2008 Phytochemical Society of North America Annual Meeting

Phytochemistry and Biology / Human Health

June 25-29, 2008
Institute of Biological Chemistry
Washington State University

Sponsors

Agilent

Elsevier

Waters

Larry A. Walker, Director
National Center for Natural Products Research, School of Pharmacy
The University of Mississippi

Mahmoud ElSohly
ElSohly Laboratories Inc.
Oxford, Mississippi

Washington State University
Institute of Biological Chemistry
Loyal and Helen Davis Endowment
CAHNRS Office of Alumni and Friends

Phytochemical Society of North America
Frank and Mary Loewus Graduate Student Travel Endowment
Arthur C. Neish Young Investigator Endowment

Organizing Committee

Norman G. Lewis, President
Daneel Ferreira
Mark Bernards

Acknowledgements

Coordination and Administration

Franck Dayan
Paula Gibbs
Hiroko Hayashi
J'Lynn Howell
Helen Miller

Design

Paula Gibbs

Table of Contents

Meeting Program

WEDNESDAY, JUNE 25		
3:00-5:00 pm	Executive Committee Meeting (Clark Hall 469)	
5:00 pm	Registration Opens (Honors Hall Lounge)	
6:00-8:00 pm	Opening Reception (Honors Hall Lounge)	

THURSDAY, JUNE 26		
SESSION 1: Phytochemicals and Human Health / Nutrition		
Chair: David R. Gang		
8:00-8:10 am	Introduction and Welcome	
8:10-8:55	**John M. Pezzuto** (University of Hawaii) *Natural product cancer chemopreventive agents*	S1-1
8:55-9:40	**J. Thor Arnason** (University of Ottawa, Canada) *Biologically active molecules from neotropical plants*	S1-2
9:40-10:25	**Augustin Scalbert** (INRA Centre de Recherche de Clermont-Ferrand/Theix, France) *Polyphenols and human health: New tools to characterize intake of the large diversity of polyphenols at the population level*	S1-3
10:25-10:40	Break	
10:40-11:10	**Jan F. Stevens** (Oregon State University) *Xanthohumol and related prenylated flavonoids from hops* (Humulus lupulus): *Beer, dietary supplements, and potential health benefits*	S1-4
11:10-11:30	**Claudia S. Maier** (Oregon State University) *Prooxidant activity of proanthocyanidins from hops* (Humulus lupulus) *causes protein carbonylation and cytoskeletal derangement in colon cancer cells*	S1-5
11:30-11:50	**Roy Navarre** (USDA-ARS, Washington State University) *Potato phytonutrient analysis and engineering*	S1-6
11:50-1:00	Lunch	
	PSNA Young Members Committee Workshop *"Ask the Editor: A panel discussion on common manuscript errors and ways to avoid them"* Panel Members: Dr Norman Lewis, Regional Editor for *Phytochemistry*, Dr Daneel Ferreira, Associate Editor for *Journal of Natural Products*, and Dr John Pezzuto, Editor in Chief for *Pharmaceutical Biology*	
SESSION 2: Plant Cell Wall Assembly		
Chair: Laurence B. Davin		
1:00-1:45 pm	**Marcia J. Kieliszewski** (Ohio University) *Extensin scaffolds and cell wall assembly*	S2-1
1:45-2:15	**Carl A. Douglas** (University of British Columbia, Canada) *Analysis of ACYL-CoA Synthetase 5 and co-expressed genes reveals a novel pathway required for pollen wall formation*	S2-2
2:15-2:45	**Mark A. Bernards** (University of Western Ontario, Canada) *Soybean root suberin and resistance to* Phytophthora sojae	S2-3
2:45-3:15	**Ann M. Patten** (Washington State University) *Advances in plant cell wall microscopy and molecular analyses*	S2-4
3:15-3:30	Break	

SESSION 3: Student and Postoctoral Posters

Chair: Mark A. Bernards

3:30 pm - on *4-minute oral summaries in the following order:*

GRADUATE AND UNDERGRADUATE STUDENTS

Patrick A. Arsenault (Worcester Polytechnic Institute) PS1-1
In planta *metabolic engineering of isoprenoids*

F. Omar Holguin (New Mexico State University) PS1-2
Accumulation pattern of methionine rich β-zein protein in Medicago sativa *(alfalfa) and the related model legume* M. truncatula *in relation to their free methionine pools*

Ray Collier (Washington State University) PS1-3
Uncovering the role of ureide transport in whole plant physiology

Jala J. Daniel (East Tennessee State University) PS1-4
Determining secondary product glucosyltransferase expression during Citrus paradisi *growth and development*

Max Feldman (Washington State University) PS1-5
Regulation of sterol biosynthesis in Arabidopis thaliana: *Experimental testing of a first generation kinetic model*

M.V.K.P. Siddhartha (East Tennessee State University) PS1-6
Heterologous expression and elucidation of biochemical function of two putative flavonoid glucosyltransferase clones (PGT2 and PGT3) from Citrus paradisi

Oliver Corea (Washington State University) PS1-7
Towards determining the individual roles of arogenate dehydratase isoforms in phenylalanine biosynthesis

Kehan Sun (Washington State University) PS1-8
Purification and characterization of recombinant mitochondrial and plastidial serine hydroxymethyltransferases from Arabidopsis

Naomi Yoshikado (Okayama University) PS1-15
Polyphenolic constituents in gambir

Maho Murasawa (Okayama University) PS1-16
Effect of tannins and related polyphenols on Pseudomonas aeruginosa

Christina M. Coleman (University of Mississippi) PS1-21
Isolation and identification of antiadhesive urinary metabolites from cranberry juice

Christina M. Coleman (University of Mississippi) PS1-22
Cranberry juice compounds with antiadhesive properties in urine

Dan J. Cuthbertson (Washington State University) PS1-23
High throughput profiling of fruits for phytochemicals related to human health

Hong Han (University of British Columbia, Canada) PS1-17
Leaf development and cuticular wax composition in Kalanchoe daigremontiana

POSTDOCTORAL RESEARCH ASSOCIATES

Daniel K. Owens (East Tennessee State University) PS1-10
Identification, recombinant expression, and biochemical characterization of a flavonol 3-O-glucosyltransferase from Citrus paradisi

Yasuhisa Kaminaga (Purdue University) PS1-11
Contribution of CoA ligases to benzenoid biosynthesis in petunia flowers

Lydia Yamaguchi (University of São Paulo, Brazil) PS1-12
Seasonal and circadian variation in biflavonoid biosynthesis in Araucaria angustifolia

Vanessa Herl (Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany) PS1-13
Molecular cloning, functional characterization and structural modeling of a Δ4,5-steroid 5β-reductase, a putative key enzyme in cardenolide biosynthesis, from Arabidopsis thaliana L.

	Mohamed M. Radwan (NCNPR, University of Mississippi) *Sorocenol G and H, two new Diels-Alder type adducts from* Sorocea muriculata *roots with anti-MRSA activity*	PS1-18
	Mohamed M. Radwan (NCNPR, University of Mississippi) *New bioactive metabolites from high potency* Cannabis sativa	PS1-19
	Safwat A. Ahmed (NCNPR, University of Mississippi) *Minor oxygenated cannabinoids from high potency* Cannabis sativa *L.*	PS1-20
6:30-9:00	Dinner and Graduate Student / Postdoctoral Poster Session (Vogel Plant Biosciences Building)	

FRIDAY, JUNE 27

SESSION 4: Metabolic Biochemistry

Chair: Jim Saunders

8:00-8:45 am	**William C. Plaxton** (Queen's University, Canada) *Metabolic biochemistry helps close the gap in plant functional genomics*	S4-1
8:45-9:30	**Suzanne Abrams** (National Research Council, Saskatoon, Canada) *Studies on ABA metabolism*	S4-2
9:30-9:50	**Hui Chen** (Washington State University) *AAE13 (At3g16170) encodes a malonyl-CoA ligase in* Arabidopsis	S4-3
9:50-10:10	**Yongzhen Pang** (Samuel Roberts Noble Foundation) *Biosynthesis and bioengineering of proanthocyanidins in model legume* Medicago truncatula	S4-4
10:10-10:25	Break	

SESSION 5: Bioinformatics

Chair: B. Markus Lange

10:25-11:10 am	**John J. Wyrick** (Washington State University) *Bioinformatics tools for investigating the regulation of plant gene expression*	S5-1
11:10-11:55	**Nicholas J. Provart** (University of Toronto, Canada) *Raising the BAR for* Arabidopsis *research: Using large-scale data sets for hypothesis generation*	S5-2
11:55-1:00	Lunch	

SESSION 6: Graduate Student Presentations

Chair: Cecilia A. McIntosh

1:00-1:15 pm	**Rigoberto Rios-Estepa** (Washington State University) *Regulation of mint essential oil biosynthesis: A second generation mathematical model explains environmental and genotypic variation*	S6-1
1:15-1:30	**Lukasz M. Kutrzeba** (University of Mississippi) *cDNA library of* Salvia divinorum *glands as a molecular tool for studying biosynthetic pathway of salvinorin A*	S6-2
1:30-1:45	**Merja Heinäaho** (University of Joensuu, Finland) *The effect of different organic farming methods on the phenolic composition of sea buckthorn berries*	S6-3
1:45-2:00	**Sashi Kasimetty** (University of Mississippi) *Antioxidant, anticancer and anticarcinogenic properties of pomegranate juice constituents and their microbial metabolites*	S6-4
2:00-2:15	**Claudia M. Cardenas** (Washington State University) *Physiological roles of HCT and HQT tobacco acyltransferases and associated metabolic processes*	S6-5
2:15-2:30	Break	

SESSION VII: Plant Phenol / Tannin Biochemistry		
Chair: Massuo Kato		
2:30-3:00 pm	**David R. Gang** (University of Arizona) *Co-regulated metabolite modules in Zingiberaceae rhizomes suggest the existence of biosynthetic modules in plant specialized metabolism*	S7-1
3:00-3:30	**Kye-Won Kim** (Washington State University) *Towards an understanding of the (+)- pinoresinol forming dirigent protein: Reaction mechanism and structure*	S7-2
3:30-4:00	**Takeshi Katayama** (Kagawa University, Japan) *Stereochemistry on the formation of syringylglycerol-8-O-4'-(sinapyl alcohol) ether from sinapyl alcohol with enzyme preparations of* Eucommia ulmoides	S7-3
4:00-4:30	**Toshiaki Umezawa** (Kyoto University, Japan) Arabidopsis thaliana *pinoresinol reductases control enantiomeric compositions of lariciresinol*	S7-4
4:30-5:00	**Juha-Pekka Salminen** (University of Turku, Finland) *Chemical structures of plant ellagitannins reveal their in vitro oxidative activity at high pH*	S7-5
5:00-5:30	**Ulrich Matern** (Philipps Universität Marburg, Germany) *Anthocyanidin synthase as a key enzyme of the flavonol pathway in* Arabidopsis thaliana	S7-6
5:30-6:30	PSNA Business Meeting	
6:30-9:00	Dinner and General Poster Session (Vogel Plant Biosciences Building)	

SATURDAY, JUNE 28		
7:30-8:00	Continental Breakfast	
SESSION 8: Metabolic Engineering and Biosynthesis		
Chair: John T. Romeo		
8:00-8:45 am	**Michel Rohmer** (Université Louis Pasteur, France) *Biosynthesis of isoprene units in plants: Last steps of the MEP pathway and cross-talk between MVA/MEP pathways*	S8-1
8:45-9:30	**Joachim Stöckigt** (Johannes Gutenberg Universität, Germany) *Structural biology in plant alkaloid biosynthesis - Why?*	S8-2
9:30-10:15	**Jacqueline V. Shanks** (Iowa State University) *'Cathedral' roseus** **and other interesting tales in plant metabolic rngineering*	S8-3
10:15-10:30	Break	
SESSION 9: Natural Products for Pest Management		
Chair: Toshiaki Umezawa		
10:30-10:55 am	**Massuo J. Kato** (Universidade de São Paulo, Brazil) *Tracking metabolic diversification in Piperaceae*	S9-1
10:55-11:20	**Charles L. Cantrell** (USDA-ARS, NPURU) *Arthropod repelling constituents from a Southern folk remedy,* Callicarpa americana	S9-2
11:20-11:45	**Eric T. Johnson** (USDA-ARS, CBRU) *Colored and white sectors of petunia flowers display differential resistance to insect herbivores*	S9-3
11:45-1:00	Lunch	

SESSION 10: Tannin Award Symposium		
Chair: Daneel Ferreira		
1:00-1:35 pm	**James A. Kennedy** (Oregon State University) *Explorations in grape and wine proanthocyanidin chemistry*	S10-1
1:35-2:10	**Nancy Terrier** (INRA, Montpellier, France) *Molecular aspects of biosynthesis of flavonoids in grape berries* ARTHUR C. NEISH YOUNG INVESTIGATOR AWARD	S10-2
2:10-2:45	**Tsutomu Hatano** (Okayama University, Japan) *Effect of phenolic compounds in traditional Sino-Japanese herbal medicines on the impairment of rat spatial cognition*	S10-3
2:45-3:00	Break	
3:00-3:35	**Ann E. Hagerman** (Miami University Ohio) *Anti-inflammatory and cytotoxic activities of polymeric polyphenolics in rabbit peripheral blood mononuclear cells*	S10-4
3:35-3:55	**Warren Grigsby** (Biopolymer Network Limited, New Zealand) *Synthesis, characterization and thermal behaviours of polyphenol stearates*	S10-5
3:55-4:30	**Stéphane Quideau** (European Institute of Chemistry and Biology, France) *C-glycosidic ellagitannins: From folk medicine to medicinal chemistry via wine sciences*	S10-6
4:30-5:20	**Takashi Yoshida** (Matsuyama University, Japan) *Progress in the chemistry of oligomeric ellagitannins in medicinal plants* TANNIN AWARD LECTURE	S10-7
6:30-9:00	Awards Banquet (The Old Post Office Wine Cellar and Gallery)	

SUNDAY, JUNE 29		
8:00-8:30	Continental Breakfast	
SESSION 11: Natural Product Biosynthesis and Metabolic Engineering		
Chair: Jim Saunders		
8:30-9:15 am	**Patrick S. Covello** (Plant Biotechnology Institute, Canada) *Artemisinin biosynthesis: The next steps*	S11-1
9:15-10:00	**Feng Chen** (University of Tennessee) *Comparative genomic, structural and biochemical study of substrate specificity evolution of the SABATH methyltransferase family*	S11-2
10:00-10:15	Break	
10:15-10:40	**Jack W. Blount** (The Samuel Roberts Noble Foundation, Inc.) *Toward an understanding of C-glycosylation of isoflavonoids in Kudzu* (Pueraria lobata)	S11-3
10:40-11:05	**Aldwin Anterola** (Southern Illinois University) *Oxylipin pathways in the moss* Physcomitrella patens*: Implications for jasmonate evolution*	S11-4
11:05-11:30	**DeYu Xie** (North Carolina State University) *Proanthocyanidin biosynthesis in transgenic tobacco plants: What molecular species is the precursor of extension units?*	S11-5
11:30-11:55	**Daniel G. Vassão** (Washington State University) *The biofuel challenge: New roles for aromatic hydrocarbons?*	S11-6
11:55-12:20	**Mylavarapu Venkatramesh** (Exelixis Plant Sciences) *NaturePOD: A technology platform for the production of Natural Products On Demand using plant cell cultures.*	S11-7
12:20-1:30	Lunch	
	Meeting Adjourns	

THURSDAY POSTER SESSION (STUDENT AND POSTDOCTORAL)

METABOLIC ENGINEERING OF NATURAL PRODUCTS

Patrick A. Arsenault (Worcester Polytechnic Institute) PS1-1
In planta *metabolic engineering of isoprenoids*

F. Omar Holguin (New Mexico State University) PS1-2
Accumulation pattern of methionine rich β-zein protein in Medicago sativa *(alfalfa) and the related model legume* M. truncatula *in relation to their free methionine pools*

NATURAL PRODUCT BIOSYNTHESIS AND BIOCHEMISTRY

Ray Collier (Washington State University) PS1-3
Uncovering the role of ureide transport in whole plant physiology

Jala J. Daniel (East Tennessee State University) PS1-4
Determining secondary product glucosyltransferase expression during Citrus paradisi *growth and development*

Max Feldman (Washington State University) PS1-5
Regulation of sterol biosynthesis in Arabidopis thaliana: *Experimental testing of a first generation kinetic model*

M.V.K.P. Siddhartha (East Tennessee State University) PS1-6
Heterologous expression and elucidation of biochemical function of two putative flavonoid glucosyltransferase clones (PGT2 and PGT3) from Citrus paradisi

Oliver Corea (Washington State University) PS1-7
Towards determining the individual roles of arogenate dehydratase isoforms in phenylalanine biosynthesis

Kehan Sun (Washington State University) PS1-8
Purification and characterization of recombinant mitochondrial and plastidial serine hydroxymethyltransferases from Arabidopsis

Renu Rawat (Washington State University) PS1-9
Flavin nucleotide metabolism: Understanding the biosynthesis and hydrolysis of FMN and FAD in plants

Daniel K. Owens (East Tennessee State University) PS1-10
Identification, recombinant expression, and biochemical characterization of a flavonol 3-O-glucosyltransferase from Citrus paradisi

Yasuhisa Kaminaga (Purdue University) PS1-11
Contribution of CoA ligases to benzenoid biosynthesis in petunia flowers

Lydia Yamaguchi (University of São Paulo, Brazil) PS1-12
Seasonal and circadian variation in biflavonoid biosynthesis in Araucaria angustifolia

Vanessa Herl (Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany) PS1-13
Molecular cloning, functional characterization and structural modeling of a Δ4,5-steroid 5β-reductase, a putative key enzyme in cardenolide biosynthesis, from Arabidopsis thaliana L.

Yang Wenyu (Donald Danforth Plant Science Center, St. Louis, MO) PS1-14
The tocotrienol form of vitamin E in Apiaceae seeds results from the activity of a dicot-type homogentisate geranylgeranyl transferase

STRUCTURE ELUCIDATION / METHODS FOR ANAYLSIS

Naomi Yoshikado (Okayama University) PS1-15
Polyphenolic constituents in gambir

Maho Murasawa (Okayama University) PS1-16
Effect of tannins and related polyphenols on Pseudomonas aeruginosa

Hong Han (University of British Columbia, Canada) PS1-17
Leaf development and cuticular wax composition in Kalanchoe daigremontiana

Mohamed M. Radwan (NCNPR, University of Mississippi) PS1-18
Sorocenol G and H, two new Diels-Alder type adducts from Sorocea muriculata *roots with anti-MRSA activity*

Mohamed M. Radwan (NCNPR, University of Mississippi) PS1-19
New bioactive metabolites from high potency Cannabis sativa

Safwat A. Ahmed (NCNPR, University of Mississippi) PS1-20
Minor oxygenated cannabinoids from high potency Cannabis sativa L.

HERBAL PRODUCTS AND NUTRACEUTICALS

Christina M. Coleman (University of Mississippi) PS1-21
Isolation and identification of antiadhesive urinary metabolites from cranberry juice

Christina M. Coleman (University of Mississippi) PS1-22
Cranberry juice compounds with antiadhesive properties in urine

Dan J. Cuthbertson (Washington State University) PS1-23
High throughput profiling of fruits for phytochemicals related to human health

NATURAL PRODUCT SYNTHESIS

Lukasz M. Kutrzeba (University of Mississippi) PS1-24
Synthesis of Salvinorin B acid, a putative biosynthetic precursor of Salvinorin A

FRIDAY POSTER SESSION (GENERAL)

NATURAL PRODUCT BIOSYNTHESIS

Hironobu Takahashi (Tokushima Bunri University, Japan) PS2-1
Biosynthesis of macrocyclic bisbibenzyl Marchantin A from liverwort Marchantia polymorpha

Glenn W. Turner (Washington State University) PS2-2
Role of the paraveinal mesophyll during vegetative storage protein accumulation and mobilization in soybean leaves

Christopher J. Mau (Washington State University) PS2-3
Taxol biosynthesis: Two new acetyl transferases

XianZhi He (The Samuel Roberts Noble Foundation) PS2-4
Generation and analysis of expressed sequence tags from the root tissues of kudzu (Pueraria lobata)*, a medicinal plant*

Masayuki Fujita (Kagawa University, Japan) PS2-5
Inhibitory interactions of quercetins against dominant glutathione S-transferases in onion bulb

Cecilia McIntosh (East Tennessee State University) PS2-6
Using graph theory models to predict secondary product glucosyltransferase function

Christopher T. Yarnes (Emory & Henry College) PS2-7
Homogenization of phenotypic variation in phenolics within hybrid oaks (Quercus grisea x Q. gambelii)

Feng Chen (University of Tennessee) PS2-8
Two poplar methyl salicylate esterases display comparable biochemical properties but divergent expression patterns

STRUCTURE ELUCIDATION / METHODS FOR ANALYSIS

Jess D. Reed (University of Wisconsin-Madison) PS2-9
Cranberry proanthocyanidin complexes with bovine serum albumin attenuate the bacterial lipopolysaccharide induced expression of iNOS and COX-2 in Raw 264.7 macrophages

Desmond Slade (University of Mississippi) PS2-10
Absolute configuration of cannabichromanone derivatives from high potency Cannabis sativa

Takashi Tanaka (Nagasaki University) PS2-11
Oligomerization of procyanidins by condensation with cinnamaldehyde in cinnamon bark

Warren Grigsby (Scion, New Zealand) PS2-12
Application of solid state NMR relaxation parameters in the characterisation of Pinus radiata *pine bark constituents*

Filippo Imperato (Università della Basilicata) PS2-13
Kaempferol 3-O-(4""-O- acetylrutinoside), a new flavonoid from the fern Dryopteris villarii

Filippo Imperato (Università della Basilicata) PS2-14
Apigenin 7-O-glucoside-4'-acetate, a new flavonoid from the fern Dryopteris villarii

Young-Soo Bae (Kangwon National University, South Korea) PS2-15
Phenolic compounds from the aerial parts of Lespedeza cuneata *G. Don*

Young-Soo Bae (Kangwon National University, South Korea) PS2-16
Antioxidant and anti-inflammatory activities of Acer tegmentosum *bark extracts*

Young-Soo Bae (Kangwon National University, South Korea) PS2-17
Evaluation of biological activities using Acer barbinerve *bark extracts*

Ann Hagerman (Miami University, Ohio) PS2-18
Isolation and characterization of soluble EGCG-protein complexes

Mark Berhow (USDA-ARS NCAUR) PS2-19
Purification of glucosinolates from Camelina sativa

Yoshiaki Amakura (Matsuyama University) PS2-20
Polyphenolics of myrtaceous plants: Eucalyptus globulus *and* Myrtus communis

Jin-Kyu Kim (Oregon State University) PS2-21
New phenolic glucoside from bark of Populus alba × glandulosa

HERBAL PRODUCTS AND NUTRACEUTICALS

Dobroslawa Bialonska (University of Mississippi) PS2-22
Effects of digestion on the bioavailability and bioactivity of punicalagins

Gloria A. Ayoola (University of Lagos, Nigeria) PS2-23
Phytochemical screening and free radical scavenging activity of Allanblackia floribunda *fruits and leaves*

Sergio Madrigal-Carballo (University of Wisconsin-Madison) PS2-24
Antioxidant capacity and polyphenol composition of pomegranate (Punica granatum) *dietary supplements*

NATURAL PRODUCT SYNTHESIS

Jannie Marais and **Vijender Adelli** (University of Mississippi) PS2-25
Synthesis of cranberry proanthocyanidins

Invited Speakers

Suzanne Abrams

Sue Abrams joined the National Research Council in Saskatoon, Saskatchewan, Canada, in 1978, having obtained a PhD in synthetic organic chemistry and postdoctoral experience in carbohydrate chemistry. Initially, as a research associate with a lipid chemist, she developed syntheses of hydroxy fatty acids important in plant waxes. In the late 1980's, she became interested in and has built a research program on the plant hormone abscisic acid. Her lab has studied metabolism of the hormone in plants and developed inhibitors of catabolic enzymes and metabolism-resistant analogs that have potential as plant growth regulators in horticulture and agriculture. Her group has synthesized hundreds of abscisic acid analogs for structure-activity studies and has developed and are employing affinity probes for identification of abscisic acid binding proteins. Recently, they have expanded their focus to develop MS-based techniques using isotopically labeled internal standards for profiling a broad range of plant hormone classes, including their metabolites in plant tissues, for functional genomics programs. Her lab provides plant hormone standards and collaborates with plant researchers in Canada, the U.S. and elsewhere. Dr. Abrams' current position is Principal Research Officer at the Plant Biotechnology Institute in Saskatoon.

J. Thor Arnason

John Arnason is Professor of Biology at the University of Ottawa and Associate Director of the Biopharmaceutical Sciences program. Arnason's interest in biologically active plants began with PDF projects on the ethnopharmacology of the Maya medicinal plants in Central America and photoactivated plant thiophenes with Neil Towers at UBC in Vancouver. His laboratory was established at the University of Ottawa in 1980 and over 50 graduate students have completed theses in phytochemistry related fields since then. He has collaborated widely with Costa Rican, Mexican, W. African and Indonesian colleagues on triterpenoid insect antifeedants and antimalarials from tropical forests. Currently, his group is undertaking ethnopharmacological studies on plants used for mental health by Maya healers and antidiabetic plants of the northern Cree. Arnason has been active in the establishment of Natural Health Products in Canada and has studied many North American species of interest to the herbal industry.

James Kennedy

James Kennedy, PhD, is currently an Associate Professor of wine chemistry in the Department of Food science and Technology at Oregon State University, as well as an adjunct professor in the Department of Horticulture. He has degrees in Chemistry (BS) and Agricultural and Environmental Chemistry (PhD) from the University of California, Davis. Kennedy has conducted research internationally as a postdoctoral research fellow at the University of Adelaide in South Australia and as a Fulbright Scholar at the Université Victor Segalen in Bordeaux, France. His research focus is on grape and wine phenolic chemistry and with a special emphasis on proanthocyanidin chemistry. His work includes research on proanthocyanidin accumulation and modification in the grape berry, extraction during wine production, and modification during wine aging. His research has led to new insights into the role that environment plays in grape proanthocyanidin chemistry as well as an improvement in our understanding of how wine production practices can be used to influence the chemistry of these sensorially important compounds.

Marcia Kieliszewski

Professor Marcia Kieliszewski has a B.A. degree in Psychology (1974) and a Ph.D. in Biochemistry (1989) both from Michigan State University. Professor Derek T.A. Lamport was her research advisor in the Plant Research Laboratory, her dissertation topic entitled 'Isolation and characterization of extensins from the graminaceous monocot Zea mays.' From Michigan State University she joined the Complex Carbohydrate Research Center at the University of Georgia (1992-1995), then the Ohio University Department of Chemistry and Biochemistry as an assistant professor. Professor Kieliszewski's research focuses on the hydroxyproline-rich glycoproteins (HRGPs) of plants, in particular the codes that dictate their extensive post-translational modifications, which includes O-glycosylation and protein inra- and intermolecular cross-linking. Most recently, Dr. Kieliszewski's research deals with the rules for HRGP self-assembly to create wall scaffolds. Her experimental approach involves designing HRGPs using a synthetic gene approach, and then expressing them in plant cells followed by isolation and biochemical characterization. This approach has been useful for elucidation the codes for Hyp glycosylation and tyrosine crosslinking, and hypothetically for tailoring HRGPs to make self-assembling scaffolds that help direct plant morphology.

John Pezzuto

Dr. 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 (Assistant Professor) 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 served as Head (1993-1995) and Interim Head (2000-2001) of the Department of Medicinal Chemistry and Pharmacognosy at the UIC College of Pharmacy, Associate Director of the UIC Cancer Center (1991-1995), and Director of the Program for Collaborative Research in the Pharmaceutical Sciences (1995-1998), the leading research unit affiliated with the UIC College of Pharmacy. He later served as Associate Dean for Research and Graduate Education, UIC College of Pharmacy (1998-2002), Deputy Director of the UIC Cancer Center (2000-2002), and Head of the Department of Medicinal Chemistry and Pharmacognosy (2001-2002). In 2002, Dr. Pezzuto accepted the position of Professor of Medicinal Chemistry and Molecular Pharmacology, and Dean of the College of Pharmacy, Nursing and Health Sciences, at Purdue University, West Lafayette, Indiana (2002-2006). He also served as Chief Scientific Officer for pharmaceutical development at McClure Park, Inc., an affiliate of the Purdue Research Foundation (2004-2006). He is currently Professor and Founding Dean of the College of Pharmacy at the University of Hawaii at Hilo (2006-present). He is an author of over 400 publications, co-inventor of several patents, the editor of three books, member of eleven editorial boards of international journals, the former editor-in-chief of the International Journal of Pharmacognosy (1990-1995), the former editor-in-chief of Combinatorial Chemistry and High Throughput Screening (1996-1997), and the current editor-in-chief of Pharmaceutical Biology. He is a full member of Subcommittee E (cancer epidemiology, prevention and control) of the National Cancer Institute (2003-present). He was the recipient of a Research Career Development Award from the National Cancer Institute (1984-1989) and a Research Fellowship from the Alexander von Humboldt Foundation (1990-1991). During the period of the Humboldt fellowship, he held a research position in the Department of Pharmaceutical Biology at the University of Munich. He has directed the research of numerous doctoral students, postdoctoral associates, and visiting scholars. He was elected Senior University Scholar at UIC in 1999, and UIC Inventor of the Year in 2000. His current research interests are predominately in the areas of biology-driven natural product drug discovery and characterization, with

primary emphasis in the fields of cancer chemotherapy, cancer chemoprevention, malaria, and AIDS. He has been supported by the National Institutes of Health continuously since 1977, and currently serves as the Principal Investigator/Program Director of a Program Project grant in cancer chemoprevention, and Program Leader for an International Collaborative Biodiversity Group. He resides in Hilo, Hawaii, with his wife and three children.

William Plaxton

Bill Plaxton received a BSc in Biochemistry in 1980 from Carleton University (Ottawa, Canada) and a PhD in Biochemistry in 1984 from the same institution. His PhD research under the supervision of Ken Storey focused on the metabolic adaptations of intertidal marine molluscs to environmental anoxia stress. He was awarded a NSERC Post-doctoral Fellowship to conduct research on plant starch metabolism with Jack Preiss (Dept. of Biochem., Michigan State Univ.). Bill began his independent career in 1986 when appointed to the faculty in the Dept. of Biology at Queen's Univ. (Kingston, Canada), and is now a Full Professor in their Plant Science research group. His research focuses on the organization and control of plant glycolysis, the biochemical adaptations of phosphate-starved plants, and enzyme control by reversible phosphorylation. This work integrates classical and modern biochemical, proteomic, and genetic tools to characterize the molecular and functional properties of key enzyme proteins. Systems being currently studied include developing and germinating castor oil seeds, and *Arabidopsis* suspension cell cultures and seedlings. These studies have significant agricultural applications including the: 1) targeted modification of storage oil versus protein levels in oilseeds such as canola or soybean, and 2) development of phosphate-efficient crops, needed to reduce mankind's rampant but inefficient use of non-renewable, unsustainable, and polluting phosphate fertilizers.

Nicholas Provart

Nicholas Provart is an assistant professor of Plant Cyberinfrastructure and Systems Biology in the Department of Cell & Systems Biology at the University of Toronto. He received his Ph.D. from the Department of Biology at the Freie Universität Berlin in 1996 under the supervision of Lothar Willmitzer. After working as a one of the founding scientists at a small plant biotechnology company in Germany for 4 years, he moved to the RNA Dynamics Group at the Torrey Mesa Research Institute of Syngenta in San Diego where he analyzed *Arabidopsis* microarray data. Since 2002 he has been at the University of Toronto, where he set up one of the first online bioinformatic resources for plants in 2003. Currently the Bio-Array Resource at bar.utoronto.ca, comprising tools for coexpression analysis of publicly-available microarray data, cis-element prediction, identifying molecular markers, generating "electronic fluorescent pictographic" (eFP) representations of gene expression, and exploring protein-protein interactions in *Arabidopsis*, is used approximately 40,000 times a month by plant researchers worldwide. Dr. Provart also runs a "wet-lab" for testing in silico-generated hypotheses related to plant abiotic stress response *in vivo*. Dr. Provart is the Director of the Collaborative Graduate Program in Genome Biology & Bioinformatics, co-developer of an Undergraduate Specialist Program in Bioinformatics & Computational Biology at the University of Toronto, and an editor for PLoS One.

Stéphane Quideau

Stéphane Quideau, trained both in synthetic organic chemistry and NMR spectroscopy, received his PhD in Natural Product Chemistry at the University of Wisconsin-Madison (USA) in 1994 under the supervision of Prof. J. Ralph. After a postdoctoral stint at The Pennsylvania State University in State College (USA) under the guidance of Prof. K. S. Feldman, he moved to Texas Tech University in Lubbock (USA) as an Assistant Professor. In 1999, he moved back to France as an Associate Professor at Bordeaux 1 University. He joined the IECB as a Group Leader in 2002. He was nominated as a Junior Member of the University Institute of France (IUF) in 2004. His team received the Young Investigators Award (ATIPE) from the CNRS in 2005, and he was promoted to the rank of Full Professor in 2005. His current fields of interest encompass structural, synthetic and biomechanistic studies of bioactive natural products, including plant polyphenols, terpenoids, alkaloids and angucycline antibiotics, the development of synthetic methodologies based on hypervalent iodine-mediated asymmetric dearomatisation of phenols, and the rational design of antigenic peptidomimetics as immunotherapeutic agents for the development of anticancer vaccines. In 2006, he received the Acros Price from the French Chemical Society and the Dr. and Mme Henri Labbé price from the French Academy of Sciences.

Michel Rohmer

Michel Rohmer was born in Strasbourg (France) in 1948. As a chemical engineer (Ecole Nationale Supérieure de Chimie de Strasbourg, 1970), he completed a PhD thesis with Professsor Guy Ourisson (Université Louis Pasteur, 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 of the Université Louis Pasteur (1974-1979). After post-doctoral work with Professor Carl Djerassi (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 Université Louis Pasteur in Strasbourg.

Michel Rohmer is a specialist of the chemistry and biochemistry of microorganisms and higher plants. He discovered the biohopanoids, a series of pentacyclic triterpenoids, corresponding to the bacterial equivalents of eukaryotic sterols. These biohopanoids are the precursors of a ubiquitous family of molecular fossils, widespread in the organic matter of sediments and widely used as biomarkers in organic geochemistry. Mevalonate was long believed to be the universal precursor of the isoprene units in living organisms. Michel Rohmer’s work on the biosynthesis of bacterial hopanoids disclosed an alternate mevalonate-independent metabolic pathway towards isoprene units. This methylerythritol phosphate pathway represents a major metabolic route, present in most bacteria and in the plastids of phototrophic organisms.

Honours and awards: Vaillant Prize, “Académie des Sciences” (1984); Gold Medal, Wallach Foundation, Mulhouse (1993); French-British Prize, the Royal Society/Académie des Sciences (1993); Humboldt–Gay-Lussac Prize, Alexander von Humboldt Foundation (1997); Member of the “Institut Universitaire de France” (1997), the “Deutsche Akademie der Naturforscher Leopoldina” (2000) and the French “Académie des Sciences” (2003); Nakanishi Prize, Chemical Society of Japan/American Chemical Society (2008); Schroepfer Medal Award, American Oil Chemists Society (2008).

Juha-Pekka Salminen

Juha-Pekka Salminen (born 1969 in Pori, Finland; married, one son) received an MSc in Environmental Chemistry in 1998 from University of Turku (Turku, Finland) and a PhD in Environmental Chemistry in 2002 from the same institution. His PhD research under the supervision of Vladimir Ossipov and Kalevi Pihlaja focused on the isolation, identification and ecological significance of birch leaf hydrolysable tannins. During his PhD work he was one of the first scientists to conduct insect feeding assays with purified, individual hydrolysable tannins. His work was awarded as the best PhD thesis during 2001-2003 in the field of Environmental Sciences, in Finland. In 2003 he was awarded a Post-doctoral Fellowship from the Academy of Finland to clarify the importance of birch leaf phenolics in plant-herbivore interactions with prof. Erkki Haukioja (Dept. Biology, Univ. Turku). Juha-Pekka was appointed in 2005 as the Docent in Chemical Ecology (equals to adjunct professor) in University of Turku. Later he has received major funding from two sources: (1) US Department of Agriculture: ”Patterns of phenolic oxidative activity: potential new traits for improving plant resistance to leaf-feeding insects” (2007 – 2009), in collaboration with Raymond Barbehenn, University of Michigan, USA, and (2) Academy of Finland: “Tannins revisited: linking chemical structure to bioactivity and ecological significance” (2007 – 2012), in collaboration with Tomas Roslin, University of Helsinki, Finland, and Anurag Agrawal, Cornell University, New York, USA. The current research of Dr. Salminen’s group focuses on (a) phenolic chemistry, especially ellagitannins, (b) chemical ecology, mainly plant-herbivore interactions, (c) tannin metabolism in plants and insects, (d) in vitro and in vivo biological activity of phenolics, especially structure-activity relationships, and (e) analytical chemistry (chromatographic techniques and mass spectrometry).

Augustin Scalbert

Augustin Scalbert was born in 1958 in Lille in the North of France. He completed agriculture science studies at the Institut National Agronomique Paris-Grignon from 1978 until graduation in 1981, and joined the Institut National de la Recherche Agronomique (www.inra.fr) the same year to do a Ph.D. (Viva 1984) on phenolic acids in wheat straw under the guidance of Bernard Monties at the Laboratoire de Chimie Biologique in Grignon (INRA).

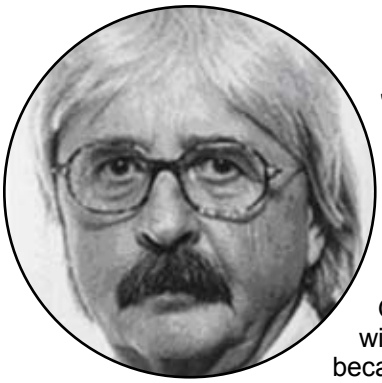
Dr. Scalbert started research on tannins in woods and barks in 1985 in the same laboratory, and a wish to do more biology research encouraged the start of research projects on the nutritional properties of dietary polyphenols in 1995. He moved in September 1999 to the Unité des Maladies Métaboliques et Micronutriments at the INRA Clermont-Ferrand, which became the Unité de Nutrition Humaine on January 1, 2006. He is presently leader of the team “Micronutrients, Metabolism and Biological Signatures” within this laboratory. Research activities aim at elucidating the bioavailability and the role of dietary polyphenols in nutrition and disease prevention, using high-throughput approaches such as functional genomics and metabolomics.

Dr. Scalbert is the author of over 80 original papers on polyphenols in different chemistry, agriculture and nutrition peer-reviewed journals, and was identified in 2005 as a Highly Cited Researcher by the Thompson Corporation (Agriculture Sciences). He is the recipient of the ICPH (International Conference on Polyphenols and Health) Award for Outstanding Flavonoid Research (sponsored by Mars Inc.) in 2007. He is also a member of the Board Committee of the Groupe Polyphénols from 1988 to 1994 and Vice-President from 1992 to 1994, and a former editor of Polyphénols Actualités, the newsletter of the Groupe Polyphénols, and editor of a book entitled Polyphenolic Phenomena published on the occasion of the 20th anniversary of the Groupe Polyphénols. He has organized several international conferences and training courses on polyphenols, including the 1st International Conference on Polyphenols and Health (Vichy, France, 2003). He edited on this occasion a supplement issue (Dietary Polyphenols and Health) published in the American Journal of Clinical Nutrition in 2005.

Dr. Scalbert is an expert or consultant for various public organizations and food industries.

Jacqueline V. Shanks

Jacqueline V. Shanks is professor in chemical engineering at Iowa State University and an adjunct professor in bioengineering at Rice University. She received her B.S. degree in chemical engineering from Iowa State University in 1983 and her Ph.D. degree in chemical engineering from the California Institute of Technology in 1989. She joined the chemical engineering department at Rice University as an assistant professor in 1988 and became associate professor in 1993. She joined the bioengineering department at Rice in 1997, and was promoted to full professor in bioengineering and chemical engineering in 1999. In Fall 1999, she joined the faculty at Iowa State University. Dr. Shanks' research interests include plant metabolic engineering of secondary metabolites, nuclear magnetic resonance spectroscopy (NMR) techniques for metabolic flux analysis, phytoremediation of explosives and related nitroaromatics, and production of valuable products from biorenewable resources. She received the National Science Foundation Young Investigator Award in 1992, and the Professional Progress in Engineering Award from Iowa State University in 1994, and the ISU University Foundation Award for Outstanding Achievement in Research in 2005. She was elected as Fellow to the American Institute of Medical and Biological Engineers in 2000. She was a member of the National Research Council Committee on Biobased Industrial Products. In the American Chemical Society, Dr. Shanks is a member of the Biochemical Technology (BIOT) and Environmental Chemistry (ENVI) Divisions. In BIOT, she served as Chair, Chair-Elect, and Past-Chair from 2000 - 2002, Alternate Councilor from 1995 - 1997, and Newsletter Editor from 1998 – 2000. She received the Van Lanen Award for service in the BIOT division in 2004. She served as Co-editor for the year 2000 of the Biochemical Engineering section of Current Opinion in Biotechnology, and as Co-editor for a 2002 issue of Metabolic Engineering devoted to Plant Metabolic Engineering. She is a member of the Editorial Advisory Board for Biotechnology Progress.



Joachim Stöckigt

Joachim Stöckigt received his Diploma (Master) in Organic Chemistry (Phosphor Chemistry) from Kiel University (Germany) , and a PhD from the Faculty of Chemistry (Münster University) on "Total Synthesis of Ergochromes from Ergot" in 1971. He has worked with Professor Meinhart H. Zenk from 1971 – 1980 at the Institute of Plant Physiology at the Faculty of Biology (Bochum University, Germany) ; in 1977/1978 with Professor A.I. Scott (Texas A & M University) and with Professor H. G. Floss at Purdue University, Faculty of Pharmacy. In 1979 he became a "Dozent" at Faculty of Biology which allowed him to establish own research and teaching at Bochum University. From 1980 – 1990 he worked at the Faculty of Chemistry and Pharmacy (Munich University) and was teaching in 1987 at Heidelberg University (Institute of Pharmacy) . In 1988 he received the "Tate and Lyle Award" of the Phytochemical Society of Europe.He became a Full Professor in 1980 at the Institute of Pharmacy (Mainz University) in the field of Pharmaceutical Biology (Pharmacognosy) and retires in 2008 from Mainz University. From 2004 – 2006 he was visiting Professor and from 2006 – 2008 Chair Professor at the College of Pharmaceutical Sciences (Zhejiang University, Hangzhou, P.R.China).

In the past his research interests were focussed on the chemistry of plant natural products, on the phytochemical characterisation of plant cell , tissue and organ culture systems, on high field in vivo NMR of alkaloid transformation, cell-free biosynthesis of lignin precursors, of eugenol, biosynthesis of chlorogenic acid, cellulose and alkaloids, especially monoterpene-derived indole alkaloids from Catharanthus roseus and from the Indian medicinal plant Rauvolfia serpentina including enzymology, molecular biology and in recent years enzyme crystallization and 3D-analysis.

John Wyrick

John Wyrick received a Ph.D. in biology from the Massachusetts Institute of Technology in 2002. As a graduate student under the direction of Dr. Richard Young, Dr. Wyrick developed and used DNA microarray technology to investigate how transcription factors regulate the expression of the yeast genome. He then worked as a postdoctoral scholar in Dr. Elliot Meyerowitz's laboratory at the California Institute of Technology. There he developed computational and bioinformatics tools to gain insight into transcriptional regulation in plants. Dr. Wyrick joined Washington State University as an Assistant Professor in the School of Molecular Biosciences in 2002. He is also a member of the Molecular Plant Sciences graduate program. Dr. Wyrick's research employs functional genomic tools to investigate the links between chromatin structure and transcription regulation. His lab also works to develop bioinformatics tools to study the transcriptional and post-transcriptional regulation of gene expression in the model plants Arabidopsis thaliana and Oryza sativa.

Takashi Yoshida

Takashi Yoshida, Prof. Emeritus of Okayama University, was born in1939 in Japan. He received degrees in Natural Products Chemistry from Faculty of Pharmaceutical Sciences, Kyoto University in Japan [M.Sc. in 1964; Ph.D. in 1969]. He was appointed as Research Associate, 1965-1970 in the same University and then as Associate Professor, 1970-1993, Professor, 1993-2005 in Faculty of Pharmaceutical Sciences, Okayama University. Since his retirement from Okayama University in March 2005, he is a Professor of Pharmacognosy at College of Pharmaceutical Sciences, Matsuyama University. He experienced postdoctoral research at the University of Alberta, Canada in 1977-1978. His research interests are on the isolation, characterization, and biological evaluation of natural products, particularly polyphenols classified as tannins in medicinal plants, foods and beverages, and he is a co-author of about 350 articles including book chapters and reviews.

Arthur C. Neish Young Investigator Award Recipient

Nancy Terrier

Nancy Terrier received an Engineer's Degree in microbiology from Ecole Nationale Supérieure Agronomique de Montpellier (Montpellier, France) in 1994 and a Ph.D. in Food Science in 1997 from the same institution. Her Ph.D. was completed under the supervision of Charles Romieu (Grape Integrative Biology, INRA National Institute of Agronomical Research, Montpellier, France) and dealt with biochemical and molecular aspects of grape acidity. She was recruited into the lab as a young scientist in 1998 to develop transcriptomic tools (EST sequencing, construction and use of oligoarrays), which were first used to study general features of grape berry development. Since 2004, Nancy has worked in the team of Véronique Cheynier (Polyphenols and Interactions team, INRA, Montpellier) and used these transcriptomic approaches in combination with metabolomics, genetics and plant transformation strategies to study the biosynthesis of flavonoid and more especially proanthocyanidins. The goal of this project is to identify new actors involved in this metabolic pathway and the mechanisms of their regulation in grape berry.

Session Abstracts

S1-1
Natural product cancer chemopreventive agents

John M. Pezzuto,[1,2,4] Ching-jer Chang,[2] Bruce A. Craig,[2] Mark Cushman,[2] William H. Fenical,[3] Harry H.S. Fong,[4] Andrew Mesecar,[4] Richard C. Moon,[1] and Richard B. van Breemen[4]

[1]School of Pharmacy, University of Hawaii at Hilo, HI; [2]Dept. of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN; [3]Scripps Institution of Oceanography, University of California, San Diego, CA; and [4]Dept. of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, IL

Natural products are of major importance in the field of cancer chemoprevention. Prominent examples include sulforaphane (cruciferous vegetables), epigallocatechin-3-gallate (green tea), curcumin (turmeric), resveratrol (grapes), and lycopene (tomatoes). For the discovery of novel natural chemopreventive agents, one approach is to evaluate crude natural products, such as plant extracts, and isolate active principles. Following biological activity, standard methods of fractionation can be employed. If a suitable receptor or target enzyme is available, the more efficient process of ultrafiltration-mass spectrometry can be used. Although edible and nonedible terrestrial plants have yielded many interesting leads, a new and exciting area of research involves exploring the biodiversity provided by microbes of the marine environment. Using a novel battery of assays, such as interaction with RxR or Keap1, and inhibition of quinone reductase 2 or NF-κB, many active leads have been identified. As a result, we are confident that a variety of novel substances of marine origin will be discovered. It is now well established that cancer chemoprevention is a viable strategy in the fight against cancer. The current armamentarium of agents has resulted largely from epidemiological observations, off-shoots of cancer therapeutic agents, or agents that were used for other therapeutic indications. With concerted effort involving a range of expertise, it is clear that new natural product chemopreventive agents with clinical potential can be uncovered using a systematic approach of drug discovery. (Supported by program project P01 CA48112 awarded by the National Cancer Institute.)

S1-2
Biologically active molecules from neotropical plants

John T. Arnason,[1] Rose Awad,[1] Tony Durst,[1] Mohamed Asim,[1] Pablo Sanchez-Vindas,[2] Luis Poveda,[2] and Victor Cal[3]

[1]Centre for Research in Biopharmaceuticals, University of Ottawa, Canada; [2]Herbario JVR, Universidad Nacional, Costa Rica; and [3]BITI, Toledo District, Belize

Our phytochemical discovery program has used a search strategy focusing on rare plants families and quantitative ethnobotany to identify neotropical plants with useful biological activities and novel phytochemicals. Study of rare families such as the Lepidobotryaceae, Canellaceae and Marcgraviaceae led to the isolation of biosynthetically unusual spiro-triterpenes with insecticidal properties, new sesquiterpenes with activity against drug resistant fungi and triterpenes with anxiolytic properties. Our ethnobotany studies in collaboration with Q'eqchi' Maya healers in Belize have identified a very large group of plants traditionally used to treat related neurological conditions such as anxiety, epilepsy, fear and depression. In the Q'eqchi' Maya ethnobotany, the frequency of traditional use of epileptic plants was found to be a significant indicator of plants active as GABA-T inhibitors, a known target for drugs treating this condition. As well, the use of plants for treatment of the culture bound syndrome, "susto" was found to be a predictor of GABAa binding activity. Some of the active principles of these plants will be discussed. These recent findings show the continuing potential of tropical plants for many new useful applications, the need to conserve biodiversity, and the importance of considering its use for local benefit.

S1-3

Polyphenols and human health: New tools to characterize intake of the large diversity of polyphenols at the population level

Augustin Scalbert, Claudine Manach, Vanessa Neveu, and Femke Vos

INRA, Centre de Clermont-Ferrand/Theix, Unité Nutrition Humaine, St Genès Champanelle, France

Polyphenols are the most abundant antioxidants in our diet and are thought to protect against a variety of chronic diseases such as cardiovascular diseases or cancers. However, the nature of the most protective polyphenols remains largely unknown. Both human intervention and epidemiological studies have dealt with a limited number of phenolic compounds due notably to the lack of tools to characterize their intake in populations. Most information on food content of the many polyphenols is scattered in a large number of publications, making it difficult to evaluate global intake. We have constructed a comprehensive database on polyphenol content in foods based on the analysis of the scientific literature. It includes over 40,000 food content values for more than 500 phenolic compounds. These data have been evaluated and aggregated to produce a food composition table which will allow the estimation of dietary intake in a population.

We also applied mass spectrometry-based metabolomic approaches to identify new polyphenol metabolites and to characterize the food metabolome, i.e. all metabolites originating from the digestion of food and recovered in biological fluids such as urine. Polyphenol and other phytochemical metabolites appear to be key features of this food metabolome. Their estimation in urine samples collected in a cohort of 154 human subjects shows that they can effectively be used as markers of food intake. These new tools will be essential to evaluate health benefits and risks of phytochemical consumption.

S1-4

Xanthohumol and related prenylated flavonoids from hops (*Humulus lupulus*): Beer, dietary supplements, and potential health benefits

Jan Frederik Stevens

College of Pharmacy and the Linus Pauling Institute, Oregon State University, Corvallis, OR

Our research group has identified some twenty prenylated chalcones and flavanones in the flowers of the female hop plant, *Humulus lupulus*. We have detected six prenylflavonoids in a variety of beers at concentrations of up to 4 mg/l. Xanthohumol (XN), the principal prenylflavonoid of hops, has received much attention in recent years due to its biological properties with relevance to human health. It has been shown by us and later by other groups that XN is a potent inducer of phase-2 enzymes that play a role in the detoxification of carcinogens. In cell culture models, XN exhibits anti-inflammatory effects through inhibition of NFκB activation, and it induces apoptosis in prostate cancer cells.

Several XN-containing dietary supplements have appeared on the market in recent years to ‘promote general health’ and to ‘fight metabolic stress.’ We have conducted a study of the pharmaco-kinetics of XN in four humans taking a single oral dose of XN. We found that XN is poorly absorbed, undergoes enterohepatic recirculation, and is (metabolically) converted into isoxanthohumol, 8-prenylnaringenin, 6-prenylnaringenin, and their glucuronides. The urinary recovery of XN and its metabolites was 1.3% of an oral dose of 10 mg XN, which showed a half-life of 32 hours. Many of the biological properties of XN can be explained by its ability to form Michael-type adducts with cellular proteins, leading to altered gene expression and protein function.

S1-5

Prooxidant activity of proanthocyanidins from hops (*Humulus lupulus*) causes protein carbonylation and cytoskeletal derangement in colon cancer cells

Woon-Gye Chung,[1] Cristobal L. Miranda,[1] Jan F. Stevens[2] and Claudia S. Maier[1]

[1]Department of Chemistry and [2]Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR

Proanthocyanidins (PCs) from various plant sources have been shown to suppress growth and induce apoptosis in diverse human cancer cells. We have used a mass spectrometry-based proteomics approach to assess the prooxidant activity of PCs from hops (*Humulus lupulus*) in human colon cancer HT-29 cells. Using one- and two-dimensional electrophoresis in combination with tandem mass spectrometry, we were able to identify protein targets of PC-induced oxidative stress response. As major targets of aldehydic lipid peroxidation products, β-actin and protein disulfide isomerase (PDIA3) were identified. Adduction of β-actin was demonstrated by the use of anti-acrolein monoclonal antibody and by anti-β-actin monoclonal antibody in Western blot analysis. PDIA3 was also detected as a target of acrolein modification by immunostaining. The proteomic analysis was complemented by measurements of cell viability, caspase-3 activity, and fluorescence microscopy for detection of ROS formation and impact of carbonylation on cytoskeleton derangement. Carbonylation of β-actin was associated with disorganization of the cytoskeleton as shown by the appearance of actin clusters in PC-treated cells. These findings suggest that hop PCs, at 50 or 100 μg/mL, induce cytotoxicity in human colon cancer cells by acting as a prooxidant leading to β-actin carbonylation and disruption of the actin cytoskeletal structure.

S1-6

Potato phytonutrient analysis and engineering

Roy Navarre,[1,2] Roshani Shakya,[2] Aymeric Goyer,[2] Joanne Holden,[1,2] Lavanya Reddivari,[3] and J. Creighton Miller[3]

[1]USDA-ARS, Prosser, WA; [2]IAREC, Washington State University, Prosser, WA; and [3]Dept. of Horticultural Sciences, Texas A & M University, College Station, TX

Potatoes have the highest per capita consumption of any vegetable, a fact which emphasizes their potential to be a key dietary source of health-promoting compounds. Only a fraction of the genetic diversity available in potato wild-species has been incorporated into modern cultivars. LCMS analysis of methanolic extracts from diverse potato germplasm was used to better understand the extent of qualitative and quantitative phytonutrient variation among potatoes. Glycoalkaloids (GAs) were one major source of diversity and ~100 different GAs were found in LCMS analysis of tuber extracts from only 7 genotypes, substantially more than expected based on the literature. Other compounds in which substantial variation was found included phenolic acids, flavonols and polyamines. Extracts from some genotypes were found to have anticancer properties when assayed against LNCaP (androgen-dependent) and PC-3 (androgen-independent) prostate cancer cells.

Genotypes with total phenolic content exceeding 10 mg/g DW were identified. Chlorogenic acid (CGA) is the most abundant tuber phenolic, has numerous health-promoting properties, and can comprise over 90% of a tuber's total phenolic content. RNAi is being used to explore the effect on tuber phenolic content of silencing hydroxycinnamoyl CoA quinate hydroxycinnamoyl transferase, which synthesizes CGA. Phytonutrient concentration was found to vary during tuber development, with younger tubers having greater concentrations of many compounds, including CGA.

The folate content of over 70 genotypes was examined and about a 3-fold range found. Overexpression of two genes involved in folate synthesis is being used in an attempt to increase folate in potatoes.

S2-1

Extensin scaffolds and cell wall assembly

Marcia J. Kieliszewski,[1] Maura C. Cannon,[2] Liwei Chen,[1] Li Tan,[1] Ben Wegenhart,[1] and Kimberly Terneus[1]

[1]Dept. of Chemistry and Biochemistry, Ohio University, Athens, OH and [2]Dept. of Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA

Cytokinesis partitions the cell by building a new cross wall in plants. How this new wall assembles at the molecular level and connects with the mother cell wall remains unclear. A lethal *Arabidopsis* embryogenesis mutant designated *root-, shoot-, hypocotyl-defective* (*rsh)* provides some clues. *RSH* encodes extensin AtEXT3, a structural glycoprotein located in the nascent cross wall or 'cell plate', cell corners, and in mature root cell walls. Electron micrographs of *rsh* mutant cells lacking RSH extensin correspond to a wall phenotype typified by incomplete cross wall assembly. Biochemical characterization of the purified glycoprotein confirmed its identity as AtEXT3, a (hydroxy)proline-rich glycoprotein (HRGP) composed of amphiphilic peptide repeats with a 28-residue periodicity: **SOOOOKKHYVYKSOOOOVKHYSOOOVYH** (O = Hyp), each repeat containing a hydrophobic isodityrosine crosslink motif (underlined). Atomic force microscopy of RSH glycoprotein imaged its propensity for self-assembly into a dendritic scaffold. Extensin peroxidase catalyzed *in vitro* formation of insoluble RSH with concomitant tyrosine crosslinking, hence this likelihood *in muro*. We suggest that self-assembling, lysine-rich extensins form positively charged scaffolds in the cell plate that react with negatively charged pectin thereby templating further orderly deposition of the new cross wall at cytokinesis.

S2-2

Analysis of *ACYL-CoA Synthetase 5* and co-expressed genes reveals a novel pathway required for pollen wall formation

SungSoo Kim, Clarice de Azevedo Souza, Michael Friedmann, Sarah McKim, George Haughn, and Carl J. Douglas

Department of Botany, University of British Columbia, Vancouver BC, Canada

Many genes of unknown specific function have been annotated in plant genome sequencing projects, for example, a family of twelve 4CL-related Arabidopsis *Acyl-CoA Synthetase* (*ACS*) genes. We identified all members of this *ACS* gene family in the fully sequenced poplar, rice, and *Physcomitrella* genomes. Phylogenetic analysis revealed an *ACS* clade that contains a single gene from each genome. Expression analyses revealed that *Arabidopsis* and poplar members of this clade (*AtACS5* and *PoptrACS13*) are flower and anther-preferred in expression. *AtACS5* is transiently and exclusively expressed in tapetum cells in the anther with maximal expression just after release of microspores from tetrads in concert with the onset of sporopollenin deposition, suggesting a potential role in pollen wall and/or sporopollenin formation. Expression of *PoptrACS13* is male flower preferred. An *ACS5* mutant is completely male sterile, fails to produce pollen grains, and pollen grains arrested in development are devoid of sporopollenin. These data suggest that *AtASC5* and similar enzymes from other species produce CoA ester intermediates used in an ancient pathway required for the biosynthesis of the sporopollenin polyester backbone. *In silico* co-expression analysis identified *Arabidopsis* genes encoding other potential enzymes and transporters in this pathway, and homologues were found in poplar, rice, *Physcomitrella*, and other plants. Reverse genetic analysis of selected co-expressed genes revealed that mutants in these genes are also compromised in male fertility and sporopollenin deposition. This work has illuminated the outlines of a novel biosynthetic pathway or pathways likely involved in generating monomer constituents of the sporopollenin polyester component of the pollen wall. We have generated working hypotheses regarding the nature of such pathways, which can now be experimentally tested.

S2-3
Soybean root suberin and resistance to *Phytophthora sojae*

Kosala Ranathunge,[1] Raymond H. Thomas,[2] Xingxiao Fang,[1] Carol A. Peterson,[1] Mark Gijzen,[3] and Mark A. Bernards[2]

[1]Department of Biology, University of Waterloo, ON, [2]Department of Biology, University of Western Ontario, London, ON, and [3]SCPFRC, Agriculture and Agri-Food Canada, London, ON, Canada

The cultivation of soybean (*Glycine max* L.) is hampered by the oomycete root pathogen *Phytophthora sojae* wherever the crop is grown. Gene-for-gene resistance to specific races of *P. sojae* has been bred into some cultivars; however, the main method for achieving field-level control is through quantitative trait loci (QTL)-mediated (i.e., horizontal) resistance. Typically, the root is the primary site of infection by *P. sojae*, and we have examined suberization in soybean roots to determine whether this natural physico/chemical barrier has any bearing on defense against root pathogens. Chemical analysis of isolated soybean epidermis and endodermis tissues demonstrated increased amounts of suberin in (1) the epidermis and adjacent cortical tissue along the root axis and (2) the endodermis as roots matured. Significantly higher amounts of suberin (P=0.05) were found in the roots of cv 'Conrad', which shows a high level of field resistance to *P. sojae*, than in those of the susceptible line 'OX760-6'. At the whole root level, a strong negative correlation between the amount of aliphatic suberin and field mortality was observed for nine independent cultivars (r = -0.89) as well as 32 recombinant inbred lines (r = -0.87) derived from Conrad and OX760-6. A time course of infection supports the hypothesis that it is the amount of pre-infection aliphatic suberin in the epidermal walls and middle lamellae that mediates field-level resistance of soybean to *P. sojae*. This idea could have broad implications for disease control as the epidermal walls of many species contain suberin.

S2-4
Advances in plant cell wall microscopy and molecular analyses

Ann M. Patten, Laurence B. Davin, and Norman G. Lewis

Institute of Biological Chemistry, Washington State University, Pullman, WA

Recent advances in imaging and localization of mRNA, proteins, and metabolites by microscopic methods are allowing for a much increased understanding of plant cell wall formation and structure. For instance, the novel application of *in situ* mRNA hybridization has lent insight on the formation of heartwood, the major structural component in western red cedar, including gene transcription of the (+) pinoresinol-forming dirigent protein, which is important in polyphenolic regio- and stereochemical specificity during heartwood lignan biosynthesis. More recently, laser microdissection has also afforded precision isolation of specific cell types for downstream analyses of RNA, protein, and metabolite compositions, which is providing novel insights into cell wall assembly. Altered cell wall anatomy and structure, such as that found in reaction wood and/or genetically-modified plant lines, can additionally now be investigated by correlation of established histochemical methods with more advanced anatomy imaging techniques such as via environmental scanning electron microscopy of fresh tissues. These advances are presented and placed in context with the current and impending global energy crises, which have prompted international research efforts to refocus on finding renewable, sustainable sources of biofuel. A major consideration currently being given is that of how lignified (lignocellulosic) plant cell walls are formed, whose efficient utilization potentially represents an attractive solution to the current energy crisis.

S4-1
Metabolic biochemistry helps to close the gap in plant functional genomics

William C. Plaxton

Depts. of Biology and Biochemistry, Queen's University, Kingston, Ontario, Canada

Remarkable insights into plant metabolism are being provided by the ever-growing collections of plant genes and bioinformatic databases, as well as by the implementation of high-throughput transcriptomic and mutant/transgenic studies. Although genomics provides a crucial blueprint and a host of powerful tools for systematic studies of metabolism, it also reveals that plant metabolism is incredibly complex and poorly understood. Up to 50% of plant genes encode unknown enzymes, whereas many annotated genes encode multiple isozymes having undefined individual properties and roles. A thorough understanding of tissue-specific plant metabolism and its control is highly relevant to the optimal application of biotechnology for improving plants via metabolic engineering. Thus, genomics should ideally be well integrated with parallel studies of the corresponding proteome, metabolome, *in vivo* metabolic fluxes, and membrane transporters/metabolic compartmentation, together with painstaking biochemical characterization of individual native enzymes. Although under-represented in current plant science research, native enzyme biochemistry helps to establish: 1) gene function, 2) enzyme physical, immunological, and kinetic/regulatory properties, 3) enzyme:enzyme interactions that may prevail *in vivo*, 4) enzyme transit/signal peptides and targeting, 5) pivotal post-translational enzyme modifications such as phosphorylation, ubiquination, or glycosylation, and 6) metabolic control. The many roles that enzyme biochemistry can play in 'helping to close the gap in functional genomics' will be illustrated using examples from our recent research. This concerns some interesting discoveries that pertain to novel phosphorylated or monoubiquinated phospho*enol*pyruvate carboxylase isoforms of developing *versus* germinated castor oil seeds, and a phosphate-scavenging purple acid phosphatase isozyme that is upregulated and differentially glycosylated by phosphate-starved *Arabidopsis thaliana*.

S4-2
Studies on ABA metabolism

Suzanne R. Abrams, Jason Boyd, Adrian Cutler, Pierre Fobert, Michele Loewen, Ken Nelson, Stacey Owen, Nirmala Sharma, David Taylor, and Irina Zaharia

Plant Biotechnology Institute, National Research Council Canada, Saskatoon, Canada

Abscisic acid (ABA) is a carotenoid-derived, monocyclic, sesquiterpenoid plant hormone that plays important roles in plants, influencing their growth, development and response to abiotic stresses. For example, transpiration, deposition of storage reserves in developing seeds, maturation of fruit, control of flowering and seed germination are mediated by ABA. Our studies are aimed at understanding the control of ABA levels in plant tissues and the biological activity of compounds in the ABA pathway. Work to develop inhibitors of ABA biosynthesis targeting the key enzyme, 9-cis-epoxycarotenoid cleavage dioxygenase, will be described, as well as ABA catabolism research, using specifically deuterated ABA analogs to uncover and study competing catabolic processes. Examples of hormone profiling of ABA catabolites for functional genomics research will be presented.

S4-3
AAE13 (At3g16170) encodes a malonyl-CoA ligase in *Arabidopsis*

Hui Chen, Hyun Uk Kim and John Browse

Institute of Biological Chemistry, Washington State University, Pullman, WA

Malonyl CoA is the precursor for fatty acid synthesis and elongation. It is also essential for the biosynthesis of phytoalexins, flavonoids and malonylated compounds. It has been widely accepted that, in plants, acetyl-CoA carboxylase (EC 6.4.1.2) is the sole source for malonyl-CoA biosynthesis. However, previous studies have suggested that malonyl-CoA might also be made directly from malonic acid by malonyl-CoA ligase (malonyl-CoA synthetase) (EC 6.2.1.14). Here, we report the cloning of the first malonyl-CoA ligase gene, *AAE13* in *Arabidopsis*, from the plant kingdom. When expressed in *E. coli*, the *AAE13* protein shows very high activity on malonic acid. *AAE13* is very specific toward malonic acid, having only ca. 10% activity on 2-methylmalonic acid and no activity on other dicarboxylic acids. Also, it has no activity on short-, medium- and long- chain carboxylic acids ranging from acetic acid to octadecanoic acid. We also measured *AAE13* activity in extracts from different *Arabidopsis* tissues and found the highest activity in the flowers, which is consistent with the highest expression of *AAE13* gene in this tissue. Using GC/MS, we identified low levels of malonic acid in flowers of wild-type *Arabidopsis*. However, in the flowers of transgenic *Arabidopsis* overexpressing the *AAE13* gene under the driven of 35S promoter, malonic acid was undetectable. When *Arabidopsis* was geminated and grown on agar plates supplemented with different concentrations of malonic acid, the growth of seedlings was significantly inhibited by malonic acid at higher concentrations. This is because malonic acid is a competitive inhibitor of succinic acid dehydrogenase. Taken together, our *in vitro* and *in vivo* experiments indicate that *AAE13* is a malonyl-CoA ligase in *Arabidopsis*.

This research is supported by NSF Arabidopsis 2010 project grant No. 0420199.

S4-4
Biosynthesis and bioengineering of proanthocyanidins in model legume *Medicago truncatula*

Yongzhen Pang, Gregory J. Peel, Shashi B. Sharma, Yuhong Tang, and Richard A. Dixon

Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK

Proanthocyanidins (PAs) possess benefits for human health and are important quality factors for forage crops. In the model legume *Medicago truncatula*, PAs are composed primarily of epicatechin units and accumulate in the seed coat, reaching maximal levels at around 20 days after pollination and the highest mean degree of polymerization after seed maturity. Levels of transcripts encoding PA pathway enzymes such as anthocyanidin reductase (ANR), which is responsible for the conversion of anthocyanidin to (-)-epicatechin, parallel the accumulation of PAs in developing seed. By expressing the *Arabidopsis* TRANSPARENT TESTA 2 (TT2) MYB family transcription factor, we obtained *M. truncatula* hairy roots that accumulate massive amounts of PAs. Microarray analysis of genes induced by TT2 in hairy roots revealed more than four hundred-fold induction of ANR, along with induction of other flavonoid pathway genes and a large number of genes of unknown function. We performed a second microarray analysis to identify genes that were preferentially expressed in the *M. truncatula* seed coat. Comparison of the two datasets defines target genes for as yet unidentified steps in PA biosynthesis and accumulation. A novel glycosyltransferase, UGT72L1, discovered from those target genes, is active specifically toward the PA precursor (-)-epicatechin, and exhibits an expression pattern in developing seeds that correlates with the presence of epicatechin glucoside and the accumulation of PAs. We propose that UGT72L1 is associated with the production of epicatechin glucoside in the seed coat as a key step in PA biosynthesis.

S5-1
Bioinformatics tools for investigating the regulation of plant gene expression

Robert T. Morris,[1] Timothy R. O'Connor,[1] Andrew J. Crofts,[2] Kelly A. Poliquin,[2] Thomas W. Okita,[2] and John J. Wyrick[1]

[1]School of Molecular Biosciences and [2]Institute of Biological Chemistry, Washington State University, Pullman, WA

In eukaryotes, gene expression is controlled by both transcriptional and post-transcriptional regulatory networks. To facilitate the study of these regulatory networks in plants, we have developed three bioinformatics tools: Athena, Osiris, and RiceRBP. Athena is a promoter database for *Arabidopsis thaliana* that contains promoter sequences for essentially all predicted genes and consensus sequences for 105 previously characterized transcription factor (TF) binding sites. Athena provides promoter analysis tools, including a visualization tool to display key regulatory elements in user-selected promoter sequences and a data-mining tool to identify promoter sequences that contain specific combinations of TF binding sites. Osiris provides a similar promoter resource for rice (*Oryza sativa L.*). Osiris contains predicted promoter sequences for all annotated rice genes and 100 TF consensus binding sequences. In addition to promoter visualization and mining tools, Osiris provides tools to visualize gene expression data for user-selected genes. Finally, the RiceRBP database provides a resource for studying post-transcriptional regulatory mechanisms in rice. The RiceRBP database contains 197 proteins experimentally identified by mass spectrometry analysis of purified RNA binding proteins from developing rice seeds. RiceRBP includes information about the localization, function, and sequence homologues of these RNA binding proteins.

S5-2
Raising the BAR for *Arabidopsis* research: Using large-scale data sets for hypothesis generation

Nicholas J. Provart

Dept. of Cell and Systems Biology, University of Toronto, Canada

We have entered the post-genomic era, where technological advances have made the generation of data about the levels and states of all biological molecules – transcripts, proteins, metabolites – in a cell or organism increasingly high-throughput and cost-effective. These data can provide a wealth of information to lab-based researchers if the data are "mined" appropriately. The complex cellular functions of an organism frequently rely on physical interactions between proteins. A map of all protein-protein interactions, an interactome, is thus an invaluable tool. I will present an interactome for *Arabidopsis thaliana* predicted from interacting orthologs in yeast, worm, fruit fly and human (joint work with Matt Geisler at Southern Illinois University and A. Harvey Millar at University of Western Australia). The *in silico* validation of these predicted interologs was established via expression and subcellular localization data. There was significant co-expression of genes whose proteins were predicted to interact. As well, interacting proteins were also significantly more likely to be found within the same subcellular location and significantly less likely to be found in conflicting localizations than randomly paired proteins. These predictions can aid researchers by extending known complexes and pathways with candidate proteins. Similarly, the ability to perform so-called "electronic Northerns," i.e. querying expression data sets with a gene of interest to see how it is responding across all treatments in the database, has proved to be of enormous utility, especially in the context of non-redundancy within gene families. In this vein, the international AtGenExpress Project and individual researchers have generated gene expression data sets from representative experiments in *Arabidopsis* and have made them available to the community. We have developed tools, available as part of the Bio-Array Resource at http://bar.utoronto.ca for exploring these and other data, to allow deeper insights into biological questions and to help guide lab-based research.

S6-1

Regulation of mint essential oil biosynthesis: A second generation mathematical model explains environmental and genotypic variation

Rigoberto Rios-Estepa,[1,2] Iris Lange,[1] Glenn W. Turner,[1] James M. Lee,[2] and Bernd M. Lange[1]

[1]Institute of Biological Chemistry and [2]School of Chemical Engineering and Bioengineering, Washington State University, Pullman, WA

We have previously generated and tested a kinetic mathematical model for essential oil biosynthesis in peppermint. The inputs for our peppermint model were developmental profiles of gene expression, enzyme concentration, enzyme activity, metabolite levels, the numbers of the specialized cells producing essential oils as variables, and kinetic properties of enzymes as static parameters. Our model predicted that, when plants were grown at low light intensities, the branch-point enzyme (+)-pulegone reductase was affected by (+)-menthofuran, a stress-induced side product, which acted as a weak competitive inhibitor of (+)-pulegone, the primary substrate for this enzyme. Further experiments with recombinant (+)-pulegone reductase demonstrated that this model prediction was correct, thus indicating the power of integrating mathematical modeling and experimentation. Here we present a second generation model to describe changes in essential oil profiles under various environmental conditions and in different transgenic lines. Model adjustments include experimentally-determined variations in the number of glandular trichomes (specialized essential oil-producing structures on leaf surfaces), the amount of essential oil stored per trichome, the distribution of glandular trichomes at different stages of leaf development, and the modulation of gene expression patterns in transgenic lines with modified essential oil composition. The implications of these findings for transgenic approaches aimed at improving essential oil yield and composition are discussed.

S6-2

cDNA library of *Salvia divinorum* glands as a molecular tool for studying biosynthetic pathway of salvinorin A

Lukasz M. Kutrzeba,[1] Jordan K. Zjawiony,[1,2] Eric McDowell,[4] Anne Descour,[4] Carol A. Soderlund,[4] David R. Gang,[4] and Franck E. Dayan[3]

[1]Department of Pharmacognosy, University of Mississippi, University, MS; [2]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, University, MS; [3]USDA-Natural Products Utilization Research Unit, University, MS; and [4]Department of Plant Sciences and BIO5 Institute, University of Arizona, Tucson, AZ

Salvinorin A, a potent and selective κOR agonist, is an active constituent *Salvia divinorum*, a hallucinogenic plant from the Mexican region of Oaxaca. We have recently shown that salvinorin A, a diterpenoid, is derived from the MEP pathway. We also proposed that its biosynthesis takes place in glandular trichomes. Here we report isolation of glandular trichomes from *S. divinorum* using an optimized bead beater method and the subsequent extraction of RNA from isolated glands yielding sufficient RNA for construction of a cDNA library, which was then used for 454 sequencing to produce an EST database representing genes expressed in the trichomes. This database was used to identify candidate genes coding for several enzymes potentially involved in the biosynthesis of salvinorin A. Our interest is focused on identification of the carboxyl methyltransferase and acetyl transferase that catalyze the steps most important for formation of salvinorin A. Preliminary results from cloning of full length cDNAs, heterologous expression of the respective recombinant proteins in *E. coli*, and the synthesis of putative substrates for the enzymes will be presented.

S6-3

The effect of different organic farming methods on the phenolic composition of sea buckthorn berries

Merja Heinäaho,[1] Ann E. Hagerman,[2] and Riita Julkunen-Tiito[3,4]

[1]Faculty of Biosciences, University of Joensuu, Finland: Present address: Lehtokatu 2, FIN-38700 Kankaanpää, Finland; [2]Department of Chemistry and Biochemistry, University of Miami, Oxford, OH, USA; [3]Faculty of Biosciences, University of Joensuu, Finland; and [4]Faculty of Biosciences, Natural Product Research Laboratories, University of Joensuu, Finland

The effects of different organic cultivation methods on the berry phenolics of two Finnish sea buckthorn (*Hippophae rhamnoides L. ssp. rhamnoides*) cultivars, "Terhi" and "Tytti", were studied in an experimental field at coastal area in Merikarvia, western Finland. Cultivation methods included different fertilizers (designed for organic cultivation), mulches (organic and plastic) and land contours (flat surface vs. ridge/planting beds). Two experiments were conducted. The first, a fertilization experiment, allowed estimation of the effects of cultivar, fertilizer, land contour and all their interactions, while the second, a mulch experiment, allowed the estimation of the effects of mulches, land contours and their interactions for the cultivar "Tytti."

Results of the HPLC (High Performance Liquid Chromatography) method indicated significant differences between the cultivars and cultivation methods. In the fertilization experiment, the number of phenolic compounds in sea buckthorn berries varied between cultivars. 15 different phenolic compounds were found in "Terhi" and 21 compounds in "Tytti." In addition, both cultivars had condensed tannins. The concentrations of quercetin derivatives 1, 2, 3, isorhamnetin 3,7-diglucoside, quercetin-3-glucoside-7-rhamnoside, hyperin, isorhamnetin 3-glucoside and flavonoid derivative 3 were higher in "Tytti" than in "Terhi." The concentrations of isorhamnetin-glucoside derivative 2 and 3 were higher in "Terhi" than in "Tytti." Flat surface increased the concentrations of individual compounds such as isorhamnetin 3,7-diglucoside, isorhamnetin-glucoside derivative 1, quercetin derivatives 2, 4 and condensed tannins.

In the mulch experiment, a total of 22 phenolic compounds and condensed tannins were found in the cultivar "Tytti." Mulches did not have any significant effect on the concentrations of phenolic compounds. However, there was significant land contour effect on the concentrations of flavonoid derivative 1, 2, quercetin derivative 2 and rhamnetin derivative 1. Their concentrations were higher when bushes were grown on the flat surface compared to that on the ridge. These results suggest sustainable methods to cultivate sea buckthorn to produce large amounts of valuable chemicals in the berries.

S6-4

Antioxidant, anticancer and anticarcinogenic properties of pomegranate juice constituents and their microbial metabolites

Sashi Kasimsetty,[1] Kesava Reddy Muntha,[4] Dobroslawa Bialonska,[1] Shabana I Khan,[3] Kristine Willett,[2,3] and Daneel Ferreira[1,3]

[1]Department of Pharmacognosy, [2]Department of Pharmacology, and [3]National Center for Natural Product Research, Research Institute for Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University, MS; and [4]Sensory Research Centre for Research and Technology, Philip Morris USA Inc, Richmond, VA

Consumption of pomegranate juice is associated with a number of health-promoting aspects, and the hydrolysable tannins present in pomegranate juice are believed to be responsible for the biological activity. These hydrolyzable tannins include gallotannins (e.g. corilagin), ellagitannins [e.g. 2,3-(S)-hexahydroxydiphenoyl-β-D-glucopyranose], and more unique gallagoyl esters (e.g. punicalagins). The tannins present in dietary supplements are also thought to be antinutrituous in nature. To determine whether health-beneficial or antinutrituous effects exist for pomegranate juice consumption, the hydrolysable tannins present in the juice were isolated, identified and studied for their biological activities. Punicalagins and punicalins, which represent the major proportion of these hydrolyzable tannins, exhibited antioxidant activity at IC_{50} values of 0.41 μM and 1.04 μM concentrations, respectively. However, it is has been recently established that hydrolyzable tannins present in pomegranate juice are not absorbed into systemic circulation. In addition, these hydrolyzable tannins were found to be metabolized by colonic microflora into simpler organic molecules called urolithins, which can then enter systemic circulation. These urolithins are believed to be responsible for the biological activities associated with pomegranate juice hydrolyzable tannins. We therefore have synthesized the major microbial metabolites of the hydrolyzable tannins, urolithin-A and urolithin-B, and these compounds are being tested for various biological activities. To date, these compounds have been found to actively inhibit the viability of HT29 colon cancer cells and also appeared to decrease the activity of elevated CYP1 enzymes. While testing is still in progress, our current results support the health-promoting properties of pomegranate juice. See also the presentation by Doroslawa *et al.,* for effects of digestion on the bioavailability and bioactivity of punicalagins.

S6-5

Physiological roles of HCT and HQT tobacco acyltransferases and associated metabolic processes

Claudia Cardenas, Michael A. Costa, Dhrubojyoti D. Laskar, Laurence B. Davin, and Norman G. Lewis

Institute of Biological Chemistry, Washington State University, Pullman, WA

Chlorogenic acid is considered to have an important role in human health as a known strong antioxidant with capacity to reduce free radicals. Chlorogenic acid plays an important function in plant stress responses, limiting leaf, flower, and fruit damage, for instance. Two enzymes have been associated with chlorogenic acid biosynthesis, as well as with a central role in the phenylpropanoid pathway, namely hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamoyl transferase (HCT) and hydroxycinnamoyl-coenzyme A quinate hydroxycinnamoyl transferase (HQT). Enzymatic studies showed that HCT catalyzes transfer of the acyl group of hydroxycinnamoyl CoA esters to either quinic or shikimic acids, whereas HQT transfers the hydroxycinnamoyl moiety to quinic acid. RNAi strategies were carried out individually to disrupt HCT or HQT gene expression and to study their physiological roles in tobacco. HQT downregulated plant lines displayed a significant decrease in chlorogenic acid levels with no notable differences on lignin total/monomeric composition as compared to WT. HCT-silenced plants, on the other hand, have a dwarfed phenotype with severe development/growth delays, and total lignin contents reduced to ~25% of WT. The lignin monomeric compositions analyzed by thioacidolysis/nitrobenzene oxidation protocols also showed a drastic decrease in guaiacyl (G) and syringyl (S) contents, whereas p-hydroxyphenyl (H) moieties increased by ~5 fold. Both HCT down and over-expression had no major effects on chlorogenic acid accumulation. These results indicate that while HQT is involved in chlorogenic acid production, the phenylpropanoid pathway leading to the biosynthesis of monolignols utilizes mainly HCT.

S7-1

Co-regulated metabolite modules in Zingiberaceae rhizomes suggest the existence of biosynthetic modules in plant specialized metabolism

Zhengzhi Xie,[1,2] Xiaoqiang Ma,[1] and David R. Gang[1]

[1]Department of Plant Sciences and BIO5 Institute, and [2]Department of Pharmaceutical Sciences, University of Arizona, Tucson, AZ

Turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*), members of the Zingiberaceae, are excellent examples of plants that produce large numbers of metabolites from diverse metabolic pathways or networks. We hypothesized that these metabolic pathways or networks contain biosynthetic modules, which lead to formation of metabolite modules—groups of metabolites whose production is co-regulated and biosynthetically linked. To test whether such co-regulated metabolite modules do exist in these plants, we performed metabolic profiling analysis on rhizome samples that were collected from different growth and development treatments, which had significant impacts on the levels of several hundred volatile and non-volatile metabolites that were detected. Importantly, one of the many co-regulated metabolite modules that were indeed readily detected in this analysis contained the three major curcuminoids in turmeric, whereas many other structurally related diarylheptanoids belonged to separate metabolite modules, as did groups of terpenoids. The existence of these co-regulated metabolite modules supported the hypothesis that the 3-methoxyl groups on the aromatic rings of the curcuminoids are formed before the formation of the heptanoid backbone during the biosynthesis of curcumin and also suggested the involvement of multiple polyketide synthases with different substrate selectivities in formation of the array of diarylheptanoids detected in turmeric. Similar conclusions about terpenoid biosynthesis could also be made. Thus, discovery and analysis of metabolite modules can be a powerful predictive tool in efforts to understand metabolism in plants.

S7-2

Towards an understanding of the (+)- pinoresinol forming dirigent protein: Reaction mechanism and structure

Kye-Won Kim, Dhrubojyoti D. Laskar, Laurence B. Davin, and Norman G. Lewis

Institute of Biological Chemistry, Washington State University, Pullman, WA

The (+)-pinoresinol forming dirigent protein is able to help engender stereoselective coupling of two achiral coniferyl alcohol derived moieties. The protein contains a number of hydrophobic residues and four different approaches have been adopted to probe both reaction mechanism and structure, i.e. site-directed mutagenesis, electron paramagnetic resonance, nuclear magnetic resonance spectroscopy, and computational structural modeling. This is also in order to establish how the unique coniferyl alcohol radical (CA·) coupling mechanism occurs.

Site-directed mutagenesis was performed to identify important amino acid residues in CA· binding and processing. We examined the stereoselectivity of twenty nine different mutants and found various residues with apparent central roles in control over stereoselective coupling and post-translational processing. These findings are discussed in terms of additional computational modeling and spectroscopic analyses.

S7-3

Stereochemistry on the formation of syringylglycerol-8-*O*-4'-(sinapyl alcohol) ether from sinapyl alcohol with enzyme preparations of *Eucommia ulmoides*

Takeshi Katayama,[1] Md. Shameul Alam,[1] Toshisada Suzuki,[1] and Nattaya Lourith[2]

[1]Faculty of Agriculture, Kagawa University, Japan and [2]School of Cosmetic Science, Mae Fah Luang University, Thailand

Arylglycerol-β(8-*O*-4')-aryl ether linkages are present in lignins and 8-*O*-4' neolignans. The intermonomer linkages are the most abundant ones in natural products except for glycosidic linkages in carbohydrates. Our group has investigated the biosynthesis of 8-*O*-4' neolignans, and Katayama and Kado found for the first time that incubation of cell-free extracts from *Eucommia ulmoides* with coniferyl alcohol (CA) in the presence of H_2O_2 gave (+)-*erythro*- and (-)-*threo*-guaiacylglycerol-8-*O*-4'-(coniferyl alcohol) ether (GGCE) and that the *erythro* isomer was preferred to the *threo* one. Lourith et al. determined the relative configuration of guaiacylglyerol-8-*O*-4'-(sinapyl alcohol) ether (GGSE) isolated from the plant by Deyama et al. as *erythro* form and found the stereoselective formation of *erythro*-GGSE and *erythro*-syringylglycerol-8-*O*-4'-(sinapyl alcohol) ether (SGSE) with optical activity by feeding experiments. In this study, enzyme reactions were carried out to clarify the stereochemistry of the formation of SGSE from sinapyl alcohol (SA) in *E. ulmoides*. Incubation of a soluble enzyme preparation with [8-^{14}C]SA in the presence of H_2O_2 gave [^{14}C]SGSE, and the (-)-*erythro* isomer was more favored than the (-)-*threo* one. Incubation of an insoluble enzyme preparation with [8-^{14}C]SA also gave [^{14}C]SGSE, and the (+)-*erythro* isomer was more favored than the (-)-*threo* one. Interestingly, the soluble preparation catalyzed the formation of (-)-*erythro* SGSE, whereas the insoluble preparation did (+)-*erythro* one. Both preparations catalyzed the diastereoselective formation of *erythro*- and *threo*-SGSEs with optical activity.

S7-4

Arabidopsis thaliana pinoresinol reductases control enantiomeric compositions of lariciresinol

Tomoyuki Nakatsubo, Masaharu Mizutani, Shiro Suzuki, Takefumi Hattori and Toshiaki Umezawa

Research Institute of Sustainable Humanosphere, Kyoto University, Japan

A lignan, lariciresinol, was isolated from *Arabidopsis thaliana* for the first time. In the *A. thaliana* genome database there are two genes (At1g32100 and At4g13660) that are annotated as pinoresinol/lariciresinol reductase (PLR). The recombinant AtPLRs showed strict substrate preference towards pinoresinol but only weak or no activity towards lariciresinol, which is in sharp contrast to conventional PLRs of other plants that can reduce both pinoresinol and lariciresinol efficiently to lariciresinol and secoisolariciresinol, respectively. Therefore, we renamed AtPLRs as *A. thaliana* pinoresinol reductases (AtPrRs). The recombinant AtPrR2 encoded by At4g13660 reduced only (-)-pinoresinol to (-)-lariciresinol, but not (+)-pinoresinol, in the presence of NADPH. This enantiomeric selectivity accords with that of other plants' PLRs so far reported, which can reduce one of the enantiomers selectively, whatever the preferential enantiomer. In sharp contrast, AtPrR1 encoded by At1g32100 reduced both (+)- and (-)-pinoresinols to (+)- and (-)-lariciresinols efficiently with comparative k_{cat}/K_m values. Analysis of lignans and spatiotemporal expression of *AtPrR1* and *AtPrR2* in their functionally deficient *A. thaliana* mutants and wild-type indicated that both genes are involved in lariciresinol biosynthesis. In addition, the analysis of the enantiomeric compositions of lariciresinol isolated from the mutants and wild-type showed that PrRs together with a dirigent protein(s) are involved in the enantiomeric control in lignan biosynthesis. Furthermore, it was demonstrated conclusively for the first time that differential expression of PrR isoforms that have distinct selectivities of substrate enantiomers can determine enantiomeric compositions of the product, lariciresinol.

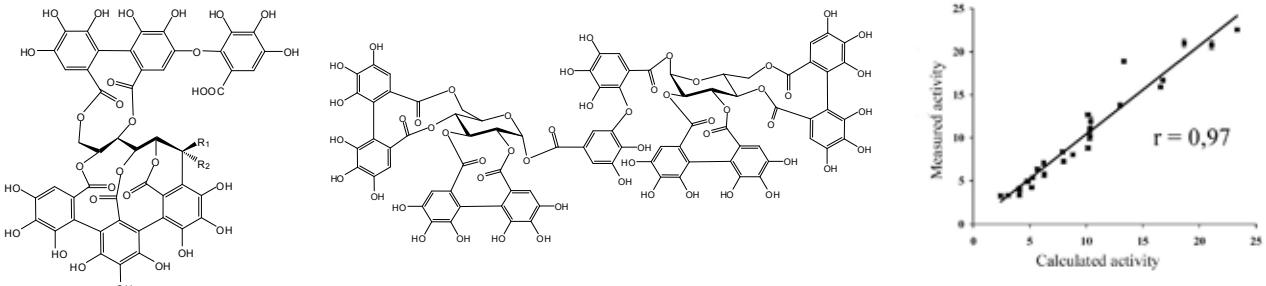
S7-5

Chemical structures of plant ellagitannins reveal their *in vitro* oxidative activity at high pH

Johanna Moilanen and Juha-Pekka Salminen

Laboratory of Organic Chemistry and Chemical Biology, Department of Chemistry, University of Turku, Finland

Chemical structures of 27 ellagitannins were systemically compared in respect of their *in vitro* oxidative activity at high pH found e.g. in lepidopteran insects. The analysis revealed over six-fold differences in the oxidative activities of individual ellagitannins which could be explained by the chemical divergences of the ellagitannins. These findings allowed the formulation of a simple equation that can be used to estimate the oxidative activities of other ellagitannins with known structures. The results suggest that, in future studies of plant-herbivore interactions, ellagitannins should be (1) taken into account as possible oxidative stress-based defenses of plants against herbivores, (2) chemically characterized from the study plants, and (3) quantified individually, not as chemically ill-defined group. These actions together with the utilization of the created equation would allow the clarification of the role of ellagitannins in plant-herbivore interactions as natural pro-oxidants.



S7-6

Anthocyanidin synthase as a key enzyme of the flavonol pathway in *Arabidopsis thaliana*

Anja Preuss, Stefan Martens and Ulrich Matern

Institut für Pharmazeutische Biologie, Philipps Universität Marburg, Germany

Anthocyanidins, proanthocyanidins and flavonols accumulate as major flavonoids in *Arabidopsis*. Three 2-oxoglutarate-dependent dioxygenases are involved in their biosynthesis, two of which, i.e. flavonol synthase (FLS) and anthocyanidin synthase (ANS), show multiple substrate and product specificities *in vitro*. The relevance of FLS or ANS *in vivo* for the formation of selected flavonoids thus needs to be examined. The *Arabidopsis* genome project revealed a small FLS gene family of *FLS*-1 and six *FLS*-like sequences while ANS is encoded by a single gene. In order to unravel the role of either enzyme in flavonol and pro-/anthocyanidin biosyntheses, we studied the functionality of recombinantly expressed FLS and FLS-like proteins as well as that of ANS. Only FLS-1 and ANS converted flavanones or dihydroflavonols to the respective flavonols, but none of the FLS-like proteins showed catalytic activity with the potential substrates in biotransformations or *in vitro*. Most notably, an *FLS*-1 mutant line still accumulated significant amounts of flavonols. The results prove that ANS is capable of catalyzing the FLS-type desaturation. The conclusion of both FLS-1 and ANS contributing to flavonol formation was supported by comparing wild-type and *FLS*-1 mutant *Arabidopsis* with the double *FLS*-1/*ANS* mutant which showed further reduced flavonol contents (Stracke et al. 2008, submitted; in cooperation with Genome Research Bielefeld University and PRI Wageningen). This corroborates for the first time the involvement of ANS in flavonol synthesis *in vivo*. Nevertheless, the remaining level of flavonols requires another marginal FLS activity which might reside in the FLS-like proteins and escaped the *in vitro* assays.

S8-1

Biosynthesis of isoprene units in plants: Last steps of the MEP pathway and cross-talk between MVA/MEP pathways

Michel Rohmer

Université Louis Pasteur/CNRS, Institut Le Bel, Strasbourg, France

Starting from pyruvate and glyceraldehyde phosphate, isopentenyl diphosphate and dimethylallyl diphosphate, the universal isoprenoid precursors are synthesized in plant plastids via deoxyxylulose phosphate, four methylerythritol derivatives and 2-methylbutene-1,4-diol diphosphate. The last two steps correspond to novel elimination/reduction reactions catalyzed by enzymes possessing a $[Fe_4S_4]$ cluster as prosthetic. They require an additional reducing system: ferredoxin in the presence of photosystems I and I and light or ferredoxin reductase/NADPH in the dark.

The methylerythritol phosphate pathway is responsible for the formation of hemi-, mono- and diterpenes, as well as the carotenoids and the prenyl chain of plastoquinone, whereas the mevalonate pathway, which is located in the cytoplasm, is involved in the biosynthesis of most sesquiterpenes and all triterpenoids, including the sterols. This dichotomy concerning the origin of the isoprene units in plant isoprenoid series is, however, not as clear-cut in plants. Cross-talk and intermediate exchanges between the two cell compartments have been often observed and may probably represent an unexplored way for the regulation of isoprenoid biosynthesis.

S8-2

Structural biology in alkaloid biosynthesis – Why?

Joachim Stöckigt

College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, People's Republic of China and Institute of Pharmacy, Johannes Gutenberg-University, Mainz, Germany

Elucidation of enzymatic biosynthesis of plant natural products resulted in remarkable progress during recent decades and broad knowledge accumulated for e.g. flavonoids, phenylpropanoids or different classes of alkaloids. There are still only a few examples known where nearly complete pathways or reaction sequences have been elaborated at the biochemical level, but functional cloning of many cDNAs coding for biosynthetic enzymes allowed their excellent purification and characterization. Sequence analysis together with site-directed mutagenesis provides preliminary but still incomplete insight into the catalyzed reactions, making mechanistic conclusions difficult. As a consequence of generating mg-amounts of plant enzymes by heterologous overexpression, the crystallization and x-ray analysis of the proteins became possible in recent years and structural biology entered more broadly the field of natural product biosynthesis.

The lecture will focus on recently obtained results in crystallization and structural analysis of enzymes catalyzing the biosynthesis of monoterpenoid indole alkaloids in cell suspension cultures of the Indian medicinal plant *Rauvolfia serpentina* (L.) Benth. ex Kurz and will address questions of why structural biology might be important in this research field.

S8-3

*Cathedral roseus**
*and other interesting tales in plant metabolic engineering

Jacqueline V. Shanks

Department of Chemical and Biological Engineering, Iowa State University, Ames, IA

Catharanthus roseus synthesizes two pharmaceutically important anticancer drugs, vinblastine (Velbe®) and vincristine (Oncovin®), via the terpenoid indole alkaloid (TIA) pathways. This talk will highlight the engineering approaches to systematically increase TIA production in *C. roseus* hairy roots and, in doing so, offers lessons in plant metabolic engineering. We have used the tools of precursor feeding studies, elicitor studies, flux analysis and expression profiling of target genes by Q RT PCR to better understand the bottlenecks within the indole and terpenoid pathways. Using this information, we then constructed transgenic *C. roseus* hairy root lines by using molecular biology tools to overexpress one or more strategic genes encoding for enzymes or transcriptional regulators in the terpenoid and/or indole pathways under the control of an inducible promoter. Transgenic lines are characterized with the previously mentioned tools, and this cycle of synthesis and analysis is run in an iterative scheme, typifying the semi-empirical nature of the metabolic engineering process. Future advances in engineering this pathway will require tool development for the analysis and synthesis steps as well as enlarging the knowledge base of this species.

S9-1
Tracking metabolic diversification in Piperaceae

Massuo J. Kato,[1] Ari S. Ferreira,[1] Lydia F. Yamaguchi,[1] Marcus T. Scott,[1] Vicente P. Emerenciano,[1] Adalberto M. Silva,[1] Alberto de Oliveira,[1] Clécio S. Ramos,[1] Joaquim V. Marques,[1] Lucas B. Navarro,[1] Karina J. M. Salazar,[1] Edgard A. Ferreira,[1] Giovana C. Freitas,[1] Anderson M. Gaia,[1] Camila Rodrigues,[1] Cristina K. Maruyama,[1] Juliana B. Reigada,[1] Antonio Salatino,[2] Maria L. Salatino,[2] Débora C. Bergamo,[3] Jonas S. Mota,[3] Fernando S. Cotinguiba,[3] Lidiane G. Felipe,[3] João M. B. Junior,[3] Maysa Furlan,[3] Vanderlan da S. Bolzani,[3] Elsie F. Guimarães,[4] Maria Claudia M. Young,[5] Sérgio A. Vanin,[6] Toshie Kawano,[7] Edson Teixeira,[7] José E. Miranda[8]

[1]Instituto de Química, Universidade de São Paulo, [2]Instituto de Biociências, Universidade de São Paulo, [3]Instituto de Química, Universidade Estadual Paulista, [4]Instituto de Pesquisas Jardim Botânico do Rio de Janeiro, [5]Instituto de Botânica, [6]Museu de Zoologia, Universidade de São Paulo, [7]Instituto Butanta, and [8]EMBRAPA, Centro Nacional de Pesquisa de Algodão, Brazil *FAPESP, CNPq*

Secondary compounds of Piperaceae species have been investigated by HPLC/ESIMS and ^{1}H NMR associated to PCA analysis. Such profiling analysis and ITS sequences revealed clades specialized in production of lignoids, benzoic acids and chromenes, piperolides and polyketides. Benzoic acids and lignoids-containing species are involved in predation specificity by Coleoptera and Lepidoptera, respectively. The investigation of seedling-adult plant chemistry revealed ubiquitous presence of amides, while in the case of lignoid-containing species (adult stages), only monomers could be detected during the early stages of development. Major bioactive compounds displayed antifungal, insecticidal, and molluscicidal activity based on natural and synthetic compounds. Taken together, the phytochemistry, biology and molecular data (ITS) pointed to some clues regarding the role of secondary compounds in Piperaceae species and raised further questions.

S9-2
Arthropod repelling constituents from a Southern folk remedy, *Callicarpa americana*

Charles L. Cantrell,[1] Jerome A. Klun,[2] Jian Chen,[3] and John Carroll[4]

[1]USDA-ARS, NPURU, University, MS; [2]USDA-ARS, Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD; [3]USDA-ARS, Biological Control of Pests Research Unit, Stoneville, MS; and [4]USDA-ARS, Animal Parasitic Diseases Laboratory, Beltsville, MD

Based on botanical lore of insect repellent properties, essential oil extracts from *Callicarpa americana* were investigated. Bioassay-guided fractionation of *C. americana* extracts using the yellow fever mosquito, *Aedes aegypti*, led to the isolation of α-humulene, humulene epoxide II, intermedeol, and a newly isolated terpenoid (callicarpenal). Heretofore, 13,14,15,16-tetranor-3-cleroden-12-al, callicarpenal, has never been identified from natural sources. In bite-deterrent studies, intermedeol and callicarpenal showed significant bite-deterring activity against *Aedes aegypti* and *Anopheles stephensi,* a transmitter of malaria. Callicarpenal and intermedeol were also evaluated in laboratory bioassays for repellent activity against host-seeking nymphs of the blacklegged tick, *Ixodes scapularis*, a known vector for Lyme disease. Lastly, the repellency of callicarpenal against workers of red imported fire ants, *Solenopsis invicta* Buren, black imported fire ants, *Solenopsis richteri*, and a hybrid of these two species was evaluated using digging bioassays.

S9-3

Colored and white sectors of petunia flowers display differential resistance to insect herbivores

Eric T. Johnson,[1] Mark A. Berhow,[2] and Patrick F. Dowd[1]

[1]Crop Bioprotection Research Unit, and [2]New Crops and Processing Technology Research Unit, USDA-ARS, Peoria, IL

Insect herbivory of crops increases the probability of fungal infection in damaged tissues. Mycotoxins, produced by some fungi, are harmful to livestock and humans. Increasing plant resistance lowers the levels of fungal infection and mycotoxin levels. Potential resistance of anthocyanins from commercial petunia flowers (*Petunia hybrida*) was examined for insecticide/antifeedant activity against corn earworm (CEW, *Helicoverpa zea*) and cabbage looper (CL, *Trichoplusia ni*). The petunia flowers studied contained a star pattern with colored and white sectors. In most cases, CEW larvae ate significantly less colored sectors than white sectors in no-choice bioassays. All CEW larvae feeding on blue sectors weighed significantly less after two days than larvae feeding on white sectors, and the weights were negatively correlated with total anthocyanin levels. CL larvae ate less of blue sectors than white sectors, and blue sectors from one petunia cultivar caused significantly higher CL mortality than white sectors. Partially purified anthocyanin mixtures isolated from petunia flowers, when added to insect diet discs at approximately natural concentrations, significantly reduced both CEW and CL larva weights compared to controls. Methanolic extracts of whole flowers of one petunia cultivar were fractioned and added to insect diet discs; the most hydrophobic fraction effectively killed both CEW and CL larvae. These studies demonstrate that the colored sectors of these petunia cultivars slow the development of these lepidopteran larvae and indicate that anthocyanins play some part in flower defense in these petunia cultivars. Production of petunia-like anthocyanins in crop plants may be able to increase insect resistance and lower fungal infection.

S10-1
Explorations in grape and wine proanthocyanidin chemistry

James A. Kennedy

Department of Food Science and Technology, Oregon State University, Corvallis, OR

Red wine astringency is an important component of overall wine quality, and proanthocyanidins are responsible for this attribute. Red wine proanthocyanidins are grape-derived (*Vitis vinifera* L.) and are extracted during wine production. Once extracted, proanthocyanidins interact and react with wine components leading to a modification in wine astringency. Studies have shown that astringency quality can vary with proanthocyanidin concentration and structure; therefore, there is a need to understand how these aspects vary with grape and wine production practices. Historically, red wine proanthocyanidins have received a considerable amount of attention from researchers not only because of their importance from a sensory standpoint but also because of their complex chemistry. Beginning with their biosynthesis in the grape and concluding with their structural change during wine aging, the results of various studies will be presented to provide an appreciation for the complexity of proanthocyanidin structure and the challenges that remain in in order to fully elucidate the relationship between red wine composition, proanthocyanidin structure, and astringency quality.

S10-2
Molecular aspects of biosynthesis of flavonoids in grape berries

Nancy Terrier

UMR Sciences for Enology, INRA, Montpellier, France

Flavonoids are a large group of secondary metabolites involved in plant defense and reproduction. *Vitis vinifera* appears as a particularly interesting model to study the biosynthesis of flavonoids. First, various flavonoids accumulate in large amounts in the berries, especially anthocyanins and proanthocyanidins. Second, several genomic tools have been developed for vines during the last decade, from the first public ESTs and microarray results until the recent release of the whole genome sequence. Thus, numerous papers have been published in the past years dealing with flavonoid biosynthesis in grape berries.

Within the EU project FLAVO, different approaches of metabolomics, transcriptomics, plant transformation and genetics have been coupled in order to decipher the missing steps in the flavonoid pathway. *Vitis* transformants overexpressing transcription factors inducing flavonoid synthesis were constructed and analyzed at the phenotypic and transcriptomic levels, allowing the identification of new putative actors of the pathway. A collection of *Vitis* cultivars and a segregating population were screened for their flavonoid compositions. Plants exhibiting extreme phenotypes were used for transcriptomic analysis. Several QTLs were detected and, interestingly, one region is involved in the control of the mean degree of polymerization of proanthocyanidins in grape skins. Recent progress on flavonoid biosynthesis will be reviewed.

S10-3

Effect of phenolic compounds in traditional Sino-Japanese herbal medicines on the impairment of rat spatial cognition

Xiu-Lan Sun,[1] Hideyuki Ito,[1] Takayoshi Masuoka,[2] Chiaki Kamei,[2] and Tsutomu Hatano[1]

[1]Department of Pharmacognosy, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, and [2]Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan

Clinical efficacy of some traditional Sino-Japanese herbal medicines on symptoms related to dementia has been revealed. The root of *Polygala tenuifolia* Willdenow (Polygalaceae) has been used as an ingredient in such medicines, and triterpene glycosides or sugar derivatives bearing hydroxy- or methoxy-cinnamic acids are characteristic constituents in the root. We examined the effects of polar fractions and those constituent acids on scopolamine-induced deficits of rat spatial cognition. Since the eight-arm radial maze task has been utilized for discrimination of the effects on both short-term and long-term memory, this method was used to see the effects. Oral administration of a glycoside-rich fraction showed a significant ameliorating effect in short-term memory, and sinapic acid was the most effective of the constituent phenolic acids. We also examined the effects of fractions obtained from *Uncaria sinensis* Havil (Rubiaceae) on the impairment of spatial cognition in rats and found effectiveness of the low-molecular-weight polyphenolic fraction, together with that of the alkaloid-enriched fraction. On the other hand, the fraction containing high-molecular-weight polyphenols did not show the significant effect. This may be attributed to the low availability of high-molecular-weight polyphenols after the oral administration.

S10-4

Anti-inflammatory and cytotoxic activities of polymeric polyphenolics in rabbit peripheral blood mononuclear cells

Kimberly N. Wisman, Akeysha A. Perkins, Melanie D. Jeffers, and Ann E. Hagerman

Department of Chemistry and Biochemistry, Miami University, Oxford, OH

Phenolic compounds are widely known for their roles as antioxidants and anti-inflammatory agents, as well as for their epidemiological association with reduced risks for certain types of diseases. In the present study we used rabbit peripheral blood mononuclear cells (PBMCs) to examine the anti-inflammatory activity and cytotoxicity of nine polyphenolics, representing several major classes of tannins and their subunits. For three of those phenolics, we also assessed activity after spontaneous oxidation. In addition, we studied the potential of those phenolic compounds to interfere with three commonly used cell viability assays. Anti-inflammatory activity was only observed with apigenin (EC_{50} 1.0 µg/mL), while most other compounds either had no affect on TNF-α or increased its production. However, (-)-epigallocatechin-3-O-gallate (EGCG), β-1,2,3,4,6-pentagalloyl-O-D glucose (PGG) and oenothein, all gallate ester-based tannins, showed a strong correlation between cytotoxicity and decreased TNF-α production. Our study also showed that many of the nine tested tannins interfered with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays which are often used to measure cell viability. Because there was no interference detected in the ATP assay, we recommend its use to measure cell viability in the presence of phenolic compounds.

S10-5
Synthesis, characterization and thermal behaviours of polyphenol stearates

Chunhua Luo,[1] Warren Grigsby,[2] Neil Edmonds,[1] Allan Easteal,[1] and Jafar Al-Hakkak[2]

[1]Department of Chemistry, The University of Auckland, and [2]Biopolymer Network Limited, New Zealand

New Zealand has a vast source of plant based lignocellulosic materials and residues. Polyphenols are extractable from a variety of such resources and can be transformed into potentially useful derivatives which can then substitute for petrochemical intermediates in a wide range of applications. We have been examining the potential of combining selected polyphenols with other bio-based materials to prepare materials for potential industrial applications. Reported is the preparation of polyphenol derivatives with saturated fatty acids. This study utilised acid chloride formation to prepare corresponding stearate esters. The products have been characterised both chemically and by the use of polymer thermal analysis. Discussed is the analysis of the esters with varying degree of substitution and the resulting impact on their inherent properties.

S10-6
C-glycosidic ellagitannins: From folk medicine to medicinal chemistry via wine sciences

Stéphane Quideau

European Institute of Chemistry and Biology, Institute of Molecular Sciences, CNRS-UMR 5255, University of Bordeaux, France

Polyphenolic nonahydroxyterphenoyl-containing *C*-glycosidic oak ellagitannins are found in wine as a result of the aging of this beverage in barrels made of oak. Once in the hydroalcoholic and slightly acidic wine solution (pH ~ 3-4), some of these natural products such as (–)-vescalagin, but not its C-1 epimer (–)-castalagin, can capture grape-derived nucleophilic entities, such as ethanol, the flavanols catechin and epicatechin, the anthocyanin oenin and the thiolic glutathione, to furnish condensation products with retention of configuration at the C-1 locus. These condensation products can contribute to the modulation of wine organoleptic properties, as evidenced by the color absorbance 23 nm bathochromic shift observed with the novel oenin-based anthocyano-ellagitannin pigment. Hydrolysis of vescalagin under solvolytic conditions furnished a novel compound that we referred to as vescalene, in addition to the known vescalin. Of pharmacological importance is the fact that several of these ellagitannins and derivatives found in wine are much more potent than etoposide (VP-16) at inhibiting topoisomerase II (top2)-mediated DNA decatenation *in vitro*. The known vescalin and the novel vescalene fully inhibited top2 at 10 mM concentration. Some of these oak ellagitannins also displayed significant anti-HSV activities against acyclovir-resistant mutants.

S10-7

Progress in the chemistry of oligomeric ellagitannins in medicinal plants

Takashi Yoshida

College of Pharmaceutical Sciences, Matsuyama University, Japan

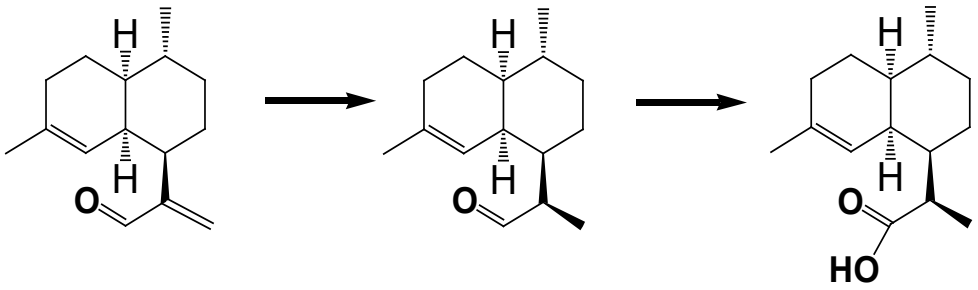
The remarkable progress made in phytochemical studies on tannin constituents of traditional medicines during the last three decades has revealed numerous novel tannins and related polyphenols from a wide array of medicinal plants long used in Japan, China and other Asian countries. Our earliest study in this field was the isolation and characterization of geraniin, a main tannin of *Geranium thunbergii* and one of the popular crude drugs in Japan, in which lay the groundwork for subsequent discoveries of many kinds of hydrolyzable tannin analogs including dimers. Several hundred hydrolyzable tannins thus characterized have enabled us to examine their various biological properties on the basis of differences of structures. Newly found biological functions of polyphenols that are associated with potent antioxidative effects have attracted increasing attention in recent years; this is because such polyphenol-rich foods and beverages are expected to be beneficial to human health, including prevention of life style-related diseases that are related to oxidative stress. Here I would like to look back over our phytochemical studies on tannins, mainly focusing on structural diversity and the chemotaxonomical significance of oligomeric ellagitannins, which constitute a notable large class among more than 500 hydrolyzable tannins.

S11-1
Artemisinin biosynthesis: The next steps

Patrick S. Covello,[1] Yansheng Zhang,[1] Keat T. Teoh,[1] Devin R. Polichuk,[1] Darwin W. Reed,[1] Lies Maes,[2] Alain Goossens,[2] Douglas J.H. Olson,[1] and Andrew R.S. Ross[1]

[1]NRC Plant Biotechnology Institute, National Research Council Canada, Saskatoon, Canada and [2]Flanders Institute for Biotechnology, Ghent University, Belgium

Artemisinin, a sesquiterpene lactone endoperoxide derived from *Artemisia annua*, forms the basis of the most important treatments of malaria in use today. In an effort to understand the biosynthesis of artemisinin in the glandular trichome of *Artemisia annua*, an expressed sequence tag approach to identifying the relevant genes was undertaken. Three genes encoding enzymes implicated in the formation of dihydroartemisinic acid from amorpha-4,11-diene have been identified. Of particular interest is the combined use of enzyme purification, mass spectrometry and ESTs to identify a cDNA encoding artemisinic aldehyde delta-11(13) reductase. The biosynthetic and biotechnological relevance of the findings will be discussed.



S11-2
Comparative genomic, structural and biochemical study of substrate specificity evolution of the SABATH methyltransferase family

Feng Chen,[1] Nan Zhao,[1] Jean-Luc Ferrer,[2] Jeannine Ross,[3] Ju Guan,[1] Yue Yang,[4] Eran Pichersky,[4] Joseph P. Noel,[3] and Xiofeng Zhuang[1]

[1]Department of Plant Sciences, University of Tennessee, Knoxville, TN; [2]Institut de Biologie Structurale, Grenoble, France, [3]The Salk Institute for Biological Studies, La Jolla, CA; and [4]Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI

The plant SABATH protein family is composed of a group of related small molecule methyltransferases (MTs) that catalyze the S-adenosyl-L-methionine dependent methylation of natural chemicals encompassing widely divergent structures. Known members of the SABATH family display strict substrate specificity. We are interested in understanding the evolution of substrate specificity of the SABATH family. SABATH genes were identified from the sequenced plant genomes including those of *Arabidopsis*, rice, poplar, and moss. Phylogenetic analysis combined with gene cloning and biochemical characterization revealed a highly conserved group of SABATH genes encoding indole-3-acetic acid (IAA) methyltransferase (IAMT). The crystal structure of *Arabidopsis* IAMT (AtIAMT1) was determined. The overall tertiary and quaternary structures of AtIAMT closely resemble the two-domain bi-lobed monomer and the dimeric arrangement, respectively. Structural modeling using AtIAMT as a template showed that the structures of IAMTs from different plant species are highly conserved. The above described evidence supports the hypothesis that IAMT is an ancient member of the SABATH family. To trace the evolutionary path of the SABATH family, a putative ancestral IAMT gene was resurrected and synthesized. *E. coli*-expressed ancestral IAMT displayed biochemical properties comparable to those of known IAMTs. Site-directed mutagenesis of the ancestral IAMT is underway to elucidate the key determinants that govern substrate specificity of SABATH proteins.

S11-3

Toward an understanding of C-glycosylation of isoflavonoids in Kudzu (*Pueraria lobata)*

Jack W. Blount, XianZhi He, and Richard A. Dixon

The Samuel Roberts Noble Foundation, Inc., Plant Biology Division, Ardmore, OK.

Kudzu (*Pueraria lobata*) is a perennial legume which was first introduced into the U.S. in 1876 by Japan at the Centennial Exposition. Since then, it has been promoted as a forage crop in the 1920s, planted by the Soil Conservation Service in the 1930s, and declared a weed by the USDA in 1972. Kudzu has been used in China as an herbal medicine for over one thousand years. The roots are a rich source of health-beneficial isoflavonoid compounds, many of which occur as either *O*-glycosides or *C*-glycosides. We are interested in the biosynthesis of the *C*-glycosides since they are non-hydrolyzable and may have better bioactivity and bioavailability than the corresponding *O*-glycosides. Puerarin (daidzein-8-*C*-glucoside) is the primary *C*-glycoside found in Kudzu root, and it has been ascribed hypotensive and cardio-protective properties as well as potential for use in the treatment of diabetes and obesity. Our project objective is to identify and characterize the Kudzu isoflavone *C*-glucosyltransferase gene(s). To this end, the poster describes HPLC analysis of the tissues available for making a subtraction library and characterizing the C-glucosyltransferase(s). These includes Kudzu roots that do, or do not, accumulate puerarin, tissues which contain varying levels of puerarin as a result of developmental stage, and callus/cell suspension cultures from these plants. Second, the talk will cover the progress made to date on the elucidation of the biochemical pathway to puerarin and other *C*-glycosides, focusing primarily on the identification of the actual substrate for *C*-glycosylation in the biosynthesis of puerarin.

S11-4

Oxylipin pathways in the moss *Physcomitrella patens:* Implications for jasmonate evolution

Aldwin Anterola,[1] Cornelia Göbel,[2] Ivo Feussner,[2] George Sellhorn,[3] and Howard Grimes[3]

[1]Southern Illinois University, Cabondale, IL, USA; [2]Albrecht-von-Haller-Institute for Plant Sciences, University of Göttingen, Germany; and [3]School of Molecular Biosciences, Washington State University, Pullman, WA, USA

Mosses and ferns have substantial amounts of very long chain polyunsaturated fatty acids, such as arachidonic and eicosapentaenoic acids, which contain 20 carbons. Flowering plants, on the other hand, typically only produce fatty acids with up to 18 carbons, such as linolenic and linoleic acids, which give rise to angiosperm oxylipins, such as jasmonic acid, via the lipoxygenase pathway. Jasmonic acid has not yet been unambiguously detected in mosses, which suggests that they may have an oxylipin pathway different from those of angiosperms. To test this hypothesis, twelve putative lipoxygenase genes from *Physcomitrella patens* were functionally expressed in *E. coli* and crude bacterial lysates were analyzed for their substrate specificities and product profiles. Among the seven recombinant lipoxygenases that were found to be active, two used arachidonic acid as the preferred substrate while the other five preferred linolenic acid. All of the seven active lipoxygenases had optimum pH at 7.0, except for one with highest activity at pH 5.0. The two arachidonic acid lipoxygenases form 12(*S*)-hydroperoxy-eicosatetraenoic acid as the main product, while the remaining five mainly produce 13(*S*)-hydroperoxyoctadecanoic acid from linolenic acid. Since mosses seem to have both C20 and C18 based oxylipins, we propose that as the flowering plants lost the C20 oxylipins in evolution, the ancestral C18 oxylipin pathway evolved to produce the octadecanoids.

S11-5

Proanthocyanidin biosynthesis in transgenic tobacco plants: What molecular species is the precursor of extension units?

DeYu Xie, Patrick Walker, Christopher Zachary, and Lili Zhou

Department of Plant Biology, North Carolina State University, Raleigh, NC

Proanthocyanidins (also called condensed tannins) are polymeric and oligomeric flavan-3-ol units. They are potent antioxidant reagents having multiple beneficial effects on human health, such as protective activities against cardiovascular diseases. In addition, they prevent ruminant animals from bloat disease. As a result, metabolic engineering of PAs in food and forage crops is of agricultural significance for the purpose of developing value-added products. We here for the first time report the molecular characterization of engineered PAs synthesized by coupled PAP1 and ANR transgenic tobacco plants (Xie et al. 2006, Plant Journal, vol. 45, p. 895-907). Different oligomeric molecular fractions of PAs were isolated from transgenic tobacco leaf tissues using column chromatography separation and characterized using HPLC-MS based profiling. The main monomeric molecules included epicatechin and epiafzelechin. *Ent*-gallocatechin was also detected. The detected dimeric PAs candidates included epicatechin-epicatechin, epiafzelechin-epicatechin, and epicatechin-epigallocatechin. Epicatechin-4 beta-8 epicatechin (Procyanidin B2) was definitively identified as one of the main dimeric PA molecules made by transgenic plants. One candidate of identified trimeric PA molecules was predicted to be epicatechin-epicatechin-epicatechin. Our results suggest that epicatechin derived from the engineered PA pathway not only serves as a starter unit but is also a likely precursor of extension units. The biosynthesis and possible mechanism of PA formation in the engineered plants will be discussed in this presentation.

S11-6

The biofuel challenge: New roles for aromatic hydrocarbons?

Daniel G. Vassão, Sung-Jin Kim, Laurence B. Davin, and Norman G. Lewis

Institute of Biological Chemistry, Washington State University, Pullman, WA

Presumed declining petroleum reserves, allied to the growing impression that existing bioenergy approaches will not satisfy our future petrochemical needs, all indicate that different (novel) methodologies will be necessary to solve this oil crisis. Additionally, currently envisaged attempts fail to address our needs for other non-fuel petroleum-derived compounds, e.g. industrial polymer feedstocks such as styrene, which comprise >10% of current petroleum use.

We are now developing new approaches to help solve these problems, building on recent biochemical advances from our laboratory leading to the production of allyl/propenyl phenols, with isolation of genes encoding two crucial enzymes of this pathway. Together, these enzymes catalyze the conversion of monolignols, widespread compounds that also serve as lignin precursors, into the liquid and combustible allylphenols.

The enzymes, an acyltransferase and a reductase, have been further biochemically characterized and incorporated into bacteria and vascular plants for proof-of-concept studies. The final compounds are volatile liquids that should be separated easily, have relatively high heats of combustion, and are structural analogues of styrene, thus being potentially useful both as a (bio)fuel and in polymer industrial chemistry.

S11-6

NaturePOD™: A technology platform for the production of *Natural Products On Demand* using plant cell cultures

Mylavarapu Venkatramesh

Exelixis Plant Sciences, Portland, OR

Large scale plant cell culture technology is well suited to the development of value-added food and feed ingredients, nutraceuticals, fatty acids, and active phytochemicals. The primary challenges in the establishment of successful plant cell culture programs are the cost of production due to low and inconsistent productivity of plant cell cultures and the lack of regulatory guidelines for products derived from cell cultures. We have used methods such as metabolic engineering and biosynthetic elicitation to enhance productivity of several commonly used plant natural products and are working to scale up several plant cell cultures to validate the commercial viability of the platform. Enabling technologies for cell line selection, maintenance, and scale up, as well as for the metabolic engineering of pathways have been established at Exelixis Plant Sciences and will be described.

Poster Abstracts

PS1-1 (Graduate Student)

In planta metabolic engineering of isoprenoids

Patrick R. Arsenault,[1] Pamela J. Weathers,[1,2] and Kristen K. Wobbe[3]

[1]Dept. of Bio. and Biotech., Worcester Polytechnic Institute, Worcester, MA; [2]Arkansas Biosciences Institute, Arkansas State University, State College, AR; and [3]Dept of Chem. and Biochem., Worcester Polytechnic Institute, Worchester, MA

Isoprenoids constitute one of the largest classes of plant secondary metabolites. Additionally, they make up a large proportion of the economically and pharmaceutically valuable plant products. In particular, the sesquiterpene artemisinin from *Artemisia annua* is of particular importance as the primary treatment for the malaria parasite *Plasmodium falciparum,* with additional activity against a variety of cancers. The biosynthetic pathway is almost entirely known to this point and we have taken this as a model for understanding the benefits and limitations of isoprenoid metabolic engineering in plant systems. Currently we are focusing on two isoprenoid rich species, *Brassica oleracea* (kale) and *Dacus carrota* (carrot). The biosynthetic genes necessary to produce the biosynthetic precursors to artemisinin have been transformed into these two species to study their response to heterologous gene expression and to evaluate the potential for *in planta* metabolic engineering. Various constructs have been created to test different strategies for maximizing expression of transgenes and increasing pathway flux, including protein fusions, targeting sequences, and a novel self cleaving peptide. In addition, comparisons of shoot and root culture systems compared to whole plant systems are being explored. *In vitro* cultures of transformed plants and regeneration of full transgenics is on-going. High producing lines are also being identified using a variety of analytical methods including HPLC-UV and LC-MS.

PS1-2 (Graduate Student)

Accumulation pattern of methionine rich β-zein protein in *Medicago sativa* (alfalfa) and the related model legume *M. truncatula* in relation to their free methionine pools

F. Omar Holguin, Suman Bagga and Champa Sengupta-Gopalan

Dept. of Agronomy and Horticulture, New Mexico State University, Las Cruces, NM

Alfalfa (*Medicago sativa*) is an important forage legume providing quality protein and is low in methionine (Met), an important amino acid. A genetic engineering approach to increase the Met content of alfalfa is to express genes encoding for high Met protein. Seed storage proteins of corn, the β- (15kD) and δ- (10 and 18kD) zeins, are very high in Met and are ideal candidates for introduction into alfalfa. The β-zein gene engineered behind the CaMV 35S promoter was introduced in alfalfa and *Medicago truncatula*, a model legume. Our analysis of these transformants shows a ~10 fold higher level of accumulation of β-zein protein and transcript in the leaves of *M. truncatula* plants when compared to alfalfa β-zein expressors. Our hypothesis is that the two *Medicago* species differ with regards to the amino acid composition and in the rate of synthesis of Met rich proteins. A metabolomic approach was used to create a model on the effects of methionine synthesis and accumulation in legumes. Comparative metabolic profiling of soluble primary and secondary polar metabolites in *M. truncatula* and alfalfa was carried out by GC-MS, UPLC and LC-MS. These analyses revealed a number of metabolic characteristics which are discretely associated with the fate of Met between species. Particular interest was focused on those metabolites associated with methionine biosynthetic pathway. These differences reveal that improving the Met content in a forage legume will require increased Met levels at the cost of decreased levels of SAM to allow for the availability of Met for protein synthesis in *M. sativa*. An understanding of the basis for the differences between *M. sativa* and the model legume, *M. truncatula*, with regards to the accumulation of the Met-rich β-zein protein will allow us to increase the Met-containing proteins in *M. sativa* using genetic engineering approaches.

PS1-3 (Graduate Student)
Uncovering the role of ureide transport in whole plant physiology

Ray Collier and Mechthild Tegeder

School of Biological Sciences, Washington State University, Pullman, WA

The ureides, allantoin and allantoic acid, are the primary nitrogen (N) transport forms in tropical legumes such as *Glycine max* and *Phaseolus vulgaris.* Following atmospheric N_2 fixation through a plant-bacteria interaction in root-located nodules, allantoin and allantoic acid are synthesized and transported via the vasculature to N sinks (e.g. developing leaves, flowers and seeds) to fuel plant metabolism and development. Recent studies have found that allantoin is also an important phytochemical stimulating the growth of phyto-friendly bacteria in the rhizosphere. Additionally, it exhibits activity against the obligate plant parasitic nematodes, *Meloidogyne incognita* and *Heterodera glycines*, both of which are important pathogens of soybean. These discoveries underscore both the importance of allantoin and allantoic acid for plant growth and the paucity of knowledge about specific biochemical interactions within plants with respect to ureides. Efforts to understand the role ureides play in tropical legumes hinge on our overall hypothesis that allantoin and allantoic acid partitioning from nodules (source) to sink organs is controlled by specific transporters and that the activity of these transporters in specific cells is crucial for efficient N distribution and usage in legumes. Putative ureide transporters (UPSs) were isolated from common bean and soybean, and their role in allantoin transport was determined by heterologous complementation of yeast transport mutants expressing the UPSs. To understand their physiological function, the location of UPS activity was resolved and the effect of UPS repression on nodule development was examined using an RNAi strategy in composite soybean plants. Currently transgenic soybean plants overexpressing or repressing *UPS* are being screened for analysis of transporter function and interrelated processes.

PS1-4 (Graduate Student)
Determining secondary product glucosyltransferase expression during *Citrus paradisi* growth and development

Jala J. Daniel,[1] Daniel K. Owens,[2] and Cecilia A. McIntosh[1-3]

[1]Department of Physiology, [2] Department of Biological Sciences, and [3]School of Graduate Studies, East Tennessee State University, Johnson City, TN

Flavonoids are secondary metabolites that are not involved directly in cellular respiration and photosynthesis but have significant roles in plant defense and human nutrition. Glucosyltransferases (GT) transfer sugars from high-energy sugar donors to other substrates. Several different kinds of flavonoid GTs exist in the tissues of grapefruit, making it a model plant for studying their structure and function. The focus of the McIntosh laboratory is to understand how flavonoid GTs influence metabolism as well as elucidating structure and function. This has led to the isolation of 8 putative flavonoid GT clones. The goal of this investigation is to determine the expression patterns of the 8 putative GTs during grapefruit growth and development by quantifying mRNA expression levels in the roots, stems, leaves. This research is designed to test the hypothesis that these 8 GTs are expressed constitutively. Alternatively, one or more could be expressed in a tissue-specific manner or developmentally regulated. Five growth stages have been defined. The first stage focuses on emerging roots; stages 2-4 focuses on leaves, stems, and roots; and stage 5 focuses on comparison of older and younger leaves. RNA was isolated using a Qiagen RNeasy Plant Mini Kit. Unique primers for each putative GT have been designed that are at least 20 nucleotides in length with similar annealing temperatures. RT-PCR will be used to semi-quantitate mRNA expression and will include determination of the linear range of amplification so that all of the 8 GT's can be compared in a semi-quantitative analysis.

PS1-5 (Graduate Student)

Regulation of sterol biosynthesis in *Arabidopis thaliana*: Experimental testing of a first generation kinetic model

Maximilian Feldman, Rigoberto Rios-Estepa, Iris Lange, and Bernd M. Lange

Institute of Biological Chemistry, Washington State University, Pullman, WA

Sterols are important structural components of the plasma membrane in all eukaryotes. In addition, certain steroids (glucocorticoids, mineralocorticoids, androgens, progestagens and vitamin D in animals; brassinosteroids in plants) have vital functions as lipid hormones. For several decades, dietary plant sterols, also referred to as phytosterols, have been used successfully and safely for lowering plasma cholesterol levels (thus reducing a well-known risk factor for atherosclerosis) and the US Food and Drug Administration and the EU Scientific Committee have sanctioned them for use in foods. Many margarines, butters, breakfast cereals and spreads are now enriched with plant-derived sterols and their esters. Phytosterols occur in small amounts in vegetable oils (usually < 1% (w/w)). Given the significance of sterols in plants and for our diet, it is especially important to define the enzymatic steps that are most critical in regulating their biosynthesis, particularly with regard to enabling molecular breeding and/or metabolic engineering approaches aimed at increasing flux toward the production of specific target sterols.

Based on information available in the literature and our own experiments, we have developed a first-generation kinetic mathematical model of the sterol biosynthetic pathway in *Arabidopsis* in which we consider tissue-specific gene expression (obtained using oligonucleotide microarrays) and metabolite patterns (obtained using GC-MS measurements), as well as kinetic properties of biosynthetic enzymes. Our model was tested and refined with experimental data from seedlings, rosette leaves, stems and seeds, with plants of different genotypes grown under various environmental conditions. Our results demonstrate that the relative importance of individual sterol biosynthetic enzymes for determining sterol composition changes in a tissue-specific fashion, and the implications of our findings for modulating sterol yield and composition in transgenic plants are discussed.

PS1-6 (Graduate Student)

Heterologous expression and elucidation of biochemical function of two putative flavonoid glucosyltransferase clones (PGT2 and PGT3) from *Citrus paradisi*

M.V.K.P Siddhartha,[1] Daniel K. Owens,[1] and Cecilia A. McIntosh[1, 2]

[1]Dept. Biol. Sciences and [2]School of Graduate Studies, East Tennessee State University, Johnson City, TN

Flavonoids are a major group of plant secondary metabolic compounds which play a variety of roles for plants that include protection against UV light, pigmentation, and flavor, and which can be important for pollination and seed dispersal, antimicrobial and antifungal properties, sexual reproduction and pollen development, and cues for microbial symbiont colonization. Flavonoids are also known to have benefits to human health due to antioxidant and anticancer properties, for instance. Glucosylation is a prominent modification reaction in flavonoid biosynthesis, and these reactions are catalyzed by glucosyltransferases (GTs). *Citrus paradisi* is an excellent model system for studying flavonoid GTs as it is known to contain GTs capable of producing flavanone and flavone 7-O-glycosides, flavonol 3-O-glucosides, chalcone glucosides, and flavonol 7-O-glucosides. For example, 40-70% of the dry weight of very young *Citrus paradisi* fruit and leaves is due to a single bitter flavanone diglycoside, naringin. Current efforts are focused on cloning, expression and characterization of putative GTs from grapefruit. The research described here is designed to test the hypothesis that grapefruit PGT2 and PGT3 clones are flavonoid glucosyltransferases. Results from experiments designed to optimize heterologous expression of soluble protein by testing media composition, culture temperature, and induction duration will be presented. Once the expression of soluble protein is optimized, expressed protein will be screened for flavonoid GT activity using a suite of flavonoids. If activity is found, the expressed enzymes will be rigorously characterized. Information on strategies for obtaining additional putative GT clones from grapefruit and results from these efforts will also be presented.

PS1-7 (Graduate Student)

Towards determining the individual roles of arogenate dehydratase isoforms in phenylalanine biosynthesis

Oliver R. A. Corea,[1] Hong Yang,[1] Man-Ho Cho,[1] Rebecca L. Hood,[2] Mark A. Bernards,[2] Susanne E. Kohalmi,[2] Laurence B. Davin,[1] and Norman G. Lewis[1]

[1]Institute of Biological Chemistry, Washington State University, Pullman, WA, USA, and [2]Department of Biology, University of Western Ontario, London, Ontario, Canada

The final two steps of Phe biosynthesis can occur via either a phenylpyruvate or an arogenate intermediate, with each route being distinguished by its enzymology; the former employing prephenate dehydratase (PDT) and the latter arogenate dehydratase (ADT). Our recent biochemical characterization of six ADT isoforms in *Arabidopsis* was the first description of an ADT in any plant species. Since these enzymes are thought to perform the critical final enzymatic step in Phe biosynthesis in plants, it is likely that their expression patterns, catalytic activities, and properties of feedback regulation will have a major influence on Phe production (i.e. in keeping up with demands of Phe for protein synthesis as well as for secondary metabolism). In order to determine the contributions of each isoform, several lines of enquiry are being employed. Quantitative RT-PCR results indicate a potential difference in the tissue-specific regulation of ADT isoforms, with ADT4 and ADT5 apparently responding to different or additional transcription cues compared to the other four ADTs. Biochemical analysis also showed differences with respect to substrate preference and catalytic efficiency between isoforms. Further work is underway to determine the specific feedback regulation properties of each isoform, and, knockout lines have been generated for each ADT isoform in *Arabidopsis*, in order to assess the function of each isoform *in planta*. Together, these studies are helping to define the metabolic networks involving Phe metabolism and its regulation.

PS1-8 (Graduate Student)

Purification and characterization of recombinant mitochondrial and plastidial serine hydroxymethyltransferases from *Arabidopsis*

Kehan Sun, Yi Zhang, and Sanja Roje

Institute of Biological Chemistry, Washington State University, Pullman, WA

Serine hydroxymethyltransferases (SHMTs) are highly conserved, ubiquitous, pyridoxal 5'-phosphate (PLP)-dependent enzymes. As key enzymes in folate-mediated one-carbon (C1) metabolism, SHMTs reversibly transfer C1 units from serine to tetrahydrofolate (THF), forming glycine and 5,10-methylene-THF. SHMTs are the entry point into the C1 metabolism for C1 groups needed to synthesize methionine, *S*-adenosylmethionine, thymidylate, purines, and pantothenate.

Mitochondria, plastids, nuclei, and the cytosol contain their own isoforms of SHMT in plants. The sequence alignment of SHMT isoforms from the same organelle but different plant species shows more similarity than the alignment of SHMT isoforms from different organelles within the same species. Our central hypothesis is that different isoforms of SHMT are not redundant and that they play distinct roles in plant metabolism due to differences in their biochemical properties and in subcellular localization. We here present our progress in biochemical characterization of two mitochondrial SHMTs and one plastidial SHMT from *Arabidopsis*.

PS1-9 (Postdoctoral)

Flavin nucleotide metabolism: Understanding the biosynthesis and hydrolysis of FMN and FAD in plants

Renu Rawat, Francisco J. Sandoval, and Sanja Roje

Institute of Biological Chemistry, Washington State University, Pullman, WA

FMN and FAD are phosphorylated derivatives of riboflavin that are synthesized by the enzymes riboflavin kinase (EC 2.7.1.26) and FAD synthetase (EC 2.7.7.2) in the presence of ATP and Mg2+. These flavin nucleotides are the cofactors for scores of enzymes that participate in vital metabolic processes in all organisms. Despite the vital roles of FMN and FAD in metabolism, much remains to be learned about the enzymes that synthesize and hydrolyze these cofactors in plants. Our long-term goal is to uncover how plants maintain intracellular levels of FMN and FAD.

Toward the goal of advancing basic understanding of FMN and FAD homeostasis in plants, we are now investigating riboflavin kinases, FAD synthetases, FMN hydrolyses, and FAD pyrophosphatases from *Arabidopsis* and pea. Our results suggest that plants have sequence homologs of riboflavin kinases from bacteria and yeast and that they have a novel type of riboflavin kinase in organelles. Our results also show that plant organelles have FMN hydrolase and FAD pyrophosphatase activities. Here we present our progress in studying enzymes involved in synthesis and hydrolysis of flavin nucleotides in plants.

PS1-10 (Postdoctoral)

Identification, recombinant expression, and biochemical characterization of a flavonol 3-*O*-glucosyltransferase from *Citrus paradisi*

Daniel K. Owens and Cecilia A. McIntosh

Department of Biological Sciences, East Tennessee State University, Johnson City, TN

Glycosylation is a predominant flavonoid modification reaction affecting the solubility, stability, and subsequent availability of the compounds. Flavonoid glycosides affect taste characteristics in citrus making glucosyltransferases interesting targets for biotechnology applications. In this work, a *C. paradisi* glucosyltransferase gene was identified, cloned, and introduced into the pET recombinant protein expression system utilizing primers designed against a predicted flavonoid glucosyltransferase gene (AY519364) from *C. sinensis*. The encoded *C. paradisi* protein is 51.2 KD with a predicted Pi of 6.27 and is 94.5% identical to the *C. sinensis* homologue. The enzyme glycosylated only the flavonol aglycones quercetin (Km=67 µM; V_{max}=20.45 pKat/µg), kaempferol (Km=12 µM; V_{max}=11.63 pKat/µg), and myricetin (Km=33 µM; V_{max}=12.21 pKat/µg) among a number of tested compounds from various flavonoid subclasses. Glycosylation occurred at the 3 position within the flavonoid backbone based on HPLC and TLC analysis with certified reference compounds. The optimum pH was 7.5 with a pronounced buffer effect noted for reactions performed in Tris-HCl buffer. The enzyme is inhibited by the metal ions Cu^{2+}, Fe^{2+}, and Zn^{2+} as well as UDP (Ki=69.5 µM) which is a product of the reaction. Treatment of the enzyme with a variety of amino acid modifying compounds suggests that cysteine, histidine, arginine, tryptophan, and tyrosine residues are important for activity. The thoroughly established biochemical functions and characteristics for this *C. paradisi* flavonol 3-*O*-glucosyltransferase adds to the growing base of glucosyltranferase knowledge and will be utilized to further investigate structure and function relationships for these types of enzymes.

PS1-11 (Postdoctoral)

Contribution of CoA ligases to benzenoid biosynthesis in petunia flowers

Yasuhisa Kaminaga,[1] Antje Klempien,[1] Dinesh A. Nagegowda,[1] Christine M. Kish,[1] Irina Orlova,[1] Goro Taguchi,[2] Eran Pichersky[2] and Natalia Dudareva[1]

[1]Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN, and [2]Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI

Biosynthesis of benzoic acid from phenylalanine requires the shortening of the propyl side chain by two carbons, which can occur via either β-oxidative or nonoxidative pathways. The first step in the β-oxidative pathway is cinnamoyl-CoA formation which could be catalyzed by a member of the 4-coumarate: CoA ligase (4CL) family that converts a range of trans-cinnamic acid derivatives into the corresponding CoA thioesters. Using a functional genomic approach, we have identified two potential CoA-ligases from the *Petunia hybrida* petal-specific cDNA library. These proteins share only 25% identity at the amino acid level and both are highly expressed in petunia corollas. Biochemical characterization of the purified recombinant proteins revealed that one of these proteins (Ph4CL) has broad substrate specificity and represents a *bona fide* 4CL, while the other is a cinnamic acid CoA ligase (PhCNL) that requires potassium ion as univalent ion cofactor. RNAi suppression of *Ph4CL* did not affect the petunia scent profile, while a down-regulation of *PhCNL* resulted in a decrease in emission of benzylbenzoate, phenylethylbenzoate and methylbenzoate without affecting benzaldehyde formation. Subcellular localization studies showed that the first step of the β-oxidative pathway of benzoic acid biosynthesis from cinnamic acid occurs in the peroxisomes.

PS1-12 (Postdoctoral)

Seasonal and circadian variation in biflavonoid biosynthesis in *Araucaria angustifolia*

Lydia F. Yamaguchi and Massuo J. Kato

Chemical Institute, University of São Paulo, Brazil

The production of biflavonoids has been proposed to carry out by oxidative coupling mediate by a putative unknown peroxidase. Leaves of coniferae *Araucaria angustifolia* contain six major amentoflavone-type biflavonoids, including amentoflavone, ginkgetin, and tetra-*O*-methylamentoflavone. These compounds are reported to possess a variety of biological activities especially as antioxidants, which is related to their capacity to suppress singlet oxygen, lipoperoxidation, and DNA oxidation. An additional mode of action is related to the ability to quench transition metals involved in free radical formation.

Seasonal and circadian rhythm were observed in the conversion of apigenin to amentoflavone and ginkgetin using enzymes from leaves of *A. angustifolia*. The reaction was followed by quantification of products by LC/ESIMS analysis. The production maximum was observed to take place at night, during which the production was at least twice higher than basal concentration. In the summer the formation was higher, indicating that the biosynthesis regulated by the seasonal rhythm and light photoperiod cycle.

Financial support: Fapesp and CNPq

PS1-13 (Postdoctoral)

Molecular cloning, functional characterization and structural modeling of a Δ4,5-steroid 5β-reductase, a putative key enzyme in cardenolide biosynthesis, from *Arabidopsis thaliana* L.

Vanessa J. Herl,[1] Gabriele Fischer,[1] Veaceslav A. Reva,[2] Martin Stiebritz,[1] Yves A. Muller,[1] Frieder Muller-Uri[1] and Wolfgang Kreis[1]

[1]Lehrstuhl für Pharmazeutische Biologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany, and [2]Universitatea de Stat din Moldova

A full-length cDNA clone that encodes a Δ4,5-steroid 5β-reductase (*At5β-StR*), a member of the short-chain dehydrogenase/reductase (SDR) family, was isolated from *Arabidopsis thaliana* leaves. The reading frame of the *At5β-StR* gene is 1167 nucleotides corresponding to 388 amino acids (MW 44 202 Da). A *Sph*I/*Sal*I *At5β-StR* fragment was cloned into the pQE vector system and afterwards transformed into *E. coli* strain M15[pREP4]. The gene was functionally expressed and the recombinant His-tagged fusion protein was characterized. *K*m values and specific activities for putative substrates as well as for the co-substrate were determined. Substrates were only reduced to 5β-pregnane-3,20-dione and Δ5,6-steroids tested were not accepted as substrates. Transcription of the gene encoding *At5β-StR* could be enhanced by osmotic stress. Protein extracts prepared from *A. thaliana* leaves also contained Δ4,5-steroid 5β-reductase activity, and the *At5β-StR* sequence from *A. thaliana* shows about 70% sequence identity to progesterone 5β-reductases isolated from various *Digitalis* (incl. *Isoplexis*) species. A three-dimensional model of *At5β-StR* highlights its close structural similarity to the related *D. lanata* enzyme. This homology also extends to the active site where single amino acid substitutions might be responsible for the increased catalytic efficiency of *At5β-StR* when compared to the activity of the, e.g. recombinant form of the *D. lanata* enzyme.

PS1-14 (Postdoctoral)

The tocotrienol form of vitamin E in Apiaceae seeds results from the activity of a dicot-type homogentisate geranylgeranyl transferase

Yang Wenyu, Rebecca E. Cahoon, and Edgar B. Cahoon

Donald Danforth Plant Science Center, St. Louis, MO

The tocotrienol form of vitamin E occurs primarily in the seed endosperm of monocots, but it is also enriched in seeds of a limited number of dicots, including tobacco, grape, and members of the Apiaceae (or Umbelliferae) family. In Apiaceae seeds, tocotrienols, rather than tocopherols, can account for >90% of the vitamin E tocochromanols. We have previously demonstrated that tocotrienols in monocot seeds derive from homogentisate geranylgeranyl transferase (HGGT). This enzyme is a variant of homogentisate phytyltransferase (HPT) that mediates the initial step in tocopherol biosynthesis. Both enzymes catalyze the condensation of homogenisate with a C20 isoprenoid diphosphate, but HGGT, in contrast to HPT, is more active with geranylgeranyl-PP than with phytyl-PP. In this study, we have isolated a cDNA for a dicot-type HGGT from the Apiaceae coriander (*Coriandrum sativum*). The coriander HGGT appears to have evolved independently of monocot HGGTs. Interestingly, the coriander HGGT shares <50% amino acid sequence identity with monocot HGGTs, yet it has many of the conserved amino acids that distinguish monocot HGGTs from HPTs. The coriander HGGT is expressed almost exclusively in seed endosperm, and the *E. coli*-expressed enzyme is approximately 60-times more active with geranylgeranyl-PP than with phytyl-PP. Furthermore, expression of the coriander HGGT transgene in the *Arabidopsis* vitamin E-null *vte2-1* mutant was accompanied by the accumulation of tocotrienols and lesser amounts of tocopherols. Partial cDNAs for HGGT-like enzymes were amplified from other Apiacieae, suggesting that the HGGT is widely-occurring in this family. Overall, these results provide biochemical and genetic evidence for the independent evolution of a dicot-type HGGT in Apiaceae that accounts for the occurrence of the tocotrienols in seeds of these plants.

PS1-15 (Graduate Student)

Polyphenolic constituents in gambir

Naomi Yoshikado,[1] Shoko Taniguchi,[1] Kayo Kuroda,[1] Kou-ichi Doi,[2] Masahiro Tanabe,[2] Takashi Shibata,[2] Takashi Yoshida,[1,3] and Tsutomu Hatano[1]

[1]Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; [2]Taiko Pharmaceutical Co., Ltd; and [3]College of Pharmaceutical Sciences, Matsuyama University, Japan

Gambir, the aqueous extract from leaves and young twigs of *Uncaria gambir* (Rubiaceae), contains large amounts of polyphenols and has been used as an astringent natural medicine in Asian countries. We determined quantities of polyphenolic constituents, such as (+)-catechin, (+)-epicatechin, and gambiriins, in various gambir products by high-performance liquid chromatography (HPLC) and their total flavan contents by the vanillin-HCl method. We also applied gel permeation chromatography (GPC) to the estimation of the amounts of polymeric polyphenols in the polyphenols.

Futhermore, we isolated five new polyphenolic constituents from gambir and their calcane-flavan and proanthocyanidin structures were established. We also re-assigned the stereochemistry of four gambiriins. From the leaves of *Uncaria gambir*, we isolated two other polyphenolic constituents in addition to two catechin dimers and dehydrodicatechin A.

PS1-16 (Graduate Student)

Effect of tannins and related polyphenols on *Pseudomonas aeruginosa*

Maho Murasawa,[1] Hideyuki Ito,[1] Sumiko Shiota,[2] Tomofusa Tsuchiya,[3] and Tsutomu Hatano[1]

[1]Department of Pharmacognosy, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; [2]Department of Pathogenic Microbiology, School of Pharmacy, Shujitsu University; and [3]Department of Microbiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan

Pseudomonas aeruginosa is widely distributed in the natural environment. This bacterium causes great risk for the compromised hosts in hospitals and often shows acquired resistance against various antibiotics, in addition to intrinsic resistance. We previously reported that tannins and related polyphenols show antibacterial effects on various bacterial species, such as MRSA and Vibrio. We thus examined their antibacterial effects on *P. aeruginosa*. Among the tannins and related polyphenols used in this study, epigallocatechin gallate (EGCG) and octyl gallate showed antibacterial effects on this bacterium. Although the minimum inhibitory concentration (MIC) of EGCG, shown by the microdilution method, was 1024 μg/ml, it inhibited pigment production completely at 64 μg/ml. Hydrolyzable tannins, pentagalloylglucose, casuarictin, geraniin, tellimagrandin I, also inhibited pigment production in *P. aeruginosa* at 8-32 μg/ml. Although the MIC of octyl gallate was 64 μg/ml, it did not inhibit pigment production at the lower (<64 μg/ml) concentrations. Since the pigments in *P. aeruginosa* represented by pyocyanin have been correlated with its pathogenicity, tannins and related polyphenols may be useful for suppressing pathogenicity.

PS1-17 (Undergraduate Student)

Leaf development and cuticular wax composition in *Kalanchoe daigremontiana*

Hong Han, Clare van Maarseveen and Reinhard Jetter

Department of Botany, University of British Columbia, Vancouver BC, Canada

Kalanchoe daigremontiana (Mother-of-thousands), a member of the Crassulaceae, shows an interesting pattern of plantlet propagation and leaf development. Gas chromatography (GC) and mass spectrometry (MS) were applied to identify and quantify the various chemical compounds in wax extracted from leaves of this plant species. The cuticular wax mixtures were found to contain triterpenoids (predominantly glutinol, germanicol, friedelin) and very-long-chain fatty acid derivatives (predominantly C33 and C35 alkanes). The total wax coverage gradually increased for 50 – 60 d of leaf development and wax biosynthesis then ceased together with epidermal cell expansion. Triterpenoid concentrations decreased from 69% to 33% during the leaf developmental period monitored while very-long-chain fatty acid derivatives increased from 22% to 46%. The epi- and intracuticular fractions of cuticular wax, from adaxial and abaxial surfaces, were analyzed by combining mechanic wax removal and extraction techniques. Triterpenoids were evenly distributed between epi- and intracuticular layers on both sides. Very-long-chain fatty acid derivatives accumulated mostly in the epicuticular wax, especially on adaxial surfaces. The study of cuticular wax composition during leaf development, together with the layered arrangement of wax, provides essential information to further understand the biosynthesis of cuticular wax and its physiological and ecological implications.

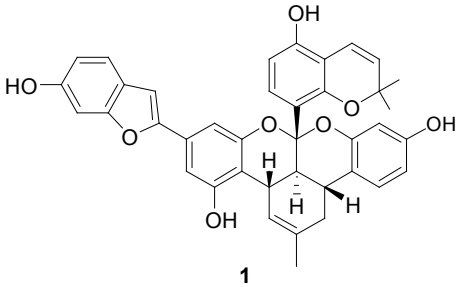
PS1-18 (Postdoctoral)

Sorocenol G and H, two new Diels-Alder type adducts from *Sorocea muriculata* roots with anti-MRSA activity

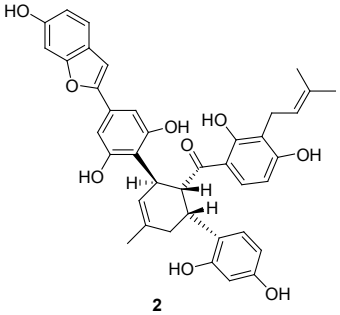
Mohamed M. Radwan,[1] Raquel Rodríguez-Guzmán,[1] Yuanqing Ding,[1] Xing-Cong Li,[1] Daneel Ferreira,[1,2] Susan P. Manly,[1] Samir A. Ross[1,2]

[1]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University, MS; [2]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS

Bioassay guided fractionation of the root extract of *Sorocea muriculata* led to the isolation of two new Diels-Alder type adducts, sorocenol G (**1**) and sorocenol H (**2**), along with oxyresveratrol, β-sitosterol, and lupeol-3-(3'-hydroxytetradecanoate). The structures were elucidated using 1D NMR, 2D NMR, GC/MS, and HRMS data and by comparison with reported data. The absolute configuration of **1** and **2** was established by the analysis of their experimental and theoretically calculated CD spectra**.** Compounds **1** and **2** showed significant and selective activity against MRSA (methicillin-resistant *Staphylococcus aureus*) with IC_{50} values of 1.5 and 0.5 μM, respectively. Compound **2** displayed antifungal activity against *C. neoformans* with an IC_{50} value of 5.1 μM.



1



2

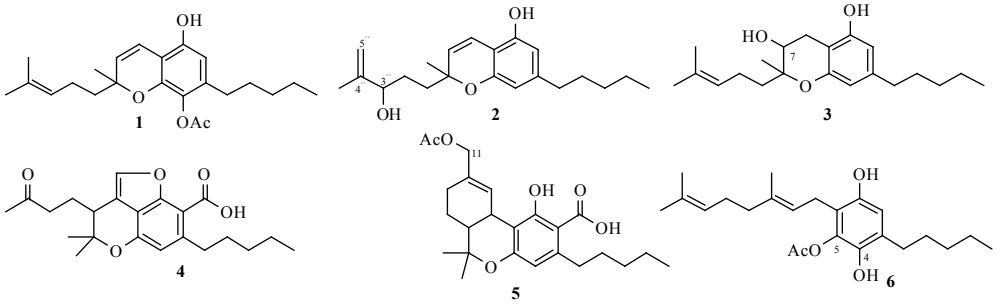
PS1-19 (Postdoctoral)

New bioactive metabolites from high potency *Cannabis sativa*

Mohamed M. Radwan,[1] Mahmoud A. ElSohly,[1,2] Desmond Slade,[1] Safwat A. Ahmed,[1] Ikhlas A. Khan[1,3] and Samir A. Ross[1,3]

[1]National Center for Natural Products Research, [2]Department of Pharmaceutics, and [3]Department of Pharmacognosy, School of Pharmacy, The University of Mississippi, University, MS

Six new metabolites (**1**-**6**) have been isolated from a high potency variety of *Cannabis sativa* L. The compounds were identified by means of HR-ES-IMS, GC-MS, UV and NMR spectroscopy as 4-acetoxycannabichromene (**1**), 3''-hydroxy-$\Delta^{(4'',5'')}$-cannabi-chromene (**2**), 7-hydroxycannabichromane (**3**), cannabicoumarononic acid A (**4**), 11-acetoxy-Δ^{9}-THC acid A (**5**) and 5-acetoxy-4-hydroxycannabigerol (**6**). The known sterol, β-sitosterol-3-*O*-β-D-glucopyranosyl-6'-acetate (**7**), was isolated for the first time from cannabis. The antimicrobial, antileishmanial and antimalarial activities of the isolates were evaluated. Compounds **1** and **6** exhibited moderate antimalarial activity, while **6** showed strong antileishmanial activity with an IC_{50} value of 4.0 ug/mL.



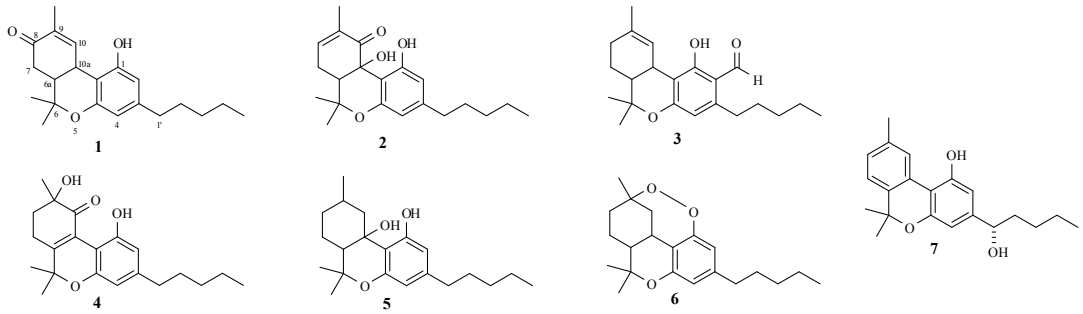
PS1-20 (Postdoctoral)

Minor oxygenated cannabinoids from high potency *Cannabis sativa* L.

Safwat A. Ahmed,[1] Samir A. Ross,[1,2] Desmond Slade,[1] Mohamed M. Radwan,[1] Ikhlas A. Khan[1,2] and Mahmoud A. ElSohly[1,3]

[1]National Center for Natural Products Research, [2]Department of Pharmacognosy, and [3]Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University, MS

Seven new oxygenated cannabinoids (**1**-**7**) were isolated from a high potency *Cannabis sativa* L. variety. Structure elucidation was achieved using spectroscopic techniques, including 1D and 2D NMR, HRMS and GCMS. These minor compounds include four tetra- (**1**-**4**), two hexahydrocannabinols (**5**-**6**) and one hydroxylated cannabinol (**7**), namely 8-oxo-Δ^{9}-THC (**1**), 10a-hydroxy-10-oxo-Δ^{8}-THC (**2**), Δ^{9}-THC aldehyde A (**3**), 9-hydroxy-10-oxo-$\Delta^{6a,10a}$-THC (**4**), 10a-hydroxyhexahydrocannabinol (**5**), 1,9-peroxyhexahydrocannabinol (**6**) and (*S*)-1′-hydroxycannabinol (**7**).



PS1-21 (Graduate Student)

Isolation and identification of antiadhesive urinary metabolites from cranberry juice

Christina M. Coleman,[1] Daneel Ferreira,[1] Amy B. Howell,[2] Jess D. Reed,[3] Christian G. Krueger[3] and Jannie P. J. Marais[4]

[1]Department of Pharmacognosy, The University of Mississippi, University, MS; [2]Marucci Blueberry and Cranberry Research and Extension Center, Rutgers University, Chatsworth, NJ; [3]Dept. of Animal Science, University of Wisconsin-Madison, Madison, WI; and [4]National Center for Natural Products Research, The University of Mississippi, University, MS

High molecular weight components of cranberry juice, specifically proanthocyanidin oligomers (PACs) which contain at least one A-type linkage, are believed to be responsible for the ability of cranberry juice (*Vaccinium macrocarpon*) to prevent urinary tract infections (UTIs). These and other high molecular weight fractions from cranberry juice have been shown to prevent the adherence of P-fimbriated uropathogenic bacteria to uroepithelial cells *in vitro.* PACs are therefore believed to be able to prevent UTIs by preventing the adherence of such bacteria to the cells lining the urinary tract. While preliminary data suggests that administration of PACs results in anti-adherence activity of urine following ingestion by both humans and swine, the active urinary metabolites from cranberry juice are currently unidentified and many questions exist regarding the potential for intact PACs to reach the urine *in vivo*. An adult female sow was therefore fed approximately 5 g lyophilized cranberry juice powder/kg/day for three days prior to collection of urine via catheter. Feeding and collection was continued for a week. A human RBC agglutination bioassay with uropathogenic P-fimbriated *E. coli* was used to identify bioactive urine fractions. Active compounds were isolated using Sephadex LH-20 as well as C-18 and HILIC HPLC, among other techniques. Identification and structural characterization were performed using NMR, MS and other spectroscopic methods. Current evidence indicates that the bioactive components may be related to oligosaccharides or amino sugars and are not obviously derived from proanthocyanidins. Information on the isolation and structural characterization of the potential cranberry juice bioactive metabolites will be presented.

PS1-22

Cranberry juice compounds with anti-adhesive properties in urine

Christina M. Coleman,[1] Daneel Ferreira,[1] Jannie P.J. Marais,[2] Amy B. Howell,[3] Ronald Cihlar,[4] and Jesse Cohen[4]

[1]Department of Pharmacognosy, The University of Mississippi, University, MS; [2]National Center for Natural Products Research, University of Mississippi, University, MS; [3]Marucci Blueberry and Cranberry Research and Extension Center, Rutgers University, Chatsworth, NJ; and [4]Department of Microbiology and Immunology, Georgetown University, Washington, DC

Cranberry juice is often used as a dietary supplement for its antioxidant properties and its ability to help prevent urinary tract infections (UTIs). It is widely believed that the major bioactive constituents of cranberry juice are flavonoids, such as condensed tannins and anthocyanins, as these compounds have well known antioxidant properties. Proanthocyanidins (PACs), a class of condensed tannins, are currently believed to be responsible for the ability of cranberry juice to prevent UTIs. While PACs have been shown *in vitro* to prevent uropathogenic P-fimbriated *E. coli* from binding to surfaces and tissues, these compounds may not be directly responsible for the ability of cranberry juice to prevent UTIs. To date, neither PACs nor closely related metabolites with anti-adhesion bioactivity have been found in urine after administration of cranberry juice. In spite of the apparent absence of PACs, urine of pigs fed cranberry juice powder has similar anti-adhesive properties as crude PAC preparations or cranberry juice fractions. Preliminary analysis of cranberry derived urine indicates that the bioactive constituents present in the urine are sugar-related, highly polar, high molecular weight compounds. These observations as well as others (see additional poster, this meeting) suggest that additional compounds may be present in cranberry juice, such as oligosaccharides or amino sugars, which may have the ability to prevent *E. coli* adhesion in the urinary tract. This presentation will therefore address ongoing fractionation of cranberry juice in an effort to identify such compounds or their related metabolic precursors.

PS1-23 (Graduate Student)

High throughput profiling of fruits for phytochemicals related to human health

Daniel J. Cuthbertson,[1] Jaime A. Yáñez,[4] Connie M. Remsberg,[4] Karina R. Vega-Villa,[4] Jody K. Takemoto,[4] Yusuke Ohgami,[4] Margaret L. Collier,[2] Preston K. Andrews,[2] John P. Reganold,[3] Neal M. Davies,[4] and Bernd M. Lange[1]

[1]Institute of Biological Chemistry, [2]Department of Horticulture and Landscape Architecture, [3]Department of Crop and Soil Sciences, and [4]College of Pharmacy, Washington State University, Pullman, WA

Consumption of fruits and vegetables has been strongly associated with reduced risk of some of the leading causes of deaths – heart disease, cancer and stroke – in industrialized nations. Thus, fruits and vegetables, which contain significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition. Several thousand phytochemicals have been identified in fruits and vegetables, but the majority remains uncharacterized both in terms of chemical structure and biological activity. Among the most prominent phytochemicals with demonstrated health-promoting effects are polyphenols (antioxidants that can prevent diseases such as cancer and coronary heart disease), lignans (phytoestrogens that have been implicated in regulating cholesterol levels, reducing the risk of certain cancers, and maintaining proper bone density post-menopause), and essential vitamins. Apples and raspberries are known to be rich in vitamins (e.g., vitamin C and folate) and polyphenolic phytochemicals with strong antioxidant activities. We have developed analytical chemistry methods to profile hundreds of phytochemicals in parallel. These complex profiles can be used to distinguish different apple varieties and, in some cases, the agricultural practices used for fruit production. Selected phytochemicals from fruit samples were assayed for antioxidant, anti-cancer and anti-adipogenic activities to test for correlations between phytochemical composition and health-promoting activities. The opportunities of utilizing our metabolomics-based integrated screening approach for discovering previously unknown health-related phytochemicals are discussed.

PS1-24 (Graduate Student)

Synthesis of Salvinorin B acid, a putative biosynthetic precursor of Salvinorin A

Lukasz Kutrzeba[1], Ruslan V. Bikbulatov[1], Joseph E. Rittiner[3], Bryan L. Roth[3], and Jordan K. Zjawiony[1,2*]

[1]Department of Pharmacognosy, and [2]National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS; [3]Department of Pharmacology, School of Medicine and Division of Medicinal Chemistry and Natural Products, School of Pharmacy, NIMH Psychoactive Drug Screening Program, University of North Carolina, Chapel Hill, NC

Salvinorin A **(1)**, a potent hallucinogen isolated from the Mexican mint *Salvia divinorum*, is a highly selective kappa-opioid receptor (κOR) agonist. 1 Biosynthesis of this neoclerodane diterpenoid proceeds via non-mevalonate pathway. 2 In the course of determination of individual steps of the pathway, we focused on characterization of two enzymes involved in the process of salvinorin A biosynthesis, carboxyl methyltransferase (CMT) and acetyl transferase (ACT) (Scheme 1). We propose that salvinorin B acid **(3a)** is a biosynthetic precursor for salvinorin A. For the purpose of this project, salvinorin A acid **(2a)** was obtained from **(1)** by reaction with LiI in pyridine. **(2a)** was then subsequently deacetylated in basic conditions (NaHCO3 in MeOH) to give mixture of salvinorin B acid and its C-8 epimer **(3b)** in 65% and 25% yield, respectively (Scheme 2). Both compounds **(3a)** and **(3b)** were characterized by 1D and 2D NMR, IR, HRMS and optical rotation. Biological activities of acids **(3a)** and **(3b)** were evaluated in vitro by κOR binding assay.

PS2-1

Biosynthesis of macrocyclic bisbibenzyl marchantin A from liverwort *Marchantia polymorpha*

Hironobu Takahashi, Saki Nagaoka, Masao Toyota and Yoshinori Asakawa

Institute of Pharmacognosy, Tokushima Bunri University, Tokushima, Japan

Marchantin A, the first characterized member of a large number of cyclic bis(bibenzyls) found in the liverwort *Marchantia polymorpha* L., shows interesting biological activities such as antifungal, antimicrobial, cytotoxicity, 5-lipooxigenase, cyclooxygenase inhibitory, and antioxidant activities. The biosynthetic pathway to marchantin A is presumed to have the two same or different bibenzyl moieties condensed by oxidative phenol coupling. Previously, Zenk *et al*. reported the biosynthetic pathway to marchantin A and C. Starting with plenylalanine, through the action of PAL, this aromatic amino acid is converted to cinnamic acid and further hydroxylated to *p*-coumaric acid, followed by reduction to dihydro-*p*-coumaric acid. An activated CoA ester condenses with three molecules of malonyl CoA to form prelunularic acid and aromatization yields lunularic acid. Two molecules of lunularic acid condense to yield marchantin C, followed by hydroxylation to marchantin A. The mechanism of this most important final coupling step is still unknown. The aim of this study is to investigate the all genes involved in the biosynthesis of marchantin A. We assembled EST data of *M. polymorpha* to get 5000 contigs and found a few full length genes. One PAL gene was cloned and the recombinant protein characterized via kinetics and physical parameters.

PS2-2

Role of the paraveinal mesophyll during vegetative storage protein accumulation and mobilization in soybean leaves

Glenn W. Turner,[1] Daniel J. Cuthbertson,[1] Siau Sie Voo,[1] Howard D. Grimes,[2] and Bernd M. Lange[1]

[1]Institute of Biological Chemistry and [2]School of Molecular Biosciences and Graduate School, Washington State University, Pullman, WA

Soybean seeds are rich in high value proteins. It has been estimated that more than 60% of the nitrogen of soybean seed storage proteins is initially metabolized in vegetative tissues prior to remobilization during seed development. This nitrogen remobilization can be disrupted by pod removal or vegetative bud removal, both of which result in a large accumulation of storage proteins within leaves. A period of vegetative bud removal (detipping) followed by a regrowth phase has been employed by several laboratories studying accumulation and subsequent mobilization of vegetative storage proteins in soybean. Our research focuses on the putative role of the paraveinal mesophyll (PVM) during this process. The PVM consists of a layer of branched but laterally elongated cells between the palisade and spongy mesophyll layers, connecting directly to these chlorenchyma tissues and to bundle sheath cells of vascular bundles. PVM cells can be distinguished from mesophyll chlorenchyma by their position, small plastids, and their strong accumulation of vacuolar proteins during bud removal. We have employed microarray analysis to evaluate transcription patterns in leaves harvested throughout a typical debudding-regrowth time-course, coupled with immunocytochemical localizations of key proteins related to PVM function. In addition, we have assessed the correlation of storage protein synthesis with dynamic changes in the pools of soluble amino acids. A model describing the current knowledge of PVM function will be presented.

PS2-3
Taxol biosynthesis: Two new acetyltransferases

Christopher J. D. Mau, Daniela Hampel, and Rodney B. Croteau

Institute of Biological Chemistry, Washington State University, Pullman, WA

Biosynthesis of the diterpenoid anti-cancer drug Taxol (generic name = paclitaxel) includes at least 19 enzymatic steps starting from geranylgeranyl diphosphate. However, the biologically active compound is just one of about 400 characterized taxane diterpenoids (taxoids) in various yew (*Taxus*) species. Taxoids which are not thought to be biosynthetic intermediates to Taxol often possess different acyl/aroyl modifications than those found in Taxol. We are searching a collection of *T. cuspidata* cDNAs for acyl- and aroyl-transferase activities that produce undesirable biologically inactive taxoids. We report the characterization of two new acetyltransferases which catalyze the transfer of two acetyl groups from acetyl CoA to the 9α- and 10β-hydroxyl groups on the functionalized taxane skeleton.

PS2-4
Generation and analysis of expressed sequence tags from the root tissues of kudzu (*Pueraria lobata*), a medicinal plant

XianZhi He, Jack W. Blount, Ji He, Patrick Zhao and Richard A. Dixon

Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK

The kudzu plant (*Pueraria lobata*) is a rich source of isoflavones and their glucosides, especially the *C*-glycosides. Its dried roots have long been widely used in traditional medicine. Although there are many reports on the isolation and analysis of kudzu plant secondary metabolites, there are few reports on the mechanisms of synthesis of the kudzu isoflavones and their glucosides at the molecular level. At this time there is no EST and genomic information reported on this medicinal plant. In this report we have generated 6,365 high-quality ESTs (average 538 bp in length) from the sequences of 7,466 randomly selected clones from a root subtraction cDNA library. The ESTs were clustered into 722 TCs and 3,913 singletons by BLAST analysis. In these 4,635 apparent unigenes, 2,023 (43.6%) were assigned to known functions and 2,612 (56.3%) do not have predicted functions. From these ESTs, 16 UDP-glycosyltransferases were identified and 9 of them have been cloned into a protein expression vector for functional analysis. One of the GTs preferred to glycosylate isoflavones such as daidzein, genistein and 6,7,4'-trihydroxyisoflavone to produce the corresponding 7-*O*-glucosides. Further characterization of these GTs is underway. We are particularly interested in the enzyme catalyzing the *C*-glycosylation of daidzein to produce puerarin, a compound with several potentially useful therapeutic properties.

PS2-5

Inhibitory interactions of quercetins against dominant glutathione *S*-transferases in onion bulb

Masayuki Fujita, M. M. Rohman and Toshisada Suzuki

Department of Plant Sciences, Faculty of Agriculture, Kagawa University, Japan

We investigated the interactions between quercetins and onion bulb glutathione *S*-transferases (GSTs), since onion is a rich source of quercetin compounds as well as sulfur compounds and since we found a high level of GST activity in the onion bulb compared to activity levels in some other vegetables in our previous studies. To examine the interactions of component GSTs, we separated five onion bulb GSTs (GSTa and GSTb as minor and GSTc, GSTd and GSTe as dominant GSTs) by DEAE-cellulose chromatography, and we tested the inhibitory effects of some precursors and glucosides of quercetin on activities of the dominant GSTs. GSTc and GSTd were strongly inhibited by the compounds, whereas the activity of GSTe was lessly sensitive to such inhibition. Among the precursors of quercetin, kaempferol, apigenin and luteolin strongly inhibited the activity of GSTc with IC_{50} values of 15.5, 33.0 and 27.0 μM, respectively and inhibited the activity of GSTd with IC_{50} values of 18.8, 32.8 and 34.0 μM, respectively. Chalcone, taxifolin and naringenin only slightly inhibited the activities of the GSTs. In the case of glucosides, quercetin-4'-glucoside showed the strongest inhibitory effect on the activities of GSTc and GSTd with IC_{50} values of 8.6 and 7.1 μM, respectively. The IC_{50} values of quercetin, quercetin-3βD-glucoside and quercetin-3,4'-diglucoside were 21.1, 76.3 and 76.0 μM, respectively, for GSTc and 20.4, 69.3 and 67.7 μM, respectively, for GSTd. Quercetin-4'-glucoside and quercetin-3,4'-diglucoside were identified as major substances inhibiting the activities of dominant GSTs in the onion bulb.

PS2-6

Using graph theory models to predict secondary product glucosyltransferase function

Debra Knisley,[1,2] Edith Seier,[1,2] Daniel Lamb,[1] Daniel K. Owens,[3] and Cecilia McIntosh[2,3]

*[1]Dept. Math., [2]Inst. Quantitative Biol., and [3]Dept. Biol. Sci., East Tennessee State University, Johnson City, TN *Current address, Blue Cross Blue Shield of TN*

Graph theory has been applied for prediction of macromolecule structure, e.g., RNA. One goal of this research is exploring application of graph theory for use of primary structure to narrow the prediction of enzyme function. Plant secondary product glucosyltransferases (GTs), specifically flavonoid GTs, are being used as the model proteins. These GTs catalyze transfer of glucose from UDP-glucose to a wide variety of secondary metabolites. Although primary structure information is available for many putative GTs through genomic and proteomic databases, a direct biochemical link between structure and function exists for only a subset of the proteins. Plant secondary product GTs contain a highly conserved signature motif known as the PSPG box; sequences vary greatly outside of this region. For example, alignment of flavonoid 3-O-GTs from *Vitis vinifera, Ipomoea purpurea* and *Perilla frutescens* show 66-75% amino acid identity within the PSPG box but only 25-31% identity overall. Therefore, primary structure alone is not a good predictor of substrate usage or specificity. We present a graph-theoretic model of 27GTs with known substrate usage, 3 of which are differentiated as having activity with non-flavonoid substrates. Highly conserved regions in the putative acceptor binding domain as well as the PSPG motif are reduced to a single vertex in order to quantify the remaining parts of the sequence. Predicted secondary structures for remaining parts of the sequence are used to generate a weighted graph and graph-theoretic measures calculated. The model reveals a predicted additional structural motif differentiating the GT's known to have activity with non-flavonoid substrates. The model is being tested and refined to look for additional discriminating characters.

PS2-7

Homogenization of phenotypic variation in phenolics within hybrid oaks (*Quercus grisea* x *Q. gambelii*)

Christopher T. Yarnes,[1] William J. Boecklen,[2] Kati Tuominen,[3] and Juha-Pekka Salminen[3]

[1]Biology Dept; Emory & Henry College, Emory, VA, USA; [2]Dept. of Biology, New Mexico State University, Las Cruces, NM, USA; and [3]Dept. of Chemistry, University of Turku, Finland

The hybridization of plants may affect ontogenetic patterns of phenolic biosynthesis with significant consequences for communities of consumers. We describe the seasonal variation of absolute and relative concentrations of 18 individual phenolics, total proanthocyanidins, and nitrogen in the *Quercus grisea x Q. gambelii* hybrid complex in central New Mexico. Expression patterns of individual compounds were most often equal between hybrid and parental taxonomic categories; non-equal patterns of expression were most often dominant (equal to one parent) toward *Q. gambelii*. These patterns of phenolic expression contrast against the more common additive patterns reported in hybrid zones of other tree species. *Q. grisea* backcrosses displayed significant developmental instability in phenolic biosynthesis relative to other hybrid oaks. Importantly, the composition and structure of phenolic phenotypes were found to vary seasonally. Throughout the growing season, the majority of variation between oak phenolic phenotypes was attributed to the relative concentrations of phenolics and nitrogen, > 93% of the total variation. The results of this study emphasize the importance of compound-specific evaluations of hybrid plant defense metabolites and metabolic variation in hybrid zones. These considerations are critical for the further empirical study of potential hybridization effects on the complex, dynamic mixtures that comprise plant defense chemistry.

PS2-8

Two poplar methyl salicylate esterases display comparable biochemical properties but divergent expression patterns

Nan Zhao,[1] Ju Guan,[1] Farhad Forouhar,[2] Timothy J. Tschaplinski,[3] Zong-Ming Cheng,[1] Liang Tong,[2] and Feng Chen[1]

[1]Department of Plant Sciences, University of Tennessee, Knoxville, TN; [2]Department of Biological Sciences, Columbia University, New York, NY; and [3]Oak Ridge National Laboratory, Oak Ridge, TN

The salicylic acid (SA) signaling pathway is critical for activating plant defenses against pathogen infections. *SABP2*, which encodes methyl salicylate (MeSA) esterase, was recently isolated from tobacco and demonstrated to be a pivotal component of the SA signaling pathway. To understand SA-mediated plant defenses in perennial woody species, two genes encoding proteins of 98% sequence identity that are highly homologous to tobacco SABP2 were identified and cloned from poplar. Proteins encoded by these two genes displayed specific esterase activity towards MeSA. They are therefore named PtSABP2-1 and PtSABP2-2, respectively. Recombinant PtSABP2-1 and PtSABP2-2 exhibited apparent Km values of 68.2 μM and 24.6 μM with MeSA, respectively. Structural modeling using the three dimensional structure of tobacco SABP2 as a template revealed that the active sites of PtSABP2-1 and PtSABP2-2 are similar to that of tobacco SABP2. Under normal growing conditions, *PtSABP2-1* showed the highest level of expression in leaves and *PtSABP2-2* was most highly expressed in roots. In leaf tissues of poplar plants under stress conditions, the expression of *PtSABP2-1* was up-regulated by a number of stress factors whereas the expression of *PtSABP2-2* was not significantly affected. *PtSABP2-1* and *PtSABP2-2* are tandem repeats, suggesting they were the consequence of a local gene duplication. A high degree of variation in their promoter regions implies that the divergence of the biological roles of the two genes is controlled at the gene expression level.

PS2-9

Cranberry proanthocyanidin complexes with bovine serum albumin attenuate the bacterial lipopolysaccharide induced expression of iNOS and COX-2 in raw 264.7 macrophages

Sergio Madrigal-Carballo,[1,2] Christian G. Krueger,[1] Linda G. Haas,[1] and Jess D. Reed[1]

[1]Dept. of Animal Sciences, University of Wisconsin-Madison, Madison, WI, and [2]National University, Heredia, Costa Rica

Tannins bind proteins through hydrogen bonding or hydrophobic interactions, forming soluble or precipitated complexes. We applied MALDI-TOF mass spectrometry to characterize complexes of cranberry proanthocyanidins (PAC) and bovine serum albumin (BSA) and tested the effects of these complexes in a cell culture model of inflammation. The solutions of BSA (Type V fatty acid free) and cranberry PAC (isolated from a preparative LH-20 column) were mixed for 1 h in different PAC-BSA ratios; aqueous acetic acid 1% v/v was used for dilutions. Analyses were performed on a Bruker Reflex III TOF mass spectrometer (Bruker, Billerica, MA). The mass spectra were acquired in linear mode over a mass range of 60-120 kDa for analyses of PAC-BSA complex and in reflectron mode for direct analyses of cranberry PAC over a mass range of 500-5000 Da. Cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) are associated with inflammation. Activated RAW 264.7 macrophages increase expression of COX-2 and iNOS in response to bacterial infection and inflammation. Macrophages actively endocytose BSA and PAC-BSA complex. We therefore tested the ability of the PAC-BSA complex to attenuate COX-2 and iNOS expression in LPS stimulated macrophages. Increasing the amount of PAC in the BSA complex caused a dose-dependent attenuation of iNOS and COX-2 expression in LPS stimulated macrophages. A 1:10 wt:wt ratio of PAC to BSA almost completely attenuated the LPS induced expression of iNOS and COX-2. We hypothesized that the BSA would block the PAC effect because complex formation would prevent PAC binding to the macrophage. However, we obtained the opposite result because the PAC-BSA complex is more active than PAC alone, suggesting that the macrophage response to LPS occurs after endocytosis of the PAC-BSA complex.

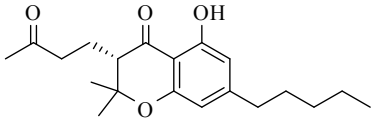
PS2-10

Absolute configuration of cannabichromanone derivatives from high potency *Cannabis sativa*

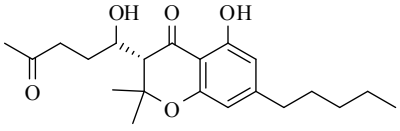
Safwat A. Ahmed,[1] Samir A. Ross,[1,2] Desmond Slade,[1] Mohamed M. Radwan,[1] Ikhlas A. Khan,[1,2] and Mahmoud A. ElSohly[1,3]

[1]National Center for Natural Products Research, [2]Department of Pharmacognosy, and [3]Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University, MS

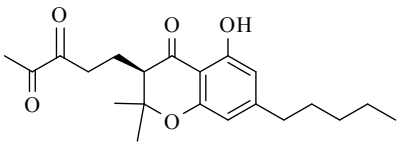
Three new cannabichromanone derivatives (**2**-**4**) were isolated from a high potency *Cannabis sativa* L. variety, along with the known cannabichromanone (**1**). Full spectroscopic data, as well as the use of electronic circular dichroism and Mosher ester analysis to determine the absolute configuration of these compounds, are reported. All isolates were tested for antimicrobial, antimalarial, antioxidant and antileishmanial activity.



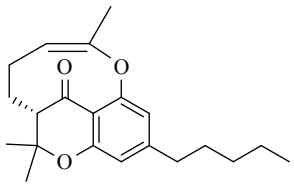
1



2



3



4

PS2-11

Oligomerization of procyanidins by condensation with cinnamaldehyde in cinnamon bark

Yosuke Matsuo, Takashi Tanaka, Yuko Yamada, and Isao Kouno

Dept. of Natural Product Chemistry, Nagasaki University, Japan

Cinnamon bark is an important spice used worldwide. It contains cinnamaldehyde, an essential oil with a conjugated aldehyde unit, and procyanidins mainly composed of (-)-epicatechin units. In this study, we demonstrated that cinnamaldehyde reacted with catechins and procyanidins at room temperature to generate condensation products including red pigments. The reaction of cinnamaldehyde with (+)-catechin yielded a complex mixture of products. Among them, four were isolated and their structures were determined by spectroscopic methods. One had two phenylpropanoid units at the C-6 and C-8 positions of the catechin skeleton. Oxidative degradation of this product yielded a red pigment with a benzopyrylium chromophore. Production of this pigment probably explained the dynamic color change of bark from white to red when it was peeled off from fresh branch. The remaining three products were isomers with two catechin and two phenylpropanoid units. The products evidenced oligomerization of catechins by relation with cinnamaldehyde. Reaction of cinnamaldehyde with procyanidin B1 also yielded condensation products. Matrix-assisted laser desorption time-of-flight mass spectral analysis of the reaction products suggested that procyanidins were oligomerized in a manner similar to the reaction with catechin. Furthermore, ^{13}C-NMR spectral comparison of the condensation products with the polymeric procyanidins obtained from commercial cinnamon bark strongly suggested that the procyanidins in the cinnamon bark were also polymerized by reaction with cinnamaldehyde.

PS2-12

Application of solid state NMR relaxation parameters in the characterisation of *Pinus radiata* pine bark constituents

Warren Grigsby, Stefan Hill, and Kirk Torr

Biomaterials Engineering Group, Scion, Rotorua, New Zealand

The composition of tree bark will vary from the base through to the canopy of the tree due to its age and thickness. We have been investigating the composition of bark obtained from *Pinus radiata* and its potential significance to industrial applications of bark extracts. We have applied ^{13}C CP/MAS NMR techniques to analyse a series of samples evaluating both tree height and geographic location within New Zealand. Generally the proportions of bark components change with increasing tree height with relatively minor influences due to geographic location. However, the use of the NMR relaxation parameter $T_{1\rho}H$ has also established differences in relative molecular motions within samples. These results are consistent with a greater degree of polymerisation of components at the base of the tree compared with bark higher up the tree. NMR analysis has been further applied to extractable components comparing extracts and extracted bark residues.

PS2-13

Kaempferol 3-*O*-(4‴-*O*-acetylrutinoside), a new flavonoid from the fern *Dryopteris villarii*

Filippo Imperato, Felicia Viggiani, Filomena Lelario, Angela Ostuni, and Antonello Lo Vecchio

Dipartimento di Chimica, Università della Basilicata, Potenza, Italy

From an ethanolic extract of the aerial parts of the fern *Dryopteris villarii,* a new flavonoid (**1**) and two known flavonoids (**2** and **3**) have been isolated by preparative paper chromatography followed by column chromatography on Sephadex-LH-20 and preparative RP HPLC. The new product (**1**) has been identified as Kaempferol 3-*O*-(4‴-*O*-acetylrutinoside) by UV spectral analysis with the customary shift reagents, total acid hydrolysis (which gave kaempferol, D-glucose and L-rhamnose), controlled acid hydrolysis (which gave kaempferol, D-glucose, L-rhamnose and rutinose (*O*-α-L-rhamnosyl-(1→6)-D-glucose)), electrospray mass spectrum (which showed a pseudomolecular ion at m/z 659 $[M+Na]^+$, a molecular ion at m/z 636 and fragment ions at m/z 449 and 287), ^1H and ^{13}C NMR spectra, and ^1H,^{13}C –COSY. A kaempferol 3-*O*-(acetylrutinoside) has previously been reported from plants but the position of acetyl group has not been established. In addition, it has been shown by HPLC analysis that this product is different from flavonoid (**1**). Flavonoids **2** and **3** have been identified as chrysin 3-*O*-rutinoside and kaempferol 3-*O* rhamnoside 7-*O*-glucoside respectively by UV spectral analysis with the customary shift reagents, total acid hydrolysid, controlled acid hydrolysis and electrospray mass spectra. Chrysin 3-*O*-rutinoside (**2**) is a new constituent of ferns whereas kaempferol 3-*O*-rhamnoside 7-*O*-glucoside (**3**) has been found previously in aerial parts of fern species *Asplenium bulbiferum* and *A. kaulfussii*.

PS2-13

Apigenin 7-*O*-glucoside 4′-acetate, a new flavonoid from the fern *Dryopteris villarii*

Filippo Imperato

Dipartimento di Chimica, Università della Basilicata, Potenza, Italy

From an ethanolic extract of aerial parts of the fern *Dryopteris villarii* (Bellardi), a new flavonoid (**1**) together with two known flavonoids (**2** and **3**) have been isolated by preparative paper chromatography followed by column chromatography on Sephadex LH-20. The flavonoid (**1**) has been identified as apigenin 7-*O*-glucoside 4′-acetate by UV spectral analysis with the customary shift reagents, acid hydrolysis, alkaline hydrolysis and electrospray mass spectrum. Only two flavone glycosides with an acyl group at position 4′ have previously been reported from plants. Flavonoids (**2**) and (**3**) have been identified as quercetin 3-*O*-rhamnoside 7-*O*-glucoside and apigenin 7-*O*-(sulphatoglucoside) respectively by UV spectral aqnalysis with the customary shift reagents, acid hydrolysis and electrospray mass spectra. Flavonoid (**2**) is a new fern constituent. Two partially characterized flavonoid *C*-glycosides (apigenin *C*-pentoside (**4**) and methoxyapigenin *C*-pentoside (**5**)) have also been found in trace amounts in this fern.

PS2-15

Antioxidant and anti-inflammatory activities of *Acer tegmentosum* bark extracts

Byung-Ho Hwang, Dong-Joo Kwon, Tae-Seong Lee, Du-Song Cha and Young-Soo Bae

Department of Wood Science and Engineering, Kangwon National University, Chuncheon, South Korea

Acer tegmentosum (Acereaceae) has been used as a traditional medicine for treatment of hepatic disorders in Korea. The purpose of this study was to evaluate the basic biological activities of *A. tegmentosum* bark extracts and to investigate the bioactive constituents. In the phytochemical study, five phenolic compounds, (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-*O*-gallate, gallic acid, and 6”-*O*-galloylsalidroside were isolated. Their structures were elucidated on the basis of chemical and spectroscopic evidence. The antioxidant activity of the above compounds was evaluated by DPPH radical scavenging method. Most compounds indicated positive antioxidant potentials compared with BHA and *α*-tocopherol as controls. In the anti-inflammatory test of the above compounds, nitric oxide (NO) assay against the Raw 264.7 (Mouse Macrophage) showed similar inhibitory potentials to NO production of the control.

PS2-16

Evaluation of biological activities using *Acer barbinerve* bark extracts

Wan-Geun Park, Dong-Joo Kwon, Sang-Yong Lee and Young-Soo Bae

Department of Wood Science and Engineering, Kangwon National University, Chuncheon, South Korea

Acer barbinerve Max. (Acereaceae) is indigenous to Korean peninsula and China and a very uncommon shrubby tree reaching around 30 feet in height. Although chemical constituents such as diarylheptanoids, rhododendrol glycoside, and tannins have been reported in the genus *Acer*, no phytochemical and bioactive studies on *A. barbinerve* have yet been reported. This work was performed to investigate the chemical constituents of the species and to evaluate the functionalities of the constituents, including crude and partitioned fractions. *A. barbinerve* bark was extracted with 70% acetone and successively partitioned with *n*-hexane, CH_2Cl_2, EtOAc and H_2O. From the EtOAc fraction, two phenolic compounds were isolated by repeating Sephadex LH-20 column chromatography and determined as methyl gallate and methyl gallate-4-*O*-*β*-D-glucopyranoside. Evaluations of the biological activity include antioxidant (electron-donating ability), anti-inflammatory and cytotoxic tests which indicated good potentials.

PS2-17

Phenolic compounds from the aerial parts of *Lespedeza cuneata* G. Don

Dong-Joo Kwon, Jae-Seon Yi, Nam-Hun Kim, and Young-Soo Bae

Department of Wood Science and Engineering, Kangwon National University, Chuncheon, South Korea

Approximately 60 species constitute the genus *Lespedeza*, which occurs from East Asia to Northeast Australia and North America. The members of this genus are tolerant of arid environments and consequently are often planted to prevent soil erosion. *L. cuneata* is a common native plant in Korea, China, India and Japan, and it was introduced to the southeastern United States in the 1800s for forage and soil conservation. The aerial parts of *L. cuneata* were collected, air-dried and extracted with 95% aqueous EtOH, and it was then successively partitioned with *n*-hexane, CH_2Cl_2, EtOAc and H_2O. Repeated Sephadex LH-20 column chromatography on the EtOAc- and H_2O-soluble fractions gave four compounds. Their structures were elucidated as quercetin, kaempferol, hirsutrin, hyperin, desmodin (apigenin-6-C-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside) and homoadonivernith (luteolin-6-C-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranoside) on the basis of spectroscopic evidences such as ^{1}H-NMR, ^{13}C-NMR, 2D-NMR and MS spectrum. Desmodin and homoadonivernith have not been reported in this plant yet.

PS2-18

Isolation and characterization of soluble EGCG-protein complexes

John D. Trombley, Megan L. Albertz, and Ann E. Hagerman

Department of Chemistry and Biochemistry, Miami University, Oxford, OH

Epigallocatechin gallate (EGCG) is a polymeric polyphenol, or tannin, found in tea and other foods. The potent antioxidant properties of polyphenols have led to the widely accepted hypothesis that dietary polyphenols may promote health by preventing oxidative stress. Like other tannins, EGCG readily binds protein and protein is abundant in the GI tract. An understanding of the physiological consequences of consuming EGCG is incomplete without an understanding of EGCG-protein complexes. We hypothesize that covalently stabilized EGCG-protein complexes will provide a useful model for exploring the physiological effects of tannins. Overnight incubation of BSA with an excess of EGCG at physiological pH and temperature yielded a product with slightly lower mobility than native BSA on SDS-PAGE. When the reaction products were electroblotted to nylon membranes, only the products with lower mobility had characteristic phenolic activity (NBT or Prussian blue staining). A 5 kDa increase in the molecular mass of the protein after incubation with EGCG was seen using MALDI-TOF. Additionally the pI (IEF gels) diminished about 0.7 pH units upon reaction of the protein with EGCG. The change in molecular mass is consistent with the covalent linkage of 10 moles EGCG with per mole of BSA, and the change in pI suggests modification of about 10 lysine residues. A persistent problem with BSA-EGCG complexes is interference by EGCG with most protein quantification methods. Binding of Ponceau S, however, is not affected by EGCG and is being used to quantify protein content. Due to the small difference in size between native BSA and BSA-EGCG, complex capillary zone electrophoresis is being used to elucidate the kinetics of complex formation, and to monitor the purity of reaction products.

PS2-19
Purification of glucosinolates from *Camelina sativa*

Mark A. Berhow, Steven F. Vaughn, Brent Tisserat, and Sandra M. Duval

USDA-ARS, Peoria, IL

Camelina sativa (Gold-of-pleasure or false flax) has been cultivated as an oilseed crop for centuries and has been used as both a fuel oil and an edible oil. Current research efforts center on its exceptionally high levels of omega-3 fatty acids, which are uncommon in vegetable oils, as well as rich levels of antioxidants such as tocopherols, which make the oil naturally stable. Interest in the use of camelina as a functional food and as a biodiesel continues to grow. Finding additional uses for the press cake material left over after oil removal will make the crop more economically competitive. The press cake contains a number of interesting phytochemicals, including the flavonoid rutin (quercetin 3-O-rutinoside) and three relatively unique glucosinolates: glucocamelinin (10-(methylsulfinyl)decyl-glucosinolate), glucoarabin (9-(methylsulfinyl)nonyl-glucosinolate) and 11-(methylsulfinyl)undecyl-glucosinolate. These glucosinolates have not been assessed for either their phytochemical activities in agriculture or their nutritional and pharmacological roles in animals due to the lack of availability of purified standards for study. Using defatted seed material, we have developed a method to isolate mg quantities of these glucosinolates using a combination of reversed phase flash and preparative HPLC methods.

PS2-20
Polyphenolics of myrtaceous plants: *Eucalyptus globulus* and *Myrtus communis*

Yoshiaki Amakura, Morio Yoshimura, Mie Tokuhara, and Takashi Yoshida

College of Pharmaceutical Sciences, Matsuyama University, Japan

As part of our continuing study on polyphenolic constituents of the myrtaceous plants, we investigated the hydrolyzable tannins and related polyphenols from *Eucalyptus globulus* and *Myrtus communis*. An aqueous methanolic extract of the *E. globulus* leaves was subjected to repeated column chromatography on Diaion HP-20, TSK-gel HW-40 and MCI GEL CHP-20P to yield a new tannin together with seventeen known compounds including flavones, flavonol glycosides, ellagic acid glucoside, chalcone glucoside, caffeic acid derivatives and hydrolyzable tannins (monomers and a dimer). The new tannin was characterized as 1,2,3,6-tetra-*O*-galloyl-β-D-galactose based on MS and NMR spectroscopic data. Most hydrolyzable tannins have glucose as sugar unit(s), whereas there are several exceptional tannins with the other sugar core such as hamamelose, allose and lyxose. This is thus the first report of the isolation of the tetra-galloyl compound with galactose core from a natural source. Similarly, ten known compounds including hydrolyzable tannins (monomers and dimers) and myricetin glycosides, were isolated from aqueous acetone extract of the leaves of *M. communis*. Antioxidant activities of the isolated compounds in this study were evaluated by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. Among the compounds evaluated, the hydrolyzable tannins such as tellimagrandins I and II, pentagalloylglucose, oenothein B and eugeniflorin D_2 exhibited more potent activity (EC_{50}: 3.98–8.00 μM) than other polyphenols such as flavonoids (EC_{50}: 12.47–134.83 μM).

PS2-21

New phenolic glucoside from bark of *Populus alba × glandulosa*

Jin-Kyu Kim,[1] Seth D. Cohen,[1] James A. Kennedy,[1] Dong-Joo Kwon,[2] and Young-Soo Bae[2]

[1]Dept. of Food Science and Technology, Oregon State University, Corvallis, OR and [2]Department of Wood Science and Engineering, Kangwon National University, Chuncheon, South Korea

The genus *Populus*, belonging to the Salicaceae family, comprises more than 100 species. Suwon poplar (*Populus alba × glandulosa*), which was hybridized from *P. alba* and *P. glandulosa*, is one of the major poplar species growing in Korea and contains a large number of phenolic compounds, especially in its bark. Phenolic glycosides occur characteristically in the bark of the family Salicaceae, including *Salix* and *Populus* species. We have previously described the isolation and structural determination of flavonoids, coumarins, salicin derivatives, and phenolic glycosides from bark of this tree. In the present study, we report that an unknown phenolic glucoside, 2-hydroxycyclohexyl-6-*O*-*p*-coumaroyl-*β*-D-glucopyranoside, was isolated from the bark of *P. alba × glandulosa* by column chromatography over Sephadex LH-20 and then Silica gel preparative TLC. The structure elucidation was done by NMR and MS spectral analyses.

PS2-22
Effects of digestion on the bioavailability and bioactivity of punicalagins

Dobroslawa Bialonska, Sashi Kanth Gupta Kasimsetty, and Daneel Ferreira

Department of Pharmacognosy and Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, Oxford, MS

Punicalagins, the most abundant tannins in the pomegranate husk, have been credited for many of the health benefits of pomegranate products. However, these compounds were not detected in the human systemic circulation after consumption of even large amounts of pomegranate products. Digestion of punicalagins involves abiotic decomposition in intestines as well as metabolic transformations by colonic bacteria. We will present data on stability of punicalagins in the digestive tract, i.e. the influence of pH, and the interaction with typical food components and digestive enzymes. We will discuss the impact of punicalagins and their decomposition products on growth of health beneficial human gut bacteria (*Lactobacillus* sp*., Bifidobacterium* sp.) and disadvantageous/harmful bacteria (*Clostridum* sp., *Bacterioides* sp.), as well as their binding to the bacterial cell wall. In addition, we will show that punicalagins may be considered as a potential treatment in prevention of ethanol teratogenicity after a preliminary study of Medaka fish embryos (an approved model in studies of human embryogenesis). See also the presentation by Kasimsetty *et al.* for antioxidant, anticancer, and anticarcinogenic properties of punicalagins and their decomposition/metabolic products.

PS2-23
Phytochemical screening and free radical scavenging activity of *Allanblackia floribunda* fruits and leaves

Gloria A. Ayoola,[1] Solomon S. Ipav,[1] Margaret O. Sofidiya,[2] Herbert A.B. Coker,[1] and Tolu O. Odugbemi[3]

[1]Dept. of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos; [2]Dept. of Pharmacognosy, Faculty of Pharmacy, University of Lagos; and [3]Dept. of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria

The phytochemical screening of the methanolic extract of *Allanblackia floribunda* (AF) fruits and leaves showed the presence of alkaloids, anthraquinones, tannins, saponins, steroids, terpenoids, flavonoids and cardiac glycosides. However, only the AF fruit contained phlobatannins and the presence of phenolics prompted us to investigate the antioxidant activity of these plant materials. Free radical scavenging activity of the extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was investigated. IC_{50} values of 0.01, 0.02 and 0.1 mg/ml were recorded for Vitamin E, AF leaves and AF fruits. Phenolic content, total flavonoids and proanthocyanidin contents were recorded as 65, 0.07 and 2.38 mg/g of powdered plant material as gallic acid, rutin and catechin equivalents respectively for AF fruits, and 12, 51.35, and 19.5 mg/g of powdered plant material respectively for AF leaves. Thus, AF leaves appear to be at least five times more potent as a free radical scavenger compared to the fruit, though half as potent as vitamin E. AF fruit is at least 10 times less potent compared to vitamin E as a free radical scavenger although it appears to contain more phenolics when compared to the leaves. The total flavonoid content and the proanthocyanidin content of the leaves are much higher when compared to the amount present in the fruits. Hence, we propose that other non-flavonoid phenolics are present in the fruits and that the difference in the amount of total flavonoid and proanthocyanidin contents is likely to be responsible for the difference in antioxidant activity.

PS2-24

Antioxidant capacity and polyphenol composition of pomegranate (*Punica granatum*) dietary supplements

S. Madrigal-Carballo,[1,3] M. Dettloff,[1] G. Rodriguez,[3] C. G. Krueger,[1] M. Dreher,[2] and J. D. Reed[1]

[1]Dept. of Animal Sciences, University of Wisconsin-Madison, Madison, WI; [2]POM Wonderful, LLC, California; and [3]National University, Heredia, Costa Rica

Improved methods for determining authenticity, standardization, and efficacy of nutritional supplements are required for growth and regulation of the market. Pomegranate nutritional supplements (PNS) are currently sold for their antioxidant content. Pomegranates contain complex mixtures of gallotannins, ellagitannins, ellagic acid, anthocyanins and other polyphenolic compounds. However, label claims on PNS may not correlate with actual tannin content or antioxidant capacity. We evaluated 28 PNS for antioxidant activity by oxygen radical absorbing capacity (ORAC) and free radical scavenging properties by diphenyl-1-picrylhydrazyl (DPPH) radical and Ferric reducing antioxidant power (FRAP). Polyphenolic composition was determined by RP–HPLC and total phenolics were estimated by the Folin-Ciocalteau assay. We found that product labels were inconsistent with polyphenol composition and antioxidant capacity. Two samples contained no detectable polyphenols, and the majority of the samples (n = 16) contained disproportionately high amounts of ellagic acid compared to pomegranate tannins, including samples in which low or no pomegranate tannins were detected. Only 4 products had tannin composition that resembled pomegranates (punicalagin, punicalin, ellagitannins and gallotannins). Results of the antioxidant assays were poorly correlated with each other and with total polyphenol content, which may be explained in part by the low solubility of ellagic acid. High levels of ellagic acid may result from adulteration with added ellagic acid or from extensive hydrolysis of the pomegranate ellagitannins during processing. Our results indicate that reliable labeling information, better standardization, improved manufacturing practices and regulation of the market is required to assure consumers of the quality of pomegranate nutritional supplements.

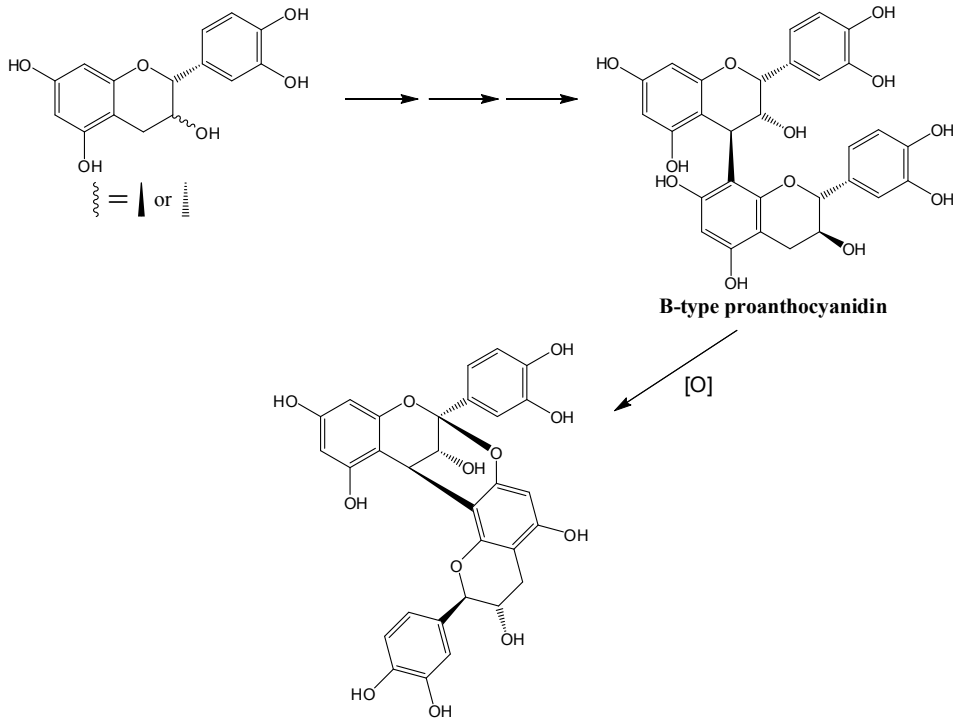
PS2-25

Synthesis of cranberry proanthocyanidins

Jannie P. J. Marais,[1] Vijender R. Adelli,[2] and Daneel Ferreira[2]

[1]National Center for Natural Products Research and [2]Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS

There is a growing body of evidence showing the beneficial effects of American cranberry (*Vaccinium macrocarpon*) juice in the human diet. Studies indicated that cranberry juice shows good antioxidant and radical scavenging ability, antitumor/anticancer/cytotoxic and antimicrobial activities, and is usually associated with the monomeric flavonoids and polyphenols present in the fruit and juice. Furthermore, cranberry juice is traditionally used to help prevent urinary tract infections, and this is claimed to be caused by the presence of A-type proanthocyanidins. Because of the complexity of the polyphenolic content and the difficulty of characterizing these compounds, we have embarked on a program investigating and developing methods to synthesize A- and B-type proanthocyanidin standards, ranging from the dimers to the icosamer level (20-mer). This included the synthesis of specific electrophilic and nucleophilic species to control the formation of the (4→8) as well as the (4→6) interflavanoid bonds to form possibly “angular” and “branched” B-type proanthocyanidins. Owing to the scant information that is available on the synthesis of A-type proanthocyanidins, initial results of the synthesis of these classes of compounds via oxidative conversion of their accompanying B-type analogs will be discussed.



Author Index

U

V

W

X

Y

Z