonol, 5,6,7-trihydroxy-3',4'-dimethoxy-8-methylflavanone and, 3',4'-dimethoxy-5,7-dihydroxy-6,8-dimethyldihydroflavonol. The occurrence of flavonoids may be associated to the original activity revealed in the in vitro assays. However, further studies must be performed in order to establish molecular synergism effects since there is no significant antineoplasic activity reported for those metabolites once isolated.

NCM 3

SIMULTANEOUS EXTRACTION AND QUANTIFICATION OF CAROTENOIDS AND TOCOPHEROLS IN BRASSICA SPECIES

Ivette Guzman, Gad Yousef, Allan Brown (North Carolina State University, Plants for Human Health Institute, Kannapolis, NC 28081, USA.)

Brassica vegetables, like broccoli and cauliflower, are consumed worldwide and are known to contain an array of bioactive compounds. Among these are two classes of photosynthetic lipid soluble compounds: carotenoids and tocopherols. They are isoprenoids with a common precursor. Carotenoids are yellow, orange and red pigments; some of which are vitamin A precursors. Tocopherols have vitamin E activity. As essential vitamins to the mammalian diet, their activities involve protecting membrane lipids from oxidative damage by quenching reactive oxygen species and protecting against degenerative diseases. Brassica species accumulate both carotenoids and tocopherols in the edible floret tissue. Due to genetic and environmental variables, carotenoid and tocopherol amounts are not constant. In order to aid breeders in the development of Brassica cultivars with high pro-vitamin A and vitamin E activity, a more efficient method was created to quantify the major accumulating carotenoids and tocopherols in broccoli and cauliflower. The novel UPLC method separates 5 carotenoids and 2 tocopherols in a 30 minute run, cutting the run time by half compared to previously published chromatographic runs. This data collected allowed us to compare amounts of β -carotene, lutein, α -tocopherol, and γ -tocopherol in *Brassica* to previous studies. We also report amounts for neoxanthin, violaxanthin, and epoxylutein. The goal is to develop a fast effective extraction and quantification method in order to screen large collections of Brassica germplasm, thus aiding breeders in producing a high pro-vitamin A and vitamin E broccoli or cauliflower.

NCM 4

DETECTION OF ADULTERATED NATURAL PRODUCT EXTRACTS CONTAINING SILDENAFIL (VIAGRA) DERIVATIVES

<u>Kimberly L. Colson</u>,¹ Joshua M. Hicks,¹ Sarah M. Luchsinger,¹ Hui Li,² Basil Desousa¹ (¹Bruker BioSpin, Billerica, 01821, USA, ²Bruker Optics, Billerica, 01821, USA.)

Adulteration of natural products with sildenafil (Viagra), sulfoaildenafil, tadalafil, vardenafil or other derivatives is common. To elude detection, adulterators regularly change the derivative supplied in the natural product sample in hopes that the presence of such adulterants go undetected due to the use of analytical methods using targeted analysis. Regenerect, which targets an audience for erectile dysfunction, was voluntarily recalled in April 2011 as a result of FDA lab analysis detecting Sulfoaildenafil in two lots (100521 and 112850). In this work, we evaluated Regenerect samples, both from a recalled lot and unrecalled lot, using ¹H NMR spectroscopy. Lot-to-lot comparisons, detection and quantification of sulfoaildenafil and component reconstruction was undertaken. Methods for automated detection of sildenafil derivatives through substructure analysis were appraised. Evaluation by NIR and IR was also performed.

HEPATOPROTECTIVE ACTIVITY OF *RHODIOLA IMBRICATA* EDGEW ACETONE EXTRACT AGAINST PARACETAMOL INDUCED HEPATOPATHY IN WISTAR RATS

Senthilkumar Ravichandran, Parimelazhagan Thangaraj (Bharathiar University, Coimbatore, Department of Botany, School of Life Sciences, Coimbatore, Tamil Nadu 641046, India.)

The dried Rhodiola rhizome was extracted in Soxhlet extractor successively with organic solvents and concentrated by rotary vacuum evaporator. The evaporated extracts thus obtained were dissolved in the respective solvents and used for antioxidant assay, acute toxicity and hepatoprotective evaluation. Based on the free radical scavenging potential, acetone extract was chosen to investigate the hepatoprotective activity. Rats weighing 150-200g were divided in to 5 groups of 6 animals in each. Group-I served as normal control received water, Group-II served as negative control, administered with Paracetamol (2g/kg), Group-III reference control, Silvmarin (25mg/kg), Group-IV & V received acetone extract for 200 and 400 mg/kg once daily for 14 days. On 14th day, blood was obtained from all animals by puncturing retro-orbital plexus for haematogram and sacrificed for biochemical and histopathological evaluation. We can conclude from this study that Rhodiola acetone extract inhibit the oxidation and maintained the activity of antioxidant enzymes to the normal level in liver with reference to the controls.

NPD 2

NEPHRO-HEPATOPROTECTIVE AND ANTIOXIDANT PROPERTIES OF A TRIHERBAL FORMULATION

<u>Onyekachi Ogbonnaya Iroanya</u>,¹ Joy Elizabeth Okpuzor,¹ Samuel Akindele² (¹University of Lagos Akoka Yaba, Department of Cell Biology and Genetics, Akoka Yaba, Lagos, 23401, Nigeria, ²Nigerian Institute of Medical Research, Department of Biochemistry, Yaba, Lagos, 234001, Nigeria.)

The nephro-hepatoprotective activity of a triherbal formulation (GOV) comprising 50 % ethanolic extract of *Gongronema latifolia*, *Ocimum gratissimum* and *Vernonia amygdalina* was studied using Wistar albino rats. The animals received a single intraperitoneal injection of CCl₄ in a dose of 1 mL/kg of a 50 % (v/v) solution in liquid paraffin. GOV dose dependently and significantly (p<0.05) attenuated the increase in serum hepatic enzyme levels after CCl₄ treatment as compared to the toxin control group. It was observed that GOV administration dose dependently resulted in a significant (p<0.05) elevation in serum levels , liver and kidney CAT, GPx, GSH, GST, SOD and total protein and significant (p<0.05) decrease in lipid peroxidation compared to the toxin control group. It also dose

dependently and significantly (p<0.05) reduced elevated serum levels of cholesterol, creatinine, triglyceride and urea and increased attenuated albumin and total protein concentrations compared to the toxin control group. Histopathologic study of the liver showed restoration of the liver architecture on administration of GOV. The results were compared to Liv 52 and silymarin. The results of this study confirmed the antioxidant and nephro-hepatoprotective activity of the triherbal formulation against carbon tetrachloride induced nephrohepatotoxicity in Wistar albino rats.

NPD 3

THE ANTIOXIDATIVE AND ANTIMICROBIAL ROLES OF ASSOCIATED FUNGI OF THE LICHEN USNEA AUSTRALIS FROM HAWAII

Dovi Kelman,¹ Jonathan D. Awaya,² Anthony D. Wright¹ (¹University of Hawaii at Hilo, College of Pharmacy, Department of Pharmaceutical Sciences, Hilo, HI 96720, USA, ²University of Hawaii at Hilo, Department of Biology, Hilo, HI 96720, USA.)

Lichens are traditionally considered as symbiotic associations between a single fungal species and either an alga or a cyanobacterium. However, it is now known that lichens provide an ecological niche for additional fungi and bacteria. Lichens have the remarkable ability to survive desiccation, extremes of temperature, UV irradiation and oxidative stress. To do this they produce a suite of secondary metabolites. In the current study, Usnea australis, commonly found on the Island of Hawaii. was investigated for antioxidant and antimicrobial properties of its microbial associates. Careful microbial isolation attempts afforded a diversity of fungal strains. Organic extracts of these fungi showed them to have considerable antioxidant activity, compared to the lichen host. Fractionation of the extracts led to the isolation of 8-methoxynaphthalen-1-ol (1), avellaneol (2) and 4-hydroxy-6-methyltetrahydro-2H-pyran-2-one (3). Compound 1 had an extremely high antioxidant activity, compared to 2 and 3. SAR results obtained for a variety of mono-methoxyl - mono-hydroxyl naphthalene derivatives will also be presented. Antimicrobial assays performed with these extracts showed them to selectively inhibit growth of a range of Gram +ve and Gram -ve bacterial pathogens. In contrast, the lichen extract was found to have no antimicrobial activity. In this presentation, aspects of the possible role of the fungal associates in providing defense against oxidative stress and pathogens to the fungal-algal association are discussed.

ANTIOXIDANT ACTIVITY OF HAWAIIAN LICHENS

<u>Dovi Kelman</u>,¹ Kimberley Johns,^{1,2} Patrick R. Wright,^{1,3} Nicole K. Tabandera,¹ Kehau A. Hagiwara,¹ Anthony D. Wright¹ (¹University of Hawaii at Hilo, College of Pharmacy, Department of Pharmaceutical Sciences, Hilo, HI 96720, USA, ²Sheffield Hallam University, Sheffield, S1 1WB, United Kingdom ³University of Freiburg, Freiburg, 79085, Germany.)

Lichens are symbiotic associations between fungi and an algal or a cyanobacterial photosynthetic partner. The symbiotic partners in lichens are subject to increased oxidative stress caused by production of reactive oxygen species (ROS), mainly through photosynthetic related processes. Lichens have the remarkable ability to survive environmental stressors such as desiccation and predation, and to produce secondary metabolites that play important ecological roles in regulation of internal water levels, UV protection, and chemical defense against oxidative stress, pathogens and herbivores. In the current study, we examined the total antioxidative potential of organic extracts of a variety of lichen species found in Hawaii. Of all extracts studied, the ones of Stereocaulon ramulosum and S. vulcani exhibited the hiahest antioxidant activity. Bioassav-guided fractionation of the extracts led to the isolation of 2,4-di-O-methyldivaric acid (1), divaricatic acid (2) and perlatolic acid (3) as the active principles from the extracts of both lichen species. Compound 1 had extremely high antioxidant activity, compared to 2 and 3. Together with antioxidant activity of compounds and extracts, structural assignment of 1 will be presented.

NPD 5

EVALUATION OF THERAPEUTIC EFFECTS OF NIGERIAN MEDICINAL PLANTS IN EXPERIMENTAL AFRICAN TRYPANOSOMIASIS

Emmanuel Olofu Ogbadoyi,¹ Adamu Yusuf Kabiru,¹ Abdulkadir Abubakar,¹ Garba Mohammed,¹ Aderonke Salako,¹ Joseph Okogun² (¹Federal University of Technology, Minna, Centre for Genetic Engineering and Biotechnology/Global Institute for Bioexploration, Minna, Niger State 920001, Nigeria, ²National Institute for Pharmaceutical Research and Development, Idu, Traditional Medicine and Medicinal Plants Research, Abuja, 900001, Nigeria.)

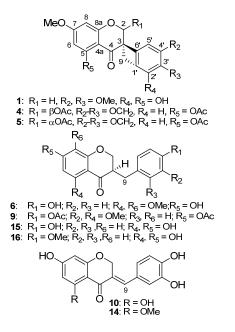
Current therapies of Human African trypanosomiasis are severely limited by the problem of increasing drug resistance. Newer, efficacious and non-toxic drugs are therefore urgently needed. Extracts of leaves and stem bark of Annona senegalensis and Eucalyptus camaldulensis, stem bark of Acasia nilotica and Tridax procumbens (whole plant) were evaluated for their therapeutic effects in model Trypanosoma brucei brucei infection in mice. The methanol extract of *E.camaldulensis* (leaf), hexane and aqueous extracts of *A. senegalensis* (stem bark) and partially purified methanol extract of stem bark of *A. nilotica* produced a complete cure of the experimental infection. Sub inoculation of healthy mice with the blood and cerebrospinal fluid of the cured mice did not result in infection. Acute toxicity studies of the extracts showed they are within safe margins. Preliminary phytochemical studies showed the presence of 7,8-dihydroxyflavone, 3hydroxyflavone, quercetin, and catechin in Τ. procumbens while 2-chloro-N-(1,3-thiozol-2yl)acetamide, 9-octadecenamide, 1-nonadecene; (Z)-9eicosene. hexadecanol. 1-pentadecanol, cis-9-octadecenoate. methylhexadecanoate; methyl methyl-n-octadecanoate, and 1-heptadecanol were detected in leaf extract of E. camaldulensis. We conclude that these extracts have enormous potential of being developed into efficacious phytomedicines for the treatment of African trypanosomiasis.

NPD 6

COX-2 SPECIFIC INHIBITORS FROM LEDEBOURIA OVATIFOLIA AND LEDEBOURIA SOCIALIS (HYACINTHACEAE:HYACINTHOIDEAE)

<u>Catherine P. Tunbridge</u>,¹ Neil R. Crouch,³ Alfred E. Thumser,² Dulcie A Mulholland¹ (¹University of Surrey, Chemical Sciences, Guildford, GU2 7XH, United Kingdom, ²University of Surrey, Biological Sciences, Guildford, GU2 7XH, United Kingdom, ³South African National Biodiversity Institute, Ethnobotany Unit, Durban, 4007, South Africa.)

A phytochemical investigation of *Ledebouria ovatifolia* and *Ledebouria socialis* yielded ten novel compounds including a cycloartane derivative, a dihydrochalcone and two xanthones along with the homoisoflavonoids depicted (**1-6**). Twenty-three known compounds were also isolated; these included three homoisoflavonoids (**7-9**) which had selective COX-2 activity at 10μ M.



MICROTITRE PLATE-BASED ANTIBACTERIAL ASSAY TOWARDS ASARUM HETEROTROPOIDES ACTIVE PRINCIPLES AGAINST HUMAN INTESTINAL BACTERIA

<u>Jun-Ran Kim</u>,^{1,2} Haribalan Perumalsamy,² Young-Joon Ahn^{1,2,3} (¹Seoul National University, Research Institute for Agricultural and Life sciences, College of Agriculture and Life sciences, Seoul, 151-921, South Korea, ²Seoul National University, Bioactive Natural Products Laboratory, Department of Agricultural Biotechnology, Seoul, 151-921, South Korea, ³Seoul National University, WCU Biomodulation Major, Department of Agricultural Biotechnology, Seoul, 151-921, South Korea.)

The growth-inhibiting activity of Asarum heterotropoides root derived materials, (-)-asarinin, α -asarone, 1,8cineole. 3-carene. methyleugenol, pellitorine. pentadecane and safrole, identified in Asarum heterotropoides roots toward 10 human intestinal bacteria was evaluated by using microtitre plate-based antibacterial assay compared to those of two commercially available antibiotics, ciprofloxacin and tetracycline. The active principles in A. heterotropoides root derived materials were identified by spectroscopic analysis: 3-carene, pellitorine and methyleugenol exhibited very strong growth inhibition and the minimum inhibition concentrations (MIC) ranged from 0.032-0.25 mg/100 µL to almost all bacteria particularly Escherichia coli and Staphylococcus aureus. The remaining active compounds asarinin, asarone, 1,8-cineole and safrole showed moderate growth inhibition (MIC 0.25-0.5 mg/100 μ L) to all bacterial species and weak growth inhibition occurred with pentadecane (4 mg/100 μ L). In the control plate well, no adverse effects were observed on growth. Among all compounds isolated, 3-carene, pellitorine and methyleugenol might play an important role in antibacterial activity.

NPD 8

GINSENOSIDES FROM HEAT-PROCESSED KOREAN GINSENG ROOTS, LEAVES AND FLOWER BUDS

Young Ho Kim (College of Pharmacy, Chungnam National University, Daejeon, 305-764, South Korea.)

Panax ginseng (C.A. Meyer, Araliaceae), an ancient and famous herbal drug in oriental traditional medicine, has been broadly used as a functional food as a boiled extract, powder, tea, tablet, capsule, etc., for thousands of years. These conventional ginseng products are reported to have a wide range of pharmacological and physiological actions, such as antiaging, antidiabetic, anticarcinogenic, analgesic, antipyretic, antistress, antifatigue, and promotion of DNA, RNA, and protein synthesis. Traditionally, ginseng has been processed to make white ginseng (WG, roots air-dried after peeling) and red ginseng (RG, roots steamed at 98-100 °C without peeling) to enhance its preservation and efficacy, which is associated with changes in chemical

constituents, especially newly formed ginsenosides as a result of the steaming process.

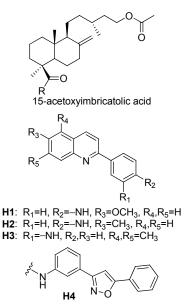
Current studies on the chemical components of the steamed ginseng roots, leaves and flower buds led to the isolation of 22 ginsenosides, including two new ginsenosides from roots, 20 ginsenosides including 6 new compounds from steamed leaves, and 20 ginsenosides, including one new compound from steamed flower buds, respectively. In addition, all of the ginsenosides were biologically evaluated thus establishing their anti-oxidant and anti-inflammatory activity and effects on human leukemia cells. Some of the ginsenosides showed specific biological activities.

NPD 9

NEW DITERPENE AND HETEROCYCLES HYBRID COMPOUNDS: SYNTHESIS AND GASTROPROTECTIVE MECHANISMS OF ACTION USING HUMAN CELL CULTURES

<u>Cristina Theoduloz</u>,¹ Gabriel Vargas,² Luis Astudillo,³ Guillermo Schmeda-Hirschmann³ (¹Universidad de Talca, Facultad de Ciencias de la Salud, Talca, Chile VII Region, ²Universidad Cientifica del Peru, Iquitos, Peru, ³Universidad de Talca, Instituto de Quimica de Recursos Naturales, Talca, Chile VII Region.)

Four new amides were prepared combining the naturally occurring labdane diterpene 15-acetoxyimbricatolic acid and the synthetic heterocycles H1-H4. The activity of the compounds was investigated on human cell culture models including basal cytotoxicity, stimulation of fibroblast and gastric epithelial cell (AGS) proliferation, protection against sodium taurocholate-induced damage on AGS cells and inhibition of the lipoperoxidation induced by tert-butylhydroperoxide in human erythrocyte membranes. Acknowledgements: FONDECYT Project Nr. 1085306.



A NANOSTRUCTURED GEL OF *LIPPIA SIDOIDES* ESSENTIAL OIL

Marco A Botelho,¹ Dinalva Brito Queiroz,³ Leonard Edward Bannet,⁴ Aarâo Lyra,¹ Rejane Andrade Carvalho,¹ Giselle Gasparino Coluchi,¹ Ana Helena Patrus,^{3,4} Ronaldo Sousa Ruela² (¹University Potiguar, School of Health, Natal, Rio Grande do Norte, 59056-010, Brasil, ²Faculdades Unidas do Norte de Minas, Post Graduation, Montes Claros, Minas Gerais, 35000000, Brasil, ³Evidence Pharmaceutical, Research & Development, Fortaleza, Ceara, 60125100, Brasil, ⁴ Santé Medical Center, Biotechnology, Sao Paulo, SP, 04516-000, Brazil.)

Objectives: The aim of this study was to compare the osteoblastic activity of a nanostrutured chitosan hydroxyapatite coated essential oil nanogel on alveolar bone resorption process.

Material and Methods: Experimental Periodontitis Disease (EPD) was induced in 30 Wistar rats subjected to ligature placement on left molars. Animals were treated with hydroxyapatite gel (HD) Saline-based gel (SG) was utilized as the negative control and doxycycline gel 10 mg/g was the positive control. Animals were randomly assigned into groups. The periodontium was examined at morphology.

Results: HD treatment reduced tissue lesion coupled to decreased myeloperoxidase activity in gingival tissue when compared to the saline gel (p<0.05). Conclusion: The HD gel was able to provide a significant alveolar restructuring in this model.

NPD 11

EVALUATION OF ANTI-POLIOVIRUS ACTIVITY OF MEDICINAL PLANTS SELECTED FROM NIGERIAN ETHNOMEDICINE

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Fourteen medicinal plants commonly used by traditional healers in Nigeria for the treatment of viral infection were investigated for *in vitro* anti-poliovirus activity, in a tissue culture system. Plants were extracted into absolute methanol and subjected to anti-viral assays. Serial two fold dilutions made from the MNTD of each extract was used to evaluate their ability to inhibit viral-induced cell death in MTT colorimetric assay. 50 % inhibitory concentration (IC₅₀) and 50 % cytotoxicity concentration (IC₅₀) were determined by statistical analyses. A selective index was calculated as the ratio of CC₅₀ to IC₅₀.

Senna siamea (bark extract) and Zephyranthes candida (whole plant) demonstrated significant *in vitro* activity with IC_{50} of $1.85 \times 10^{-3} \ \mu g/mL$ and $1.21 \times 10^{-1} \ \mu g/mL$

respectively. Bioassay-guided fractionation indicated that activities were retained in the chloroform fraction of *Z. candida* as well as hexane and chloroform fractions of *S. siamea* with IC_{50} of $1.2 \times 10^{-6} \ \mu g/mL$, $2.3 \times 10^{-1} \ \mu g/mL$, $5.1 \times 10^{-1} \ \mu g/mL$ respectively.

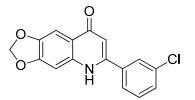
These results support the traditional use of *Senna* siamea and *Zephyranthes candida* as antiviral agents and suggest that they could possibly provide a template for anti-poliovirus drug development.

NPD 12

NOVEL QUINOLONE CMQ INDUCES APOPTOSIS AND MITOTIC CATASTROPHE IN PROSTATE CANCER CELLS VIA REACTIVE OXYGEN SPECIES-AND MITOCHONDRIA-DEPENDENT PATHWAYS

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Quinolone derivatives have been shown to confer various pharmacological activities. In this study, we investigated the effect of 2-(3-chlorophenyl)-6,7methylenedioxyquinolin-4-one (CMQ) as a drug candidate for antitumor activities in p53-expressing LNCaP cells and p53-null PC-3 cells of prostate cancers. CMQ-1 inhibited tumor cell growth via microtubuledepolymerization and G2/M cell cycle arrest in both cell types. Intriguingly, CMQ triggered a strong apoptotic activity in LNCaP cells but induced mitotic catastrophe in the tested PC-3 cells. The cell cycle blockade in both cell types was found to be associated with an elevated level of reactive oxygen species (ROS), followed by activation of the mitochondrial apoptotic pathway. As a result, cytochrome c, Smac and apoptosis-inducing factor (AIF) were released from mitochondria into the cytosol, with subsequent consecutive activation of caspases-9 and -3. In addition, CMQ significantly activated caspase-8 in LNCaP cells but not in PC-3 cells. Intraperitoneal injection of CMQ significantly suppressed tumor growth in SCID mice bearing LNCaP or PC-3 xenografts. Our findings suggest that CMQ can display differential antitumor activities in different prostate cancers.



2-(3-chlorophenyl)-6,7methylenedioxyquinolin-4-one (CMQ)

A MECHANISTIC ANALYSIS OF BRYONOLIC ACID TRANSCRIPTIONAL CONTROL: PERTURBATION OF INFLAMMATORY AND ANTIOXIDANT GENES *IN VITRO* AND *IN VIVO*

Tonibelle N. Gatbonton-Schwager, John J. Letterio, Gregory P. Tochtrop (¹Case Western Reserve University, Departments of Pharmacology, Pediatrics, and Chemistry, Cleveland, OH 44106, USA.)

The aim of this study is to characterize the mechanisms mediating the anti-inflammatory activity of bryonolic acid (BA) and validate the utility of BA as a tool to explore the relationships between triterpenoid structure and activity. Here we show that BA reduces the inflammatory mediator, nitric oxide (NO) by suppressing the expression of the inflammatory enzyme inducible nitric oxide synthase (iNOS) in LPS-activated RAW 264.7 macrophage cells. In addition, BA robustly induces the antioxidant protein, heme oxygenase-1 (HO-1) in vitro and in vivo in an Nrf2-dependent manner. Further analysis of Nrf2 target genes (NQO-1, CAT, GCLC and GR) revealed a selectivity for the timing and level of gene induction by BA in treated macrophages with distinct patterns for Nrf2-regulated antioxidant genes. These findings are significant as this is the first study to show mechanistic insights for the anti-inflammatory activity of BA through suppression of iNOS and induction of HO-1. Our study validates the use of BA as a tool to explore the role of the triterpenoid scaffold as a of determinant the anti-inflammatory and chemopreventive properties of these molecules. Further, understanding how selective gene modulation is controlled by the triterpenoid skeletal structure will aid in the design of selective therapeutics that minimize undesirable effects that may often be the consequence of interactions with multiple downstream targets.

NPD 14

ANTIDIABETIC ACTIVE FRACTIONS FROM MOMORDICA BALSAMINA FRUIT PULP

<u>Ajudhia Nath Kalia</u>, Shruti Aggarwal, Pradeep Kamboj (ISF College of Pharmacy, Dept. of Pharmacognosy, Moga, 142001, Punjab, India.)

The present study was designed to isolate and identify the anti-diabetic potential fraction from the methanol extract (ME) of *Momordica balsamina* fruit pulp (MBFP). Charantin and vicine (anti-diabetic compounds) in the *n*butanol fraction of the ME were identified on the basis of HPTLC and spectral data. Administration of the *n*butanol fraction in an OGTT-model improved glucose tolerance of normal rats. In STZ induced diabetic rats, a fraction showed significant (p<0.05) anti-hyperglycemic and anti-hyperlipidimic activities in a time dependent manner and were on par with the standard anti-diabetic drug metformin (500 mg/kg). In conclusion, the antidiabetic potential of MBFP may be attributed due to the presence of charantin, vicine and other phenolic compounds. NPD 15

MELANIN PRODUCTION ENHANCEMENT OF HUMAN TYROSINASE PLASMID (PAH7/TYR) BY TAT AND AN ENTRAPMENT IN ELASTIC CATIONIC NIOSOMES

Narinthorn Khositsuntiwong,¹ Aranya Manosroi,^{1,2} Friedrich Goetz,³ Rolf G. Werner,⁴ Worapaka Manosroi,⁵ Jiradej Manosroi^{1,2} (¹Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand, ²Natural Products Research and Development Center, Science and Technology Research Institute/Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand, ³Faculty of Biology, University of Tübingen, Department of Microbial Genetics, Tübingen, 72076, Germany, ⁴Boehringer-Ingelheim, Ingelheim am Rhein, 55216, Germany, ⁵Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand.)

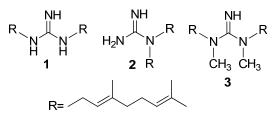
HIV-1 Tat peptide (T), human tyrosinase plasmid (pAH7/Tyr, P) and elastic cationic niosomes (E) complexes (TPE at T/P/E ratio of 0.5:1:160 w/w) exhibited the highest gene expression, as determined by tyrosinase enzyme activity and melanin production in melanoma ($B_{16}F_{10}$) cells of about 12 and 13 fold increases relative to the control, respectively. The remaining plasmid in TPE complexes at 8-week of storage were 45, 32 and 28% for TPE that kept at 4±2, 25±2 and 45±2°C respectively.

NPD 16

SYNTHESIS OF ALKALOIDS FROM *PTEROGYNE NITENS* TUL. AND ITS ANTITUMORAL ACTIVITY IN NUDE MICE BALB/C

Juliana Maria Bozeto,¹ <u>Mauro Cafundo Morais</u>,¹ Flavio Campos Monteiro,¹ Luis Octavio Regasini,² Dulce Helena Siqueira Silva,² Vanderlam Bolzani,² Christianne Pienna Soares¹ (¹School of Pharmaceutical Sciences, ²Institute of Chemistry, São Paulo State University -UNESP, Araraquara, SP 14801-902, Brazil.)

The phytochemical study of leaves from *Pterogyne nitens* Tul. (Fabaceae) afforded guanidine alkaloids nitensidine A (**1**), nitensidine B (**2**) and nitensidine C (**3**). Nitensidine A was selected as prototype compound for antitumor activity, and a synthetic geranylated derivative has been prepared. The synthetic guanidine alkaloid was evaluated towards apoptosis in SiHa cells (80 % at 0.6 μ M) and nude mice xenograft model. Tumor growth was 74.2 % in animals treated with saline, whereas animals treated with nitensidine A at 0.244 mg/kg b.w. was 13.8 % (P<0.001).



WNT/β-CATENIN SIGNALING MEDIATES THE ANTITUMOR ACTIVITY OF MAGNOLOL IN COLORECTAL CANCER CELLS

Hyen Joo Park,¹ You-Jin Kang,² Hwa-Jin Chung,¹ Ki-Dae Kim,¹ <u>Sang Kook Lee</u>¹ (¹Seoul National University, College of Pharmacy, Seoul, 151-742, South Korea, ²Ewha Womans University, College of Pharmacy, Seoul, 120-750, South Korea.)

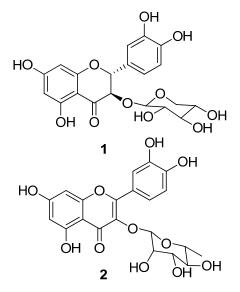
Abnormal activation of the canonical Wnt/beta-catenin pathway and up-regulation of the beta-catenin/T-cell factor (TCF) response to transcriptional signaling play a critical role early in colorectal carcinogenesis. Therefore, Wnt/beta-catenin signaling is considered an attractive target for cancer chemotherapeutics or chemopreventive agents. Small molecules derived from the natural products were used in our cell-based reporter gene assay to identify potential inhibitors of Wnt/beta-catenin signaling. Magnolol, a neolignan from the cortex of Magnolia obovata, was identified as a promising candidate. as it effectively inhibited beta-catenin/TCF reporter gene (TOPflash) activity. Magnolol also suppressed Wnt3a-induced beta-catenin translocation and subsequent target gene expression in HEK293 cells. To further investigate the precise mechanisms of action in the regulation of Wnt/beta-catenin signaling by magnolol, we performed Western blot analysis, real-time reverse transcriptase-polymerase chain reactions, and an electrophoretic mobility shift assay in human colon cancer cells with aberrantly activated Wnt/beta-catenin signaling. Magnolol inhibited the nuclear translocation of beta-catenin and significantly suppressed the binding of beta-catenin/TCF complexes onto their specific DNAbinding sites in the nucleus. These events led to the down-regulation of beta-catenin/TCF-targeted genes downstream such as c-mvc. matrix metalloproteinase (MMP)-7, and urokinase-type plasminogen activator (uPA) in SW480 and HCT116 human colon cancer cells. Magnolol also exhibited antitumor activity in a xenograft-nude mouse model bearing HCT116 cells. These findings suggest that the growth inhibition of magnolol against human colon cancer cells can be partly attributed to the regulation of the Wnt/beta-catenin signaling pathway.

NPD 18

CONSTITUENTS OF CHAMAECYPARIS OBTUSA WITH INHIBITORY ACTIVITY ON ALDOSE REDUCTASE AND SORBITOL ACCUMULATION

<u>Jin-Kyu Kim</u>,¹ Dong-Joo Kwon,^{2,5} Min-Young Lee,³ Soon-Sung Lim,^{1,3,4} Young-Soo Bae,⁵ Jun-Hyung Cho⁶ (¹Hallym University, Institute of Natural Medicine, Chunchon, 200-702, South Korea, ²Hallym University, Biomedical Science, Chunchon, 200-702, South Korea, ³Hallym University, Center for Efficacy Assessment and Development of Functional Foods and Drugs, Chunchon, 200-702, South, ⁴Hallym University, Food Science and Nutrition, Chunchon, 200-702, South Korea, ⁵Kangwon National University, Forest Biomaterials Engineering, Chunchon, 200-701, Republic of Korea, ⁶Kangwon National University, Paper Science and Engineering, Chunchon, 200-701, South Korea.)

Taxifolin-3-O- α -D-xylopyranoside (1) and quercitrin (2) were isolated from an EtOAc-soluble extract of the leaves of *Chamaecyparis obtusa*. Quercitrin was found to possess a potent inhibitory activity of human recombinant aldose reductase *in vitro*, its IC₅0 value being 11.5 mM. Kinetic analysis showed that quercitrin exhibited uncompetitive inhibition against DL-glycealdehyde. Also, quercitrin suppresses sorbitol accumulation in rat lens under high glucose conditions, demonstrating the potential to prevent sorbitol accumulation *ex vivo*. These results suggest that this compound may be a promising agent in the prevention or treatment of diabetic complications.



NPD 19

RESORCINOL AND FLAVONOIDS COMPOUNDS FROM ONONIS NATRIX

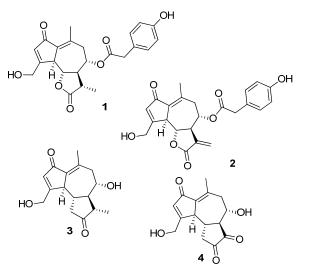
Adnan Jathlan Al-Rehaily,¹ Mohammad S Ahmed,¹ Jamal Mustafa,¹ Muhammad Yousaf,¹ Mohammed A Al-Yahya,¹ Mansour S Al-Said,¹ Shabana I Khan,² Ikhlas A Khan² (¹King Saud University, Department of Pharmacognosy, Riyadh, 2457, Central Kingdom of Saudi Arabia, ²University of Mississippi, The National Center for Natural Product Research, School of Pharmacy, Oxford, MS 38677, USA.)

Phytochemical study of the acetonitrile extract of the aerial parts of *Ononis natrix* has resulted in the isolation and identification of 17 resorcinol derivatives and 4 flavonoids. Among these we could succeed in identification of two new compounds and their structures are illustrated below. The isolated natural products were evaluated for their antimicrobial, antimalarial, antitrypanosomal, cytotoxic and antioxidant activity.

ISOLATION OF SESQUITERPENE LACTONES FROM ROOTS OF CICHORIUM INTYBUS L. WITH LEISHMANICIDAL ACTIVITY

Thirumurugan Rathinasabapathy, <u>Patricio Rojas-Silva</u> Alexander Poulev, Slavko Komarnytsky, Ilya Raskin (Rutgers, The State University of New Jersey, Plant Biology and Pathology Department, New Brunswick, NJ 08901, USA.)

In our effort to seek novel leishmanicidal bioactives from medicinal plants, we isolated four sesquiterpene lactones from the roots of Cichorium intybus (Asteraceae), known as chicory, and tested their bioactivity against Leishmania tarentolae. Chicory roots infusion has been used as an effective antimalarial treatment in Afghanistan, a country where the prevalence of Leishmaniasis is also very high. Dried and powdered chicory roots were extracted with methanol for 24 hours at room temperature, and then the methanolic extract was filtered, concentrated and dried under vacuum. Next, the methanolic extract was partitioned with *n*-hexane and ethyl acetate. The ethyl acetate extract was then fractionated using fast centrifugal partition chromatography (FCPC) yielding 12 different fractions that were further purified by preparative HPLC. Fraction 5 yielded two compounds identified by LC-MS as 11(S),13-dihydrolactucopicrin (1) and lactucopicrin (2), respectively. Fractions 8 and 9 were combined and purified together to yield two compounds identified by LC-MS as 11(S),13-dihydrolactucin (3) and lactucin (4). All the compounds were confirmed by ¹H-NMR. The IC₅₀ leishmanicidal activity for 2 was the highest (24.8 μ M), while the remaining compounds showed low activity >50 μ M. This study provides new perspectives on the development of sesquiterpene lactones into novel leishmanicidal drugs and supports lactucopicrin as a candidate for further studies.



NPD 21

METABOLITES FROM THE SILKWORM (*BOMBYX MORI* L.) DROPPINGS PROMOTE THE ACTIVITY OF HO-1 AND SIRT1

<u>Ji-Hae Park</u>, Jin-Gyeong Cho, Dae-Young Lee, Do-Gyeong Lee, Su-Yeon Kim, Nam-In Baek (Graduate School of Biotechnology & Graduate School of Oriental Medicinal Materials & Processing, Kyung Hee University, Yongin, 446-701, South Korea.)

Silkworm droppings are excrements of the silkworm. Bombyx mori L. whose alcohol extracts improve some skin troubles caused by atopy. So, this study was initiated to isolate the principal compounds to manifest the activity of silkworm droppings. Dried and powdered silkworm droppings were extracted with 80% ag. MeOH, and the concentrated extract was partitioned with EtOAc, *n*-BuOH and H₂O, successively. The repeated silica gel and ODS column chromatography of the EtOAc and n-BuOH fractions led to isolation of 25 metabolites. From the result of spectroscopic data including NMR. EI/MS. FAB/MS. polarimetry, and IR six lignans, five flavonoids. seven megastigmene norsesquiterpenes, and seven hydroxyl fatty acids were identified. Three compounds have never been reported in nature, and the other twenty-two compounds were also isolated for the first time from silkworm droppings. Seven compounds among them increased expression of heme oxygenase-1 (HO-1) in HepG2 cells, and two compounds increased the expression of SIRT1 in HepG2 and HEK239 cells, respectively. The enzymes are involved in suppression of inflammatory mediators or factors that may be used to improve atopy-related symptoms.

NPD 22

A BOTANIC LEAD OF ATTENTION DEFICIT HYPERACTIVITY DISORDER

<u>Che-Yi Lin</u>, Jui-Ching Chen, Cheng-Po Huang, Chien-Chang Wu, Yuan-Ling Ku (Medical and Pharmaceutical Industry Technology and Development Center, New Taipei, 24886, Taiwan.)

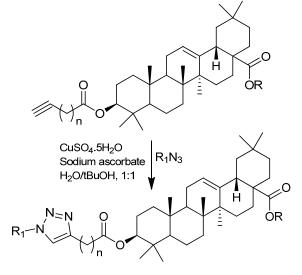
PDC-1421, an extract acquired by a single Traditional Chinese Medicine, demonstrated a great response in tetrabenazine-induced hypothermia. We distinguish the main mechanism of PDC-1421 from the pharmacology in central nervous system. The IC₅₀ of norepinephrine transporter (NET), Dopamine transporter (DAT), and Serotonin transporter is 1.27, 76.4, and greater than 300 μ g/mL, respectively. The IC₅₀ of NE uptake is 0.704 μ g/mL in HEK293 cells, but the IC₅₀ of both dopamine and serotonin uptake are more than 100 μ g/mL. In this study, we identified PDC-1421 as a major NET inhibitor and minor DAT inhibitor for pharmacodynamics. Currently, stimulant drugs that are effective against attention deficit hyperactivity disorder (ADHD) are thought to work by altering the levels in either norepinephrine or the synergistic effect of dopamine and norepinephrine. These results indicated that PDC-1421 may be designated as the botanic lead of ADHD.

1,2,3-TRIAZOLE-SUBSTITUTED OLEANOLIC ACID DERIVATIVES: SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY

<u>Mariano Walter Pertino</u>,¹ Cecilia López,¹ Cristina Theoduloz,² Guillermo Schmeda-Hirschmann¹ (¹Universidad de Talca, Instituto de Química de Recursos Naturales, Talca, VII Region, Chile, ²Universidad de Talca, Facultad de Ciencias de la Salud, Talca, VII Region, Chile.)

Starting from the naturally occurring triterpene oleanolic acid, alkyl esters were prepared and treated with different aromatic azides to produce "hybrid" compounds using click chemistry. The antiproliferative activity of the new triterpene derivatives was evaluated towards normal lung fibroblasts (MRC-5), gastric epithelial adenocarcinoma (AGS), promyelocytic leukemia (HL-60), lung cancer (SK-MES-1), and bladder carcinoma (J82) cells. Acknowledgements: This work was supported by

Acknowledgements: This work was supported by FONDECYT N $^{\circ}$ 11100046.



NPD 24

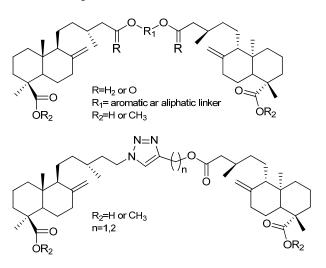
NEW DIMERIC DITERPENES: SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY

<u>Mariano Walter Pertino</u>,¹ Marco Francisco Bastias,¹ Cristina Theoduloz,² Guillermo Schmeda-Hirschmann¹ (¹Universidad de Talca, Instituto de Química de Recursos Naturales, Talca, VII Region, Chile, ²Universidad de Talca, Facultad de Ciencias de la Salud, Talca, VII Region, Chile.)

Several labdane diterpenes have shown biological activity, including antiproliferative effects. Little has been done on the synthesis of dimeric diterpenes using different linkers. The diterpenes labd-8(17)-en-15-hydroxy-19-oic acid (imbricatolic acid) and labd-8(17)-en-15,19-dioic acid (junicedric acid) were used as terpene moieties to prepare dimeric compounds. The new compounds include ethers and esters with different 'linkers' (spacers) as well as 1,2,3-triazole-substitute

derivatives prepared by click chemistry. The antiproliferative activity of the new compounds was assessed against normal lung fibroblasts (MRC-5) and four different cancer cell lines including: gastric epithelial adenocarcinoma (AGS), promielocytic leukemia (HL-60), lung cancer (SK-MES-1), and bladder carcinoma (J82) cells.

Acknowledgements: This work was supported by FONDECYT grants 11100046 and 1085306.



NPD 25

HAGININ A, A PTP1B INHIBITOR FROM THE BRANCH OF *LESPEDEZA CYRTOBOTRYA*, STIMULATES GLUCOSE UPTAKE THROUGH AMPK ACTIVATION IN SKELETAL MUSCLE CELLS

<u>Jin Hee Kim</u>,¹ Eun Sook lee,¹ Min Kyun Na² (¹Daegu Hanny University, Department of Herbal Skin Care, Gyeongsan, 712-715, South Korea, ²Yeungnam University, College of Pharmacy, Gyeongsan, 712-749, South Korea.)

Protein tyrosine phosphatase 1B (PTP1B) negatively regulates insulin signaling, and PTP1B inhibitors have been seen as promising therapeutic agents against obesity and type 2 diabetes. We identified haginin A, an isoflavonoid compound, as one of the major components of the branch of Lespedeza cyrtobotrya, and an inhibitor of PTP1B by screening an extract library of Korean natural plants. Haginin A inhibits PTP1B activity with IC₅₀ values of 3.75 \pm 0.6 μ M in a competitive inhibitor of PTP1B, with a K_i value of 2.5 µM. Also, haginin A dosedependently stimulated glucose uptake in differentiated L6 rat myoblast cells. Western blotting analyses revealed that haginin A increased the phophorylation level of AMPK and acetyl-CoA carboxylase (ACC). In addition, it enhanced insulin-mediated Akt activation. In summary. AMPK activation was involved in effects of haginin A on glucose transport activation and insulin sensitivity. Haginin A can be further developed as potential compound for anti-diabetic therapy.

ANTIVIRAL ACTIVITIES OF THE AQUEOUS EXTRACT FROM PEAT MOSS AGAINST INFLUENZA VIRUS *IN VITRO* AND *IN VIVO*

<u>Ha-Na Youn</u>,¹ Yu-Na Lee,¹ Dong-Hun Lee,¹ Jae-Keun Park,¹ Seong-Su Yuk,¹ Yong-Kee Jeong,² Joong-Bok Lee,¹ Seung-Yong Park,¹ In-Soo Choi,¹ Chang-Seon Song,¹ Kwon-Ho Jo³ (¹Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, Seoul, 143-701, South Korea, ²Department of Biotechnology, College of Natural Resources and Life Science, Dong-A University, Busan, 604-714, South Korea, ³Blue Bio Industry Regional Innovation Center (BBI-RIC) Dongeui University, #24 Gaya-dong, Busanjin, Busan, 604-714, South Korea.)

Peat moss (PM) is the decomposing, dead, parts of sphagnum moss that usually are found deep in a bog. It has long been used as folk medicine to treat bacterial infection. Here we tested the inhibitory activity of water extract from PM towards influenza A virus in vitro and in vivo. In vitro anti-influenza virus activities of PM extract evaluated using influenza A/NWS/33 (H1N1) virus by the Neutral Red assay on MDCK cells. PM extract exhibited inhibitory activities against A/NWS/33 (H1N1) with 50% effective concentration (EC₅₀) values ranging from 3.2 to 10 μ g/ml. The mean 50% cytotoxic concentration (CC₅₀) value of PM extract in the MDCK cells showed 930.30 μ g/ml. The antiviral activity of PM extract was further evaluated using murine influenza virus infection model. The mice were infected intranasally with influenza A/NWS/33 (H1N1) virus, and the extracts were orally administered at 10 and 100 mg/kg once daily for 5 days beginning 4 h pre-virus exposure. In this infection model, PM extract was significantly effective at 100 mg/kg in increasing survival rate (40%) of infected mice, whereas all of the mice in the control group were died. The dose of 10mg/kg also increased the survival rate (20%) and the survival times of infected mice, although not reaching statistical significance. In the present study, peat moss playing a role as antiviral inhibitor during influenza virus infection was considered to be less toxic and highly protective against influenza infection. Therefore, peat moss may warrant further evaluation as a possible therapy for influenza.

NPD 27

ECKLONIA CAVA EXTRACT PREVENTS AGGREGATION OF β-AMYLOID AND REDUCES β-AMYLOID MEDIATED NEURONAL DEATH.

Young Eun Jeon, Xing Fu Yin, Dan Bi Choi, Min Ju Kim, II-Jun Kang (Department of Food Science and Nutrition & Hallym International Medical Tourism Education Center, Hallym University, Chuncheon, 200-702, South Korea.)

 β -amyloid (β A) is a major pathogenic peptide for Alzheimers disease (AD) and is generated by the processing of amyloid precursor protein (APP). The β A monomers aggregate into oligomeric and fibrillar forms which have been implicated as the toxic species inducing the neuronal dysfunction. Brown algae Ecklonia cava is known for its anti-oxidant and anti-inflammatory functions. Therefore, we tested the effect of E. cava extract on the production and aggregation of βA peptides. The extract of E. cava reduced BA secretion from HEK293 cells expressing APP with Swedish mutation and increased soluble APPB and C-terminal fragment- β (CTF β), of which activity was similar to BACE (β -site of APP cleaving enzyme) inhibitors. Furthermore, the extract inhibited βA oligomerization, particularly mid-size oligomer formation, confirmed by the ultrastructural morphology. Congo red, thioflavin T assays, and electron microscopy showed that the extract inhibited BA fibril formation effectively. Finally, the extract protected primary cortical neurons from various BAinduced cell deaths, especially oligomer-induced death. Although further study is needed to test the effectiveness of the extract in vivo, our results demonstrate, for the first time, that the extract of E. cava could be used as an anti- β A agent for AD therapeutics.

NPD 28

NELUMBO NUCIFERA RHIZOME EXTRACT AMELIORATES THE SCOPOLAMINE-INDUCED REDUCTIONS OF CELL PROLIFERATION, NEUROBLAST DIFFERENTIATION AND BDNF LEVELS

Xing Fu Yin, Young Eun Jeon, Dan Bi Choi, Il-Jun Kang (Department of Food Science and Nutrition & Hallym International Medical Tourism Education Center, Hallym University, Chuncheon, 200-702, South Korea.)

This study examined the effects of Nelumbo nucifera rhizome extracts (NRE) on cell proliferation and neuroblast differentiation in the hippocampal dentate gyrus (DG) of a rat model of scopolamine-induced amnesia. Immunohistochemical markers included Ki67, an endogenous marker for active cell cycle, and doublecortin (DCX), a marker for immature neurons and migratory neuroblasts. Scopolamine was administered for 28 days via an ALzet minipump (44 mg/mL delivered at 2.5 μ L/h). NRE was administered by gavage, 1 g/kg per day for 28 days. The administration of scopolamine significantly reduced the number of Ki67- and DCXimmunoreactive cells in the DG, whereas scopolamine did not induce any significant changes in mature neurons. The administration of NRE significantly ameliorated the scopolamine-induced reduction of Ki67and DCX-immunoreactive cells in the DG. In addition, the administration of NRE significantly restored the scopolamine-induced reduction of brain-derived neurotrophic factor in DG homogenates. These results suggest that NRE can ameliorate the scopolamineinduced reductions of cell proliferation, neuroblast differentiation and BDNF levels.

ANTIMICROBIAL EVALUATION OF A FOCUSED NARINGENIN AND RESVERATROL CHEMICAL LIBRARY

Dianqing Sun,¹ Julian G Hurdle,² Robin E Lee,³ Li Shen,¹ Tamara P Kondratyuk,¹ Richard E Lee,³ Mark Cushman,⁴ John M Pezzuto¹ (¹University of Hawaii at Hilo, College of Pharmacy, Hilo, HI 96720,USA, ²University of Texas at Arlington, Department of Biology, Arlington, TX 76019, USA, ³St Jude Children's Research Hospital, Department of Chemical Biology and Therapeutics, Memphis, TN 38105, USA, ⁴Purdue University, College of Pharmacy, West Lafayette, IN 47907, USA.)

Flavonoids are a class of important naturally occurring secondary metabolites in plants which have been reported to mediate a variety of biological responses. One such compound, (2S)-naringenin was recently reported to show good antituberculosis activity (MIC = 2.8 µg/mL) (Chem. Biodivers., 2010, 1814). Inspired by this report, a small focused flavonoid and resveratrol library was screened against a panel of Gram-positive and -negative bacterial pathogens. Among the naringenin, resveratrol, and analogs evaluated, abyssinone II, a naturally occurring flavonoid bearing a lipophilic prenyl group, demonstrated relatively good activity against M. tuberculosis (H37Rv), E. faecalis (ATCC 29212), S. aureus (N315), and S. pneumoniae (HM162), with MIC values of 50, 25, 12.5 and 25 µg/mL, respectively. However, racemic naringenin only showed marginal activity in our TB assay (MIC = 200 μ g/mL). None of the tested compounds was active against Gramnegative bacteria. Based on these data, abyssinone II was selected as a chemical starting point for further medicinal chemistry optimization in an attempt to identify advanced experimental candidates with antimicrobial therapeutic potential.

NPD 30

PRODUCTION AND IDENTIFICATION OF BIOLOGICALLY ACTIVE COMPOUNDS OF FUNGI ASPERGILLUS SPP.

Pervin Basaran, Mehmet K. Özcan (Suleyman Demirel University, Department of Food Engineering, Isparta, 32100, Turkey.)

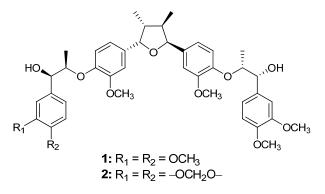
Fungal-derived biologically active secondary metabolite production can be down or up-regulated by signals or substrates from the plant sources. In this study, HPLC Mass Spectroscopy Time-Of-Flight (HPLC-MS-TOF) was employed for the identification of novel compounds produced by *Aspergillus* grown on specific plant substrates under defined conditions. Fungal cells of selected species were extracted and the crude extract was dissolved in acetonitrile and formic acid mixture in ethyl acetate. The HPLC system consisted of binary pump, degasser, a reversed phase column and autosampler working with a diodearray detector. The MS was in positive ionisation mode, capillary voltage 3kV, collision energy ranged from 50 to 100 V working at 300° C desolving temperature, and the peaks between 120-950 were recorded. Aspergillus strain tested here was able to produce two major pharmaceutically active compounds, fumagillol (C₁₆H₂₆O₄) and italicic acid (C₁₅H₁₆O₆). These compounds show anticancer activity by blocking endothelial cell proliferation, they are effective inhibitor of angionenesis and several semi-synthetic analogues are currently being tested in clinical trials for the treatment of cancer.

NPD 31

NEUROPROTECTIVE EFFECTS OF ETHYL ACETATE EXTRACT OF *CODONOPSIS LANCEOLATA* ON ISCHEMIC DAMAGE IN GERBIL

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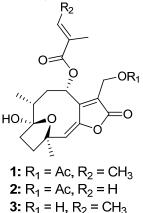
We observed the neuroprotective effects of ECLs treatment on ischemic damage in the gerbil hippocampal CA1 region four days after an ischemic insult. Among the 10 ECLs, Ethyl acetate Extracts of Raw and Steamed Codonopsis lanceolata (EERCL and EESCL) showed significant neuroprotection; the percentage of neurons remaining after treatment with EERCL and EESCL was 72.7% and 68.4% of that seen in the shamischemia group, respectively. The administration of EERCL and EESCL significantly decreased the reactive gliosis of microglia compared with that seen in the vehicle-treated ischemia group. In addition, SOD1 and BDNF immunoreactivity in the EERCL- and EESCLischemia groups were markedly increased compared with that in the vehicle-treated ischemia group. These results suggest that the administration of EERCL and EESCL can reduce ischemic neuronal loss potentially by maintaining SOD1 and BDNF immunoreactivity in the ischemic hippocampal CA1 region.



BIOLOGICALLY ACTIVE CONSTITUTES FROM THE FLOWER OF VERNONIA CINEREA

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Bioassay-guided fractionation of the methanol extract of *Vernonia cinerea* (Asteraceae) led to the isolation of three sesquiterpene lactones, 8α -tigloyloxyhirsutinolide-13-O-acetate (1), 8α -tigloyloxyhirsutinolide (2), and 8α -(2-methylacryloyloxy)-hirsutinolide-13-O-acetate (3), along with two flavonoids (4 and 5) and phthalic acid (6). The structure and absolute stereochemistry of these compounds (1-3) were determined on the basis of 1D and 2D NMR experiments. All six compounds inhibited LPS induced NO formation with cultured RAW 264.7; compounds 1-3 were the most active (IC₅₀ = 1.9, 6.6, and 5.7 μ M, respectively).



NPD 33

INHIBITION OF SREBP-1C-DEPENDENT HEPATIC STEATOSIS BY SAUCHINONE, AN AMPK-ACTIVATING LIGNAN IN SAURURUS CHINENSIS

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The building-up of fat in the liver results from delivery of a large amount of fatty acids to hepatocytes and impaired export of triglyceride, which is eventually related with chronic liver diseases in association with systemic metabolic dysfunction. Sauchinone as an AMPK-activating lignan in *Saururus chinensis* has been shown to prevent iron-induced oxidative stress and liver injury. Previously, we identified the role of AMPK in LXRa-mediated SREBP-1c-dependent lipogenesis. Since sauchinone as an antioxidant has the efficacy to activate AMPK, this study investigated its effects on SREBP-1c-dependent lipogenesis in hepatocytes, and high fat diet (HFD)-induced hepatic steatosis and oxidative injury. Sauchinone prevented the ability of T0901317 to activate SREBP-1c. repressina transcription of the fas, acc, scd-1, abca1, and LXRa genes. Consistently, HFD feeding to mice caused fat accumulation in the liver with SREBP-1c induction, which was attenuated by sauchinone treatment. Also, sauchinone had the ability to inhibit oxidative stress as shown bv decreases in TBARS formation. nitrotyrosinylation, and 4-hydroxynonenal production. Moreover, it prevented not only liver injury, but also AMPK inhibition elicited by HFD feeding. These results demonstrate that sauchinone has the capability to inhibit LXRa-mediated SREBP-1c induction and SREBP-1cdependent hepatic steatosis, thereby protecting hepatocytes from oxidative stress induced by fat accumulation.

NPD 34

EVALUATION OF SMOKING CESSATION AGENTS FROM NATURAL PRODUCTS

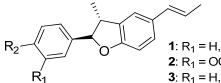
Danny Tudor,¹ Devin Harden,^{1,2} Aaron Behm,² David Montgomerie,² Chad Ahia,¹ Supakit Wongwiwatthananukit,¹ Anthony Otsuka,¹ Leng Chee Chang¹ (¹College of Pharmacy, University of Hawaii Hilo, Hilo, 96720, USA, ²Dept. of Biology, University of Hawaii Hilo, Hilo, HI 96720, USA.)

C. elegans is a useful model for the study of nicotinedependent behaviors. We hypothesized that current smoking cessation drugs (bupropion, varenicline, and mecamylamine) would exhibit similar effects in C. elegans. An egg-laying (el) assay was used to study each drug's ability to inhibit the el response in worms after being acutely exposed to nicotine. Egg-laying is dependent on the nicotinic acetylcholine receptor system. All three drugs produced a significant decrease in el behavior when worms were subsequently exposed to nicotine, compared with a control group. The ability of drugs to behaviorally block treated worms from seeking nicotine was studied in a chemotaxis assay. We hypothesized that C. elegans cohorts naïve to nicotine will be attracted to the nicotine side of the plate, but worms that were treated with drugs should be equally attracted to both the nicotine and non-nicotine sides of the plate. The nicotine side was significantly favored by naïve worms compared with the non-nicotine side. Compared to naïve worms, those worms treated with all three drugs were less attracted toward the nicotine side of the plate. As a negative control, worms treated with the green tea compound (i.e., EGCG), were significantly attracted to the nicotine side of the plates. We discovered that worms treated with Phytolacca latbenia or Fagonia critica crude extracts were significantly less attracted toward the nicotine side of the plate.

CYTOTOXIC DIHYDROBENZOFURANS FROM MITREPHORA WANGII HU

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(+)-(*2R*, *3R*)-2,3-Dihydro-2-(4-hydroxyphenyl)-3-methyl-5[1-(*E*)-propenyl] benzofuran or conocarpan (1) and two methoxyl derivatives (2 and 3) were isolated from the leaf hexane extract of *Mitrephora wangii* Hu. Compounds 1 and 2 exhibited significant inhibitory activity against *Streptomyces* 85E in the hyphaeformation inhibition assay, with clear zones of inhibition of 21 and 11 mm, respectively. Compounds 1 and 2 further inhibited growth of human leukemic monocyte lymphoma (U937) cells with IC₅₀ values of 6.3 and 5.9 μ g/ml, respectively. Compound 1 also demonstrated a strong inhibitory activity against human lung carcinoma (LU-1) cells with the IC₅₀ value<5 μ g/ml. Moreover, compound 3 was isolated from plant and from this genus as *2R*, *3R* configuration for the first time.



1: R₁ = H, R₂ = OH **2**: R₁ = OCH₃, R₂ = OH **3**: R₁ = H, R₂ = OCH₃

NPD 36 UNCOVERED THERAPEUTIC POTENTIAL OF MARINE RED ALGAE FROM NEW CALEDONIA

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Marine plants, such as algae, have been investigated for decades and have been shown to be an important source of molecules with therapeutic, agrochemical and botanical potential. To date, little is known regarding bioactivities of red algae from New Caledonia. In order to evaluate their potential, we collected several species of red algae based on availability and easy access. Extractions, partitions and fractionations were successively done and samples were screened for therapeutic potential against a wide range of bacterial pathogens and human cancer cell lines. For the first time, we have uncovered the biological activities of several species with some of them showing cellular selectivity. Our findings may lead to the discovery, identification and isolation of new molecules/leads to serve as candidates for the development of novel therapeutics.

NPD 37

DEVELOPMENT OF POTENTIAL CNS THERAPEUTICS DERIVED FROM THE ALKALOID CYTISINE

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(-)-Cytisine, a potent nAChR ligand, and its structural frameworks (3,7-diazabicyclo[3.3.1]nonane and 2pyridone) serve as invaluable templates in the development of novel bioactive compounds. In this study, cytisine isolated from Laburnum anagyroides and also its 3,7-diazabicyclo[3.3.1]nonane scaffold were used to develop new compounds applying e.g. the hybrid or twin drug approach with other natural products. The compounds synthesized were tested for their affinities for different nAChR subtypes using radioligand binding assays. A broad spectrum of affinities (e.g. K_i values for $\alpha 4\beta 2^*$: <1 nM to >10,000 nM) provided important insights into structure-affinity relationships. The novel compounds could be useful for further development of therapeutics to treat disorders involving nAChR dysfunction.

Funding: This work was supported by P20RR016467

NPD 38

ANTI-PROLIFERATIVE DINEOLIGNANS FROM SAURURUS CHINENSIS AGAINST HUMAN CANCER CELL LINES

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Activity-guided fractionation of an EtOAc-soluble fraction of Saururus chinensis afforded two anti-proliferative dineolignans, manassantin A (1) and B (2) along with four flavonoids and four aristolactams. Their chemical structures were identified by spectroscopic methods. Compounds 1 and 2 were evaluated for their antiproliferation activities against 28 human cancer cell lines and 2 human normal lung cell lines using MTS assay. Compounds 1 and 2 showed a potent anti-proliferation activity against cervical (C33a, $IC_{50} = 0.015 \ \mu M$ for 1; 0.277 μ M for 2) and lung (NCI-H460, IC₅₀ = 0.049 μ M for 1; 1.368 μ M for 2) cancer cell lines without any remarkable cytotoxic effect on normal lung cell lines $(IC_{50} > 10 \mu M)$. C33a cells treated with these compounds showed marked ERK inhibition possibly due to Raf inactivation. Together, these data demonstrated the identification of anti-proliferative dineolignans and its possible mechanism of action.

INHIBITION OF LXRα-MEDIATED HEPATIC STEATOSIS BY LIQUIRITIGENIN, A LICORICE FLAVONOID, IN ASSOCIATED WITH NRF2 ACTIVATION

<u>Young Woo Kim</u>,¹ II Je Cho,¹ Sang Geon Kim,² Sang Chan Kim¹ (¹Daegu Haany University, College of Oriental Medicine, Daegu, 706-060, South Korea, ²Seoul National University, College of Pharmacy, Seoul, 151-742, South Korea.)

Nonalcoholic fatty liver disease is considered as a major hepatic constituent of the metabolic syndrome. LXRa functions a major regulator of lipid homeostasis through activation of SREBP-1c, which promotes hepatic steatosis and steatohepatitis. Nrf2 is the crucial transcription factor necessary for the induction of antioxidant enzymes. This study investigated the potential of liquiritigenin (LQ), a hepatoprotective flavonoid in licorice, to inhibit LXRa-induced hepatic steatosis, and the underlying mechanism of the action. LQ treatment attenuated fat accumulation and lipogenic gene induction in the liver of mice fed a high fat diet. Also, LQ had the ability to inhibit oxidative liver injury, as shown by decreases in thiobarbituric acid reactive substances formation and nitrotyrosinylation. Moreover, LQ treatment antagonized T0901317-mediated SREBP-1c activation, and transactivation of the lipogenic target genes. LQ was found to activate Nrf2, and the ability of LQ to inhibit LXRa-mediated SREBP-1c activation was reversed by a deficiency of Nrf2, which supports the inhibitory role of Nrf2 in LXRq-dependent lipogenesis. Consistently, treatment with other Nrf2 activators or forced expression of Nrf2 also inhibited LXRα-mediated SREBP-1c activation. Our results demonstrate that LQ has an efficacy to activate Nrf2, which contributes to inhibiting the activity of LXRa that leads to SREBP-1c induction and hepatic steatosis.

NPD 40

RESVERATROL PREVENTS MITOCHONDRIAL DYSFUNCTION THROUGH PARP-LKB1 ACTIVATION

<u>Mi Jeong Jo</u>,¹ Sang Mi Park,¹ II Je Cho,¹ Sang Geon Kim,² Seon Young Jee¹ (¹Daegu Haany University, Department of Oriental Medicine, Daegu, 706-060, South Korea, ²Seoul National University, Department of Pharmacy, Seoul, 151-742, South Korea.)

This study investigated the potential of resveratrol to protect against the mitochondrial impairment induced by arachidonic acid (AA)+iron and the underlying mechanism for this cytoprotection. Resveratrol treatment inhibited apoptosis, ROS production, and GSH depletion elicited by AA+iron in HepG2 cells. Also, resveratrol attenuated superoxide generation and inhibited mitochondrial dysfunction induced by AA+iron. Overall, AMPK activation by resveratrol contributed to cell survival, as supported by the reversal of mitochondrial membrane potential by either overexpression of a dominant negative mutant of AMPK or compound C treatment. Resveratrol increased inhibitory phosphorylation of GSK3beta downstream of AMPK, which contributed to mitochondrial protection and cell survival. Likewise, siRNA knockdown of LKB1 reduced the ability of resveratrol to protect cells from mitochondrial dysfunction. Furthermore, this LKB1dependent mitochondrial protection resulted from poly(ADP-ribose)polymerase (PARP) activation, but not SIRT1 activation, as supported by the experiment using 3-aminobenzamide, а PARP inhibitor. Other polyphenols, such as apigenin, genistein, and daidzein, did not activate AMPK, nor did they protect mitochondria against AA+iron. Thus, resveratrol protects cells from AA+iron-induced ROS production and mitochondrial AMPK-mediated through dysfunction inhibitory phosphorylation of GSK3beta downstream of PARP-LKB1 pathway.

NPD 41

ANTI-INFLAMMATORY EFFECTS OF ISATIDIS RADIX AND ITS ACTIVE COMPONENT, TRYPTANTHRIN IN LIPOPOLYSACCHARIDE-ACTIVATED RAW264.7 MACROPHAGE CELLS

Mi Jeong Jo, Sang Mi Park, Sook Jahr Park, Jong Rok Lee, Sung Hui Byun, <u>Sang Chan Kim</u> (Daegu Haany University, College of Oriental Medicine, Daegu, 706-060, South Korea.)

Isatidis Radix, the dried root of *Isatis indigotica*, is categorized as a fever-reducing agent in East Asian traditional herbal medicine. The present study was conducted to evaluate the anti-inflammatory effects of Isatidis Radix and its components tryptanthrin and indigo in lipopolysaccharide (LPS)-activated Raw264.7 cells.

LPS-induced nitric oxide (NO) and prostaglandin E2 (PGE2) production were dose dependently decreased by the treatment of Isatidis Radix water extract (IRE) and tryptanthrin, while indigo had no effect. The inhibition of NO production by IRE and tryptanthrin was due to suppression of iNOS expression mediated from the inhibition of nuclear factor- κ B (NF- κ B) nuclear translocation and inhibitory- κ B α phosphorylation, as determined by Western blot analysis. In case of PGE2 inhibition, IRE and tryptanthrin did not reduce COX-2 expression, but they showed inhibitory effect of COX-2 activity. In addition, IRE inhibited production of inflammatory cytokines IL-1 β and TNF- α .

These findings suggest that Isatidis Radix could produce an anti-inflammatory effect through inhibition of iNOS expression and COX-2 activity via NF- κ B pathway, and that tryptanthrin might be one component contributing to anti-inflammation of IRE.

RED GINSENG EXTRACT PREVENTS CCI₄-INDUCED LIVER FIBROSIS

<u>Sang Mi Park</u>, Mi Jeong Jo, Sung Hui Byun, Sang Chan Kim, II Je Cho (Daegu Haany University, Department of Oriental Medicine, Daegu, 706-06, South Korea.)

Korean Red Ginseng, the processed root of Panax ginseng Meyer, has been frequently used for various therapeutic purposes in oriental medicine and is now widely used around the world. The present study investigated the possible preventive effect of Red Ginseng Extract (RGE) for the treatment of liver fibrosis. We injected mice with multiple doses of carbon tetrachloride (CCl₄) for 4 weeks and then used the animal to determine whether RGE treatment therapeutically improved liver functions and resolved fibers accumulated in the liver. Multiple CCl₄ injections caused elevated levels of ALT, AST and collagen accumulation. In contrast, concomitant treatment with RGE (30, 100, and 300 mg/kg) significantly reduced them in a dose-dependent manner. In histopathological analysis, RGE treatment decreased the percentages of degenerative regions, numbers of degenerative hepatocytes and collagen deposited percentages in hepatic parenchyma. In addition, RGE inhibited the mRNA level of transforming growth factor beta 1, plasminogen activator inhibitor 1 genes in fibrogenic liver. Moverover, RGE dose-dependently reduced the number of alpha smooth muscle actin-positive cells in liver tissue. Taken together, these results demonstrate that RGE can protect the CCl₄-induced liver fibrosis, partly via hepatic stellate cell inactivation.

TRADITIONAL CREE ANTI-DIABETIC MEDICINE: ADVANCED METABOLOMIC ASSESSMENT IN EXPERIMENTAL MODELS OF DIABETES AND ALZHEIMER'S DISEASE

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Type II diabetes (T2D) may be a risk factor for Alzheimer's Disease (AD), where AD patients have elevated insulin levels and decreased insulin sensitivity. However, the exact mechanism linking the two disease states remains to be elucidated. AD09, a plant used by the Cree of Eevou Istchee in the treatment of diabetes, was used to treat TgCRND8 transgenic mice ectopically expressing human amyloid precursor protein with Swedish (KM670/671NL) and Indiana (V717F) mutations. TgCRND8 mice and their non-transgenic littermates were fed the AD09 EtOH extract, containing quercetin glycosides, morroniside and goodyeroside as well as other phenolics, at a concentration of 250 mg/kg daily for 2 months. The mice were then exposed to a variety of behavioural tests and glucose and insulin challenge to measure their metabolic response. We found that AD09 may increase insulin resistance in Tg mice while having no impact on glucose mobilization, with no effect observed in NonTg mice with mild behavioural impact on indices of learning, memory, anxiety, and mobility. (Supported by CIHR TGF-96121 and MOP6286 to JTA and SALB).

M 2

ANALYSIS OF *GINKGO BILOBA* LEVOPIMARADIENE SYNTHASE (LPS) PROMOTER IN *ARABIDOPSIS THALIANA*

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The major constituents of *G. biloba* are diterpeneoid ginkgolides which have potent anti-platelet antagonist factor activity. Levopimaradiene synthase (LPS) catalyzes the first committed step in ginkgolide biosynthesis pathway by converting geranylgeranyl pyrophosphate into levopimaradiene. A 2.2 kb promoter region of *GbLPS* was isolated and fused to a beta-glucuronidase (GUS) reporter gene. In *Arabidopsis thaliana*, the *GbLPS* promoter exhibited activities in developing young tissues. When cotyledons were fully open, the growth stage 1, GUS was intensively

expressed in cotyledons. When two rosette leaves appeared, the growth stage 1.02, GUS expression was seen in the rosette leaves while GUS expressions in cotyledons disappeared. At 5-rosette leaf stage, growth stage 1.05, GUS expression was observed only in the newly formed leaves. In flower development, GUS was expressed in floral buds, carpel and growing ovaries. However, during the maturation of seed, GUS expression was disappeared. In the roots, GUS was constitutively expressed in the vascular tissues before emergence. After inflorescence inflorescence emergence, GUS was expressed in the newly formed roots.

Acknowledgement: Financial Support by Systems and Synthetic Agrobiotech Center is appreciated.

М 3

UNRAVELING THE BIOSYNTHETIC CAPACITY OF MONOTERPENES IN SPECIALIZED EPITHELIAL CELLS OF GRAPEFRUIT PEEL

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The essential oil of citrus peel, which consists primarily of monoterpenes, is synthesized in specialized epithelial cells lining secretory cavities. In order to obtain a quantitative understanding of essential oil formation in the secretory cavities, we have used grapefruit (Citrus paradisi) as our model for monoterpenoid essential biosynthesis in the genus Citrus. We first determined the relationship between distribution and size of secretory cavities and essential oil production at different stages of fruit development. Essential oil biosynthesis starts when secretory cavities are formed at the very early stage of fruit formation. The oil production then enters an exponential phase when secretory cavities are expanding (fruit sizes between 30 and 100 mm), before slowing down to a linear increase. Second, we also investigated the secretory cavity content using microcapillaries and subsequent chemical analyses. Our results indicate that about 95% of the oil is composed of monoterpenoids (with about 90% of limonene), about 5% is accounted for coumarins and methylated flavonoids, and less than 0.01% is due to the accumulation of fatty acids and sterols. Third, a cell type-specific transcript analysis was performed with isolated epithelial cells that actively synthesize essential oil. Cells were harvested using laser-capture microdissection and global gene expression patterns in these cells were assessed using Affymetrix GeneChip Citrus Genome Arrays. Transcript abundances of selected genes were also evaluated by quantitative real-time PCR. These analyses showed that genes involved in monoterpene biosynthesis are coordinately expressed in epithelial cells, concomitant with developmental changes in the accumulation of essential oil. Our goal is the integration of developmental, biochemical, and anatomical data sets into a comprehensive mathematical model of Citrus peel essential oil biosynthesis.

METABOLITE PROFILING OF TRITERPENE SAPONINS IN *MEDICAGO TRUNCATULA* CORE COLLECTION

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Medicago truncatula is a model legume species that produces structurally diverse triterpene saponins with a wide range of bioactivities. Triterpene saponins were profiled in an *M. truncatula* core collection containing 63 lines which were determined to represent the major genetic diversity found in the USDA germplasm collection. High resolution metabolic profiling was used to assess the metabolic diversity of triterpene saponins in aerial and root tissues of the diverse lines using UPLC-QTof-MS. Principal component analysis revealed substantial biochemical variation in triterpene saponin content in the core collection. Comparative metabolite analyses revealed the total highest triterpene saponin level in core line 23, which was 7 times higher than that of the lowest line (core line 28). In addition, differential accumulation of 6 specific aglycone classes was observed in the core lines. As a more specific example, the highest accumulation of medicagenic acid saponins was identified in core line 23, which contained 124 times more medicagenic acid than the lowest accumulating line (core line 43). The metabolic profiling results provide high resolution triterpene saponin phenotypes for the core lines. The identified hyper- and hypo-saponin accumulating lines will be used for comparative gene expression analyses to identify putative genes involved in triterpene saponin biosynthesis.

M 5

VIRUS-INDUCED GENE SILENCING OF CYTOCHROMES P450 PUTATIVELY INVOLVED IN ALKALOID BIOSYNTHESIS IN OPIUM POPPY

<u>Guillaume Beaudoin</u>, Peter Facchini (University of Calgary, Department of Biological Sciences, Calgary, AB T2N1N4, Canada.)

Benzylisoquinoline alkaloids are nitrogenous, lowmolecular weight compounds found in approximately 20% of plant species. These include the analgesics morphine and codeine, the anti-cancer compound noscapine, the vasodilator papaverine, and the antimicrobial agent sanguinarine. Each of these pathways has been shown or is predicted to involve at least one cytochrome P450 (CYP). However, only one CYP in morphine branch pathway and two CYPs in the sanguinarine branch pathway have been characterized. Several CYP gene candidates showing a correlation between transcript and specific alkaloid accumulation in opium poppy plants and cell cultures have been identified. Virus-induced gene silencing has been used to demonstrate the *in planta* relevance of our

candidates. Preliminary results suggest that the silencing of some of these have an effect on the accumulation of sanguinarine, as well as perhaps other alkaloids in the roots of opium poppy. Additional studies used to characterize these enzymes will be discussed.

M 6

BIOAVAILABILITY STUDIES OF VITAMIN C IN AMLA AND ITS COMBINATIONS

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In-vivo bioavailability of Vitamin C in different combinations with Amla (A) was compared with Synthetic Vitamin C (SVC) using HPLC in New Zealand Rabbits. SVC, A, Amla + Piperine (A+P) and Amla + Ginger (A+G) 100 mg/kg, were administered orally and the serum samples were analysed at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24h after treatment. The results showed that at 6h A+P combination has higher concentration of Vitamin C (212.53 μ g/ml) and this may be due to presence of Piperine since it is a bioavailability enhancer. It can be concluded that A+P combination can be an alternative to Synthetic Vitamin C.

Μ7

COMBINED GENOMIC-METABOLOMIC APPROACH FOR THE DIFFERENTIATION OF GEOGRAPHICAL ORIGINS OF NATURAL PRODUCTS

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The correct identification of the geographical origins of natural products is essential to quality control, as their physiological effects correlate with chemical components. In this study, we applied both genomics and metabolomics to the origin identification of 101 deer antler samples from Canada. New Zealand, and Korea. The genomics identified deer species in each country but failed to categorize all the samples, due to the presence of identical species in different countries. For identical species, NMR-based metabolomics gave clean separations, compounds specific to each country were identified, and the validity was confirmed by prediction analysis. As the genomics provided unambiguous readouts for different species, and the metabolomics cleanly distinguished among identical species from different countries, their combined use could be a robust method for origin-identification even in difficult cases. We believe the method to be generally applicable to many herbal medicinal products for which various species are grown internationally.

ARABIDOPSIS FIBER-REDUCED (SND1/NST1) MUTANTS: NANOINDENTATION PROBING OF MECHANICAL PROPERTIES IN DISTINCT CELL WALL TYPES

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Nanoindentation analyses were used to measure mechanical properties within specific cell walls of wildtype (WT) and several Arabidopsis transcription factor mutant lines involved in cell-wall (fiber cell) development. These tests facilitated measurement of elastic modulus (E) and hardness (H) of individual cell wall types [interfascicular fibers (if), xylary fibers (xf) and xylem vessels (xv)] from stem cross- sections. Reductions in E values of xf and if of snd1/nst1 were noted at the single cell level as compared to WT E values. Effects on elastic modulus within cell walls were observed from early to mature developmental stages (4, 5 and 8 weeks). Moreover, effects on physiological/mechanical properties were also observed on E values of xv of snd1/nst1 at the mature stage as compared to WT E values. The snd1/nst1 line displayed a prostrate phenotype with reductions in estimated lignin contents up to 53% of WT level In addition, E values were determined on complementation of Arabidopsis with the NAC homologues from poplar, namely PtNAC1, PtNAC6, PtNAC7, PtNAC13 and PtNAC17. Pyrolysis GC/MS analysis of laser microdissected vb and if also further confirmed differences in lignin composition in their specific cell wall types.

М9

PROMOTER ANALYSIS OF MULTI COPY *GINKGO BILOBA* 1-HYDROXY-2-METHYL-2-(*E*)-BUTENYL-4-DIPHOSPHATE REDUCTASE (IDS) GENE

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Isoprenoids are synthesized by condensation of fivecarbon (isoprene) units, which are derived from two distinct routes in plants: cytosolic mevalonate (MVA) and plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) pathways. 1-hydroxy-2-methyl-2-(*E*)-butenyl-4-diphosphate reductase (IDS) of *Ginkgo biloba* is an enzyme at the final step of the MEP pathway. The gene of the enzyme was cloned as a multi-copy gene in gymnosperms *Ginkgo biloba*. To evaluate the function of each isogene, the role of promoter of the isogene was ex-amined in *Arabidopsis*. Promoters of *GbIDS* series, *GbIDS1, 2, and 2-1* were cloned and fused to GUS. The *GbIDS1* pro::GUS fusion showed expression in most tissues except for roots, petals, and stigma. The *GbIDS2*pro::GUS fusion showed expression in the young leaves and tissues, and internodes where the flower and shoot branched. There was no GUS expression observed in roots and reproductive tissues of the plants. The results alluded household and specific roles of *GbIDS1*, and *2* respectively.

Acknowledgement: Financial support by Systems and Synthetic Agrobiotech Center is appreciated

M 10

FUNCTIONAL CHARACTERIZATION OF *MEDICAGO TRUNCATULA* MYB TRANSCRIPTION FACTORS: MTMYB2 AND MTMYB70

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The family of MYB transcription factors is one of the most abundant classes of transcription factors in plants, and the subfamily containing the two-repeat R2R3 DNAbinding domain is the largest. A number of R2R3 MYB proteins have been shown to regulate the biosynthesis of phenolic compounds, including lignin. Researchers are interested in reducing lignin in plants because lignin interferes with cellulosic ethanol production. In other to understand the role of MYB transcription factors on the control of lignin biosynthesis during xylem formation, fulllength cDNA of 21 MYB transcription factors of Medicago truncatula were isolated using the primers specific to the conserved MYB domains via RACE. For functional characterization of MYB transcription factor 2 and 70, overexpression constructs were individually transformed into the Arabidopsis thaliana. Among them, 35S::MtMYB2 and 35S::MtMYB70 displayed phenotypic changes relative to wild-type plants, which were alteration stem strength and the lignin contents. So, we generated MtMYB2 and MtMYB70 transgenic poplar using hybrid poplar (Populus alba x P. glandulosa). The existence of a single MtMYB gene in the hybrid poplar genome was supported by Southern hybridization. And we selected lines according to Northern blot analysis of MtMYB expression. Thus, the expression analysis of the lignin biosynthetic genes could be found genes encoding enzymes specific to lignin biosynthesis. In addition, histochemical analyses would be clearly demonstrated difference of lignin and cellulose deposition. In case of MtMYB70, we supposed to MtMYB70 binding DNA motif using the universal protein binding microarray (PBM). These results suggest that MtMYB genes play an important role in the biosynthesis of lignin and the regulation of secondary cell wall formation.

GRANDISIN IN VITRO METABOLISM USING BIOMIMETICAL MODELS

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As part of our ongoing project on the biomimetic oxidation reactions of natural products, we present the biomimetic oxidation of grandisin catalyzed by the Jacobsen reagent and the metabolism of this lignan by bacteria from pig ceacum. The samples were analyzed by using a validated UPLC-DAD methodology. The results observed with the Jacobsen reagent indicate the presence of five products, which generally resulted from the addition of hydroxyl groups. The major yielded compound was a di-hydroxylated product. Two minor signals related to a possible water elimination from the hydroxylate and tri-hydroxylate products were also observed. A third minor signal resulted from the oxidative cleavage of grandisin as previously observed with fungus metabolism. A fourth signal was related to a tetra-hydroxylated product. The results noted in pig ceacum model showed that no grandisin was metabolized at any time period studied. These are important findings for the in vivo study of metabolism.

Acknowledgements: FAPESP, CAPES and CNPq for the financial support.

M 12

PISUM SATIVUM IS A NOVEL BIOINFORMATICS PLATFORM TO STUDY PROANTHOCYANIDIN BIOSYNTHESIS

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Proanthocyanidins (PAs) are flavonoid polymers with strong antioxidant capabilities. Recent studies suggest human consumption of PAs convey numerous health benefits. The complete biosynthesis of PAs remains to be elucidated. Currently, *Arabidopsis thaliana* and *Medicago truncatula* are the model organisms used to study PAs. In both species, PA polymers are composed of 2,3-*cis*-flavan-3-ol monomers. There are numerous examples in nature of PA polymers composed of mixtures of 2,3-*cis*- and 2,3-*trans*-monomers. We initiated studies of PA metabolism in five *Pisum sativum*

(pea) cultivars to understand PA diversity and to unravel unknown aspects of PA biosynthesis. Due to extensive breeding, various pea cultivars with distinctly different PA profiles, or lacking PAs, are readily available. Comparative genomics of different pea cultivars would allow us to gain unique insights into PA biosynthesis. Pyrosequencing and a microarray were performed to study differences in seed coat gene expression of five pea cultivars. Accordingly, quantitative PCR analyses confirmed the reliability of the genomics approach. Our results show comparative genomics can be utilized to explain differences in metabolic profiles naturally occurring within a species. Bioinformatics analysis implicated involvement of several previously unknown genes in PA biosynthesis. Work is currently in progress to characterize the roles of these new genes in PA biosynthesis.

M 13

METABOLOMIC CONSEQUENCES IN AROGENATE DEHYDRATASE AND NAC TRANSCRIPTION FACTOR MODIFIED ARABIDOPSIS THALIANA

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Metabolomics represents one of the most powerful tools to probe the overall effects of gene down-regulation and gene-knockouts in transgenic plants at all stages of growth and development. This is of particular importance in helping assess the overall effects on metabolism of transgenic lines engineered to be more susceptible for saccharification/fermentation for renewable bioenergy/bioproducts. We thus describe herein the effects of genetically modulating members of the arogenate dehydratase (ADT) gene family, which represent the final step in phenylalanine biosynthesis in higher plants, as well as that of various NAC transcription factors involved in secondary wall formation in fiber cells of Arabidopsis. The purpose was to establish global effects on metabolism when generating transgenic lines altered in lignin compositions and contents. Of these manipulations, ADT is an important upstream biochemical step, whose different members of its gene family differentially modulate carbon flux into the phenylpropanoid pathway, whereas the transcription factors of interest control secondary wall formation in fiber cells. Accordingly, numerous T-DNA knockouts of the six-member ADT family and of various NAC TF genetically modified Arabidopsis plant lines were subjected to metabolomic analyses to establish the effects on overall metabolism, particularly in those lines having different levels of lignin reduction, with the results so obtained discussed.

CHARACTERIZATION OF ARABIDOPSIS THALIANA SERINE HYDROXYMETHYLTRANSFERASES FROM MITOCHONDRIA AND THE CYTOSOL

Zhaoyang Wei,¹ Kehan Sun,¹ Francisco Sandoval,² Sanja Roje^{1,2} (¹Graduate Program in Molecular Plant Sciences, Washington State University, Pullman, WA 99164, USA, ²Institute of Biological Chemistry, Washington State University, Pullman, WA 99164, USA.) Serine hydroxymethyltransferases (SHMT) catalyze the reversible conversion of L-serine and (6S)-H4PteGlun to glycine and (6S)-5,10-CH2-H4PteGlun. This reaction is the major one-carbon unit source for a series of essential metabolic processes, and it plays a central role in photorespiration. Arabidopsis genome encodes seven SHMT isoforms localized in the cytosol, mitochondria, plastids and nuclei, adapted to their specific physiological functions. Knowledge of the biochemical properties of each isoform is critical to understand and manipulate the one-carbon pathway in plants, which is an important target for nutritional enhancement of crops. We functionally expressed and purified three recombinant Arabidopsis SHMTs, two from mitochondria and one from the cytosol. Biochemical properties were studied with respect to enzyme oligomerization state, Michaelis-Menten kinetic parameters, and impact of the folate polyglutamyl tail length.

M 15

MERGER OF PRIMARY AND SECONDARY METABOLISM: ENDOGENOUS TURNOVER PATHWAY OF CYANOGENIC GLUCOSIDES ENABLES SORGHUM TO CHANNEL NITROGEN FROM DEFENSE COMPOUNDS INTO PRIMARY METABOLISM

Nanna Bjarnholt,¹ David P. Dixon,² Robert Edwards,³ Lene Dalsten,¹ Mohammed Saddik Motawia,¹ Kirsten Jørgensen,¹ Birger Lindberg Møller¹ (¹Plant Biochemistry Laboratory and the VKR Research Centre Pro-Active Plants, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark, ²School of Biological & Biomedical Sciences, University of Durham, South Road, Durham DH1 3LE, United Kingdom, ³CNAP, Department of Biology (Area 8), University of York, HeslingtonYO10 5DD, United Kingdom.)

Numerous plants produce cyanogenic glucosides (CGs), which release toxic hydrogen cyanide (HCN) upon cleavage by endogenous plant β -glucosidases as part of the plants' chemical defense. Several crops, including sorghum (*Sorghum bicolor*), contain so high amounts of CGs that they may cause intoxications of humans or animals. As part of our efforts to create plants with reduced amounts of CGs for safe consumption, we aim

to understand the role the compounds play in plants. In the germinating sorghum seedling, the total amount of the CG dhurrin initially increases up to 3-5 % of plant dry weight, followed by a decrease after 3-4 days. Our hypothesis is that when the seedling has past the earliest vulnerable growth stages where it needs the CGs for defense, it channels the CG nitrogen back into primary metabolism, and we have found that sorghum has the necessary tools for this. At pH > 5 dhurrin and alutathione (GSH) react spontaneously to form a conjugate where one GSH substitutes the glucose moiety of dhurrin. This conjugate is a substrate for glutathione-S-transferases of the lambda class (GSTLs) which perform an unusual reductive cleavage of the conjugate to produce p-hydroxyphenyl acetonitrile (pOHPCN). As we have previously demonstrated, pOHPCN is catabolized by sorghum-specific nitrilase 4 homologs (NIT4s) to release the nitrogen as ammonia. The decrease in dhurrin content during seedling development is accompanied by an increase in total activity of the GSTL+NIT4A/NIT4B2 complex in the developing sorghum seedlings, demonstrating that this is likely to be the pathway acting in planta.

M 16

BIOAVAILABILITY OF TART CHERRY ANTHOCYANINS

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Tart cherry fruits produce several types of biologically active compounds, including anthocyanins and several other flavonoids. These phytochemicals have been studied for their potential health effects. Therefore, our overall goal was to advance knowledge of the value of tart cherry for their prospective health benefits, including several types of chronic diseases. Here we report the production of anthocyanins in tart cherry fruits and on modes of action of its major phytopharmaceutical compounds at target sites. There are important concerns about bioavailability, preservation and processing of tart cherry fruits for future consumption and uses. Any therapeutic effects that anthocyanins have are dependent on sufficient bioavailability both as exposure to cells and as exposure to a whole organism through the diet. The in vivo bioavailability of anthocyanins following ingestion of tart cherry fruits, and their potential biological effects, has not been well characterized. In the present study, the bioavailability of tart cherry anthocyanin compounds was determined in both human subjects and rats. The consumption of tart cherries resulted in the appearance of different proportions of anthocyanin derivatives (unmodified anthocyanins, methylated anthocyanins and glucurono-conjugated derivatives).

METABOLOMIC ANALYSIS OF ANTIOXIDANT PHYTONUTRIENTS IN THE *HIGH PIGMENT* (*hp*-1, *hp*-2) PHOTOMORPHOGENIC MUTANTS OF TOMATO

Ryne Ramaker, James W. Emmet, <u>Stephen C. Grace</u> (University of Arkansas at Little Rock, Department of Biology, Little Rock, 72204, USA.)

We used targeted and untargeted metabolomic analysis to determine antioxidant content across different metabolite classes in three tomato cultivars and two high pigment mutants (hp-1 and hp-2) that exhibit altered photomorphogenic responses. Levels of chlorogenic acid (CGA), rutin, lutein, β -carotene, and lycopene were determined by HPLC-PDA while untargeted metabolic profiling was performed by ESI-LCMS. Green fruits contain substantially higher levels of CGA and rutin, while β -carotene and lycopene were subtantially higher in red fruits, indicating a shift in resource allocation from phenylpropanoids to carotenoids durina fruit development. We also observed a strong correlation between CGA and rutin levels across all genotypes, particularly in green fruits, suggesting that synthesis of hydroxycinnamic acids and flavonoids is coordinately regulated. The hp-2 mutant contained significantly higher levels of CGA and rutin than the other cultivars, particularly in green fruits, as well as higher levels of β carotene. The antioxidant content of tomato fruits was strongly correlated with CGA and rutin levels. Correlation and discriminant function analysis were used to identify metabolic relationships among different antioxidant classes in tomato. This study provides a framework for future studies focused on engineering a more nutritious tomato through selective breeding, genetic engineering, and optimal growth conditions.

M 18

METABOLOMICS AND PHARMACOGNOSY OF MULLEINS (VERBASCUM L.)

<u>Milen Ivanov Georgiev</u> (Institute of Biology, Leiden University, Natural Products Laboratory, Leiden, 2333 CC, The Netherlands.)

The genus Verbascum L. comprises of about 360 species of flowering plants in the Scrophulariaceae family. The leaves, flowers and whole aerial parts of have been used in the traditional folk medicine for centuries, for treatment of a wide range of human ailments, inter alia bronchitis, tuberculosis, asthma, and different inflammations. We report the application of ¹H NMR metabolic fingerprinting in tandem with principal component analyses in five different Verbascum species. V. xanthophoeniceum and V. nigrum accumulate higher amounts of the pharmaceuticallyimportant harpagoside (\sim 0.5% on dry weight basis) and verbascoside, forsythoside B and leucosceptoside B (in total 5.6 - 5.8% on dry weight basis), which underlines the possibility for their application in pharmaceutical The anti-inflammatory activities of V. industry. xanthophoeniceum were evaluated using several in vitro and in vivo assays. Based on the obtained results it was concluded that V. xanthophoeniceum could serve as a promising source of active compounds with antiinflammatory action, particularly in complementmediated disorders.

This work has been supported by a Marie Curie Fellowship of the European Community programme "Intra-European Fellowships" project SYSBIOPRO under contract number PIEF-GA-2009-252558.

BIOACTIVE SECONDARY METABOLITES FROM THE XISHA MARINE SPONGES

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As part of our ongoing natural products discovery program from marine invertebrates, the extracts from fifteen sponges, Phyllospogina foliascens, Phakellia fusca, Theonella swinhoei, Haliclona oculata, Plakortis simplex, Aplysinopsis sp., Pseudoceradna sp., Agelas clathrodes, Stelletta tenuis. Callyspongia sp., Halichondria rugosa, Acanthella sp., Mycale fibrexilis, Craniella australiensis, Hippospongia lachne, collected from Xisha Islands in the South China Sea, were selected for more detailed chemical investigation. Chromatographic purification of the extracts resulted in the isolation of 240 secondary metabolites, including alkaloids, norditerpene peroxides, cyclopeptides, sesquiterpenes. plakortones, and bromotyrosine derivatives. Their structures were determined by 2D NMR, MS, CD, Mosher's method, and X-ray diffraction experiments. Eighty four of these metabolites are new compounds, containing 5 novel compounds bearing unprecedented skeletons. Some cyclopeptides showed potent cytotoxicity against tumor cell line SPC-A-1, most of the diterpenes containing isocyano, isothiocyano, thiocyano, and formamide functionalites showed potent antimalarial activities. antifouling and some sesquiterpenes also exhibited potent inhibitory activities on BEL-7402, SPC-A-1, HAC, Colon-26 and other tumor cell lines, meanwhile some plakortones showed moderate anti-inflammatory activities.

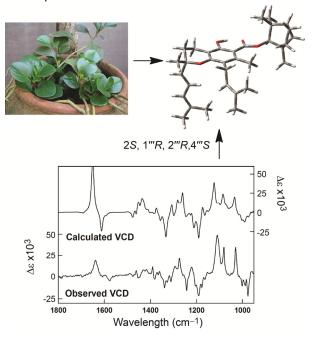
P 2

ABSOLUTE CONFIGURATION DETERMINATION OF COMPLEX CHIRAL NATURAL PRODUCT MOLECULES USING VIBRATIONAL CIRCULAR DICHROISM (VCD): A CASE STUDY

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The determination of absolute configuration of chiral molecules is a long-standing problem in natural product chemistry. Herein we report the use of VCD spectroscopy and DFT calculations to assign the absolute configuration of six isomeric monoterpene chromane esters isolated from *Peperomia obtusifolia* (Piperaceae). This work reinforces the capability of VCD

to determine unambiguously the absolute configuration of structurally complex molecules in solution, without the need of crystallization, derivatization or UV-Vis chromophores.



Ρ3

BIOORGANIC STUDIES ON JASMONATE GLUCOSIDE, A PUTATIVE TRIGGER FOR ION CHANNEL ACTIVATION IN PLANT

<u>Minoru Ueda</u> (Tohoku University, Sendai, 980-8578, Japan.)

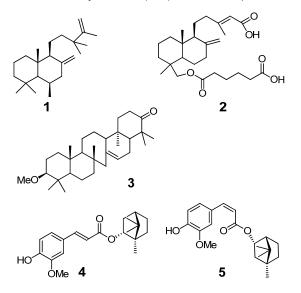
Jasmonates are ubiquitously occurring plant growth regulators with high structural diversity that mediate numerous developmental processes and stress responses. We identified 12-O-a-D-glucopyranosyljasmonic acid (GJA) as the bioactive metabolite inducing nyctinastic leaf-closure of Samanea saman. We demonstrate that leaf-closure of isolated Samanea pinnae is induced upon stereospecific recognition of (-)-GJA. Similarly, rapid and cell-type-specific shrinkage of extensor motor cell protoplasts was selectively initiated by (-)-GJA. (-)-GJA was inactive with respect to activation of typical JA responses, such as induction of JA-responsive genes, accumulation of plant volatiles considered to be mediated by COI1-dependent fashion. Furthermore, application of selective inhibitors indicated that leaf movement is mediated by potassium fluxes initiated by opening of potassium-permeable channels. Additionally, GJA has been identified as a trap-closing chemical factor of the Venus Flytrap (4): trap-snapping movement of Dionaea can be triggered by GJA without external stimuli. This movement is also known to be classified in an ion-channel regulated behavior. Collectively, our data point to the existence of an ionchannel activation mechanism triggered by GJA in plants.

Ρ4

CHEMICAL CONSTITUENTS OF EAST EUROPEAN FOREST SPECIES

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This work is part of the EU FP7 (FP7-KBB-2008-2B-227239) *ForestSpecs* project whose aim is to utilize diverse types of wood residues from the forestry industry. A comparative analysis was carried out of the performance of the MARS microwave extraction system against traditional extraction methods, Soxhlet extraction and shaker extraction. A number of compounds have been isolated and identified, including the novel labdane diterpenoid 6β ,13-dihydroxy-14-oxo-8(17)-labdene (1) from *L. gmelinii* and the novel pumilanoic acid (2) from *P. pumila*, the known and quite unusual serratane triterpenoid, 3β -methoxyserrat-14-en-21-one (3) as well as *E*- and *Z*-bornyl ferulate (4, 5) also from *P. pumila*.

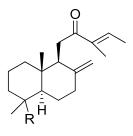


P 5 RESIN DITERPENES FROM AUSTROCEDRUS CHILENSIS

<u>Guillermo Schmeda-Hirschmann</u>,¹ Veronica Rachel Olate,¹ Olatz Goikoetxeaundia² (¹Universidad de Talca, Instituto de Quimica de Recursos Naturales, Talca, Chile VII Region, ²Euskal Herriko Unibertsitatea, Facultad de Ciencias Químicas, Donostia-San Sebastián, 20018, Spain.)

From the resin of the Chilean gymnosperm *Austrocedrus chilensis* (D. Don) Florin et Boutelje (Cupressaceae), 16 diterpenes belonging to the labdane, abietane and isopimarane skeletons were isolated and identified by spectroscopic and spectrometric methods. Some 14 diterpenes are reported for the first time for the species and the diterpene 12-oxolabda-8(17),13E-dien-19 oic acid is described for the first time as a natural product.

Single drop resin samples were collected from female and male adult trees and the diterpene composition analyzed by GC-MS and ¹H NMR. Multiple samples from the same individuals were compared according to tree gender and season (late spring, summer, winter) to disclose similarities and differences. Acknowledgements: FONDECYT Nr. 1085306.



14, R = COOH **14a**, R = COOCH₃ **14b**, minor (*Z*) isomer

P 6

LARVICIDAL ACTIVITY OF COMPOUNDS IDENTIFIED IN PONGAMIA PINNATA SEED AGAINST AEDES AEGYPTI AND CULEX PIPIENS PALLENS

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The toxicity of materials derived from the seeds of Pongamia pinnata Pierre to third instar larvae of Aedes aegypti and Culex pipiens pallens was examined using a direct contact bioassay. Results were compared with those of the currently used insecticide: fenthion and temephos. The active principles of P. pinnata were identified as karanjin (1), pongamone (2), palmitic acid (3) and karanjachromene (4), by spectroscopic analysis. The seed steam distillate compounds were identified by GC-MS, as oleic acid, elaidic acid, arachidonic acid, octadecanamide and behenic acid. Based on 24h LC₅₀ values, karanjin (14.61 and 16.13 ppm) was the most toxic compound but less effective than fenthion (0.0031 and 0.068 ppm) and temephos (0.016 and 0.056 ppm) against Ae. aegypti and Cx p. pallens. Moderate toxicity was shown by pongamone (34.50 and 39.53 ppm), palmitic acid (36.93 and 42.96 ppm), and karanjachromene (43.05 and 48.95 ppm). *P. pinnata* seed derived materials, particularly karanjin, merit further study as potential mosquito larvicides for the control of mosquito populations in light of global efforts to reduce the level of highly toxic synthetic larvicides in the aquatic environment.

Ρ7

QUANTITATIVE ANALYSIS OF COMPOUNDS IN FERMENTED INSAMPAEDOK-SAN AND ITS NEUROPROTECTIVE ACTIVITY IN HT22 CELLS

Bohyoung Lee,¹ Jin Bae Weon,¹ Jin Yeul Ma,² Hye Jin Yang,¹ Eun young Mok,¹ Choong Je Ma¹ (¹Kangwon National University, Department of Biomaterials Engineering, Division of Bioscience and Biotechnology, Chuncheon, 200-701, South Korea, ²Korea Institute of Oriental Medicine, Daejeon, 305-811, South Korea.)

Insampaedok-san (IS) is a traditional medicine used as a treatment for colds. We investigated the consituents, neuroprotective activity and anti-oxidative activity in IS and its fermentation proeduct with Lactobacillus (FIS). Contents of four marker compounds (ferulic acid, hesperidin, 6-gingerol and glycyrrhizin) and other compounds in Insampaedok-san (IS) and fermented Insampaedok-san (FIS) were measured and compared by established HPLC-DAD. Neuroprotective activity of IS and FIS was evaluated and compared by cytoprotective effect against glutamate induced neurotoxicity in HT22 cells. Anti-oxidative activity of IS and FIS was compared by DPPH free radical, hydroxyl radical and hydrogen peroxide scavenging activity tests. Contents of three compounds, ferulic acid and glycyrrhizin decreased, but 6-gingerol was increased by fermentation. FIS showed more potent neuroprotective activity than IS. As a results of DPPH, hydroxyl radical and hydrogen peroxide scavenging test, anti-oxidative activity of IS slightly was increased by fermentation. In conclusion, fermentation with Lactobacillus could alter contents of compounds in IS and improve neuroprotective activity and antioxidative activity of IS.

P 8

QUANTITATIVE ANALYSIS OF THE EIGHT MAJOR COMPOUNDS IN THE SAMSOEUM USING A HPLC COUPLED WITH DAD AND ESI-MS

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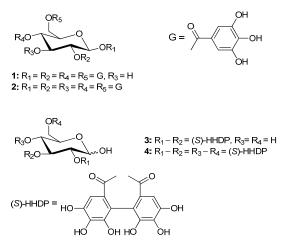
The simultaneous determination of eight major compounds, ginsenoside Rg3, caffeic acid, puerarin, costunolide, hesperidin, naringin, glycyrrhizin and 6gingerol in the Samsoeum using a high-performance liquid chromatography (HPLC) coupled with diode array detection (DAD) and an electrospray ionization mass spectrometer (ESI-MS) was developed for an accurate and reliable quality assessment. Eight compounds were qualitatively identified based on their mass spectra and by comparing with standard compounds and quantitatively analyzed by HPLC-DAD. Separation of eight compounds was carried out on a LUNA C₁₈ column (S-5 μ m, 4.6 mm I.D. 250 mm) with gradient elution composing composed of acetonitrile and 0.1% trifluoroacetic acid (TFA). The data showed good linearity (R² >0.9996). The limits of detection (LOD) and the limits of quantification (LOQ) were less than 0.53 μ g and 1.62 μ g, respectively. Inter- and intra- day precisions (expressed as relative standard deviation (RSD) values) were 1.94 and 1.91%, respectively. The recovery of the method was in the range of 94.24 - 107.90%. The established method is effective and could be applied to quality control of Samsoeum.

Ρ9

POLYPHENOLIC SECONDARY METABOLITES FROM JUGLANS MANDSHURICA MAX.

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Juglans mandshurica has a narrow distribution in Korea and northeastern China. Its leaves, roots and seeds have been used as a traditional medicine for esophageal and cardiac diseases, gastroenteritis, diabetes, and lung cancer. The present study reports the isolation of 17 polyphenolic secondary metabolites from the stem bark of J. mandshurica. These compounds were identified as aromadendrin, taxifolin, ampelopsin, kaempferol. quercetin, myricetin, afzelin, astragalin, quercitrin, hirsutrin, myricitrin, gallic acid and ellagic acid, including 1,2,4,6-tetra-O-galloyl-α-D-glucose (**1**), 1,2,3,4,6-penta-O-galloyl- α -D-glucose (2), (S)-2,3-HHDP-D-glucose (3), and pedunculagin (4). Their structures were elucidated by means of 1D, 2D-NMR and HR-MS analyses. Compounds 1, 2, 3 and 4 were reported from this plant for the first time.



CHALCONE GLYCOSIDES FROM *BRASSICA RAPA* L. EHIDABENIF AND THEIR SYNTHETIC ANALOGUES INHIBIT LPS-INDUCED NO PRODUCTION.

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Phytochemical investigation of the aerial parts of *Brassica rapa* L. ehidabenif resulted in isolation of new chalcone glycosides, 4'-O- β -D-glucopyranosyl-4-hydro-xy-3f-methoxychalcone (A1), 4'-O- β -D-glucopyranosyl-3',4-dimethoxychalcone (A2), and 4,4'-di-O- β -D-glucopyranosyl-3'-methoxychalcone (A3) along with known glycosides. Among the isolates, chalcone glycoside (A2) inhibited LPS-induced NO production in microglia HAPI cells. Moreover, structure-activity relationship studies on synthesized chalcone glycoside analogues showed that 4'-O- β -D-glucopranosyl-3'-methoxychalcone (A11), which has no functional groups in the B-ring, inhibited NO production more potently than A2.

Project supported by the NSF Hawaii EPSCoR Program under National Science Foundation award EPS-0903833.

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PHYTOCHEMICAL STUDY OF THE NATIVE HAWAIIAN PLANT, *METROSIDEROS POLYMORPHA*

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Metrosideros polymorpha Gaudich, known in Hawaii as 'ôhi'a lehua, is a flowering, evergreen tree in the myrtle family, Myrtaceae. M. polymorpha is endemic to the six largest Hawaiian Islands and is the most common native tree in Hawaii. It is a slow growing species, tolerating a wide range of temperature, rainfall and soil conditions, and is the largest component of lowland and montane wet and mesic forests, dry forests, sub-alpine shrublands and new lava flows. As the name implies, the species is able to assume a variety of forms from scrubby brush to a tree of 20-25 m in height depending on growing conditions. Despite its common occurrence, little is known about the chemistry of this species. In the present investigation, a methanol extract of the dried leaves of *M. polymorpha* was fractionated by solvent partition followed by countercurrent chromatography, preparative thin layer chromatography and HPLC to afford a range of flavonoids and flavonoid glycosides as well as other secondary metabolites.

P 12

DIRECT IDENTIFICATION OF PHENOLIC CONSTITUENTS IN *FABIANA IMBRICATA* INFUSIONS BY HPLC-DAD AND HPLC-MSN

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A simple and direct method was developed for the qualitative analysis of polyphenols in infusions from the Chilean crude drug 'Pichi' or 'Pichi romero' (Fabiana imbricata R. et. P., Solanaceae) by HPLC-DAD and HPLC-MS. The phenolic constituents identified in the plant infusions were chlorogenic acid. hydroxyacetophenone, scopoletin and quercetin derivatives. The glycosides were mainly glucosides of phydroxyacetophenone and scopoletin while di- and triglycosides of guercetin were the main flavonoids. The results suggest that the medicinal properties reported for this infusion should be attributed to the presence of several phenolics with known anti-oxidant, diuretic and anti-inflammatory activity. The HPLC trace obtained can be useful for a rapid authentication of the crude drug as well as for qualitative analysis and differentiation of plant populations in the plant distribution range.

Acknowledgements: Project PBCT PSD-50 and Programa de Investigacion en Productos Bioactivos, Universidad de Talca.

P 13

PIGMENT ANALYSIS OF SPATHES IN ANTHURIUM SPECIES

Benjamin R. Clark,¹ Jon Y. Suzuki,² Barbara J. Bliss,² Robert P. Borris¹ (¹College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720, USA, ²USDA-ARS-Pacific Basin Agricultural Research Center, Tropical Plant Genetic Resources and Disease Research Unit, Hilo, HI 96720, USA.)

Anthurium is a genus of popular ornamental plants in the Araceae that produce highly colored, modified leaf structures known as spathes. While it is known that anthocyanins are the primary pigments responsible for the striking red and orange colors observed in anthuriums, most pigment analyses have relied on older techniques such as thin layer chromatography. We describe here the analysis of pigments from a number of *Anthurium* varieties using LC-MS, which revealed the presence of a range of pelargonidin and cyanidin glycosides. Of particular interest was the detection of numerous pre-pigments in the form of flavonoids, flavonols, proanthocyanidins and their glycosides, which may provide tools for greater control of spathe color through applied molecular genetics.

Project supported in part by the NSF Hawaii EPSCoR Program under National Science Foundation award EPS-0903833

QUANTITATIVE ANALYSIS OF CURCUMIN AND RELATED COMPOUNDS IN *CURCUMA LONGA* BY HPLC AND LC-MS

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Turmeric, Curcuma longa L. (Zingiberaceae), is a wellknown medicinal plant in many cultures and is used for a variety of pharmacological activities. Many of these biological activities are attributed to the presence of curcumin and related diphenylheptanoids. Curcumin is found in turmeric along with its desmethoxy and bisdesmethoxy analogs. Like most natural products, the concentration of these interesting compounds is expected to vary depending on growing conditions and time of harvest. In the present work, conditions for the extraction of curcumin and its analogs on an analytical scale have been optimized, and spectrophotometric and chromatographic methods (HPLC, LC-MS) for the determination of total curcumin analogs as well as the individual diphenylheptanoids have been established. These methods were then applied to the analysis of field samples of turmeric grown in a variety of locations on the Island of Hawaii.

Project supported in part by grants from Western Sustainable Agriculture Research & Education (SARE), USDA, award FW10-056 and the NSF Hawaii EPSCoR Program under National Science Foundation award EPS-0903833.

P 15

EFFECT OF GYOJYA-NA EXTRACTS ON UVB-INDUCED APOPTOSIS IN HACAT CELL

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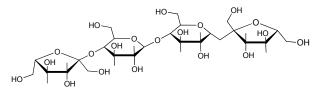
The ascetic green (Gyojya-na) is a hybrid plant from ascetic garlic (Allium victorialis L. subsp. Platyphyllum Hulten) and leek (Allium tuberosum Rottler et Spreng), and is cultivated around Nagai City, Yamagata prefecture as a special regional farm product. At present. Gyojya-na is still cultivated at about 3000 kg/year. In order to explore the cell biological effects of Gyojya-na extracts, we examined a rescue effect of UVB-induced apoptosis in human keratinocytes (HaCaT cells). HaCaT cells were UVB irradiated (400mJ/cm²) and cell survival was measured by the MTT method. Some Gyojya-na extract fractions extracted by hexane partially inhibited UVB-induced cell death. Thus, a UVB-induced cell death inhibitor may exist in the extracts. Using NMR. MS and IR, we analyzed the component to identify chemical structures at present. Because recent studies suggested that upregulation of MAP kinase or Akt suppressed UVBinduced apoptosis in HaCaT cells, a component extracted from Gyojya-na may be able to activate or inactivate signaling pathways such as ERK1/2, JNK2/3, p-38 MAP kinase or Akt. When the component structure is confirmed, the mechanisms of apoptosis inhibition in HaCaT cells through the intercellular signaling pathways can be studied.

P 16

A TETRASACCHARIDE ISOLATED FROM THE FRUITS OF TAXUS CUSPIDATA

<u>Se-Yeong Park</u>¹, Chang-Kiu Lee², Young-Soo Bae¹ (¹Kangwon National University, Forest Biomaterials Engineering, Chunchon, 200-701, South Korea, ²Kangwon National University, Chemistry, Chunchon, 200-701, South Korea.)

One tetrasaccharide was isolated from the H_2O soluble extract of the fruits of *Taxus cuspidata* by Sephadex LH-20 column chromatographic purification. Its structure was elucidated by 1D, 2 D-NMR and MALDI-TOF MS, including acid hydrolysis, acetylation and permethylation.



P 17 DIFFERENTIAL LIPID PROFILES OF MICROALGAE CO-CULTIVATED WITH MARINE BACTERIA

Dagmar Enss, <u>Heiko Rischer</u> (VTT Technical Research Centre of Finland, Espoo, 02044, Finland.)

Monoalgal cultures isolated from the environment and commonly used for scientific investigations are generally not axenic but contain bacteria. Relatively stable associations (sometimes as symbiosis) of bacteria and algae are known from ecological research, but have not been studied with respect to algal lipid production. However, it is known that the organisms influence each other's physiology or even exchange metabolites. Since mutually beneficial coexistence could even have an economic impact on biotechnological applications we investigated axenic algae, isolated native bacteria and diverse co-cultivations in respect to their respective lipid accumulation.

A first screen based on GC-FID total lipid analysis indeed revealed specific species combinations resulting in higher lipid concentrations compared to the individual cultures alone or the native community. A more thorough LC-MS/MS investigation comprising more than 200 lipids focused on particular differences in the metabolic profiles of these combinations. Qualitative changes in the lipid profiles were recorded suggesting metabolic interaction between the algae and the bacteria.

DIARYLHEPTANOID SULFATES AND RELATED COMPOUNDS FROM THE *MYRICA RUBRA* BARK

<u>Morio Yoshimura</u>, Yoshiaki Amakura, Takashi Yoshida (Department of Pharmacognosy, College of Pharmaceutical Sciences, Matsuyama University, Matsuyama, 790-8578, Japan.)

The EtOAc and *n*-BuOH soluble portions obtained from the 80% EtOH extract of *Myrica rubra* bark were separately purified by a combination of chromatography over Diaion HP-20, Toyopearl HW40, Sephadex LH-20 and/or Silica gel to yield three new compounds, myricanol 11-O-sulfate (1), juglanin B 11-O-sulfate (2), and myricanone 5-O-(6'-O-galloyl)-glucoside (3) together with 11 known compounds. Each structure was elucidated on the basis of spectral analyses (NMR, MS, IR, and [α]_D).

P 19

CHEMICAL CHARACTERIZATION OF THREE SAMPLES OF BRAZILIAN PROPOLIS BY NMR AND GC/MS

<u>Caroline Cristina Fernandes-Silva</u>,¹ Antonio Salatino,¹ Giuseppina Negri,² Maria Luiza Faria Salatino¹ (¹University of São Paulo, Botany, São Paulo, SP 05508-090, Brazil, ²Federal University of São Paulo, Psicobiology, São Paulo, SP 04023-062, Brazil.)

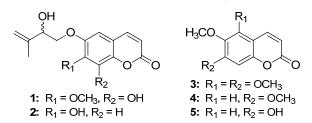
Ethyl acetate fractions of three samples of Brazilian propolis, corresponding to the states of Ceará, Santa Catarina and Paraná were analyzed by NMR and GC/MS. The NMR analysis of propolis from Ceará suggests the presence of carbonylic and aromatic compounds, and methoxylated compounds with long carbon chains. The CG/MS data indicated the presence of triterpenoids such as lupenone and lupeol. The NMR analysis of the propolis from Santa Catarina showed higher diversity of carbonyl compounds and aromatic substances, methylenic and methyl groups linked to oxygen, mainly between 100.4 e 94.6 ppm, indicating the presence of flavonoids or another phenolic compounds, most of them methoxylated, such as ketone esters and/or acids. The CG/MS data are in agreement with NMR evidence, indicating the presence of the cinnamic and ferulic acids (phenylpropanoids), αamyrine (triterpenoid) and pinocembrin (flavonoid). The NMR analysis of the propolis from Paraná indicated the presence of carbons linked to oxygen, suggesting the presence of other classes of phenolics, perhaps flavonoids and lignans. In the CG/MS analysis no flavonoids were found, however, the presence of methoxylated substances with ketone carbonyl, such as 1-hydroxy-3(4-hydroxy-3-methoxyphenyl) 2-propanone, as suggested by NMR analysis were observed. Thus, the differences in the chemical composition of the three Brazilian propolis studied in the present work are obvious.

P 20

COUMARINS FROM THE MALAGASY CEDRELOPSIS RAKOTOZAFYI (PTAEROXYLACEAE)

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The genus *Cedrelopsis* is endemic to Madagascar and comprises eight species, four of which have been examined phytochemically: *C. gracilis*, *C. microfoliata*, *C. grevei* and *C. longibracteata*. The plant *Cedrelopsis rakotozafyi* has been investigated and yielded seven compounds: a new coumarin, 8-hydroxy-7-methoxy-6-(2-hydroxy-3-methylbut-3-enoxy)-2H-1-benzopyran-2-one (1) along with four known coumarin derivatives (2-5) and two known triterpenoids, lupeol and α -amyrin.

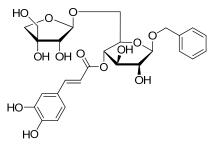


P 21

PHENOLIC COMPOUNDS FROM SUNFLOWER (HELIANTHUS ANNUUS) SEEDS

Yoshiaki Amakura, Morio Yoshimura, <u>Takashi Yoshida</u> (Department of Pharmacognosy, College of Pharmaceutical Sciences, Matsuyama University, Matsuyama, 790-8578, Japan.)

A new compound, benzylalcohol α -D-apiofuranosyl-(1 \rightarrow 6)- α -D-(4-O-caffeoyl) glucopyranoside (1), was isolated together with eight known phenolic compounds from the seeds of sunflower (*Helianthus annuus*). The known compounds were characterized by spectroscopic methods as caffeic acid, methyl caffeoate, methyl chlorogenate, chlorogenic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and eriodictyol 5-O- α -D-glucoside. The anti-oxidative effect of these phenolic constituents was also evaluated on the basis of oxygen-radical absorbance capacity (ORAC), and caffeic acid derivatives were shown to be major antioxidants in the seeds.



LUTEOLIN ENHANCES TUMOR NECROSIS FACTOR-RELATED APOPTOSIS-INDUCING LIGAND-MEDIATED APOPTOSIS OF SK-BR3 HUMAN BREAST CANCER

<u>An Keun Kim</u>, Eun Young Kim (College of Pharmacy, Sookmyung Women's University, Seoul, 140-742, South Korea.)

The dietary flavonoid luteolin has been reported to induce apoptosis in various cancer cells, whereas it has no effect on normal cells. Here, we investigated the effect of luteolin on tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis in TRAIL-resistant SK-BR3 human breast cancer cells. Combination of luteolin with TRAIL inhibited SK-BR3 cell growth much more strongly than each agent alone. Long-term toxicity was determined by clonogenic survival assay and enhanced apoptosis was confirmed by PARP-cleavage. Combined treatment with luteolin and TRAIL markedly reduced the clonogenic capacity of cells and activated PARP-cleavage. The apoptotic mechanism induced by combination involved the activation of caspases. The luteolin and TRAIL cooperatively activated caspase-3, -6, -8 and -9. Moreover, combined treatment induced Bid activation, Bcl-2 protein down-regulation. The expression of FLIPs was also down-regulated by TRAIL/luteolin combination. Taken together, the results indicate that luteolin/TRAIL combination could sensitize SK-BR3 human breast cancer cells to TRAIL-induced apoptosis by stimulating caspase-signaling pathway and by regulating the survival proteins.

P 23

SYNTHESES OF MACROCYCLIC ENGELHARDIONE ANALOGS AS POTENTIAL ANTITUBERCULOSIS AGENTS

Li Shen, <u>Dianqing Sun</u> (University of Hawaii at Hilo, College of Pharmacy, Hilo, HI 96720, USA.)

Engelhardione was originally isolated from the roots of Engelhardia roxburghiana (Juglandaceae). This naturally occurring macrocyclic compound belongs to a broad family of secondary plant metabolites called diarylheptanoids, which have been shown to mediate diverse biological activities. Engelhardione was reported to have potent in vitro activity against Mycobacterium tuberculosis strain H37Rv (MIC = $0.2 \mu g/mL$). As part of our continuous effort to develop new antituberculosis agents, we have employed this emerging natural product lead as a chemical starting point for subsequent structure-activity relationship (SAR) studies. Recently, we reported the first total synthesis of engelhardione. and this effort ultimately led to the structural revision of this macrocyclic natural product. The correct structure of the reported engelhardione should be that of pterocarine (L. Shen, D. Sun, Tetrahedron Lett., in press). Synthesis

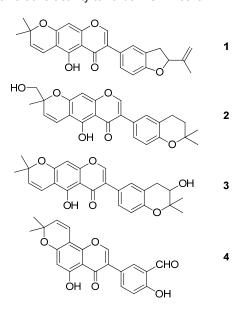
of engelhardione was achieved using a series of aldol condensation reactions and selective hydrogenation to generate the key linear building block, 1,7diphenylheptan-3-one derivative, followed by the macrocyclic Ullmann condensation and appropriate deprotection to afford engelhardione. Using this developed synthetic scheme, diversified macrocyclic engelhardione analogs were subsequently synthesized for antituberculosis screening. Microwave-assisted organic synthesis (MAOS) of this intramolecular macrocyclization will also be presented.

P 24

ISOLATION, CHARACTERIZATION, AND BIOACTIVITIES OF PRENYLATED ISOFLAVONOIDS FROM RHYNCHOSIA EDULIS

Bernhard W.G. Vogler,¹ Ifedayo V. Ogungbe,¹ Gabrielle M. Hill,¹ Rebecca A. Crouch,¹ Meenakshi Nagarkoti,¹ William A. Haber,² William N. Setzer¹ (¹University of Alabama in Huntsville, Department of Chemistry, Huntsville, AL 35899, USA, ²Missouri Botanical Garden, St Louis, MO 63166, USA.)

Four new prenylated isoflavones, rhynchosins A-C (1-3) and rhynchosinal (4), were isolated by bioassay-quided fractionation of the dichloromethane bark extract of Five Rhvnchosia edulis. previously described compounds, scandenal, ulexin B, cajanone, cajanin, and cyclochandalone. were also isolated. These isoflavonoids showed weak inhibitory activity towards rhodesain, the major cathepsin-L like protease in Trvpanosoma brucei. Thev also have weak antiproliferative activity towards MCF-7 cells.



PHENOLIC GLYCOSIDES FROM THE STEM OF STEWARTIA PSEUDOCAMELLIA MAXIM.

Jong Hwan Kwak,¹ Kyoung Hwa Hong,¹ Jong Jin Bae,¹ Ok Pyo Zee,¹ Hana Youn,² Yu-na Lee,² Chang-seon Song² (¹Sungkyunkwan University, School of Pharmacy, Suwon, Gyeonggi-do, 440-746, South Korea, ²Konkuk University, College of Veterinary Medicine, Seoul, 143-701, South Korea.)

Stewartia pseudocamellia Maxim. is a deciduous tree in the family Theaceae which is distributed in southern Korea. Its fruit or the bark of stems and roots have been used as a folk medicine for treatment of circulatory disorders, paralysis of the limbs, legs and arms, and several pains. In our continuing studies to find bioactive compounds from natural sources, we have found that the MeOH extract of S. pseudocamellia has antiviral activity against influenza A(H1N1) virus. The MeOH extract of S. pseudocamellia was consecutively partitioned with hexane, CH₂Cl₂, EtOAc and *n*-BuOH to give five fractions. Among these fractions, the n-BuOH fraction was subjected to column chromatographic separation. Two new phenolic glycosides were isolated from the *n*-BuOH fraction, and the structures were determined from their spectral data. The tructures of two new compounds, named stewartiaside A and pseudocamelliaside, were established as 1-(2'-methoxy, 4',6'-dihydroxy-2'-methoxy)phenyl), 3-hydroxy-5-(4"hydroxyphenyl) pentan-1-one 3-O-β-D-glucopyranoside and 2, 3, 5-trimethoxy 1-O-B-D-glucopyranoside 6'-Ogallate, respectively.

P 26

LIGNAN DERIVATIVES FROM POLAR EXTRACTS FROM STEMS AND DRIED EXUDATE OF FIVE SPECIES OF BURSERACEAE

Juan Diego Hernández,¹ Luisa Urania Román,¹ Carlos Martin Cerda-García-Rojas,³ Jesús Martín Torres-Valencia,² Pedro Joseph Nathan³ (¹Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Químico-Biológicas, Morelia, Michoacán 58030, México, ²Universidad Autónoma del Estado de Hidalgo, Area Académica de Química, Pachuca, Hidalgo 42184, México, ³Centro de Investigación y Estudios Avanzados del IPN, Química, México, D.F. 07000, México.)

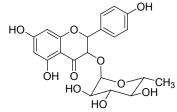
Species of the genus Bursera contain monoterpenes, sesquiterpenes, diterpenes, triterpenes and lignan derivatives. Many species have been used in traditional medicine. From the apolar and polar extracts of stems and the dried exudate of Bursera ariensis, B. bolivarii, B. discolor, B. fagaroides, and B. multifolia, were isolated and identified ariensin and ariensin В and podophyllotoxin related lignans, like deoxypodophyllotoxin, morelensin, yatein, and 8'desmethoxyyatein, whose spectroscopic properties were compared with those of podophyllotoxin. All the species were collected in several localities of Mexico.

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A FLAVONOID GLYCOSIDE FROM EPERUA GLABRIFLORA (DUCKE) COWAN.

Lidiam Maia Leandro, <u>Valdir F. Veiga Junior</u> (Universidade Federal do Amazonas, Department of Chemistry, Manaus, Amazonas 69079000, Brazil.)

The phytochemical study of the ethyl acetate extract of stem bark of the Amazonian Leguminoseae *Eperua glabriflora* afforded a 3-*O*-rhamnosylflavonol identified as engeletin.

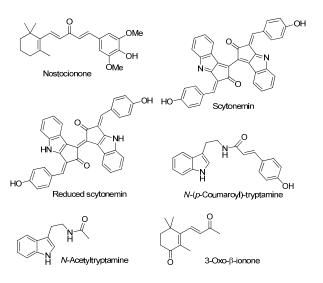


P 28

ISOLATION OF SECONDARY METABOLITES FROM MICROALGAE

Yuji Muramatsu, Hitoshi Sato, Masayuki Ninomiya, <u>Mamoru Koketsu</u> (Gifu University, Department of Materials Science and Technology, Faculty of Engineering, Gifu, 5011193, Japan.)

The extracts of two terrestrial alga *Nostoc commune* Vauch. and *Nostoc flagelliforme* showed high antioxidant activities. We isolated two β -ionone derivatives, nostocionone and 3-oxo- β -ionone along with four indole alkaloids: scytomemin, reduced scytonemin, *N*-(pcoumaroyl)tryptamine, and *N*-acetyltryptamine from the extract of *N. commune* and *N. flagelliforme*. Nostocionone and reduced scytonemin showed strong anti-oxidant activities.

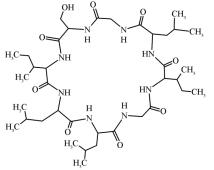


A CYCLOPEPTIDE ISOLATED FROM JATROPHA RIBIFOLIA (POHL) BAILL AND ITS SOLID PHASE SYNTHESIS

<u>Meri Emili Pinto Ferreira</u>,¹ Eduardo Maffud Cilli,¹ Joseam Fechine Tavares,² Marcelo Sobral da Silva,² Vanderlan da Silva Bolzani¹ (¹Universidade Estadual Paulista "Júlio de Mesquita Filho", UNESP Cep. 14800-900, Araraquara, São Paulo, Brazil, ²Laboratório de Tecnologia Farmacêutica, UFPB, cx. Postal 5009, João Pessoa, Paraíba, Brazil.)

Species of *Jatropha*, Euphorbiaceae family, is well known due to their several biologically active compounds. Their cyclic peptides are also an interesting class of secondary metabolites due to structural features and some pharmacological properties¹. This work deals with the isolation and structure elucidation of a new cyclic peptide from *Jatropha ribifolia*, collected in the city of João Pessoa, located in the Northeast of Brazil. Two grams of crude extract of *J. ribifolia* were submitted to HPLC, and a new peptide was isolated along with known diterpenes. The synthesis of the new cyclic peptide was accomplished by solid-phase peptide synthesis (SPPS)-Fmoc/tBu, Some biological activities are being evaluated.

¹Picchi, D.G. Altei, W.F., Saito, M.S., Bolzani, V.S., Cilli, E.M. Cyclic peptide from plant biomass: chemical features and diversity, biosynthesis and biological activities, Química Nova, v. 32, n. 5, p. 1262-1277, 2009.





P 30

A THYMOL DERIVATIVE FROM AGERATINA GLABRATA

Armando A. Talavera,¹ Guadalupe P. Garcia,² Mario H. Gómez,¹ Hugo G. Garcia,¹ Isabel G. Martinez,² Thomas Scior,² <u>Rosa del Rio</u>¹ (¹Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Químico Biológicas, Morelia, Michoacán 58030, México, ²Benémerita Universidad Autónoma de Puebla, Facultad de Ciencias Químicas, Puebla, 72570, México.)

Ageratina glabrata, H.B.K (syn. Eupatorium glabratum, Kunth) is widely distributed throughout Mexico and popularly known as "chamizo blanco" and "hierba del golpe" for its traditional use as external analgesic remedy. NMR and mass analyses identified a thymol derivative. The dichloromethane extract of *A. glabrata* leaves was fractionated by column chromatography affording a pure compound 10-benzoyloxy-6,8,9-trihydroxythymol isobutyrate The ¹³C NMR spectrum was in agreement with the proposed structure.

P 31

ESSENTIAL OIL COMPOSITION AND SECRETORY STRUCTURES OF *GRAMMOSCIADIUM SCABRIDIUM* BOISS.

<u>Hamzeh Amiri</u>,¹ Shirin Dadfar,² Niliofar Khoshbakht², Ali Zarei,¹ AbloInaser Mohammadi¹ (¹Lorestan University, Khoram-Abad, 465, Iran, ²Islamic Azad University, Broujerd, 32465, Iran.)

The genus Grammosciadium DC. (Apiaceae) consists of three species in the Flora of Iran. Generally, they occur in temperate or temperate-cold pasturelands and are considered to be attractive animal foods. G. scabridium were collected from the southwest mountains of Khoram-Abad in Lorestan province. Collected plant materials were dried in the shade and were hydrodistilled using a Clevenger apparatus. The oil was analyzed by capillary GC and GC/MS. The anatomical studies were carried out using a staining method with brown bismark and methyl green. GC and GC/MS analysis of the essential oil resulted in the identification of 36 compounds, representing 89.3% of the total oil. Major constituents of the oil were 2-cyclohexen-1-one limonene (17.09%), (29.53%), apiol (11.78%), hexadecanoic acid (5.62%), and spathulenol (3.24%).

P 32

T-FBS-02 IS A LIVER X RECEPTOR-α LIGAND AND STIMULATES REVERSE CHOLESTEROL TRANSPORT WITHOUT INDUCING HEPATIC LIPOGENESIS

Minh-Hien Hoang, Hee-jin Jun, Yaoyao Jia, <u>Sung-Joon</u> <u>Lee</u> (Korea University, Food Bioscience and Technology, Seoul, 136-713, South Korea.)

Aim to investigate the ligand activity of T-FBS-02 on liver X receptors (LXRs). Methods and results T-FBS-02 bound directly to LXR- α but not LXR- β in a reporter gene assay, time-resolved fluorescence resonance energy transfer analysis, and limited protease digestion experiment. T-FBS-02 stimulation in macrophages stimulated cellular cholesterol efflux dose-dependently, with induction of ATP-binding cassette transporter A1 and G gene and protein expression. In hepatocytes, T-FBS-02 significantly induced Insig-2a levels and delayed nuclear translocation of sterol regulatory element-binding protein 1 (SREBP-1) protein, resulting in a dose-dependent reduction in the expression of genes in fatty acid synthesis and cellular lipid levels.

Conclusion: T-FBS-02, a direct ligand for LXR- α , activates reverse cholesterol transport in macrophages without inducing hepatic lipogenesis. The induction of Insig-2a suppressed the nuclear translocation of SREBP-1c.

PRELIMINARY PHYTOCHEMICAL EVALUATION AND SEED PROXIMATE ANALYSIS OF SURIB (SESBANIA LEPTOCARPA DC.)-SUDAN

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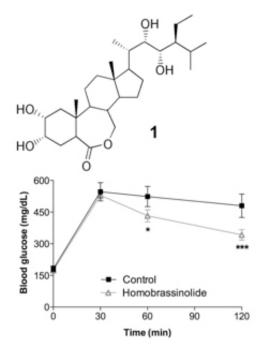
Surib (Sessbania leptocarpa) of the family Leguminosae is a wild plant widely spread in Gezira scheme-Sudan. Different parts of Surib have been used traditionally for various ailments especially in Africa where the plant spread widely. Seed comprises the major plant part and its use in human food and animal feed was not yet through evaluated. The preliminary phytochemical screening was performed using the conventional chemical tests using precipitation and color reagents as appropriate, (A.O.A.C., 1980) were used for the determination of the proximate seed composition. Proximate analysis of the seed revealed that carbohydrates and crude fibers constitute about 80% while proteins and fats values were 5.25 and 6.13% respectively. It could be concluded and recommended that the nutrient value of Surib seed is negligible for its low content of proteins and fatty substances and thus it is of no use as animal feed or human food.

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BRASSINOSTEROID DECREASES HYPERGLYCEMIA IN A DIET-INDUCED OBESITY MOUSE MODEL

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The prevalence of obesity is increasing globally, and obesity is a major risk factor for metabolic syndrome. Previously we observed that daily oral administration of homobrassinolide (1) to healthy rats resulted in a slight decrease in fasting blood glucose accompanied by reduction of hepatic expression of PEPCK and G6Pase mRNAs, as well as hepatic AMPK activation. In H4IIE rat hepatoma cells, 1 reduced glucose production and decreased expression of PEPCK and G6Pase in cAMPstimulated upregulation bioassay. Acute, single-dose administration of 50-300 mg/kg of 1 resulted in a significant reduction in the fasting blood glucose in high fat diet-induced obese C57BL/6J mice. Animals receiving 50 mg/kg of 1 for 8 weeks showed 23% lower fasting blood glucose levels and enhanced insulin sensitivity. Gene expression for hepatic PEPCK and G6Pase was significantly decreased. Thus, the data demonstrate that homobrassinolide improves glucose metabolism and increases insulin sensitivity in an animal model of obesity and insulin resistance.





CAFFEINE ATTENUATES THE CYTOTOXIC ACTIVITIES OF INTERCALATING AROMATIC ALKALOIDS

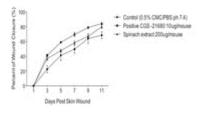
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Many anti-tumor drugs function by intercalating into DNA. The xanthine alkaloid caffeine can also intercalate into DNA as well as form π - π molecular complexes with other planar alkaloids and anti-tumor drugs. The presence of caffeine could interfere with the intercalating anti-tumor drug by forming π - π molecular complexes with the drug, thereby blocking the planar aromatic drugs from intercalating into the DNA and ultimately lowering the toxicity of the drug to the cancer cells. The cytotoxic activities of several known DNA intercalators (berberine, camptothecin. chelerythrine, ellipticine. and sanguinarine) on MCF-7 breast cancer cells, both with and without caffeine present (200 μ g/mL) were determined. Significant attenuation of the cytotoxicities by caffeine was found. Computational molecular modeling studies involving the intercalating anti-tumor drugs with caffeine were also carried out using density functional theory (DFT) and the recently developed M06 functional. Relatively strong π - π interaction energies between caffeine and the intercalators were found. suggesting an "interceptor" role of caffeine protecting the DNA from intercalation.

WOUND HEALING ACTIVITY OF PHYTOECDYSTEROIDS

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Ecdysteroids are polyhydroxylated derivatives of 5acholestane, structurally similar to cholesterol-derived animal steroid hormones. Plants are natural sources of ecdysteroids. Edible plants, such as Spinacia oleracea (spinach). contain considerable amounts of ecdysteroids, such as 20-hydroxyecdysone (1). Ajuga turkestanica, an herb from the basil family native to Uzbekistan, contains high levels of the turkesterone (2). Since the increasing interest in the identification of biologically active natural products in traditionally used botanicals, the objective of this investigation was to evaluate extracts and identify biologically active compounds using an in vitro skin fibroblast migration and proliferation. Compounds 1 and 2 were the most active among the tested ecdysteroids. We also employed a skin closure model in CD-1 mice to show that spinach extract significantly accelerated cutaneous wound closure. These compounds and the methodologies employed can be used for further studies on the biological role of ecdysteroids, as well as the potential application of crop plants which contain high ecdysteroid levels.



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POLYPHENOL SYNERGISM AUGMENTS THERAPEUTIC POTENTIAL OF RESVERATROL: UPDATING OF THE FRENCH PARADOX

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Resveratrol, one of the minor components of red wine, has been considered as the molecule responsible of French paradox. Resveradox has also been described as a cardioprotective, anti-inflammatory, antioxidant and chemopreventive agent, however is a molecule with high absorption and very low bioavailability. This molecule seems also to have high therapeutic potential in obesity. Other polyphenols -Catechins and flavonols are other major polyphenols found in red wine and grapes. The purpose of this work was to assess if polyphenol blending, mimicking the grape total polyphenol content, could increase the biological activities of resveratrol. The catechin-quercetin-resveratrol blend, upregulates NO synthase expression in a bigger extent than resveratrol stand alone. This blending have more antioxidant effect and a similar lipolytic/anti-obesity effect compared to resveratrol stand alone. Moreover the catechinflavonoid-resveratrol composition seems to be more bioavailable than resveratrol standalone, because of the inhibition of the sulfation of resveratrol in the liver. The results presented suggest that resveratrol-polyphenol synergism could contribute to the therapeutic potential. Therefore resveratrol synergized by polyphenols may be the true cause of the French Paradox.

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CELASTRUS ACULEATUS MERR. SUPPRESSES AUTOIMMUNE ARTHRITIS IN RATS BY INHIBITING PATHOGENIC T-CELL AND ANTIBODY RESPONSES

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Rheumatoid arthritis (RA) is one of the major autoimmune diseases of global prevalence. The prolonged use of conventionally-used drugs is associated with severe adverse reactions. Therefore, safer and less expensive therapeutic products are continually being sought. Celastrus aculeatus Merr. (Celastrus) is a traditional Chinese medicinal herb that has been used in folk medicine for centuries for the treatment of rheumatic conditions. Celastrol represents one of the bioactive components of Celastrus. We examined the anti-arthritic activity of Celastrus and Celastrol, as well as the immunological basis of their action using the rat adjuvant-induced arthritis (AA) model of human RA. Lewis rats were treated with Celastrus (1.5/3g/Kg) or Celastrol (1 mg/Kg) daily beginning at the onset of arthritis and then continued throughout the observation period. The severity of clinical and histological arthritis was graded, and the levels of specific disease-related parameters were measured. Celastrus/Celastrol inhibited the severity of ongoing AA, along with significant reduction in the level of proinflammatory cytokines: IL-17, IL-6 and IFN-g; transcription factors: STAT3 and ROR-gt; pathogenic antibodies; MMP9 activity; and phospho-ERK. Thus, Celastrus suppresses the mediators of immune pathology in arthritis, and it offers a promising alternative/adjunct treatment for RA.

COMPARISON OF ANTI-HEPATOFIBROTIC EFFECT: ARTEMISIA CAPILLARIES VS. ARTEMISIA IWAYOMOGI

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Artemisia capillaries and Artemisia iwayomogi have been indiscriminately utilized for variously liver disorders as a traditional hepatotherapeutic medicine in Asian countries including Korea. In the present study, antihepatofibrotic effect of water extract of Artemisia capillaries (AC) and Artemisia iwayomogi (AI) were compared in carbon tetrachloride-induced liver fibrosis rat model. AI (50 mg/kg) significantly attenuated the CCl₄-induced excessive release of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin (p < 0.05) in serum, and hydroxyproline and malondialdehyde contents (p < 0.05) in liver tissue. Further, AI markedly ameliorated total antioxidant capacity (TAC), Glutathione (GSH), Superoxide dismutase (SOD) (p <0.05) in liver tissue. Unexpectedly AC didn't change any of the above parameters. Meanwhile, histopathological and immunohistochemical analysis revealed that AI drastically reduced the inflammation, necrosis, collagen accumulation and activation of hepatic satellite cells in liver tissue rather than AC treatment. Several fibrosisrelated genes such as transforming growth factor β , platelet-derived growth factor β and connective tissue growth factor and α -smooth muscle actin were more prominently down-regulated by AI than AC treatment.

Taken together, our results clarified that AI has more potentially hepatoprotective and anti-fibrotic properties rather than AC through enhancing antioxidant capacity and down-regulation of fibrogentic cytokines.

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NANOTECHNOLOGY IN PHYTOMEDICINES

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Objectives: The aim of this study was to compare the antiplaque and antigingivitis effectiveness of an essential oil-containing nanostructured gel.

Material and Methods: Thirty five qualifying subjects, aged 18-65 years, were randomized into two groups: essential oil nanostructured gel (Cepakill^{®;} Antiseptic); 0.12% chlorhexidine nanostructured gel (Nano gel^{®;}). At

baseline, subjects received a complete oral soft tissue examination and scoring of plaque index (PI), gingival index (GI), and gingival bleeding index (GBI). Subjects started locally applying twice daily with their respective nanostructured gel as an adjunct to their usual mechanical oral hygiene procedures. Subjects were reexamined at 7 days. The treatment groups were compared with respect to baseline clinical variables.

Results: From the 35 subjects at baseline, 32 were evaluated after 7 days of treatment. There were no statistically significant differences among the two groups from baseline and after 7 days treatment.

Conclusion: This 1 week controlled clinical study demonstrated that the essential oil nanostructured gel and the chlorhexidine nanostructured gel had comparable antiplaque and antigingivitis activity.

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SYNERGISTIC EFFECTS BETWEEN AN AQUEOUS EXTRACT OF *PANAX QUINQUEFOLIUS* AND TOBRAMYCIN AGAINST *PSEUDOMONAS AERUGINOSA* IN A RAT MODEL OF LUNG INFECTION

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This study describes the immunomodulatory and antimicrobial properties of the North American ginseng extract (Panax quinquefolius) in synergy with the antibiotic tobramycin to combat Pseudomonas aeruginosa in vivo. The infection was induced in male Sprague-Dawley rats under anesthesia by intratracheal instillation of P. aeruginosa in agar-beads (107 colony forming units (CFU). Starting on day 7 post-infection, animals were treated daily, for three consecutive days with saline, tobramycin (300 μ g/kg b.wt. intratracheal) and/or aqueous extract of ginseng (100 mg/kg b.wt. subcutaneous): animals were killed 24 h after the third drug instillation. The infection in the control group was successfully maintained after 10 days of infection (6.0 x 106 CFU). Treatment of infected animals with tobramycin (6.6 x 104 CFU), ginseng (5.3 x 104 CFU), or tobramycin and ginseng (2.0 x 103 CFU) reduced the bacterial infection in the lungs. Cytokine analysis revealed a reduction of pro-inflammatory cytokines (IL-2, IL-4, IL-6, IL-12p70, IFN-α, GM-CSF, TNF-α) in infected animals treated with the ginseng extract. Taken together, the North American ginseng extract promoted the killing of P. aeruginosa and attenuated the inflammatory response. (Support provided by the Ministry of Research & Innovation, The Ontario Research Fund-Research Excellence program).

THE ANTI-ULCER ACTIVITIES OF THE OIL EXTRACT OF BALANITES AEGYTIACA SEED IN GUINEA PIGS

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Peptic ulcer is caused by an uncontrollable increase in gastric acid secretion from the gastric glands, which are located in the body of the stomach. This study was carried out to investigate the anti-ulcer activities of the oil extract of Balanites aegytiaca seed in guinea pigs. Thirty guinea pigs weighing between 200g to 440.8g were randomly assigned into six groups of five animals each. Gastroduodenal ulceration was induced using pyloric ligation and histamine injection after fasting the animals for 24hrs and orally administering both the oil extract and omeprazole 1h prior to ulcer induction. The anti-ulcer effect of the oil was compared with the standard (control), omeprazole (10mg/kg body weight) given orally. The severity of the ulcers was scored using standard method following the sacrifice of the animals. The histopathology of the lesion was also studied. Balanites aegyptiaca oil significantly (P < 0.05) reduced the volume of gastric secretion in histamine induced ulcer model. It also reduced the mean severity of ulcer scores and ulcer index in both pyloric ligation and histamine induced models. The oil extract increased the percentage protection in both models of experiment. However, omeprazole at the dose rate of 10mg/kg body weight gave better protection. The results showed that B. aegypitiaca oil possesses anti-secretory and cytoprotective activity which can be employed in the prevention, treatment and relief of gastric ulcer symptoms.

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THE ALTERATION OF COMPONENTS IN THE FERMENTED HWANGRYUNHAEDOK-TANG AND ITS NEUROPROTECTIVE ACTIVITY

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Hwangryunhaedok-tang is a traditional herbal prescription that has sedative activity, hypotensive and anti-bacterial effects. In this study, we investigated the alteration of contents of components in Hwangryunhaedok-tang, antioxidant activitv and activity by fermentation neuroprotective with Lactobacillus acidophilus KFRI 128. Contents of three marker compounds (geniposide, berberine and palmatine) unknown and compounds in the Hwangryunhaedok-tang (HR) and the fermented Hwangryunhaedok-tang (FHR) were measured and

compared using a photodiode HPLC-DAD. The antioxidant activity of HR and FHR were determined by DPPH free radical and hydrogen peroxide (H_2O_2) scavenging assay. Also, the neuroprotective activities of HR and FHR against glutamate-induced oxidative stress in a mouse hippocampal cell line (HT22) were evaluated by MTT assay. The contents of geniposide and palmatine decreased, but not the content of berberine was increased in the FHR. The contents of unknown compounds (1), (2), (3), (4) and (5) in the HR were altered by fermentation. Electron donating activity (EDA, %) value of FHR was higher than HR for DPPH radical scavenging activity and H2O2 scavenging activity, respectively. In the MTT assay, FHR showed more potent neuroprotective activity than HR by 513.9%. Clearly the fermentation converts compounds in HR and enhances the antioxidant and neuroprotective activity.

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IN VITRO AND *IN VIVO* ANTITUMOR EFFECTS OF DEOXYELEPHANTOPIN ON HUMAN BREAST CANCER CELLS

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Breast cancers are very common for women and a major cause of death in women worldwide. They can be often highly resistant to current chemotherapy, and thus new therapeutics are desirable. In this study, we evaluated the effect of deoxyelephantopin (DET). а phytocompound extracted from Elephantopus scaber (Asteraceae), as a possible anti-tumor phytomedicine against human breast cancer cells, MDA-MB-231. We observed that DET can effectively suppress the growth of test tumor cells in vitro using cell-apoptosis assay. Upon DET treatment, transforming growth factor-beta (TGF-beta) level was significantly decreased in test cells. DET apparently can inhibit cell growth by inducing a G2-M phase cell cycle arrest and apoptosis in test cells, and the clonogenicity of these cells was also reduced in a concentration-dependent manner. DET can also significantly inhibit the invasion and migration of MDA-MB-231 cells. The effect of DET on suppression of NF- κ B, via activation by TNF- α , was examined using electrophoretic mobility shift analysis (EMSA). Decreased level on expression of phospho-NF-kappaB and the downstream molecules of NF-kB signaling pathway, including survivin, Bcl-2, MMP-9 and VEGF, were observed in DET-treated cells. Under in vivo conditions, DET significantly inhibited tumor growth and the myeloid derived suppressor cells (MDSCs) population in nude mice experiment. Taken together, our findings suggest that DET may warrant systematic investigation for potential application to chemoprevention or control of breast cancers.

ADJUVANT EFFECT OF SPECIFIC MICROTUBULE-DEPOLYMIRIZING AGENTS ON DENDRITIC CELL-BASED CANCER VACCINES

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Damage-associated molecular patterns (DAMPs) are associated with immunogenic cell death (ICD). Specific microtubule-depolymerizing agents (MDAs) such as colchicine have been shown to confer anti-cancer activity and also trigger activation of DCs. In this study, we evaluated the ability of three MDAs (colchicine and two 2-phenyl-4-quinolone analogues) to induce ICD in test tumor cells, and activate DCs. The three test phytochemicals considerably increased the expression of DAMPs including HSP70. HSP90 and HMGB1, but it had no effect on expression of calreticulin (CRT). DC vaccines pulsed with MDA-treated tumor cell lysates (TCLs) had a significant effect on tumor suppression, cytotoxic T-lymphocyte activity, and survival rate of test mice. In vivo antibody depletion experiments suggested that CD8+ and NK cells, were the main effector cells responsible for the anti-tumor activity. In addition, culture of DCs with GM-CSF and IL-4 significantly increased the production of IL-12 and decreased production of IL-10. MDAs also induced phenotypic maturation of DCs and augmented CD4⁺ and CD8⁺ T-cell proliferation. Specific MDAs including the clinical drug, colchicine, can induce immunogenic cell death in tumor cells, and DCs pulsed with MDA-treated TCLs can generate potent anti-tumor immunity in mice. This approach may warrant future clinical evaluation as a cancer vaccine.

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ANTIOXIDANT ACTIVITY OF A HPLC-MS/MS FINGERPRINTED ETHYL ACETATE FRACTION DERIVED FROM CATTLEY GUAVA LEAF AND ITS POTENTIAL ACTIVITY TOWARDS H₂O₂ INDUCED DNA DAMAGE IN HEPG2 CELLS

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The ethyl acetate fraction of the leaf extract of thE exotic fruit, Cherry guava, growing in Jeju Island of S. Korea was evaluated for its abilities to protect cell viability, scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH), alkyl and hydroxyl free radicals. The protective effect was measured against apoptotic cell death induced by H_2O_2 in human liver carcinoma HepG-2 cells. In addition, we performed a HPLC-MS/MS fingerprint of the ethyl

acetate fraction of leaf extract to determine the possiblephytochemicals that are responsible for the biological activity.

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ANTI-DIABETIC EFFECT OF PICEATANNOL, A STILBENOID, IN VITRO AND IN VIVO

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Piceatannnol (PIC) has been found to suppress an increase in fasting blood glucose levels and to improve impaired glucose tolerance in type 2 diabetic model db/db mice. In L6 myotubes, PIC dose-dependently and significantly promoted glucose uptake. It was demonstrated to increase the phosphorylation of AMP-activated protein kinase (AMPK) and the translocation of glucose transporter 4 (GLUT4) to plasma membrane by Western blotting. The stimulatory effect of PIC on the translocation of GLUT4 was also confirmed by transfecting Halo Tag-glut4 vector to L6 cells and visualization. PIC is suggested to show anti-diabetic effect by, at least in part, stimulating AMPK-dependent glucose uptake in muscles.

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ANTI-UROLITHIATIC SCREENING OF AERIAL PARTS OF *ERYTHRINA STRICTA*

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The anti-urolithiatic activity of Erythrina stricta (aerial parts) was evaluated by a calculi-producing diet model. Calcium oxalate nephrolithiasis was induced by injecting sodium oxalate (7mg/100g/day, i.p.) for 7 days. A significant increase in the serum ASAT, ALAT, ALP levels was observed in the control group receiving sodium oxalate. In addition, animals of the control group showed a decrease in the level of serum enzymatic catalase and significant increase in the levels of thiobarbituric acid reactive substances (TBARS) in kidney homogenates. Oral administration of a 70% ethanol extract (500mg/kg/day, b.w.) and ethyl acetate fraction (200mg/kg/day, b.w.) along with sodium oxalate in treated groups, showed a significant dose dependent restoration of all altered serum and homogenate enzymatic parameters. Further, histological estimation of kidneys in treated groups strongly inhibited the growth of calculi within the tubule and reduced necrosis of tubular epithelial cell. The results indicate that the aerial parts of Erythrina stricta are endowed with anti-urolithiatic activity as evidenced by an inhibitory effect on crystal growth and the improvement of kidney function and architecture.

OCCURRENCE OF LIGNOIDS IN SCHILLERIA AND MACROSTACHYS CLADES OF PIPER

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The phylogenetic analysis in *Piper* species using ITS as molecular marker distinguished clades specialized in the production of benzoic acids/chromenes, amides and lignoids. The chemical profiles of these species were determined by analysis of crude extracts by ¹H NMR and HPLC combined with principal component analysis (PCA) and hierarchical cluster analysis (HCA). It is remarkable that lignans of dibenzylbutyrolactone and furofuran types occurr in *Macrostachys*, while *Schilleria* contain dimers of propenylphenols such as tetrahydrofurans and conocarpans. The sequences of dirigent protein genes involved in the stereoselectivity of dimerization step indicated consistencies among *Piper* species but without resolution between clades.

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THE ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES FROM LEAVES AND STICKS OF ILLICIUM VERUM

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In this study, we compared the antibacterial and antioxidant activities of supercritical fluid (SFE) and 95% ethanol extracts from leaves and sticks of Illicium verum. The obtained results revealed that the SFE extracts showed better antimicrobial activity than the alcoholic extracts against the clinical antibiotic resistant pathogens with minimum inhibitory concentration (MIC) value at 0.1 mg/mL. The extracts obtained from sticks showed better antimicrobial activity than the extracts of leaves, although the leaf extracts possess a broader antimicrobial spectrum against all the test strains. Moreover, the chemical components of SFE extracts, anethole, anisyl aldehyde and anisyl acetone, provided the antibacterial activity of Illicium verum leaves, and anethole was the major antibacterial substance of Illicium verum sticks. For the antioxidant activity determination, the alcoholic extracts of leaves and sticks showed better antioxidant activities than the extracts of SFE. Overall, the results revealed that the extracts of *Illicium verum* leaves and sticks have the potential to be developed as natural antibiotics and antioxidants.

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HYPOLIPIDEMIC EFFECT OF *DIOSCOREA OPPOSITA* ON DIET-INDUCED OBESITY IN MICE

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Plants belonging to the genus Dioscorea have long been used as edible tuber crops in many tropical and subtropical areas, and as a traditional herbal medicine in oriental countries including Korea, China, and Japan. In this study, a number of experiments were carried out to evaluate the hypolipidemic effect of the *n*-BuOH-soluble extract from Dioscorea opposita rhizomes against highfat induced mice in vivo. The body weights, parametrial adipose tissue weights, and the levels of TG, TC and LDL-cholesterol in blood serum of female ICR mice were significantly decreased by feeding a high-fat diet with BuOH extract for 8 weeks. In addition, the anti-obesity effects of the phenolic compounds isolated from BuOH extract were assessed using pancreatic lipase in vitro. The seventeen compounds isolated from D. opposita remarkably reduced pancreatic lipase activities. These results suggested that hypolipidemic effects of D. opposita in high-fat diet induced mice may be due to the inhibition of intestinal absorption of dietary fat.

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CY-FBS-01, IS A PAN-AGONIST FOR PPARS

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Cy-FBS-01, a natural flavonoid, has anti-atherogenic activity both in vitro and in vivo; however, its molecular target has not been clearly understood. We investigated the ligand binding of CY-FBS-01 to peroxisome proliferator-activated receptors (PPARs) and its effects on lipid metabolism in vitro. CY-FBS-01 directly bound to all PPAR subtypes in surface plasmon resonance assay, and induced transactivation activity in reporter gene assav and time-resolved fluorescence resonance energy transfer analyses. CY-FBS-01 significantly reduced cellular lipid concentrations in lipid-loaded hepatocytes as well. Hepatic transcriptome profiling in lipid-loaded hepatocytes revealed that the net effects of CY-FBS-01 in lipid metabolism pathways were similar to those of fenofibrate and statin, normalizing the expressions in lipid metabolism gene expressions. CY-FBS-01 induced unique target genes of PPARs in the hepatocytes as well. CY-FBS-01, a phytochemical flavonoid abundant in fruit and vegetable and is a pan-agonist for PPARs.

LUTEOLIN SENSITIZES SK-HEP1 HUMAN HEPATOCELLULAR CARCINOMA CELLS TO TRAIL-INDUCED APOPTOSIS THROUGH UPREGULATION OF DEATH RECEPTORS

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Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a promising candidate for cancer treatment since it selectively induces apoptosis in many cancer cells. However, the cytotoxic effect of TRAIL is limited in some TRAIL-resistant cancer cells. Luteolin, a flavonoid found in many plants, exerts various biological and pharmacological activities. In the present study, we examined the effect of luteolin on TRAIL-induced apoptosis in human hepatocellular carcinoma (HCC) SK-Hep1 cells. Combined treatment of luteolin and TRAIL induced marked decrease of cell viability. Cleavage of PARP and activation of effector caspases (caspase-3, 6, and 7) demonstrated that the combined effect of luteolin and TRAIL is mediated through apoptosis. Treatment with luteolin in combination with TRAIL induced the activation of death receptor pathway-related proteins like caspase-8, DR4 and DR5. The synergy effect of TRAIL/luteolin combination was markedly blocked in the presence of DR4/Fc and DR5/Fc chimeric proteins. Our results indicate that luteolin sensitizes SK-Hep1 HCC cells through the death receptor signaling pathway.

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PHYTOCHEMICAL SCREENING AND APHRODISIAC STUDY OF MALLOTUS MUTICUS AND RHIZOPHORA MUCRONATA EXTRACTS

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Preliminary phytochemical investigation of crude ethanolic extract of Mallotus muticus and Rhizophora mucronata showed the presence of saponin, flavonoids, triterpenes/steroid, tannins and polyphenols. The aphrodisiac potential of M. muticus and R.mucronata were studied on male rats by evaluating their sexual behavior activity and total free serum testosterone level. Based on the results obtained, the total free serum testosterone levels increased throughout the period of administration of both crude extracts compared to negative control. The significant increase in testosterone concentration is observed in both extracts, M. muticus at 400 mg/kg (p<0.05) and R. mucronata at 300 mg/kg (p<0.05). For sexual behavior activity, the numbers and latency of mounting, intromission and ejaculation were recorded. The results have shown that both extracts

stimulate sexual parameters in rats' sexual behavior. Acute oral toxicity study showed that a high dose of the both extracts 2000 mg/kg of body weight caused no mortality and did not demonstrate any abnormal behaviors.

P 55

PHYTOCHEMICAL SCREENING OF MANGROVE EXTRACTS FOR APHRODISIAC POTENTIALS

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Phytochemical screenings of 6 mangrove extracts namely *R.apiculata, R.mucronata, B. parviflora, M. muticus, D. trifoliata and A. aureum* reveals the presence of saponins, flavonoids, triterpenes/ steroids, tannins and polyphenolic compounds. Preliminary investigation of the aphrodisiac potentials of these extracts was done by screening serum testosterone level in treated rats. A significant increase in serum testosterone level with *M. muticus* was observed. The significant increase in serum testosterone level to the high presence of saponins and steroidal compounds found in the leaves extract.

T 1

FUNCTIONAL GENOMICS TO ELUCIDATE NEW ENZYMES IN BENZYLISOQUINOLINE ALKALOID BIOSYNTHESIS

<u>Thu-Thuy T. Dang</u>, Peter James Facchini (University of Calgary, Department of Biological Sciences, Calgary, AB T2N 1N4, Canada.)

Opium poppy (*Papaver somniferum*) contains more than 80 benzylisoguinoline alkaloids (BIAs) including the pharmacologically active compounds morphine, codeine, noscapine, papaverine and sanguinarine. Of these, the biosynthesis of morphine and codeine are best understood. However, the biosynthetic pathways leading to most other BIAs are not very well characterized. Stem cDNA libraries of eight opium poppy cultivars displaying different BIA profiles were analyzed using 454 pyrosequencing to generate transcript profile databases. Comparative transcript and metabolite profiling revealed several differentially expressed genes that often correlated with the occurrence of specific alkaloids. Candidate genes were silenced via virus-induced gene silencing approach to test their involvement in specific pathways, and the encoded enzymes produced in Escherichia coli were biochemically characterized. The nature of these enzymes and their metabolic functions will be discussed.

Т2

TRANSCRIPTION PROFILE OF CYTOCHROME P450-HYDROXYLASES POTENTIALLY INVOLVED IN PLAUNOTOL BIOSYNTHESIS IN *CROTON STELLATOPILOSUS* OHBA

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Plant cytochromes P450 are involved in a wide range of biosynthetic reactions. In Croton stellatopilosus which produces an antipeptic acyclic diterpenoid, plaunotol, a geranylgeraniol-18cytochrome P450-dependent hydroxylase activity has been shown to catalyze the last step of the plaunotol pathway. In this study, a PCRbased approach was used to isolate cDNA of C. stellatopilosus cytochrome P450-hydroxylases. The results showed that two core fragments, namely cyp97_5.4 and cyp79_7.8 belonging to the P450 families of cyp97 and cyp79, respectively, were expressed in high level during the development of leaves. Both gene expressions were correlated with pluanotol content found in same leaves. There results suggested that cyp97 5.4 or cyp79 7.8 might be directly involved in the biosynthesis of plaunotol in C. stellatopilosus.

Т3

INTEGRATION OF TRANSCRIPT AND METABOLITE PROFILING IN CELL CULTURES OF 18 PLANT SPECIES FROM FOUR FAMILIES THAT PRODUCE BENZYLISOQUINOLINE ALKALOIDS

<u>Scott Cameron Farrow</u>, Jillian M. Hagel, Peter J. Facchini (University of Calgary, Biological Sciences, Calgary, Alberta T2N1N4, Canada.)

Benzylisoquinoline alkaloids (BIAs) are a large and diverse class of plant secondary metabolites that often possess potent pharmacological properties. In an effort to better understand BIA metabolism, we have generated expressed sequence tag databases for 18 species of BIA-producing cell cultures. We also used HLPC-MS to accurately characterize the alkaloid profiles of each cell culture. The integration of metabolite and transcript profiles provides a valuable repository for the discovery of BIA biosynthetic genes. Using these integrated databases, we have identified numerous gene candidates responsible for most known and several uncharacterized BIA biosynthetic enzymes. The public availability of these resources, and their utility to gene discovery and a better understanding of BIA metabolic networks will be discussed.

Т4

ADVANCED PROTEOME ANALYSIS OF AROGENATE DEHYDRATASE KNOCKOUT MUTANTS IN *ARABIDOPSIS THALIANA*

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Arogenate dehydratases (ADTs) are a class of enzymes involved in production of phenylalanine (which, in addition to its involvement in protein biosynthesis, is the main starting metabolite in the phenylpropanoid pathway) in plant systems. Our group has recently produced several ADT knockout mutant combinations in Arabidopsis. Knockout lines displaying the most significant physiological and lignin compositional changes were further selected for gel-free high throughput proteome analysis. Relative proteome changes were analyzed in isolated chloroplasts as well as at the whole cell level during four different time points (2, 4, 6, and 8 weeks) in leaves, stems, and roots. Initial results have provided evidence that the six ADT isoenzymes in Arabidopsis are present at a low abundance in relation to the entire proteome and that the individual expression of each ADT may be regulated in a spatial and temporal manner. These results are discussed in terms of effects overall to the plant proteome that such mutations have.

BIO 1

MICROBIAL PRODUCTION OF PLANT ISOQUINOLINE ALKALOIDS

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The secondary metabolites of higher plants include diverse chemicals, such as alkaloids, isoprenoids and phenolic compounds (phenylpropanoids and flavonoids). Although these compounds are widely used in human health and nutrition, at present they are mainly obtained by extraction from plants and extraction yields are low because most of these metabolites accumulate at low levels in plant cells. Recent advances in synthetic biology and metabolic engineering have enabled tailored production of plant secondary metabolites in microorganisms, but these methods often require the addition of expensive substrates. Previously we reported the microbial system to produce reticuline, the key intermediate for producing the isoquinoline alkaloids, from dopamine. Isoquinoline alkaloids, such as morphine, codeine, papaverine, berberine and so on, are synthesized from reticuline and used for the medicine. In this technology, reticuline and the related alkaloids, scoulerine and magnoflorine, are produced with the combination of the Micrococcus luteus and plant enzymes. Here we report further development of an Escherichia coli fermentation system that yields plant alkaloids from simple carbon sources, using selected enzymes to construct a tailor-made biosynthetic pathway. In this system, engineered cells cultured in growth medium without additional substrates produce the plant isoquinoline alkaloids. The fermentation platform offers opportunities for low-cost production of many diverse alkaloids. Recent progresses in molecular characterization of isoquinoline alkaloid biosynthesis are also discussed.

BIO 2

APPLICATION OF HYDROXYPROLINE-O-GLYCOSYLATION FOR ENHANCED PLANT-BASED PRODUCTION

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Hydroxyproline-O-glycosylation involves posttranslational hydroxylation of proline to hydroxyproline (Hyp) and subsequent glycosylation, a modification that is unique to plants and green algae. Our earlier work with synthetic genes encoding various Hyp-rich glycoproteins (HRGPs) expressed in plant cells elucidated a Hyp-O-glycosylation 'code', that is a peptide sequence directs the Hyp-O-glycosylation; specifically contiguous Hyp residues, as in X-Hyp-Hyp are sites of oligoarabinosylation; in contrast, clustered noncontiguous Hyp residues, as in X-Hyp-X-Hyp repeats are mainly sites of highly branched arabinogalactan polysaccharide addition, where X is often Ser or Ala. These results demonstrated the feasibility of Hyp-Obased glycoprotein design in plants and triggered the following applications: 1) by introducing a Hyp-Oalvcosvlation tag to recombinant proteins expressed in tobacco BY-2 cells, we dramatically enhanced the production of secreted proteins up to 1500 fold; 2) using the same approach, we significantly improved the yields of recombinant proteins transiently expressed in Nicotiana benthamiana; 3) by engineering specific Hyp-O-based 'designer' biopolymers into plants, we could reconstruct the plant cell walls for improved biomass processability.

BIO 3

TOOLS FOR DEVELOPING GLYCOSYL-TRANSFERASE ASSAYS AND METHODS FOR XYLAN PROFILING IN PLANTS

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Hemicelluloses are polysaccharides in plant cell walls that have β -(1 \rightarrow 4)-linked backbones and include xyloglucans, xylans, mannans, glucomannans and mixed-linkage glucans. These are synthesized by glycosyltransferases (GTs)that catalyze the transfer of sugar from a glycosyl donor to a suitable acceptor. Most naturally occurring GT acceptors do not contain a chromophore or fluorophore which precludes detection of primary product of GT reaction by spectroscopic techniques. Many GT assays therefore make use of chemically modified donor or acceptor analogues like radiolabeled sugar donors. Availability of such chemically modified analogues is a major problem. We are interested in L-arabinosyltransferases which use UDP-L-arabinofuranose as substrate, a compound that has not been made in radiolabeled form. Here, we present LC-MS as an efficient tool to assay GTs that are heterologously expressed in tobacco leaves. A GT family of particular interest is GT61, which is highly expanded in grasses and proposed to be involved in adding arabinose sidechains onto xylan. We have developed methods to profile xylans using sequential extraction, enzymatic hydrolysis, HPAEC, and LC-MS. With these methods, we have shown specific changes to the profile in some rice GT61 mutants. The key differences in structure of xylan from GT61 mutants and wild type will be discussed.

BIO 4

LIPID, MONOSACCHARIDES AND VITAMIN E CHEMISTRY OF *STICHOCOCCUS BACILLARIS* STRAIN SIVA2011: SOURCE FOR BIODIESEL AND VALUE-ADDED PRODUCTS

<u>Sivakumar Ganapathy</u>,¹ Jennifer Gidden,² Jianfeng Xu,¹ Gregory Phillips¹, Jackson Lay² (¹Arkansas State University, Arkansas Biosciences Institute, Jonesboro, AR United States 72401, ²University of Arkansas, Arkansas Statewide Mass Spectrometry Facility, Fayetteville, AR 72701, USA.)

Fossil fuel is currently the accepted primary source of energy in the world. Negative effects from global warming and increased costs have been predicted as we continue to burn this fuel and deplete a diminishing resource. Microalgae are one of the proposed platforms for lipid-based fuel. It consumes large amounts of CO2 for growth, which makes it an attractive renewable option for next generation energy production. Stichococcus sps. are becoming recognized for their potential as biodiesel and bio-based organic product precursors sources. We have isolated a new S. bacillaris strain, siva2011, for biofuel production. It is comparable to S. bacillaris based on the 18S region of the nuclear rDNA, as well as unique fatty acid methyl esters, monosaccharides and its vitamin E profile. We present growth and vitamin E kinetics, lipid chemistry and bioprocess engineering data for this strain. The mass spectral characterizations of extracts of S. bacillaris strain siva2011 have been useful for monitoring the potential of this organism for producing a high quality biofuel and other value-added products.

BIO 5

THE EXTRACTION OF HIGH VALUE PHYTOCHEMICALS IN THE CONTEXT OF A BIOREFINERY

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To increase biorefinery revenues, phytochemicals can be extracted prior or even after the biochemical conversion of biomass to biofuels or products. Water, at temperatures between 65°C and 100°C, is an excellent extraction solvent because residual water will not interfere with leading biochemical conversion pretreatments. Herbaceous biomass, switchgrass, and woody biomass, sweetgum, are biorefinery feedstocks that contain valuable phytochemicals. The water from 90°C switchgrasss extracts contained guercitrin and rutin at concentrations of 193 and 186 mg kg^{-1} of dry biomass, respectively. The centrifugal partition chromatography-purified quercitrin and rutin fractions decreased by 78 and 86%, respectively, the oxidation of low density lipoprotein. Sweetgum bark and heartwood extracted in 65°C water yielded 1.7 mg g⁻¹ and 0.2 mg g⁻¹ of shikimic acid, respectively. The addition of this 65°C water-based extraction step coupled to pretreatment with

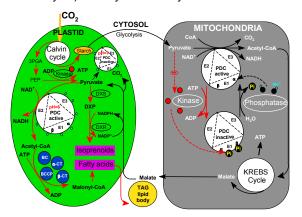
0.98% H₂SO4 at 130°C for 50 min resulted in 21% and 17% increases in xylose percent recovery from bark and heartwood, respectively, as compared to direct pretreatment. These results indicate that, in addition to recovering shikimic acid, the 65°C wash step also increases xylose recovery, demonstrating that this could be integrated to a biorefinery operation.

BIO 6

ENGINEERING OF NON-MEVALONATE PATHWAY FOR THE ENHANCEMENT OF OIL CONTENT IN MICROALGAE CHLAMYDOMONAS REINHARDTII

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Microalgae have a strong potential as vectors for biofuels since their oil content may exceed 70% (w/wDW), as compared with 5% for the best agricultural oil crops. Algae accumulate lipids when cultivated under stress conditions, such as nitrogen depletion and highlight. However, while nutrient limitation can effectively increase lipid content, it can also decrease overall cellular activity leading to reduced cell proliferation. In order to optimize metabolic flux networks that increase the conversion efficiency of solar energy to oil without compromising overall productivity, we aimed to identify and reengineer enzymes that sit at key regulatory pathways. The isoprenoid pathway was chosen in this study since it shares a common intermediate substrate (i.e. pyruvate) with lipid synthesis. Secondly, it uses only the MEP pathway for the biosynthesis of isoprenoids, including sterols in the cytosol (figure). We hypothesized that reducing the utilization of pyruvate in the isoprenoid synthesis pathway can result in the overproduction of fatty acids. In order to test this hypothesis, we produced knockdown mutants using constitutive and inducible promoters in expression RNAi vectors. Experiments are currently underway to test their ability to produce fatty acids under both favorable (low light and nitrogen replete) and stress conditions. Additionally, а complementary chemogenomic approach was conducted to understand the side effects of specific inhibitors of MEP pathway (i.e. ketoclomazone and fosmidomycin) on fatty acids biosynthesis.



BIO 7

OPTIMIZATION OF POTASSIUM HYDROXIDE PRETREATMENT FROM STEAM-EXPLODED SOYBEAN HULL USING RESPONSE SURFACE METHODOLOGY

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Soybean hulls represent the major by-product of soybean processing industry and constitutes about 8% of the whole seed. In this study, we have evaluated the potential of soybean hull as a biomass resource as feedstock for the production of bioethanol. Soybean hull is a lignocellulosic material containing about 50.8% cellulose, 14.5% hemicellulose, and 11.5% lignin. The impact of varying pretreatment parameters (temperature, time, and concentration) on acid hydrolysis of soybean hull were investigated. The important independent variables for pretreatment were selected as reaction temperature, reaction time and potassium hydroxide concentration. The pretreatment condition for maximizing the solid recovery, the cellulose recovery and the lignin removal was optimized using RSM (Response Surface Methodology). An optimum cellulose recovery was found with pretreatment conditions of 70°C reaction temperature, 198 min reaction time, 0.6% potassium hydroxide concentration.

BIO 8

COMBINATIONS OF STEAM EXPLOSION AND CHEMICAL PRETREATMENT FOR FERMENTATION SUGAR PRODUCTION FROM *MISCANTHUS SINENSIS*

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Miscanthus sinensis is an interesting raw material for bioethanol production, it is a high yield low maintenance plant with a high cellulose and hemicellulose content. In this study we used steam explosion and chemical pretreatment to evaluate the combination of the pretreatment for bioethanol production with *Miscanthus sinensis*. A combined pretreatment involved sequential treatments by steam explosion (severity log Ro 4.38 and severity log Ro 4.68) and chemical pretreatment (aqueous alkali and organosolv). The steam explosionorganosolv (dioxane:water) pretreatment conditions for *Miscanthus sinensis* were optimized to obtain higher sugar yield. The steam explosion (severity log Ro 4.38)organosolv (dioxane:water) pretreated material resulted in 79% lignin removal, a cellulose yield of more than 84% and 32% of hemicelluloses recovery. As a result, from 100 g of raw material, 26.8 g (80.7%) of glucose was recovered of 33.2 g available cellulose.

BIO 9

BIOSYNTHESIS OF NOVEL NATURAL NUTRACEUTICALS AND ANTIOXIDANTS: A BIOTECHNOLOGICAL APPROACH

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There has been a growing interest in the use of nutraceuticals as food supplements as well as natural bio-ingredients in food industries. The numerous health benefits of the ω -3 polyunsaturated fatty acids (PUFAs) have been recognized in the modulation of risk of a variety of diseases and disorders. Selected endogenous oils, such as fish oil is rich in ω -3 PUFAs especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are believed to play roles in the prevention of cardiovascular disease and in improving human immune functioning in adults. On the other hand, phenolic compounds represent another important group which possesses antioxidant and functional properties. The incorporation of phenolic acids into triacylglycerols could potentially result in novel structured phenolic lipids, with enhanced anti-oxidative and functional properties. The presented work aimed at the optimization of an environmentally-friendly biotechnological process of selected phenolic lipids in solvent-free medium, by lipase-transesterification of edible oils and endogenous phenolic extracts. The bioconversion yield was determined. In addition, the biosynthesized novel biomolecules of phenolic lipids were characterized in terms of their chemical structures and their antioxidant potentials. This presentation is will cover the overall work carried out in our laboratory that aimed at the development of biotechnological processes, using enzyme technology, for the production of added-value novel nutraceuticals and anti-oxidant biomolecules.