Société Phytochimique de L'Amérique du Nord

Sociedad Fitoquímica de América del Norte

Newsletter

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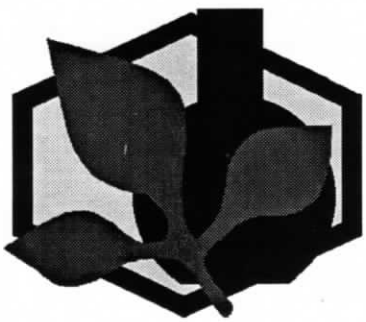
PSNA Newsletter

Editor: **Dr. W. Dennis Clark**



The Phytochemical Society of North America is a nonprofit scientific organization whose membership (currently over 400) is open to anyone with an interest in phytochemistry and the role of plant substances in related fields. Annual membership dues are U.S. $20 for regular members and $10 for student members. Annual meetings featuring symposium topics of current interest and contributed papers by conference participants are held throughout the United States, Canada, and Mexico. Still a specialist organization despite its broadened interests, PSNA meetings are small enough to offer informality and intimacy that are conducive to the exchange of ideas. A newsletter is circulated to members several times a year to keep them informed of upcoming meetings and developments within the society. If you would like additional information about the PSNA or if you have material to be included in the newsletter, please contact the PSNA Secretary. Annual dues and changes in addresses should be sent to the PSNA Treasurer. Also see the PSNA homepage, currently at: http://www.fiu.edu/orgs/psna.

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From the Editor

As always, the summer issue of the *Newsletter* provides the program and abstracts for our annual meeting. This year we are meeting in that quiet hotbed of scientific research, Pullman, Washington. I am sure that you will find the facilities and environment of Pullman and of Washington State University both relaxing and stimulating. The program committee, Norman G. Lewis and G.H. Neil Towers has assembled an excellent group of speakers and have organized an intellectually vigorous conference.

I would like to compliment my colleagues for helping me make the transition to Secretary of PSNA and Editor of the *Newsletter* as smooth as possible. I am also indebted to Dr. Kelsey Downum for maintaining and nurturing our electronic presence on the World Wide Web (see address inside front cover).

The size of this program and abstract volume precludes me from including additional information from the world of phytochemistry, but I look forward to the PSNA membership for sending me information for future editions of the newletter regarding upcoming meetings and other significant events of interest to our readership. I also appleal for volunteers to submit accounts of their research (ca. 500 words) that would appropriate for publication in the *Newsletter*.

The Editor

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA
SOCIEDAD FITOQUÍMICA DE AMÉRICA DEL NORTE
SOCIÉTÉ PHYTOCHIMIQUE DE L'AMÉRIQUE DU NORD

1998 MEETING

WASHINGTON STATE UNIVERSITY
PULLMAN, WASHINGTON, U.S.A.
JULY 26-31, 1998

"Phytochemicals in Human Health Protection, Nutrition and Plant Defense"

Program Committee

Norman G. Lewis • G. H. Neil Towers

Invited Speakers

Yoshinori Asakawa
Tokushima Bunri University
PHYTOCHEMISTRY OF BRYOPHYTES: BIOLOGICALLY ACTIVE TERPENOIDS AND AROMATIC COMPOUNDS FROM LIVERWORTS

Rodney Croteau
Washington State University
TAXOL BIOSYNTHESIS: PROSPECTS FOR PATHWAY ENGINEERING

Daneel Ferreira
University of the Orange Free State
RECENT ADVANCES IN THE CHEMISTRY OF PROANTHOCYANIDINS

Jeffrey B. Harborne
The University of Reading
PHYTOCHEMICALS AND PLANT DEFENSE AGAINST MICROBES

W. E. (Ted) Hillis
CSIRO
THE FORMATION OF HEARTWOOD AND ITS EXTRACTIVES: AN OVERVIEW

Ulrich Matern
Philipps-University Marburg
CURRENT ASPECTS OF COUMARIN BIOSYNTHESIS AND APPLICATION

Junya Mizutani
Plant Ecochemicals Research Center
PLANT ECOCHEMICALS FROM THE VIEWPOINT OF PLANT DEFENSE

Lilian U. Thompson
University of Toronto
ROLE OF LIGNANS IN CARCINOGENESIS

Meinhart H. Zenk
Universität München
BIOSYNTHESIS OF PHARMACEUTICALLY USEFUL ALKALOIDS

Gordon M. Cragg
National Cancer Institute
NATURAL PRODUCT DRUG DISCOVERY AND DEVELOPMENT: THE NCI ROLE

Richard A. Dixon
Samuel Roberts Noble Foundation
MOLECULAR CONTROLS FOR ISOFLAVONOID BIOSYNTHESIS IN RELATION TO PLANT AND HUMAN HEALTH

Georg G. Gross
Universität Ulm
BIOSYNTHESIS, BIODEGRADATION, AND CELLULAR LOCALIZATION OF HYDROLYZABLE TANNINS

Edwin Haslam
Sheffield University
PRINCIPLES OF MOLECULAR RECOGNITION: POLYPHENOLS

Peter J. Houghton
King's College London
BIOLOGICALLY ACTIVE COMPOUNDS FROM *BUDDLEJA* SPECIES

Jerry L. McLaughlin
Purdue University
SIMPLE (BENCH-TOP) BIOASSAYS AND THE ISOLATION OF NEW CHEMICALLY DIVERSE ANTITUMOR AND PESTICIDAL AGENTS FROM HIGHER PLANTS

Dirk Selmar
Technical University
CYANIDE IN FOODS: BIOLOGY OF CYANOGENIC GLUCOSIDES AND RELATED NUTRITIONAL PROBLEMS

G. H. Neil Towers
University of British Columbia
NATURAL PRODUCTS AND PHYTOMEDICINE

PROGRAM SUMMARY

SUNDAY, JULY 26

3:00-9:00 REGISTRATION (West End of Compton Union Building)

4:30-5:30 EXECUTIVE MEETING (Compton Union Building, Room 224)

6:00-9:30 WELCOME RECEPTION/SALMON BARBECUE (Area between Compton Union Building and New Library. Alternate site: Compton Union Building, Cascade Room)

MONDAY, JULY 27

8:00-8:20 WELCOME AND OPENING REMARKS: Congressman G. R. Nethercutt **(TENTATIVE)**

SYMPOSIUM SESSION 1 ***Drug Discovery and Pathway Engineering***
Moderator: ***Richard A. Dixon***

8:20-9:05 ***Symposium Paper 1*** - NATURAL PRODUCT DRUG DISCOVERY AND DEVELOP-MENT: THE NCI ROLE. Gordon M. Cragg

9:05-9:55 ***Symposium Paper 2*** - BIOSYNTHESIS OF PHARMACEUTICALLY USEFUL ALKALOIDS. Meinhart H. Zenk

9:55-10:15 BREAK

10:15-11:05 ***Symposium Paper 3*** - TAXOL BIOSYNTHESIS: PROSPECTS FOR PATHWAY ENGINEERING. Rodney Croteau

11:05-11:25 ***Oral Paper 1*** - REGULATION OF ALKALOID AND AMIDE BIOSYNTHESIS IN OPIUM POPPY AND OTHER PLANTS. Peter J. Facchini and Min Yu

11:25-11:45 ***Oral Paper 2*** - MODIFICATION OF NICOTINE AND CHLOROGENIC ACID POOLS IN TOBACCO LINES TRANSFORMED WITH HETEROLOGOUS TDC AND TYDC GENES. Gabriel Guillet, Julie Poupart, Juan Basurco and Vincenzo De Luca

11:45-12:05 ***Oral Paper 3*** - THE TERMINAL *O*-ACETYLTRANSFERASE OF VINDOLINE BIOSYNTHESIS DEFINES A NEW CLASS OF PROTEINS RESPONSIBLE FOR COENZYME A-DEPENDENT ACYL TRANSFER. Benoit St-Pierre, Pierre Laflamme, Anne-Marie Alarco and Vincenzo De Luca

12:05-12:25 ***Oral Paper 4*** - CELLULAR SPECIALIZATION FOR THE VINDOLINE BIOSYNTHETIC PATHWAY IN THE LEAVES OF *CATHARANTHUS ROSEUS*. Benoit St-Pierre, Felipe Vazquez Flota and Vincenzo De Luca

12:25-1:30 LUNCH

SYMPOSIUM SESSION 2 ***Polyphenols in Human Health Protection and Plant Defense***
Moderator: ***Ragai H. Ibrahim***

1:30-2:15 ***Symposium Paper 4*** - PRINCIPLES OF MOLECULAR RECOGNITION: POLYPHENOLS. Edwin Haslam

2:15-3:00 ***Symposium Paper 5*** - RECENT ADVANCES IN THE CHEMISTRY OF PROANTHO-CYANIDINS. Daneel Ferreira

3:00-3:20 BREAK

3:20-4:05 ***Symposium Paper 6*** - MOLECULAR CONTROLS FOR ISOFLAVONOID BIOSYNTHE-SIS IN RELATION TO PLANT AND HUMAN HEALTH. Richard A. Dixon, P. Canovas, X.-Z. He, C. Lamb, F. McAlister and C.L. Steele

4:05-4:25 ***Oral Paper 5*** - FLAVONOIDS INTERACTIONS WITH P-GLYCOPROTEIN, A NEW HOPE FOR ANTICANCER CHEMOTHERAPY. Denis Barron, A. Di Pietro, G. Comte, J.M. Jault, H. Cortay, J.B. Daskiewicz, G. Conseil, G. Dayan and C. Bayet

4:25-4:45 ***Oral Paper 6*** - PROPERTIES OF A FLAVONOL 3-*O*-HETERODISACCHARIDE GLYCOSIDASE FROM *FAGOPYRI ESCULENTUM* (BUCKWHEAT). Wolfgang Kreis and Andreas Baumgertel

4:45-5:05 ***Oral Paper 7*** - PHENOLIC COMPOUNDS FROM *CYCLOPIA INTERMEDIA* (HONEYBUSH TEA). E. Vincent Brandt, B. Irene Kamara, Elizabeth Joubert, Jacobus A. Steenkamp and Daneel Ferreira

MONDAY, JULY 27

SYMPOSIUM SESSION 2	***Polyphenols in Human Health Protection and Plant Defense***
Moderator:	***Ragai H. Ibrahim***
5:05-5:25	***Oral Paper 8*** - TOWARDS THE ISOLATION OF AN ISOFLAVONE SYNTHASE cDNA: THE FIRST STEP IN THE SYNTHESIS OF THE PHYTOALEXIN MEDICARPIN AND CANCER-PREVENTATIVE ISOFLAVONOIDS. Christopher L. Steele and R. A. Dixon
6:00-7:30	DINNER (Compton Union Building, Ball Room)
POSTER SESSION 1	***Phytochemical Pathways Useful in Medicine and Plant Defense***
Moderators:	***Michael A. Costa/David R. Gang***
8:00-10:00 PM	COMPTON UNION BUILDING, BALL ROOM
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Poster Paper 5	EVALUATION OF CHEMICALS DERIVED FROM AGRICULTURAL PRODUCTS FOR CHEMOPROTECTIVE ACTIVITY. Mark A. Berhow, S. F. Vaughn, M. J. Plewa, E.D. Wagner, L. Rayburn and E. Gugger
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Poster Paper 27	CONFORMATIONAL DYNAMICS OF PROANTHOCYANIDINS: PHYSICAL AND COMPUTATIONAL APPROACHES. Fred L. Tobiason, R.W. Hemingway and T. Hatano
Poster Paper 29	MEMBERS OF THE MULTIGENE FAMILY ENCODING BLACK CHERRY (*PRUNUS SEROTINA*) (*R*)-(+)-MANDELONITRILE LYASE ARE DIFFERENTIALLY EXPRESSED. Zihua Hu and Jonathan E. Poulton

MONDAY, JULY 27

POSTER SESSION 1 ***Phytochemical Pathways Useful in Medicine and Plant Defense***
Moderators: ***Michael A. Costa/David R. Gang***

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Poster Paper 53 IDENTIFICATION OF COMPONENTS FROM VOLATILE OILS: SPECTROSCOPICAL ANALYSIS OF SESQUITERPENE MIXTURES. Cláudia B. Brochini, Cecilia V. Núñez, Isabel C. Moreira and Mariana H. Chaves

Poster Paper 55 CHEMICAL CONSTITUENTS FROM *BRILLANTAISIA PALISATII* LIND. E.A.P. Galo, C.S. Silveira, S.C. Morgado, Davyson de Lima Moreira and F.S. Menezes

Poster Paper 57 NINETEEN NEW STEROIDAL GLYCOSIDES FROM *ALLIUM* PLANTS: ISOLATION, STRUCTURAL ELUCIDATION AND EFFECTS ON BLOOD COAGULABILITY. Junpeng Peng, Xinsheng Yao and John Ralph

TUESDAY, JULY 28

SYMPOSIUM SESSION 3 ***Medicinals/Nutriceuticals and New Genetic Engineering Targets***
Moderator: ***Laurence B. Davin***

8:00-8:50 ***Symposium Paper 7*** - BIOLOGICALLY ACTIVE COMPOUNDS FROM *BUDDLEJA* SPECIES. Peter J. Houghton

8:50-9:40 ***Symposium Paper 8*** - ROLE OF LIGNANS IN CARCINOGENESIS. Lilian U. Thompson

9:40-10:00 BREAK

10:00-10:25 ***Oral Paper 9*** - FORMATION OF CANCER-PREVENTING LIGNANS IN PLANTS: ENGINEERING THEIR METABOLIC PATHWAYS. Michael A. Costa, Zhi-Qiang Xia, Joshua D. Ford, Huai-Bin Wang, David R. Gang, Laurence B. Davin and Norman G. Lewis

10:25-10:50 ***Oral Paper 10*** - GENETIC ENGINEERING OF RESVERATROL ACCUMULATION IN ALFALFA. Nancy L. Paiva and John D. Hipskind

TUESDAY, JULY 28

SYMPOSIUM SESSION 3 ***Medicinals/Nutriceuticals and New Genetic Engineering Targets***
Moderator: ***Laurence B. Davin***

10:50-11:10 ***Oral Paper 11*** - NATURAL AND SYNTHETIC PHENYLPROPENOIDS AS DETOXICATION ENZYME INDUCERS AND FREE RADICAL SCAVENGERS. Albena T. Dinkova-Kostova and Paul A. Talalay

11:10-11:30 ***Oral Paper 12*** - SHOTGUN CLONING OF WHOLE PATHWAYS FROM A PEPPERMINT GLAND cDNA LIBRARY. B. Markus Lange, Mark R. Wildung, Einar Stauber and Rodney Croteau

11:30-11:50 ***Oral Paper 13*** - CYTOTOXIC CONSTITUENTS OF *FORSYTHIA* SPECIES. Katsuya Endo

11:50-12:10 ***Oral Paper 14*** - FLAX SEED LIGNANS, STEREOCHEMISTRY, QUANTITATION AND BIOLOGICAL ACTIVITY. Alister D. Muir and Neil D. Westcott

12:10-12:30 ***Oral Paper 15*** - CHEMICAL CHARACTERIZATION OF FLAX AND THE EFFECT OF EXTRACTS ON RETTING ENZYMES. W. Herbert Morrison, D. E. Akin, G. R. Gamble and G. Henriksson

12:30-1:30 LUNCH

SYMPOSIUM SESSION 4 ***Biochemistry of Plant Phenolics in Cell Wall Structure and Defense Including Heartwood Formation***
Moderator: ***Edwin Haslam***

1:30-2:15 ***Symposium Paper 9*** - BIOSYNTHESIS, BIODEGRADATION, AND CELLULAR LOCALIZATION OF HYDROLYZABLE TANNINS. Georg G. Gross

2:15-3:00 ***Symposium Paper 10*** - THE FORMATION OF HEARTWOOD AND ITS EXTRACTIVES: AN OVERVIEW. W.E. (Ted) Hillis

3:00-3:15 BREAK

3:15-3:35 ***Oral Paper 16*** - MAPPING HEARTWOOD FORMATION THROUGH THE LIGNAN PATHWAY. David R. Gang, Masayuki Fujita, Laurence B. Davin and Norman G. Lewis

3:35-3:55 ***Oral Paper 17*** - THE CHEMISTRY OF HEARTWOOD FORMATION IN NORTHERN WHITE CEDAR (*THUJA OCCIDENTALIS*). Herbert L. Hergert

3:55-4:15 ***Oral Paper 18*** - CONCOMITANT GLUCOSYLATION ALONG THE LIGNIFICATION PATHWAY IN CONIFERS – REGULATORY ASPECT OR METABOLIC ACCIDENT? Hartmut Förster, Valerie Steeves and Rod A. Savidge

4:15-4:35 ***Oral Paper 19*** - CONIFERYL ALCOHOL OXIDASE IS A RESIDUAL ACTIVITY OF A CELL WALL-LOCATED STRONGLY BASIC PEROXIDASE. A. Ros Barceló

4:35-4:55 ***Oral Paper 20*** - *IN VITRO* PRODUCTION OF LIGNANS BY A PEROXIDASE FROM THE ENDOPLASMATIC RETICULUM OF *ZEA MAYS* L. AND POSSIBLE BIOLOGICAL FUNCTIONS. Wolfgang Gröger, H. Förster, M. Völker, A. Schierhorn, A. Porzel and U. Pommer

4:55-5:15 ***Oral Paper 21*** - STEREOCHEMICAL DIVERSITY IN LIGNAN BIOSYNTHESIS. Toshiaki Umezawa, T. Okunishi, K. Mikame, S. Suzuki and M. Shimada

5:15-5:35 ***Oral Paper 22*** - FORMATION OF (+)-*ERYTHRO* AND (-)-*THREO*-GUAIACYL-GLYCEROL-β-CONIFERYL ETHERS AND (+)-SYRINGARESINOL BY ENANTIOSELECTIVE PHENOXY RADICAL COUPLING REACTIONS. Takeshi Katayama, Yuki Kado and Atsushi Ogaki

5:35-5:55 ***Oral Paper 23*** - BIOSYNTHESIS OF LIGNANS IN *MAGNOLIA KOBUS* DC. VAR. *BOREALIS* SARG. Shuji Ozawa and Teruhisa Miyauchi

6:00-7:30 DINNER (Compton Union Building, Ball Room)

TUESDAY, JULY 28

POSTER SESSION 2 ***Phytochemical Pathways Useful in Medicine and Plant Defense***
Moderators: ***Joshua D. Ford/Anastasia L. Crowell***

8:00-10:00 P.M. COMPTON UNION BUILDING, BALL ROOM

Poster Paper 2 COMPOUNDS REMOVED FROM PLANT SURFACES AS POTENTIAL ANTICANCER AGENTS. Alicja M. Zobel, J.M. Lynch, M. Podbielkowska, E. Kupidlowska and K. Wierzchowska-Renke

Poster Paper 4 POTENTIAL CANCER CHEMOPREVENTIVE PRENYLFLAVONOIDS FROM HOPS (*HUMULUS LUPULUS*). Jan Fred Stevens, Alan W. Taylor, Cristobal L. Miranda, Donald R. Buhler and Max L. Deinzer

Poster Paper 6 DEVELOPMENT OF AN ELISA METHOD FOR THE DETERMINATION OF SAIKOSAPONIN A, AN ACTIVE COMPONENT OF *BUPLEURUM FALCATUM* L., AND ITS APPLICATIONS. Chung Ki Sung, Jeong Mee Lee, Da Woon Jung, Baik Hwang, Jun Cheul Ahn, Keon-Hyoung Song and Chang-Koo Shim

Poster Paper 8 ANTIOXIDANT ACTIVITY OF BRAZILIAN PLANT EXTRACTS: DPPH SCAVENGING TEST. Luciana Mensor, S. G. Leitão, F. S. Menezes, C. S. Coube and G. G. Leitão

Poster Paper 10 THE ROLE OF STRUCTURE AND SOLUBILITY IN DETERMINING THE ANTIMICROBIAL ACTIVITY OF TERPENOIDS AGAINST SELECT HUMAN PATHOGENS. S. Griffin, S. Grant Wyllie, J. Markham and D.N. Leach

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Poster Paper 14 SEVEN KEY PHYTOCHEMICAL CONSTITUENTS OF EDIBLE AND MEDICINAL HERBS: UNDERSTANDING TRADITIONAL MEDICINE. Suzanne Diamond

Poster Paper 16 EFFECTS OF VARIOUS PREGNANES, 23-NOR-5-CHOLENIC ACIDS AND THEIR FUCOSIDES ON CARDENOLIDE ACCUMULATION IN SHOOT CULTURES OF *DIGITALIS LANATA* EHRH. Wolfgang Kreis, Melitta Luta, Werner Haussmann and Andreas Hensel

Poster Paper 18 BIOSYNTHESIS OF ANTIOXIDANT LIGNANS IN *SESAMUM INDICUM*. Ying Jiao, Laurence B. Davin and Norman G. Lewis

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Poster Paper 24 BIOSYNTHESIS OF THE SPERMIDINE ALKALOID LUNARINE. Silvia Sagner

Poster Paper 26 A TRANSGENIC APPROACH TO UNDERSTANDING POTENTIAL METABOLIC CHANNELING INVOLVED IN ISOFLAVONE *O*-METHYLATION DURING MEDICARPIN BIOSYNTHESIS IN ALFALFA (*MEDICAGO SATIVA*). XianZhi He and R.A. Dixon

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Poster Paper 34 DEFENSE MECHANISMS OF GRAPEVINE BERRIES DURING THEIR DEVELOPMENT, IN RELATION WITH SALICYLIC ACID TREATMENTS. Elena Kraeva, Alain Deloire, Jacques Bierne and Claude Andary

Poster Paper 36 KENAF: A SOURCE OF POTENT PHYTOALEXINS. Robert D. Stipanovic, A.A. Bell and L.S. Puckhaber

TUESDAY, JULY 28

POSTER SESSION 2 ***Phytochemical Pathways Useful in Medicine and Plant Defense***
Moderators: ***Joshua D. Ford/Anastasia L. Crowell***

Poster Paper 38 THE MODE OF CHEMICAL DEFENSE CHANGES IN WHITE POPLAR (*POPULUS ALBA*) LEAVES DURING PLANT ONTOGENY. Riitta Julkunen-Tiitto

Poster Paper 40 MOSQUITOCIDAL ACTIVITY OF AN ACETYLENIC COMPOUND FROM *CRYPTOTAENIA CANADENSIS* (APIACEAE). Ute Eckeñbach, R. L. Lampman, D. De Groat, D. S. Seigler, J. Ebinger and R. J. Novak

Poster Paper 42 ANTIFEEDANT ACTIVITY OF PHOTOGEDUNIN DERIVATIVES AND LIMONOIDS FROM *CEDRELA SALVADORENSIS* (MELIACEAE) ON FALL ARMYWORM *SPODOPTERA FRUGIPERDA*. Carlos Céspedes A., José Calderón P., Laura Lina, Eduardo Aranda and Blas Lotina-Hennsen

Poster Paper 44 PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITY OF *ECHINACEA* spp. Shannon E. Binns, Bibiana Purgina, Bernard R. Baum, John T. Arnason, Dennis V.C. Awang and John Livesey

Poster Paper 46 TB-TYPE OF PHOTOSYNTHETIC ASSIMILATION OF CARBON. Vasile V. Trif and Eleonora Trif

Poster Paper 48 OLIGOMERIC PROTERACACINIDINS WITH AN EXCEPTIONAL VARIATION OF INTERFLAVANYL BONDS FROM THE HEARTWOOD OF *ACACIA GALPINII*. Elfranco Malan, Daneel Ferreira, Johan Coetzee and Linette Bennie

Poster Paper 50 A NEW *C*-GLYCOSYLFLAVONE FROM THE FERN *PTERIS VITTATA*. Filippo Imperato and A. Telesca

Poster Paper 52 SIGNIFICANCE OF FLAVONOID LEVELS IN A PHENETIC TAXONOMY OF THE GENUS *BETULA*. Boguslaw Wilkomirski

Poster Paper 54 SECONDARY METABOLITES OF *ARAUCARIA ANGUSTIFOLIA* (BERT.) O. KUNTZ. Fabiana N. Fonseca, Ary J. S. Ferreira, Patricia Sartorelli, Norberto P. Lopes, Eny I. S. Floh, Walter Handro and Massuo J. Kato

Poster Paper 56 VOLATILE COMPOUNDS FROM BRAZILIAN MONIMIACEAE. Gilda G. Leitão, Ana Cláudia F. Amaral, Simone S. V. Soares, Naomi K. Simas and Thelma B. M. Brito

WEDNESDAY, JULY 29

SYMPOSIUM SESSION 5 ***Bioactive Molecules/Engineered Foods***
Moderator: ***Vincenzo De Luca***

8:00-8:50 ***Symposium Paper 11*** - SIMPLE (BENCH-TOP) BIOASSAYS AND THE ISOLATION OF NEW CHEMICALLY DIVERSE ANTITUMOR AND PESTICIDAL AGENTS FROM HIGHER PLANTS. Jerry L. McLaughlin

8:50-9:40 ***Symposium Paper 12*** - PHYTOCHEMISTRY OF BRYOPHYTES: BIOLOGICALLY ACTIVE TERPENOIDS AND AROMATIC COMPOUNDS FROM LIVERWORTS. Yoshinori Asakawa

9:40-10:00 BREAK

10:00-10:40 ***Symposium Paper 13*** - CYANIDE IN FOODS: BIOLOGY OF CYANOGENIC GLUCOSIDES AND RELATED NUTRITIONAL PROBLEMS. Dirk Selmar

10:40-11:05 ***Oral Paper 24*** - FUSION OF TWO ENZYMES OF SESQUITERPENE BIOSYNTHESIS AND STUDIES ON CHANNELING OF AN INTERMEDIATE METABOLITE. Maria C. Cordeiro, Per Mercke and Peter E. Brodelius

11:05-11:25 ***Oral Paper 25*** - EFFECTS OF DIGITOXIGENIN, DIGOXIGENIN, AND VARIOUS CARDIAC GLYCOSIDES ON CARDENOLIDE ACCUMULATION IN SHOOT CULTURES OF *DIGITALIS LANATA* EHRH. Wolfgang Kreis and Christoph Theurer

11:25-11:45 ***Oral Paper 26*** - ANTI-INFLAMMATORY ACTIVITY OF *ANGELICA* AND *HERACLEUM* SPECIES IN TERMS OF INHIBITORY EFFECT ON 5-LIPO-OXYGENASE AND CYCLO-OXYGENASE. Jinghua Liu, S. Zschocke, E. Reininger and R. Bauer

11:45-1:00 LUNCH

WEDNESDAY, JULY 29

SYMPOSIUM SESSION 6 ***Plant Defense and Signaling Processes***
Moderator: ***Stewart A. Brown***

1:00-1:50 ***Symposium Paper 14*** - CURRENT ASPECTS OF COUMARIN BIOSYNTHESIS AND APPLICATION. Ulrich Matern

1:50-2:35 ***Oral Paper 27*** - *PHYTOPHTHORA INFESTANS* STIUMULATED BIOSYNTHESIS OF HYDROXYCINNAMIC ACID AMIDES IN *SOLANUM TUBEROSUM* AND CLONING OF A cDNA ENCODING THE INVOLVED TYRAMINE HYDROXYCINNAMOYL-TRANSFERASE. Dieter Strack

2:35-2:55 ***Oral Paper 28*** - UV-B IRRADIATION ACTIVATES A 48 KDA MBP KINASE AND ENHANCES THE WOUND RESPONSE IN TOMATO LEAVES. Johannes Stratmann and Clarence A. Ryan

2:55-3:15 BREAK

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4:15-4:35 ***Oral Paper 32*** - CLONING, CHARACTERIZATION AND IMMUNOCYTOCHEMICAL LOCALIZATION OF A WOUND-INDUCIBLE TYPE-I SERINE CARBOXY-PEPTIDASE FROM TOMATO PLANTS. Daniel S. de Moura, Daniel R. Bergey and Clarence A. Ryan

4:35-4:55 ***Oral Paper 33*** - PHENYLPHENALENONE-TYPE PHYTOALEXINS FROM BANANA FRUITS. Nobuhiro Hirai, T. Kamo, N. Kato, M. Tsuda, D. Fujioka and H. Ohigashi

4:55-5:15 ***Oral Paper 34*** - PURIFICATION, CLONING, AND HETEROLOGOUS EXPRESSION OF A FLAVONOL-3-*O*-GALACTOSYLTRANSFERASE FROM *PETUNIA HYBRIDA* POLLEN. Keith D. Miller and Loverine P. Taylor

5:15-6:45 PSNA General Annual Meeting

6:45-8:00 DINNER (Compton Union Building, Ball Room)

SYMPOSIUM SESSION 7 ***Post-doctoral and Graduate Student Research - Best Poster Competition***
Moderator: ***Jonathan E. Poulton***

8:00-10:00 P.M. COMPTON UNION BUILDING, BALL ROOM

Poster BPC 1 TRITERPENOID TYPE PHYTOALEXINS FROM NECTARINE FRUITS. El Hassane Lahlou, Nobuhiro Hirai and Hajime Ohigashi

Poster BPC 2 DEHYDRODICONIFERYL ALCOHOL METABOLISM IN *PINUS TAEDA*: GENE CLONING, ENZYME PURIFICATION AND PROPERTIES. Hiroyuki Kasahara, David R. Gang, Laurence B. Davin and Norman G. Lewis

Poster BPC 3 CHARACTERIZATION OF RADISH SEEDLING THIOGLYCOLIC LIGNIN BY FT-IR SPECTROSCOPY AND AN IMPROVED METHOD FOR ITS PREPARATION. Minghua Chen and Jerry W. McClure

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Poster BPC 6 A BIOSYNTHETIC PRECURSOR OF PHENYLPHENALENONES IN BANANA FRUITS. Tsunashi Kamo, Nobuhiro Hirai, Mitsuya Tsuda, Daie Fujioka and Hajime Ohigashi

Poster BPC 7 PHYTOCHEMICAL INVESTIGATION OF *PIPER LHOTZKYANUM* KUNTH. Davyson de Lima Moreira, M.A.C. Kaplan and E.F. Guimarães[2]

WEDNESDAY, JULY 29

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Moderator: ***Jonathan E. Poulton***

Poster BPC 8 STUDIES ON THE MODE OF ACTION OF THIARUBRINE A, AN ANTIBIOTIC POLYINE FROM THE ASTERACEAE. Caroline Aemisegger, Jon Page , Philippe Matile and G. H. Neil Towers

Poster BPC 9 SYNTHESES OF IRREVERSIBLE FLAVONOID INHIBITORS OF P-GLYCOPROTEIN. J.B. Daskiewicz, G. Comte, D. Barron and A. Di Pietro

Poster BPC 10 DEVELOPMENTAL REGULATION OF MONOTERPENE BIOSYNTHESIS IN THE GLANDULAR TRICHOMES OF PEPPERMINT (*MENTHA* X *PIPERITA* L.). Marie Rufener, Jonathan Gershenzon and Rodney Croteau

Poster BPC 11 FUNCTIONAL ARCHITECTURE OF TYROSINE/DOPA DECARBOXYLASE AND BERBERINE BRIDGE ENZYME GENE PROMOTERS FROM OPIUM POPPY. Sang-Un Park and Peter J. Facchini

Poster BPC 12 GUINEA-BISSAU'S PLANTS ACTIVE AGAINST *NEISSERIA GONORRHOEAE.* Olga M. D. Silva, E. Ferreira, L. Porém, S. Franco, N. Romão, M. Vaz Pato and E. T. Gomes

Poster BPC 13 EFFECT OF FEEDING SALICYLIC ACID ON THE SECONDARY METABOLISM IN CULTURED CELLS OF *CATHARANTHUS ROSEUS.* R.A. Budi Muljono, R. Verpoorte and J.J.C. Scheffer

Poster BPC 14 PHENOLIC CONSTITUENTS FROM THE FRUITS OF *PHYLLANTHUS EMBLICA AND ITS APPLICATION IN* CHINA. Yingjun Zhang and Chongren Yang

Poster BPC 15 DETERMINATION OF AN ELM HYBRID BY COMPARING ITS FLAVONOID STRUCTURES WITH THE PARENTS'. Ingrid Jordon

THURSDAY, JULY 30

SYMPOSIUM SESSION 8 ***Plant Defenses***
Moderator: ***Eric E. Conn***

7:45-8:30 ***Symposium Paper 15*** - PLANT ECOCHEMICALS FROM THE VIEWPOINT OF PLANT DEFENSE. Junya Mizutani

8:30-9:15 ***Symposium Paper 16*** - PHYTOCHEMICALS AND PLANT DEFENSE AGAINST MICROBES. Jeffrey B. Harborne

SYMPOSIUM SESSION 9 ***Postdoctoral and Graduate Student Oral Presentations - Best Paper Competition***
Moderator: ***Eric E. Conn***

9:15-9:35 ***Oral BPC 1*** - DIRIGENT PROTEIN AND PINORESINOL/LARICIRESINOL REDUCTASE. TISSUE SPECIFIC EXPRESSION OF GENES AND SUBCELLULAR LOCALIZATION OF ENZYMES: A COMPARISON WITH LIGNIFICATION. Vincent Burlat, Mi Kwon, Laurence B. Davin and Norman G. Lewis

9:35-9:55 ***Oral BPC 2*** - REGULATION OF DESACETOXYVINDOLINE 4-HYDROXYLASE. THE SECOND TO LAST ENZYME IN VINDOLINE BIOSYNTHESIS. Felipe A. Vazquez-Flota and Vincenzo De Luca

9:55-10:15 ***Oral BPC 3*** - DOES THIS PLANT HAVE A NOVEL POLYKETIDE SYNTHASE? Amrita Singh and Brian Ellis

10:15-10:30 BREAK

10:30-10:50 ***Oral BPC 4*** - SYSTEMIC WOUND-INDUCIBLE EXPRESSION OF A NOVEL POLYGALACTURONASE GENE IN TOMATO LEAVES. Martha Orozco-Cardenas, Daniel R. Bergey and Clarence A. Ryan

10:50-11:10 ***Oral BPC 5*** - TRACING THE SIGNAL: CHARACTERIZATION OF THE BBE SIGNAL PEPTIDE. David A. Bird and Peter J. Facchini

11:10-11:30 ***Oral BPC 6*** - IMMUNOCHEMICAL METHODS FOR THE STUDY OF GOSSYPOL. Xi Wang and Leslie C. Plhak

11:30-11:50 ***Oral BPC 7*** - RATE-LIMITING PROCESSES IN MONOLIGNOL FORMATION IN *PINUS TAEDA*. Aldwin M. Anterola, Hendrik van Rensburg, Laurence B. Davin and Norman G. Lewis

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THURSDAY, JULY 30

SYMPOSIUM SESSION 9 ***Postdoctoral and Graduate Student Oral Presentations - Best Paper Competition***
Moderator: ***Eric E. Conn***

11:50-12:10 ***Oral BPC 8*** - MOLECULAR AND BIOCHEMICAL ANALYSIS OF A 'HOMOLOG' GENE TO ACETYL COENZYME A:DEACTEYLVINDOLINE-*O*-ACETYLTRANSFERASE (DAT) FROM *CATHARANTHUS ROSEUS* (L.) G. DON. Pierre Laflamme, Benoit St-Pierre and Vincenzo De Luca

12:10-12:30 ***Oral BPC 9*** - EVIDENCE FOR A NOVEL MODE OF ACTION OF STEROIDAL ALKALOIDS. Anil T. Mangla and W. David Nes

12:30-12:50 ***Oral BPC 10*** - IDENTIFICATION AND QUANTIFICATION OF ONION (*ALLIUM CEPA* L.) HYBRID PARENT FLORAL VOLATILES AND THEIR EFFECT ON HONEY BEE ATTRACTIVENESS. Erin M. Silva and Bill B. Dean

12:50-1:50 LUNCH

2:00 Depart for Tour and Banquet

6:00-10:00 BANQUET (Cavanaugh's Inn at the Park, Skyline Room, Spokane, WA)

Symposium Paper 17 - NATURAL PRODUCTS AND PHYTOMEDICINE. G. H. Neil Towers

Awards Ceremony

FRIDAY, JULY 31

SYMPOSIUM SESSION 10 ***Plant Survival Strategies: Defenses and Attractants***
Moderator: ***G.H. Neil Towers***

8:00-8:30 ***Oral Paper 35*** - THE CLITOCYBULOLS, A NEW SKELETAL TYPE OF SESQUITERPENE. William A. Ayer

8:30-9:00 ***Oral Paper 36*** - BIOLOGICAL ACTIVITIES OF TURKISH MEDICINAL PLANTS AGAINST AGRICULTURAL PESTS. Bilge Sener, Nurgün Erdemoglu, William S. Bowers and Philip H. Evans

9:00-9:30 ***Oral Paper 37*** - CLONING AND CHARACTERIZATION OF POLYPHENOL OXIDASE, A POTENTIAL ANTI-HERBIVORE PROTEIN OF HYBRID POPLAR. C. Peter Constabel, Mary Christopher and Lynn Yip

9:30-10:00 ***Oral Paper 38*** - MOLECULAR BIOLOGY OF FLORAL SCENT. Natalia Dudareva, Jihong Wang, Leland Cseke and Eran Pichersky

10:00-10:20 BREAK

10:20-10:40 ***Oral Paper 39*** - THE CHEMISTRY OF PLANTS AND FRUITS USED IN FUR-RUBBING BY ANIMALS. Manuel Aregullin, Mary Baker, Matthew E. Gompper and Eloy Rodriguez

10:40-11:00 ***Oral Paper 40*** - SUBTERRANEAN TERMITE FEEDING STIMULANTS ISOLATED FROM ASSOCIATED FUNGI IN LABORATORY COLONIES. Brice A. McPherson and David L. Wood

11:00-11:20 ***Oral Paper 41*** - POLYGODIAL, AN ANTI-*CANDIDA* DRIMANE SESQUITERPENE DIALDEHYDE. Chris Lunde and Isao Kubo

11:20-11:40 ***Oral Paper 42*** - OCCURRENCE AND CHARACTERIZATION OF DIMBOA SPECIFIC GLUCOSYLTRANSFERASE IN MAIZE DURING JUVENILE STAGE OF GROWTH. Kenkichi Ebisui, Atsushi Ishihara and Hajime Iwamura

11:40-12:00 ***Oral Paper 43*** - NATURALLY OCCURRING QUINOLS AND QUINONES AS STUDIED BY ELECTRON SPIN RESONANCE. Jens A. Pedersen

12:00-12:20 ***Oral Paper 44*** - CHARACTERIZATION OF LIGNANS BY ELECTROSPRAY MASS SPECTROSCOPY. James Kerwin and Norman G. Lewis

12:20-1:15 LUNCH

FRIDAY, JULY 31

SYMPOSIUM SESSION 11 ***Maintaining Health with Phytochemicals***
Moderator: ***Michael A. Costa***

1:15-1:45 ***Oral Paper 45*** - MEDICINAL PLANTS OF THE STOLLMANN WILDERNESS. Suzanne Diamond

1:45-2:15 ***Oral Paper 46*** - A COLLABORATIVE APPROACH TO THE SUSTAINABLE DEVELOPMENT OF PHYTOCHEMICAL PRODUCTS FROM NATIVE CANADIAN PLANTS. Robin J. Marles, Faiz Ahmad, Natalie Tays, Jack Moes, Clayton Jackson, Clarence Marzolf and Markus Schmülgen

2:15-2:45 ***Oral Paper 47*** - CITRUS LIMONOIDS: FUNCTIONAL CHEMICALS IN AGRICULTURE AND FOODS. Shin Hasegawa, Gary Manners, Luke Lam and Edward Miller

2:45-3:05 ***Oral Paper 48*** - PHYTOMEDICINES WITH HOLISTIC APPROACH, PROVEN EFFICACY AND SAFE IN CLINICAL, *IN VIVO* AND *IN VITRO* STUDIES. S. Farooq

3:05-3:25 ***Oral Paper 49*** - STUDIES ON ARJUNA EXTRACT: A CARDIOTONIC. W. Madhulatha, P. Neelakanta Reddy and V. S. Sundara Rao

3:25-3:45 BREAK

6:00-7:30 DINNER (Compton Union Building, Ball Room)

7:30 MEETING ADJOURNED

SYMPOSIUM SESSION 1 ***DRUG DISCOVERY AND PATHWAY ENGINEERING***
Moderator: ***Richard A. Dixon***

MONDAY, 8:20-9:05

NATURAL PRODUCT DRUG DISCOVERY AND DEVELOPMENT: THE NCI ROLE

Symposium Paper 1

Gordon M. Cragg,
Natural Products Branch,
Developmental Therapeutics Program,
Division of Cancer Treatment and Diagnosis,
National Cancer Institute,
Bethesda, MD 20892, USA.

Over 60% of the drugs in commercial use for the treatment of cancer have their origin in nature, and, over the past 40 years, the National Cancer Institute (NCI) has supported the exploration of nature as a source of novel, potential agents. Over 400,000 extracts of plants, marine organisms and microbes, collected from many regions of the world, have been screened for anti-tumor activity. Since 1986, collections have been performed in over 25 source countries located in tropical and sub-tropical regions, and the NCI has established policies of equitable collaboration and compensation for interaction with these mainly developing countries. Collections are continuing on a reduced scale in these regions, while being replaced with collaborative agreements with qualified source country organizations, and have been expanded to include the continental United States. The NCI has played a major role in the discovery and development of many of the available commercial and investigative anti-cancer agents, mainly through collaboration with the world-wide scientific community. Increasing emphasis is being placed on such collaboration, and mechanisms for supporting drug discovery and development programs will be discussed.

MONDAY, 9:05-9:55

BIOSYNTHESIS OF PHARMACEUTICALLY USEFUL ALKALOIDS

Symposium Paper 2

Meinhart H. Zenk,
Lehrstuhl für Pharmazeutische Biologie,
Universität München,
Karlstr. 29.
80333 Munich, GERMANY.

Alkaloids play a considerable role among natural products used in medicine. With the advent of molecular biology it is now possible to engineer pathways for these natural products to conform with human needs. The prerequisite, however, is to first map out the complete pathway for a given compound at the metabolite and enzyme level. Examples will be given for the isoquinoline pathway, leading to morphine both in the poppy plant and in mammals. The biosynthesis of colchicine, which is characterized by a unique tropolone ring, will be discussed in detail as well as galanthamine, a potential drug directed against Alzheimer's disease. The formation of the taxane nucleus, present in the anticancer drug taxol, which is formed by the alternative terpenoid pathway involving 1-deoxyxylulose will be presented. The necessity to completely understand pathways leading to alkaloids prior to successful modification of plants and plant cell cultures will be discussed.

MONDAY, 9:55-10:15

BREAK

MONDAY, 10:15-11:05

TAXOL BIOSYNTHESIS: PROSPECTS FOR PATHWAY ENGINEERING

Symposium Paper 3

Rodney Croteau,
Institute of Biological Chemistry,
Washington State University,
Pullman, WA 99164-6340,
USA.

The biosynthesis of taxol and related taxoids in yew (*Taxus*) species involves the cyclization of the common isoprenoid precursor geranylgeranyl diphosphate to taxa-4(5),11(12)-diene followed by extensive oxidative modification of this diterpene olefin intermediate and elaboration of side chains. This sequence of reactions leading to taxol requires in excess of a dozen distinct enzymatic steps. The taxadiene synthase from Pacific yew has been purified and characterized, and the mechanism of the cyclization examined. A PCR strategy was employed to isolate the corresponding cDNA, which was confirmed by functional expression in *E. coli*, sequenced and shown to resemble other terpenoid cyclases of plant origin. The order of early hydroxylation steps in the conversion of taxadiene to taxol is proposed. Two of these hydroxylation steps, catalyzed by cytochrome P450 enzymes, have been deciphered. The initial hydroxylation of taxadiene proceeds with allylic rearrangement to yield taxa-4(20),11(12)-dien-5α-ol, which is a substrate for subsequent acyl CoA-dependent transacylation. A homology-based, PCR cloning strategy is being exploited to isolate cDNAs encoding P450 hydroxylases from *Taxus* for expression using yeast. Demonstration of the early steps of taxol biosynthesis suggests that the complete pathway can be defined by a systematic, stepwise approach at the cell-free enzyme level. When combined with *in vivo* studies to determine contribution to pathway flux, slow steps can be targeted for gene isolation and subsequent overexpression in *Taxus* to improve the yield of taxol and related compounds.

MONDAY, 11:05-11:25

Oral Paper 1

Peter J. Facchini and Min Yu,
Department of Biological Sciences,
University of Calgary,
2500 University Drive N.W.
Calgary, Alberta, T2N 1N4,
CANADA.

REGULATION OF ALKALOID AND AMIDE BIOSYNTHESIS IN OPIUM POPPY AND OTHER PLANTS

Aromatic amines are involved in the biosynthesis of many plant secondary metabolites, including alkaloids and amides. In opium poppy, tyramine and dopamine are incorporated into both benzylisoquinoline alkaloids and hydroxycinnamic acid amides. We have demonstrated a temporal correlation between the biosynthesis of alkaloids and amides, the accumulation of tyrosine/dopa decarboxylase (TYDC) and berberine bridge enzyme (BBE) mRNAs, and the induction of tyramine hydroxycinnamoyl transferase (THT) activity, in elicited opium poppy cultures. The THT enzyme was also purified and characterized. Members of the large opium poppy *tydc* gene family exhibit specific developmental and inducible expression patterns in opium poppy plants and cell cultures. The precise differential regulation of opium poppy *tydc* promoters is conserved in tobacco transformed with *tydc* promoter-reporter gene fusions. Our data suggest ubiquitous and fundamental roles for tyramine in plant development and defense responses, and suggest highly coordinated mechanisms of regulation in species, such as opium poppy, that produce multiple secondary products from aromatic amines.

MONDAY, 11:25-11:45

Oral Paper 2

Gabriel Guillet,
Julie Poupart, Juan Basurco and Vincenzo De Luca,
Institut de Recherche en Biologie Végétale,
Université de Montréal,
4101 rue Sherbrooke est,
Montréal, Québec, CANADA
H1X 2B2.

MODIFICATION OF NICOTINE AND CHLOROGENIC ACID POOLS IN TOBACCO LINES TRANSFORMED WITH HETEROLOGOUS TDC AND TYDC GENES

Tryptophan and tyrosine decarboxylase (TDC and TYDC) clones which had been obtained from *Catharanthus roseus* and *Papaver somniferum*, were used to generate transgenic tobacco lines with reduced tryptophan and tyrosine pools, respectively. While seedlings of the individual lines contained chlorogenic acid and nicotine levels comparable to those of untransformed controls, crosses of TDC with TYDC transgenic plants yielded a line which had enhanced levels of these metabolites. In contrast, nicotine and chlorogenic acid levels were significantly decreased in mature leaves of all transgenic lines, compared to non-transformed controls. The differences observed in seedlings and in mature tobacco plants suggest that regulatory processes controlling the levels of tryptophan and tyrosine in tobacco are affected by phenological development.

MONDAY, 11:45-12:05

Oral Paper 3

Benoit St-Pierre, Pierre Laflamme, Anne-Marie Alarco and Vincenzo De Luca,
Institut de Recherche en Biologie Végétale, Université de Montréal,
4101 rue Sherbrooke est,
Montréal, Québec, CANADA
H1X 2B2.

THE TERMINAL *O*-ACETYLTRANSFERASE OF VINDOLINE BIOSYNTHESIS DEFINES A NEW CLASS OF PROTEINS RESPONSIBLE FOR COENZYME A-DEPENDENT ACYL TRANSFER

The gene encoding acetyl CoA:deacetylvindoline 4-*O*-acetyltransferase (DAT) (EC 2.3.1.107) which catalyzes the last step in vindoline biosynthesis was isolated and characterized. The genomic clone contained an open reading frame encoding a 50 kD polypeptide which included the sequences of nine tryptic fragments derived from the purified DAT heterodimer. However, cleavage of DAT protein to yield a heterodimer appears to be an artifact of the protein purification procedure, since the size of the protein (50 kD) cross-reacting with anti-DAT antibody in seedlings and in leaves of various ages also corresponds to the size of the enzymatically active recombinant gene product. Studies with the intact plant and with developing seedlings showed that induction of DAT mRNA, protein and enzyme activity occurred preferentially in vindoline accumulating tissues such as in leaves and in the cotyledons of light treated etiolated seedlings. The ORF of DAT showed significant sequence identity to 19 other plant genes, whose biochemical functions were mostly unknown. The Mr of approximately 50 kD, a HXXXDG triad, and a DFGWGKP consensus sequence are highly conserved among the 20 plant genes and these criteria may be useful to identify this novel family of acyltransferase.

MONDAY, 12:05-12:25

Oral Paper 4

Benoit St-Pierre, Felipe Vazquez Flota and
Vincenzo De Luca,
Institut de Recherche en Biologie Végétale,
Université de Montréal,
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Montréal, Québec, CANADA
H1X 2B2.

CELLULAR SPECIALIZATION FOR THE VINDOLINE BIOSYNTHETIC PATHWAY IN THE LEAVES OF *CATHARANTHUS ROSEUS*

Leaves and roots of *Catharanthus roseus* produce a wide range of monoterpenoid indole alkaloids. The enzymology and regulation of this pathway have been studied in detail in our laboratory, and elsewhere. However little is known about its cellular localization. We have studied the cell-specific expression of four genes involved in the early and late stages of vindoline biosynthesis by *in situ* hybridization. The precursors of terpenoid indole alkaloids are derived from the shikimate pathway through the action of tryptophan decarboxylase (TDC), and from the mevalonate pathway by the action of several enzymes that convert geraniol into secologanin. The enzymatic condensation of tryptamine and secologanin is catalysed by strictosidine synthase (SS) to yield strictosidine, the general precursor of most terpenoid indole alkaloids. Expression of TDC and SS which appear to be coordinately regulated, were restricted to particular leaf cells. The last two steps in vindoline biosynthesis are catalyzed by the desacetoxyvindoline 4-hydroxylase (D4H) and deacetylvindoline 4-O-acetyltransferase (DAT). Although these transcripts were localized in the same cell type, they differed from the location of TDC and SS expression. These results imply that intercellular movement of a vindoline pathway intermediate must occur to allow operation of this pathway in *Catharanthus* leaves.

MONDAY, 12:25-1:30 **LUNCH**

SYMPOSIUM SESSION 2 ***POLYPHENOLS IN HUMAN HEALTH PROTECTION AND PLANT DEFENSE***

Moderator: ***Ragai H. Ibrahim***

MONDAY, 1:30-2:15

Symposium Paper 4

Edwin Haslam,
Department of Chemistry,
University of Sheffield,
Sheffield, S3 7HF,
UNITED KINGDOM.

PRINCIPLES OF MOLECULAR RECOGNITION: POLYPHENOLS

Insofar as the possible modes of physiological action of polyphenols are concerned, present evidence suggests that they have the *potential* to act in three general areas, namely: transition metal ion complexation, as anti-oxidants in cellular pro-oxidant states and by association with proteins and peptides. The principles which underly the means whereby polyphenols may recognize and associate with other molecules will be outlined and the significance of such processes in the development of astringency will be discussed.

MONDAY, 2:15-3:00

Symposium Paper 5

Daneel Ferreira,
Department of Chemistry,
University of the Orange Free State,
P.O. Box 339,
Bloemfontein, 9300,
SOUTH AFRICA.

RECENT ADVANCES IN THE CHEMISTRY OF PROANTHOCYANIDINS

In spite of the rapid recent advances in the chemistry of the oligomeric proanthocyanidins, the appropriate literature is still characterized by a predominant analytical approach. Development towards understanding of the chemical dynamics of this complex group of natural products, *i.e.* enantioselective synthesis of the electrophilic and nucleophilic constituent flavanyl units, manipulation of some crucial bonds in the molecular tannin framework and experiments designed to simplify oligo- and polymeric structures, will be discussed. This knowledge is a prerequisite to eventually understand the interaction of these compounds with other biomolecules and hence their effects on human health.

MONDAY, 3:00-3:20 **BREAK**

MONDAY, 3:20-4:05

Symposium Paper 6

Richard A. Dixon[1],
P. Canovas[2], X.-Z. He[1],
C. Lamb[2], F. McAlister[1] and
C.L. Steele[1],
[1]Samuel Roberts Noble Foundation,
Ardmore, OK 73402, USA;
[2]Salk Institute for Biological Studies,
La Jolla, CA 92037, USA.

MOLECULAR CONTROLS FOR ISOFLAVONOID BIOSYNTHESIS IN RELATION TO PLANT AND HUMAN HEALTH

Isoflavonoids function in plant-microbe interactions as antimicrobial phytoalexins and regulators of bacterial nodulation genes, and dietary isoflavones have been ascribed strong cancer chemopreventative activity in humans. Many of the genes encoding enzymes for the elaboration of isoflavonoids from phenylpropanoid precursors have been isolated. These provide a means to develop novel transgenic plants with improved disease resistance and, perhaps, value added health benefits for humans. We will describe recent progress on the molecular biology of isoflavone formation and *O*-methylation. The isoflavonoid pathway is transcriptionally induced in response to elicitation or infection. We have cloned two transcription factors, a basic leucine zipper protein and a homolog of the human Ku autoantigen, involved in activation of expression of the chalcone synthase gene. We will describe how phosphorylation of these factors may control their activity following elicitation.

MONDAY, 4:05-4:25

Oral Paper 5

Denis Barron[1], A. Di Pietro[2], G. Comte[1], J.M. Jault[2], H. Cortay[2], J.B. Daskiewicz[1], G. Conseil[2], G. Dayan[2] and C. Bayet[1], [1]Plant Biochemistry Laboratory, UPRESA CNRS 5013, Department of Chemistry and Biochemistry, Claude Bernard University-Lyon 1, Building 303, 43 Bd. du 11 Novembre 1918, 69622 Villeurbanne Cedex, FRANCE; [2]Institute of Biology and Chemistry of Proteins (UPR CNRS 412), 7 Passage du Vercors, 69367 Lyon Cedex 07, FRANCE.

FLAVONOIDS INTERACTIONS WITH P-GLYCOPROTEIN, A NEW HOPE FOR ANTICANCER CHEMOTHERAPY

P-glycoprotein is a plasma membrane transporter often overexpressed in cancer cells submitted to prolonged chemotherapy. It extrudes the cytotoxic drugs at the expense of ATP hydrolysis and confers a multidrug resistance (MDR) cellular phenotype. On the other hand, flavonoid compounds have been shown to inhibit a number of ATP dependent enzymes, due to an analogy of rings A and C of flavonoids with the adenine ring of ATP. As a consequence, flavonoid compounds are able to reverse the chemoresistance process by inhibition of P-glycoprotein activity. The presence of hydroxyl groups at positions 5 and 3, as well as hydrophobic substituents on ring A, are important parameters for binding affinity to the protein. The methods developed by our group for the organic synthesis of hydrophobic prenyl flavonoids will be reviewed. Both A- and B-ring prenylations will be considered. Introduction of C_5, C_{10} and C_{15} terpene units will be covered.

MONDAY, 4:25-4:45

Oral Paper 6

Wolfgang Kreis and Andreas Baumgertel, Lehrstuhl für Pharmazeutische Biologie, FAU Erlangen-Nürnberg, Staudtstr. 5, D-91058 Erlangen, GERMANY.

PROPERTIES OF A FLAVONOL 3-*O*-HETERODISACCHARIDE GLYCOSIDASE FROM *FAGOPYRI ESCULENTUM* (BUCKWHEAT)

The dried herb of *Fagopyrum esculentum* Moench (Polygonaceae) contains high amounts of rutoside; the drug is used in the treatment of vascular diseases and circulatory disorders. The title enzyme was isolated from this source. The enzymatic hydrolysis of rutoside was maximal at pH 4.8 and 30°C reaching specific activities of about 50 nkat/mg protein in the partially purified extract. The Km for rutoside was 0.123 mM. The energy of activation was determined as 32 kJ/mol. Quercitrin and isoquercitrin were not deglycosylated. Rutinose was identified as the only sugar released during the enzymatic quercetin formation. Enzymatic degradation of biologically active drug constituents may occur at various stages of a pharmaceutical process. As shown here, specific enzymes may even be active in ethanolic extracts or at low temperatures.

MONDAY, 4:45-5:05

Oral Paper 7

E. Vincent Brandt, B. Irene Kamara, Elizabeth Joubert[†], Jacobus A. Steenkamp and Daneel Ferreira, Department of Chemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein, 9300, SOUTH AFRICA; [†]Infruitec, Private Bag X5013, Stellenbosch, 7500, SOUTH AFRICA.

PHENOLIC COMPOUNDS FROM *CYCLOPIA INTERMEDIA* (HONEYBUSH TEA)

The leaves and stems of *Cyclopia intermedia*, one of the woody legumes endemic to the Cape fynbos region of South Africa, are used to brew a traditional herbal tea with a pleasant honey flavour. In order to support the establishment of the honeybush tea industry as a viable agricultural enterprise we initialized an investigation concerning the phenolic constituents of the plant. The processed leaves and stems were thus found to contain a range of flavanones, flavones, isoflavones, xanthones, coumestans, a 4-hydroxycinnamic acid and the inositol, (+)-pinitol.

The substantial number of compounds with pharmacological properties may contribute towards appeal of the tea as a health beverage and our current efforts are focused on the phenomenon of its natural sweetness as well as the potential of the various compounds to act as anti-oxidants or to exhibit alternative health promoting properties. Availability of sufficient quantities of especially isoflavones and coumestans, therefore, currently rely on their syntheses *via* the appropriate chalcones, in the case of the coumestans by a novel single step synthesis believed to proceed by a sequential autoxidation initiated by $Tl(NO_3)_3$.

MONDAY, 5:05-5:25

Oral Paper 8

Christopher L. Steele and
Richard A. Dixon,
Samuel Roberts Noble
Foundation,
Ardmore, OK 73402,
USA.

TOWARDS THE ISOLATION OF AN ISOFLAVONE SYNTHASE cDNA: THE FIRST STEP IN THE SYNTHESIS OF THE PHYTOALEXIN MEDICARPIN AND CANCER-PREVENTATIVE ISOFLAVONOIDS

The first committed step to isoflavone production is the conversion of liquiritigenin or naringenin to the unstable intermediate hydroxyisoflavone by isoflavone synthase, IFS. IFS has been shown to be a member of the cytochrome P450 hydroxylase superfamily; as such it has at least four highly conserved amino acid motifs that allows for a similarity/PCR approach for cDNA isolation. In addition, treatment of alfalfa (*Medicago sativa* L.) suspension cell cultures with a crude elicitor preparation from the bean pathogen *Colletotrichum lindemuthianum* has been shown to induce IFS activity which peaks at 10 hours and rapidly declines after twenty hours. Using a cDNA library made with mRNA extracted from elicited cell cultures as template and degenerate oligonucleotides as primers, PCR products were cloned. Following the screening of eighty subclones, six unique P450-like clones were identified, 2.2, 3.3, 17.2, 1.3, 9.3, and 1.4. Northern blot analysis using total RNA isolated from cell suspension cultures with and without elicitation revealed that three clones (2.2, 3.3, and 1.3) are inducible, two (1.4 and 17.2) are constitutively expressed , and one (9.3) failed to hybridize. Full length cDNA clones have been isolated and sequenced for all of the inducible cDNAs. Probing the cDNA library under low stringency conditions produced an additional P450-like cDNA which is 98% identical to pTF26, an unknown P450-like cDNA previously isolated from alfalfa. These P450-like cDNAs are currently being functionally expressed in several systems to allow enzymatic assays to identify the IFS cDNA.

MONDAY 6:00-7:30 **DINNER - COMPTON UNION BUILDING, BALL ROOM**

POSTER SESSION 1 ***PHYTOCHEMICAL PATHWAYS USEFUL IN MEDICINE AND PLANT DEFENSE***

Moderators: ***Michael A. Costa/David R. Gang***

MONDAY, 8:00-10:00 PM **COMPTON UNION BUILDING - BALL ROOM**

Poster Paper 1

J.W. Bok[1], L. Lermer[1], J. Chilton[2], H.G. Klingeman[3] and G.H.Neil Towers[1],
[1]Department of Botany, University of British Columbia, Vancouver, B.C., V6T 1Z4, CANADA;
[2]North American Reishi, Box 1780, Gibsons, British Columbia, VON 1VO, CANADA;
[3]Department of Medicine, Terry Fox Laboratory, University of British Columbia, 601 West 10th, Vancouver, B.C., V5X 1C3, CANADA.

ANTITUMOR STEROLS FROM THE MYCELIA OF *CORDYCEPS SINENSIS*

Activity guided fractionations led to the isolation of two novel antitumor compounds 5α,8α-epidioxy-24(R)-methylcholesta-6,22-dien-3β-D-glucopyranoside and 5,6-epoxy-24(R)-methylcholesta-7,22-dien-3β-ol from the methanol extract of *Cordyceps sinensis*. Two previously known compounds, ergosteryl-3-O-α-D-glucopyranoside and 22-dihydroergosteryl-3-O-β-D-glucopyranoside were also isolated. The structures of the first two novel compounds were established by 1D and 2D NMR spectroscopic techniques, with the first synthesized in order to confirm the identity of the sugar moiety by chemical correlation. The glycosylated form of ergosterol peroxide was found to be a greater inhibitor to the proliferation of K562, Jurkat, WM-1341, HL-60 and RPM-8226 tumor cell lines by 10 to 40% at 10 μg/ml than its previously identified aglycone, 5α,8α-epidioxy-24(R)-methylcholesta-6,22-diene-3β-ol.

Poster Paper 3

Debora C. Baldoqui[1], Massuo J. Kato[2], Alberto J. Cavalheiro[1], Vanderlan S. Bolzani[1], Maria C. M. Young[3] and Maysa A. Furlan[1],
[1]Instituto de Qu'mica, Universidade Estadual Paulista, 14800-900 Araraquara;
[2]Instituto de Qu'mica, Universidade de São Paulo, 05599-970, São Paulo;
[3]Seção de Fisiologia e Bioqu'mica de Plantas, Instituto de Botânica, 01051 São Paulo, SP, BRAZIL.

EVALUATION OF DNA-DAMAGING ACTIVITY OF BENZOPYRAN AND PRENYLATED BENZOIC ACID FROM *PIPER ADUNCUM* SW

Piperaceae species have proven to be a rich source of biologically active compounds such as lignoids, flavonoids, amides and alkaloids. Our previous research on *Piper hispidum* SW led to the isolation of the antifungal amide *N*-[7-(3',4'-methylenedioxyphenyl)-2*Z*,4*Z*-heptadienoyl]-pyrrolidine. From the leaves of *P. aduncum*, a plant species used as remedy for stomach aches, three benzopyrans and a prenylated benzoic acid derivative were isolated by chromatographic techniques and had their structures determined based on spectroscopic data. Two of the benzopyrans showed selective activity toward the mutant yeasts of *Saccharomyces cerevisiae*. (Funding provided by FAPESP, PADCT and CNPq)

Poster Paper 5

Mark A. Berhow[1], S.F. Vaughn[1], M.J. Plewa[2], E.D. Wagner[2], L. Rayburn[2] and E. Gugger[3], [1]USDA, ARS, PWA NCAUR, Bioactive Agents Research, 1815 N. University St., Peoria, IL 61604, USA; [2]Department of Crop Sciences, 1101 W. Peabody Dr., University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA; [3]Archer Daniels Midland Company, 1001 Brush College Rd., Decatur, IL 62521, USA.

EVALUATION OF CHEMICALS DERIVED FROM AGRICULTURAL PRODUCTS FOR CHEMOPROTECTIVE ACTIVITY

New research projects have been initiated to identify naturally occurring chemicals in plants and agricultural products which have biological activity. Following leads from scientific observations reported in the literature, or those observed by our research group, agricultural products will be examined to determine their chemical modes of action. A number of projects are underway. An extract prepared from soybean "soy solubles" left over from the processing of soybeans has been shown to have potent chemo-protective activity in anti-mutagen bioassays. The chemical agent(s) responsible for this are being determined. Initial work indicates that the active agents may be related to either the isoflavonoids or the saponins or both. The extract is being broken down into its chemical components by a series of fractionation steps and the chemical composition will be confirmed by UV absorption, mass spectrometry and NMR. The chemoprotective activity is being determined by an advanced cell assay, COMET, which measures the ability of added chemicals to protect cell DNA from damage by potent carcinogens.

Poster Paper 7

Yves Sauvaire, C. Broca, Y. Baissac, R. Gross, P. Petit and G. Ribes, Université de Montpellier, Place E. Bataillon, 34095 Montpellier, FRANCE.

4-HYDROXYISOLEUCINE: AN INTERESTING INSULINOTROPIC AMINO ACID

Non insulin-dependent diabetes mellitus (NIDDM) has a very marked economic impact because of its high frequency. Thus, it seems worthwhile to search for new antidiabetic agents. We have demonstrated that 4-hydroxyisoleucine (4-OH-Ile), an amino acid extracted and purified from fenugreek seed (*Trigonella foenum-graecum* L.), is an interesting component since it stimulates *in vitro* insulin secretion at micromolar concentrations and only in the presence of elevated glucose concentrations. Fenugreek seeds contain two isomers of 4-OH-Ile which were isolated and identified as 2S, 3R, 4S (major) and 2R, 3R, 4S (minor). *In vivo* studies showed that only the major isomer is active at micromolar concentrations. *In vivo* studies were performed in normal rats and dogs by intravenous (IVGTT) or oral (OGTT) glucose tolerance tests. During IVGTT in normal rats, 4-OH-Ile (18 mg/Kg) enhanced insulin secretion and thus improved glucose tolerance (area under the curve/30 min: 5421 ± 125 mg/dl versus 6459 ± 67 mg/dl in controls, $p<0.01$). A similar effect was obtained during OGTT in normal dogs (area under the hyperglycemic curve/45 min: 5027 ± 107 mg/dl versus 6290 ± 140 mg/dl in controls, $p<0.01$). In conclusion, our results demonstrate that 4-0H-Ile (2S, 3R, 4S) is an insulinotropic agent of potential interest in the treatment of NIDDM.

Poster Paper 9

E. Cartron, M.A. Carbonneau, J.P. Cristol, G. Fouret, B. Descomps, Claude Andary* and C.L. Léger, Laboratoire de Biologie et Biochimie des Lipides, Faculté de Médecine; *Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Université Montpellier I, 34060 Montpellier, FRANCE.

PHENOLIC COMPOUNDS OF VARIOUS ORIGINS HAVE DIFFERENTIAL EFFECTS ON THE LOW-DENSITY LIPOPROTEIN ANTIOXIDANT AND ANTI-ATHEROGENIC STATUS

Some epidemiological studies showed a negative correlation between polyphenol consumption and the incidence of cardiovascular diseases in humans, which can be explained from the viewpoint of the oxidative theory of atherosclerosis. So we decided to study the *in vitro* effect of natural phenolic mixtures (originated from red wine or olive oil waste waters) or purified phenolic compounds (+)-catechin, (–)-epicatechin, phenolic acids belonging to the benzoic and cinnamic series, caffeoyl quinic- and tartric-derivatives) on LDL 5-μM Cu^{2+}-oxidizability. Differential antioxidant effects of these substances were shown. Some of them had a most efficient antioxidant effect at submicromolar concentrations, which suggests a relevant physiological anti-atherogenic action of these substances.

Poster Paper 11

Suzana G. Leitão[1], F.P.G. Melo[1], T.C. Santos[1], F. Delle Monache[1], J.L.S. Gonçalves[2] and M.D. Wigg[2],
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Faculdade de Farmácia, UFRJ,
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BRAZIL;
[2]Depto de Virologia, Instituto de Microbiologia,
UFRJ, Rio de Janeiro,
21941-590, BRAZIL.

ANTIVIRAL ACTIVITY OF FLAVONOID-RICH EXTRACTS FROM *VITEX* AND *CONGEA*

Vitex species (*ca.* 250 species, Verbenaceae) are small trees or shrubs widespread in Brazil. The genus *Congea* (Verbenaceae) comprises approximately 10 species occurring from the Northeast India to the Malayan peninsula. *V. polygama* was collected at Marica, Rio de Janeiro and *V. cymosa* at Corumba, Mato Grossso do Sul, Brazil. *C. tomentosa* was collected at the Rio de Janeiro Botanical Garden. Plant material was extracted with either ethanol or ethanol: H_2O (1:1) and partitioned between water and organic solvents. Ethyl acetate extracts from all plants were evaluated for their antiviral activity. Before antiviral tests the maximun non-toxic concentrations of the extracts were determinated using HEp-2 cells. The anti-HSV-1 (*Herpes simplex* Virus Type-1) and anti-BVDV (Bovine Viral Diarrhea Virus) activities were analyzed through determination of the percentage of inhibition calculated in treated and untreated cells. Antiviral activities for HSV-1 were: *V. polygama* - 85.2 % (fruits) and 73.7 % (leaves); *V. cymosa* - 94.4 % (leaves); and *C. tomentosa* - 63.7 % (leaves). For BVDV, antiviral activities were: *V. polygama* - 99.6 % (fruits) and 96.9 % (leaves) and *C. tomentosa* - 80.0 % (leaves). Fractionation of the ethyl acetate extracts from all plants led to the isolation of *C*-glucosyl flavonoids (*Vitex*) and acylglucosylflavonoids (*Vitex* and *Congea*).

Poster Paper 13

Elfranco Malan[1],
Daneel Ferreira[1] and
Pricilla Swartz[2],
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University of the Orange Free State,
P.O. Box 339,
Bloemfontein, 9300, RSA;
[2]National Botanical Institute,
Private Bag X101,
Pretoria, 0001, RSA.

COMPARATIVE MORPHOLOGICAL AND CHEMICAL CHARACTERISTICS OF *ACACIA KARROO*

Acacia karroo is one of the most widespread trees in Africa occurring in many different habitats which result in morphological differences. Botanists proposed that *A. karroo* be subdivided into several subspecies. Concoctions or infusions of the bark, heartwood, leaves and gum are used for a range of ailments. The gum is also used in the food industry. Chemical investigation produced a range of differently substituted flavonoids, partially *O*-methylated flavonoids, lignoids, terpenoids and 3,10-dihydroxy-9-*O*-(6′-hydroxy-7′-*O*-methyl-2′-hydroxymethyldihydrobenzo-furan-3-yl)-dibenz- [b,d]-pyran-6-one and its 10-*O*-methyl analogue. These metabolites were distributed differently in the tree specimens from four different localities and therefore support the idea of subspecies. The chemical results also support the use of concoctions and infusions in folk medicine in the various habitats of *A. karroo*.

Poster Paper 15

Blas Lotina-Hennsen[1],
Katia Robles-Garcia[1],
Beatriz King-Diaz[1],
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Facultad de Química, UNAM,
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MEXICO.

INHIBITORY EFFECTS OF SESQUITERPENE LACTONES FROM *COSMOS PRINGLEI* (ASTERACEAE) ON PHOTOSYNTHESIS IN SPINACH CHLOROPLASTS

Cosmos pringlei (Asteraceae) is a medicinal plant employed by the tarahumara indians for the treatment of gastric ulcer and other ailments. Bioactivity-guided fractionation of the active extract led to the isolation of dehydrocostus lactone, 14-*iso*valeryloxy-costunolide and costunolide. These lactones act as phytogrowth inhibitors to *Echinocloa crusgalli* and *Amaranthus hypochondriacus*. The effect of these lactones on several photosynthetic activities on isolated spinach chloroplasts was also investigated. The results indicated that the first two acted as uncouplers, because they enhanced both basal and phosphorylating electron flow from H_2O to Paraquat and the activity of the light Mg^{+2}-ATPase. In addition, dehydrocostus lactone inhibited ATP synthesis without affecting uncoupled electron flow, but 14-*iso*valeryloxy-costunolide inhibited both ATP synthesis and uncoupled electron flow. Therefore, it acts as an uncoupler-Hill reaction inhibitor. On the other hand, constunolide was characterized as an energy transfer inhibitor, because it inhibitied photophosphorylation, Mg^{+2}-ATPase activity and phosphorylating electron transport without affecting basal and uncoupled electron transport.

Poster Paper 17

Alicja M. Zobel[1], J.M. Lynch[1] and K. Wierzchowska-Renke[2], [1]Department of Chemistry, Trent University, Peterborough, Ontario, CANADA K9J 7B8; [2]Pharmacy, Medical Academy, 08-416 Gdansk, POLAND.

UV-A AND METALS INFLUENCING PHENOLIC COMPOUNDS IN MEDICINAL AND AGRICULTURAL PLANTS

UV-A radiation caused qualitative and quantitative changes of phenolic compounds in all 20 investigated species of plants of which some were medicinal (*Pastinaca sativa, Angelica archangelica, Achillea mellifolia, Ruta graveolens, Heracleum mantegazianum, Equisetum arveuse*) and some agricultural (*Melilotus albus, Brassica napus, Zea mays*). Changes were in coumarins, flavonoids, and simple phenolic acids, all of which absorbed 280 and 325 nm radiation. Changes to compounds on the surface of the leaves could increase absorption of UV-A thus, increase the defense system of a plant by preventing UV-A penetration through the epicuticular waxes and epidermal layer.

Poster Paper 19

James A. Saunders and Monica J. Pedroni, Climate Stress Lab, USDA/ARS, Bldg. 50, Rm. 100, Beltsville, MD 20705, USA.

AFLP DNA ANALYSIS OF OPIUM POPPY

There are several different types of DNA analysis procedures that have been used to identify and characterize cultivars for genetic diversity. Each procedure has it own requirements, sensitivity, and reliability. Amplified Restriction Fragment Length Polymorphism (AFLP) is the newest DNA analysis procedure. It combines assay flexibility, with a high degree of sensitivity and reproducibility to yield significantly more information about the DNA under study than older techniques. We have applied this procedure to more than 70 different lines of opium poppy in an effort to characterize the genetic diversity of the collection. Cultivars of opium poppy sharing a common heritage could be easily distinguished from lines of a more distant genetic background. Analysis of the genetic relatedness was performed on autoradiographic banding patterns using commercially available software. This data was used to generate a similarity dendrogram depicting the predicted phylogenic relationship of the opium poppy cultivars. This type of analysis allowing unambiguous cultivar identification can be accomplished with limited fresh leaf material. Experiments are underway to attempt genetic analysis of opium gum samples for forensic use.

Poster Paper 21

Ufuk Koka, Zhi-Qiang Xia, Laurence B. Davin and Norman G. Lewis, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340, USA.

DEFINING THE BIOCHEMICAL PATHWAY TO THE ANTITUMOR LIGNAN, PODOPHYLLOTOXIN

Podophyllotoxin is a very important antitumor agent isolated from plants. Etoposide and teniposide, which are the semisynthetic glucoside derivatives of epipodophyllotoxin, are widely used clinically against small cell lung cancer, testicular cancer, lymphoma and leukemia. We are working on the biosynthesis of podophyllotoxin as a first step towards biotechnological exploitation of the pathway to these medicinally important lignans. Previous work has suggested a biochemical precursor relationship between matairesinol and podophyllotoxin. The biosynthetic pathway to matairesinol has been established in *Forsythia* species. Pinoresinol synthase, pinoresinol reductase and secoisolariciresinol dehydrogenase have been purified from *Forsythia intermedia* and characterized in this laboratory. Since 5-methoxypodophyllotoxin also occurs in *Linum flavum*, we characterized pinoresinol reductase and secoisolariciresinol dehydrogenase from this species for comparative purpose. Administration of matairesinol to *Linum flavum* roots resulted in the build-up of an intermediate identified as 7-hydroxy matairesinol.That this was an intermediate in the biosynthetic pathway to 5-methoxy podophyllotoxin was established by incubating [7-^{3}H]-7-hydroxy matairesinol with *L. flavum* roots, where it was found that it was incorporated into 5-methoxy podophyllotoxin in 1.3 %. The main objective of our research is to identify all the biosynthetic intermediates between pinoresinol and podophyllotoxin, and characterize the enzymes and identify the genes involved. Future studies will use both *Linum flavum* and *Podophyllum hexandum* cell suspension cultures to further establish the biosynthetic steps.

Poster Paper 23

Dangyang Ke,
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OVER-EXPRESSION OF CAROTENOID GENES DRAMATICALLY INCREASES CAROTENOID LEVELS IN CANOLA SEEDS

Phytoene synthase (PS) and other carotenoid genes from the bacterium, *Erwinia*, were over-expressed in canola (*Brassica napus* L.) seeds under a seed-specific promoter (napin) for molecular farming of carotenoid compounds. The PS alone caused over 50-fold increases in concentration of total carotenoids, mainly beta and alpha-carotene and significant amounts of phytoene. Northern analysis and carotenoid accumulation time course profile indicated that the PS gene was turned on around 21 days post-anthesis (DPA). Total chlorophyll concentration of 35 DPA young seeds and levels of gamma-tocopherol and total tocopherols in mature seeds from transgenic plants were reduced. We are also studying the effects of combining PS with phytoene desaturase and/or geranylgeranyl pyrophosphate synthase on carotenoid profile and levels of chlorophylls and tocopherols.

Poster Paper 25

Santokh Singh[1], J.W. Bok[1],
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^{15}N NUCLEAR MAGNETIC RESONANCE STUDIES OF NITROGEN RECYCLING DURING PHENYLALANINE METABOLISM IN *LENTINUS LEPIDEUS*

The metabolic fate of the phenylalanine ammonia-lyase-generated ammonium ion in *Lentinus lepideus*, a wood-destroying basidiomycete was investigated. [^{15}N]-L-Phenylalanine was administered to four-day old mycelium of *L. lepideus* in the dark. Analyses of the ^{15}N-labeled metabolites by ^{15}N-nuclear magnetic resonance spectroscopy indicate that this nitrogen is first incorporated into the amide moiety of L-glutamine and then into L-glutamate by the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway. ^{15}N is also incorporated into γ-aminobutyric acid and alanine by a direct transamination at the level of phenylalanine. A new nitrogen recycling mechanism during phenylalanine metabolism in fungi is proposed.

Poster Paper 27

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CONFORMATIONAL DYNAMICS OF PROANTHOCYANIDINS: PHYSICAL AND COMPUTATIONAL APPROACHES

The interaction of plant polyphenols with proteins accounts for a good part of their commercial (e.g. leather manufacture) and biological (e.g. anti-microbial activity) significance. The interplay between observations of physical data such as crystal structure, NMR analyses, and time-resolved fluorescence with results of computational chemistry approaches has been essential to any success we have had in this effort. Examples of critical steps that demonstrate the importance of combining physical data with computational chemistry, are summarized. Both measurement of physical properties and computational studies are required if we are to make progress in our understanding of the interactions between polyphenols and proteins.

Poster Paper 29

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MEMBERS OF THE MULTIGENE FAMILY ENCODING BLACK CHERRY (*PRUNUS SEROTINA*) (*R*)-(+)-MANDELONITRILE LYASE ARE DIFFERENTIALLY EXPRESSED

In rosaceous stone fruits, the flavoprotein (*R*)-(+)-mandelonitrile lyase (MDL; EC 4.1.2.10) catalyzes the final step in the degradation of the cyanogenic glucosides, (*R*)-amygdalin and (*R*)-prunasin, to the defense compound HCN. In black cherry (*Prunus serotina* Ehrh.) seeds, MDL exists as several isoforms whose chemical nature and physiological significance were unclear. We report here the sequences of five distinct MDL cDNAs, designated MDL1-MDL5. As expected, all encode a signal sequence, a likely FAD-binding site, and several potential N-glycosylation sites. Genomic sequences corresponding to MDL1-MDL4 were also characterized. All four MDL genes are interrupted at identical positions by three short introns, suggesting that they share common ancestry. Exhibiting 75-88% amino acid identity, they appear to represent members of a multigene family. Southern analysis employing both conserved coding region probes and gene-specific probes revealed approximately 8 members within this family. Northern analysis showed that the MDL genes are differentially expressed in black cherry organs. *mdl1* and *mdl2* are highly expressed in immature seeds. *mdl1* is also expressed in seedling leaves and roots but at much lower levels, whereas *mdl2* was undetectable in post-embryonic tissues. In contrast, *mdl3* expression was undetectable in developing seeds but was observed at low levels in roots. Finally, *mdl4* is expressed only in post-embryonic tissues.

Poster Paper 31

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[2]Biologie de la Rhizosphère, INRA, Route de St Cyr, F-78026 Versailles, FRANCE.

PHYTOPHTHORA RESISTANCE THROUGH PRODUCTION OF ELICITIN, A FUNGAL PROTEIN ELICITOR, IN TRANSGENIC TOBACCO PLANTS

Elicitins are 10 kDa proteins secreted by *Phytophthora* fungi that elicit an incompatible hypersensitive reaction, leading to resistance against certain plant pathogens. Various natural molecules, site-directed mutated elicitins and synthetic peptides were previously shown to differently induce necrotic effects and tobacco defense genes. We observed that induction of necrosis and resistance are borne by several different amino acid residues which were identified and located in the 3D structure we recently obtained. Transformation of tobacco with a gene encoding b cryptogein produced an intracellular accumulation of elicitin in the plant before attack. This indeed provided the protein signal not produced by the pathogen which resulted in resistance to the pathogen, *Phytophthora parasitica* var *nicotianae*. Resistance was improved when the foreign gene was in the heterozygous state, and a single amino acid substitution that reduced the necrotic effects of the protein also conferred some resistance.

Poster Paper 33

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BIOSYNTHETIC PATHWAY OF OAT PHYTOALEXIN, AVENANTHRAMIDE

Biosynthetic pathway of oat phytoalexins, avenanthramides, was investigated by feeding experiments. Labeled putative precursors were administered to oat leaf segments treated with penta-*N*-acetylchitopentaose as an elicitor. Among tested compounds, oat leaf segments incorporated [ring-UL-^{14}C]anthranilic acid and [2,3,4,5,6-^{2}H]L-phenylalanine into avenanthramides with low dilution of isotopes. [2,6-^{2}H]L-Tyrosine was not incorporated into avenanthramides. These findings corroborated that avenanthramides were biosynthesized from anthranilic acids and hydroxycinnamic acids derived from phenyalanine. In addition to phenylalanine, sodium [$^{13}C_2$]acetate and [1,2-^{13}C]*p*-coumaric acid were incorporated into avenanthramide L, which indicated that avenalumoyl (5-(4-hydroxyphenyl)-penta-2,4-dienoyl) moiety of avenanthramide L was biosynthesized from *p*-coumaric acid by elongation of side chain with an acetate unit.

Poster Paper 35

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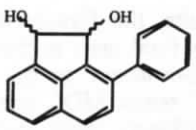
PHENYLPHENALENONIC PHYTOANTICIPINS FROM THE RESISTANT *MUSA* SELECTED HYBRID SH-3481

A new acenaphthylene derivative and two new dimeric phenylphenalenones have been isolated from healthy rhizomes of musa selected hybrid resistant cultivar SH-3481. The structure of the new substances were elucidated using spectroscopy data. Seven previously reported phenylphenalenone type phytoalexins were isolated as constitutive natural antibiotics, and as phytoanticipins from this resistant banana cultivar. This suggests that this type of phytoalexin plays an important role as part of the resistant mechanism to fungal diseases in this important alimentary resource botanic family.



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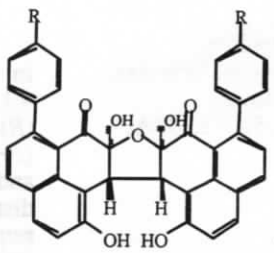

2: R=H
3: R=OH

Poster Paper 37

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BIOSYNTHESIS OF THE ISOPRENE UNITS OF CHAMOMILE SESQUITERPENES

The biosynthetic pathways that are involved in the formation of the isoprene units of the sesquiterpenes of chamomile flowers (*Matricaria recutita)* have been investigated by applying a stable isotope labeling technique. ^{13}C labeling of the sesquiterpenes has been achieved by injection of a solution of [1-^{13}C]-glucose in the anthodia of the plant. The sesquiterpenes bisabololoxide A, and chamazulene have been isolated from the hydrodistillate of the labeled flowers. Analysis of labeling patterns and absolute ^{13}C abundances by quantitative ^{13}C NMR spectroscopy showed that the biogenetically first two isoprene building blocks are predominantly formed via the new glyceraldehyde/pyruvate pathway, whereas the third unit is of mixed origin, derived from both the mevalonic acid pathway and the glyceraldehyde/pyruvate pathway.

Poster Paper 39

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ISOLATION AND CHARACTERIZATION OF A FLAVANONE-7-*O*-GLUCOSYLTRANSFERASE (7GT) ACTIVITY FROM *PETUNIA HYBRIDA*

Citrus spp. are known for the accumulation of flavanone glycosides (e.g., naringin comprises up to 70% of the dry weight of very young grapefruit). In contrast, petunia utilizes naringenin for production of more flavonol glycosides and anthocyanins. This investigation addressed the question of whether or not petunia is capable of glucosylation of naringenin and, if so, what are the characteristics of this flavanone glucosylating enzyme. Petunia leaf tissue contains a 7GT activity, although at 100-fold lower levels than grapefruit. This activity was purified 90-fold via ammonium sulfate fractionation followed by FPLC on Superose 12 and Mono Q yielding three peaks of activity. All peaks glucosylated flavanone, flavonol, and flavone substrates. Peaks I and II were more specific and peak I was significantly more active. Enzyme activity was not effected by Ca^{2+}, Mg^{2+}, AMP, ADP, or ATP. Petunia 7GT was 10,000 times more sensitive to UDP than the flavanone-specific 7GT in grapefruit. These and other results suggest that different flavonoid accumulation patterns in these two plants may be partially due to the different relative levels and biochemical properties of their 7GT enzymes.

Poster Paper 41

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IS METHYL GALLATE A NATURAL CONSTITUENT OF MAPLE (GENUS ACER) LEAVES?

Methyl gallate is found in extracts of silver maple leaves (*Acer saccharinum* L.) (Bailey et al., 1986, J. Nat. Prod. 49:1149-1150) but, as Haslam (1965, Phytochemistry 4:495-498) suggested, this compound may well be an artifact of extraction in methanol due to methanolysis of digallic acid in the extract. Given the current interest in the biological activity of methyl gallate (e.g., Mendez and Mato, 1997, Phytochemistry 44:41-43; Nagami et al., 1995, J. Food Sci. 60:653), it is important to establish whether methyl gallate is a natural constituent of the leaves that may function as a defensive compound against herbivorous insects. In the present study, leaves of three *Acer* species (family Aceraceae) indigenous to North America, *A. rubrum* L. (red maple), *A. saccharum* Marsh. (sugar maple) and silver maple were extracted either with methanol or ethanol. The extracts were then analyzed by HPLC. Methyl gallate was present in methanolic extracts, but was also found in ethanolic extracts. In addition, methyl gallate was present in extracts of red maple leaves made with diethyl ether, methylene chloride, acetonitrile, or ethyl acetate. The conclusion is inescapable that methyl gallate is both an artifact of isolation in methanol and a natural constituent of maple leaves. The implication that this compound is found in the leaves of red maple in particular is under investigation for its possible role in the innate resistance of this species to feeding by larvae of forest tent caterpillar.

Poster Paper 43

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EFFECTS OF SOME SECONDARY METABOLITES FROM *CEDRELA SALVADORENSIS* (MELIACEAE) IN PHOTOSYNTHESIS ON CHLOROPLASTS OF *SPINACEA OLERACEA L.*

Bioactivity-directed fractionation of the dichloromethane extract of the heartwood of *Cedrela salvadorensis* L. resulted in the isolation of two epimeric photogedunins and in the acquirement of two bioactive epimeric photogedunin acetates at the 23-OH position. The dichloromethane extract, the epimeric mixture and both photogedunin acetates caused partial inhibition of germination, radical growth and shoot development. Both acetates photoinhibited photophosphorylation, H^+-uptake and non-cyclic electron flow from water to methylviologen (basal, phosphorylating and uncoupled) requiring three minutes of pre-illumination; therefore, they behave as Hill reaction inhibitors. Photogedunin acetate inhibits PSII electron flow from H_2O to DCBQ without affecting PSI electron transport from reduced DCPIP to MV and PSII from water to SiMo. Therefore, its target is localized at the Q_B level. Another compound inhibits PSII electron flow from reduced DCPIP to MV, PMS_{red} to MV and $TMQH_2$ to MV without affecting PSII electron flow, interacting at b_6f level and in the span of P_{700} to F_x.

Poster Paper 45

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CHANGES IN ARGININE, PAL ACTIVITY, AND NEMATODE BEHAVIOR IN SALINITY-STRESSED CITRUS

De novo arginine biosynthesis is a response of citrus to a range of stresses. Stress in plants often enhances susceptibility to herbivory and pathogenic attack. Using a citrus and nematode (*Tylenchulus semipenetrans*) system, we investigated the effects of salinity on nematode behavior, amino acids (particularly arginine), and phenylalanine ammonia lyase (PAL) activity. We tested the hypothesis that under salinity stress, citrus grows more slowly and produces arginine in response to high levels of *in vivo* ammonia, resulting in lower PAL activity and increased susceptibility to nematode attack. After 30 days of high salinity (0.1 M NaCl), plants exhibited a 38% reduction in growth, 35% reduction in PAL activity, and had 54% higher infection rates. PAL was inversely correlated ($P < 0.05$) with salinity level and with increase in arginine.

Poster Paper 47

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INSECTICIDAL ACTIVITY OF SECONDARY METABOLITES ISOLATED FROM *GUTIERREZIA MICROCEPHALA* ON FALL ARMYWORM *SPODOPTERA FRUGIPERDA*

Gutierrezia microcephala (Asteraceae), commonly known as broomwood, grows in arid regions of the central and north of Mexico and in the southwestern United States. The diverse extracts and flavonoids have been used on human health and on animals. Several species of Asteraceae were investigated, and from *G. microcephala* (J.E. Smith) flavonoids, labdanes and other metabolites were isolated. A phytochemical study of the aerial parts of *G. microcephala*, collected in north Mexico (Highway Monterrey-Saltillo, Km 240), was undertaken because no biological studies had been carried out before. During the present study, we isolated some flavonoids which had been reported to possess cytotoxic activity. In search of the mechanisms through which this plant shows its alellopathic effects, we found its interactions on growth and its effect on feeding of fall army worm (FAW). In a concentration-dependent manner two compounds, 5,7,4'-trihydroxy-3,6,8-trimethoxyflavone and methyl esters of bacchabolivic acid, produce high mortality with a LD_{50} of 200μM; lower concentrations (from 10 to 100μM) inhibit the feeding and delay the time of pupation and adult emergence. The methoxyflavone is an antifeedant and also delays times of pupation and adult emergence.

Poster Paper 49

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METHYL-ESTERS OF *p*-CINNAMIC ACID FROM PERENNIAL RYEGRASS AS INHIBITORS OF PHOTOPHOSPHORYLATION AND PHOTOSYNTHESIS IN SPINACH CHLOROPLASTS

Perennial ryegrass (*Lolium perenne* vars. Embassy and Nui), infected with endophyte fungi *Acremonium lolii*, grow in soils of southern Chile. This grass shows a strong allelopathic effect on associated flora. We have phytochemically investigated these varieties. We isolated two known alkaloids, as well as the methyl ester of 3,4-dihydroxy-*trans*-cinnamic acid and the methyl ester of 4-hydroxy-*trans*-cinnamic acid; their structures were corroborated by IR, UV, MS and NMR data. Our data indicated that these compounds show inhibitory effects on seeds of *Physalis ixocarpa, Trifolium alexandrinum, Lolium multiflorum* and *Triticum vulgare*, both in respiration and germination bioassays. In addition, we carried out photosynthesis assays, and only the dihydroxy compound inhibited photophosphorylation (synthesis of ATP) and partial phosphorylating activity in photosynthesis. The results show that the monohydroxy compound partially inhibits phosphorylating activity.

Poster Paper 51

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THE FIRST *O*-GALLOYL *C*-GLYCOSYLFLAVONES

The range of naturally occurring *C*-glycosylflavonoids is extended by identification of a series of *O*-galloyl *C*-glycosylflavones from *Pelargonium reniforme*, comprising the 8-*C*-glucosyl derivatives 2''-*O*-galloylvitexin and 2''-*O*-galloylorientin and their 6-*C* analogues 2''-*O*-galloylisovitexin and 2''-*O*-galloylisoorientin. Their structures were established from spectroscopic studies. Differentiation between *C*-glycosylation at the C-6 and C-8 positions was facilitated by conspicuous doubling of signals associated with the presence of 8-*C*-substitution due to steric interactions, in contrast to the absence of similar phenomena in instances of 6-*C*-glucosylation.

Poster Paper 53

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IDENTIFICATION OF COMPONENTS FROM VOLATILE OILS: SPECTROSCOPICAL ANALYSIS OF SESQUITERPENES MIXTURES

A method to fractionate volatile oils and to identify their sesquiterpenic constituents is described. The fractionation process includes flash chromatography over silica gel and chromatography over silica gel/$AgNO_3$, utilising pentane, CH_2Cl_2 and/or acetone as eluents. GC chromatograms were obtained in order to get the relative percentage and the retention time of each constituent in the volatile oils, as well as to analyse and combine the fractions eluted from the columns. This procedure affords mixtures of sesquiterpenes which are analysed by GC/MS, ^{13}C and ^{1}H NMR. The volatile oils from some species (leaves and stem bark of *Guarea guidonia* - Meliaceae, leaves and fruits of *Xylopia emarginata* and leaves of *Porcelia macrocarpa* - Annonaceae) have already been examined by this method and several sesquiterpenes were identified, including new ones. (FAPESP, CNPq)

Poster Paper 55

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Produtos Naturais, UFRJ.

CHEMICAL CONSTITUENTS FROM *BRILLANTAISIA PALISATII* LIND

Brillantaisia palisatii Lind. belongs to the family Acanthaceae, order Scrophulariales, superorder Lamiiflorae (*sensu* Dahlgren). This family consists of 250 genera with 2500 species spread over tropical regions, in the Mediterranean, United States of America and Australia. The chemistry of Acanthaceae shows a great diversity of classes such as alkaloids, lignans, flavonoids and terpenoids. Fresh leaves of *B. palisatii* collected near the Botanical Garden (Rio de Janeiro State, Brazil) were submitted to steam distillation in a modified Clavenger extractor. The essential oil obtained showed the presence of five aliphatic alcohols (3-hexen-1-ol - 29.67%), five monoterpenes (α-pinene - 8.63%) and seven sesquiterpenes (isocaryophyllene - 7.81%). Dried and ground leaves were further submitted to an extraction procedure using ethanol. The ethanolic extract was dried under reduced pressure and further suspended in water. This suspension was submitted to a liquid-liquid partition with hexane, dichloromethane, ethyl acetate and butanol. From the ethyl acetate partition it was possible to isolate and to identify verbascoside, a phenylpropanoid glucoside. From the same extract was also isolated a flavonol bearing two rhamnose residues.

Poster Paper 57

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NINETEEN NEW STEROIDAL GLYCOSIDES FROM *ALLIUM* PLANTS: ISOLATION, STRUCTURAL ELUCIDATION AND EFFECTS ON BLOOD COAGULABILITY

To find naturally occurring substances to prevent and treat thrombosis and cancer, more than 30 natural compounds (mainly glycosides) were isolated from 6 medicinal plants, including *Allium chinense* G. Don, *A. macrostemon* Bunge, *A. sativum* L, *Bolbostemma paniculatatum* (Maxim.) Franquet, *Rhamnella gilgitica* Mansfeld et Melch, and *Rhodiolacrenulata* SH. Fu. Of these, 19 have been identified as new steroidal saponins by means of chemical evidence and spectral analyses, such as 2D-NMR and FAB-MS; six possessed strong inhibitory effects on platelet aggregation, seven showed significant prolongation of coagulation time on blood coagulation and 10 promoted fibrinolysis activity. Recent screens show four have strong cytotoxicity (IC50 below 10 mg/ml). It is noteworthy that tubeimoside A, B and C showed the most remarkable effects on blood coagulability and cytotoxicity. These results are consistent with the traditional use in Chinese medicine of these plants to treat breast cancer and thrombosis-like diseases.

SYMPOSIUM SESSION 3 — *MEDICINALS/NUTRICEUTICALS AND NEW GENETIC ENGINEERING TARGETS*

Moderator: *Laurence B. Davin*

TUESDAY, 8:00-8:50

BIOLOGICALLY ACTIVE COMPOUNDS FROM *BUDDLEJA* SPECIES

Symposium Paper 7

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The genus *Buddleja* has been used in traditional medicine for wound healing, for treating skin diseases and as a liver-protectant. Phytochemical studies using cultured hepatocytes for bioassay-guided fractionation have revealed that flavonoids and caffeic acid derivatives are responsible for liver-protectant activity and that similar compounds are responsible for wound-healing effects. Recent studies have shown the presence of antifungal activity against dermatophytes using serial dilutions against cultured microorganisms. This activity has been traced to terpenoids present in the stem, bark and roots. Some of these compounds are novel, and their structures were determined by spectroscopic methods. The results of studies carried out give a scientific base for the ethnopharmacological uses of this genus as well as providing some novel potential lead compounds.

TUESDAY, 8:50-9:40

ROLE OF LIGNANS IN CARCINOGENESIS

Symposium Paper 8

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Mammalian lignans, primarily enterodiol (ED) and enterolactone (EL), are produced from plant lignans such as secoisolariciresinol diglucoside (SDG) and matairesinol, respectively, after the action of bacterial flora in the colon of human or animals. They are hypothesized to have anticancer effects because of their chemical structural similarity to estrogen, high excretion in individuals who are of low risk of cancer, and their biological properties. Flaxseed, the richest source of lignans, (1) reduced the incidence, size and number of mammary tumors in carcinogen-treated rats, (2) regressed the growth of mammary tumors, (3) reduced the development and growth of colon tumors, and (4) reduced the incidence of lung metastasis of melanoma cells. The SDG, ED and EL produced similar effects. Thus it is concluded that the mammalian lignans derived from the SDG can influence the carcinogenesis process.

TUESDAY, 9:40-10:00 — **BREAK**

TUESDAY, 10:00-10:25

FORMATION OF CANCER-PREVENTING LIGNANS IN PLANTS: ENGINEERING THEIR METABOLIC PATHWAYS

Oral Paper 9

Michael A. Costa,
Zhi-Qiang Xia,
Joshua D. Ford,
Huai-Bin Wang,
David R. Gang,
Laurence B. Davin and
Norman G. Lewis,
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Washington State University,
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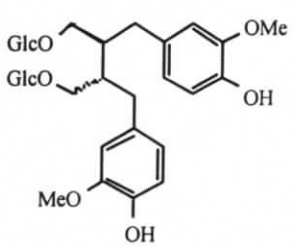
The ability to bioengineer metabolic pathways of cancer-preventing lignans for the purpose of increasing their levels in commonly consumed food plants is becoming a reality. This is being made possible because of two factors: 1) the increased knowledge and understanding that we are accumulating about lignan precursors and biosynthetic pathways, which will allow us to alter strategic points for increased formation; and 2) data accumulating from numerous studies showing the beneficial effects afforded to human health, especially on cancer-related illnesses, from consuming these lignan-derived compounds. Of particular interest are the mammalian lignans, or phytoestrogens, enterolactone and enterodiol, produced by the intestinal microflora upon conversion of the lignan-derived substrates consumed, such as secoisolariciresinol diglucoside and matairesinol. These compounds reportedly have cancer chemopreventive properties. The genes producing specific enzymes in the lignan biosynthetic pathways leading to these products are now being isolated, along with their promoters, with the goal of increasing levels of these phytoestrogen precursors and directing their location of biosynthesis and accumulation in normally consumed foods such as cereal grains. The fact that these lignan biosynthetic pathways are naturally present in many important food crops, such as wheat, flax, sesame, and numerous fruits and vegetables, allows for the potentially feasible manipulation of the enzyme components and locations of product accumulation through genetic engineering. First attempts are now being made to manipulate foods in schemes designed to protect good health by increasing the levels of pinoresinol synthase, pinoresinol/lariciresinol reductase, and secoisolariciresinol dehydrogenase in wheat and flax. The "nutraceutical" substances formed by these enzymes will provide a novel approach to prevention of diseases such as cancer by allowing for increased intake of biologically active compounds found in natural plant material that is consumed in the normal daily diet.



TUESDAY, 10:25-10:50

Oral Paper 10

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John D. Hipskind,
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Ardmore, OK, 73401 USA.

GENETIC ENGINEERING OF RESVERATROL ACCUMULATION IN ALFALFA

Resveratrol is an important phytoalexin in a diverse array of plant species, but is not naturally found in alfalfa (*Medicago sativa*). Agar-plate bioassays indicate that resveratrol inhibits the growth of several alfalfa pathogens, suggesting that production of this foreign phytoalexin in alfalfa may increase pathogen resistance. We have engineered the production of resveratrol (3, 5, 4′-trihydroxystilbene) in alfalfa by introducing the resveratrol synthase gene from peanut (*Arachis hypogaea*). Unlike previous reports using similar genes in tobacco, we have found high levels of a resveratrol-sugar conjugate accumulating in the leaves, not free resveratrol, and have observed no adverse effects on the transformants. Pathogen challenge experiments are underway, in comparison with wild-type plants. Resveratrol has also been reported to have cancer chemopreventative and other beneficial activities. This material will allow controlled diet studies in mice to assess, for example, the utility of engineering beneficial phenolics into commonly consumed food plants to prevent tumorigenesis.

TUESDAY, 10:50-11:10

Oral Paper 11

Albena T. Dinkova-Kostova and
Paul A. Talalay,
Department of Pharmacology and Molecular Sciences,
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USA.

NATURAL AND SYNTHETIC PHENYLPROPENOIDS AS DETOXICATION ENZYME INDUCERS AND FREE RADICAL SCAVENGERS

It is widely recognized that protection against the toxic and neoplastic effects of carcinogens can be successfully achieved by induction of tissue levels of Phase 2 detoxication enzymes (e.g., glutathione transferases, UDP-glucuronosyltransferases, epoxide hydrolase and NAD(P)H: quinone reductase). Structural features that are characteristic of Phase 2 enzyme inducers can be found in various classes of plant phenylpropenoids, some of which have been shown to inhibit tumor formation in animals. Surprisingly, there is no systematic information on what structural elements are responsible for the difference in the degree of potency. This study represents a detailed stucture-activity analysis on a series of naturally occurring and synthetic substituted phenylpropenoids of two types: (a) Ar-CH=CH-CO-R where R = OH, OCH_3, CH_3, or Ar; and (b) *bis*(benzylidene)cycloalkanones. It was found that introduction of *ortho*-hydroxyl groups on the aromatic rings of these phenylpropenoids dramatically enhanced their potencies as inducers for quinone reductase. Unexpectedly, the *bis*(benzylidene)cycloalkanones that induced quinone reductase most potently also powerfully quenched the chemiluminescence of lucigenin evoked by superoxide radicals generated by the xanthine/xanthine oxidase reaction. Moreover, the order of inducer potency appears to be correlated with the ability to quench superoxide radicals. This observation suggests that the regulation of Phase 2 enzymes may involve both electrophilic and radical quenching mechanisms.

TUESDAY, 11:10-11:30

Oral Paper 12

B. Markus Lange,
Mark R. Wildung,
Einar Stauber,
Rodney Croteau,
Institute of Biological Chemistry,
Washington State University,
Pullman, WA 99164-6340,
USA.

SHOTGUN CLONING OF WHOLE PATHWAYS FROM A PEPPERMINT GLAND cDNA LIBRARY

As part of an ongoing effort to isolate isoprenoid biosynthetic genes we have employed a cDNA library derived from peppermint (*Mentha x piperita*) oil gland secretory cells, a plant cell type highly specialized for essential oil production. After sequencing 1100 clones from this library, we have obtained nearly the entire set of known genes of isoprenoid biosynthesis (IPP isomerase; GPP synthase; FPP synthase; GGPP synthase; monoterpene, sesquiterpene and diterpene synthases; limonene-3-hydroxylase), and have characterized the first gene of the plastidial non-mevalonate pathway for isopentenyl diphosphate biosynthesis (1-deoxyxylulose-5-phosphate synthase). We have also cloned enzymes involved in the supply of carbon precursors and energy for the non-photosynthetic plastids of peppermint glandular trichomes. Among the clones sequenced so far, we have found most of the genes of the oxidative pentose phosphate pathway and plastidial glycolysis, as well as potential transporters for adenylate, hexose and triose phosphates. Additionally, a variety of regulatory enzymes has been identified. The peppermint gland cDNA library thus provides an ideal system for further investigations to understand the complex metabolism of non-green plastids and essential oil formation.

TUESDAY, 11:30-11:50

CYTOTOXIC CONSTITUENTS OF FORSYTHIA SPECIES

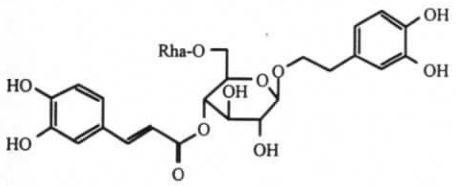
Oral Paper 13

Katsuya Endo,
Tohoku College of Pharmacy,
Komatsushima,
Sendai 981-8558, JAPAN.

Constituents of *Forsythia suspensa* and *F. viridissima* have been tested for their cytotoxic effects to understand and evaluate the pharmacological quality of the Oriental medicines.

Forsythoside A, the major phenolic glycoside of *F. suspensa* leaves, showed wide and fairly potent cytotoxic effects to P-388 (IC50 5.5 μg/ml), MKN-28 (18.4 μg/ml), HOC-21 (19.0 μg/ml), MB-231 (35.2 μg/ml), MB-435S (20.0 μg/ml), L-1210 (6.1 μg/ml), colon 26 (11.3 μg/ml) and Meth A (10.1 μg/ml), but not to MCF-7 (greater than 50). It did not affect the viability of non-cancer cells like human fibroblasts and rat hepatocytes at 100 μg/ml.

Acteoside, the major phenolic glycoside of *F. viridissima* leaves and isomeric to forsythoside A, was similarly effective, but somewhat less potent. Other homologous phenolic glycosides from these plants such as forsythosides B, C and F, and a lignan glucoside, arctiin, as well as a terpene glucoside, forsythid, were also found active. Biochemical studies are in progress, in order to elucidate the mechanism of the cytotoxicity.



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TUESDAY, 11:50-12:10

FLAX SEED LIGNANS, STEREOCHEMISTRY, QUANTITATION AND BIOLOGICAL ACTIVITY

Oral Paper 14

Alister D. Muir and
Neil D. Westcott,
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Research Centre, Agriculture &
Agri-Food Canada,
107 Science Place,
Saskatoon ,SK S7N 0X2,
CANADA.

The principal lignan of flax (*Linum usitatissimum*) is (+) secoisolariciresinol diglucoside (SDG) which occurs naturally as a component, along with caffeic, ferulic and *p*- coumaric acids, of an aqueous alcohol soluble complex. (-) SDG is also present at approximately 1% of the (+) isomer. The (+) and (-) isomers of SDG can be resolved by RP-HPLC. The concentration of SDG varies significantly between cultivars ranging from 0.6 to 1.75% of the weight of the seed. Both (+) and (-) SDG exhibit antioxidant properties. Recent studies of the biological activity of SDG in animal models will also be reviewed.

TUESDAY, 12:10-12:30

CHEMICAL CHARACTERIZATION OF FLAX AND THE EFFECT OF EXTRACTS ON RETTING ENZYMES

Oral Paper 15

W. Herbert Morrison[1],
D.E. Akin[1], G.R. Gamble[1] and
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Flax, the source of linen, must undergo a process called retting to free the fibers from the plant. This process primarily degrades pectin which acts as a binder to hold the bast tissue to the core. Chemical and instrumental techniques have been used to identify phenolics, waxes and cutin constituents of flax bast and the location of phenolics associated with the bast. Microspectrophotometric examination of the bast has shown that the phenolics, possibly lignin, are located primarily at cell corners and stain positive with acid phloroglucinol. Sequential extraction of flax bast tissue with hexane, propanol, methanol and water yielded an extract containing flavonoids and hydroxy-methoxy cinnamic acids linked to oligosaccharides and hydroxy acids through glycoside linkages which have been shown to inhibit activity of the enzymes required for retting.

TUESDAY, 12:30-1:30 **LUNCH**

SYMPOSIUM SESSION 4 — ***BIOCHEMISTRY OF PLANT PHENOLICS IN CELL WALL STRUCTURE AND DEFENSE INCLUDING HEARTWOOD FORMATION***

Moderator: ***Edwin Haslam***

TUESDAY, 1:30-2:15

Symposium Paper 9

Georg G. Gross,
Universität Ulm,
Abteilung Allgemeine Botanik,
D-89069 Ulm, GERMANY.

BIOSYNTHESIS, BIODEGRADATION, AND CELLULAR LOCALIZATION OF HYDROLYZABLE TANNINS

Enzyme studies have shown that 1,2,3,4,6-pentagalloyl-β-D-glucose (PGG), the common and immediate precursor of both gallotannins and ellagitannins, was formed by a series of transgalloylation steps utilizing β-glucogallin (1-*O*-galloyl-β-D-glucose, βG) as general acyl donor. This mechanism also applied to the subsequent transformation of PGG to higher substituted complex gallotannins, as shown by the isolation of several βG-dependent acyltransferases. In contrast, the oxidation of PGG to ellagitannins remained still highly enigmatical. Recent experiments with [U-^{14}C] PGG on this challenging question are reported. In these investigations, an esterase, classified as 'plant tannase', of eventual ecological significance was isolated. Monospecific antibodies were raised against PGG as tannin model compound, and against the enzyme synthesizing this metabolite from tetragalloylglucose. The results of current immuno-histochemical studies with these reagents on the distribution of hydrolyzable tannins and their sites of synthesis are presented.

TUESDAY, 2:15-3:00

Symposium Paper 10

W.E. (Ted) Hillis,
CSIRO Division of Forestry and Forest Products,
Clayton, Vic. 3169,
AUSTRALIA.

THE FORMATION OF HEARTWOOD AND ITS EXTRACTIVES: AN OVERVIEW

The amount of symmetrically shaped heartwood formed in a tree of a given diameter is controlled by several inherent biological factors, and these will be summarized. Variable amounts and different types of heartwood extractives are characteristic of particular genera and species. These extractives are formed in the transition zone adjacent to the heartwood during a period of increased metabolic activity of the parenchyma cells which then die. The ethylene formed at the same time could be involved in the reduction of moisture content characteristic of the transition zone. Several types of irregularly shaped discolored woods, sometimes known as false heartwood, found in sapwood are the result of different biological factors. The containment of insect and fungal attack results from the formation of increased amount of extractives, with compositions different from those of the host species. A few species can produce either at the heartwood periphery pure or almost pure compounds in isolated tracheids or vessels or also in heartwood cavities. Thus these compounds can be biosynthesized from carbohydrates without formation of waste products.

TUESDAY, 3:00-3:15 — **BREAK**

TUESDAY, 3:15-3:35

Oral Paper 16

David R. Gang[1],
Masayuki Fujita[2],
Laurence B. Davin[1] and
Norman G. Lewis[1],
[1]Institute of Biological Chemistry,
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USA;
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JAPAN.

MAPPING HEARTWOOD FORMATION THROUGH THE LIGNAN PATHWAY

Heartwood formation often results in massive deposition of non-lignin phenolic "extractive" metabolites, the type and extent of which can vary with plant source. These often complex mixtures of phenolic "extractive" metabolites are laid down in a *post-lignification* process at the "transition" zone, separating the sapwood from the heartwood, and then diffuse into the lignified cell wall. In this study, the commercially important western red cedar (*Thuja plicata*) was used as a model plant to study non-lignin phenolic deposition (>20% lignans by weight) during heartwood formation. From previous investigations using *Forsythia sp.*, it was established that entry into this lignan pathway occurs via stereoselective coupling of *E*-coniferyl alcohol substrates to give pinoresinol, via participation of a dirigent (guiding) protein. The genes encoding the dirigent protein have since been obtained from *Forsythia sp.*, western red cedar and western hemlock (*Tsuga heterophylla*). Genes encoding pinoresinol/lariciresinol reductases were also cloned from western red cedar, and corresponding recombinant proteins have been produced in *E. coli*. Interestingly, two distinct enzyme types were identified, with one catalyzing the conversion of (+)-pinoresinol into (–)-secoisolariciresinol and the other utilizing the opposite antipodes, i.e., converting (–)-pinoresinol into (+)-secoisolariciresinol. Thus, the early steps of the biochemical pathway, at both the gene and enzyme levels, leading to massive formation of lignans, such as plicatic acid, in western red cedar heartwood have been defined and demonstrate the exquisite control involved in phenolic coupling and the subsequent metabolic events *in planta*, in contrast to the dogma of random assembly and deposition.

TUESDAY, 3:35-3:55

Oral Paper 17

Herbert L. Hergert,
Repap Technologies Inc.,
PO Box 766,
Valley Forge, PA 19482, USA.

THE CHEMISTRY OF HEARTWOOD FORMATION IN NORTHERN WHITE CEDAR (*Thuja occidentalis*)

Northern white cedar is used for many in-ground applications because the heartwood (up to 85% of a mature tree) is resistant to attack by pathogens. Chemical analyses of the heartwood shows the presence of 1.2% sesquiterpenes (mainly occidol and occidentalol), plicatin (0.4%), other related lignans (0.5%), and phenolic polymers (3.1%) which are present only in trace amounts in sapwood. The heartwood also has some other "non-lignin" components, presumed derived from cedar-specific lignans. The phenolic compounds are deposited in pit torii and presumably resist in the invasion of fungal hyphae.

TUESDAY, 3:55-4:15

Oral Paper 18

Hartmut Förster[1],
Valerie Steeves[2] and
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Faculty of Forestry and
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CANADA.

CONCOMITANT GLUCOSYLATION ALONG THE LIGNIFICATION PATHWAY IN CONIFERS – REGULATORY ASPECT OR METABOLIC ACCIDENT?

Uridine 5`-diphosphoglucose:coniferyl alcohol glucosyltransferase (CAGT), the enzyme catalyzing synthesis of coniferin, syringin and most probably being involved in the synthesis of cinnamyl aldehyde glucosides, was investigated throughout an annual cycle of cambial growth and dormancy in *Pinus strobus* L. Enzymatic products yielded by partially purified CAGT of the cambial zone and developing xylem were isolated by HPLC and identified using GC-MS and ^{1}H-nmr. Usually, monitored cinnamyl alcohol glucosides (coniferin, syringin, *p*-coumaryl alcohol glucoside) are considered to be precursors indicating progress and developmental stage of lignification. However, CAGT competes with CAD and peroxidases for coniferyl alcohol, the former blocking lignification and the latter enabling the aldehyde to be polymerized into lignin. In addition, CAGT evidently competes with CAD for cinnamyl aldehydes forming the corresponding glucosides, as shown here for the first time. Considering all of the above, it seems probable that the restriction of CAGT activity exclusively to the period of active cambial growth is not merely an accident of secondary metabolism. Coniferin as well as the other glucosides formed during lignification could well have an important role in influencing the nature of primary metabolism underlying seasonal cambial growth, and CAGT activity may be essential for the cambium to perpetuate itself as a meristem when its derivatives are actively differentiating into xylem or phloem.

TUESDAY, 4:15-4:35

Oral Paper 19

A. Ros Barceló,
Department of Plant Biology,
University of Murcia,
E-30100 Murcia, SPAIN.

CONIFERYL ALCOHOL OXIDASE IS A RESIDUAL ACTIVITY OF A CELL WALL-LOCATED STRONGLY BASIC PEROXIDASE

Coniferyl alcohol oxidase (CAO) activity was determined in cell walls from several species belonging to the family Asteraceae. In the cases studied, CAO activity was partially located ionically bound to cell walls, and was due to a strongly basic peroxidase (BP). This BP was apparently responsible for the capacity of cell walls to oxidize coniferyl alcohol in the absence of H_2O_2, as tested by means of an *in situ* inactivation assay, and showed a visible spectrum typical of a high spin ferric secretory plant peroxidase. CAO was a residual activity of this BP, constituting less than 5% of the activity shown in the presence of H_2O_2. The *in vivo* importance of this CAO activity was studied by means of histochemical tests. The results obtained suggest that CAO activity of this BP should be considered as an evolutive residue rather than as a evolutionary gain.

TUESDAY, 4:35-4:55

Oral Paper 20

IN VITRO PRODUCTION OF LIGNANS BY A PEROXIDASE FROM THE ENDOPLASMATIC RETICULUM OF *ZEA MAYS* L. AND POSSIBLE BIOLOGICAL FUNCTIONS

Wolfgang Gröger[1],
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A lot of plants naturally contain lignans derived from cinnamyl alcohols of the lignin biosynthesis. We describe here a basic peroxidase associated with the endoplasmatic reticulum of *Zea mays* L. which is able to metabolize phenylpropanoid acids to lignans. Hence, *p*-coumaric-, caffeic-, ferulic-, and sinapic acids are catalysed to dimeric lignans of the diarylbutane- and dibenzylbutyrolactone-type. A typical co-substrate of this reaction is hydrogen peroxide. The products of the cinnamic acids are dimeric compounds with a conjugated ring system forming diarylbutane structures and represent an alternative pathway for phenylpropanoid monomers in the secondary metabolism. The turnover of ferulic acid could be shown in etiolated as well as green and elicitated seedlings. In comparison to etiolated plants the green and elicitated plants exhibit higher peroxidase activities. The specific catalytic activity for the maize-sort "BASTION" was determined with 40.66 μkat x mg^{-1}. The catalyzing peroxidase can be induced by different stressors like heat or wounding. Corresponding products were isolated by preparative HPLC showing small antiviral activities. The occurrence of lignans in monocots formed from cinnamic acids suggest their influence on lignification and composition of the cell wall. This is considered to play an important role for digestibility of plants by consuments as well as being a component of plant defense against pathogen attacks.

TUESDAY, 4:55-5:15

Oral Paper 21

STEREOCHEMICAL DIVERSITY IN LIGNAN BIOSYNTHESIS

Toshiaki Umezawa,
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K. Mikame,
S. Suzuki and
M. Shimada,
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Mechanisms in lignan biosynthesis have been receiving widespread interest in many aspects such as biochemical and stereochemical, since lignans have various biological activities and unique stereochemical properties. In relation to the stereochemical mechanisms, enantiomeric compositions of lignans vary with plant sources. However, no satisfactory explanations have been proposed to account for stereochemical differences to produce lignans having various enantiomeric compositions.

In the present investigation, lignans (pinoresinol, lariciresinol, secoisolariciresinol, and dibenzylbutyrolactone lignans such as matairesinol and arctigenin) were isolated from various plant species, and their enantiomeric compositions were determined by chiral HPLC and GC-MS. Also, enantioselective formation of the lignans were demonstrated with enzyme preparations from some of the plants. Based on the enantiomeric compositions of the naturally occurring and enzymatically formed lignans, stereochemical mechanisms of lignan biosynthesis are discussed; there is a stereochemical diversity in lignan biosynthesis in different plant species.

TUESDAY, 5:15-5:35

Oral Paper 22

FORMATION OF (+)-*ERYTHRO* AND (-)-*THREO*-GUAIACYLGLYCEROL-β-CONIFERYL ETHERS AND (+)-SYRINGARESINOL BY ENANTIOSELECTIVE PHENOXY RADICAL COUPLING REACTIONS

Takeshi Katayama,
Yuki Kado and
Atsushi Ogaki,
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Kagawa University,
Miki-cho, Kagawa 761-0795,
JAPAN.

Lignans and neolignans exhibit a diversity of biological activity implicated in natural plant defenses and important pharmacological action in mammals. However, biosynthesis of the neolignans and syringyl lignans have been unclear. The purpose of this investigation is to clarify the enantioselective formation of the neolignans and the syringyl lignans. Incubation of cell-free extracts from *Eucommia ulmoides* with coniferyl alcohol in the presence of hydrogen peroxide gave (+)-*erythro* and (–)-*threo*-guaiacylglycerol-β(8-*O*-4')-coniferyl ethers (34-38% e.e.). Further experiments indicated for the first time that the 8-*O*-4' neolignan formation was enantioselective. Although pinoresinol yielded at the same time was racemic, (+)-pinoresinol and (+)-syringaresinol formation was shown by feeding experiments in the plant. Following administration of [U-^{14}C]phenylalanine and [8-^{14}C]sinapyl alcohol to excised shoots of *Liriodendron tulipifera*, (+)-[^{14}C]syringaresinol (8-44% e.e.) was obtained both as itself and as the aglycone. The result suggested the presence of an enantioselective coupling of sinapyl alcohol radicals.

TUESDAY, 5:35-5:55

Oral Paper 23

Shuji Ozawa and
Teruhisa Miyauchi,
Faculty of Agriculture,
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Sapporo 060-8589, JAPAN.

BIOSYNTHESIS OF LIGNANS IN *MAGNOLIA KOBUS* DC.VAR.*BOREALIS* SARG.

As part of our studies of lignan biosynthesis in woody plants, we investigated the formation of lignans in *Magnolia kobus* var. *borealis*. This plant contains various furofuran lignans. The lignan contents varied according to tissue type and season. From *in vivo* labelling experiments of *M. kobus* var. *borealis*, it was established that deuterated coniferyl alcohol had been incorporated into pinoresinol, but not fargesin and kobusin. Next, we examined *O*-methylation reactions of phenolic furofuran lignans by incubation with cell-free extracts from *M. kobus* var. *borealis*. It was shown that the cell-free extracts catalysed the *O*-methylation reactions of pinoresinol and syringaresinol, suggesting the conversions of pinoresinol into eudesmin, and syringaresinol into yangambin, respectively. The *in vitro* transmethylation reactions observed were slightly enantionselective with the (+)-forms being the preferred substrates. Purification and characterization of the *O*-methyltransferase(s) is under active investigation.

TUESDAY, 6:00-7:30 **DINNER - COMPTON UNION BUILDING BALL ROOM**

POSTER SESSION 2 ***PHYTOCHEMICAL PATHWAYS USEFUL IN MEDICINE AND PLANT DEFENSE***

Moderators: ***Joshua D. Ford/Anastasia L. Crowell***

TUESDAY, 8:00-10:00 PM **COMPTON UNION BUILDING - BALL ROOM**

Poster Paper 2

Alicja M. Zobel[1], J.M. Lynch[1],
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E. Kupidlowska[2] and
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COMPOUNDS REMOVED FROM PLANT SURFACES AS POTENTIAL ANTI-CANCER AGENTS

Mixtures of phenolic compounds removed from surfaces of several plants retarded mitotic numbers when investigated using Levan's test. The strongest mitodepressive reaction had *Brassica oleracea* extracts and *Taxus baccata*. *Ruta graveolens* extracts from the surfaces of leaves were of a similar potency as the extracts from the interior of the leaves. Ultrastructural changes observed in the electron microscope were mostly to the endomembrane system of endoplasmic reticulum and plasmalemma and to the matrix of mitochondria which became more electron dense than control ones and increased in size. Changes to mitochondria suggested that the energy status of treated cells was affected.

Poster Paper 4

J. Fred Stevens[1],
Alan W. Taylor[1],
Cristobal L. Miranda[2],
Donald R. Buhler[2] and
Max L. Deinzer[1],
Departments of [1]Chemistry and
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POTENTIAL CANCER CHEMOPREVENTIVE PRENYLFLAVONOIDS FROM HOPS (*HUMULUS LUPULUS*)

Ten prenylflavonoids (PFs) were isolated from hops and identified (*Phytochemistry* 44, 1575, 1997), seven of which were investigated for cancer chemopreventive activity. Xanthohumol (XN) and related PFs were quantified in hops and beer, and their fate was monitored during the brewing process by LC-MS. Hop PFs of the chalcone type were largely converted into their isomeric flavanones during wort boiling. A typical American beer was found to contain *ca* 0.5 mg/l of the flavanone, isoxanthohumol (IX), and trace quantities of XN, 6- and 8-prenyl-naringenin and 6-geranylnaringenin. Six hop PFs were tested for their ability to inhibit the proliferation of human breast cancer (MCF-7) and colon cancer (HT-29) cells *in vitro*. XN, IX and dehydrocycloXN were most active against MCF-7 cells (IC_{50} 13-16 μM 2 days after exposure), whereas the PFs tested failed to inhibit growth of the HT-29 cells. XN and IX displayed no toxicity towards rat hepatocytes at 10 μM after 6 or 24 hours of exposure. The inhibition of carcinogen metabolism by five hop PFs was also evaluated. Desmethylxanthohumol and IX were most effective in inhibiting the metabolic activation of two procarcinogens (AFB1 and DMBA) mediated by cyto-chrome P450. The results indicate that some hop PFs have potential cancer chemopreventive properties in humans, but it is too early to speculate about the impact of dietary intake of PFs (beer consumption) on human health.

Poster Paper 6

Chung Ki Sung,
Jeong Mee Lee,
Da Woon Jung,
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Jun Cheul Ahn[2],
Keon-Hyoung Song[3] and
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Jeonbuk 590-170, KOREA;
Coll. of Pharmacy[3],
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Seoul 151-742, KOREA.

DEVELOPMENT OF ELISA METHOD FOR THE DETERMINATION OF SAIKOSAPONIN A, AN ACTIVE COMPONENT OF *BUPLEURUM FALCATUM* L., AND ITS APPLICATIONS

Saikosaponin a (Sa) is one of major active components of *Bupleuri Radix*(root of *Bupleurum falcatum* L.), one of the famous Oriental drugs used mainly for treatment of liver diseases. Quite recently Oriental drug prescription containing this drug was reported to have caused serious side-effects including even death, which demands a new pharmacodynamic study on this drug. Regardless of this report, this drug shows a strong antiinflammatory effect, and has been used for the treatment of hepatitis and related liver diseases. For the supply of this drug, the cultivation for mass production in the field and on the tissue culture system have been studied. For the purpose of determination of Sa in biological fluids and plant samples, we established a highly sensitive and specific ELISA system using Sa specific antibody raised by immunization of Sa-BSA conjugate to rabbit, peroxidase conjugated anti-rabbit second antibody and tetramethyl benzidine as substrate. The measuring range extends from 50pg/ml to 20 ng/ml. The method showed minor cross reactivity with saikosaponin d(0.3%), which differs from Sa on the stereochemistry of the 16-hydroxyl group. We applied this method to determine Sa in plant samples like callus, hairy root and Oriental drug prescription and also in biological fluid like serum taken for phamaco-kinetic study.

Poster Paper 8

Luciana Mensor, S. G. Leitão,
F. S. Menezes, C. S. Coube and
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ANTIOXIDANT ACTIVITY OF BRAZILIAN PLANT EXTRACTS: DPPH SCAVENGING TEST

Extracts from ten Brazilian plants from four different families were tested against DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) free-radicals in six different concentrations (250, 125, 50, 25, 10 and 5 μg/ml). The antioxidant effect of an extract corresponds to its intensity of quenching DPPH. The antioxidant activity of plant extracts belonging to Verbenaceae family were very high, comparable with standard antioxidants such as rutin and *Ginkgo biloba* (Tebonin Oral Solution®), both used as antioxidants for medical purposes. The EC_{50} values were obtained by linear regression curves. Experiments were made in triplicate and all plant extracts showed a dose-response curve. *Lantana trifolia* (Verbenaceae) leaf extract showed the highest activity of all plants studied (93.5 % at 125 μg/ml). Further investigation of this extract by TLC sprayed with DPPH reagent showed that substances responsible for antioxidant activity are phenolic.

Poster Paper 10

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Hawkesbury, Richmond,
AUSTRALIA.

THE ROLE OF STRUCTURE AND SOLUBILITY IN DETERMINING THE ANTIMICROBIAL ACTIVITY OF TERPENOIDS AGAINST SELECT HUMAN PATHOGENS

The Minimum Inhibitory Concentration (MIC) of over fifty terpenoids, several aromatic ethers and two ionones have been determined against *P. aeruginosa, E. coli, S. aureus* and *C. albicans* using an agar plate dilution method. The water solubility of the compounds has also been measured. Hierarchical cluster analysis was used to group the compounds into five groups according to their activity against the four microorganisms. Of these five groups, those containing hydrocarbons and acetates were relatively inactive. This may be attributed to their low water solubility. Each of the remaining four groups, all containing oxygenated terpenes, showed characteristic but distinct activity behaviour patterns towards the four test organisms. Functional group and water solubility differences do not appear to account for all aspects of these relationships. This and other data will be discussed as a contribution towards a more detailed understanding of relationships between the structure of terpenes and their antimicrobial activity against a range of pathogenic organisms.

Poster Paper 12

Peter J. Houghton,
Pharmacognosy Research Laboratories,
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King's College London, Manresa Road,
London SW3 6LX, UK.

ACTIVITY OF ALKALOIDS FROM ANGOSTURA BARK (*GALIPEA OFFICINALIS*) AGAINST *MYCOBACTERIUM TUBERCULOSIS*

The threat of drug-resistant tuberculosis makes the discovery of novel antitubercular compounds imperative. The activity of an ethanolic extract of *Galipea officinalis* bark demonstrated by a preliminary screen was traced to the alkaloids present. Fractions and derived compounds were tested for activity against *M. tuberculosis* cultured in Middlebrook medium using serial dilutions and growth was observed after 14 days. Alkaloids were found in the most active fractions and their structures were deduced by mass and NMR spectroscopic methods. Some of the isolated quinoline alkaloids showed significant activity, but the greatest activity was shown by a polar fraction which is still being investigated.

TUESDAY, 5:35-5:55

Oral Paper 23

Shuji Ozawa and
Teruhisa Miyauchi,
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Hokkaido University,
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BIOSYNTHESIS OF LIGNANS IN *MAGNOLIA KOBUS* DC.VAR.*BOREALIS* SARG.

As part of our studies of lignan biosynthesis in woody plants, we investigated the formation of lignans in *Magnolia kobus* var. *borealis*. This plant contains various furofuran lignans. The lignan contents varied according to tissue type and season. From *in vivo* labelling experiments of *M. kobus* var. *borealis*, it was established that deuterated coniferyl alcohol had been incorporated into pinoresinol, but not fargesin and kobusin. Next, we examined *O*-methylation reactions of phenolic furofuran lignans by incubation with cell-free extracts from *M. kobus* var. *borealis*. It was shown that the cell-free extracts catalysed the *O*-methylation reactions of pinoresinol and syringaresinol, suggesting the conversions of pinoresinol into eudesmin, and syringaresinol into yangambin, respectively. The *in vitro* transmethylation reactions observed were slightly enantionselective with the (+)-forms being the preferred substrates. Purification and characterization of the *O*-methyltransferase(s) is under active investigation.

TUESDAY, 6:00-7:30 **DINNER - COMPTON UNION BUILDING BALL ROOM**

POSTER SESSION 2 ***PHYTOCHEMICAL PATHWAYS USEFUL IN MEDICINE AND PLANT DEFENSE***

Moderators: ***Joshua D. Ford/Anastasia L. Crowell***

TUESDAY, 8:00-10:00 PM **COMPTON UNION BUILDING - BALL ROOM**

Poster Paper 2

Alicja M. Zobel[1], J.M. Lynch[1],
M. Podbielkowska[2],
E. Kupidlowska[2] and
K. Wierzchowska-Renke[3],
[1]Department of Chemistry,
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[3]Pharmacy, Medical Academy,
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COMPOUNDS REMOVED FROM PLANT SURFACES AS POTENTIAL ANTI-CANCER AGENTS

Mixtures of phenolic compounds removed from surfaces of several plants retarded mitotic numbers when investigated using Levan's test. The strongest mitodepressive reaction had *Brassica oleracea* extracts and *Taxus baccata*. *Ruta graveolens* extracts from the surfaces of leaves were of a similar potency as the extracts from the interior of the leaves. Ultrastructural changes observed in the electron microscope were mostly to the endomembrane system of endoplasmic reticulum and plasmalemma and to the matrix of mitochondria which became more electron dense than control ones and increased in size. Changes to mitochondria suggested that the energy status of treated cells was affected.

Poster Paper 4

J. Fred Stevens[1],
Alan W. Taylor[1],
Cristobal L. Miranda[2],
Donald R. Buhler[2] and
Max L. Deinzer[1],
Departments of [1]Chemistry and
[2]Agricultural Chemistry,
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Corvallis, OR 97331, USA.

POTENTIAL CANCER CHEMOPREVENTIVE PRENYLFLAVONOIDS FROM HOPS (*HUMULUS LUPULUS*)

Ten prenylflavonoids (PFs) were isolated from hops and identified (*Phytochemistry* 44, 1575, 1997), seven of which were investigated for cancer chemopreventive activity. Xanthohumol (XN) and related PFs were quantified in hops and beer, and their fate was monitored during the brewing process by LC-MS. Hop PFs of the chalcone type were largely converted into their isomeric flavanones during wort boiling. A typical American beer was found to contain *ca* 0.5 mg/l of the flavanone, isoxanthohumol (IX), and trace quantities of XN, 6- and 8-prenyl-naringenin and 6-geranylnaringenin. Six hop PFs were tested for their ability to inhibit the proliferation of human breast cancer (MCF-7) and colon cancer (HT-29) cells *in vitro*. XN, IX and dehydrocycloXN were most active against MCF-7 cells (IC_{50} 13-16 μM 2 days after exposure), whereas the PFs tested failed to inhibit growth of the HT-29 cells. XN and IX displayed no toxicity towards rat hepatocytes at 10 μM after 6 or 24 hours of exposure. The inhibition of carcinogen metabolism by five hop PFs was also evaluated. Desmethylxanthohumol and IX were most effective in inhibiting the metabolic activation of two procarcinogens (AFB1 and DMBA) mediated by cyto-chrome P450. The results indicate that some hop PFs have potential cancer chemopreventive properties in humans, but it is too early to speculate about the impact of dietary intake of PFs (beer consumption) on human health.

Poster Paper 6

Chung Ki Sung,
Jeong Mee Lee,
Da Woon Jung,
Baik Hwang[1],
Jun Cheul Ahn[2],
Keon-Hyoung Song[3] and
Chang-Koo Shim[3],
Coll. of Pharmacy,
Coll. of Natural Sciences[1],
Chonnam National Univ.,
Kwangju 500-757, KOREA;
Dept. of Biology[2],
Seonam Univ.,
Jeonbuk 590-170, KOREA;
Coll. of Pharmacy[3],
Seoul National Univ.,
Seoul 151-742, KOREA.

DEVELOPMENT OF ELISA METHOD FOR THE DETERMINATION OF SAIKOSAPONIN A, AN ACTIVE COMPONENT OF *BUPLEURUM FALCATUM* L., AND ITS APPLICATIONS

Saikosaponin a (Sa) is one of major active components of *Bupleuri Radix*(root of *Bupleurum falcatum* L.), one of the famous Oriental drugs used mainly for treatment of liver diseases. Quite recently Oriental drug prescription containing this drug was reported to have caused serious side-effects including even death, which demands a new pharmacodynamic study on this drug. Regardless of this report, this drug shows a strong antiinflammatory effect, and has been used for the treatment of hepatitis and related liver diseases. For the supply of this drug, the cultivation for mass production in the field and on the tissue culture system have been studied. For the purpose of determination of Sa in biological fluids and plant samples, we established a highly sensitive and specific ELISA system using Sa specific antibody raised by immunization of Sa-BSA conjugate to rabbit, peroxidase conjugated anti-rabbit second antibody and tetramethyl benzidine as substrate. The measuring range extends from 50pg/ml to 20 ng/ml. The method showed minor cross reactivity with saikosaponin d(0.3%), which differs from Sa on the stereochemistry of the 16-hydroxyl group. We applied this method to determine Sa in plant samples like callus, hairy root and Oriental drug prescription and also in biological fluid like serum taken for phamaco-kinetic study.

Poster Paper 8

Luciana Mensor, S. G. Leitão,
F. S. Menezes, C. S. Coube and
G. G. Leitão,
Depto de Produtos Naturais e Alimentos,
Faculdade de Farmácia, UFRJ,
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Nucleo de Pesquisas em Produtos Naturais, UFRJ, BL.H,
Rio de Janeiro,
21941-590, BRAZIL.

ANTIOXIDANT ACTIVITY OF BRAZILIAN PLANT EXTRACTS: DPPH SCAVENGING TEST

Extracts from ten Brazilian plants from four different families were tested against DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) free-radicals in six different concentrations (250, 125, 50, 25, 10 and 5 μg/ml). The antioxidant effect of an extract corresponds to its intensity of quenching DPPH. The antioxidant activity of plant extracts belonging to Verbenaceae family were very high, comparable with standard antioxidants such as rutin and *Ginkgo biloba* (Tebonin Oral Solution®), both used as antioxidants for medical purposes. The EC_{50} values were obtained by linear regression curves. Experiments were made in triplicate and all plant extracts showed a dose-response curve. *Lantana trifolia* (Verbenaceae) leaf extract showed the highest activity of all plants studied (93.5 % at 125 μg/ml). Further investigation of this extract by TLC sprayed with DPPH reagent showed that substances responsible for antioxidant activity are phenolic.

Poster Paper 10

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J. Markham and
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AUSTRALIA.

THE ROLE OF STRUCTURE AND SOLUBILITY IN DETERMINING THE ANTIMICROBIAL ACTIVITY OF TERPENOIDS AGAINST SELECT HUMAN PATHOGENS

The Minimum Inhibitory Concentration (MIC) of over fifty terpenoids, several aromatic ethers and two ionones have been determined against *P. aeruginosa, E. coli, S. aureus* and *C. albicans* using an agar plate dilution method. The water solubility of the compounds has also been measured. Hierarchical cluster analysis was used to group the compounds into five groups according to their activity against the four microorganisms. Of these five groups, those containing hydrocarbons and acetates were relatively inactive. This may be attributed to their low water solubility. Each of the remaining four groups, all containing oxygenated terpenes, showed characteristic but distinct activity behaviour patterns towards the four test organisms. Functional group and water solubility differences do not appear to account for all aspects of these relationships. This and other data will be discussed as a contribution towards a more detailed understanding of relationships between the structure of terpenes and their antimicrobial activity against a range of pathogenic organisms.

Poster Paper 12

Peter J. Houghton,
Pharmacognosy Research Laboratories,
Department of Pharmacy,
King's College London, Manresa Road,
London SW3 6LX, UK.

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The threat of drug-resistant tuberculosis makes the discovery of novel antitubercular compounds imperative. The activity of an ethanolic extract of *Galipea officinalis* bark demonstrated by a preliminary screen was traced to the alkaloids present. Fractions and derived compounds were tested for activity against *M. tuberculosis* cultured in Middlebrook medium using serial dilutions and growth was observed after 14 days. Alkaloids were found in the most active fractions and their structures were deduced by mass and NMR spectroscopic methods. Some of the isolated quinoline alkaloids showed significant activity, but the greatest activity was shown by a polar fraction which is still being investigated.

Poster Paper 14

Suzanne Diamond,
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SEVEN KEY PHYTOCHEMICAL CONSTITUENTS OF EDIBLE AND MEDICINAL HERBS: UNDERSTANDING TRADITIONAL MEDICINE

Humans evolved utilizing many plants, both foods and medicines, that our current society has forgotten about. Around the turn of the century it is estimated that our European ancestors utilized more than 100 different plant foods, compared to this modern day where 80% of the diet comes from just 30 foods. Because plants are exposed to the sun all day, every day, they are loaded with antioxidants that protect them from U.V. rays that would otherwise damage and burn their tissues. People are actually reliant upon the diversity of compounds that many plants contain for their own health. Myriad edible and medicinal herbs from nature can fortify the diet while at the same time, the antioxidant compounds they possess will not only slow the aging process, but can balance critical hormone levels and blood sugar, alleviate depression, reduce stress, strengthen capillaries and blood vessels, improve circulation and brain function, balance acid/alkaline levels of the body, improve inter-cellular communication and soothe cell membranes especially of the respiratory and digestive systems. A seven faceted approach to health and healing designed to amend the common deficiencies of the modern diet will be presented. Fundamental Diet Requirements include: 1. Anthocyanins; 2. Omega-3 Essential Fatty Acids; 3. Phytoestrogens; 4. Bitters; 5. Mucilage (Mucopolysaccharides); 6. Trace Elements, Minerals and Other Nutrients; 7. Fructo-oligosaccharides and other soluble fibres.

Poster Paper 16

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Werner Haussmann and
Andreas Hensel,
Lehrstuhl für Pharmazeutische Biologie,
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EFFECTS OF VARIOUS PREGNANES, 23-NOR-5-CHOLENIC ACIDS AND THEIR FUCOSIDES ON CARDENOLIDE ACCUMULATION IN SHOOT CULTURES OF *DIGITALIS LANATA* EHRH

Various pregnanes, such as 21-hydroxypregnenolone, cortexone and 5β-pregnan-3β,14β,21-triol-20-one, as well as 23-nor-5,20(22)*E*-choladienic acid-3β-ol were administered to shoot cultures of *Digitalis lanata*. Cardenolide formation was clearly enhanced under these conditions, glucodigifucoside being one of the major products. In order to check the effect of other close precursurs to that compound, the β-D-fucosides of some of the substances mentioned above were synthesized. The feeding of 21-hydroxypregnenolone β-D-fucoside resulted in an 25-fold increase of glucodigi-fucoside as compared to a control treated with the corresponding aglycone, 21-hydroxypregnenolone. It is concluded that glycosylation may occur prior to butenolide ring formation and that fucosylation is a rate-limiting step in cardiac glycoside biosynthesis.

Poster Paper 18

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BIOSYNTHESIS OF ANTIOXIDANT LIGNANS IN *SESAMUM INDICUM*

Sesamum indicum seeds accumulate the antioxidant lignans, (+)-sesamin and (+)-sesamolin. It was established that 8-week old *S. indicum* plants contain seeds which catalyzed different lignan transformations (methylenedioxy bridge formation and oxygen insertion, respectively) depending upon the seed stage of maturity, *i.e.* lignan formation was developmentally regulated. In the study of the metabolic fate of (±)-[3,3′-O^{14}CH$_3$]pinoresinols in mature seeds of *S. indicum*, only the (+)-enantiomer was metabolized *in vivo*, giving radiolabeled (+)-piperitol, (+)-sesamin, and (+)-sesamolin, respectively. Microsomal preparations catalyzed the O_2/NADPH/ cytochrome P-450 dependent transformation of (+)-pinoresinol into (+)-piperitol, and (+)-piperitol into (+)-sesamin. Preliminary characterization of this cytochrome P-450 will be discussed.

Poster Paper 20

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CLONING, EXPRESSION AND PARTIAL CHARACTERIZATION OF A SESQUITERPENE CYCLASE FROM *ARTEMISIA ANNUA* L

The antimalarial sesquiterpene, artemisinin, is produced by *Artemisia annua* L. The first committed step in the biosynthesis is cyclization of farnesyldiphosphate (FDP) by a cyclase; a key enzyme of artemisnin biosynthesis and therefore a good target for metabolic engineering. The molecular cloning of this sesquiterpene cyclase (germacranadiene synthase; GS) from *A. annua* is the aim of this project. Upregulation of GS in transgenic plants will be attempted to increase the yield of artemisinin. A cDNA encoding a sesquiterpene cyclase has been isolated from a cDNA library made from young leaves of *A. annua* using a tobacco sesquiterpene probe. The deduced amino acid sequence showed 30-45% identity to other plant sesquiterpene cyclases. Subcloning of the open reading frame into an *E. coli* expression vector was carried out. After induction with IPTG, an active soluble protein was obtained which efficiently converts FDP to olefin(s) and geranyl di-phosphate to a lesser extent. The enzyme was purified by ion-exchange chromatography. The partially purified enzyme is used to generate product(s) for identification by GC-MS. It is also used to determine kinetic data for the enzyme. The metal ion requirements for the enzyme are evaluated.

Poster Paper 22

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GIBBERELLINS(GA): METABOLITES FROM FEEDS OF [^{13}C,^{3}H]GA_{24} TO SEEDLING SHOOTS OF MAIZE (*ZEA MAYS*)

Our previous isolation and biosynthetic studies (see poster and Kobayashi, *et al*, Plant Physiol. *110*, 413-418 1996) have demonstrated the presence of the early 13-hydroxylation pathway in seedling shoots of maize. The isolation of GA_{15}, GA_{24}, GA_9, GA_4 and GA_7 from maize seedlings suggests the presence of a second, hypothetical, biosynthetic pathway, the non-early 3,13-hydroxylation pathway (see poster). While our metabolic studies provide no evidence for the presence of this second pathway, we have previously demonstrated metabolism of its presumed members to those of the early 13-hydroxylation pathway. Thus GA_{15} was metabolized to GA_{44}, GA_9 to GA_{20} and GA_4 to GA_1 (but not GA_7 to GA_3). We here present evidence for the metabolism of [^{13}C,^{3}H]GA_{24} to [^{13}C]GA_{17}, [^{13}C]GA_{19} and [^{13}C]GA_{20}, with no evidence of metabolism to [^{13}C]GA_9. Thus the members of the presumptive non-early 3,13-hydroxylation pathway are connected to the early 13-hydroxylation pathway by 13-hydroxylation, resulting in a metabolic grid. (Supported by grant #MCB-960446.) (See also ASPP abstract in Plant Physiol. *117,* 1998 supplement.)

Poster Paper 24

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BIOSYNTHESIS OF THE SPERMIDINE ALKALOID LUNARINE

The seeds of *Lunaria annua* L. (Cruciferae) contain lunarine as the major alkaloid. Lunarine belongs to the quite rare class of macrocyclic polyamine alkaloids and is composed of spermidine connected with two molecules of *p*-coumaric acid. Most interesting in the biosynthesis is the phenolic coupling of the two *p*-coumaric acid moieties which resembles the formation of Pummerer's ketone. Lunarine had already been shown to be biosynthesized from the labeled precursor L-phenylalanine (1). We could show that *trans*-cinnamic acid and *p*-coumaric acid as well as spermidine and putrescine serve also as precursors of this alkaloid. Furthermore we could identify bis(*p*-coumaroyl)spermidine as the penultimate precursor of the alkaloid undergoing a final stereoselective phenoloxidative coupling reaction. The biosynthetic route and the enzymes involved in the phenolic coupling have been studied. The biosynthesis of lunarine is discussed in the light of cytochrome P-450 enzymes being involved in phenolic coupling reactions.

Reference

[1] Poupat, C., Kunesch, G. (1971) C. R. Acad. Sc. Paris, Série C **273**, 433-436.

Poster Paper 26

XianZhi He and
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A TRANSGENIC APPROACH TO UNDERSTANDING POTENTIAL METABOLIC CHANNELING INVOLVED IN ISOFLAVONE *O*-METHYLATION DURING MEDICARPIN BIOSYNTHESIS IN ALFALFA (*MEDICAGO SATIVA*)

Medicarpin, the major phytoalexin in alfalfa, is synthesized via the isoflavonoid branch of phenylpropanoid metabolism. The methyl group at the 9-position of medicarpin is generally accepted to arise at an early step via the methylation of the 4'-position (B-ring) of daidzein. Surprisingly, the isoflavone-*O*-methyltransferase (IOMT) activity, which is co-stimulated with all other enzyme activities leading to medicarpin biosynthesis, methylates the A-ring hydroxyl group of daidzein in vitro. We proposed that the IOMT may be part of a metabolic channel and that, as a consequence, the IOMT can methylate the B-ring hydroxyl of daidzein *in vivo*. To test this hypothesis directly, we have cloned the IOMT using information from internal amino acid sequence of the purified enzyme. The activity of the enzyme *in vivo* is being addressed in transgenic alfalfa by loss of function (antisense and potential co-suppression) studies, and by gain of function studies in transgenic bean (*Phaseolus vulgaris*), a species that does not appear to carry out 4'-*O*-methylation of its isoflavonoids.

Poster Paper 28

Chong-Uk Park,
Margaret Essenberg and
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LOCATION AND TIME OF APPEARANCE OF A PHYTOALEXIN-BIOSYNTHETIC ENZYME RELATIVE TO HYPERSENSITIVE CELL DEATH AT *XANTHOMONAS* INFECTION SITES IN COTTON COTYLEDONS

Objectives were to learn where phytoalexins are synthesized in cotton foliar tissue and to test the hypothesis that earliness of phytoalexin biosynthesis correlates with level of bacterial blight resistance. Leafy cotyledons were infiltrated with *X. campestris* pv. *malvacearum* and at intervals, tissue samples were excised for histochemistry. Hypersensitively responding (HR) cells were detected by their autofluorescence. Bacteria were detected by immunogold staining. The first enzyme committed to sesquiterpenoid phytoalexin biosynthesis, (+)-delta-cadinene synthase (CDN1), was detected by immunofluorescent staining. Mock-inoculated tissues exhibited no staining for CDN1, and inoculated susceptible Ac44 exhibited very little. In two resistant lines, CDN1 appeared in all HR cells and in a few adjacent living cells during the first 3 days postinoculation, but by 6 days, all CDN1-containing mesophyll cells were dead. HR cells and CDN1-containing cells were observed sooner in highly resistant Im216 than in moderately resistant OK1.2. Supported by NRICGP/USDA and by the Okla. Agric. Exp. Sta.

Poster Paper 30

Pascale Jäger-Vottero,
Ying Jiao and
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INDUCTION OF A LIGNAN-DERIVED EXTRACELLULAR PRECIPITATE IN *PINUS TAEDA* L. CELL SUSPENSION CULTURES: A COMPARISON WITH HEARTWOOD METABOLITE FORMATION

When *Pinus taeda* L. cell suspension culture cells were transferred from a modified Brown and Lawrence medium containing 2,4 D, to an 8% sucrose solution, an induction of monolignol, lignan and oligomeric lignan biosynthesis occurred. Within 6 days, sequential metabolite accumulation (monomers then dimers) ultimately gave rise to formation of an extracellular precipitate in the medium. Both their excretion and metabolic (e.g. of dehydrodiconiferyl alcohol) profiles showed striking similarity to that accompanying heartwood metabolite deposition. Addition of potassium iodide (20 mM), a hydrogen peroxide scavenger, also revealed that the absence of hydrogen peroxide prevented formation of the extracellular precipitate, whereas supplementation of the 8% sucrose media with either pinoresinol or dehydrodiconiferyl alcohol prior to cell transfer shifted formation of hydrogen peroxide 24 hours earlier, suggesting that hydrogen peroxide synthesis was induced by the presence of the lignans in the extracellular medium. Interestingly, dehydrodiconiferyl alcohol was actively metabolized, being converted into its dimethylated and allylic double bond reduced analogues. These results are discussed in terms of a comparison with that of induction of heartwood formation.

Poster Paper 32

Gerardo Rodriguez and
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PURIFICATION OF POLYPHENOL OXIDASE (PP0) AND ISOLATION AND STRUCTURAL IDENTIFICATION OF SUBSTRATES FROM RED CLOVER (*TRIFOLIUM PRATENSE* L.)

Red clover silage has a lower extent of microbial proteolysis in the silo and rumen than alfalfa. The difference is caused by the presence of a soluble polyphenol oxidase and a high content of phenolic substrates. Polyphenol oxidase (PPO) catalyzes the oxidation of phenols to quinones that react with amino, sulphydryl, thioester, phenolic, indol and imidazol groups on proteins and inactivate proteases. Two phenyl-propanoid compounds (clovamide and phaselic acid) were isolated by low pressure column chromatography and identified using ^{1}HNMR and ^{13}CNMR. Both phenolic compounds are potential substrates for PPO as indicated by their rapid disappearance during the oxidation process. PPO was partially purified using detergent and ammonium sulfate fractionation and chromatography on sephadex G-150. Crude PPO showed pH optima of 6.5 with catechin and 10.5 with 4-*ter*butylcatechol. Partially purified PPO catalizes the reaction of *ter*-butylcatechol and O_2 to form two different quinones as shown by high performance liquid chromatography (HPLC). PPO purification on a size exclusion column (Protein PaK 300sw, HPLC) gave three main fractions. Fraction 1 had an activity of 5.68 absorbance units per mg of protein (abs/mgp), fraction 2 had 10.3 abs/mgp and fraction 3 had 148.8 abs/mg.

Poster Paper 34

Elena Kraeva[1,3], Alain Deloire[1], Jacques Bierne[2] and
Claude Andary[3],
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[2]Laboratoire de Biologie Cellulaire et Moléculaire, Université de Reims-Champagne-Ardenne, 51687 Reims;
[3]Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, 34060 Montpellier, FRANCE.

DEFENSE MECHANISMS OF GRAPEVINE BERRIES DURING THEIR DEVELOPMENT, IN RELATION WITH SALICYLIC ACID TREATMENTS

Veraison, a key stage of grape berry development, represents the beginning of ripening and is characterized by berry tissue softening, accumulation of sugar and amino acids, loss of acidity and, in black varieties, the synthesis of anthocyanin pigments. During the growth period (before ripening), salicylic acid (SA) treatment provoked cell necrosis of the berry skin. The necrotic area of each berry was limited and stabilized 12 hours after SA treatment. Histological observation, made at this time, showed that the necrosis concerned only a limited number of cells from the exocarp and the outer mesocarp. Moreover SA treatment influenced the phenolic compound profile, particularly condensed tannins. These molecules seemed to reduce the oxidant effect of SA. When the SA treatment was made at veraison and during ripening, no cell necrosis was observed. We have also shown that, before ripening, SA treatment and wounding induce β-1,3-glucanase mRNA synthesis. At this stage, no glucanase mRNA expression was detected, but this enzyme was constitutively expressed during ripening.

Poster Paper 36

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KENAF: A SOURCE OF POTENT PHYTOALEXINS

New phytoalexins isolated from kenaf include hibiscanal, *o*-hibiscanone (3,8-dimethyl-1,2-naphthoquinone, *o*-HBQ), and 3-hydroxy-*p*-hibiscanone. At 10 µg/ml, *o*-HBQ killed 94% of the mycelial samples of the plant pathogen, *Verticillium dahliae*. The phytoalexins from kenaf appear to be derived from δ-cadinene through loss of an isopropyl group.

Poster Paper 38

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THE MODE OF CHEMICAL DEFENSE CHANGES IN WHITE POPLAR (*POPULUS ALBA*) LEAVES DURING PLANT ONTOGENY

The effect of plant ontogeny on the production of defense components was investigated in the leaves of a wild white poplar (*Populus alba* L.) clone. A number of phenolic glucosides, cinnamic acid derivatives, flavonoid-glycosides and condensed tannins was analyzed in five most apical leaves of current-growth shoots of seedlings, saplings and an adult tree. In the leaves of seedling (one year old) and sapling (3 years old) toxins such as salicin, salicortin and tremulacin predominated. By contrast, salicortin and tremulacin were absent but condensed tannins were very abundant in the leaves of the adult tree. The mode of chemical defense based on high molecular weight secondary metabolites appears to be minor compared to lower molecular weight components, such as salicortin and flavonoid-glycosides in juvenile white poplar plants.

Poster Paper 40

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Survey, Champaign, IL, USA.

MOSQUITOCIDAL ACTIVITY OF AN ACETYLENIC COMPOUND FROM *CRYPTOTAENIA CANADENSIS* (APIACEAE)

As a part of our program for identifying mosquitocidal compounds from plants, an acetylenic compound was isolated from *Cryptotaenia canadensis*. The plant was extracted with boiling 80% methanol and the methanol was removed under vacuum. The extract was partitioned into chloroform and water. Both phases were tested against 3rd and 4th instar larvae of *Culex pipiens* (Culicidae/Diptera) at concentrations between 5-50 ppm. The dose-response curve of the active chloroform-phase was determined with a probit analysis (SPSS). The chloroform-phase was further purified by the treatment with activated charcoal. A high resolution MS indicated the empirical formula to be $C_{17}H_{24}O_2$. The exact structure was elucidated with GC-MS-EI and GC-MS-CI fragmentation, UV-spectrometry, 1H-NMR and 13C-NMR spectrometric data.

Poster Paper 42

Carlos Céspedes A.[1],
José Calderón P.[1],
Laura Lina[3],
Eduardo Aranda[3] and
Blas Lotina-Hennsen[2],
[1]Instituto de Química;
[2]Facultad de Química, UNAM,
C.P. 04510, MEXICO D.F.;
[3]UAEM, Cuernavaca, MEXICO.

ANTIFEEDANT ACTIVITY OF PHOTOGEDUNIN DERIVATIVES AND LIMONOIDS FROM *CEDRELA SALVADORENSIS* (MELIACEAE) ON FALL ARMYWORM *SPODOPTERA FRUGIPERDA*

Extracts of *Cedrela salvadorensis* Standley (Meliaceae) have insecticidal activity and resulted in the isolation of gedunin, cedrelanolide, and two epimeric photogedunins which were separated as their acetates. The epimeric photogedunin and its acetates were assayed against *S. frugiperda*. These compounds show a comparable effect on the fall armyworm to toosendanin, a commercial insecticide derived from *Melia azedarach*, in a range of 30 to 100 ppm. The compounds caused larval mortality as well as growth reduction and increased the development time of survivors in a concentration-dependent manner. Additionally, it is possible to observe in many of the treated groups a significant time delay of pupation and adult emergence. The LD50 of the photogedunin acetate mixture is of 200 uM (104 ppm), with respect to minor concentrations. It is possible to observe the time delay of pupation with deformation of pupa and time delay of adult emergence with fly deformation and infertility.

Poster Paper 44

Shannon E. Binns, Bibiana
Purgina, Bernard R. Baum,
John T. Arnason, Dennis V.C.
Awang and John Livesey,
Biology Dept.,
University of Ottawa,
Ottawa, ON K1N 6N5,
CANADA.

PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITY OF *ECHINACEA* spp.

Native populations of *Echinacea* spp. (Compositae) have been assessed for their alkylkamide, polyacetylene and phenolic contents by HPLC as part of a taxonomic study of the genus. Hexane fractions of ethanolic extracts from specific plant organs contained polyacetylenes and were found to have phototoxic activity against clinical fungal isolates. Chemical variation and degradation within ethanolic extracts of *Echinacea* spp. were observed over time. Also, the lipophilic and hydrophilic constituents found in ethanolic extracts of young seedlings were markedly different from those in older plants of the same population.

Poster Paper 46

Vasile V Trif and
Eleonora Trif,
Australian Group of
Photosynthesis,
Centre for Physiology &
Biochemistry of Vegetables,
59 Pix Road,
Elizabeth Field, 5113 S.A. ,
AUSTRALIA.

TB-TYPE OF PHOTOSYNTHETIC ASSIMILATION OF CARBON

This study reveals the adaptation of various plants to the gaseous hydrocarbon environments by the so-called TB-type assimilation. Kept for 542 days in assimilation chambers deprived of CO_2 and O_2, but filled (100%) with gaseous hydrocarbons, the plants developed new stalks and leaves. The rate of chlorophyll biosynthesis is greater than for plants kept in air. The chloroplasts extracted from various leaves of plants under investigation were examined by EPR spectroscopy. The EPR spectra consists in the superposition of two types of free radicals: (i) the light induced ones with g_1 =2.004 and ΔB =0.9 mT; (ii) the dark stable ones with g_2 = 2.006 and ΔB = 4.2 mT. The intensity of signal (i) is gradually reduced and the signal (ii) broadens from ΔB = 3.7 mT for plants stored in CH_4 for 1-4 days, to ΔB = 5.0 mT for those stored for 60 days. The newly formed leaves exhibit only a photosensitive signal with g_1 = 2.004 and ΔB = 0.71 mT. The successive alteration of the free radical spectra could be related to the steps in suspending the water photodissociation occurring in the PS II photosystem as C_nH_{2n+2} becomes source both for H^+ and e^-. The photoactivity of PS II can be restored partially if a part of chlorophyll molecules are replaced by the pigment P671, a Mn(II)-containing chlorophyll-like phephytin.

Poster Paper 56

VOLATILE COMPOUNDS FROM BRAZILIAN MONIMIACEAE

Gilda G. Leitão,
Ana Cláudia F. Amaral,
Simone S. V. Soares,
Naomi K. Simas and Thelma B. M. Brito,
NPPN-UFRJ (Natural Products Research Center, Federal University of Rio de Janeiro),
Bl. H, CCS,
Ilha do Fundão, 21941-590,
Rio de Janeiro, RJ,
BRAZIL.

The family Monimiaceae comprises 34 genera and *ca.* 440 species distributed mainly in the warmer parts of the Southern Hemisphere. Leaves of plants from this family usually have many spherical secretory oil cells. The presence of common monoterpenes as pinene, elemene, terpinolene and sesquiterpenes as spathulenol and germacrene, among others, has been reported in *Doryphora, Hedycarya, Laurelia, Peumus* and *Xylosma*. The present report shows the constituents of the essential oils extracted by hydrodistillation from seven individuals of this family : *Siparuna arianeae* (leaves), *S. apiosyce* (leaves from male and female trees, young stems, bark), and leaves of *Mollinedia gilgiana, M. glaziovii, M. salicifolia* and *M. shottiana,* which were characterized by GC-MS analysis and Kovats' indices.The common compounds of these species were identified as elemene, pinene, copaene, bisabolol and spathulenol. The latter appears as the major compound among the *Mollinedia* and *Siparuna* studied. This is in accordance with literature data covering other species of the family.

SYMPOSIUM SESSION 5 *BIOACTIVE MOLECULES/ENGINEERED FOODS*
Moderator: *Vincenzo De Luca*

WEDNESDAY, 8:00-8:50

Symposium Paper 11

SIMPLE (BENCH-TOP) BIOASSAYS AND THE ISOLATION OF NEW CHEMICALLY DIVERSE ANTITUMOR AND PESTICIDAL AGENTS FROM HIGHER PLANTS

Jerry L. McLaughlin,
Professor of Pharmacognosy,
Department of Medicinal Chemistry and Molecular Pharmacology,
School of Pharmacy and Pharmacal Sciences,
Purdue University,
West Lafayette, IN 47907-1333, USA.

Four simple (bench-top) bioassays have served well for the detection and fractionation monitoring of new plant antitumor and pesticidal agents. These are: (1) lethality to the larvae of brine shrimp (*Artemia salina*), (2) the inhibition of crown gall tumors induced by plasmid transfer and expression from *Agrobacterium tumefaciens* on discs of potato (*Solanum tuberosum*) tubers, (3) the inhibition or stimulation of frond proliferation of duckweed (*Lemna minor*) and (4) lethality to the larvae of yellow fever mosquitoes (*Aedes aegyptii*). Since 1984, over 320 chemically diverse bioactive plant components have been isolated and characterized in our laboratory using these methods. Recently isolated bioactive compounds from the Meliaceae, Lauraceae, Euphorbiaceae, Rutaceae, and other plant families will be presented, but our most exciting recent leads have been with the potent acetogenins from the Annonaceae. These compounds are powerful inhibitors of mitochondrial electron transport systems and of the NADH oxidase that is prevalent in the plasma membranes of tumorous cells. The consequence is ATP depletion, and this is especially toxic to multiple drug resistant tumor cells and pesticide resistant insects that possess ATP-dependent xenobiotic efflux systems. Results of structural activity relationship studies (in drug resistant cancer cells, isolated mitochondria, mosquito larvae, cockroaches, and submitochondrial enzyme preparations) will be presented. (Previously aided by RO1 grant no. CA30909 from NCI/NIH).

WEDNESDAY, 8:50-9:40

Symposium Paper 12

PHYTOCHEMISTRY OF BRYOPHYTES: BIOLOGICALLY ACTIVE TERPENOIDS AND AROMATIC COMPOUNDS FROM LIVERWORTS

Yoshinori Asakawa,
Faculty of Pharmaceutical Sciences,
Tokushima Bunri University,
Yamashiro-cho,
Tokushima 770-8514, JAPAN.

A number of bryophytes are medicinal plants and are said to possess certain biological activities, (cytotoxic, antimicrobial, antidotal, antipyretic, antirhinitic, sedative and antiseptic activities, etc.) and other beneficial effects (e.g. for cardiopathy, hemostasis, pulmonary tuberculosis and neurasthenia, etc.). We have been interested in their biological activities and have studied *ca.* 800 species of bryophytes. Most liverworts contain mainly sesqui- and diterpenoids and lipophilic aromatic compounds, several of which showed allergenic contact dermatitis, cytotoxicity against P-388, melanoma and KB cell, antimicrobial, antifungal, antitumor promoting and anti-HIV-I activity, insect antifeedant, molluscicidal and piscicidal activity, inhibitory activity against cathepsins B and L, plant growth, superoxide anion radical release, 5-lipoxygenase, cyclo-oxygenase, calmodulin and DNA polymerase, and neurite sprouting and muscle relaxing activities. The isolation, structures and biological activity of unique terpenoids and aromatic compounds from bryophytes will be discussed.

WEDNESDAY, 9:40-10:00 **BREAK**

WEDNESDAY, 10:00-10:40

Symposium Paper 13

CYANIDE IN FOODS: BIOLOGY OF CYANOGENIC GLUCOSIDES AND RELATED NUTRITIONAL PROBLEMS

Dirk Selmar,
Botanical Institute,
Technical University, 4,
38106 Braunschweig,
GERMANY.

Cyanogenic glucosides are widely spread in the plant kingdom. They are present in more than 3000 plant species, including nearly all food plants. Upon tissue disruption, cyanogenic glucosides are hydrolyzed, resulting in the liberation of HCN. In general, the actual concentrations of cyanogens in plant tissues are very low. Thus, the amount of HCN liberated is very small. In most foods, the concentration of cyanide derived from cyanogenic glucosides is less than 100 µg per kg fresh weight. With regard to the lethal HCN-dose for humans (60 mg), these amounts are almost negligible. In contrast, more than 100 mg of HCN may be liberated per kg fresh weight in strongly cyanogenic plants. In order to avoid cyanide intoxications, food from such plants (e.g. cassava, bamboo) must be processed and detoxified before usage. In this lecture, an overview on the biology of cyanogenic glucosides and the health problems related to foods derived from cyanogenic plants will be presented. In this context, problems due to acute intoxication by food from highly cyanogenic plants will be discussed, as well as the chronic effects caused by the long term usage of food containing only minor amounts of cyanide.

WEDNESDAY, 10:40-11:05

Oral Paper 24

Maria C. Cordeiro,
Per Mercke and
Peter E. Brodelius,
Department of Plant
Biochemistry,
Lund University,
P.O.Box 117,
S-22100 Lund, SWEDEN.

FUSION OF TWO ENZYMES OF SESQUITERPENE BIOSYNTHESIS AND STUDIES ON CHANELLING OF AN INTERMEDIATE METABOLITE

The aim of this project is to test if chanelling of intermediates can be achieved by fusion of enzymes located on each side of a branching point in a biosynthetic pathway. As a model we have chosen a fusion of farnesyldiphosphate synthase (FDPS) from *Artemisia annua* with a sesquiterpene cyclase (*epi*-aristolochene synthase; *e*AS) from tobacco. The intermediate farnesyl-diphosphate (FDP) is a substrate for other competing enzymes. A FDPS clone was obtained by PCR from a cDNA library from *A. annua*. The tobacco cyclase clone was kindly supplied by Dr. J. Chappell. Two fusions have been constructed, *i.e.* FDPS-*e*AS and *e*AS-FDPS. The stop codon of the N-terminal enzyme was removed and replaced by a linker between the two open reading frames. These fusions and the single cDNA clones were separately introduced into an *E. coli* expression vector. Active proteins were obtained after induction with IPTG. Kinetic properties of the various proteins is under investigation. These investigations include studies on the chanelling of FDP by the bifunctional enzymes *in vitro*. Next, the fusion proteins will be introduced into tobacco and the effects on the biosynthesis of *epi*-aristolochene will be investigated.

WEDNESDAY, 11:05-11:25

Oral Paper 25

Wolfgang Kreis and
Christoph Theurer,
Lehrstuhl für Pharmazeutische
Biologie,
FAU Erlangen-Nürnberg,
Staudtstr. 5,
D-91058 Erlangen, GERMANY.

EFFECTS OF DIGITOXIGENIN, DIGOXIGENIN, AND VARIOUS CARDIAC GLYCOSIDES ON CARDENOLIDE ACCUMULATION IN SHOOT CULTURES OF *DIGITALIS LANATA* EHRH

Various cardenolide genins and cardenolide glycosides were administered to *Digitalis lanata* shoot cultures to investigate conversion reactions related to the formation and rearrangement of the sugar side chain of Digitalis glycosides. Digitoxigenin was converted to digoxigenin, digitoxigen-3-one, 3-epidigitoxigenin, as well as various cardenolide fucosides and digitalosides. Digitoxosylated cardenolides were not formed, although the shoot cultures were capable of producing these compounds. Exogenous cardenolide fucosides were not converted into cardenolide digitoxosides. We assume that cardenolide fucosides and digitoxosides are formed *via* different biosynthetic routes and that cardenolide genins can be fucosylated but not digitoxosylated. Digitoxosylation may only occur at an earlier stage in the cardenolide pathway.

WEDNESDAY, 11:25-11:45

Oral Paper 26

Jinghua Liu[1], S. Zschocke[2],
E. Reininger[2] and R. Bauer[2],
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Columbia University,
Havemeyer Hall, 3000,
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10027, USA;
[2] Institute of Pharmaceutical
Biology,
University of Duesseldorf,
Universitaetsstr.1,
D-40225 Duesseldorf,
GERMANY.

ANTI-INFLAMMATORY ACTIVITY OF *ANGELICA* AND *HERACLEUM* SPECIES IN TERMS OF INHIBITORY EFFECT ON 5-LIPOOXYGENASE AND CYCLO-OXYGENASE

Duhuo has been used in traditional Chinese medicine as a remedy for arthritic disease. We have identified more than forty compounds, mostly coumarins from Radix *Angelicae pubescentis* (Duhuo in Chinese). Various substitutes from the genera *Angelica*, and *Heracleum* were analyzed for their constituents and inhibitory effects on cyclooxygenase (COX-1) and 5-lipoxygenase (5-LO). HPLC analysis showed that osthol, columbianedin, columbianetin acetate and angelol-type coumarins are the principal constituents of Radix *Angelicae pubescentis* while the constituents of the substitutes from *Angelica* and *Heracleum* species were found to be mainly furanocoumarins and falcarindiol. Linoleic acid confirmed by GC from all of the species turned out to be the most active constituent in the extract exerting COX-1 inhibition and also has a strong inhibitory activity on 5-LO. Osthol was another major inhibitor of 5-LO in Radix *Angelicae pubescentis*, while falcarindiol was mainly responsible for the 5-LO inhibitory effect in the substitutes.

WEDNESDAY, 11:45-1:00 **LUNCH**

SYMPOSIUM SESSION 6 ***PLANT DEFENSE AND SIGNALING PROCESSES***
Moderator: ***Stewart A. Brown***

WEDNESDAY, 1:00-1:50

CURRENT ASPECTS OF COUMARIN BIOSYNTHESIS AND APPLICATION

Symposium Paper 14

Ulrich Matern,
Institute of Pharmaceutical Biology,
Philipps-University,
Deutschhausstrasse 17 A,
D-35037 Marburg, GERMANY.

Coumarins accumulate constitutively in many plants, and several of these metabolites exhibit significant bioactivities *in vitro*, e.g. inhibition of cytochrome P450 monooxygenase and gyrase, benzodiazepine receptor affinity or suppression of HIV, as well as *in vivo*, i.e. against oedema. In some plants, coumarins and furanocoumarins accumulate also upon insect attach, fungal infection or jasmonate treatment, and their function as phytoalexins or oviposition stimulators has been proposed. The biosynthesis of furanocoumarins can be induced, furthermore, in plant cell cultures by the addition of fungal elicitors, and microsomal fractions of such induced cells. This enabled very recently the mechanistic investigation of the reactions converting umbelliferone to (+)-marmesin and psoralens. The results suggest the oxidative cleavage of a carbon-carbon single bond in (+)-marmesin to yield psoralen *via* β-cleavage and formation of a resonance-stabilized intermediate.

WEDNESDAY, 1:50-2:35

Oral Paper 27

PHYTOPHTHORA INFESTANS STIMULATED BIOSYNTHESIS OF HYDROXYCINNAMIC ACID AMIDES IN _SOLANUM TUBEROSUM_ AND CLONING OF A cDNA ENCODING THE INVOLVED TYRAMINE HYDROXYCINNAMOYLTRANSFERASE

Dieter Strack,
Institut für Pflanzenbiochemie (IPB), Weinberg 3,
D-06120 Halle (Saale),
GERMANY.

Treatment of suspension-cultured potato cells with an elicitor from *Phytophthora infestans* induced the biosynthesis of hydroxycinnamic acid amides and the activities of the involved enzymes. The pivotal enzyme was characterized as hydroxycinnamoyl-CoA:tyramine hydroxycinnamoyltransferase (THT; EC 2.3.1.110), catalyzing the formation of various hydroxycinnamic acid amides. Using degenerated primers derived from amino acid sequences of THT peptides, a THT-specific fragment was obtained by RT-PCR. A cDNA library was screened with the PCR fragment as a probe and several THT-specific cDNAs were isolated. Based on sequence analysis, one clone (pTHT3), covering the complete coding region, was used for expression of THT in *E. coli*. Protein extracts of bacteria containing pTHT3 showed high THT activity. Gel filtration revealed that the active enzyme was a dimer. Genomic Southern analyses indicated that in potato THT is encoded by a multigene family.

WEDNESDAY, 2:35-2:55

Oral Paper 28

UV-B IRRADIATION ACTIVATES A 48 KDA MBP KINASE AND ENHANCES THE WOUND RESPONSE IN TOMATO LEAVES

Johannes Stratmann and
Clarence A. Ryan,
Institute of Biological Chemistry,
Washington State University,
Pullman, WA 99164-6340,
USA.

Signals are generated in response to wounding, pathogen infection or UV-light that induce systemic wound response proteins (SWRPS) like proteinase inhibitors (PIs) which are involved in the plant defense against herbivorous insects and pathogens. These signals comprise systemin, an 18 aa phloem mobile polypeptide, the oligosaccharides chitosan and polygalacturonic acid and a rapid hydraulic signal generated by wounding. An early event after the perception of all these elicitors is the rapid systemic activation of a 48 kDa MBP kinase upstream of the octadecanoid pathway. Here we report that unlike all other above mentioned signals, UV-B (280 - 320 nm) irradiation of 2-3 week old tomato plants activates a 48 kDa MBP kinase without a concomitant induction of PI synthesis. However, when plants were first irradiated with UV-B light and subsequently wounded, the PI accumulation in the wounded and the systemic unwounded leaves increased 2 - 4 fold compared to wounding alone. Currently the biochemical mechanisms underlying this enhancing effect of UV-B irradiation on the wound response are being further investigated. *[Supported in part by grants from the College of Agriculture and Home Economics (CAHE), Washington State University, Pullman, and from the National Science Foundation].*

WEDNESDAY, 2:55-3:15 **BREAK**

WEDNESDAY, 3:15-3:35

Oral Paper 29

Hubert Gagnon and
Ragai Ibrahim,
Plant Biochem Lab, Concordia University, Montreal, Quebec, CANADA H3G 1M8.

ALDONIC ACIDS: A NOVEL FAMILY OF NOD-GENE INDUCERS IN THREE *RHIZOBIUM* spp.

Molecular signals, such as the flavonoid (or non-flavonoid) nod gene inducers and the bacterial lipochitin oligosaccharides (LCOs) are known to act as modulators of species-specificity in the early stages of Rhizobium-legume interactions. We found that in white lupin (*Lupinus albus*) the naturally occurring 4-C sugar acids (erythronic or tetronic acids) act as nod gene inducers of *R. lupini*, and that lupiwighteone (a monoprenyl isoflavone) exerts a synergistic effect with the carbohydrate-like inducers, as measured by the increased □-galactosidase activity of *R. lupini* strains harboring nodC::lacZ fusions, and by radiolabel incorporation into LCOs. Similar experiments confirmed the role of tetronic acid as a nod gene inducer of both *R. loti* and *R. meliloti*. Luteolin, the known inducer of *R. meliloti* also acts in synergism with tetronic acid. These results are discussed in relation to the impact of these unusual signal molecules on our knowledge of flavonoid signaling in Rhizobium-legume symbiosis.

WEDNESDAY, 3:35-3:55

Oral Paper 30

Javier Narváez-Vásquez and
Clarence A. Ryan,
Institute of Biological Chemistry,
Washington State University,
Pullman, WA 99164-6340,
USA.

ACTIVATION OF PHOSPHOLIPASE A BY WOUNDING, SYSTEMIN AND OLIGOSACCHARIDE ELICITORS IN EXCISED TOMATO PLANTS

A lipid-mediated signaling cascade regulates the response of plants to wounding by herbivory. Here we report that wounding causes a rapid increase in the activity of a phospholipase A (PLA) in leaves, as measured by the accumulation of [^{14}C]-Choline-labeled lysophosphatidyl choline (LPC). The radioactivity in the LPC pool peaked at about 15 min, then rapidly declined by 30 min, and increased again after 60 min. However, supplying young excised tomato plants with systemin, an 18-amino acid polypeptide elicitor of the systemic wound response, induced a PLA activity that persisted over a long period of time (over 2 h). The inactive systemin analog, Ala17-Systemin, and jasmonic acid did not cause the accumulation of radiolabel LPC, but LPC increased when the oligosaccharide elicitors polygalacturonic acid and chitosan were supplied to the excised plants. These data corroborate our working model in which the release of linolenic acid from plant membranes by PLA is one of the early steps in the activation of defense genes in plants in response to herbivory. *(Supported in part by the College of Agriculture and Home Economics, Washington State University and a grant from the National Science Foundation).*

WEDNESDAY, 3:55-4:15

Oral Paper 31

Bong-Seok Ku and
Soo-Un Kim,
Division of Applied Biology and Chemistry and The Research Center for New BioMaterials in Agriculture,
Seoul National University,
Suwon 441-744, KOREA.

DIFFERENTIAL ACTIVATION OF DAHP SYNTHASE AND ISOCHORISMATE SYNTHASE ON RESPONSE TO CHITOSAN ELICITATION IN SUSPENSION CELL CULTURE OF MADDER, *RUBIA AKANE*

Elicitation by chitosan in madder cell culture was studied in terms of enzyme activity and signal transduction. Chitosan added at 25 mg/mL to the culture stimulated higher accumulation of the pigments. On treatment with the elicitor, Mn-dependent deoxyarabinoheptulose phosphate synthase was activated while Co-dependent form was suppressed. Isochorismate synthase exhibited higher activity compared to the control throughout the culture period. We tried to correlate the enhanced pigment accumulation with signal transduction pathway in the cell. Treatment of chitosan increased hydrogen peroxide content of the cell suggesting an initial event on chitosan treatment was production of hydrogen peroxide. Level of jasmonic acid was found enhanced until 20 days after the treatment. To confirm the role of jasmonate in the signal transduction, LOX inhibitor salicylhydroxamic acid and chitosan were added to the culture to result in the lowered production of pigment as well as jasmonate. Staurosporin, a protein kinase inhibitor, lowered the production of jasmonic acid and the pigments, whereas okadaic acid, an inhibitor of phosphatase increased the level of jasmonic acid and the pigments. Therefore, oxygen burst, jasmonate biosynthesis and protein phosphorylation were involved in the elicitation process in the madder cell. (Supported by RCNBMA)

WEDNESDAY, 4:15-4:35

Oral Paper 32

Daniel S. de Moura,
Daniel R. Bergey and
Clarence A. Ryan,
Institute of Biological Chemistry,
Washington State University,
Pullman, WA 99164-6340,
USA.

CLONING, CHARACTERIZATION AND IMMUNOCYTO-CHEMICAL LOCALIZATION OF A WOUND-INDUCIBLE TYPE-I SERINE CARBOXYPEPTIDASE FROM TOMATO PLANTS

In solanaceous plants, mechanical wounding and/or herbivory release the polypeptide hormone systemin which activates a lipid-based signaling cascade leading to the systemic synthesis of a group of proteins called Systemic Wound Response Proteins, or SWRPs. A 25kD SWRP, which we purified and partially sequenced, revealed significant similarity to the β subunit of a type-I serine carboxypeptidase (serCPD). Oligonucleotide primers were designed to amplify a specific cDNA fragment by PCR. The PCR product was used as a probe to screen a wound-induced tomato leaf cDNA library. A 1.8 kb cDNA clone was sequenced and, like the cereal type-I serCPDs, the deduced polypeptide consists of both α and β subunits that flank a putative internal propeptide. Tomato serCPD mRNA levels increase both locally and systemically in young plants within 2-3 hours after wounding, systemin or methyl jasmonate treatment. Immunoblot analyses showed that serCPD protein levels increase within 4-6 hours. Immunocytochemical localization reveal that serCPD is localized in the vacuole, in the same proteinaceous aggregates as inhibitors I and II were previously localized. The role of tomato type-I serCPD in wound response signalling is not known, but may include either the nonspecific turnover of cellular proteins, or the specific processing and release of active systemin from its precursor, prosystemin. [Supported in part by grants from the Brazilian government-CNPq (DSM), the National Institute of Health (DRB), and the National Science Foundation (CAR)].

WEDNESDAY, 4:35-4:55

Oral Paper 33

Nobuhiro Hirai[1,2], T. Kamo[1,2],
N. Kato[1], M. Tsuda[1],
D. Fujioka[3] and H. Ohigashi[1],
[1]Graduate School of Agriculture,
Kyoto University,
Kyoto 606-8502, JAPAN;
[2]CREST, Japan Science and Technology Corporation;
[3]Osaka Co-operative Wholesale Society.

PHENYLPHENALENONE-TYPE PHYTOALEXINS FROM BANANA FRUITS

Unripe banana fruits show resistance to growth of fungal hyphae, and the pathogen is quiescent until fruits ripen, suggesting that unripe fruits produce phytoalexin. We isolated seventeen phenylphenalenone-type phytoalexins, including five new compounds from the peel of unripe banana (*Musa acuminata* [AAA] cv. Buñgulan) fruit which had been injured and then inoculated with conidia of *Colletotrichum musae*. These phenylphenalenones can be classified into four types: 4-phenyl-phenalenone, 9-phenylphenalenone, 2-phenyl-1,8-naphthalenedicarboxylic acid, and an intermediate type. An antifungal test on the phytoalexins showed that a phenolic hydroxyl group was essential for the activity. A ripening treatment decreased contents of the phenylphenalenones, suggesting that the resistance to fungal growth is caused by the phenylphenalenones.

WEDNESDAY, 4:55-5:15

Oral Paper 34

Keith D. Miller and
Loverine P. Taylor,
Plant Biochemistry Research and Training Center and Department of Genetics and Cell Biology,
Washington State University,
Pullman, WA 99164-6340,
USA.

PURIFICATION, CLONING, AND HETEROLOGOUS EXPRESSION OF A FLAVONOL-3-*O*-GALACTOSYLTRANSFERASE FROM *PETUNIA HYBRIDA* POLLEN

In petunia and maize pollen, flavonols are required for pollen germination. Exogenously added flavonol aglycones can biochemically complement transgenic plants lacking flavonoids. In wild type pollen, only flavonol glycosides are detected. It is unknown if flavonol aglycones or the glycosides induce pollen germination. As a first step in identifying the active compound, a gametophytically expressed flavonol-3-*O*-galactosyltransferase (F3GalTase) was isolated from immature petunia pollen, purified, and partially sequenced. Degenerate oligonucleotide primers were designed and a RACE PCR protocol was used to obtain an 800 bp 5'RACE product which was used to screen a pollen cDNA library. A 1.43 kbp cDNA was obtained with a single 1.35 kbp ORF which encodes a 451 amino acid polypeptide of 49 kDa. The translated cDNA contains a putative signal sequence comprising the first 30 amino acids which after cleavage would result in a polypeptide of 46 kDa, similar in size to the native protein subunit. Heterologous expression of the cDNA in *E. coli* resulted in the production of an active F3GalTase which used UDP-galactose and kaempferol or quercetin to form the respective flavonol galactosides, proving the identity of the isolated cDNA.

WEDNESDAY, 5:15-6:45 **PSNA GENERAL ANNUAL MEETING - TODD HALL ROOM 130**

WEDNESDAY, 6:45-8:00 **DINNER - COMPTON UNION BUILDING - BALL ROOM**

SYMPOSIUM SESSION 7 — *POST-DOCTORAL AND STUDENT RESEARCH - BEST POSTER COMPETITION*

Moderator: *Jonathan E. Poulton*

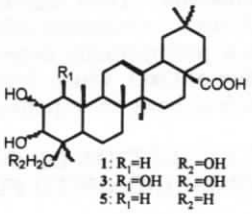
WEDNESDAY, 8:00-10:00 — **COMPTON UNION BUILDING - BALL ROOM**

Poster BPC 1

El Hassane Lahlou[1],
Nobuhiro Hirai[1,2] and
Hajime Ohigashi[2],
[1]CREST, Japan Sci. Tech. Corp.;
[2]Graduate School of Agric.,
Kyoto Univ.,
Kyoto 606-8502, JAPAN.

TRITERPENOID TYPE PHYTOALEXINS FROM NECTARINE FRUITS

Seven triterpenoids (**1-7**), were induced and isolated from Nectarine(*Prunus persica* cv. Fantasia) immature fruits inoculated with *Colletotrichum musae,* of which two are new (**3**,**4**). The structure of substances were established by chemical and spectroscopic means. Compounds **1**,**2**,**5**,**6** and **7** were previously reported as constituve natural products from other plants, but were never described as phytoalexins. All these compounds showed antifungal activity against the fungi mentioned above.



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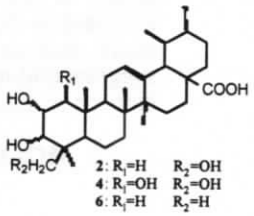
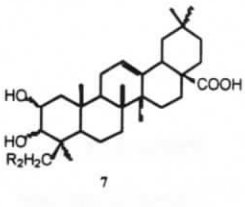
3: R_1=OH R_2=OH
5: R_1=H R_2=H



2: R_1=H R_2=OH
4: R_1=OH R_2=OH
6: R_1=H R_2=H



7

Poster BPC 2

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DEHYDRODICONIFERYL ALCOHOL METABOLISM IN *PINUS TAEDA*: GENE CLONING, ENZYME PURIFICATION AND PROPERTIES

Dehydrodiconiferyl alcohol (DHDCA) is a 8,5′-linked lignan that is ubiquitous in the plant kingdom. However, little is known about its biosynthesis and subsequent metabolism. Loblolly pine (*Pinus taeda*), has been shown to contain both DHDCA and its 7′,8′-(allylic) reduced derivative, dihydrodehydrodiconiferyl alcohol. In the search in diverse plants for enzymes that catalyze the formation and subsequent reduction of a related lignan, the 8,8′-linked pinoresinol, a cDNA library was constructed from suspension culture cells of *P. taeda* and a gene for a reductase homologous to pinoresinol-lariciresinol reductase (PLR) was isolated. This, however, when expressed in *E. coli*, was unable to reduce either pinoresinol or lariciresinol. When assayed for an ability to reduce DHDCA, we found that this new recombinant enzyme is able to reduce the benzylic ether of DHDCA, quite analogously to the reaction catalyzed by PLR. This finding further demonstrates the evolution and conservation of the lignan pathway throughout the plant kingdom and supports the contention that phenolic coupling and subsequent metabolism is under strict biochemical control.

Poster BPC 3

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CHARACTERIZATION OF RADISH SEEDLING THIOGLYCOLIC LIGNIN BY FT-IR SPECTROSCOPY AND AN IMPROVED METHOD FOR ITS PREPARATION

Thioglycolic acid lignins (LTGAs) are generally considered virtually unaltered with regard to its native structure. The preparation of LTGAs is a quantitative technique that is often used to measure lignin in small samples of herbaceous tissues. FT-IR spectra of LTGA from 7-d-old radish seedlings first homogenized in 50% aqueous MeOH showed major peaks due to protein contamination (~1630, ~1530 cm-1, amide I and II bands) interfering with lignin peaks (~1595, ~1510, ~1420, aromatic skeletal vibration). However, when samples were first homogenized in KPO_4 buffer to remove soluble proteins, washed with 1M NaCl to remove ionically bound proteins, and incubated with cellulase to digest nonlignified cells, FT-IR spectra were comparable to those of Kraft hardwood LTGA and with published hardwood lignin FT-IR spectra. Unless steps are taken to remove protein from such herbaceous samples, lignin can be overestimated by as much as 8 fold.

Poster BPC 4

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EXPRESSION OF OXALATE OXIDASE IN FUNGUS-INFECTED BEAN TISSUES

Many economically important plant pathogenic fungi secrete oxalic acid as part of the tissue invasion process. The relationship between oxalic acid secretion by the phytopathogenic fungus, *Sclerotinia sclerotiorum,* and production of oxalate oxidase (OxO) by *Phaseolus* species infected with the fungus was investigated. Oxalate oxidases, which catalyze the degradation of oxalic acid to CO_2 and H_2O_2, have been well-characterized in monocots; however, although genes encoding OxO-like proteins have been identified in dicots, it is not known if their products have OxO activity. This study demonstrates that OxO protein and associated activity accumulate in dicot tissues in response to fungal infection. SEM observations of calcium oxalate surrounding infection hyphae and microautoradiographic studies of infected leaves previously fed with $^{45}Ca^{2+}$-Cl_2, showed that fungal oxalic acid chelates calcium from host tissues. Activity assays and Western immunoblots demonstrated that OxO is expressed in bean tissues in response to infection. TEM immunocytochemistry showed that OxO was localized to the host cell wall surrounding infection hyphae. This study confirms that oxalic acid secretion is a pathogenicity determinant in some pathogenic fungi, and suggests that up-regulation of OxO, which degrades oxalic acid, is one line of defense induced in dicot hosts such as bean, in response to pathogen attack.

Poster BPC 5

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MOLECULAR EVOLUTION OF *O*-METHYLTRANSFERASES: FROM LIGNIN TO FLORAL SCENT

O-methyltransferases play important roles in plant secondary metabolism. Two *O*-methyltransferases—IEMT and COMT—have been isolated from *Clarkia breweri*, an annual California plant. IEMT catalyzed the formation of two floral scent compounds, methyleugenol and methylisoeugenol, from eugenol and isoeugenol, respectively. COMT methylates caffeic acid and 5-hydroxyferulic acid to produce ferulic and sinapic acids, respectively. IEMT and COMT from *C. breweri* are 83% identical in the protein level. In *in vitro* mutagenesis experiments, we showed that an IEMT mutant with as few as 5 closely spaced residues replaced with the corresponding residues of COMT has almost exclusive COMT activity, and this activity can be enhanced by the additional mutation of 2 neighboring residues. This result indicates that new methyltransferases could evolve by mutation at relatively small number of positions in the polypeptide chain.

Poster BPC 6

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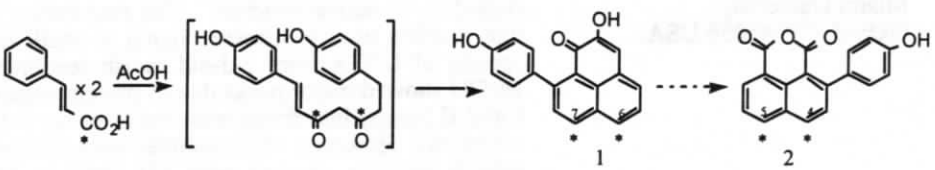
A BIOSYNTHETIC PRECURSOR OF PHENYLPHENALENONES IN BANANA FRUITS

Unripe fruits of banana (*Musa acuminata*) produce phenylphenalenone-type phytoalexins against infection by *Colletotrichum musae*. Two molecules of phenylpropanoid are supposed to be intermediates of phenylphenalenone in Haemodoraceae family. We investigated the biosynthetic precursor of phenylphenalenones in banana fruits. Typical phytoalexins in banana fruits, hydroxyanigorufone (**1**) and 2-(4'-hydroxyphenyl)-1,8-naphthalic anhydride (**2**), were isolated from the peels of fruits supplied with [1-^{13}C]cinnamic acid. The ^{13}C NMR spectra showed that the signals of C-6 and C-7 of **1**, and those of C-4 and C-5 of **2** were enhanced by 56-62%, indicating that two molecules of cinnamic acid are the precursor of phenylphenalenone also in *Musa*.



Poster BPC 7

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PHYTOCHEMICAL INVESTIGATION OF *PIPER LHOTZKYANUM* KUNTH.

Piper lhotzkyanum Kunth belongs to the family Piperaceae and is commonly found in humid forests with low incidence of light in Southeast Brazil. Recently, many chromenes and benzoic acid derivatives have been isolated and identified in Piperaceae species. These kinds of compounds have been to have shown interesting insecticidal properties. Leaves of *P. lhotzkyanum* were collected near Teresópolis and submitted to successive extraction with hexane and methanol. The methanol extract was partitioned with hexane, dichloromethane, ethyl acetate and butanol. The hexane extract from leaves of *P. lhotzkyanum* was purified by chromatographic methods resulting in isolation of a new chromene: lhotzchromene; two known prenylated benzoic acid derivatives isomers, previously isolated from leaves of *P. murrayanum*: (E) and (Z) of 4-hydroxy-3-(3',7'-dimethyl-1'-oxo)-2',6'-octadienylbenzoic acid, and a mixture of hydroxylated sesquiterpenes: spathulenol, guaiol, *epi*-γ-eudesmol, hinesol, β-eudesmol, α-eudesmol along with the acyclic diterpene phytol. The dichloromethane partition of the methanol extract was fractionated in silica gel column to afford a *C*-glucosyl flavone and a dihydrocinnamic acid derivative. The isolated substances were identified using spectroscopic analysis. The mixture was analysed by GC/MS and the substances were identified by comparison of the mass spectra and retention indices (RI) with literature records.

Poster BPC 8

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STUDIES ON THE MODE OF ACTION OF THIARUBRINE A, AN ANTIBIOTIC POLYINE FROM THE ASTERACEAE

Thiarubrine A, a dithiacyclohexadiene polyine isolated from the roots of *Ambrosia chamissonis* (Asteraceae) exhibits strong phototoxicity as well as light-independent activity against microorganisms. Little is known about how thiarubrines exert their toxic effects. In this study the light–independent effect of thiarubrine A on the respiration and morphology of yeast cells has been investigated. Cellular respiration of *Saccahromyces cerevisiae* was found to be inhibited almost immediately after the addition of the dithiine. Transmission electron microscopy revealed dramatically deformed mitochondria in treated yeast cells. Thus it is likely that the mitochondria are a target for the thiarubrines. Further investigations showing interactions between biologically important thiols and thiarubrine A indicate that other targets may also be involved.

Poster BPC 9

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[2]Institute of Biology and Chemistry of Proteins (UPR CNRS 412), 7 Passage du Vercors, 69367 Lyon Cedex 07, FRANCE.

SYNTHESES OF IRREVERSIBLE FLAVONOID INHIBITORS OF P-GLYCOPROTEIN

P-glycoprotein (Pgp), which is often involved in multidrug resistance belongs to the superfamilly of ABC (ATP binding cassette) transporters. Little is known about the structure and the mechanism of these transporters. In order to get information on the structure of the binding sites and identify the amino acids involved in the interaction of Pgp with inhibitors, the preparation of labelled irreversible inhibitors is necessary. The strategy is based on the formation of covalent bonds between the labelled inhibitor and the protein, followed by proteolysis and identification of labelled peptide fragments. Thus we decided to undertake the synthesis of a collection of azidoflavonoids, where the azido group is introduced at positions of the flavonoid ring that are supposed to play a role in binding. The synthetic scheme first involves the obtainment of nitroflavones which are reduced to aminoflavones and finally converted to corresponding azido derivatives. The preparation of a number of such derivatives of flavones, chalcones and β-hydroxychalcones, as well as the characterization of their binding to Pgp will be presented.

Poster BPC 10

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Jonathan Gershenzon and
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USA.

DEVELOPMENTAL REGULATION OF MONOTERPENE BIOSYNTHESIS IN THE GLANDULAR TRICHOMES OF PEPPERMINT (*MENTHA X PIPERITA* L.)

Monoterpenes are the major components of the essential oil from peppermint (*Mentha x piperita* L.). The monoterpenes arise from non-mevalonate isopentenyl diphosphate via the acyclic, ten-carbon intermediate, geranyl diphosphate. (-)-Limonene synthase, an enzyme that has been purified and cloned, effects cyclization of geranyl diphosphate to (-)-limonene. Limonene synthase was used as a probe to investigate the temporal regulation of monoterpene biosynthesis in peppermint. Changes in limonene synthase activity over this period closely paralleled the rate of monoterpene biosynthesis. The profiles of total limonene synthase protein (detected on immunoblots) and steady-state limonene synthase mRNA corresponded closely to changes in limonene synthase activity suggesting control at the level of gene expression. The major monoterpene products in peppermint, menthol and menthone, are synthesized from limonene by a series of conversions which include hydroxylation, redox reactions involving both double bonds and the oxygenated carbon, and a double bond migration. *In vitro* enzyme activities for these conversions are uniformly high during the first few weeks of leaf development, but then decline to very low levels. Steady state mRNA levels (detected by northern analysis) for monoterpene biosynthetic enzymes (1-deoxyxylulose 5-phosphate synthase, geranyl diphosphate synthase, limonene-3-hydroxylase, NADPH-cytochrome P450 reductase) follow a similar pattern to that of limonene synthase. Monoterpene biosynthetic enzymes in peppermint are developmentally regulated most likely at the level of gene expression.

Poster BPC 11

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FUNCTIONAL ARCHITECTURE OF TYROSINE/DOPA DECARBOXYLASE AND BERBERINE BRIDGE ENZYME GENE PROMOTERS FROM OPIUM POPPY

Tyrosine/dopa decarboxylase (TYDC) and the berberine bridge enzyme (BBE) represent the entry point and a key branch point, respectively, in the biosynthesis of the antifungal benzylisoquinoline alkaloid sanguinarine in select species of the Papaveraceae and Fumareaceae. TYDC and BBE mRNAs accumulate in cultured opium poppy cells after wounding or elicitor treatment. Deletion analysis of the promoters from the *tydc6*, *tydc7*, and *bbe1* genes was used to identify regulatory domains necessary for expression of the β-glucuronidase (GUS) reporter gene in a transient expression system based on microprojectile bombardment of opium poppy cell cultures. Functionally identified regulatory domains were further analyzed by electrophoretic mobility shift assay to characterize the transcription factors that bind to each promoter element. Both *tydc* and *bbe* genes are induced by a signaling pathway that likely involves jasmonic acid (JA) as a second messenger. Inhibitors of JA biosynthesis block the induction of *tydc* and *bbe* genes, and the accumulation of sanguinarine. Other experiments suggest a role for Ca^{2+} in the induction of sanguinarine biosynthesis.

Poster BPC 12

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[2]Sector de Resistência aos Antibióticos, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon, PORTUGAL.

GUINEA-BISSAU'S PLANTS ACTIVE AGAINST *NEISSERIA GONORRHOEAE*

In sequence of our studies on plants used by Fulani traditional healers against venereal diseases (in Guinea-Bissau) and previously tested against *Neisseria gonorrhoeae* strains (with different susceptibilities to penicillin and tetracycline) extracts showing promising results were studied.
In this communication we report:

- the chemical characterization by LC-UV and TLC of three of the most active extracts (*Guiera senegalensis* leaves and *Terminalia macroptera* roots and leaves);
- the identification of major compounds (ellagitannins and flavonoids) of *T. macroptera* leaves ethanol extract; and
- the bioguided fractionation study of *Guiera senegalensis* extract, and chemical profile of the active fractions.

Poster BPC 13

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EFFECT OF FEEDING SALICYLIC ACID ON THE SECONDARY METABOLISM IN CULTURED CELLS OF *CATHARANTHUS ROSEUS*

Cell suspension cultures of *Catharanthus roseus* produced 2,3-dihydroxybenzoic acid (2,3-DHBA) after elicitation with a fungal cell wall preparation of *Phytium aphanidermatum*, and showed concomitantly an induction of isochorismate synthase (ICS, EC 5.4.99.6). To study whether salicylic acid (SA) is a precursor of 2,3-DHBA in *Catharanthus roseus*, SA was fed to the cell cultures of this plant. Over the first 24 h, the uptake and metabolism of SA were rapid, with SA glycosides and methyl salicylate as metabolites in the cells, but 2,3-DHBA was not detected. After 72 hours, induction of cell death was observed. Moreover, the feeding of SA to the cells resulted in induction of anthranilate synthase (AS, EC 4.1.3.27), but the level of ICS remained unchanged. The accumulation of 2,3-DHBA and the induction of ICS in the cell cultures in the presence of SA together with the fungal elicitor were similar to those in the cells elicited only by *P. aphanidermatum*. SA which could be an intermediate in the biosynthesis of 2,3-DHBA in plants, was not found to be directly involved as a precursor of 2,3-DHBA.

Poster BPC 14

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PHENOLIC CONSTITUENTS FROM THE FRUITS OF *PHYLLANTHUS EMBLICA* AND ITS APPLICATION IN CHINA

Phyllanthus emblica L. (Euphorbiaceae) is a small shrub widely distributed from South Asia *via* Southeast Asia to tropical and subtropical areas of China. It has been used not only as Ayurvedic medicine by Indian, but also as traditional Chinese herbal medicine by Han people as well as ethnic-medicine by Zang, Meng, Dai and other minorities in China, for the treatment of inflammation and pyreticosis for a long time. Many studies lead to a further understanding about this plant, such as its function as tonic, anti-hepatitis, anti-tumor, anti-aging, anti-radiation and anti-mutation. In China, there have been several medicines, health foods and beverage developed from this plant. From its fruits, four phenolic constituents, corilagin, putrajivain A, 1,6-O-di-galloyl-β-D-glucose and chebulagic acid were isolated in our laboratory. Among them, the yield of putrajivain A was more than 0.43%, which has been reported as a potent inhibitory substance for HIV reverse transcriptase.

Poster BPC 15

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DETERMINATION OF AN ELM HYBRID BY COMPARING ITS FLAVONOID STRUCTURES WITH THE PARENTS'

Flavonoid structure can be used to determine hybridization of plants. I am looking at *Ulmus pumila* (Siberian elm) and *Ulmus rubra* (red elm) as parents and a suspected hybrid called *Ulmus X ytnotha*. Their should be either new flavonoids or a different combination of them in the hybrid species. Documenting hybrids is a necessary practice when trying to define evolutionary and successional patterns. This field is called plant chemotaxonomy. Flavonoids are commonly used for this purpose because of their chemical stability and ubiquity in the plant kingdom. When isolating the substances of interest, flavonoids in this case, one needs a basic understanding of their chemistry. I am using previously established qualitative and quantitative phytochemical methods. After extraction of the flavonoids from the plant material with a Soxhlet apparatus, isolation can be carried out in various ways. The methods I am using include a combination of NMR (Nuclear Magnetic Resonance) and UV Vis (Ultra Violet - Visible) spectroscopy, paper, thin layer, column, and high pressure liquid chromatography.

SYMPOSIUM SESSION 8 ***PLANT DEFENSES***
Moderator: ***Eric E. Conn***

THURSDAY, 7:45-8:30

PLANT ECOCHEMICALS FROM THE VIEWPOINT OF PLANT DEFENSE

Symposium Paper 15

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Green plants produce a variety of so-called secondary metabolites which may play important roles in complex interactions among living organisms, such as plant-plant, plant-microorganism and plant-insect in the natural environment. The following topics will be discussed from the viewpoints of phytochemistry and plant defense. (1) Sesquiterpene lactones and their derivatives from *Chloranthus* spp. (Chloranthaceae), (2) oligostilbenes from *Carex* spp. (Cyperaceae), (3) sesquiterpenes from *Rosa rugosa* (Rosaceae), (4) inducibly produced antimicrobial compounds and their formation mechanisms in wild and crop plants (*Taraxacum officinale, Iris pseudocorus, Glycine max* and *Capsicum annuum*), and (5) some antifeeding compounds from Nigerian and Japanese plants (*Aristolochia albida, Skimmia japonica, etc.*).

THURSDAY, 8:30-9:15

PHYTOCHEMICALS AND PLANT DEFENSE AGAINST MICROBES

Symposium Paper 16

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Phytochemicals are involved in protecting higher plants against pathogenic fungi and bacteria either as static or dynamic barriers. Some toxins are present at the surface of plant tissues, while others may occur in bound form within the same tissues. Yet others may be elicited *de novo* during the infection process in the well known phytoalexin response. More than one biosynthetic pathway may be so elicited. A range of terpenoid, phenolic, nitrogen and sulfur based structures can be formed. There are also structural and proteinaceous barriers to plant infection. Hence, there are difficulties in determining the relative contributions of phytochemicals to defense in any given plant-microbial interaction. The role of phytochemicals in contributing to disease resistance will be assessed in studies of plants in the Gramineae, Leguminosae and Rosaceae.

SYMPOSIUM SESSION 9 ***POSTDOCTORAL AND GRADUATE STUDENT ORAL PRESENTATIONS-BEST PAPER COMPETITION***
Moderator: ***Eric E. Conn***

THURSDAY, 9:15-9:35

DIRIGENT PROTEIN AND PINORESINOL/LARICIRESINOL REDUCTASE. TISSUE SPECIFIC EXPRESSION OF GENES AND SUBCELLULAR LOCALIZATION OF ENZYMES: A COMPARISON WITH LIGNIFICATION

Oral BPC 1

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Institute of Biological Chemistry,
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Pullman, WA 99164-6340,
USA.

The dirigent protein controlling the outcome of stereoselective coupling of *E*-coniferyl alcohol gives pinoresinol (as its (+)- or (–)-antipode, according to the species),[1] which can then be further processed with the corresponding enantiospecific (+)- or (–)-pinoresinol/lariciresinol reductase(s).[2] The genes for each of these proteins/enzymes have been cloned, and the recombinant proteins expressed in functional form. This provided the opportunity to clearly establish whether monolignol coupling and subsequent metabolic events involved in lignin and lignan formation differed biochemically, temporally and spatially. Accordingly, a combination of tissue printing, *in situ* hybridization and immunolabeling studies were carried out in order to define the tissue specific expression of these genes as well as the subcellular localization of the corresponding proteins/enzymes. These results were compared with those obtained with immunoprobes directed against different synthetic lignin polymers,[3] concerning the specific ultrastructural localization of different types of lignins.

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2. Gang, D.R.; Fujita, M.; Davin, L.B.; Lewis, N.G. The "Abnormal Lignins": Mapping Heartwood Formation through the Lignan Biosynthetic Pathway. *In*: Lewis, N.G.; Sarkanen, S. (Ed.) *Lignin and Lignan Biosynthesis*; ACS Symp. Series, Washington, DC, 697 : 389-421, 1998.
3. Burlat, V., Ambert, K.; Ruel, K.; Joseleau, J.-P. Relationship between the Nature of Lignin and the Morphology of Degradation Performed by White-rot Fungi, *Plant Physiol. Biochem.*, 35(8) : 645-654, 1997.

THURSDAY, 9:35-9:55

Oral BPC 2

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REGULATION OF DESACETOXYVINDOLINE 4-HYDROXYLASE THE SECOND TO LAST ENZYME IN VINDOLINE BIOSYNTHESIS

The expression of desacetoxyvindoline 4-hydroxylase (D4H) which catalyzes the second to last reaction in vindoline biosynthesis in *Catharanthus roseus* appeared to be under complex multilevel developmental and light regulation. Developmental studies with etiolated- and light-treated seedlings suggested that while light had little effect on the levels of *d4h* transcripts, those of D4H protein and enzyme activity could be increased, depending on seedling development, up to 9- and 8-fold, respectively, compared to etiolated seedlings. However, light treatment could stop and reverse the decline of *d4h* transcripts at later stages of development. Repeated exposure to light also was required to maintain the levels of enzyme activity observed throughout development. Further studies showed that phytochrome appeared to be involved in the activation of D4H. Additional studies also confirmed that different major isoforms of D4H protein exist in etiolated- (pI 4.7) and light-grown (pI 4.6) seedlings, suggesting that a component of the light-mediated activation of D4H may involve an undetermined post-translational modification. The biological reasons for this complex control of vindoline biosynthesis may be related to the need to produce structures which could sequester away from cellular activities the cytotoxic vinblastine and vincristine dimers which are derived partially from vindoline.

THURSDAY, 9:55-10:15

DOES THIS PLANT HAVE A NOVEL POLYKETIDE SYNTHASE?

Oral BPC 3

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Polyketide synthases form a diverse family of condensing enzymes that catalyze the initial reaction in the biosynthesis of biologically and pharmaceutically significant natural products. In plants, chalcones and stilbenes are the best investigated examples of this group. In raspberry, the major aroma compound of the fruit, *p*-hydroxy-phenylbutan-2-one (pHPB) is a polyketide derivative and the enzyme (pHPB-3-ene-2-one synthase) catalyzing the initial reaction of this pathway may be a novel polyketide synthase (1).
Sequence alignment of chalcone, stilbene, and acridone synthases reveal highly conserved regions that may be the basis for the similar catalytic properties shown by these different enzymes. To capture the array of polyketide synthase genes, we have used the conserved regions to design degenerate PCR primers. In the present study, we have identified four closely-related members of this gene family in *Rubus idaeus*.

1. Borejsza, W. and Hrazdina, G.1996. Aromatic Polyketide Synthases. Plant Physiology 110: 791-799.

THURSDAY, 10:15-10:30 **BREAK**

THURSDAY, 10:30-10:50

SYSTEMIC WOUND-INDUCIBLE EXPRESSION OF A NOVEL POLYGALACTURONASE GENE IN TOMATO LEAVES

Oral BPC 4

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Daniel R. Bergey and
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Pullman, WA 99164-6340,
USA.

In tomato plants, subfamilies of the polygalacturonase (PG) catalytic subunits have been found only in fruit and abscission zones. We have cloned and sequenced cDNAs encoding a novel subfamily of the catalytic subunit (tomLPG-1) from tomato leaves. A regulatory β-subunit cDNA was also cloned from tomato leaves that is identical to the fruit β-subunit. We are investigating wound-inducible expression of both tomLPG-1 and the regulatory subunit using Northern, immunoblot and activity assays. Northern analyses show that mRNA levels of the catalytic and regulatory subunit increase between 1-3 hours both locally and systemically after wounding, and continue to increase up to 12 hours after treatment. In immunoblots, antisera raised against a tomato fruit catalytic subunit cross-reacted with leaf protein extracted from plants overexpressing prosystemin. PG activity increased 2-3 fold in both wounded and unwounded (systemic) leaves of wild-type tomato plants. Maximal enzyme activity occurred between 4-5 hours and declined to background levels by 8 hours. The deduced amino acid sequence of tomLPG-1 shares 40.8% and 36.9% identity, respectively, with the PG's expressed in tomato abscission zones and fruit. Thus, wound-inducible tomLPG-1 represents a new family of PG's in tomato plants. *(Supported in part by NSF grant IBN9601099 Washington State University, CAHE, Project #1291)*

THURSDAY, 10:50-11:10

Oral BPC 5

David A. Bird and
Peter J. Facchini,
Department of Biological Sciences,
University of Calgary,
Calgary, Alberta, T2N 1N4,
CANADA.

TRACING THE SIGNAL: CHARACTERIZATION OF THE BBE SIGNAL PEPTIDE

The berberine bridge enzyme (BBE) represents an important branch point in the biosynthesis of sanguinarine since its substrate, (*S*)-reticuline, is also an intermediate in morphine biogenesis. BBE is targeted to a subcellular compartment that has not been well-characterized. A series of gene fusions encoding 10-, 15-, 25-, and 50-amino acids from the *N*-terminus of BBE fused to β-glucuronidase (GUS) were transcribed and translated *in vitro* in the presence of dog microsomes. Each gene fusion was also introduced into opium poppy cell cultures via particle bombardment. *In vitro* and *in vivo* studies have demonstrated that the *N*-terminal signal peptide consists of less than 25 residues and targets BBE to a microsomal cell fraction. Sucrose density gradient centrifugation has shown that a 50-amino acid *N*-terminal peptide targets GUS to both the endoplasmic reticulum and a subcellular fraction of higher density. The precise and final subcellular location of BBE might be determined by additional targeting information within the protein or mRNA. Important implications of the subcellular targeting and localization of BBE on the regulation of benzylisoquinoline alkaloid biosynthesis will be discussed.

THURSDAY, 11:10-11:30

Oral BPC 6

Xi Wang and
Leslie C. Plhak,
Louisiana Agricultural Experiment Station,
Louisiana State University,
Baton Rouge, LA 70803, USA.

IMMUNOCHEMICAL METHODS FOR THE STUDY OF GOSSYPOL

Analysis of gossypol in cottonseed products is a concern because of the existence of two enantiomeric forms of gossypol and the diversity of bound gossypol. Antibodies to gossypol are being developed as tools for studying "free" and "bound" forms of gossypol. The relatively small size of gossypol (MW 518), requires that it is bound to a protein carrier in order to be immunogenic in animals. Several methods have been investigated to obtain pure and characterized gossypol-protein conjugates including direct reaction or using bifunctional linking arms. In a feasibility study, rabbits were immunized using gossypol-LPH conjugates for the production of polyclonal antibodies. The sera obtained after each boost were screened for anti-gossypol antibodies using an antibody-capture non-competitive ELISA. Signal vs. background absorbances increased as the number of immunizations increased, indicating that anti-gossypol antibodies were produced. Monoclonal antibodies are also being developed for specific forms of gossypol.

THURSDAY, 11:30-11:50

Oral BPC 7

Aldwin M. Anterola,
Hendrik van Rensburg,
Laurence B. Davin and
Norman G. Lewis,
Institute of Biological Chemistry,
Washington State University,
Pullman, WA 99164-6340,
USA.

RATE-LIMITING PROCESSES IN MONOLIGNOL FORMATION IN *PINUS TAEDA*

Cell suspension cultures of *Pinus taeda* were induced to form monolignols, *p*-coumaryl and coniferyl alcohols, by placing them in 8% sucrose containing 20mM KI. The cells were exogenously supplied with saturating amounts of monolignol precursors and the effect on both cellular and extracellular phenylpropanoid metabolites was observed, in order to determine possible rate-limiting steps of the phenylpropanoid pathway.

It was observed that only the administration of *p*-coumaryl and coniferyl aldehydes were able to stoichiometrically increase the amount of *p*-coumaryl and coniferyl alcohols in the medium, exhibiting immediate conversion of substrate to the product. Exogenously supplied Phe slightly increased monolignol formation, while none of the phenylpropanoic acids that were administered (i.e., cinnamic, *p*-coumaric, caffeic and ferulic acids) increased the amount of monolignols beyond basal levels. Analysis of the cellular extracts revealed accumulation of cinnamic and *p*-coumaric acids, when cells were exogenously supplied with Phe or cinnamic acid, respectively. On the other hand, the amounts of caffeic and ferulic acids remained at basal levels when *p*-coumaric or caffeic acids were added, respectively.

Thus, the two hydroxylation reactions (cinnamic to *p*-coumaric acid and *p*-coumaric to caffeic acid) and CoA ligation are the major "rate-limiting" steps of the phenylpropanoid pathway. The reduction of *p*-coumaryl or coniferyl aldehyde is not "rate-limiting," as has often been proposed.

THURSDAY, 11:50-12:10

Oral BPC 8

Pierre Laflamme,
Benoit St-Pierre and
Vincenzo De Luca,
Institut de Recherche en
Biologie Végétale,
Université de Montréal,
4101 rue Sherbrooke est,
Montréal, Québec, CANADA
H1X 2B2.

MOLECULAR AND BIOCHEMICAL ANALYSIS OF A 'HOMOLOG' GENE TO ACETYL COENZYME A:DEACTEYLVINDOLINE-*O*-ACETYLTRANSFERASE (DAT) FROM *CATHARANTHUS ROSEUS* (L.) G. DON

During isolation of the DAT gene from *Catharanthus roseus*, a homolog gene was also observed. Sequence analysis of this 'homolog' gene revealed a *ca.* 63% homology with that of DAT. This genomic clone contained a 1.32 kb open reading frame for a *ca.* 50 kDa protein. The deduced amino acid sequence of 443 amino acids displayed 78% homology with that of DAT. *Escherichia coli* transformed with the pQE30, HIS-tagged expression vector harbouring the ORF expressed *O*-acetyltransferase activity with different substrate specificity than DAT. Present biochemical and molecular studies of this DAT 'homolog' are focusing on its biological role and its pattern of regulation and distribution throughout the mature plant and young seedlings with respect to DAT.

THURSDAY, 12:10-12:30

Oral BPC 9

Anil T. Mangla and
W. David Nes,
Department of Chemistry &
Biochemistry,
Texas Tech University,
Lubbock, Texas, 79409-1061,
USA.

EVIDENCE FOR A NOVEL MODE OF ACTION OF STEROIDAL ALKALOIDS

As part of a program on natural products that may have antifungal and therapeutic value, we have undertaken a reexamination of the biochemistry and physiology of steroidal alkaloids. Using *Prototheca wickerhamii*, a non-photosynthetic yeast-like microorganism that operates a cycloartenol-ergosterol based pathway (Nes, W.D. et al. *PNAS* (1990) 87, 7565), we studied the effect of 4 nitrogen-containing azasteroids; solasodine (1), solanidine (2), tomatine (3) and 25-azacycloartenol (4) on cell growth and sterol biosynthesis. The compounds inhibited *P. wickerhamii* growth at 1_{50} values that ranged from 35 nM (4) to 5 μM (2 and 3). In each treatment, we found a direct relationship between growth inhibition and sterol production. We observed that 1, 2 and 4 inhibited ergosterol production by inhibiting the activity of sterol methyl transferase (SMT) activity, thereby inducing an accumulation of cellular cycloartenol; 3 inhibited growth by an unknown mechanism, probably by sterol-complexing with tomatine. Kinetic studies with the soluble SMT enzyme confirmed that 1, 2 and 4 inhibited enzyme activity and 3 had no effect on enzyme activity, suggesting new roles for 1 and 2.

THURSDAY, 12:30-12:50

Oral BPC 10

Erin M. Silva and
Bill B. Dean,
Department of Horticulture and
Landscape Architecture,
Washington State University,
24106 North Bunn Road,
Prosser, WA 99350, USA.

IDENTIFICATION AND QUANTIFICATION OF ONION (*ALLIUM CEPA* L.) HYBRID PARENT FLORAL VOLATILES AND THEIR EFFECT ON HONEY BEE ATTRACTIVENESS

Flower volatiles were collected from nine onion hybrid parents of varying attractiveness to the honey bee and analyzed by gas chromatography-mass spectrometry. Differences were found in the volatile profiles of each of the hybrid parents in regards to the specific volatiles emitted and the quantity of volatiles produced. Twenty-two compounds were identified tentatively and 8 identifed positively and quantified from the onion flower headspace samples. The majority of compounds contained sulfur with the remaining falling into the classes of terpenoids, aldehydes, and acids. The more attractive hybrid parents tended to produce greater quantities of volatiles while the lesser attractive parents produced a lower quantity of volatiles. Further testing using specific compounds identified in the onion flower scent will determine if a correlation exists between the presence of a volatile/mixture of volatiles at a particular concentration and the relative bee attractiveness of the onion flower.

THURSDAY, 12:50-1:50 **LUNCH**

THURSDAY, 2:00 **DEPART FOR TOUR AND BANQUET - Meet Tour Bus in Front of Compton Union Building**

THURSDAY, BANQUET

NATURAL PRODUCTS AND PHYTOMEDICINE

Symposium Paper 17

G.H. Neil Towers,
University of British Columbia,
Dept. of Botany,
6270 University Boulevard,
Vancouver, B.C., V6T 1Z4,
CANADA.

Many natural products of plants and fungi display enhanced biological activities in the presence of UV and/or visible light. The recent photochemistry and photobiology of some of these powerful photosensitizers and their potential in photomedicine will be reviewed. The review includes a discussion of the polyynes and their sulfur derivatives, the perylenequinones, alkaloids, furanocoumarins, furanochromones, furanoquinolines and porphyrins. Because of the increasing appearance of drug-resistant microorganisms, the search for new antibiotics from biological sources has intensified. In almost all of the recent screenings of plants and microorganisms for new biological activities including those againt infectious organisms, the effects of light on the activities have been completely ignored. It is timely therefore to review this subject. My review will focus on some of our most recent work which has been supported by the Canadian Bacterial Diseases Network.

SYMPOSIUM SESSION 10
Moderator:

PLANT SURVIVAL STRATEGIES: DEFENSES AND ATTRACTANTS
G.H.N. Towers

FRIDAY, 8:00-8:30

Oral Paper 35

William A. Ayer,
Department of Chemistry,
University of Alberta,
Edmonton, AB, CANADA T6G 2G2.

THE CLITOCYBULOLS, A NEW SKELETAL TYPE OF SESQUITERPENE

In one square meter of soil, approximately 1-3 million nematodes are found. Many of them are important plant parasites. It has recently been observed that nematodes are quickly killed by the fungus *Clitocybula oculus*. We have grown this fungus and have isolated 3 sesquiterpene metabolites, clitocybulol A, B, and C. The structures of these compounds will be described, as well as bioassays against one species of nematode. The biosynthesis of these compounds is currently under investigation and will be discussed.

FRIDAY, 8:30-9:00

Oral Paper 36

Bilge Sener[1], Nurgün Erdemoglu[1], William S. Bowers[2] and Philip H. Evans[2],
[1]Dept. of Pharmacognosy, Faculty of Pharmacy, Gazi University, P.O. Box 143, 06572 Maltepe-Ankara, TURKEY;
[2]Laboratory of Chemical Ecology, Dept. of Entomology, University of Arizona,
Tucson, AZ 85721, USA.

BIOLOGICAL ACTIVITIES OF TURKISH MEDICINAL PLANTS AGAINST AGRICULTURAL PESTS

Insect pest infestation of cultivated crops continues to be the principal limitation to increased agricultural production of food and fiber. Despite serious environmental concerns over their use and abuse, insecticides remain the first line of defense against herbivorous insects and disease vectors. Recent studies reveal that plants possess many subtle defenses that interfere with pest growth, and lack any toxicity to higher animals. To investigate the biological actions of Turkish medicinal plant extracts, we have developed a variety of screening assays. The results of 270 organosoluble extracts prepared from Turkish medicinal plants will be reported.

FRIDAY, 9:00-9:30

Oral Paper 37

C. Peter Constabel,
Mary Christopher, and
Lynn Yip,
Department of Biological Sciences,
University of Alberta,
Edmonton, AB T6G 2E9,
CANADA.

CLONING AND CHARACTERIZATION OF POLYPHENOL OXIDASE, A POTENTIAL ANTI-HERBIVORE PROTEIN OF HYBRID POPLAR

Poplar is being extensively used as a model system for woody plant biology, and we are using the poplar hybrid *Populus trichocarpa X P. deltoides* to investigate anti-herbivore defense mechanisms of trees. Mechanical wounding or herbivore damage of young hybrid poplar leaves stimulates the rapid accumulation of foliar polyphenol oxidase (PPO) activity. This enzyme oxidizes plant phenolics, and the resulting quinones can negatively impact the growth and performance of folivore insects. In hybrid poplar PPO induction is systemic, and PPO accumulates in wounded as well as unwounded leaves on wounded plants, supporting the proposed defensive role for this protein. In order to study the regulation of PPO expression in hybrid poplar, we have isolated and characterized a cDNA encoding hybrid poplar PPO. RNA hybridization experiments using the PPO cDNA as a probe demonstrate that the wound-induction of PPO activity is preceded by a corresponding increase in PPO mRNA. We are now studying the expression of this gene in both healthy and damaged plants. Supported by NSERC Canada.

FRIDAY, 9:30-10:00

Oral Paper 38

Natalia Dudareva,
Jihong Wang, Leland Cseke and
Eran Pichersky,
Biology Department,
University of Michigan,
Ann Arbor, MI 48109, USA.

MOLECULAR BIOLOGY OF FLORAL SCENT

The biogenesis of floral scent has been investigated in a model organism, *Clarkia breweri*. Genes encoding enzymes which catalyze the formation of linalool, methyleugenol, and benzyl acetate have been isolated and characterized. These genes are all expressed coordinately in floral tissues and their expression levels parallel levels of the corresponding biosynthetic enzymes. Generally, petals have the highest levels of enzyme activities and gene expression. The *Clarkia* genes encoding scent enzymes belong to various gene families—terpene synthases, methyltransferases, and acyltransferases—whose members are involved in many pathways of secondary metabolism.

FRIDAY, 10:00-10:20

BREAK

FRIDAY, 10:20-10:40

Oral Paper 39

Manuel Aregullin[1],
Mary Baker[2], Matthew E. Gompper[3] and Eloy Rodriguez[1],
[1]Phytochemical Laboratory, L.H. Bailey Hortorium, Division of Biological Sciences, Cornell University, Ithaca, NY 14853, USA;
[2]Department of Anthropology, University of California, Riverside, CA 92521, USA;
[3]Department of Zoology, University of Tennessee, Knoxville, TN 37996, USA.

THE CHEMISTRY OF PLANTS AND FRUITS USED IN FUR-RUBBING BY ANIMALS

The behavior associated with the utilization of plants and fruits for fur-rubbing purposes appears to be a wide spread trait in different animal species. In a continued effort to study the zoopharmacognosy (animal self-medication) of diverse animal groups, we have investigated the chemistry of resins of *Trattinnickia aspera* (Burseraceae) used by coatis (*Nasua narica*) in Barro Colorado Island, Panama, and the chemistry of species of *Citrus* (Rutaceae) and *Piper* (Piperaceae) used by capuchin monkeys (*Cebus capucinus*) in Costa Rica. The major constituents identified in the plants used by the animals were triterpenes, sesquiterpenes and furanocoumarins. In this presentation the chemistry, bioactivity and repellent properties of the fur-rubbing resins and oils will be discussed.

FRIDAY, 10:40-11:00

Oral Paper 40

Brice A. McPherson and
David L. Wood,
Department of Environmental Science, Policy, and Management, Division of Insect Biology,
University of California,
Berkeley, CA 94720 USA.

SUBTERRANEAN TERMITE FEEDING STIMULANTS ISOLATED FROM ASSOCIATED FUNGI IN LABORATORY COLONIES

The influences of substrate chemistry on subterranean termite (Isoptera: Rhinotermitidae) feeding behavior are largely unknown. The presence of fungi may significantly alter the preference, nutritional status, and even toxicity of dead wood. Fungus-colonized filter paper taken from laboratory *Reticulitermes hesperus* cultures yielded both stimulatory and deterrent extracts in cellulose TLC feeding assays for the 3 principal North American *Reticulitermes* species. A zygomycete soil fungus isolated from the termite colonies that produced the filter paper extracts was stimulatory for these termite species. Open column chromatography and HPLC revealed multiple sources of stimulatory activity in this fungus. In contrast to the fungus-cultivating members of the Termitidae, no obligate associations with fungi are known for the Rhinotermitidae. This appears to be the first evidence that a zygomycete can affect termite behavior.

FRIDAY, 11:00-11:20

Oral Paper 41

Chris Lunde and
Isao Kubo,
Department of Environmental Science, Policy and Management,
University of California,
Berkeley, CA 94720-3112, USA.

POLYGODIAL, AN ANTI-*CANDIDA* DRIMANE SESQUITERPENE DIALDEHYDE

Polygodial showed potent fungicidal activity against yeast-like fungi *Candida albicans*, *Candia utilis*, *Cryptococcus neoformans* and also filamentous fungi including *Trichophyton mentagrophytes*, *Trichophyton ruburum*, and *Penicillium marneffei*. The antifungal activity of polygodial was generally not reduced by several susceptibility-testing conditions such as medium, incubation temperature, inoculum size, and medium pH. However, polygodial's antifungal activity was strongly increased at acidic conditions. Unlike amphotericin B, polygodial did not show any hemolytic activity and also its antifungal activity was not diminished in the presence of ergosterol. Based on killing kinetics, polygodial showed strong and fast fungicidal activity against *Candida albicans* under growing conditions. The fungicidal activity of polygodial was strongly increased at non-growing conditions.

FRIDAY, 11:20-11:40

Oral Paper 42

Kenkichi Ebisui,
Atsushi Ishihara and
Hajime Iwamura,
Division of Applied Life Sciences,
Graduate School of Agriculture, Kyoto University,
Kyoto 606-8502, JAPAN;
CREST, Japan Science and Technology Corporation (JST).

OCCURRENCE AND CHARACTERIZATION OF DIMBOA SPECIFIC GLUCOSYLTRANSFERASE IN MAIZE DURING JUVENILE STAGE OF GROWTH

DIMBOA, a dominant defense compound in maize, occurs as glucoside. Upon infection or by insect attack, the glucoside is hydrolyzed to produce the aglycone. We have found that during non-autotrophic growth stage free DIMBOA occurs transiently, together with a glucosidase specific for DIMBOA-G, and suggested that this process is a defense system specific for the vulnerable, juvenile stage. In this study, we examined if a specific glucosyltransferase occurs to produce the precursor glucoside. Shoots of maize seedlings grown for 36 hr after germination were extracted, and the extracts were purified by ion-exchange chromatography and gel filtration techniques using DIMBOA as substrate. The characterization revealed that a glucosyltransferase highly specific to DIMBOA occurs concurrently with the transient occurrence of DIMBOA-G and DIMBOA.

FRIDAY, 11:40-12:00

Oral Paper 43

Jens A. Pedersen*,
Department of Chemistry,
Aarhus University,
140 Langelandsgade,
DK-800 Aarhus C, DENMARK

*Present address: Department of Botany, University of British Columbia, Vancouver, BC, CANADA V6T 2B1.

NATURALLY OCCURRING QUINOLS AND QUINONES AS STUDIED BY ELECTRON SPIN RESONANCE

In this paper I shall outline how I have applied a simple procedure using electron spin resonance (ESR) techniques to detect, identify and partly quantify quinones and quinols in crude plant extracts. The compounds are converted to the corresponding semiquinone radicals, subsequently observed by ESR. Compounds lacking an *ortho* or a *para* dihydroxy grouping, e.g. recorcinols or monohydric phenols are not observed due to their short lifetime as radicals. Non-phenolic compounds are ignored by the spectrometer. The number of quinones/quinols which has been studied by ESR run far beyond one thousand.[1] However, the number of naturally occurring compounds amenable for study by the technique run into thousands.

Example of compounds studied are dihydric phenolics from the leaves of 170 Ecuadorian *Lycopodium* specimens and from 590 specimens of 91 genera of Gesneriaceae[2]. Naphtho- and anthraquinones from leaves and roots have been studied in a number of specimens e.g. of *Galium, Drosera* and *Ceratostigma*. Some rules for obtaining the absolute identity of these quinones from their ESR spectra have been established.[3,4]

The scope and limitation of the technique will be given, both as an analytical tool in the structural study of the title compounds, as well as a tool for furnishing data of chemotaxonomic value.

1 J.A. Pedersen, Handbook of EPR Spectra from Quinols and Quinones, CRC Press, Boca Raton, FL 1985.
2 L.P. Kvist and J.A. Pedersen, Biochem. Syst. Ecol. 14, 385, 1986.
3 J.A. Pedersen, J. Chem. Soc., Faraday Trans., 88, 3423, 1992.
4 D. Stockfisch, M. Kaaber, J.A. Pedersen, Magn. Res. Chem. 34, 619, 1996.

FRIDAY, 12:00-12:20

Oral Paper 44

James Kerwin[1] and
Norman G. Lewis[2],
[1]Botany Department,
Box 351330,
University of Washington,
Seattle, WA 98195, USA;
[2]Institute of Biological Chemistry,
Washington State University,
Pullman, WA 99164-6340, USA.

PROFILING LIGNANS USING ELECTROSPRAY MASS SPECTROMETRY

Electrospray mass spectromety (ESMS), a soft method for structural analysis of biological compounds, was first used routinely for peptide analysis in the early 1990's. Techniques were subsequently developed for analysis of other compound classes including carbohydrates, lipids and polyphenols. The advantages of this method of MS analysis include sensitivity (low picomolar), and the ability to rapidly characterize complex biological samples without preliminary chromatographic separation. Lignan analysis using ESMS and collision-induced mass spectrometry (ESMS/MS) allowed delineation of isobaric species and identification of product/ions characteristic of this compound class. By using conditions that promoted in-source fragmentation of precursor ions, ESMS/MS of several classes of lignans (diarylbutanes, dibenzylbutyrolactones and furofurans), unique product ions for each class were identified. These diagnostic ions can be used for analysis of complex mixtures of plant polyphenols using precursor ion scanning. Although ESMS and ESMS/MS spectra were very similar for several isobaric species; (pinoresinol and matairesinol; *p*-coumarylresinol and dehydrodicoumaryl alcohol) distinctive product ions were present for each compound. This MS technique can be used for rapid identification of complex mixtures of picomolar concentrations of plant polyphenols with minimal preliminary chromatographic separations.

FRIDAY, 12:20-1:15 **LUNCH**

SYMPOSIUM SESSION 11 *MAINTAINING HEALTH WITH PHYTOCHEMICALS*
Moderator: *Michael A. Costa*

FRIDAY, 1:15-1:45

Oral Paper 45

MEDICINAL PLANTS OF THE STOLTMANN WILDERNESS

Suzanne Diamond,
Flora Manufacturing and Distributing Ltd.,
7400 Fraser Park Dr.,
Burnaby, BC, CANADA.

Many plants of the Stoltmann Wilderness, located two and a half hours northwest of downtown Vancouver, hold great promise as powerful phytomedicines of the future. The majority of these potential medicines have yet to be fully investigated for their active ingredients. Preliminary studies on the dried berries of blueberries and mountain bilberries in the region (including *Vaccinium alaskensis*, *V. membranaceum* and *V. ovalifolium*) show anthocyanin levels far greater than that of the European bilberry, *Vaccinium myrtillus* L. A 25% anthocyanin extract of *V. myrtillus* is a major phytopharmaceutical drug in trade with indications for treating retinal eye disorders, capillary fragility, poor circulation, varicose veins, cataracts, menstrual disorders, edema, peptic ulcers, skin and connective tissue disorders, inflammatory conditions, atherosclerosis and diabetic microangiopathy. The potential economic value of these and other plants from the region are disregarded by the provincial government which is already subsidizing the forest industry. The ecosystem of the Sims Creek and Upper Elaho Valley where most of the research has been carried out includes thousand year old red cedars, yellow cedars and Douglas fir stands. Aggressive clearcut logging practices by Interfor are quickly liquidating these valley bottom forests with all of their biological diversity and with it many potential medicines may be lost forever.

FRIDAY, 1:45-2:15

Oral Paper 46

A COLLABORATIVE APPROACH TO THE SUSTAINABLE DEVELOPMENT OF PHYTOCHEMICAL PRODUCTS FROM NATIVE CANADIAN PLANTS

Robin J. Marles[1], Faiz Ahmad[1], Natalie Tays[2], Jack Moes[3], Clayton Jackson[4], Clarence Marzolf[5] and Markus Schmülgen[6],
[1]Botany Dept., Brandon University, Brandon, MB;
[2]Otetiskiwin School, Nisichawasik First Nation, Nelson House, MB;
[3]Manitoba Agriculture, Brandon, MB;
[4]Manitoba Crop Diversification Centre, Carberry, MB;
[5]Medicinal and Aromatic Plants Association of Manitoba, Portage la Prairie, MB;
[6]Food Development Centre, Portage la Prairie, MB, CANADA.

Sustainable development should be a cultural, ecological, and economic concept. Our approach to the discovery of new plants as sources of valuable phytochemicals involves direct participation of First Nations researchers in ethnobotanical research, ensuring the preservation of traditional knowledge within aboriginal communities and their role in future development. As most native plant species cannot withstand economic levels of harvest from the wild, ecological sustainability requires the selection and characterization by phytochemical and genetic methods of suitable populations from which cultivated varieties can be developed. Agronomic studies must determine the appropriate conditions and techniques for cultivation and harvest of these new crops, for which joint efforts between government researchers, producers and processors are essential to find pragmatic solutions. Economic sustainability requires the identification of target markets and stringent quality control from start to finish to maintain a superior herbal or nutraceutical product that stands above its numerous competitors. Collaboration is clearly the best route to success.

FRIDAY, 2:15-2:45

Oral Paper 47

CITRUS LIMONOIDS: FUNCTIONAL CHEMICALS IN AGRICULTURE AND FOODS

Shin Hasegawa,
Gary Manners, Luke Lam* and Edward Miller**,
WRRC, ARS, USDA,
800 Buchanan St.,
Albany, CA 94710, USA;
*LKT Laboratories, St. Paul, MN, USA;
** Baylor College of Dentistry, Dallas, TX, USA.

Citrus limonoids are a unique group of chemically related and highly oxygenated triterpenoids which occur, with a few exceptions, only in species of the *Citrus* genus and species of genera related to *Citrus*. They are one of the two bitter principles in citrus juices. They induce the detoxifying enzyme, glutathione *S*-transferase and possess anticarcinogenic activity in laboratory animals. They inhibit the proliferation and reduce the viability of human breast cancer cells in culture. They also display natural pest control properties. The biochemical analysis of 36 limonoid aglycones and 17 glucosides, which have been isolated from *Citrus* and its hybrids, has shown that citrus limonoids are excellent chemotaxonomic markers for plant varietal identification. These observations demonstrate the importance of citrus limonoids in the agricultural and food industries, and in human health.

FRIDAY, 2:45-3:05

Oral Paper 48

S. Farooq,
321-D, Okhla Village,
New Delhi - 110025,
INDIA.

PHYTOMEDICINES WITH HOLISTIC APPROACH, PROVEN EFFICACY AND SAFE IN CLINICAL, *IN VIVO* AND *IN VITRO STUDIES*

A comparative study in experimental animals was done using modern and traditional prepared (Aasava & Arishta) phytomedicines with Holistic approach of total extraction (Aqueous & Alcoholic) from minor forest plants. These were tested for standardization, therapeutic value, efficacy, *in vitro* cytological changes and toxicity. The study involved biochemical changes sialic acid, cholesterol, glucose and haematological changes, including total leukocyte count, haemoglobin, red blood cell count, and haematocrit. The observations were made from the content of trachea and blood of experimental animals - albino rats and rhesus monkeys. These parameters were tested for side effects also as per OECD Guidelines. The total extract preparation was found effective and potent with no side effects.

FRIDAY, 3:05-3:25

Oral Paper 49

W. Madhulatha,
P. Neelakanta Reddy and
V. S. Sundara Rao
Central Leather Research Institute,
Adyar, Chennai - 600 020,
INDIA.

STUDIES ON ARJUNA EXTRACT - A CARDIOTONIC

Indian system of medicine, mostly uses plant products, either in the form of whole powder or as an extract. During our studies on tannins of indigenous tanning material, we found that most of them are used in Indian medicine, either as a curative or preventive medicine. The studies on biological and therapeutic properties of tannins opened up new vistas in recent years, emphasizing Naturopathy as a less harmful treatment. However, the combination of different groups of natural products, which could be extracted out of various plants and their curative effects described in Indian medicine are intriguing and needs further studies.

Terminalia arjuna Bedd. which belongs to Combretaceae family is a popular tanning material in India. The bark is reported to be cardiotonic as well as cardio stimulant, although it is not effective for aortic diseases as it increases blood pressure. Our studies aimed at isolation and identification of major compounds responsible for the above properties. As a prelude to the studies, the bark extract is fractionated into major fractions containing triterpenoids (I), monomeric flavonoids (II), low (III) and high molecular weight tannins (IV) using solvent extraction and chromatographic methods. These fractions were individually tested *in vivo* for their effect on cardio vascular system and collagen metabolism of heart muscle. The experiment was carried out on albino rats by oral administration of the fractions. The results indicate that the tannins have definite role to play in the properties of the drug. This paper reports and compares the results of the effectiveness of the individual fractions.

FRIDAY, 3:25-3:45 **BREAK**

FRIDAY, 6:00-7:30 **DINNER - COMPTON UNION BUILDING, BALL ROOM**

FRIDAY, 7:30 **MEETING ADJOURNED**

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_____ Volume 29 (1994)	**Phytochemistry of Medicinal Plants** (List $71.00, PSNA $42.60)
_____ Volume 28 (1993)	**Genetic Engineering of Plant Secondary Metabolism** (List $89.50, PSNA $53.70)
_____ Volume 27 (1992)	**Phytochemical Potential of Tropical Plants** (List $79.50, PSNA $47.70)
_____ Volume 26 (1991)	**Phenolic Metabolism in Plants** (List $89.50, PSNA $53.70)
_____ Volume 25 (1990)	**Modern Phytochemical Methods** (List $85.00, PSNA $51.00)
_____ Volume 24 (1989)	**Biochemistry of the Mevalonic Acid Pathway to Terpenoids** (List $85.00, PSNA $51.00)
_____ Volume 23 (1988)	**Plant Nitrogen Metabolism** (List $89.50, PSNA $53.70)
_____ Volume 22 (1987)	**Opportunities for Phytochemistry in Plant Biotechnology** (List $59.50, PSNA $35.70)
_____ Volume 21 (1986)	**Phytochemical Effects of Environmental Compounds** (List $75.00, PSNA $45.00)
_____ Volume 20 (1985)	**The Shikimic Acid Pathway** (List $55.00, PSNA $33.00)
_____ Volume 19 (1984)	**Chemically Mediated Interactions Between Plants and Other Organisms** (List $45.00, PSNA $27.00)
_____ Volume 18 (1983)	**Phytochemical Adaptations to Stress** (List $49.50, PSNA $29.70)
_____ Volume 16 (1981)	**Cellular & Subcellular Localization in Plant Metabolism** (List $39.50, PSNA $23.70)

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Société Phytochimique de L'Amérique du Nord

Sociedad Fitoquímica de América del Norte

Newsletter

● Volume 38, Number 2 ● December 1998 ●

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Dr. James Saunders (1999)
Dr. Kelsey Downum (2000)
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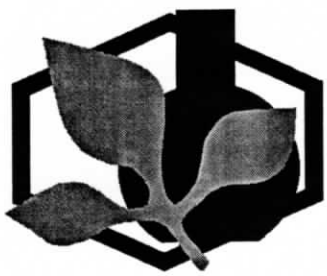
Dr. Nikolaus H. Fischer (2002)

PSNA Newsletter

Editor: **Dr. W. Dennis Clark**



The Phytochemical Society of North America is a nonprofit scientific organization whose membership (currently over 400) is open to anyone with an interest in phytochemistry and the role of plant substances in related fields. Annual membership dues are U.S. $20 for regular members and $10 for student members. Annual meetings featuring symposium topics of current interest and contributed papers by conference participants are held throughout the United States, Canada, and Mexico. Still a specialist organization despite its broadened interests, PSNA meetings are small enough to offer informality and intimacy that are conducive to the exchange of ideas. A newsletter is circulated to members several times a year to keep them informed of upcoming meetings and developments within the society. If you would like additional information about the PSNA or if you have material to be included in the newsletter, please contact the PSNA Secretary. Annual dues and changes in addresses should be sent to the PSNA Treasurer. Also see the PSNA homepage, currently at:
http://ls.la.asu.edu/psna.

Table of Contents

From the Editor

This end-of-year issue is full of good news about the Society and its members. Our annual meeting was a very successful one, which began with a welcome via satellite by Congressman George R. Nethercutt, Jr., and was highlighted by a wonderful banquet dinner that was punctuated by a lavish tribute to Professor G.H.N. Towers. In between, the meeting organizers, Norman Lewis and Neil Towers, provided us with an illustrious international group of invited speakers from seven countries. Each speaker gave us an excellent career-level overview of his or her topic and a clear peek into the leading research on it. The absence of Professor Jeffrey Harborne due to illness caused some consternation. And the quiet announcement by Professor Jerry McLaughlin of his impending retirement, beginning two days after the end of the meeting, will cause us to miss his wit and wisdom in phytochemistry.

Behind the scenes, President Vince De Luca announced a proposal for raising financial support for endowing a Young Investigator Award, the details of which are presented later in this newsletter. The post-meeting membership increased to above the 400-member level once again, aided by the efforts of the conference organizing team.

The conference venue at Washington State University presented us with a quiet yet inspiring location. It is commendable that WSU has built such a powerful scientific research center, which serves so well as a host institution for conferences such as ours. The wonderful dinners and free-flowing beer and wine certainly added to the enjoyment of many conference participants. And, as far as I could tell, the graduate students who served as our shuttle drivers were always reliable and happy to be of service.

We introduce some new features with this issue of the newsletter, in an effort to emphasize the "news" of the Society and its members. These include announcements, photographs, and brief comments here and there among the more formal, regular offerings of the newsletter. We encourage the membership to contribute to these features in future issues by sending information directly to the secretary by email at dennis.clark@asu.edu or by fax at (602) 965-6899.

We are now looking forward to our 1999 meeting at Concordia University, Montréal,Canada, in July. The organizing committee of Vincenzo De Luca, Ragai Ibrahim, and Luc Varin have already put together an excellent program. More details can be found in this newsletter.

The Editor

Minutes of the 1998 Executive Committee Meeting

The meeting began at 4:30 PM on Sunday, July 26. Present were Vincenzo De Luca, Susan McCormick, John Romeo, Cecilia McIntosh, and Dennis Clark. President De Luca opened the meeting with a discussion of the loss of members as reported by the Treasurer, Cecilia McIntosh. The PSNA formerly had about 485 members, but as of 1998 this number had dropped to about 285. McIntosh reported that spikes in membership occurred from presenters at the annual meeting who join but do not renew their membership after the first year. De Luca commented that the loss of members may be due to lower funding in phytochemistry, in spite of the ongoing demand for phytochemists in industry and in phytomedicine. The membership also suffered a drop because of the 1997 meeting that was held jointly with the Phytochemical Society of Europe. John Romeo suggested that having our meeting overseas was, relative to attracting new members, like not having a meeting at all. De Luca advised that we should send membership applications to all conference presenters who are not members. The organizers of this year's meeting are making a concerted effort to sign up new members from among the presenter pool when they check in at the registration table. McIntosh said that the society website might be useful for recruiting new members.

Vince De Luca proposed that PSNA change the term of the President to 2 years instead of 1 so each new President could have more time to both figure out and carry out the duties of the position better. Although this seemed like a reasonable suggestion, any change in terms of officers must be coordinated with the concomitant terms of the President-Elect and the Past-President.

Cecilia McIntosh mentioned that the society rents out the member mailing list to other societies and might with to do so in exchange for putting a flier about our upcoming meeting in another society's mailing.

Vince De Luca proposed that the society raise $50,000 to endow a young investigator symposium at each annual meeting. This symposium will be named after Arther Neish and will be held for the first time at the 1999 meeting, depending on the success of obtaining financial support. John Romeo voiced support for the proposal and recommended that the PSNA seed the fund with an initial contribution of $10,000. De Luca will seek additional support for the endowment until the $50,000 goal is reached.

All of the officers discussed the need to locate a site for the annual meeting in 2000. Dr. Rachel Mata of UNAM invited the society to meet in Mexico City again. The 1994 meeting in Mexico was very successful and well-attended by many UNAM phytochemists, several of whom joined the society. However, the general consensus was that we have had too many non-U.S. meetings since 1994 and that a U.S. venue would be preferable for 2000. The USDA center in Beltsville, Maryland, would be a good site for another PSNA meeting. (This was later confirmed as the tentative site for the 2000 meeting in discussion with Jim Saunders.)

John Romeo reported that the society's agreement with Plenum Press for publishing the *Recent Advances in Phytochemistry* series is coming to the end of the latest 5-year contract period. Personnel changes at Plenum will probably result in the offer of a less-favorable new contract, so we may want to seek a new publisher.

The Treasurer, Cecilia McIntosh presented her interim financial report, which the committee accepted (see elsewhere in this newsletter).●

Minutes of the 1998 Annual Business Meeting

President Vincenzo De Luca called the meeting to order at 5:15 PM on Wednesday, July 29. He first called upon Cecilia McIntosh to give the Treasurer's report. The report, presented elsewhere in this issue, was distributed to the membership and accepted. Dennis Clark presented the Secretary's report by discussing the frequency of the newsletter, which will be twice in 1998, 3 times in 1999, and probably should be quarterly

thereafter. Clark also stated that the society website will be moved to Arizona State University in the Fall, and requested the need from the membership for information about upcoming meetings of other societies, about news of members of PSNA, and about research summaries that can be included in the newsletter. John Romeo presented the report of the Editor-in-Chief. The PSNA and Phytochemical Society of Europe will share royalties from volume 32 of *Recent Advances in Phytochemistry*. New editorial personnel at Plenum Press may result in changes in our contract for the *Recent Advances* series. Romeo predicted that this would be a great year for sales of the series. De Luca announced the election of Susan McCormick to the office of President-Elect. He then introduced the issue of extended terms for the president's office, an idea that was postponed until the details of overlapping incoming and outgoing officers can be worked out.

Vince De Luca presented a proposal to endow the young investigators' symposium, which began in 1995. He asked that members solicit donations to build a fund of $50,000. The symposium, depending on the success of fundraising, will be named in honor of Arthur Neish and will be offered at the 1999 meeting in Montréal.

The 1999 meeting will be held at Concordia University, Montréal, Canada, July 10-13. The main theme will be "Origin of Metabolism" revolving around common chemical reactions. The dates were chosen by the organizing committee (V. De Luca, R. Ibrahim, L. Varin) in part for the opportunity to experience the Montréal Jazz Festival on Sunday, July 11.

The President introduced the subject of future meetings by proposing that we should have 2 U.S. meetings for every 1 non-U.S. meeting. This is based on the need of the society to influence membership through a minimum frequency of meetings in the U.S., because that is where the bulk of our membership resides. Currently, the Executive Committee is exploring the details of holding the 2000 meeting at the USDA center in Beltsville, Maryland. Jim Saunders has confirmed the availability of resources for this meeting, probably for sometime in June. Initially, the suggested theme will be, "Phytochemical Research by Molecular Methods." Rachel Mata has offered to host the meeting in 2001 in Mexico City, but the Executive Committee has not yet taken action on this offer. One suggestion is that we hold a joint meeting there with the American Society of Pharmacognosy.

A general discussion ensued regarding the make-up of PSNA and the need to both diversify to attract a wider variety of members as well as to focus on our strenghs in phytochemistry to maintain our identity. The nature of our identity was discussed relative to the roles and definitions of phytochemistry. This discussion wound down without a general consensus from the meeting participants.

President De Luca offered hearty thanks to the meeting organizers, Norman Lewis and Neil Towers and the team of hard-working staff and graduate students at WSU. The meeting was adjourned shortly before 6:30 PM.●

Financial Support for the 1998 Meeting

The PSNA offers its special thanks to the following organizations for helping defray some of the costs associated with the 1998 meeting: Applied Phytologics, Inc.; Eisig-Tode Foundation; Elsevier Science; Federal Express; Hewlett Packard; Monsanto Company; Packard Instrument Company; PerSeptive Biosystems; Thermoquest; Washington State University, College of Agriculture and Home Economics; Waters Corporation.●

PSNA 1997 Financial Report

January 1, 1997 - December 31, 1997

RECEIPTS

Membership dues	$ 3965.00
Plenum Publishing - royalties, page charges on RAP	4658.91
Interest on checking account	117.00
Rental of mailing list	200.00
CD interest	1614.33
1996 meeting refund	3012.67
TOTAL RECEIPTS	$13568.17

EXPENDITURES

Executive Committee expenses	
Treasurer (ballots, dues notices)	$ 279.98
Editor, RAP	1000.00
Travel - Executive Committee	1500.00
Foreign check service charge	8.00
Checking account service charges	45.15
Speaker travel - 1996 meeting	1400.00
TOTAL EXPENDITURES	$ 5263.13

ASSETS

Checking account	$14926.14
Savings (CDs)	32543.64
TOTAL ASSETS	$47543.64

S. McCormick, C. McIntosh

PSNA 1998 Interim Financial Report

January 1, 1998 - July 20, 1998

RECEIPTS

Membership dues	$ 3020.00
Plenum Publishing	
Royalties	5411.47
page charges on RAP	2266.00
Interest on checking account	15.12
Interest on TN FAIR account	257.75
Rental of mailing list	150.00
1997 meeting refund	NA
TOTAL RECEIPTS	$11120.34

EXPENDITURES

Executive Committee expenses	
Treasurer (dues notices)	$ 104.20
Editor, RAP	1000.00
Secretary	2000.00
Checking account service charges (Peoria)	45.15
Check printing (TN)	14.73
Pullman meeting advance	7500.00
TOTAL EXPENDITURES	$ 10642.03

ASSETS

Checking account	$ 4589.35
FAIR account	28520.81
Savings (CDs)	13000.00*
TOTAL ASSETS	$46110.16

*Face value; mature 8/8/98

C. McIntosh

Student Awards, 1998 Meeting

Best Paper: Aldwin M. Anterola, Washington State University
Rate-Limiting Processes in Monolignol Formation in Pinus taeda

and

Erin M. Silva, Washington State University
Identification and Quantification of Onion (Allium cepa L.) Hybrid Parent Floral Volatiles and Their Effect on Honey Bee Attractiveness

Best Poster: Sarah E. Keates, Washington State University
Expression of Oxalate Oxidase in Fungus-Infected Bean Tissues

and

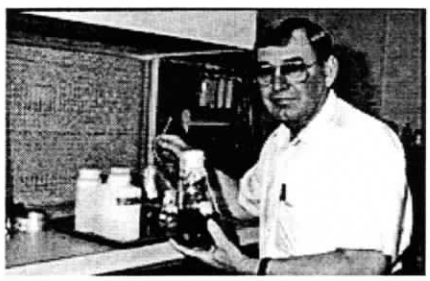
Jihong Wang, University of Michigan
Molecular Evolution of O-Methyltransferases: From Lignin to Floral Scent

Travel Awards:

Caroline Aemisegger, University of British Columbia
Retno Andayani
David Bird, University of Calgary
Jean-Baptiste Daskiewicz, Claude Bernard University
Minghua Chen, Miami University
Gabriel Guillet, Université de Montréal
Pierre Laflamme, Université de Montréal
Sang-Un Park, University of Calgary
Olga M.D. Silva, CECF, Faculty of Pharmacy
Amrita Singh, University of British Columbia
Felipe Vasquez-Flota, Université de Montréal
Jihong Wang, University of Michigan
Xi Wang, Lousiana State University
Anil T. Mangla, Texas Tech University

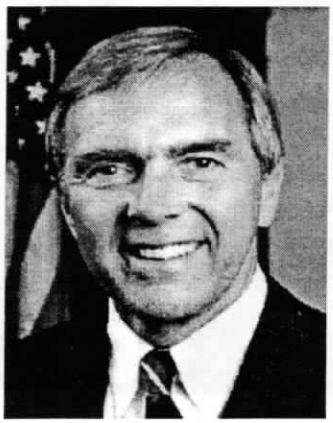
Mabry Wins Pergamon Phytochemistry Prize

Professor Tom J. Mabry, Lifetime Member and first president of the PSNA, has received the prestigious Pergamon Phytochemistry Prize for 1998. During his 35-year career at the University of Texas at Austin, Professor Mabry has published over 700 research papers, edited 13 books, and co-authored two phytochemical classics, *Systematic Identification of Flavonoids* and *Sesquiterpene Lactones*. He has also made important contributions to the teaching of phytochemistry and has trained over 60 graduate students in his laboratories at Austin. His high quality scientific publications and books have guided numerous scientists throughout the world. Congratulations, Tom!



Conference Highlights 1998

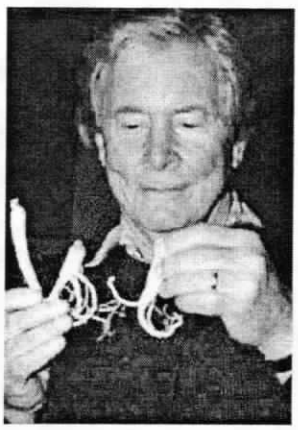
The 1998 annual meeting of the PSNA at Washington State University, July 26-31, began with a wonderful salmon barbecue, accompanied by good local microbrew beer and an excellent selection of wines. The opening session the next day was graced by the positive words of U.S. Congressman George R. Nethercutt, Jr. (Washington 5th District) in his welcoming remarks to the conference via live satellite broadcast.



Congressman Nethercutt came through as a strong advocate of scientific research and a good friend of phytochemistry (i.e., takes his ginkgo supplements).

The symposium consisted of a high-level cast of contributors from 7 countries, whose topics centered around the conference theme, *Phytochemicals in Human Health Protection, Nutrition and Plant Defense*. The PSNA is especially appreciative of the efforts by the conference organizers, headed by

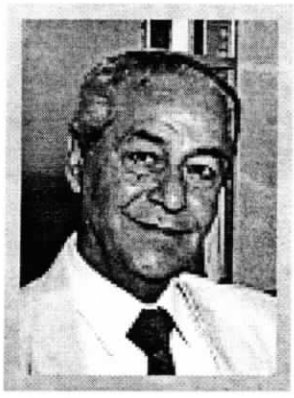
Norman Lewis and Neil Towers, in securing the speakers for such an excellent set of presentations. In addition, the graduate students and staff of the Institute of Biological Chemistry put in yeoman's efforts before and during the conference, further ensuring its success.



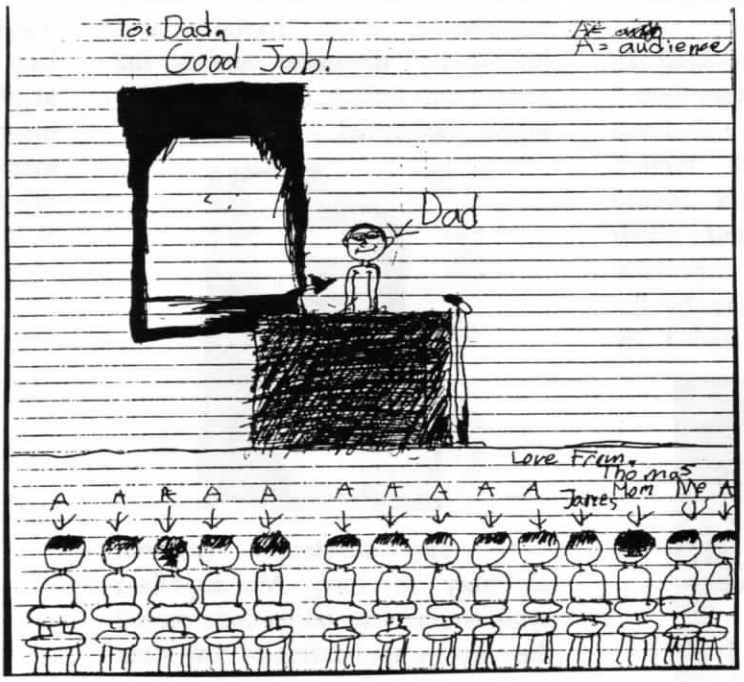
Oral paper sessions took place without a hitch, accompanied by the visage of Stewart Brown watching the slide presentations from the back of the meeting room with binoculars. Poster sessions were staged under the most genteel conditions imaginable for a scientific conference, thereby stimulating much friendly and informative discourse among the participants before, during, and after dinner was served.

The substantial research summary in Jerry McLaughlin's symposium presentation underscored the creative application of new ideas in the search for anti-cancer drugs from plants. But perhaps the most lasting memory of the 1998 conference comes from Neil Towers' banquet address, Norman Lewis' tribute to him during the evening's proceedings, and the dance exhibition by Neil's youngest daughter. On the occasion of his 75th birthday, Neil certainly left one and all with the impression that he is not done yet - he's still having fun.

Toward the end of the week, thoughts and plans began to turn to the 1999 meeting at Concordia University in Montréal. The meeting organizers, Vince De Luca, Ragai Ibrahim, and Luc Varin, have already planned another exceptional program. The meeting information and registration materials are included elsewhere in this issue of the newsletter. See you in Montréal!



Two views of the 1998 PSNA conference participants: Group Photo (above), and Group Sketch with Dad Presenting, by Thomas Marles (below).



PHYTOCHEMICAL SOCIETY OF NORTH AMERICA
SOCIÈTÉ PHYTOCHIMIQUE D'AMÉRIQUE DU NORD
SOCIEDAD FITOQUÌMICA DE L'AMÉRICA DEL NORTE

CONCORDIA UNIVERSITY, MONTRÉAL, CANADA, July 10-13, 1999

"Evolution of Plant Metabolic Pathways"

ABSTRACT SUBMISSION FORM (Please Type)

Name: _____

Professional Address: _____

_____

City/Ville/State/Estado: _____

Country: _____ **Postal Code**: _____

Phone: _____ **Fax**: _____ **E-mail**: _____

Preferred presentation medium: _____ **Oral** _____ **Poster**

Please indicate if you wish to be considered for one or both of the following awards (intended for graduate students and recent Ph.D.'s) - please include a maximum one-page CV.

_____ Travel Assistance, _____ Best Paper/Poster, _____ Date of Ph.D.

Abstracts* must be received by **March 30, 1999**. Two unfolded copies of the Abstract and a **diskette** of the abstract (**MS-Word or Word Perfect**) should be forwarded by Air Mail to:

Dr. Ragai Ibrahim, Biology Dept., Concordia University, 1455 De Maisonneuve Blvd. West, Montréal (P. Québec), Canada H3G 1M8.

*Type your Abstract on plain paper, **not exceeding** 16.5 x 7.5 cm (6.5 x 3.0 inch).
No extension beyond this size limit ! Thank you.

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1.	Chateau Versailles 1808 Sherbrooke St. West chateau@surfsup.net	(514) 933-3611 (T) 933-6867 (F) (800) 361-7129 (Canada) (800) 361-3664 (USA)	$ 88.00 S/D Incl. Cont. Bkfst.
2.	Hotel Europa 1240 Drummond St. hotel@europahotelmtl.com	(514) 866-0492 (T) 861-4089 (F) (800) 361-3000	$85.00 S/D $ 105.00 (two beds) Cont. Bkfst. $5.95
3.	Le Nouvel Hotel 1740 Boul. René Lévesque www.lenouvelhotel.com	(514) 931-8841 (T) 931-3233 (F) (800) 363-6063	$ 85.00 (S) $ 95.00 (D) Incl. Cont. Bkfst.
4.	Manoire Le Moyne (Suites) 2100 De Maisonneuve Bd. W. www.ibst.com lemoyne@ibst.com	(514) 931-8861 (T) 931-7726 (F) (800) 361-7191	$ 89.00 Junior suite $104.00 Deluxe suite (S/D) Incl.compl. kitchen
5.	Days Inn 1005 Guy St. www.interresa.ca/hotel/daysincv	(514) 938-4611 (T) 938-8718 (F) (800) 567-0880	$ 129.00 (S) $ 139.00 (D)
6.	Hotel Comfort Suites 1214 Crescent St. comfmon1@netcom.ca	(514) 878-2711 (T) 878-0030 (F) (800) 465-6116	$ 105.00 (S/D) Incl. Cont. Bkfst.
7.	Hotel Montéal-Crescent 1377 Bd. René Lévesque	(514) 938-9797 (T/F) (800) 361-5064	$ 75.00 (S/D) $ 85.00 (two beds)
8.	Hotel Chateau Berri 756 Berri St. (Metro: Champs de Mars)	(514) 844-0767 (T) 844-4230 (F) (888) 909-6161	$85.00/95.00 (S/D) Incl. Cont. Bkfst. chateauberri@giminc.com
9.	Hotel Saint-Denis 1254 Saint-Denis St. (Metro: Berri-UQAM)	(514) 849-4526 (T) 849-4529 (F) (800) 363-3364	$ 59.00 (S/D) www.hotel-st-denis.com
10.	YMCA Residences 1450 Stanley St. (Free sport facilities)	(514) 849-8393 (T) 849-7821 (F) ymca_residence_montreal@ymca.ca	$39.00/ 56.00/ 65.00 (S/D/triple, male/female) $3.00Bkfst, 6.00 L/D www.sympatico.ca/ymca
11.	Concordia University Residences (Hingston Hall) 7141 Sherbrooke St. W. (Metre: Vendome ->#105 Bus)	(514) 848-4755 jjpeters@alcor.concordia.ca	$25.00 Students $30.00 Non-student (Shuttle bus from Resid. to Hall Bldg. Meeting site)

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9.	Hotel Saint-Denis 1254 Saint-Denis St. (Metro: Berri-UQAM)	(514) 849-4526 (T) 849-4529 (F) (800) 363-3364	$ 59.00 (S/D) www.hotel-st-denis.com
10.	YMCA Residences 1450 Stanley St. (Free sport facilities)	(514) 849-8393 (T) 849-7821 (F) ymca_residence_montreal@ymca.ca	$39.00/ 56.00/ 65.00 (S/D/triple, male/female) $3.00Bkfst, 6.00 L/D www.sympatico.ca/ymca
11.	Concordia University Residences (Hingston Hall) 7141 Sherbrooke St. W. (Metre: Vendome ->#105 Bus)	(514) 848-4755 jjpeters@alcor.concordia.ca	$25.00 Students $30.00 Non-student (Shuttle bus from Resid. to Hall Bldg. Meeting site)

Local Hotel Listing (Cont'd)

Except #8, #9 and #10, all hotels are within walking distance (5-12 min.) from the Hall Building of Concordia University (1455 De Maisonneuve Blvd. West) where the sessions will be held.

PLEASE RESERVE AS EARLY AS POSSIBLE - Montréal Hotels are usually fully booked by early May ! RESERVE BEFORE THAT DATE !

Please mention **"CONCORDIA UNIVERSITY - PSNA'99"** at the time of reservation, in order to get the preferred rates listed.

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