

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA

Newsletter

Volume 32
Number 3

February 1993

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Dr. Constance Nozzolillo (1993, Chair) **Dr. G.H. Neil Towers** (1994)

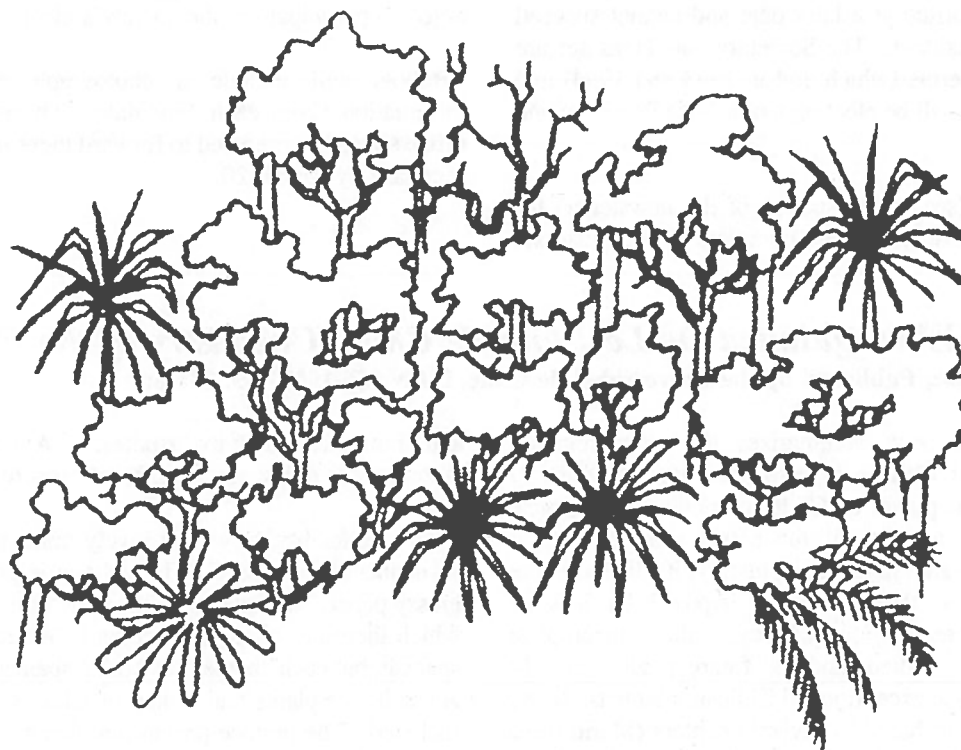
Dr. Jonathan Poulton (1995) **Dr. David Seigler** (1996)

Dr. Brian Ellis (1997)

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, the role of plant substances, and related fields. Annual membership dues are \$20.00 for regular members and \$10.00 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. 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 Society secretary. Annual dues and changes in addresses should be sent to the Society treasurer.

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA NEWSLETTER



FEBRUARY, 1993

VOLUME 32, NUMBER 3

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CALL FOR NOMINATIONS

The PSNA constitution specifies that members are responsible for nominating candidates for election of officers. A new Vice President (President-Elect) is elected each year and automatically becomes President the next year or any time that the office of President may be vacated. The President of PSNA is not eligible for re-election to this office at a later date and cannot succeed himself (herself) as President. The Secretary and Treasurer are elected for three-year terms (which end in 1993 and 1994) and may be re-elected. We will be electing a new Vice President and a Secretary this year.

A form is provided (see center section of the newsletter) for submitting your nominations. Please complete the nomination

form and mail it to Murray Isman, Department of Plant Science, University of British Columbia, Vancouver, B.C. V6T 1Z4, Canada. Nomination forms must be mailed by March 10, 1992. Election ballots will be distributed to members in April. All members, including those who joined in the past year, are urged to participate in the society's election process.

Ballots will include a photograph of and biographical information about each candidate. Those agreeing to run for office should be prepared to forward these materials to the PSNA secretary by March 20.

BOOK REVIEW: *Quimica De La Flora de Chile (Chemistry of the Flora of Chile)*, Ed. by Orland Munoz, Published by the Universidad de Chile, ISBN 956-190174-9, 351 pp.

This beautifully done book summarizes the most relevant phytochemical work of Chilean investigators over the past 20 years. Emphasis is on plants of Chile, particularly endemics. The 18 chapters give an idea of the actual state of natural products chemistry research in Chile: its history; its diversity; the major accomplishments; the limitations imposed by lack of technology and foreign dependency; the incomplete representation of flora studied; and the future goals. The 14 contributors are, with one exception, all Chilean scientists. Many of the authors have contributed to several chapters (Mario Silva to 5; Juan Garbarino, Magalis Bittner, and Orlando Munoz to 4 each).

Of the postulated 5971 species of mainland Chilean plants, 177 species belonging to 69 genera in 13 families (Aristolochiaceae, Berberidaceae, Bignoniaceae, Celastraceae, Compositae, Elaeocarpaceae, Nolanaceae, Rhamnaceae, Scrophulariaceae, Solanaceae, Amaryllidaceae, Lycopodiaceae, and Podocarpaceae) are reported on in this volume. The studies vary in scope. Chapters range from extensive surveys of chemicals known in a given family (a very nice review of Chilean work in the Asteraceae compiled by Cecilia Labbe D. of the University of Chile at Santiago covers 73 publications on 86 species) to brief reports (new terpenoids from 3 species of the genus *Nolana*) or reviews of families poorly represented in Chile (Eleocarpaceae) or families not yet sufficiently studied (Rhamnaceae) or a single genus within a family (*Berberis* alkaloids). In addition to the Asteraceae, there is significant chemical distribution work reported from the Bignoniaceae, Rhamnaceae, Scrophulariaceae, and Solanaceae. I found particularly interesting a chapter by Silva, Bittner, and Pacheco on flavonoids and terpenes of two Composite genera, *Robinsonia* and *Dendroseris* on the Juan Fernandez archipelago. Two chapters venture beyond straight chemical reporting to descriptions of chemical ecological work

and biological activity studies. Apparently only Silva's laboratory is doing any significant bioactivity work.

Each chapter begins with a lovely color photograph of a plant subsequently discussed. The volume is printed on high quality glossy paper. The quality of the structures, figures and tables (of which there are many) is excellent. All chapters are written in Spanish, but each chapter has both a Spanish and English abstract. An index of plants and a map of Chile showing its regions are included. The preface provides a nice historical summary of 50 years of phytochemical work in Chile, singling out the contributions of Drs. Herbert Appel, Ennio Bianchi, Juan Garbarino, Mario Silva, O. Cori, Bruce, Cassels, and Mariano Castillo among others. The concluding chapter "Y ahora Que...?" poses the question of future directions, emphasizing the need for technological equipment for Chilean labs, less dependence on projects of foreign scientists, and the need to complete a chemical inventory so plants can be protected and exploited sensibly. There is clearly much left to do. Less than 5% of Chilean plants have been phytochemically studied! Several families have not been significantly examined at all, including the Umbelliferae, Cactaceae, Papilionaceae, Caryophyllaceae, Labiatae, Euphorbiaceae, and 70% of the Compositae.

This book is a nice beginning, and it can serve as a useful model for others in Latin America where communication among phytochemists is often less than ideal. It will also be a useful reference book for libraries and individuals working with these particular families. I congratulate the editor Professor Munoz in pulling this all together.

Reviewed by John Romeo, Department of Biology, University of South Florida, Tampa, FL 33620.

NEW PSNA MEMBERS

The following are new members of the society. We welcome your participation in society business and at PSNA meetings. New members are encouraged to participate in elections and are urged to vote.

J.A. Aladesanmi
Dept. Pharmacognosy
Faculty of Pharmacy
Obafemi, Awaolowo University
Ile-Ife, Osun, NIGERIA

Denise E. Blume
Natural Products Inc.
115 Meadowview Dr.
Boone, NC 28607

Ben Bowen
Dept. of Biotechnology Research,
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P.O. Box 38
Johnston, IA 50131

Jack Cazes
107 Society Hill
Cherry Hill, NJ 08003-2402

Margaret A. Clarke
1100 Robert E. Lee Blvd.
New Orleans, LA 70124

Vincent Coleman
3104 Larkspur Circle
College Station, TX 77845

John P. Devlin
Boehringer Ingelheim Pharmaceuticals
900 Ridgebury Rd., P.O. Box 368
Ridgefield, CT 06877-0368

Francesca Faini de Castri
Fac. Ciencias, Depto. Quimica
Las Palermas 3425
Nunoa, Casilla 653
Santiago CHILE

Craig D. Dodson
Dept. Chemistry
Univ. Nebraska at Kearney
Kearney, NE 68849-5320

Imperato Filippo
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Potenza 85100
ITALY

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Harrison, NY 10528

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Agridyne Technologies Inc.
417 Wakara Way
Salt Lake City, UT 84108

Lucrecia Gonzalez
915 NW 106 Ave Circle
Miami, FL 33172

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Forest Products Lab
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Madison, WI 53705-2398

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Tucson, AZ 85721

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St. Louis, MO 63118

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USDA-ARS-NCAUR
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Peoria, IL 61604

Chung Ill-Min
AK-102, Turner Hall
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Univ. Illinois
Urbana, IL 61801

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Biol. Dept.
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Montreal, Quebec H3G 1M8
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Kanata ONT K2K 1S7
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Suzana Guimaraes Leitao
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3650 Nicholson Dr., Apt. 2130
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Pennsylvania State Univ.
University Park, PA 16802

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Malvern, PA 19355

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Faculty of Agriculture
Hokkaido University
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Sapporo 060, JAPAN

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Evanston, IL 60201

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Tampa, FL 33620

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Calvex
Baton Rouge, LA 70802

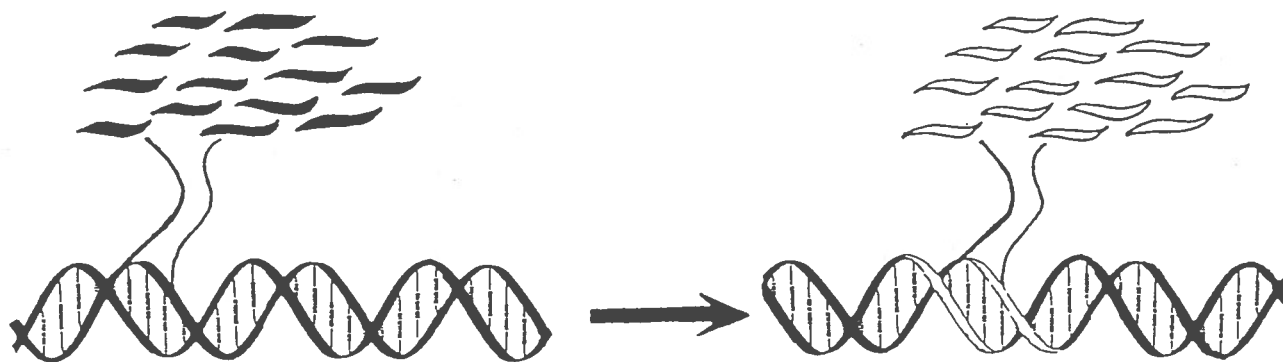
Christine K. Shewmaker
Calgene, Inc.
1920 Fifth St.
Davis, CA 95616

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Dept. Biological Sciences
Goucher College
Baltimore, MD 21204

Erica Szenasy
Ottawa-Carleton Institute of Biology
University of Ottawa
30 Marie Curie
Ottawa, ONT K1N 6N5
CANADA

Heekyung Tak
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Baton Rouge, LA 70802

Marie-Rose Van Calsteren
Agriculture Canada-Food Research
3600 Casavant Blvd. West
St. Hyacinthe, Quebec J2S 8E3
CANADA



REPORT OF THE TREASURER

The Treasury of the Phytochemical Society of North America ended the year with \$26,420.69 in accounts in Peoria, a significant decrease from last year. The attached financial statement shows the major source of receipts during 1992 were from membership dues (\$5,033.00) and royalties from the sales of *Recent Advances in Phytochemistry* \$4,084.88). Due to the increase in dues, revenue from membership dues grew during 1992 by a modest amount (\$768.00), however membership numbers dropped significantly to 345 (including 56 new members) at the end of 1992.

The largest expenditures during 1992 were directed to the annual meetings: \$11,892.20 for meeting-related expenses for the 1992 meeting in Miami, including \$5,000 for travel of Phytochemical Society of Europe members, \$1,490 for student travel awards and \$4668.70 for travel of Symposium speakers. \$5,500 was advanced for the 1993 meeting at Asilomar.

Savings are divided between two rising interest CD's (\$2,305.15 earning 6.75% and \$23,434.29 earning 6.00%). The remaining funds are in a money market checking account currently paying 2.75%.

Three final notes: 1) The membership directory is in final preparation and will be mailed soon along with a dues notice for 1993; 2) members that expect to retire during the next year are eligible for "emeritus status" and exemption of annual dues - please contact me if you are retiring but wish to remain a member so I don't continue sending dues notices; 3) if your address, phone number or other information has changed, please let me know as soon as possible so that the mailing list and data base can be updated.

Respectfully submitted,

Susan P. McCormick, Treasurer

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA NOMINATIONS FORM FOR 1993

I nominate _____ for Vice President (President-Elect) 1993-94.

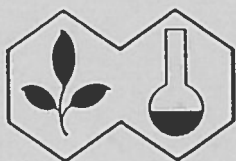
I nominate _____ for Secretary, 1993-96

Fold here

Fold here and tape to seal

Mail to Dr. Murray B. Isman

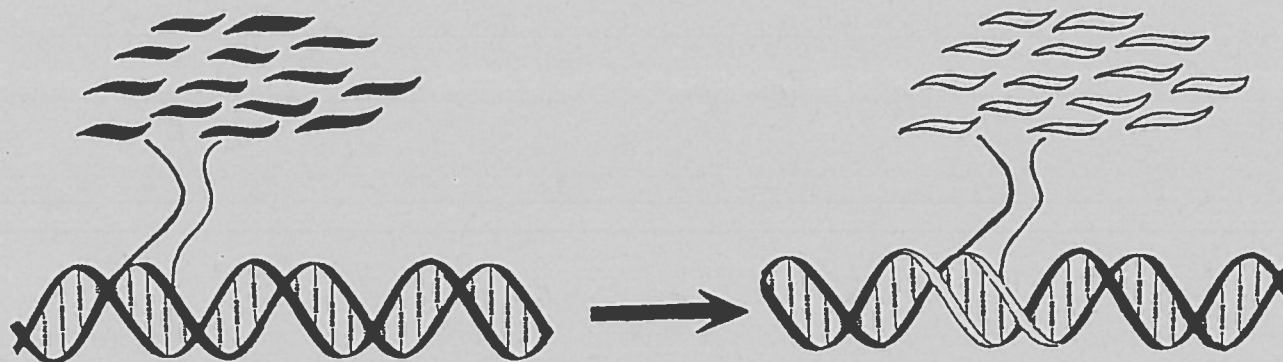
Address on reverse side



*Phytochemical Society
Of North America*

**Dr. Murray B. Isman
Department of Plant Science
University of British Columbia
Vancouver, B.C. V6T 1Z4
CANADA**

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA
ANNUAL MEETING, JUNE 27-JULY 1, 1993
ASILOMAR CONFERENCE CENTER, PACIFIC GROVE, CA



**SYMPOSIUM ON GENETIC ENGINEERING
OF PLANT SECONDARY METABOLISM**

INVITED SPEAKERS AND TOPICS

NEAL COURNEY-GUTTERSON, DNA Plant Technology Corp., ANTHOCYANINS

RODNEY CROTEAU, Washington State Univ., MONOTERPENES

RICHARD DIXON, The Noble Foundation, ISOFLAVONOIDS AND LIGNIN

RAGAI IBRAHIM, Concordia Univ., FLAVONOIDS

RICHARD JORGENSON, Univ. of California, Davis, DEVELOPMENT OF PATTERNS

TONI KUTCHAN, Univ. Munchen, BENZYLISOQUINOLINE ALKALOIDS

SALLY McCAMMON, USDA, Washington, D.C., ETHICAL/REGULATORY CONCERNS

YVES POIRIER, Michigan State Univer., PLASTIC POLYMERS

RICHARD ROBINS, AFRC Inst. of Food Res., POLYAMINES AND NICOTINE ALKALOIDS

RON SEDEROFF, North Carolina State Univ., LIGNIN

JOHN STEFFENS, Cornell Univ., POLYPHENOL OXIDASE

WILLIAM STROHL, Ohio State Univ., POLYKETIDE ANTIBIOTICS IN *Streptomyces*

Asilomar

408 - 372 - 8016

Betty Forbes
Pat Kaufman

**PHYTOCHEMICAL SOCIETY OF NORTH AMERICA
ANNUAL MEETING, JUNE 27-JULY 1, 1993
ASILOMAR CONFERENCE CENTER, PACIFIC GROVE, CA**

The program will include symposium sessions, contributed papers and a poster session. The meeting site is one of the most beautiful locations along the scenic California coastline and will provide a wonderful environment for a stimulating conference.

Urge your students to apply for travel grants and best student paper awards. Now is a good time to encourage students to join the PSNA. An application form is on the inside of the back cover of every newsletter and copies of the PSNA brochure are available from the secretary.

Please note that the conference is scheduled for Sunday to Thursday. Thus if you wish to obtain reduced air fares that require a Saturday stay over, accommodations at a hotel or motel in the Monterey area must be obtained. The Asilomar Conference Center cannot provide accommodations for extra days before or after the meeting. See phone numbers listed under Lodging and Meeting Facilities for information about hotels and motels in the Monterey area.

For further information on any aspect of the meeting, contact Dr. Gary Kuroki, DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, CA 94608. Telephone: (510) 547-2395; FAX: (510) 547-2817.

PROJECTED SCHEDULE

Sunday, June 27	3:00-6:00 PM - Registration 6:00-7:00 PM first meal (dinner) 7:00-10:00 PM - Reception
Monday, June 28	AM - Symposium presentations (3 talks) PM - Contributed papers Evening - Poster session - Posters will remain up through Wednesday
Tuesday, June 29	AM - Symposium presentations (3 talks) PM - Contributed papers Evening - Banquet
Wednesday, June 30	AM - Symposium presentations (3 talks) PM - Contributed papers, business meeting Evening - Monterey Bay Aquarium
Thursday, July 1	AM - Symposium presentations (3 talks) Last meal, lunch 12:00 noon - check out

IMPORTANT DEADLINES

April 10, 1993	Deadline for submission of housing/registration forms (without late charge). Deadline for submission of abstracts.
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Please note that registration forms and abstracts should be mailed to Dr. Gary Kuroki. Housing forms should be mailed to the Asilomar Conference Center.

TRAVEL TO PACIFIC GROVE

Pacific Grove is located on the Monterey Peninsula near California's famous Highway 1. The Monterey/Pacific Grove area is 124 miles south of San Francisco, and 77 miles southwest of San Jose. Detailed maps will be printed in the program of abstracts.

Air. The Monterey Airport is served by American, United and U.S. Air flights from San Francisco, San Jose or Los Angeles. (Delta also flies out of L.A.). Although commuter flights from San Francisco or San Jose to Monterey are expensive (\$150 to \$300), there is negligible cost if flights are added on to coast to coast flights. Consult with your travel agent to determine add on costs. Asilomar may be reached from the Monterey Airport by taxi (fare is about \$18). There is no shuttle service between the airport and Asilomar.

Bus. Greyhound provides nonstop service from the San Francisco airport to Monterey for \$18.75 one way. Bus transportation from the San Jose airport to Monterey is about \$13.00 one way. Check with your travel agent for schedules.

Car. From San Jose take Highway 101 south to Highway 156 west, then south to Monterey/Pacific Grove. For a more scenic route take Highway 17 south to Santa Cruz, then Highway 1 south to Monterey. From San Francisco you may drive down the coast on scenic Highway 1, or drive south on Highway 101 through San Jose as described above.

Rental cars. Avis is offering special rates with unlimited mileage to PSNA conferees using the Avis Worldwide Discount Number. These rates are in effect one week prior to, and following, the meeting. Be sure to mention the Avis Worldwide Discount Number: B569801. Call 1/800-331-1600 for information and reservations.

LODGING AND MEETING FACILITIES AND MEALS

All conference facilities as well as room and board will be provided by the Asilomar Conference Center. Rooms have been reserved for 150 participants. The housing rates include lodging and all meals for the duration of the conference beginning with dinner on the 27th and ending with lunch on the 1st. Lodging rates are very reasonable and you are strongly encouraged to stay at this beautiful facility.

Should you decide to stay off conference grounds either during the meeting (there is a \$6.00 per day surcharge from conferees not staying at Asilomar) or before and after the meeting, you will have to make your own arrangements. Asilomar has only a limited number of rooms available outside of the conference dates. Phone numbers for Asilomar and for information about additional lodging are given below.

Asilomar Conference Center
(408) 372-8016

Pacific Grove Chamber of Commerce
(408) 373-3304

Monterey Chamber of Commerce
(408) 648-5350

All meals will be provided by Asilomar. Please indicate if you are a vegetarian on the housing form. The banquet will offer a choice of meat, fish or vegetarian entrees.

SPECIAL EVENTS AND LEISURE ACTIVITIES

The special event this year will be a private viewing of the Monterey Bay Aquarium. This is one of Monterey's most famous attractions and is typically very crowded. However, we will have the unique opportunity to visit the aquarium during evening hours. A dessert party in front of the Kelp Forest display is planned and we are sure you will enjoy this very special event. A welcome reception is planned for Sunday the 27th, after dinner. The banquet is traditionally held on the last evening of the meeting. However, due to scheduling of the aquarium visit, it will be held on Tuesday, the 29th. Accompanying persons are welcome to attend all of these events. The Monterey Bay area has numerous activities for everyone including scuba diving, snorkeling, sea kayaking, bicycle riding, walking tours, hiking and golfing at the famous Pebble Beach Country Club. Additional attractions include driving along the beautiful Highway 1 (including the nearby 17 mile drive), Big Sur, Carmel, Cannery Row and the Point Lobos Wildlife Reserve.

**PHYTOCHEMICAL SOCIETY OF NORTH AMERICA
ANNUAL MEETING JUNE 27-JULY 1, 1993
ASILOMAR CONFERENCE CENTER, PACIFIC GROVE, CALIFORNIA**

CALL FOR ABSTRACTS

Registrants are invited to present contributed papers or posters on any topic related to phytochemistry. Please read the instructions carefully, and note the abstract deadline of **April 10, 1993**. All abstracts should be sent to **Dr. Gary W. Kuroki, DNA Plant Technology Corp., 6701 San Pablo Ave., Oakland, CA 94608; Tel. (510) 547-2395; FAX (510) 547-2817.**

GENERAL INFORMATION. Oral presentations will be limited to 15 minutes (12 min for presentation and 3 min for questions). A standard 35 mm slide projector and overhead projector will be provided. Additional equipment may be arranged by special request. Please indicate such requests on the abstract form. Due to the limited time available for presented papers, it may be necessary to restrict the number of oral presentations. If this happens, some authors may be assigned to a poster session. Should this be necessary, authors will be notified 4 weeks in advance of the meeting.

POSTERS. Individuals presenting posters will have a space 1.2 m high by 2.4 m wide (4 X 8 ft). Posters should contain lettering and photos that can be seen from a distance, and information should be arranged in vertical columns so participants can read the poster without having to repeatedly move from left to right. Materials must be anchored by push pins. **TAPE IS NOT ALLOWED AS IT DAMAGES THE BOARDS.** Please provide your own pins.

ABSTRACTS. Abstracts should be submitted on the enclosed Abstract Submission Form and must fit entirely within a rectangle 16.5 x 7.6 cm (6.5 X 3 inches) when printed using Courier or Prestige Elite 10 cpi nonproportional fonts or typing elements. You may use the box provided, or use a plain piece of white paper, but the abstract must fit within the allotted space. Two copies of the abstract (unfolded) should be forwarded to Dr. Gary W. Kuroki. Please follow the example below when preparing your abstract.

HOW TO PREPARE THE ABSTRACT, AND SUGGESTIONS TO IMPROVE THE QUALITY OF ORAL PRESENTATIONS.

David N. Kuhn and Kelsey R. Downum, Dept. of Biol. Sci., Florida International University, Miami, FL, USA 33199.

An informative abstract will contain a concise statement of the problem/objectives, experimental methods, and results. The text should cite data from representative experiments, and should state findings and draw conclusions. Statements such as, "Such-and-so will be discussed," are not recommended.

Use one idea per slide. For a 35 mm slide, the narrow dimension of your copy should be 0.7 X the long dimension; double space lines, and use bold type. If more than a 4.5" X 3" area is used for typed copy, the final slide will not be suitable!

TRAVEL ASSISTANCE. Graduate students and recent Ph.D.'s (within one year of graduation) are eligible for partial travel assistance (up to 50% of air fare or equivalent). Everyone applying for travel assistance must be a member of the PSNA, and present a paper (oral or poster) at the meeting.

BEST PAPER/POSTER AWARDS. Cash awards (100.00 each) will be given for the outstanding oral and poster presentations by graduate students or recent Ph.D.'s. Please indicate if you wish to be considered for one or both awards on the Abstract Submission Form.

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA
ANNUAL MEETING
ASILOMAR CONFERENCE CENTER, PACIFIC GROVE, CALIFORNIA
JUNE 27-JULY 1, 1993

ABSTRACT SUBMISSION FORM

Name: _____

Address: _____

City, State, Postal Code: _____ Country (non-U.S.) _____

Phone: _____ FAX number: _____

Preferred presentation medium: ORAL POSTER

Special equipment needed: _____

Please indicate if you would like to be considered for one or both of the following awards (intended for graduate students and recent Ph.D.'s):

Travel Assistance Best Paper/Poster Date of Ph.D. (if recent Ph.D.)

ABSTRACTS MUST BE RECEIVED BY APRIL 10, 1993. SEND OR FAX TO: DR. GARY W. KUROKI, DNA PLANT TECHNOLOGY CORP., 6701 SAN PABLO AVE., OAKLAND, CA 94608. Call (510) 547-2395 if you have questions. (FAX 510-547-2817).

Type your abstract in the box provided or on plain paper. Be sure that no part of the abstract extends beyond the 16.5 x 7.6 cm (6.5 x 3 in) size limit.

PSNA FINANCIAL REPORT

January, 1992 - December, 1992

RECEIPTS

Membership Dues	5,033.00
Royalties <i>RAP</i>	4,084.88
Interest on Checking	394.78
Mailing List Rental	100.00
Funds from 6-month CD	5,000.00
Total Receipts	\$14,612.66

EXPENDITURES

Advance for 1993 Meeting	5,500.00
1992 Meeting Expenses	7,000.00
1992 Speaker Travel	4,668.70
1992 PSE Travel	5,000.00
1992 Student Travel	1,490.00
1992 EC Travel	436.50
1992 Best paper/poster awards	300.00
Executive Committee Expenses	
Secretarial	2,000.00
Editor-In-Chief	2,000.00
Treasurer	246.82
Checking Service Charges	45.22
Total Expenditures	\$28,687.24

CHECKING SUMMARY

Receipts	\$14,612.66
Expenditures	\$28,687.24
Net Gain (Loss)	(\$14,074.58)

ASSETS

Checking	\$ 588.78
Savings	25,831.91
Total	\$26,420.69



MEETINGS AND PROGRAMS OF INTEREST

TWELFTH ANNUAL SYMPOSIUM: CURRENT TOPICS IN PLANT BIOCHEMISTRY, MOLECULAR BIOLOGY AND PHYSIOLOGY: University of Missouri-Columbia, March 31-April 3, 1993. Topics: Plant Protein Phosphorylation, Plant Protein Kinases and Phosphatases, Plant G-Proteins. For further information contact Dr. Doug Randall, 117 Schweitzer Hall, University of Missouri-Columbia, Columbia, MO 65211. (Tel. 314-882-7796, FAX 314-882-5635).

MOLECULAR GENETICS OF PLANT-MICROBE INTERACTIONS: East Brunswick, New Jersey, April 21-25, 1993. For further information, contact Rutgers's Center for Agricultural Molecular Biology, Rutgers, The State University of New Jersey, Cook College, New Brunswick, NJ 08903-0231. (Tel. 908-932-8165; FAX 908-932-6535).

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA: Asilomar, CA, June 27-July 1, 1993. The symposium topic will be "Genetic Engineering of Plant Secondary Metabolites." For further information contact Dr. Brian E. Ellis, Department of Plant Science, University of British Columbia, Vancouver, B.C., Canada V6T 2A2 (Tel. 604-228-3451) or Dr. Gary Kuroki, DNA Plant Technology Corp., 6701 San Pablo Ave., Oakland, CA 94608-1239 (Tel. 510-547-2395, FAX 510-547-2817).

SEVENTH INTERNATIONAL SYMPOSIUM ON IRON NUTRITION AND INTERACTIONS IN PLANTS: Zaragoza, Spain, June 27-July 2, 1993. The aim of this symposium is to bring together scientists from a broad range of disciplines related to iron nutrition and interactions in plants in order to provide opportunities for researchers to exchange new knowledge, ideas, experiences and techniques. For further information contact Dr. Javier Abadia, Aula Dei Experimental Station (SIC, Apdo 202, 50080 Zaragoza, Spain (FAX (+34)-76-575620).

PLANT GROWTH REGULATOR SOCIETY 20TH ANNUAL MEETING: St. Louis, MO, August 6-9, 1993. The meeting will feature symposia and research reports. A symposium on "Plant Growth Regulators in Post-harvest Biology and Technology of Horticultural Crops" will be organized by A. Kader, Univ. California, Davis. A second symposium on "The Role of Plant Growth Regulators in the Development and Reproduction of Cereal Plants" will be organized by D. Ho, Washington Univ., St. Louis. Research reports are invited in all areas of plant growth regulation and will be published in the Society's proceedings. Prizes of \$300 and \$100 will be awarded for the two best student papers. For further information, contact Dr. Louise Ferguson, Program Chair, Univ. California, Kearney Agricultural Research Center, 9240 S. Riverbend Ave., Parlier, CA 93648. (Tel. 209-891-2500).

XV INTERNATIONAL BOTANICAL CONGRESS: Tokyo, Japan, August 28-September 3, 1993. The scientific program will include about 240 symposia and more than 1,000 posters in the following divisions: 1. Systematics and Evolution, 2. Structure and its Dynamics, 3. Phytochemistry and Natural Products, 4. Metabolism and Bioenergetics, 5. Developmental Botany, 6. Ecology and Environmental Botany, 7. Genetics, 8. Biotechnology and Breeding. For further information, contact the Congress Secretariat, XV International Botanical Congress Tokyo, c/o Department of Botany, Faculty of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

FOURTH INTERNATIONAL CONGRESS ON PLANT MOLECULAR BIOLOGY: Amsterdam, The Netherlands. June 19-24, 1994. The Congress will be composed of Plenary Sessions, Concurrent Symposia, Poster Sessions and Interactive Workshops. The first program announcement will be mailed in the fall of 1992. For further information, contact the Congress Secretariat, RAI Organisatie Bureau Amsterdam by Europaplein 12, 1078 GZ Amsterdam, The Netherlands. (Tel. 31-0-20-549-12-12; FAX 31-0-20-646-44-69).

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E. VILLARREAL-ROSALES, P. METZGER & E. CASADEVALL (France), Ether lipid production in relation to growth in *Botryococcus braunii*.

Indexed/Abstracted in: *Current Awareness in Biological Sciences (CABS), Curr Cont ASCA, Chem Abstr, BIOSIS Data, PASCAL-CNRS Data, CAB Inter, Cam Sci Abstr, Curr Cont/Agri Bio Env Sci, Curr Cont/Life Sci, Curr Cont Sci Cit Ind, Curr Cont SCISEARCH Data*

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Volume 33
Number 1

June 1993

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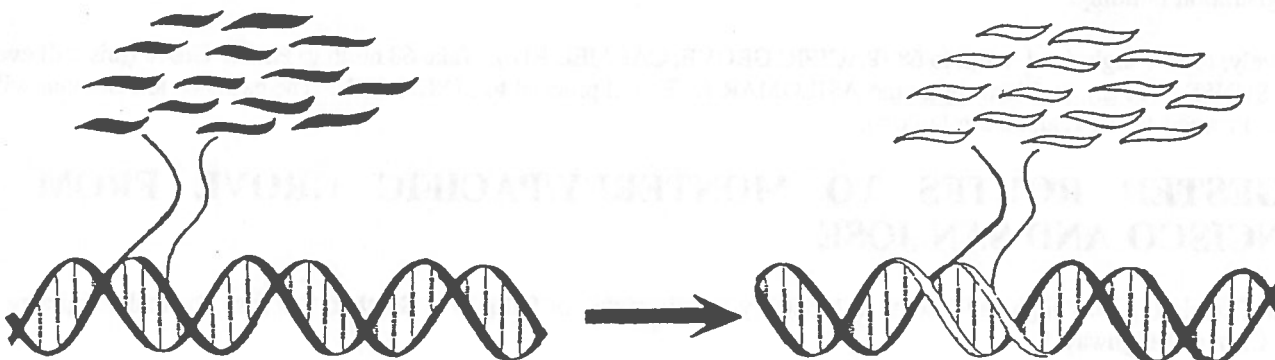
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The **Phytochemical Society of North America** is a nonprofit scientific organization whose membership (currently over 400) is open to anyone with an interest in phytochemistry, the role of plant substances, and related fields. Annual membership dues are \$20.00 for regular members and \$10.00 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. 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 Society secretary. Annual dues and changes in addresses should be sent to the Society treasurer.

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA NEWSLETTER



JUNE, 1993

VOLUME 33, NUMBER 1

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IMPORTANT NOTICE TO MEMBERS WHO WILL ATTEND THE PSNA MEETING IN ASILOMAR

Each year a number of members arrive at the meeting without their programs. Each year extra copies of the summer issue of the newsletter are printed and available for forgetful members. Because of budget constraints, this year members will be charged \$5.00 for a second copy of the program. Remember to bring your program.

THANKS TO DONORS

Financial support for the Asilomar meeting from the following companies and organizations is acknowledged with appreciation by the PSNA:

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DIRECTIONS TO ASILOMAR FROM MONTEREY

From highway 1 take the DEL MONTE AVE exit into Monterey. When you get to the marina veer right onto LIGHTHOUSE AVE. Proceed through the tunnel and continue on. When you reach David Ave., LIGHTHOUSE will turn into CENTRAL AVE. Follow CENTRAL until it intersects with LIGHTHOUSE again. Turn right onto LIGHTHOUSE until you reach ASILOMAR AVE. Turn left onto ASILOMAR AVE. and proceed south until you reach SINEX AVE. The entrance to Asilomar will be on your right. Proceed to the registration building.

Alternatively, follow highway 1 south to 68 (PACIFIC GROVE, CARMEL RD.). Take 68 north to Pacific Grove (this will eventually turn into SUNSET AVE.), and turn right onto ASILOMAR AVE. and proceed to SINEX AVE. The entrance to Asilomar will be on your left. Proceed to the registration building.

SUGGESTED ROUTES TO MONTEREY/PACIFIC GROVE FROM SAN FRANCISCO AND SAN JOSE

From San Francisco follow highway 1 south to Monterey (scenic route), or follow 101 South to San Jose, then take highway 17 west to Santa Cruz and highway 1.

For a faster route, take 101 south from San Francisco through San Jose to 156 west (do not take 156 east). This will take you to highway 1. Proceed on 1 south to Monterey.

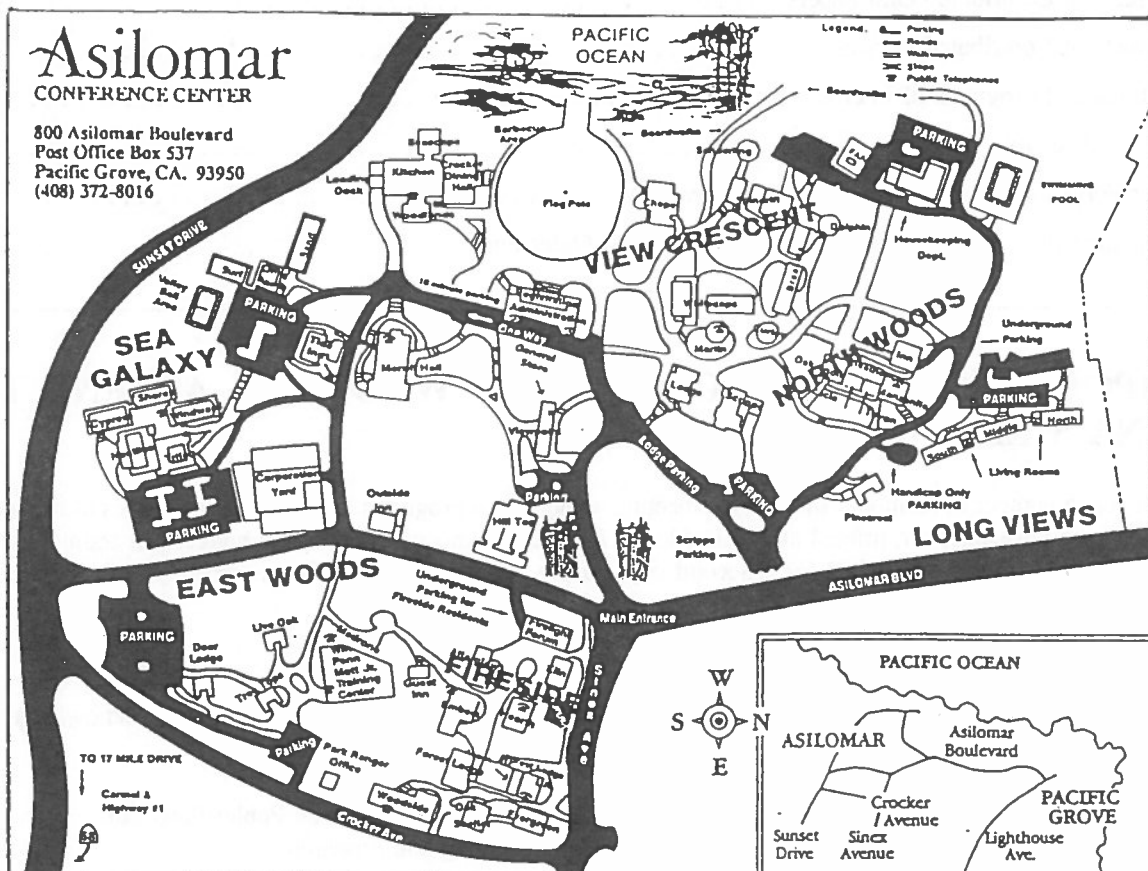
IMPORTANT PHONE NUMBERS:

Asilomar Conference Center
408/372-8016

Monterey Chamber of Commerce
408/648-5350

Pacific Grove Chamber of Commerce
408/373-3304

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GENETIC ENGINEERING OF PLANT SECONDARY METABOLISM

1993 PSNA ANNUAL MEETING

SUNDAY, JUNE 27

- 3:00 Registration
6:00 Dinner
7:00 Reception
(Posters may be mounted anytime after 3:00 p.m.)

MONDAY, JUNE 28

- 7:30 Breakfast

MORNING SESSION I - Brian Ellis, Chair

- 8:15 Welcome - Jim Saunders, President, PSNA

- 8:30 **Symposium Paper 1** - THE BIOSYNTHESIS OF TROPANE ALKALOIDS: STUDIES LEADING TOWARDS THE GENETIC ENGINEERING OF AN ALKALOID PATHWAY. Richard J. Robins, Nicholas J. Walton, Adrian J. Parr, Michael J.C. Rhodes, Anthony Michael, John D. Hamill and Sylvie Rabot

- 9:20 **Symposium Paper 2** - MOLECULAR GENETIC TECHNIQUES APPLIED TO THE ANALYSIS OF ENZYMES OF ALKALOID BIOSYNTHESIS. Toni M. Kutchan

- 10:10 Coffee Break

- 10:30 Contributed Paper 1 - CYTOCHEMICAL AND IMMUNOLocalIZATION OF POLYAMINES IN WHITE SPRUCE IN TISSUE CULTURE. Vindhya Amarsinghe and John E. Carlson

- 10:45 Contributed Paper 2 - MOLECULAR GENETICS OF THE BETALAIN PATHWAY IN HIGHER PLANTS AND FUNGI. Jean-Pierre Zryd, Ursula Hinz, Laurence Bindschaedler, Lukas Müller

- 11:00 Contributed Paper 3 - THE PHENOLIC DOMAIN OF SUBERIN. Mark A. Bernards, Jaroslav Zajicek and Norman G. Lewis

- 11:15 Contributed Paper 4 - SUBSTITUTED TRUXILLIC ACIDS: SYNTHESIS AND ANALYSIS IN PLANT CELL WALLS. Jean I.M. Rajaonarivony, Norman G. Lewis, and G.H.N. Towers

- 12:00 Lunch (all posters should be up by 1:20)

AFTERNOON SESSION I - Jonathan Poulton, Chair

- 1:20 **Symposium Paper 3** - POLYKETIDE ANTIBIOTICS IN *STREPTOMYCES*. Richard W. Plater and William R. Strohl

- 2:10 Contributed Paper 5 - CHANGES IN FLAVAN-3-OL COMPOSITION OF SAINFOIN LEAF POLYMERS DURING LEAF DEVELOPMENT. Mohammed R. Koupai-Abyazani, John McCallum, Alister D. Muir, Bruce A. Bohm, G.H.N. Towers, and Margaret Y. Gruber

- 2:25 Contributed Paper 6 - EVIDENCE FOR A GENE CLUSTER INVOLVING TRICHOHECENE PATHWAY BIOSYNTHETIC GENES IN *FUSARIUM SPOROTRICHIOIDES*. Thomas M. Hohn, Susan P. McCormick, and Anne E. Desjardins

- 2:40 Contributed Paper 7 - INVESTIGATION OF THE FACTORS CONTROLLING PTEROCARPAN STEREOCHEMISTRY IN LEGUMES. Nancy L. Paiva and Richard A. Dixon
- 2:55 Contributed Paper 8 - PHARMACOLOGY OF GINGER: MODULATION OF EICOSANOID PRODUCTION. K.C. Srivastava and T. Mustafa
- 3:10 Coffee Break

Best Paper Competition

- 3:30 Contributed Paper 9 - BENZYLIC ETHER REDUCTASES IN *FORSYTHIA* SP.: ENZYMOLOGY AND MECHANISM OF ACTION. Albena Dinkova, Alex Chu, Laurence B. Davin and Norman G. Lewis
- 3:45 Contributed Paper 10 - CLONING AND REGULATION OF FLAVONOL 3-SULFOTRANSFERASE FROM *FLAVERIA BIDENTIS*. Sirinart Ananvoranich, Patrick Bulick and Ragai K. Ibrahim
- 4:00 Contributed Paper 11 - FUNGAL PATHOGENS OF PEA HAVE EVOLVED BIOCHEMICALLY DIFFERENT, BUT HIGHLY SPECIFIC CYTOCHROME P450S TO DETOXYFY THE PHYTOALEXIN PISATIN. Helga L. George and Hans D. VanEtten
- 4:15 Contributed Paper 12 - THE ISOLATION OF FUNCTIONAL RNA FROM WOUNDED GRAND FIR (*ABIES GRANDIS*) STEMS FOR THE STUDY OF MONOTERPENE BIOSYNTHESIS. Christopher L. Steele, Efraim Lewinsohn and Rodney Croteau
- 4:30 Contributed Paper 13 - A PHAGOSTIMULANT FUNGAL METABOLITE FOR THE WESTERN SUBTERRANEAN TERMITE *RETICULITERMES HESPERUS* BANKS. Brice A. McPherson, David L. Wood, and Isao Kubo
- 5:00 Contributed Paper 14 - GENETIC VARIABILITY OF SINAPINE IN *BRASSICA* AND *SINAPIS* SPECIES. Wang, X., McGregor, D.I., and Downey, R.K.
- 5:15 Contributed Paper 15 - LARVAL DEFENSE IN *LEMA* (CHRYSOMELIDAE: CRIOCERINAE). Timothy C. Morton and Fredric Vencil
- 6:00 Dinner

POSTER SESSION

7:00 - 10:00 p.m.

Authors for Posters 1-13 are asked to be present at their posters from 7:00 - 8:30 p.m.

(* denotes posters that are part of the Best Poster Competition)

- * Poster 1 - VASCULAR DEVELOPMENT AND LIGNIFICATION IN *ARABIDOPSIS THALIANA*. D.P. Dharmawardhana, B.E. Ellis and J.E. Carlson
- * Poster 2 - A NEW APPROACH FOR TROPANE ALKALOID OVERPRODUCTION FROM HAIRY ROOTS OF *HYOSCYAMUS MUTICUS*. Fabricio Medina-Bolivar and Hector Flores
- * Poster 3 - ANTIBIOTIC ACTIVITY OF THE THIARUBRINES ISOLATED FROM *AMBROSIA CHAMISSONIS* ROOTS AND ROOT CULTURES. Shona Ellis, Zyta Abramowski, and G.H. Neil Towers
- * Poster 4 - PURIFICATION AND INDUCTION OF PHENYLALANINE AMMONIA-LYASE FROM THE CORN SMUT FUNGUS *USTILAGO MAYDIS*. Seong Hwan Kim, James W. Kronstad and Brian E. Ellis

- * Poster 5 - EXPRESSION OF PHENYLALANINE AMMONIA-LYASE IN ELICITOR-TREATED *PINUS BANKSIANA* CELL SUSPENSION CULTURES. Monica L. Lam and Brian E. Ellis
- * Poster 6 - GENETIC MANIPULATION AND PRODUCTION OF ALIZARIN IN *RUBIA PEREGRINA*. L. A.U.H. Lodhi, S.A. Coomber and B.V. Charlwood
- * Poster 7 - CYTOKININ-STIMULATED PUTRESCINE SYNTHESIS IN CULTURED GRAPE ROOTS. Mary Lou Mendum and Douglas O. Adams
- * Poster 8 - INVOLVEMENT OF PLANT CELL MEMBRANE PROTEINS DURING EARLY DEFENSE RESPONSES OF ALFALFA CULTIVARS TO *COLLETOTRICHUM TRIFOLII*. Liliana Di Nola-Baron and Nichole R. O'Neill
- * Poster 9 - DIHYDROFLAVANOL AND 3,4-DIOL REDUCTASE ACTIVITIES IN SAINFOIN LEAVES. Santokh Singh, John McCallum, Mohammed R. Koupai-Abyazani, G.H. Neil Towers, Alister D. Muir, Bruce A. Bohm and Margaret Y. Gruber
- * Poster 10 - PHENOLICS AND ACYLATED FLAVONOL GLYCOSIDES FROM PINES THAT INHIBIT GYPSY MOTH LARVAE DEVELOPMENT. Clifford Beninger, Mamdouh Abou-Zaid and Blair Helson
- * Poster 11 - TANNINS FROM BARK OF *ACACIA* TREES. Karin E. Readel, David S. Seigler, H. David Clarke, John E. Ebinger and Juan F. Hernandez
- * Poster 12 - 4-COUMARATE:CoA LIGASE FROM LOBLOLLY PINE XYLEM: ISOLATION, CHARACTERIZATION AND COMPLEMENTARY DNA CLONING. Kui Shin Voo, Ross W. Whetten, David M. O'Malley, and Ronald R. Sederoff
- * Poster 13 - DETERMINATION OF SINAPINE FOR RAPESEED BREEDING. Wang, X., McGregor, D.I., and Downey, R.K.
- Poster 14 - SULFATED FLAVONOL-SPECIFIC BINDING TO NUCLEAR PROTEINS IN *FLAVERIA*. Jacques Grandmaison and Ragai Ibrahim
- Poster 15 - DIFFERENTIAL HYBRIDIZATION STRATEGY FOR THE CLONING OF PLANT ISOFLAVONE PRENYLTRANSFERASES (IPTs). Sylvie Attucci, Patrick Gulick & Ragai Ibrahim
- Poster 16 - PICOTAG METHOD FOR ANALYSIS OF FREE AMINO ACIDS IN PLANTS: SOME APPLICATIONS. Ramon A. Razal, Norman G. Lewis, and G.H. Neil Towers.
- Poster 17 - CHARACTERIZATION OF UDP-GLUCOSE: CYANIDIN 3-O-GLUCOSYLTRANSFERASE IN *VITIS VINIFERA* CELL SUSPENSION CULTURES. Chi Bao Do and François Cormier
- Poster 18 - EXPRESSION OF TRICHODIENE SYNTHASE FROM *FUSARIUM SPOROTRICHIOIDES* IN TRANSFORMED TOBACCO CELL SUSPENSION CULTURES. Michael Zook, Thomas M. Hohn, and Ray Hammerschmidt
- Poster 19 - ISOPENTENYL DIPHOSPHATE ISOMERASE AND FARNESYL DIPHOSPHATE SYNTHASE ACTIVITIES IN ELICITED *CINCHONA ROBUSTA* CELL SUSPENSION CULTURES. A. Ramos-Valdivia, R. van der Heijden and R. Verpoorte
- Poster 20 - IMMUNOAFFINITY PURIFICATION OF THE GLUCOSINOLATE BIOSYNTHETIC ENZYME, THIOHYDROXIMATE S-GLUCOSYLTRANSFERASE, FROM *BRASSICA*. J.W.D. GrootWassink, D.W. Reed and A.D. Kolenovsky
- Poster 21 - INVESTIGATION OF ALTERED SECONDARY METABOLISM BY TRYPTOPHAN DECARBOXYLASE (TDC)-EXPRESSING TOBACCO SEEDLINGS. Juan Basurco & Vincenzo de Luca
- Poster 22 - IMPROVEMENT OF CANOLA MEAL BY REDIRECTION OF TRYPTOPHAN AWAY FROM MUSTARD OIL GLYCOSIDES. Supa Chavadej, Normand Brisson and Vincenzo De Luca

Poster 23 - ISOLATION AND CHARACTERIZATION OF A FLAVONOL SYNTHASE cDNA CLONE FROM PETUNIA. Timothy A. Holton, Filippa Brugliera and Yoshikazu Tanaka

Poster 24 - ELICITOR REGULATION OF ENZYME ACTIVITIES FROM DIFFERENT METABOLIC PATHWAYS IN *CATHARANTHUS ROSEUS* CELL CULTURES. Paulo R. H. Moreno, Robert van der Heijden and Robert Verpoorte

Poster 25 - A NEW ANTIBIOTIC FROM *RHUS GLABRA*. Geeta Saxena, G.H.N. Towers and R.E. W. Hancock

Poster 26 - A NEW C-GLYCOSYLFLAVONE FROM THE FERN *ASPLENIUM VIVIPARUM*. Filippo Imperato

Poster 27 - CLONING AND CHARACTERIZATION OF 4-COUMARATE:COENZYME A LIGASE (4CL) GENES FROM POPLAR AND *ARABIDOPSIS*. Sandra Allina, Diana Lee, and Carl J. Douglas

Poster 28 - NEW LIMONOIDS FROM THE WOOD AND LEAVES OF *CHISOCHETON MICROCARPUS* AND *CHISOCHETON MACROPHYLLUS* (MELIACEAE). P.J. Gunning, L.B. Jeffs, M.B. Isman and G.H.N. Towers

Poster 29 - ELECTRICALLY INDUCED PROTOPLAST FUSION FOR PRODUCTION OF LEPTINES IN THE LEAVES OF CULTIVATED POTATO. Jianping Cheng and James A. Saunders

Poster 30 - EVALUATION OF PHENOLICS AND ACYLATED FLAVONOL GLYCOSIDES FROM *ACER* AS POTENTIAL SOURCE OF RESISTANCE TO FOREST TENT CATERPILLAR. Mamdouh Abou-Zaid and Blair Helson

Poster 31 - POLYACETYLENE INDUCTION IN SAFFLOWER. Lauralyn Beaverson and K.R. Downum

Poster 32 - EFFECTS OF OZONE ON PHENYLPROPANOID METABOLISM IN SOYBEAN. Fitzgerald L. Booker

Poster 33 - ISOLATION, IDENTIFICATION, AND QUANTITATION OF C₁₆, C₁₈, AND C₂₀-ESTERS OF P-COUMARIC AND FERULIC ACIDS IN THE LATEX OF SWEETPOTATO CULTIVARS. Maurice C. Snook

Poster 34 - NEW FLAVONOL-C-GLYCOSIDES FROM CORN (*Zea mays* L.) FOR THE CONTROL OF THE CORN EARWORM (*Helicoverpa* *Zea*). Maurice C. Snook

Poster 35 - CHARACTERIZATION OF THE PEA MITOCHONDRIAL PHOSPHATE TRANSPORTER. Cecilia A. McIntosh and David J. Oliver

Poster 36 - ISOLATION OF LIMONOATE DEHYDROGENASE (LDH) *ARTHROBACTER GLOBIFORMIS*. Charles Suhayda, Shin Hasegawa, Terres Ronneberg and Chi Fong

Poster 37 - FERULIC ACID 5-HYDROXYLASE. Lloyd Yu

Poster 38 - THE LATE BIOSYNTHETIC STEPS OF THE 6a-HYDROXY PTEROCARPAN PISATIN. Greg DiCenzo and Hans VanEtten

Poster 39 - TEMPORAL AND SPATIAL EXPRESSION OF AMYGDALIN HYDROLASE IN ROSACEOUS STONE FRUITS (*PRUNUS SEROTINA* AND *P. DOMESTICA*). Chun Ping Li and Jonathan E. Poulton

Poster 40 - TISSUE AND SUBCELLULAR LOCALIZATION OF PRUNASIN HYDROLASE IN YOUNG STEMS AND LEAVES OF BLACK CHERRY (*PRUNUS SEROTINA* EHRH.). Elisabeth Swain and Jonathan E. Poulton

Poster 41 - PUTATIVE FAD-BINDING SITE OF *PRUNUS SEROTINA* (R)-(+)-MANDELONITRILE LYASE AS DEDUCED FROM THE NUCLEOTIDE SEQUENCE OF A FULL-LENGTH cDNA CLONE. I-Ping Cheng and Jonathan E. Poulton

Poster 42 - ANTHOCYANIN CONTENT AND ANTHOCYANOPLAST FORMATION IN RED CABBAGE SEEDLINGS IN RESPONSE TO MINERAL NUTRIENT DEFICIENCIES. C. Nozzolillo and M. Hodges

Poster 43 - VOLATILE COMPOUNDS OF THE LIPOXYGENASE PATHWAY INVOLVED IN *ASPERGILLUS FLAVUS*/COTTON PLANT INTERACTIONS. H.J. Zeringue, Jr.

TUESDAY, JUNE 29

7:30 Breakfast

MORNING SESSION II - Laurence Davin, Chair

8:30 **Symposium Paper 4** - GENETIC ENGINEERING OF ANTHOCYANINS. Neal Courtney-Gutterson

9:20 **Symposium Paper 5** - DIVERSE PATTERNS OF ENDOGENOUS CHALCONE SYNTHASE GENE EXPRESSION AND ANTHOCYANIN PIGMENTATION CAN BE ELICITED BY DEVELOPMENTAL IMPRINTS IMPOSED ON AN ECTOPIC CHALCONE SYNTHASE TRANSGENE. Richard Jorgensen

10:10 Coffee Break

10:30 Contributed Paper 16 - BIOGENETIC INTERPRETATION OF PIGMENT METABOLISM IN ROSES AND CARNATION FLOWER MUTANTS. Maurice Jay, Nadine Chirol & Jean-Phillippe Biolley

10:45 Contributed Paper 17 - POLLINATION AND WOUNDING STIMULATE KAEMPFEROL ACCUMULATION IN STIGMAS OF *PETUNIA HYBRIDA*. Thomas Vogt, Peggy Pollak and Loverine Taylor

11:00 Contributed Paper 18 - FLORAL FLAVONOIDS AND THEIR MODIFICATION IN LISIANTHUS. Kathy E. Schwinn, Kevin M. Davies, Robyn M. Miller, Simon C. Deroles, J. Marie Bradley, David G. Manson and Nigel K. Given

11:15 Contributed Paper 19 - COMPLEMENTATION OF THE ANTHOCYANIN-2 MUTATION OF *PETUNIA*. Kevin M. Davies, Kathy E. Schwinn, David G. Manson, Robyn M. Miller, Simon C. Deroles and J. Marie Bradley

12:00 Lunch

AFTERNOON SESSION II - Vincenzo DeLuca, Chair

1:20 **Symposium Paper 6** - ENGINEERING ALTERED GLUCOSINOLATE BIOSYNTHESIS BY TWO ALTERNATIVE METABOLIC PATHWAYS. Ragai Ibrahim and Vincenzo DeLuca

2:00 Contributed Paper 20 - BIOSYNTHESIS OF ACYLGLUCOSE BY *LYCOPERSICON PENNELLII* GLANDULAR TRICHOMES. G.S. Ghangas and J.C. Steffens

2:15 Contributed Paper 21 - ASPECTS OF HYDROXYMETHYLGLUTARYL-COENZYME A METABOLISM IN *CATHARANTHUS ROSEUS*. Robert van der Heijden, Veronika de Boer-Hlupá, Robert Verpoorte and Johann A. Buine

- 2:30 Contributed Paper 22 - PARTIAL PURIFICATION AND CHARACTERIZATION OF A WOUND-INDUCIBLE DITERPENE CYCLASE FROM GRAND FIR. Brigitte Stofer Vogel, Roy LaFever and Rodney Croteau
- 2:45 Contributed Paper 23 - MODULATION OF MONOTERPENE CYCLASE ACTIVITIES IN GRAND FIR CALLI BY PLANT GROWTH REGULATORS AND AUTOCLAVED FUNGAL EXTRACTS. Efraim Lewinson, Eric Worden, and Rodney Croteau
- 3:00 Coffee Break
- 3:20 Contributed Paper 24 - MEASUREMENT OF THE RATE CONTROLLING STEPS OF MONOTERPENE BIOSYNTHESIS IN PEPPERMINT. David McCaskill and Rodney Croteau
- 3:35 Contributed Paper 25 - MONOTERPENE CYCLASES AND THE REGULATION OF MONOTERPENE BIOSYNTHESIS IN PEPPERMINT. J. Gershenzon, W.R. Alonso and R. Croteau
- 3:50 Contributed Paper 26 - PROMOTER ANALYSIS OF AN HMGR GENE FROM *CAMPTOTHECA*. Ronald J. Burnett, Igancio E. Maldonado-Mendoza and Craig L. Nessler
- 4:05 Contributed Paper 27 - ACCUMULATION AND BIOSYNTHESIS OF CAMPTOTHECIN AND 10-OH-CAMPTOTHECIN IN *CAMPTOTHECA ACUMINATA* PLANTS. Melina Lopez-Meyer, David D. Henning and Thomas D. McKnight
- 4:30 PSNA *Business Meeting*
- 6:00 Banquet and awards

WEDNESDAY, JUNE 30

- 7:30 Breakfast

MORNING SESSION III - Kelsey Downum, Chair

- 8:30 **Symposium Paper 7** - GENETIC MANIPULATION OF LIGNIN AND PHENYLPROPANOID-DERIVED COMPOUNDS INVOLVED IN INTERACTIONS WITH MICROORGANISMS. Richard A. Dixon, Maria J. Harrison, Weiting Ni, Abraham Oommen and Nancy L. Paiva
- 9:20 **Symposium Paper 8** - REGULATION OF LIGNIN BIOSYNTHESIS IN LOBLOLLY PINE. Ross Whetten, David O'Malley, Wuli Boa, Kui Shin Voo, Wei Wei Liu, John MacKay, Chen-Loung Chen, and Ron Sederoff
- 10:10 Coffee Break
- 10:30 Contributed Paper 28 - MANIPULATION OF GENES INVOLVED IN LIGNIN BIOSYNTHESIS. Wolfgang Schuch
- 10:45 Contributed Paper 29 - LIGNIFICATION IN CELL CULTURES OF *PINUS TAEDA*. Mark A. Bernards, Maysa Furlan and Normal G. Lewis
- 11:00 Contributed Paper 30 - PHENYLPROPANOID COUPLING ENZYMES IN LIGNAN/LIGNIN SYNTHESIS: NON-STEREOSELECTIVE COUPLING. Laurence B. Davin, Paul W. Paré and Norman G. Lewis
- 11:15 Contributed Paper 31 - SOLUBILIZATION AND PARTIAL PURIFICATION OF A CONIFERYL ALCOHOL STEREOSELECTIVE COUPLING ENZYME. Paul W. Paré, Laurence B. Davin and Normal G. Lewis

12:00 Lunch

AFTERNOON SESSION III - Jonathan Gershenzon, Chair

- 1:20 **Symposium Paper 9**, SYSTEM FOR ENVIRONMENTAL EVALUATION OF TRANSGENIC PLANTS. Sally L. McCammon
- 2:10 Contributed Paper 32 - ANTHRAQUINONE CONSTITUENTS OF *CASSIA ALATA*. Adetunji J. Aladesanmi, Erastus O. Ogunti and Joseph J. Hoffmann
- 2:25 Contributed Paper 33 - MOLECULAR ANALYSIS OF MAJOR LATEX PROTEIN GENE FAMILY SUPPORTS THE TRIPLOID-HYBRID ORIGIN OF OPIUM POPPY. Craig L. Nessler
- 2:40 Contributed Paper 34 - NATURALLY OCCURRING TYROSINASE INHIBITORS. Ikuyo Kinst-Hori, Yoshihiro Yokokawa, and Isao Kubo
- 2:55 Contributed Paper 35 - LOCATION ON TREE INFLUENCES COMPOSITION OF TRITERPENOIDS IN GRAPEFRUIT EPICUTICULAR WAX. Harold E. Nordby and Roy E. McDonald
- 3:10 Coffee Break
- 3:30 Contributed Paper 36 - TAXANE METABOLITES. Alex Chu, Maysa Furlan, Jaroslav Zacijek, Norman G. Lewis and Rodney Croteau
- 3:45 Contributed Paper 37 - DEVELOPMENTAL AND CELLULAR REGULATION OF TAXANE PRODUCTION IN FIELD AND CULTURED SHOOTS OF *TAXUS* SPP. Eric Zeldin, David Ellis, William Russin, Marion Brodhagen, Ray Evert and Brent McCown
- 4:00 Contributed Paper 38 - TAXOL FROM *AGROBACTERIUM*-TRANSFORMED ROOT CULTURES. Y. Yoke Plaut-Carcasson, Lella Benkrima, Margaret Dawkin, Lihong Sun, Nicholas Wheeler, Alvin Yanchuk, Santosh Misra
- 4:15 Contributed Paper 39 - A ¹³C-PULSE-LABEL STUDY OF SESQUITERPENOID PHYTOALEXIN BIOSYNTHESIS IN *GOSSYPIUM HIRSUTUM* COTYLEDONS. Piotr M. Gorski, Thayne E. Vickstrom, Margaret L. Pierce, and Margaret Essenberg
- 4:30 Contributed Paper 40 - CHARACTERIZATION OF ENZYMES INVOLVED IN BIOSYNTHESIS OF LONG CHAIN LIQUID WAXES IN JOJOBA (*SIMMONDSIA CHINENSIS*) SEEDS. Jim Metz, Kathy Lardizabal and Mike Lassner
- 5:30 Dinner
- 7:00 Monterey Bay Aquarium
"Kelp Forest Dessert Party"

THURSDAY, JULY 1

7:30 Breakfast

MORNING SESSION IV - Gary Kuroki, Chair

- 8:30 **Symposium Paper 10** - ANALYSIS AND MODIFICATION OF POLYPHENOLOXIDASE EXPRESSION. J.C. Steffens
- 9:20 **Symposium Paper 11** - GENETIC ENGINEERING OF MONOTERPENE METABOLISM IN *MENTHA*. Rodney Croteau

- 10:10 Coffee Break
- 10:30 Contributed Paper 41 - CHARACTERIZATION AND INDUCTION OF TWO CYTOCHROME P-450-DEPENDENT MONOOXYGENASES INVOLVED IN RESIN ACID BIOSYNTHESIS IN GRAND FIR. Christoph Funk and Rodney Croteau
- 10:45 Contributed Paper 42 GENETIC MANIPULATION OF PHYTOALEXIN COMPOSITION IN *GOSSYPIUM*: EFFECTS ON DISEASE RESISTANCE. Alois A. Bell and M.E. Mace
- 11:00 Check-out
- 12:00 Lunch and Farewell



GENETIC ENGINEERING OF PLANT SECONDARY METABOLISM

SYMPOSIUM ABSTRACTS

Symposium Paper 1 - Monday, 8:30

THE BIOSYNTHESIS OF TROPANE ALKALOIDS: STUDIES LEADING TOWARDS THE GENETIC ENGINEERING OF AN ALKALOID PATHWAY

Richard J. Robins, Nicholas J. Walton, Adrian J. Parr, Michael J.C. Rhodes, Anthony Michael, John D. Hamill and Sylvie Rabot. Institute of Food Research, AFRC, Norwich Laboratory, Norwich, U.K. NR4 7UA

Tropane alkaloids are produced in members of the Solanaceae. Two of them, hyoscyamine and hyoscyne, have importance in pharmaceutical applications as muscle relaxants or sedatives. Currently these are prepared from field grown plants of various *Datura* species and hybrids, or of *Duboisia*. The biosynthesis of the tropane ring involves a pathway from arginine and/or ornithine, forming the common intermediate, tropane. This compound is stereospecifically reduced to either tropine, used to form the tropine esters - including hyoscyamine and hyoscyne, or pseudotropine, used to form the recently-identified calystegins. These polynhydroxyalkaloids may have anti-glucosidase activity.

Most natural sources produce a relatively complex spectrum of alkaloids. In addition, hyoscyne is in greater demand but its precursor, hyoscyamine, tends to be obtained in greater yield. Thus, this pathway offers a realistic target for genetically engineering the quality and quantity of the alkaloids produced.

Recent studies on the tropane alkaloids have identified the following catalytic steps as apparently crucial to limiting the flow of metabolites into the tropane aromatic esters, hyoscyamine and hyoscyne. These are:

1. The supply of putrescine from arginine/ornithine;
2. The methylation of putrescine to form N-methylputrescine;
3. The esterification of tropine with an aromatic acid;
4. The introduction of the 6,7-epoxide in hyoscyne.

The present state of knowledge of the physiological processes involved in the regulation of the pathway and the purification and properties of the pertinent enzymes will be presented. Data indicating what steps should be amenable to genetic manipulation will be considered. Progress in using the expression of heterologous genes in the formation of the related alkaloid, nicotine, will be discussed.

The metabolism of hyoscyamine to scopolamine has been examined in detail by Yamada and colleagues at Kyoto University, Japan. A gene coding for this conversion has been isolated and expressed in tobacco and *Atropa belladonna*. The effect this expression has on the accumulation in cultures and whole plants of these alkaloids will be presented.

Symposium Paper 2 - Monday, 9:20

★ Elegant work - dynamic speaker.

MOLECULAR GENETIC TECHNIQUES APPLIED TO THE ANALYSIS OF ENZYMES OF ALKALOID BIOSYNTHESIS

Toni M. Kutchan, Laboratorium für Molekulare Biologie, Universität München, Karlstrasse 29, 8000 München 2, Germany

Many of the enzymes of alkaloid biosynthesis are not only regulatory enzymes, but are also catalyzing chemical reactions that are either not reproducible in organic chemistry or are not to be found elsewhere in nature outside of the plant species which produces that particular alkaloid. Traditionally, these enzymes have been very difficult to purify in quantities that permit a detailed biochemical analysis of the reaction mechanism. Through the use of molecular genetic techniques, these enzymes can be over-expressed in heterologous systems and analyzed in more detail at the biochemical level.

The berberine bridge enzyme, a key enzyme of benzophenanthridine alkaloid biosynthesis, has been analyzed at the level of cDNA. The enzyme has been heterologously expressed in insect cell cultures using a baculovirus-based expression system. In this manner, milligram quantities of homogeneous enzyme have been purified and used in a biochemical analysis of the reaction mechanism.

Symposium Paper 3 - Monday, 1:20

POLYKETIDE ANTIBIOTICS IN *STREPTOMYCES*

Richard W. Plater and William R. Strohl. Department of Microbiology, The Ohio State University

Streptomycetes are mycelial bacteria that undergo morphological and biochemical development somewhat analogous to that of filamentous fungi, during which various members synthesize approximately seventy percent of the naturally-derived antibiotics known today. Among the antibiotics produced by streptomycetes are several polyketide antibiotics, which are synthesized from the condensation of carboxylic acid units by polyketide synthases by a mechanism that is analogous to fatty acid biosynthesis. The result is that intermediates are produced which typically contain alternating methylene and carbonyl groups at an early point during their synthesis. Enzymatic and chemical reactions on the polyketide chain, including reduction of the keto groups, dehydration and cyclization reactions, and a variety of post-assembly modifications (e.g., oxidations, reductions, hydroxylations, O-methylations, glycosylations) allow polyketides to encompass a wide spectrum of chemical structures.


Streptomycete polyketide synthases can be split into two broad classifications, Type I and Type II enzymes. Type I polyketide synthases consist of large polypeptides containing multifunctional domains arranged in complex modules. These enzymes are somewhat analogous to yeast-like fatty acid synthetases in both structure and function. The Type I polyketide synthases produce complex molecules, often constructed with several different extender units that require differential steps at each round of chain extension. Polyketide natural products synthesized by Type I polyketide synthases include the macrolide antibiotics erythromycin, oleandomycin, avermectin, and the immunomodulatory agent PK-506. The complex polyketide synthases appear to be required to accommodate the intricate incorporation and modification of the different extender units into the growing molecules. (continued on p. 12)

Type II polyketide synthases function as multienzyme complexes, typically containing three or more separate polypeptides. The polyketides produced by Type II enzymes include those which are relatively simple, constructed with a limited array of extender units (primarily malonyl-CoA), usually contain aromatic rings, and appear to have similar modes of formation. These include aromatic polyketide natural products such as the benzoisochromane quinones (e.g., actinorhodin), anthracyclines (e.g., daunomycin, aclacinomycin), and tetracyclines. We will analyze and compare the early steps in the formation of polyketides by both Type I and Type II enzymes, with an emphasis on the potential for domain switching and hybrid metabolite formation through genetic engineering.

Symposium Paper 4 - Tuesday, 8:30

GENETIC ENGINEERING OF ANTHOCYANINS

Neal Courtney-Gutterson, DNA Plant Technology Corp., 6701 San Pablo Ave, Oakland CA 94608 USA


organized - good speaker

Anthocyanin biosynthesis and its involvement in determining flower color is among the best studied of secondary metabolic processes. The extensive biochemistry of anthocyanins has enabled us to begin manipulating flower color in ornamental plants by directed genetic engineering.

Flower color is a result of complex interactions of primary anthocyanin pigments with flavonoid copigments and ionic components of the solution in which the pigments are found. Many genes determining anthocyanin biosynthesis have been isolated and characterized from a range of plants. For example, genes encoding the core enzymes of the flavonoid biosynthetic pathway (e.g., chalcone synthase, chalcone isomerase and flavanone hydroxyltransferase), the enzymes specific for anthocyanin biosynthesis (e.g., dihydroflavonol reductase and UFGT) and the enzymes which interconvert anthocyanidin pigments (e.g., flavonoid-3',5'-hydroxylase) have been cloned. Tools to reduce expression of plant genes and to enhance expression of plant genes have been developed in the past decade, so that modification of flower color can be approached directly.

Elimination or reduction of pigment has been demonstrated by reducing expression of chalcone synthase (CHS) and dihydroflavonol reductase (DFR) genes, using *Petunia hybrida* as a model system. A novel method for reducing expression of plant genes was discovered through work to manipulate expression of chalcone synthase in petunia. This method, known as sense suppression, has been utilized now to modulate flower color in chrysanthemum and rose. Starting with a rose variety producing deep red flowers, individual transgenic plants producing flowers with a range of pink colors have been obtained. This approach is a very useful way to alter flower color without modifying other desirable traits of the plant for commercial purposes. In addition, sense suppression can be used as a tool for studying the biochemistry of plant pathways.

The alteration of flower color was first demonstrated through the production of pelargonidin in petunia, which only produces cyanidin and delphinidin derivatives naturally. Such an approach, in which a dihydroflavonol reductase gene with substrate specificity different from that of the target plant was used successfully, should be applicable to other ornamental plants as well.

The long-term goal of producing blue roses has moved recently from the realm of the breeder's brush to that of the molecular geneticist's restriction enzymes. A number of genes encoding functions essential for blue flower color have been identified in petunia. These include functions responsible for synthesis of delphinidin (found in the flowers of 90% of all blue-flowering plants), for synthesis of flavonol copigments and for modulation of vacuolar pH, a key determinant of flower color.

The ability to regulate the amount of anthocyanin pigment produced in flowers, the specific pigment produced, the production of copigment and the internal composition of the vacuole is opening up the possibility of truly *engineering* flower color.

Symposium Paper 5 - Tuesday, 9:20

DIVERSE PATTERNS OF ENDOGENOUS CHALCONE SYNTHASE GENE EXPRESSION AND ANTHOCYANIN PIGMENTATION CAN BE ELICITED BY DEVELOPMENTAL IMPRINTS IMPOSED ON AN ECTOPIC CHALCONE SYNTHASE TRANSGENE

Ramberg - disorganized

Richard Jorgensen, Department of Environmental Horticulture, University of California, Davis

The endogenous chalcone synthase gene can be used as a reporter for the epigenetic state of an ectopic chalcone synthase transgene in petunia and transgene epialleles are useful for probing the developmental determination of CHS gene expression. The CHS transgene is capable of suppressing the expression of the homologous, endogenous CHS gene(s) by an unknown mechanism that is probably dependent on the expression of the transgene and that often causes the coordinate suppression of the transgene as well (see abstract of Courtney-Gutterson). Interference with endogenous CHS expression results in a block in anthocyanin pigmentation which is most easily visualized in flower corollas; in many cases, transgene loci elicit strikingly complex pigmentation patterns, rather than uniformly white flowers. These patterns are organized spatially in three dimensions and appear to be determined by cells at the edges of petals or in the vasculature, but in a variety of different ways. The epigenetic metastability of some transgenes is being exploited to perturb these patterns genetically and so to gain some understanding of how they are determined developmentally. The long term goal is to identify the developmental factors that can induce new epialleles and control the expression of both the endogenous CHS gene and the CHS transgene, as well as to determine the means by which the expression of one gene interferes with the expression of the other.

Symposium Paper 6 - Tuesday, 1:20

ENGINEERING-ALTERED GLUCOSINOLATE BIOSYNTHESIS BY TWO ALTERNATIVE METABOLIC PATHWAYS

Ragai Ibrahim and Vincenzo De Luca, Plant Biochemistry Lab, Concordia University, Montréal, Canada H3G 1M8 and IRBV Université de Montréal, Canada H1X 2B2

Plants possess a remarkable ability to synthesize a variety of secondary metabolites, of which glucosinolates (G1s) represent a classical example. G1s owe their structural diversity to their amino acid precursors, but have in common a sulfate group derived from 3'-phosphoadenosine 5'-phosphosulfate (PAPS). In order to reduce G1s accumulation in *Brassica*, two strategies were designed to compete for the precursors involved in their biosynthesis. One strategy utilizes the tryptophan decarboxylase gene (PNAS 86: 2582, 1989) which results in the accumulation of tryptamine, an insect antifeedant. The other introduces the flavonol sulfotransferase gene (PNAS 89: 1286, 1992) which diverts PAPS towards the synthesis of flavonol sulfates, rather than G1s. The results of both strategies will be discussed in relation to metabolic engineering of plant secondary products.

Symposium Paper 7 - Wednesday, 8:30

GENETIC MANIPULATION OF LIGNIN AND PHENYLPROPANOID-DERIVED COMPOUNDS INVOLVED IN INTERACTIONS WITH MICROORGANISMS

Richard A. Dixon, Maria J. Harrison, Weiting Ni, Abraham Oommen and Nancy L. Paiva. Plant Biology Div., Noble Foundation, Ardmore, OK 73402 USA

In legumes, the phenylpropanoid pathway leads to the production of antimicrobial phytoalexins and rhizobial *nod* gene inducers, as well as to the flavonoid pigments and the structural polymer lignin found in most plant species. We have recently cloned genes from alfalfa encoding enzymes specific for these functionally diverse branch pathways. Expression of heterologous isoflavone reductase (IFR) and pterocarpan synthase genes in alfalfa could alter phytoalexin stereochemistry and enhance antimicrobial activity. Peanut is being evaluated as a source of these genes, and the alfalfa IFR promoter studied for its performance in transgenic systems. Overexpression of chalcone O-methyltransferase may lead to more efficient nodulation. Expression of antisense caffeic acid O-methyltransferase reduced lignin content in transgenic tobacco and alfalfa.

Symposium Paper 8 - Wednesday, 9:20

REGULATION OF LIGNIN BIOSYNTHESIS IN LOBLOLLY PINE

Ross Whetten, David O'Malley, Wuli Boa, Kui Shin Voo, Wei Wei Liu, John MacKay, Chen-Loung Chen, and Ron Sederoff. Forest Biotechnology Group, College of Forest Resources, North Carolina State University, Raleigh, NC, 27695-8008

Good source organized, Hummer

The enzymatic pathway for biosynthesis of lignin has not been fully established for any higher plant. The pathway for the synthesis of the major monolignol precursor in conifers, coniferyl alcohol, is better defined. Three of the enzymes from the pathway for precursor biosynthesis have been purified, characterized and cloned from loblolly pine. These enzymes are, phenylalanine ammonia-lyase (PAL), 4-coumarate:coenzyme A ligase (RCL), and cinnamyl alcohol dehydrogenase (CAD). These enzymes act at key steps in precursor biosynthesis. A main objective has been to identify the proteins and genes essential for the biosynthesis of lignin in loblolly pine. This information is necessary for studies of the regulation of biosynthesis and for developing strategies for genetic engineering of lignin content or quality.

In contrast to work done in angiosperms, at least two of the pine lignin biosynthetic enzymes appear to be coded by single genes, rather than gene families. What is not known is the pathway for biosynthesis after the formation of the monolignol precursor. The transport, storage, and enzymatic mechanism of polymerization are not established. Coniferin is thought to be an abundant storage molecule for lignin precursors, but it is not known how it is stored and transported to the lignifying zone. Equally important, we do not know what enzymes are necessary and sufficient for polymerization of lignin. Although peroxidases have long been thought to be essential for polymerization, recent results also implicate a laccase. A laccase has been purified from differentiating xylem of loblolly pine. It is only found in lignifying tissues. In xylem where fractionation can be done, it is only found in association with the cell walls. The purified enzyme is able to synthesize DHP *in vitro*.

The differentiating xylem of loblolly pine has proven to be a good system for biochemical characterization of enzymes in lignin biosynthesis. Recent advances in genomic mapping in conifers should allow mapping of the genes involved in lignin biosynthesis, and provide further insight into studies of gene function and regulation.

x Role of phenolics in development
Proteins in wood cell wall x-linked.

Symposium Paper 9 - Wednesday, 1:20

SYSTEM FOR ENVIRONMENTAL EVALUATION OF TRANSGENIC PLANTS

Sally L. McCammon, Animal and Plant Health Inspection Service, United States Department of Agriculture, Washington, D.C. 20250

The function of a regulatory system is to facilitate safe technology transfer. The Animal and Plant Health Inspection Service (APHIS) certifies, based on its authority under the Federal Plant Pest Act of 1957 and the Plant Quarantine Act of 1912, that no plant pest potential is present for transgenic plants imported, moved interstate, or released into the environment. Thus, genes implicated in pathogenesis from the donor, the vector, or the vector agent do not become part of the heritable characteristics of the plant. Once authority to evaluate plant pest potential is invoked, the United States Department of Agriculture (USDA) is also responsible, under the National Environmental Policy Act, to evaluate effects on the environment.

Many of the genes being introduced into plants are for traditional purposes and some of them are for novel purposes. APHIS has reviewed approximately 400 applications to field test transgenic plants containing new genes for insect tolerance, virus tolerance, fungal tolerance, heavy metal resistance, selective markers, pharmaceutical products, and increased or altered product quality. To date, only single or a few genes have been inserted. Those genes affecting product quality have included different strategies for control of ripening, such as modification of degradative enzyme production and modified ethylene and cytokinin regulation, as well as oil modification, flower color, and increased solids and seed storage protein. Pharmaceutical products have or will include monoclonal antibodies to human and animal disease agents and enzymes and immune stimulants.

The purpose of an environmental assessment made by APHIS is not to "prove safety" as is done in traditional risk assessments where a specific risk to human health has been identified, such as pathogens, carcinogens, or pesticides. In these assessments, the hazard is known and the assessment is done to quantify the probability of occurrence, using the model risk = hazard X exposure.

An environmental assessment is done with transgenic plants in order to see if identified concerns for plants are associated with the plant in the environment. Thus, an environmental assessment is a qualitative evaluation that allows an "informed decision" to be made as to whether an event is likely. The major environmental issues for crop plants are weediness, nontarget effects (especially on endangered or beneficial species), plant pest potential, and gene transfer. Exposure occurs if plants are released into the environment and become established or can interbreed with wild relatives. A hazard may be identified by evaluating the gene x plant x environment interaction and comparing with the parental variety. There is no a priori assumption of risk.

The issues of the use of the environment are beginning to be defined socially. However, a regulatory system cannot resolve conflicts; social, moral, or economic, and typically characterized by polarization of beliefs. It can assist in resolving questions associated with development, use, and commercialization of the product of biotechnology. A regulatory system for new products must include the flexibility to account for initial testing, development, commercialization, and international acceptance.

Symposium Paper 10 - Thursday, 8:30



Interesting stuff - boring speaker

ANALYSIS AND MODIFICATION OF POLYPHENOLOXIDASE EXPRESSION

J.C. Steffens, Dept. of Plant Breeding, Cornell University, Ithaca, NY 14853-1902.

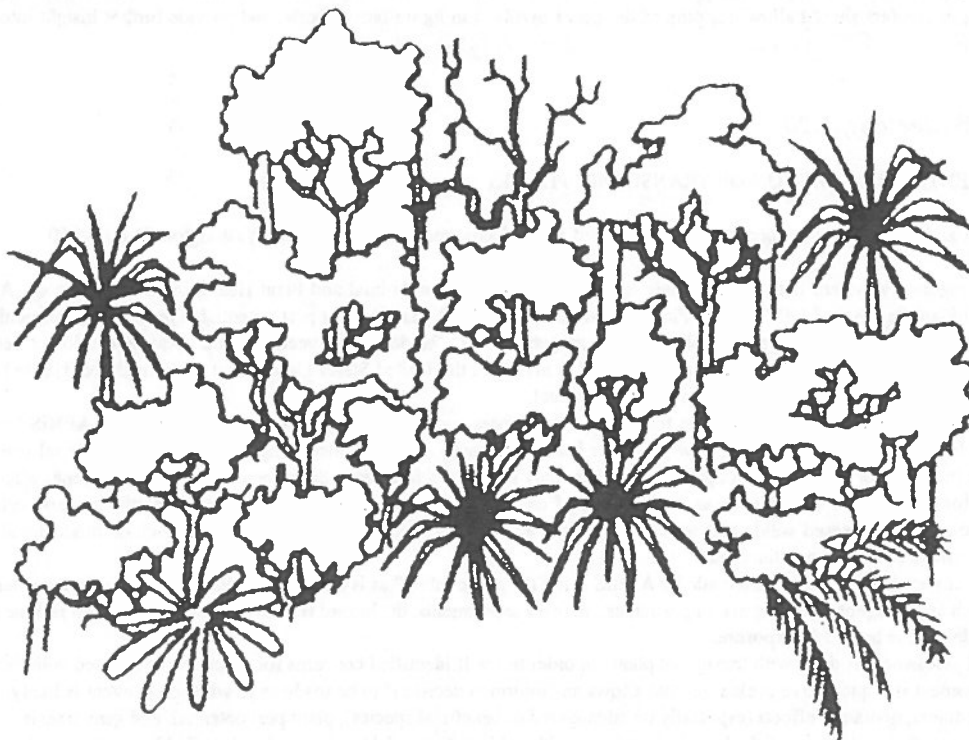
Polyphenol oxidase (PPO) catalyzes the oxidation of mono- and diphenols at the expense of O_2 . The quinonoid reaction products of PPO are strong electrophiles and undergo secondary reactions which covalently modify a variety of cellular constituents. Although PPO is one of the first enzymes known to biochemistry, its role in the biology of plants remains unclear. Until recently, few of the many hypotheses of PPO function have been testable. Transgenic plants provide a direct means to test proposed functions for PPO, and also allow evaluation of approaches to modify PPO expression for increased postharvest quality or herbivore resistance.

Symposium Paper 11 - Thursday, 9:20

GENETIC ENGINEERING OF MONOTERPENE METABOLISM IN *MENTHA*

Rodney Croteau, Institute of Biological Chemistry, and Department of Biochemistry and Biophysics, Washington State University, Pullman, WA 999164-6340

Most monoterpenes are derived by oxygenation of a parent olefin that is formed by cyclization of the ubiquitous isoprenoid precursor geranyl pyrophosphate. The commercial mint (*Mentha*) species produce either C_3 -oxygenated or C_6 -oxygenated *p*-menthane monoterpenes as the major essential oil components. The biosynthetic origin of both of these monoterpene families from the common olefin precursor (-)-limonene is reviewed. The purification and properties of the geranyl pyrophosphate: limonene cyclase, and of the two regiospecific cytochrome P-450 limonene hydroxylases catalyzing oxygen insertion at C_3 or C_6 of the cyclic skeleton, are reported. The isolation of the cDNA species encoding these enzymes, the first of monoterpene metabolism to be cloned, is described and the results of sequence comparison studies are noted. The organization and regulation of monoterpene metabolism in *Mentha* are delineated and the roles of the cyclase and hydroxylases in the control of essential oil yield and composition are defined. Prospects for genetic engineering of monoterpene production in *Mentha* species are discussed.



CONTRIBUTED PAPERS

(starred abstracts are entered in the Best Contributed Paper competition)

Oral Paper 1 - Monday, 10:30

CYTOCHEMICAL AND IMMUNOLocalIZATION OF POLYAMINES IN WHITE SPRUCE IN TISSUE CULTURE

Vindhya Amarsinghe and John E. Carlson. Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., Canada V6T 1Z3

Polyamines were localized in embryogenic callus and proembryos of white spruce with o-phthalaldehyde and polyclonal antibodies raised against putrescine, spermidine or spermine conjugated to bovine serum albumin via heterobifunctional agents. Rapidly dividing cells in embryogenic callus and in proembryos showed higher levels and greater localization of polyamines than non-dividing or terminally differentiated cells. Nuclei in rapidly dividing cells showed higher levels of cytochemical staining than in the cytoplasm, but DNase-I pretreatment was required to demonstrate the same with immunolocalization. However, with both methods, localization was always excluded from condensed chromatin. Parallel quantifications have also been conducted using HPLC.

Oral Paper 2 - Monday, 10:45

MOLECULAR GENETICS OF THE BETALAIN PATHWAY IN HIGHER PLANTS AND FUNGI

Jean-Pierre Zryd, Ursula Hinz, Laurence Bindschaedler, Lukas Müller, Laboratoire de Phytogénétique Cellulaire, Université de Lausanne, CH 1015 Lausanne, Switzerland.

Betalain pigments replace anthocyanidins in families of the order Caryophyllales and are present in some fungi. We have studied the genetics of those pigments in *Portulaca grandiflora* and identified three important loci. One of them, named C, codes for the enzyme DOPA-4,5-dioxygenase. We have purified this enzyme from the fungus *Amanita muscaria* and obtained cDNA clones. The absence of cleavage signal sequence and of post transcriptional modification points to a cytoplasmic localization of the enzyme. Immunological analysis indicates a strong homology between fungal and higher plant enzymes. A *Portulaca* cDNA bank is being tested by PCR and positive clones identified. We are investigating sequence homology between fungi and plants. The first enzyme of the biosynthetic pathway in *Amanita muscaria*, a specific tyrosine hydroxylase, has also been purified and shown to be distinct from fungal tyrosinases and plant polyphenol oxidases.

Oral Paper 3 - Monday, 11:00

THE PHENOLIC DOMAIN OF SUBERIN

Mark A. Bernards¹, Jaroslav Zajicek² and Norman G. Lewis¹, ¹Institute of Biological Chemistry and ²NMR Spectroscopy Center, Washington State University, Pullman, WA, USA 99164-6340.

Suberin is a complex heteropolymer assumed to comprise both aliphatic and aromatic domains, where the aliphatic moiety is ester-linked to a "lignin-like" phenolic backbone. Importantly, no direct evidence has been put forward in support of this model. [1-¹³C] and [2-¹³C]-phenylalanines were individually administered to wound healing potato tuber slices and, following uptake and metabolism, the carbon-13 enriched suberins partially purified. The carbon-13 enriched suberin preparations were analyzed using solid-state ¹³C-NMR spectroscopy, which revealed for the first time direct evidence for the nature of the monomer(s) and covalent linkage(s) in the suberin polymer.

Oral Paper 4 - Monday, 11:15

SUBSTITUTED TRUXILLIC ACIDS: SYNTHESIS AND ANALYSIS IN PLANT CELL WALLS

Jean I.M. Rajaonarivony¹, Norman G. Lewis², and G.H.N. Towers¹. ¹Botany Department, University of British Columbia, V6T 1Z4, ²Institute of Biological Chemistry, Washington State University, Pullman, WA, USA 99163-6340

Irradiation of a non-crystalline mixture of E-p-coumaric acid and E-ferulic acid, produced three types of substituted cyclobutane dimers, identified by NMR and MS. In the cell walls of Kikuyu Grass (*Pennisetum clandestinum*) grown under similar high light intensity, E-p-coumaric and E-ferulic were the main phenolic acids. The Z-esters were also present and 12 types of the dimers (truxillic acids and truxinic acids) were found in substantial amounts. We also report that truxillic acids occur in a wide variety of plant species including barley, corn, and tobacco, and that their chemistry is not uniform throughout a plant.

The analysis of truxillic acids in plant cell walls is relevant to agriculture as it is generally assumed that their presence affect the digestibility of grasses by ruminants and insects.

Oral Paper 5 - Monday, 2:10

CHANGES IN FLAVAN-3-OL COMPOSITION OF SAINFOIN LEAF POLYMERS DURING LEAF DEVELOPMENT

Mohammed R. Koupai-Abyazani, John McCallum, Alister D. Muir, Bruce A. Bohm, G.H.N. Towers, and Margaret Y. Gruber. Botany Department, University of British Columbia, Vancouver, BC V6T 1Z4 and Agriculture Canada, 107 Science Place, Saskatoon, SK, S7N 0X2.

Condensed tannin polymers were isolated from Sainfoin (*Onobrychis viciifolia* Scop.) at different stages of leaf development. Analysis of the phloroglucinol degradation products by RP-HPLC indicated that catechin, epicatechin, galocatechin and epigallocatechin were present as terminal groups, while all but catechin were incorporated as extension units. During leaf development the proportion of *cis*-isomers decreased from 83 to 48% while the proportion of tri-OH B-ring flavan-3-ols increased from 60 to 90% indicating that biosynthesis of these compounds is a dynamic process.

Oral Paper 6 - Monday, 2:25

EVIDENCE FOR A GENE CLUSTER INVOLVING TRICHOECENE PATHWAY BIOSYNTHETIC GENES IN *FUSARIUM SPOROTRICHOIDES*

Thomas M. Hohn, Susan P. McCormick, and Anne E. Desjardins. Mycotoxin Research Unit, USDA/ARS, National Center for Agricultural Utilization Research, Peoria, IL, 61604 USA.

Trichothecenes are toxic sesquiterpenoids produced by several genera of fungi and by two species of the plant genus *Baccharis*. Two overlapping cosmid clones carrying the *Tax5* gene have been isolated and shown to complement T-2 toxin deficient mutants (*Tax3-1* and *Tax4-1*) blocked at different steps in the trichothecene biosynthetic pathway. All of the cosmid transformed mutants produced 2- to 10- fold more trichothecenes than the parent strain. DNA fragments that complement either the *Tax3-1* or *Tax4-1* mutants were identified in the region of overlap between the two cosmid clones. The *Tax4* gene has been characterized and shown to encode a polypeptide with significant homology to cytochrome P450 enzymes. These results suggest that two or more genes involved in the biosynthesis of trichothecenes are closely linked to *Tax5* in *Fusarium sporotrichioides*.

Oral Paper 7 - Monday, 2:40

INVESTIGATION OF THE FACTORS CONTROLLING PTEROCARPAN STEREOCHEMISTRY IN LEGUMES

Nancy L. Paiva and Richard A. Dixon, Plant Biology Division, The Noble Foundation, P.O. Box 2180, Ardmore, Oklahoma 73402.

Pterocarpanes are produced in many legume species, particularly in response to pathogen attack. Many species accumulate only "-" (6aR, 11aR) pterocarpanes, some accumulate "+" (6aS, 11aS) pterocarpanes, while a few accumulate mixtures of "+" and "-". Several authors have proposed that the enzymes isoflavone reductase (IFR) and pterocarpan synthase (PTS) control the stereochemistry of the pterocarpanes. As part of a long-term project to change alfalfa from a (-)-medicarpin plant to a (+)-medicarpin plant to improve its fungal resistance, we have been examining a (+) medicarpin-forming cultivar of peanut as the possible donor for the required (+)-pterocarpan genes. Assays on a number of different tissues, however, have only revealed the presence of "-" IFR activities, the same as in alfalfa. A possible explanation for this apparent contradiction will be outlined.

Oral Paper 8 - Monday, 2:55

PHARMACOLOGY OF GINGER: MODULATION OF EICOSANOID PRODUCTION

K.C. Srivastava and T. Mustafa, Institutes of Community Health and Biology, Odense University, Winsløwparken 17, DK-5000 Odense, Denmark

The rhizome of ginger in traditional medicine is reported to have several medicinal properties. The scientific investigations relating to consumption of fresh or powdered rhizome by humans and *in vitro* effects of aqueous and organic extracts will be briefly reviewed. Pungent components of ginger inhibit cyclooxygenase and lipoxygenase activity in arachidonic acid (AA) metabolism, and probably thereby reduce inflammation and relieve pain in rheumatic disorders and migraine headache. Ginger is reported to reduce nausea, vertigo and vomiting in humans. However, the mechanism of action is not yet understood. Effects on the gastrointestinal system include increase of bile secretion and antiemetic action. As ginger modulates eicosanoid production, it may serve to provide clue(s) to drugs directed to AA pathway enzymes as pharmacological targets.

* Oral Paper 9 - Monday, 3:30 *

BENZYLIC ETHER REDUCTASES IN *FORSYTHIA* SP.: ENZYMOLOGY AND MECHANISM OF ACTION

Albena Dinkova, Alex Chu, Laurence B. Davin and Norman G. Lewis, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

Pinoresinol is a pivotal intermediate in the biosynthesis of the dibenzylbutyrolactone lignan class, such as matairesinol or trachelogenin which has pronounced activity against the HIV virus. (-)-Matairesinol is formed from pinoresinol via an enantiospecific NAD(P)H-dependent reduction to give (+)-lariciresinol and (-)-secoisolariciresinol, and subsequent dehydrogenation. To our knowledge, these reductive steps are the first examples of benzylic ether reductions in plants. Pinoresinol reductase has been purified using a combination of affinity, hydrophobic, anion exchange, and gel filtration chromatography; its properties, and the stereospecificity of the reduction step, have been established.

* Oral Paper 10 - Monday, 3:45 *

CLONING AND REGULATION OF FLAVONOL 3-SULFOTRANSFERASE FROM *FLAVERIA BIDENTIS*

Sirinart Ananvoranich, Patrick Bulick and Ragai K. Ibrahim, Plant Biochem. Lab., Concordia University, Montreal, Quebec, Canada H3G 1M8.

Flavonol 3-sulfotransferase (3ST) catalyses the 3-phosphoadenosine-5'-phosphosulfate-dependent sulfation of flavonols. The fact that 2,4-D induces 3ST activity, whereas quercetin 3-sulfate reduces enzyme activity in cell cultures of *F. bidentis*, prompted us to study the regulation of this enzyme. A cDNA clone encoding 3ST was isolated from *F. bidentis* and used as a probe for Northern blot analysis. The changes in 3ST activity paralleled those of 3ST mRNA levels in 2,4-D treated cultures. These results will be discussed in relation to the possible involvement of 3ST in the regulation of polar auxin transport.

* Oral Paper 11 - Monday, 4:00 *

FUNGAL PATHOGENS OF PEA HAVE EVOLVED BIOCHEMICALLY DIFFERENT, BUT HIGHLY SPECIFIC CYTOCHROME P450S TO DETOXYIFY THE PHYTOALEXIN PISATIN

Helga L. George and Hans D. VanEtten*, Dept. of Biol. Sciences, Univ. of Cal., Santa Barbara, CA 93106, *Dept. of Plant Pathology, Univ. of Arizona, Tucson, AZ 85721

Garden pea (*Pisum sativum*) produces the isoflavonoid phytoalexin pisatin, which is toxic to most fungi. Most fungi that are pathogens of pea are able to detoxify pisatin by demethylation. The characteristics of pisatin demethylase have been studied most thoroughly in *Nectria haematococca*; the enzyme was shown to be a cytochrome P450 and to be necessary for pathogenicity on pea. We have characterized the biochemical properties of the demethylase from whole cell and microsomal preparations of *Fusarium oxysporum* f. sp. *pisi*, *Mycosphaerella pinodes*, and *Ascochyta pisi*. The enzymes were all induced by pisatin, but they varied in their substrate and induction specificity, sensitivity to P450 inhibitors, and sensitivity to glucose repression. Despite these differences, all of the enzymes were shown to be cytochrome P450s, and they all had low K_m 's towards (+) pisatin. This indicates that fungal pathogens of pea have evolved different, but highly specific, cytochrome P450s to detoxify pisatin.

* Oral Paper 12 - Monday, 4:15 *

THE ISOLATION OF FUNCTIONAL RNA FROM WOUNDED GRAND FIR (*ABIES GRANDIS*) STEMS FOR THE STUDY OF MONOTERPENE BIOSYNTHESIS.

Christopher L. Steele, Efraim Lewinsohn and Rodney Croteau, Inst. of Biol. Chem., Washington State University, WA, USA 99164-6340

Grand Fir stems possess five wound inducible monoterpene synthases. In order to isolate the cDNAs encoding these proteins and to study their transcriptional regulation, a fast and reliable RNA isolation method was developed. Woody stems are frozen, pulverized, and extracted in the presence of PVP and RNase inhibitors. The extracts are clarified and polysaccharides removed with 10% ethanol. Nucleic acids are precipitated with isopropanol and pelleted through CsCl. The yields (25 ug RNA/g FW) and quality of the RNA were ascertained by spectrophotometry, electrophoresis and *in vitro* translation. This protocol allows the examination of wound inducible gene expression in grand fir stems.

* Oral Paper 13 - Monday, 4:30 *

A PHAGOSTIMULANT FUNGAL METABOLITE FOR THE WESTERN SUBTERRANEAN TERMITES *RETICULITERMES HESPERUS* BANKS.

Brice A. McPherson, David L. Wood, and Isao Kubo, Dept. of Entomological Sciences, University of California, Berkeley, CA 94720, USA.

Methanolic extracts of fungi associated with colonies of *R. hesperus* in northern California were found to elicit feeding in laboratory assays. Feeding behavior was quantified using cellulose thin layer chromatography (TLC) plates as the feeding substrate. Bioassay-directed fractionation using primarily size-exclusion chromatography yielded a potent phagostimulatory component, active at less than 10 micrograms per 1 cm² TLC plate.

Development of effective subterranean termite baits will require that foragers exhibit preference for baits over other cellulosic materials. A phagostimulant active in the microgram range may provide the key to creation of selective baits.

* Oral Paper 14 - Monday, 4:45 *

GENETIC VARIABILITY OF SINAPINE IN *BRASSICA* AND *SINAPIS* SPECIES

Wang, X.¹, McGregor, D.I.², and Downey, R.K.², ¹Department of Plant Science, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4. ²Agriculture Canada Research Station, Saskatoon, SK, Canada, S7N 0X2

Significant genetic variation of sinapine was found among 25 strains and cultivars of *Brassica carinata* Braun., *B. juncea* (L.) Coss., *B. napus* L., *B. nigra* L. Koch, *B. rapa* L. and *Sinapis alba* L. Sinapine content in the seed ranged from 16.6 to 112 µg per seed and sinapine concentration of oil-extracted meal was from 16.2 to 24.3 mg/g oil-extracted meal. The sinapine content in seed was positively correlated with the seed size but sinapine concentration of the oil-extracted meal was not related to seed size. Environmental effects on the variation of sinapine were also significant and appeared to be related to seed development. However, since environmental effects are small, variation in sinapine content was mainly due to genetic variability.

* Oral Paper 15 - Monday, 5:00 *

LARVAL DEFENSE IN *LEMA* (CHRYSOMELIDAE: CRIOCERINAE)

Timothy C. Morton and Fredric Vencl, Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, N.Y., USA 11794-5245

Lema feed on several families of noxious plants, including Solanaceae and Commelinaceae in N.Y. Frass accumulates on the dorsum (due to a dorsally situated anus) and forms a fecal shield. In preliminary experiments, larvae were reared in the lab on lettuce or their natural host. A bioassay for testing efficacy of defense was developed using the generalist predator *Formica rufa*, the wood ant. Larvae were placed in an arena near an ant colony, those remaining in the arena after the 5 minute trial were considered defended. Larvae reared on *Solanum* or *Commelina* were never captured, larvae reared on lettuce were always captured. Fecal shields of defended larvae could be transferred to lettuce reared larvae and confer protection. This indicates that larval defense is plant derived (not autogenous). Experiments for this field season (May & June) will identify those plant compounds involved in defense using artificial diet supplemented with either 1) plant fractions or 2) pure compounds.

Oral Paper 16 - Tuesday, 10:30

BIOGENETIC INTERPRETATION OF PIGMENT METABOLISM IN ROSES AND CARNATION FLOWER MUTANTS

Maurice Jay, Nadine Chirol & Jean-Philippe Biolley, Labo Biol Micromoléculaire et Phytochimie, Université Claude Bernard, Lyon I, F-69622 Villeurbanne, France

In the red rose "Mme Delbard", the flavonoid pattern is mainly based on B-ring-substituted compounds. Spontaneous mutations gave rise to colour phenotypes with markedly reduced dihydroflavonol 3'-hydroxylase activity. Therefore, the predominance of kaempferol over perlargonidin suggests a more active flavonol synthase than dihydroflavonol reductase (DHFR), while the reverse is the case for dihydroquercetin in the parent variety. In the yellow carnation "Londorqa", mutants with chalcone isomerase activity exhibited heterogeneity at the loci encoding DHFR activity, as indicated by white- and orange-coloured phenotypes. These results will be discussed in relation to the effect of mutation on flavonoid biogenesis.

Oral Paper 17 - Tuesday, 10:45

POLLINATION AND WOUNDING STIMULATE KAEMPFEROL ACCUMULATION IN STIGMAS OF *PETUNIA HYBRIDA*

Thomas Vogt, Peggy Pollak and Loverine Taylor, Department of Horticulture, Washington State University, Pullman, WA 99164-6414

Flavonol aglycones have been shown to be essential for germination of pollen in *Petunia hybrida* and *Zea mays* [1]. Within 24 hours of pollination with flavonol-deficient white pollen from transgenic petunia, wildtype petunias accumulate high amounts of the flavonol, kaempferol, in the outer-most cells of the stigma.

The kaempferol allows the otherwise sterile pollen grains to germinate and restores pollen tube growth, leading to successful fertilization. This mechanism may be important in terms of evolution, since it ensures that fertility is not solely dependent on the flavonoid glycoside content of each individual pollen grain.

1. Mo, Y, Nagel, C. and Taylor, L. (1992) PNAS 89, 7213-7217

Pleiotropic - Flavonoid color/pollen tube growth
(2=3 OH at 3)
Pollination & wounding
→ same effect

Oral Paper 18 - Tuesday, 11:00

FLORAL FLAVONOIDS AND THEIR MODIFICATION IN *LISIANTHUS*

Kathy E. Schwinn, Kevin M. Davies, Robyn M. Miller, Simon C. Deroles, J. Marie Bradley, David G. Manson and Nigel K. Given, New Zealand Institute for Crop & Food Research Limited, Private Bag 4005, Levin, New Zealand; Kenneth R. Markham, Industrial Research Ltd., PO Box 31-310, Lower Hutt, New Zealand.

We are interested in producing novel flower colours in *lisianthus* (*Eustoma grandiflorum*) by manipulation of the flavonoid biosynthetic pathway. *Lisianthus* flowers normally produce anthocyanins and flavonols that are galactosylated at the 3-positions. 50% of the flavonols are acylated. A small amount of non-acylated flavonols glucosylated at the 3-position are also present. Novel anthocyanin(s) glucosylated at the 3-position were produced in the flowers by insertion of an *Antirrhinum majus* cDNA clone for a flavonoid glucosyltransferase under the control of the CaMV 35S promoter. In addition, a decrease in the amount of acylated flavonols was correlated with an increase in the amount of glucosylated flavonols.

Oral Paper 19 - Tuesday, 11:15

COMPLEMENTATION OF THE ANTHOCYANIN-2 MUTATION OF *PETUNIA*

Kevin M. Davies, Kathy E. Schwinn, David G. Manson, Robyn M. Miller, Simon C. Deroles and J. Marie Bradley, New Zealand Institute for Crop & Food Research Limited, Private Bag 4005, Levin, New Zealand.

"Mitchell" petunia has white flowers due to the *an2* mutation, which is believed to affect a regulatory gene, as several steps in the conversion of dihydroflavonols to anthocyanins are inhibited. We have overcome the *an2* mutation by introducing the *Antirrhinum majus* dihydroflavonol 4-reductase (DFR) cDNA under the control of the CaMV 35S promoter. As the mutation is complemented by the presence of the DFR alone, this step must be the only complete block in anthocyanin biosynthesis caused by *an2*. The activity of the UDP-glucose: flavonoid glucosyltransferase in "Mitchell" flowers was 10% that of wild-type plants. However, anthocyanin derivatives were formed from introduced leucopelargonidin. Thus, whilst some of the other anthocyanin biosynthetic steps following DFR are also inhibited by the *an2* mutation, they retain sufficient activity to allow anthocyanin synthesis to occur.

Oral Paper 20 - Tuesday, 2:00

BIOSYNTHESIS OF ACYLGLUCOSE BY *LYCOPERSICON PENNELLII* GLANDULAR TRICHOMES

G.S. Ghangas and J.C. Steffens, Dept. of Plant Breeding, Cornell University, Ithaca, NY 14853-1902

Glandular trichomes of wild tomato secrete 2, 3, 4 tri-O-acylglucoses possessing short to medium chain length fatty acids (C₄-C₁₂). When ¹⁴C-isobutyrate is administered to *L. pennellii* leaves the label is converted to 1-O-isobutyryl-β-D-glucose. This is followed by the appearance of further acylated and more hydrophobic glucose esters of isobutyrate. *L. pennellii* extracts catalyzed the formation of 1-O-isobutyryl-β-D-glucose from isobutyrate and UDPG, and detached *L. pennellii* trichomes catalysed the transfer of the isobutyryl moiety from synthetic 1-O-isobutyryl-β-D-glucose to D-glucose. Detached *L. pennellii* trichomes also catalysed the formation of diacylglucose and triacylglucose via transfer of the isobutyryl moiety from 1-O-[¹⁴C-isobutyryl]-β-D-glucose to mono- or diacylglucoses.

Oral Paper 21 - Tuesday, 2:15

ASPECTS OF HYDROXYMETHYLGLUTARYL-COENZYME A METABOLISM IN *CATHARANTHUS ROSEUS*

Robert van der Heijden*, Veronika de Boer-Hlupá, Robert Verpoorte and Johannis A. Buine**. *Division of Pharmacognosy, Leiden/Amsterdam Center for Drug Research, Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands. **Department of Microbiology and Enzymology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands.

Mevalonate is a central building block for a vast array of plant primary and secondary metabolites. At present much research is focused on the regulation of the mevalonate biosynthesis in order to manipulate the accumulation of the mevalonate derived metabolites. An important intermediate in the biosynthesis of mevalonate is 3-hydroxymethylglutaryl-coenzyme A (HMG-CoA). Some enzymes involved in the metabolism of HMG-CoA are now being studied in *Catharanthus roseus*, a model system for studies on the regulation of indole alkaloid biosynthesis. Spectrophotometric and some newly developed HPLC methods are used to assay the activities of these enzymes in various *C. roseus* tissues and cultures. High acetoacetyl-CoA thiolase activity was found in stems and latex and, under certain conditions, in suspension cultured cells. The acetoacetyl-CoA thiolase and HMG-CoA lyase activities have been partially purified.

Oral Paper 22 - Tuesday, 2:30

PARTIAL PURIFICATION AND CHARACTERIZATION OF A WOUND-INDUCIBLE DITERPENE CYCLASE FROM GRAND FIR

Brigitte Stofer Vogel, Roy LaFever and Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

The production of oleoresin, a mixture of cyclic monoterpene olefins and diterpene resin acids, is an important component of the defense response of conifers against herbivore and pathogen attack. A diterpene cyclase activity converting geranylgeranyl pyrophosphate to abietadiene (the olefin precursor of abietic acid) is greatly increased in Grand Fir (*Abies grandis*) sapling stems in response to wounding. This diterpene cyclase was partially purified by anion-exchange, hydrophobic interaction and hydroxyapatite chromatography. The enzyme requires Mg²⁺ (Mn²⁺) for activity, exhibits a K_m value of 3.9 μM for geranylgeranyl pyrophosphate and showed a pH optimum at 7.2.

Oral Paper 23- Tuesday, 2:45

MODULATION OF MONOTERPENE CYCLASE ACTIVITIES IN GRAND FIR CALLI BY PLANT GROWTH REGULATORS AND AUTOCLAVED FUNGAL EXTRACTS

Efraim Lewinson, Eric Worden, and Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

The biosynthesis of oleoresin, a complex mixture of monoterpenes and resin acids, is wound-inducible in the stems of Grand Fir (*Abies grandis*) saplings. To test whether non-differentiated tissue mimicked this wound response, calli were generated from de-barked stem pieces. Mechanical damage to the calli had no effect but calli transferred to medium devoid of growth regulators, or containing autoclaved fungal extracts, showed increased (8- to 20-fold) levels of limonene, α- and γ-terpinene, and terpinolene cyclase activities compared to controls. This result indicates a different pattern of regulation of monoterpene cyclases in calli compared to saplings where the major wound-inducible cyclase produces α- and β-pinene.

Oral Paper 24 - Tuesday, 3:20

MEASUREMENT OF THE RATE CONTROLLING STEPS OF MONOTERPENE BIOSYNTHESIS IN PEPPERMINT

David McCaskill and Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Monoterpenes are synthesized by glandular trichomes of peppermint (*Mentha x piperita*). Secretory cells isolated from these trichomes are semi-permeable because of the conditions of their isolation. Exploiting this permeability with pulse labelling experiments allowed for the measurement of the flux of intermediates through the mevalonic acid (MVA) pathway *in situ*. Little is known of the regulation of isoprenoid biosynthesis, including the rate controlling steps of the MVA pathway. Isolated secretory cells were pulse labelled with either [¹⁴C]pyruvate or [¹⁴C]MVA. The radiolabelled intermediates were extracted, purified and subsequently quantitated by radio-HPLC. Based on the steady state metabolite levels observed, both HMG-CoA reductase and geranyl pyrophosphate synthase appear to represent rate controlling steps.

Oral Paper 25 - Tuesday, 3:35

MONOTERPENE CYCLASES AND THE REGULATION OF MONOTERPENE BIOSYNTHESIS IN PEPPERMINT

J. Gershenzon, W.R. Alonso and R. Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

The ability to genetically engineer monoterpene production in commercial mint cultivars is contingent upon a detailed understanding of the factors that regulate monoterpene formation in plants. The first committed step of monoterpene biosynthesis in peppermint is the cyclization of geranyl pyrophosphate (GPP) to the olefin, 4*S*-limonene, a reaction catalyzed by GPP:4*S*-limonene cyclase. Changes in the activity of this enzyme (measured in cell-free extracts) were found to correspond very closely to changes in the overall rate of monoterpene biosynthesis (measured *in vivo* from a pulse of ¹⁴CO₂), suggesting that this monoterpene cyclase might have an important role in controlling the rate of monoterpene formation. Using immunocytochemical methods, GPP:4*S*-limonene cyclase was observed to be localized exclusively in the leucoplasts of the glandular trichome secretory cells. This result, in conjunction with previous reports, indicates that several key reactions of monoterpene biosynthesis are localized in the plastid compartment.

Oral Paper 26 - Tuesday, 3:50

PROMOTER ANALYSIS OF AN HMGR GENE FROM *CAMPTOTHECA*

Ronald J. Burnett, Igancio E. Maldonado-Mendoza and Craig L. Nessler, Dept. of Biology, Texas A&M University, College Station, TX, USA 77843-3258

Camptothecin, a potent anticancer monoterpene indole alkaloid, is extracted from the seeds and bark of the Chinese tree, *Camptotheca acuminata*. The synthesis of camptothecin depends on the availability of mevalonate to form secologanin, a key intermediate in indole alkaloid production. We have cloned and sequenced a *Camptotheca* gene encoding HMGR (3-hydroxy-3-methylglutaryl-Coenzyme A reductase). Promoter fragments from this gene, *hmg1*, were fused with the β-glucuronidase (GUS) reporter gene and transformed into tobacco. Deletion analysis shows that a 165 bp 5' fragment is sufficient to confer wound induction and methyl jasmonate (MeJA) suppression of the *hmg1*:GUS gene. Fine mapping of the promoter by gel shift analysis and DNase I footprinting have further defined the elements which respond to wounding and MeJA within this promoter fragment.

Oral Paper 27 - Tuesday, 4:05

ACCUMULATION AND BIOSYNTHESIS OF CAMPTOTHECIN AND 10-OH-CAMPTOTHECIN IN *CAMPTOTHECA ACUMINATA* PLANTS

Melina Lopez-Meyer, David D. Henning and Thomas D. McKnight, Dept. of Biology, Texas A&M University, College Station, TX 77843

Camptothecin (CPT) is an anti-cancer indole alkaloid produced by the Chinese tree *Camptotheca acuminata*. We used HPLC to analyze the accumulation of CPT and 10-OH-CPT in 11-month old trees. CPT is present in all tissues, but it accumulates to highest levels in the upper growing regions. 10-OH-CPT is much less abundant (less than 10% of CPT) and is present in highest amounts in the bark, in lower amounts in the leaves, and usually is not detectable in wood. Several tissues could incorporate labelled tryptamine precursor into the next intermediate in the CPT pathway, strictosidine, but only bark and wood could produce strictosamide, the compound immediately following strictosidine.

Oral Paper 28 - Wednesday, 10:30

MANIPULATION OF GENES INVOLVED IN LIGNIN BIOSYNTHESIS

G. J. Schuch

Wolfgang Schuch, ZENECA Seeds, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY Great Britain

Lignin is the second most abundant biopolymer (after cellulose). It is of critical importance during plant growth and development. In addition, it is also of economic importance as it has to be removed from trees during the pulp production process in order to gain access to cellulose used in paper making. We have focussed our research on the isolation and manipulation of genes involved in lignin biosynthesis in order to make improvements to the paper making process.

As the first step towards this objective we have purified cinnamyl alcohol dehydrogenase, (CAD) one of the enzymes specific to lignin biosynthesis and cloned its cDNA. We have investigated the expression of CAD using tissue printing and Northern analysis. In order to manipulate lignin biosynthesis, we have constructed chimeric antisense and partial-sense genes and have introduced these into tobacco. Transgenic tobacco plants have been identified in which CAD enzyme has been downregulated by approx. 90% of control activity.

Oral Paper 29 - Wednesday, 10:45

LIGNIFICATION IN CELL CULTURES OF *PINUS TAEDA*

Mark A. Bernards, Maysa Furlan and Norman G. Lewis, Institute of Biological Chemistry, Washington State University, Pullman, WA, USA 99164-6340.

Central enigmas in lignin synthesis revolve around (1) the nature of the monomer (i.e., monolignol glucosides or monolignols) being transported to the site of lignin deposition, and (2) the mechanism of polymer synthesis (i.e., whether only peroxidase/H₂O₂ is required or if oxidases such as laccase are also cooperatively involved). To address these problems, we have developed a cell culture system using *Pinus taeda* cell suspension cultures in which the formation of both wall associated and extracellular "lignins" are induced. This culture system allows us to address questions of monomer transport and polymer synthesis, with a particular emphasis placed upon initial coupling steps.

Oral Paper 30 - Wednesday, 11:00

PHENYLPROPANOID COUPLING ENZYMES IN LIGNAN/LIGNIN SYNTHESIS: NON-STEREOSELECTIVE COUPLING

Laurence B. Davin, Paul W. Paré and Norman G. Lewis, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

"Insoluble residues" from *Forsythia* sp. catalyze pinoresinol synthesis when incubated with coniferyl alcohol. Interestingly, (1) the (+)-enantiomeric form predominates (~70%) and (2) addition of NAD/malate (which stimulates H₂O₂ synthesis) apparently increases the amount of (+)-pinoresinol formed. We have now solubilized both non-stereoselective and stereoselective coupling enzymes from the *Forsythia* residues: the former has been purified using a combination of gel filtration, anionic, and cationic exchange, affinity, and chromatofocusing chromatography. This enzyme requires no exogenously supplied cofactors and catalyzes the formation of racemic (±)-pinoresinols, (±)-dehydrodiconiferyl alcohols, and related lignans.

Oral Paper 31 - Wednesday, 11:15

SOLUBILIZATION AND PARTIAL PURIFICATION OF A CONIFERYL ALCOHOL STEREOSELECTIVE COUPLING ENZYME

Paul W. Paré, Laurence B. Davin and Normal G. Lewis, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

The central enigma in lignin and neolignan biosynthesis concerns the enzymology of coupling of achiral precursors, e.g. coniferyl alcohol and the formation of optically active products such as (+)-pinoresinol found, for example, in *Forsythia intermedia*. We report here the solubilization and partial purification of a coniferyl alcohol coupling enzyme which catalyzes the formation of (+)-pinoresinol. This enzyme, isolated from *F. intermedia* stems upon removal of soluble and membrane bound enzymes, has provided the first evidence for stereoselective coupling; it catalyzes formation of (+)-8,8' - ¹⁴C]pinoresinol from [8-¹⁴C] coniferyl alcohol in the absence of exogenously provided cofactors.

Oral Paper 32 - Wednesday, 2:10

ANTHRAQUINONE CONSTITUENTS OF CASSIA ALATA

Adetunji J. Aladesanmi, Erastus O. Ogunti and Joseph J. Hoffmann¹, Dept. of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria, ¹Bioresources Research Facility, Office of Arid Lands Studies, University of Arizona, 250 East Valencia Road, Tucson, AZ 85706, U.S.A.

Further phytochemical investigation of the leaves of *Cassia alata* Linn. Caesalpinaceae have yielded six anthraquinones out of which two are new natural products.² The compounds have been isolated from the combined CHCl₃ and EtOAc extracts of the leaves. The new compounds have been identified by their chemical and spectroscopic properties.

²E.O. Ogunti, A.J. Aladesanmi and S.A. Adesanya, *Fitoterapia*, **LX11**, 537 (1991).

Oral Paper 33 - Wednesday, 2:25

MOLECULAR ANALYSIS OF MAJOR LATEX PROTEIN GENE FAMILY SUPPORTS THE TRIPLOID-HYBRID ORIGIN OF OPIUM POPPY

Craig L. Nessler, Dept. of Biology, Texas A&M University, College Station, TX, USA 77843-3258

Opium poppy, *Papaver somniferum*, is the sole natural source of several medicinal alkaloids including codeine and morphine. Its alkaloid-rich latex contains an abundant group of laticifer-specific, low molecular-weight polypeptides called the major latex proteins (MLPs). Southern analysis shows that MLPs are encoded by a family of at least eight genes which can be divided into two distinct subfamilies. This report describes two new member of the MLP gene family (*gMLP 146*; *gMLP149*) that are physically linked and separated by approximately 5.5 kb. Comparison of their nucleotide and predicted amino acid sequence with other MLP genes indicates that both new MLP genes belong to the *gMLP15* subfamily. The organization of the MLP gene family is consistent with the proposed triploid-hybrid origin of opium poppy.

Oral Paper 34 - Wednesday, 2:40

NATURALLY OCCURRING TYROSINASE INHIBITORS

Ikuyo Kinist-Hori, Yoshihiro Yokokawa, and Isao Kubo. Div. of Entomology & Parasitology, Coll. of Natural Resources, UC Berkeley, Berkeley, CA 94720

Tyrosinase inhibitors may be used as alternative insect control agents. This led us to search for tyrosinase inhibitors from plants. We found phenolic compounds isolated from various parts of cashew, *Anacardium occidentale* (Anacardiaceae) to be active against mushroom tyrosinase.

Among the compounds tested, cardols, anacardic acids, and methyl cardols, exhibited inhibitory activity. The most effective compounds were cardols, followed by anacardic acids.

Oral Paper 35 - Wednesday, 2:55

LOCATION ON TREE INFLUENCES COMPOSITION OF TRITERPENOID IN GRAPEFRUIT EPICUTICULAR WAX

Harold E. Nordby and Roy E. McDonald, USDA, ARS, SAA, U.S. Horticultural Research Laboratory, 2120 Camden Rd., Orlando, FL, USA 32803

A grapefruit's peel becomes pitted or brown-stained when stored at low, nonfreezing temperatures, a disorder termed chilling injury (CI). Fruit from the exterior canopy of the tree are more prone to this disorder than interior canopy fruit. Epicuticular wax composition has been related to CI development. Grapefruit from interior and exterior tree canopies were studied over an 8-month season for their epicuticular wax composition and CI development during cold storage. The levels of five of the 29 triterpenoids observed by gas and thin layer chromatography differed between interior and exterior canopy fruit showing a possible seco-triterpene relationship with CI.

Oral Paper 36- Wednesday, 3:30

TAXANE METABOLITES

Alex Chu, Maysa Furlan, Jaroslav Zacijek, Norman G. Lewis and Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

Taxol, a highly functionalized diterpene isolated in small amounts from the Pacific yew, is FDA approved for clinical use in treating patients with ovarian cancer. A better understanding of the biosynthesis of this highly oxygenated diterpene and the regulation of its formation is needed. We are currently defining the number, types, and sequences of enzymatic steps involved in the transformation of the C₂₀ isoprenoid branch-point intermediate, geranylgeranyl pyrophosphate, to taxol in order to determine the pathway and rate limiting step(s). Twelve new taxane metabolites have been identified; their structures and putative biosynthetic significance will be described.

Oral Paper 37 - Wednesday, 3:45

DEVELOPMENTAL AND CELLULAR REGULATION OF TAXANE PRODUCTION IN FIELD AND CULTURED SHOOTS OF *TAXUS* SPP.

Eric Zeldin¹, David Ellis¹, William Russin², Marion Brodhagen¹, Ray Evert² and Brent McCown¹. Depts. of Horticulture¹ and Botany², University of Wisconsin, Madison, WI, USA 53706

The developmental and tissue/cell specific regulation of taxane production in *Taxus* spp. was examined to develop *in vitro* strategies to enhance production. There is no strong developmental regulation in field shoots, with taxane accumulation occurring at all stages of shoot elongation. In addition, newly isolated *Taxus* shoots in culture produce taxane levels similar to that of well-established cultures; however, levels of taxol are lower and levels of 7-epi-10-deacetyl taxol are higher in culture than in field needles. Anti-taxol antibodies bound specifically with vacuolar tannin bodies in phloem-associated parenchyma cells, indicating that regulation of taxane production or sequestration may occur at the cellular level.

Oral Paper 38 - Wednesday, 4:00

TAXOL FROM *AGROBACTERIUM*-TRANSFORMED ROOT CULTURES

Y. Yoke Plaut-Carcasson^{1, 2}; Lella Benkrima¹; Margaret Dawkin; Lihong Sun¹; Nicholas Wheeler³; Alvin Yanchuk⁴; Santosh Misra⁵. 1. Celex Laboratories Inc., Box 5764 Station B, Victoria BC V8R 6S8 Canada; 2. Corresponding author; 3. Weyerhaeuser Company, Centralia Washington USA; 4. B.C. Ministry of Forests Victoria BC Canada; 5. University of Victoria, BC Canada

We began work one year ago on the use of *Agrobacterium rhizogenes* to transform *Taxus brevifolia* and other *Taxus* species to produce *in vitro* root culture lines that can grow rapidly and be cloned repeatedly and indefinitely. Transformed root cultures synthesizing significant concentrations of taxol can be another long term renewable source of taxol.

Taxus embryos are dissected from seeds and germinated seedlings infected with *A. rhizogenes* using *Nicotiana* suspension cultures as cell feeder plates or with plant phenolics known to induce *vir* genes. We now have several lines of 'hairy root' cultures growing rapidly in suspension, its opines identified and their taxol concentrations being analyzed using HPLC. We use as many *Taxus* seeds as can be obtained in order to select the most active cultures.

Oral Paper 39 - Wednesday, 4:15

A ¹³C-PULSE-LABEL STUDY OF SESQUITERPENOID PHYTOALEXIN BIOSYNTHESIS IN *GOSSYPIUM HIRSUTUM* COTYLEDONS.

Piotr M. Gorski, Thayne E. Vickstrom, Margaret L. Pierce, and Margaret Essenberg, Dept. of Biochem. & Mol. Biol., Okla. Agric. Exp. Sta., Okla. State Univ., Stillwater, OK 74078-0454, USA.

The objective was to learn when phytoalexin biosynthesis occurs during the hypersensitive response of cotton cotyledons to *Xanthomonas campestris* pv. *malvacearum*. Attached cotyledons were infiltrated with 10 mM [1, 2-¹³C₂] acetate or unlabeled acetate for 4-hr pulses at various times after inoculation. The phytoalexin 2,7-dihydroxycadalene and its 7-methyl ether were quantitated, and their 70 eV EI/mass spectra were recorded. Biosynthesis was most rapid as necrotic cells appeared (35-85 hr) but continued after the number of dead cells stabilized. Results suggest that biosynthesis occurs in hypersensitively responding cells and continues in neighboring cells that are not destined to die.

Oral Paper 40 - Wednesday, 4:30

CHARACTERIZATION OF ENZYMES INVOLVED IN BIOSYNTHESIS OF LONG CHAIN LIQUID WAXES IN JOJOBA (*SIMMONDSIA CHINENSIS*) SEEDS.

Jim Metz, Kathy Lardizabal and Mike Lassner, Calgene, Inc., 1920 5th Street, Davis, CA 95616

The North American desert bush, jojoba, synthesizes and stores, in its seeds, long chain liquid waxes. These waxes, which can comprise up to 60% of the mature seed's dry weight, are oxygen esters derived from long chain (primarily C20:1 to C24:1) alcohols and acyl-CoA. The basic enzymatic pathway involved in wax synthesis has been described (Ohlrogge, et al., 1978, Lipids 13:203-210). We are interested in two enzymatic activities which occur at the end of this pathway. These activities are associated with an NADPH specific acyl-CoA reductase (which converts fatty acyl-CoAs to the corresponding alcohols) and an acyl-CoA:fatty alcohol acyl-transferase (which catalyzes synthesis of wax esters from the alcohol and a fatty acyl-CoA). Both the reductase and acyl-transferase are associated with a membrane fraction in developing jojoba embryos. Reductase activity has been shown to be associated with a 56kD polypeptide, and a cDNA encoding this polypeptide has been cloned. The acyl-transferase has been solubilized and partially purified via chromatography. These data, in combination with a substrate tagging strategy, suggest that the activity is associated with a 57kD polypeptide.

Oral Paper 41 - Thursday 10:30

CHARACTERIZATION AND INDUCTION OF TWO CYTOCHROME P-450-DEPENDENT MONOOXYGENASES INVOLVED IN RESIN ACID BIOSYNTHESIS IN GRAND FIR

Christoph Funk and Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Oleoresin, composed mainly of monoterpenes (turpentine) and diterpenes (rosin), plays an important role in defense responses of conifers. Abietic acid, a major constituent of both grand fir (*Abies grandis*) and lodgepole pine (*Pinus contorta*) rosin is synthesized from geranylgeranyl pyrophosphate via abietadiene, abietadienal and abietadienal. The two oxidations converting the olefin to abietadienal, have been demonstrated in cell-free stem extracts of both conifers. Dependence of these microsomal activities on oxygen and NADPH, as well as inhibition by CO (light reversible) and substituted imidazole inhibitors, showed the enzymes to be two distinct cytochrome P-450-dependent monooxygenases. Both enzymes are induced in grand fir stems after wounding, and the end product, abietic acid, accumulates.

Oral Paper 42- Thursday 10:45

GENETIC MANIPULATION OF PHYTOALEXIN COMPOSITION IN GOSSYPIUM: EFFECTS ON DISEASE RESISTANCE

Alois A. Bell, R.D. Stipanovic and M.E. Mace, USDA, ARS, Southern Crops Research Laboratory, College Station, TX, USA 77845

Sesquiterpenoid aldehyde phytoalexins in cultivated Upland cotton (*Gossypium hirsutum*) were changed qualitatively by introducing genes from the wild species *Gossypium raimondii* and *Gossypium sturtianum* via multiple species hybrids. Two genes from *G. raimondii* introduced a methoxyl group at the C-7 position of the hemigossypol phytoalexin, while the gene from *G. sturtianum* increased methylation of the 3-hydroxyl group. The foreign genes caused qualitative changes in phytoalexin composition in both leaves and stems, but changes were more pronounced in leaves. Both qualitative changes decreased the toxicity of phytoalexins to the wilt fungi and increased the susceptibility of cotton to disease. The implications of these results for qualitatively improving cotton phytoalexins will be discussed.

POSTERS

(starred posters are entered in the Best Poster competition)

* Poster 1 *

VASCULAR DEVELOPMENT AND LIGNIFICATION IN *ARABIDOPSIS THALIANA*

D.P. Dharmawardhana, B.E. Ellis and J.E. Carlson. Biotechnology Laboratory and Department of Plant Science, University of British Columbia, Vancouver, B.C., Canada V6T 1Z4

The timing and quality of lignin deposition during *Arabidopsis* development have been determined histochemically and biochemically. Lignin can first be detected in tracheary elements of the embryo radicle at approximately 36 hours post-imbibition. During subsequent development, lignification proceeds in a temporally coordinated manner closely following the secondary cell wall thickening in vascular tissue in a cell-by-cell sequence. Interestingly, we observed developmental regulation in the type of lignin deposited in the flowering shoot. Lignin deposited up to 20-21 days after germination (5 days after bolting) is predominantly of the guaiacyl type. Syringyl type lignin appeared only after differentiation of fibers between the vascular bundles in the flowering stem. This was initially detected histochemically, but confirmed by chemical analysis.

* Poster 2 *

A NEW APPROACH FOR TROPANE ALKALOID OVERPRODUCTION FROM HAIRY ROOTS OF *HYOSCYAMUS MUTICUS*

Fabricio Medina-Bolivar¹ and Hector Flores^{1,2} ¹Graduate Program in Plant Physiology and ²Dept. of Plant Pathology/Biotechnology Institute, The Pennsylvania State University, University Park, PA 16802

In vitro hairy root cultures of *Hyoscyamus muticus* have been shown to accumulate higher levels of the tropane alkaloid hyoscyamine than do cell suspension cultures. We have observed that the hairy root and cell suspension phenotypes are readily interconverted by manipulating the levels of 2,4-D in the medium. Using this approach we have obtained three hairy root-derived cell suspension lines which can grow over 400 µm of p-fluorophenylalanine (PFP) and regenerated hairy roots from these cultures. The hairy root-derived cell suspension PFP-resistant lines accumulated cinnamoyl-putrescines. These compounds share the same biosynthetic precursors with hyoscyamine. One hairy root clone, derived from the PFP-resistant cell suspension cultures, showed significantly higher levels of hyoscyamine than the parental hairy roots. This result is consistent with our hypothesis that the overproduced precursors found in the cell suspension can be diverted into the tropane alkaloid pathway in the regenerated hairy roots. (Supported by NSF BCS-9110288).

* Poster 3 *

ANTIBIOTIC ACTIVITY OF THE THIARUBRINES ISOLATED FROM *AMBROSIA CHAMISSONIS* ROOTS AND ROOT CULTURES

Shona Ellis, Zyta Abramowski, and G.H. Neil Towers, Dept. of Botany, University of British Columbia, Vancouver, BC., Canada V6T 1Z4.

Eight thiarubrines (dithiacyclohexadiene polyynes) have been isolated from *Ambrosia chamissonis* roots and root cultures. Their activities were compared against several bacteria and fungi. Both light dependent and independent activities have been observed. All are toxic toward the gram-positive bacteria and fungi tested. Activity against the gram-negative bacteria is more restricted. Cross-sections revealed the localization of the compounds in transgenic and non-transgenic root cultures.

* Poster 4 *

PURIFICATION AND INDUCTION OF PHENYLALANINE AMMONIA-LYASE FROM THE CORN SMUT FUNGUS *USTILAGO MAYDIS*

Seong Hwan Kim, James W. Kronstad and Brian E. Ellis, Department of Plant Science, University of British Columbia, Vancouver, BC, Canada, V6T 1Z4

Phenylalanine ammonia-lyase (PAL, EC.4.3.1.5) has been purified from liquid-cultured cells of the phytopathogenic fungus *Ustilago maydis* strain 001, by use of heat treatment, ammonium sulfate precipitation, chromatography with DEAE cellulose and gel filtration on Bio-Rad A-0.5m column. PAL activity in *U. maydis* was highly increased in L-tryptophan-treated cells, but was suppressed in glucose-treated cells. PAL activity was higher in cells grown in potato dextrose broth-, casamino acid-, peptone- and yeast extract-supplemented media than in those grown in tryptone- and beef extract-treated media. The purified PAL is currently being characterized and the PAL cDNA will be subsequently cloned.

* Poster 5 *

EXPRESSION OF PHENYLALANINE AMMONIA-LYASE IN ELICTOR-TREATED *PINUS BANKSIANA* CELL SUSPENSION CULTURES

Monica L. Lam and Brian E. Ellis, Dept. of Plant Science, University of British Columbia, Vancouver, BC, Canada V6T 1Z4

Phenylalanine ammonia-lyase (PAL), the first enzyme in the lignin biosynthetic pathway, catalyzes the conversion of phenylalanine to cinnamic acid. Despite the importance of PAL in phenylpropanoid metabolism, its regulation is not well understood. Transient increases in PAL activity are observed in *Pinus banksiana* cell suspension cultures treated with an ectomycorrhizal fungal elicitor. As PAL is inducible in this interaction, it is a convenient system for studying PAL regulation.

We have purified a partial PAL cDNA clone from a library prepared from elicitor-treated pine cells. We are investigating PAL regulation by manipulating the chemical environment of elicitor-treated cells with exogenously applied PAL inhibitors and with phenylpropanoid compounds which are reported to act as regulatory signals in angiosperms. PAL transcription rates and changes in PAL activity are being monitored to examine the effect of these chemicals on the induction of PAL in elicitor-treated cells.

* Poster 6 *

GENETIC MANIPULATION AND PRODUCTION OF ALIZARIN IN *RUBIA PEREGRINA*. L.

A.U.H. Lodhi, S.A. Coomber and B.V. Charlwood. Plant Tissue Culture and Molecular Sciences Group, School of Life Sciences, Kings College London, Campden Hill Road, London W8 7Ah.

Rubia peregrina L. is a member of Rubiaceae family and is widely distributed in temperate to torrid zones. The roots of the plant have been used since ancient times for dyeing and medicinal purposes. The undifferentiated callus of *Rubia peregrina* L. has been initiated from stems and leaves of the explant. A rapid, high performance liquid chromatography (HPLC) method was developed for the qualitative and quantitative analysis of alizarin from the crude plant extract. *Agrobacterium* mediated transformed hairy roots were produced *in vitro*. The putative transformed roots were subjected to histochemical analysis and amplification of TL, NPTII and GUS regions by Polymerase Chain Reaction (PCR).

Isochorismate hydroxymutase, the enzyme catalyzing the conversion of chorismate into isochorismate is responsible for the key step in the production of alizarin. The gene coding for this enzyme has been amplified by PCR from *E. coli* and is being cloned into the plant transformation vector. The transformed roots are being produced to study the expression of chimeric gene.

* Poster 7 *

CYTOKININ-STIMULATED PUTRESCINE SYNTHESIS IN CULTURED GRAPE ROOTS

Mary Lou Mendum and Douglas O. Adams, Department of Viticulture and Enology, University of California, Davis

Polyamines are believed to influence developmental processes such as flowering and embryogenesis in plants. In cultured root segments from two grape species (*Vitis vinifera* and *Vitis champinii*), the putrescine concentration increased as the concentration of the cytokinin benzyladenine was increased from 0.0 to 10 μ M. The auxin naphthalene acetic acid had little effect on putrescine levels except in the *Vitis vinifera* cultivar 'Cabernet Sauvignon'. Putrescine accumulation in the *Vitis vinifera* cultivar 'Chardonnay' changed between four and seven weeks of culture. Kinetin also increased putrescine levels, but 2-isopentenyl adenine did not.

Addition of arginine to the culture medium reduced growth, but did not increase putrescine. However, in the presence of agmatine, putrescine increased tenfold in the cultured tissue. Agmatine with either BA or kinetin increased putrescine more than either cytokinin alone or agmatine alone, suggesting that the two cytokinins increased the root segments' capacity to convert agmatine to putrescine.

We are currently measuring the activity of the first enzyme in the putrescine synthesis pathway, arginine decarboxylase, in BA-treated grape leaves.

* Poster 8 *

INVOLVEMENT OF PLANT CELL MEMBRANE PROTEINS DURING EARLY DEFENSE RESPONSES OF ALFALFA CULTIVARS TO COLLETOTRICHUM TRIFOLII

Liliana Di Nola-Baron and Nichole R. O'Neill. USDA, Agricultural Research Service, Beltsville, Maryland 20705.

The temporal differences in cell membrane protein composition and in mRNA synthesis between resistant and susceptible clones of alfalfa (*Medicago sativa*) to the anthracnose disease were studied *in vivo* upon inoculation with race 1 or race 2 of *Colletotrichum trifolii*. Immediately after inoculation several changes in protein composition occur in the cell membranes of the plants. In particular, the accumulation of a 29 KD protein, which is associated with a concomitant increase in mRNA synthesis, seems to be directly related to the resistance response in the alfalfa clones. The recognition response is race-cultivar specific, and it is triggered only in an "alive" interaction between the plant and the pathogen. These results indicate that cellular membranes in plants may play an important role in the early recognition process between fungal pathogens and plants.

* Poster 9 *

DIHYDROFLAVANOL AND 3,4-DIOL REDUCTASE ACTIVITIES IN SAINFOIN LEAVES

Santokh Singh, John McCallum, Mohammed R. Koupai-Abyazani, G.H. Neil Towers, Alister D. Muir, Bruce A. Bohm and Margaret Y. Gruber, Dept. of Botany, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada and Research Station, Agriculture Canada, 107 Science Place, Saskatoon, SK, Canada

Soluble enzyme extracts from sainfoin leaves (*Onobrychis viciifolia* Scop., cv. Melrose) catalyzed the NADPH-dependent reduction of (+)-dihydromyricetin to 2,3-*trans*-3,4-*cis*-leucodelphinidin and (+)-gallocatechin. The activity of these reductase declined with an increase in leaf maturity. The enzyme preparation was also capable of reducing (+)-dihydroquercetin to (+)-catechin. Formation of (-)-epigallocatechin and condensed tannins could not be detected in these assays.

* Poster 10 *

PHENOLICS AND ACYLATED FLAVONOL GLYCOSIDES FROM PINES THAT INHIBIT GYPSY MOTH LARVAE DEVELOPMENT

Clifford Beninger, Mamdouh Abou-Zaid and Blair Helson, Forestry Canada, FPMI, P.O. Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7

Needles (1 kg. fr. wt.) from mature trees of *Pinus strobus* L. (eastern white pine), *P. resinosa* Ait. (red pine), *P. sylvestris* L. (scotch pine) and *P. banksiana* Lab. (jack pine) (family *Pinaceae*) were collected from Kirkwood Forest near the city of Sault Ste. Marie, Ontario and extracted with 70% EtOH. The ethanolic extracts were freeze-dried and fractionated using a Buchner funnel packed with 200 g polyvinylpyrrolidone (PVPP) with water followed by increasing concentrations of ethanol. Fractions were further separated by PVPP column chromatography. Final purification was achieved on Sephadex LH-20 column using MeOH. Pure compounds were subjected to chemical and physical investigations (UV, ¹H-NMR, ¹³C-NMR and FAB-MS). Six phenolics and acylated flavonol glycosides were isolated and identified. Crude pine extracts containing these compounds incorporated into artificial diet at concentrations of 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mg/g were evaluated for their effect on growth and mortality of *Lymantria dispar* L., (gypsy moth). All concentrations significantly reduced growth of larvae after only 10 days of feeding.

* Poster 11 *

TANNINS FROM BARK OF ACACIA TREES

Karin E. Readle¹, David S. Seigler¹, H. David Clarke¹, John E. Ebinger¹ and Juan F. Hernandez². Department of Plant Biology, University of Illinois, Urbana, IL 61801. ²Centro de Investigaciones y Asistencia Tecnologica de Estado Guanajuato, Apartado Postal 890, Leon, Guanajuato, Mexico.

The percentage of tannins in bark of several species of *Acacia* from Mexico was determined by casein precipitation. Extracts with 70% acetone were concentrated and redissolved in water. Total phenolic content was determined before and after precipitation with casein by the Folin-Denis analysis. Percentages of tannins in these species generally ranged from 1-8% and were consistent with amounts previously reported in the literature. Addition of sodium bisulphite to facilitate the dissolution of the samples prior to assays (as is commonly used in the leather industry) resulted in amounts 2-3 times greater than those with water alone. These data suggest that many literature values for tannins are substantially lower than the actual amounts present.

* Poster 12 *

4-COUMARATE:CoA LIGASE FROM LOBLOLLY PINE XYLEM: ISOLATION, CHARACTERIZATION AND COMPLEMENTARY DNA CLONING

Kui Shin Voo¹, Ross W. Whetten², David M. O'Malley², and Ronald R. Sederoff². Departments of ¹Genetics and ²Forestry, P.O. Box 8008, North Carolina State University, Raleigh, North Carolina 27695-8008 Tel: 919-515-7800

4-Coumarate:CoA ligase (4CL, EC 6.2.1.12) catalyzes a reaction at a branch point of the general phenylpropanoid pathway that supplies precursors for lignin biosynthesis and several other plant secondary metabolic pathways. 4CL from loblolly pine (*Pinus taeda* L.) was purified to homogeneity from differentiating xylem. The pine enzyme has an apparent molecular weight of 64 kD and is similar in size and kinetic properties to the 4CL previously characterized from spruce and parsley. The enzyme has high affinity for caffeic acid ($K_m = 9.10 \pm 0.3 \mu$ M), 4-coumaric acid ($K_m = 6.83 \pm 0.7 \mu$ M), and ferulic acid ($K_m = 9.10 \pm 0.7 \mu$ M), but has a low affinity for sinapic acid. 4CL is inhibited by naringenin and coniferin, which are end products of phenylpropanoid metabolism. Although the lignin composition in compression wood is different from that of normal wood, there is no evidence for another form of 4CL enzyme in compression wood. cDNA clones for 4CL were

obtained by screening a xylem expression library with polyclonal antibodies and by PCR derived probes made from conserved 4CL sequences. Identity of these clones was verified by comparison of DNA sequence and experimentally determined pine 4CL peptide sequences.

* Poster 13*

DETERMINATION OF SINAPINE FOR RAPESEED BREEDING

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Ion-exchange, UV spectrophotometric and HPLC methods were evaluated for selection in breeding for low sinapine canola. Sinapine determinations of 180 samples from *Brassica carinata* Braun., *B. juncea* (L.) Coss., *B. napus* L., *B. nigra* L Koch, *B. rapa* L. and *Sinapis alba* L. Both methods showed a close correlation, $r = 0.724$. Using the HPLC and the ion-exchange UV spectrophotometric methods, 40 and 120 samples, respectively, could be analyzed by one person per 8 hour day. A UV fluorescence method developed to screen sinapine content in rapeseed/canola seed was able to detect as little as 1.2 µg sinapine in one seed. Using this rapid and inexpensive method, one person could semi-quantitatively analyze about 600 seeds or "half-seeds" per 8 hour day.

Poster 14

SULFATED FLAVONOL-SPECIFIC BINDING TO NUCLEAR PROTEINS IN *FLAVERIA*

Jacques Grandmaison and Ragai Ibrahim, Plant Biochem Lab, Dept of Biology, Concordia University, Montréal, QC, Canada H3G 1M8

Immunocytochemical localization of flavonol 3-sulfotransferase (F3ST) and its product flavonol 3-sulfate (F3S) in *Flaveria*, revealed that 90% of labelling is associated with the nuclear compartment. These observations were corroborated by analysis of the substrate (Quercetin, Q), the product (Q3S) and F3ST activity in cell organelles. Purification of the nuclear Q3S-binding proteins was achieved by successive chromatography on Superose 12, Q3S-Sepharose and Mono Q columns, using Q³²S as a marker. SDS-PAGE of the binding proteins exhibited two apparently homogenous species with M_r of 45 and 36 kD. Competition assays of the purified proteins with several related phenolic compounds revealed a high binding affinity for Q3S and Q. The putative significance of these nuclear binding proteins will be discussed.

Poster 15

DIFFERENTIAL HYBRIDIZATION STRATEGY FOR THE CLONING OF PLANT ISOFLAVONE PRENYLTRANSFERASES (IPTs)

Sylvie Attucci, Patrick Gulick & Ragai Ibrahim, Plant Biochemistry Lab, Dept of Biology, Concordia University, Montréal, QC, Canada H3G 1M8

IPTs catalyze the prenylation of positions 6, 8, and/or 3' of the isoflavones genistein and 2'-OH-genistein in white lupins (*Lupinus albus*). Prenylated isoflavones have been shown to possess phytoalexin activity, which seems to increase with the level of prenylation. IPT activity can be detected in root extracts, as confirmed by HPLC analysis. In order to isolate cDNA clones encoding IPTs, oligonucleotides derived from conserved sequences among other PTs were used as primers for PCR amplification of gene fragments from lupin root cDNA. The PCR-amplified products were cloned and the resulting library was screened by differential colony hybridization with root-, hypocotyl- and leaf first strand -cDNA probes. Further analysis of the positive clones will be presented.

Poster 16

PICOTAG METHOD FOR ANALYSIS OF FREE AMINO ACIDS IN PLANTS: SOME APPLICATIONS

Ramon A. Razal¹, Norman G. Lewis², and G.H. Neil Towers¹. ¹Department of Botany, University of British Columbia, Vancouver, BC Canada V6T 1Z4; ²Institute of Biological Chemistry, Washington State University, Pullman, WA, USA 99163

Pre-derivatization with phenylisothiocyanate followed by high performance liquid chromatography on a Waters Picotag system (Cohen *et al.* Nature 320:769-770) was used in analyzing extracts of free amino acids from various plant and fungal sources. Various amino acids, including phenylalanine and tyrosine, were detected and differences were noted in their pools in the different plant materials. One application for which the method was tested was in determining the levels of aroenate, an amino acid which serves as an intermediate in the biosynthesis of the aromatic amino acids, phenylalanine and tyrosine, in the various plant materials. Although the method was amenable to detecting aroenate in fungal cultures, no detectable levels of aroenate were found in the plants investigated.

Poster 17

CHARACTERIZATION OF UDP-GLUCOSE: CYANIDIN 3-O-GLUCOSYLTRANSFERASE IN *VITIS VINIFERA* CELL SUSPENSION CULTURES

Chi Bao Do and François Cormier, Food Research and Development Centre, Agriculture Canada, 3600 Casavant Blvd. West, Saint-Hyacinthe, Quebec, Canada J2S 8E3

In pigmented cells of *Vitis vinifera* suspension culture (cell line # 13.1) anthocyanin accumulation was promoted by cultivation in a production medium which contained a high carbohydrate concentration, i.e. 277 mM glucose, and a low nitrate concentration (6.25 mM). Under such culture conditions the activity of UDP-glucose: cyanidin 3-O-glucosyltransferase was characterized by the occurrence of a maximum which preceded the onset of anthocyanin accumulation by 1 day. Purification of the enzyme was performed using gel filtration, ion-exchange chromatography and chromatofocusing. Under actual conditions, enzyme preparation was purified 6000 fold. By improving the conditions of chromatofocusing we expect to obtain an enzyme preparation purified to homogeneity.

Poster 18

EXPRESSION OF TRICHODIENE SYNTHASE FROM *FUSARIUM SPOROTRICHIOIDES* IN TRANSFORMED TOBACCO CELL SUSPENSION CULTURES

¹Michael Zook, ²Thomas M. Hohn, and ¹Ray Hammerschmidt, ¹Dept. Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824. ²Mycotoxin Research Unit, USDA/ARS, NCAUR, Peoria, IL, 61604 USA

The gene encoding trichodiene synthase, a sesquiterpene cyclase from the fungus *Fusarium sporotrichioides*, was used to transform tobacco (*Nicotiana tabacum*). Immunoblot analysis demonstrated the presence of variable levels of trichodiene synthase in five different transformant cell lines. The production of trichodiene in transformant cell lines containing elevated levels of sesquiterpene cyclase activity was demonstrated by GC/MS analysis. *In vivo* labelling with [³H]mevalonate and [³H]trichodiene has demonstrated that the level of trichodiene produced is proportional to the level of trichodiene synthase and has revealed the presence of a novel metabolite in transformant cell lines with trichodiene synthase activity. Structural analysis suggests that this metabolite is a monooxygenated derivative of trichodiene.

Poster 19

ISOPENTENYL DIPHOSPHATE ISOMERASE AND FARNESYL DIPHOSPHATE SYNTHASE ACTIVITIES IN ELICITED *CINCHONA ROBUSTA* CELL SUSPENSION CULTURES

A. Ramos-Valdivia, R. van der Heijden and R. Verpoorte. Division of Pharmacognosy, Leiden/Amsterdam Center for Drug Research. Gorlaeus Laboratories, P.O. Box 9502, 2300 RA Leiden, The Netherlands

IPP-isomerase and FPP-synthase are involved in the biosynthesis of terpenoids. IPP-isomerase also provide C₅-units for the biosynthesis of "Rubia type" anthraquinones. Elicitation of cell suspension cultures of *Cinchona robusta* with a *Phytophthora cinnamomi* preparation resulted in a rapid induction of anthraquinone biosynthesis. In these cultures IPP-isomerase activity was induced. Simultaneously FPP-synthase activity was inhibited and culture growth suppressed. These results support the hypothesis that by regulation of IPP-isomerase and FPP-synthase activities the channelling of C₅-units towards anthraquinone biosynthesis can be influenced.

Poster 20

IMMUNOAFFINITY PURIFICATION OF THE GLUCOSINOLATE BIOSYNTHETIC ENZYME, THIOHYDROXIMATE S-GLUCOSYLTRANSFERASE, FROM *BRASSICA*

J.W.D. Groot Wassink, D.W. Reed and A.D. Kolenovsky, National Research Council, Saskatoon, Saskatchewan, Canada S7N 0W9

Crop variety breeding of oilseed brassicas (canola: *Brassica napus* and *Brassica campestris*) continues to be concerned with elimination of the antinutritional glucosinolates (sulfated S-glucosyl thiohydroximates). Germplasm with extremely low or zero levels of glucosinolates is not readily available, but might be genetically engineered.

Purifying thiohydroximate S-glucosyltransferase (S-GT), the second last biosynthetic enzyme, proved to be very difficult due to low abundance and instability. Screening of several *Brassica* sources showed that environmentally stressed cauliflower produced extra high levels of glucosinolates and S-GT. Chromatographic and electrophoretic enrichments were insufficient to achieve homogeneity; the resolving power of monoclonal antibody was required. Enzyme was absorbed from crude tissue extracts onto agarose-antibody matrix, with elution (10-20% activity recovery) yielding two proteins (~2000-fold purification) of which one corresponded to S-GT.

Poster 21

INVESTIGATION OF ALTERED SECONDARY METABOLISM BY TRYPTOPHAN DECARBOXYLASE (TDC)-EXPRESSING TOBACCO SEEDLINGS

Juan Basurco & Vincenzo de Luca, Institut de Recherche en Biologie Végétale, Université de Montréal, Montréal, Canada

Transformed tobacco seedlings expressing a novel TDC activity have been analyzed for altered secondary metabolism. Transgenic and non-transformed control seeds were germinated in the absence of light and etiolated seedlings were harvested at different stages of growth. HPLC analyses of seedling extracts revealed that tryptamine-accumulating transgenic seedlings lacked a major metabolite compared with nontransformed control seedlings. Light treatment of seedlings resulted in rapid disappearance of this metabolite and further accumulation of tryptamine. These results will be discussed in relation to the ability of TDC to redirect the shikimate pathway towards the production of tryptophan-derived secondary metabolites.

Poster 22

IMPROVEMENT OF CANOLA MEAL BY REDIRECTION OF TRYPTOPHAN AWAY FROM MUSTARD OIL GLYCOSIDES

Supa Chavadej¹, Normand Brisson² and Vincenzo De Luca¹, Departments of Biology¹ and Biochemistry², Université de Montréal,

Cruciferous plants and vegetables accumulate over a hundred different mustard oil glycosides which are derived directly from the protein amino acids, methionine, phenylalanine and tryptophan. Transgenic Canola plants that redirect tryptophan into tryptamine were engineered by introduction of a plant gene which encodes tryptophan decarboxylase. Plants containing this gene accumulated reduced levels of tryptophan-derived indole glucosinolates. The indole glucosinolate content of mature seeds from transgenic plants was only 3.3% of that found in seeds of non-transformed plants, thus providing a method to remove these undesirable byproducts and to increase the value of the Canola meal which is produced after extraction of oil from seed.

Poster 23

ISOLATION AND CHARACTERIZATION OF A FLAVONOL SYNTHASE cDNA CLONE FROM PETUNIA

Timothy A. Holton, Filippa Brugliera and Yoshikazu Tanaka, Calgene Pacific Pty Ltd, 16 Gipps Street, Collingwood, VIC, Australia

Flavonols are a class of flavonoids that are mostly colourless but can exert considerable effects on flower colour by co-pigmentation with anthocyanins. Flavonols are produced by the action of flavonol synthase (FLS) on dihydroflavonols. FLS is a dioxygenase which requires 2-oxoglutarate, ascorbate and Fe²⁺ ions as co-factors. All of the 2-oxoglutarate dependent dioxygenases cloned so far share a few small pockets of sequence conservation. Degenerate oligonucleotides designed to two of these regions were used as primers to amplify dioxygenase sequences, including FLS, from petunia by PCR. Antisense FLS expression in petunia led to a reduction in flavonol synthesis and a corresponding change in flower colour.

Poster 24

ELICTOR REGULATION OF ENZYME ACTIVITIES FROM DIFFERENT METABOLIC PATHWAYS IN *CATHARANTHUS ROSEUS* CELL CULTURES

Paulo R. H. Moreno, Robert van der Heijden and Robert Verpoorte, Div. Pharmacognosy, Gorlaeus Lab, Leiden/Amsterdam Center for Drug Research, P.O. Box 9502, 2300 RA Leiden, The Netherlands

Cell suspension cultures of *Catharanthus roseus* were elicited with an autoclaved cell-free filtrate of *Pythium aphanidermatum*. The regulation of alkaloid, terpenoid and phenolic biosynthetic pathways was studied through assaying the activity of the following enzymes: anthranilate synthase (AS), tryptophan decarboxylase (TDC), strictosidine synthase (SSS), strictosidine-β-glucosidase (SG), isopentenyl diphosphate isomerase (IPP-isomerase), geraniol-10-hydroxylase (G10H), chorismate mutase (CM) and phenylalanine ammonia lyase (PAL). After elicitation, AS and TDC were induced resulting in an increase of tryptamine in the cells. Also SSS was induced, SG was not changed. Ajmalicine accumulation was not increased compared with control cells. An increased amount of phenolic compounds was found in the medium, however neither CM nor PAL were induced after elicitor treatment. The enzyme activities of IPP-isomerase and G10H were not induced after elicitation. This findings suggested that the secoiridoid pathway was not modulated by elicitors.

Poster 25

A NEW ANTIBIOTIC FROM *RHUS GLABRA*

Geeta Saxena, G.H.N. Towers¹ and R.E. W. Hancock², ¹Department of Botany and ²Microbiology, University of British Columbia, Vancouver, BC Canada V6T 1Z4

From the antibiotic screening of 100 medicinal plants of British Columbia, *Rhus glabra* (Anacardiaceae) exhibited both the widest zones of inhibition in a disc assay and the broadest spectrum of activity including both Gram-positive and Gram-negative bacteria. Further isolation of the active components resulted in the identification of a new antibiotic from this plant.

Poster 26

A NEW C-GLYCOSYLFLAVONE FROM THE FERN *ASPENIUM VIVIPARUM*

Filippo Imperato, Dipartimento di Chimica, Università della Basilicata, 85100 Potenza, Italy

From an ethanolic extract of aerial parts of the fern *Asplenium viviparum* a new flavonoid has been isolated by preparative paper chromatography followed by Sephadex LH-20 column chromatography. UV spectral analysis with the customary shift reagents, chromatographic behaviour, colour reactions and treatment with 3N HCl suggested that the isolated substance may be a C-glycosylflavone. Degradation with hydriodic acid gave apigenin. FeCl₃ oxidation gave xylose. ¹H NMR and FAB mass spectrum showed that the isolated compound is 3-C-xylosylapigenin 7-methyl ether which is a new natural product.

Poster 27

CLONING AND CHARACTERIZATION OF 4-COUMARATE:COENZYME A LIGASE (4CL) GENES FROM POPLAR AND *ARABIDOPSIS*

Sandra Allina, Diana Lee, and Carl J. Douglas, Dept. of Botany, University of British Columbia, Vancouver, BC Canada V6T 1Z4

We are interested in the role of 4-coumarate:coenzyme A ligase (4CL) in regulating the biosynthesis of phenylpropanoid compounds. In poplar, enzymological studies (Grand et al. 1983. *Planta* 158:225) suggested the presence of multiple 4CL isoforms with different substrate specificities, while 4CL has not been studied in *Arabidopsis*. We used heterologous 4CL probes to isolate putative 4CL clones from hybrid poplar (*Populus trichocarpa* X *deltoides*) and *Arabidopsis* cDNA libraries. DNA sequence analysis showed that these clones encode authentic 4CL. Genomic Southern blots indicated that 4CL is encoded by a single gene in *Arabidopsis* but is encoded by a multi-gene family in poplar. Consistent with this, we found at least two classes of poplar 4CL cDNA clones which do not cross-hybridize at high stringency. We hypothesize that in poplar, divergent 4CL gene family members encode enzymes with different enzymatic properties (e.g. preferentially utilize differently methoxylated cinnamic acid derivatives as substrates) and that the differential expression of these genes may help to regulate the biosynthesis of different phenylpropanoid compounds in poplar. In contrast, we hypothesize that in *Arabidopsis* there is a single 4CL form which efficiently utilizes several cinnamic acid derivatives as substrates.

Poster 28

NEW LIMONOIDS FROM THE WOOD AND LEAVES OF *CHISOCHETON MICROCARPUS* AND *CHISOCHETON MACROPHYLLUS* (MELIACEAE)

P.J. Gunning¹, L.B. Jeffs¹, M.B. Isman² and G.H.N. Towers¹, Departments of ¹Botany and ²Plant Science, University of British Columbia, Vancouver, BC, Canada

Screening of members of the Mahogany family (Meliaceae) for insecticidal activity led to the identification of methanolic extracts of some *Chisocheton* species as having strong activity against the variegated cutworm *Peridroma saucia*. Isolation of the constituents of two members of this genus, *C. microcarpus* and *C. macrophyllus* led to the identification of new limonoids.

Poster 29

ELECTRICALLY INDUCED PROTOPLAST FUSION FOR PRODUCTION OF LEPTINES IN THE LEAVES OF CULTIVATED POTATO

Jianping Cheng and James A. Saunders, SARL, Plant Science Institute, USDA, ARS, Beltsville, MD, USA 20705

Leptines are a group of glycoalkaloids found in certain selections of *Solanum chacoense*. These glycoalkaloids have insecticidal activity against the Colorado potato beetle (CPB) which causes serious loss in potato production in North America. Because leptines are not present in tubers, it is desirable to incorporate this character into the cultivated potato, *S. tuberosum* for CPB resistance. In this study, protoplasts from a dihaploid of *S. tuberosum* were electrically induced to fuse with protoplasts from *S. chacoense*, 55-1, which contains leptines in the leaves and is resistant to CPB. Interspecific somatic hybrids were regenerated and identified by using a combination of hybrid vigour characteristics, morphological traits and isozyme patterns. Preliminary work on insecticidal activity against CPB in these somatic hybrids is promising, indicating that incorporation of leaf production of leptines into the cultivated potato may lead to an environmentally sound measure for potato protection against CPB.

Poster 30

EVALUATION OF PHENOLICS AND ACYLATED FLAVONOL GLYCOSIDES FROM *ACER* AS POTENTIAL SOURCE OF RESISTANCE TO FOREST TENT CATERPILLAR

Mamdouh Abou-Zaid and Blair Helson, Forestry Canada, FPML, P.O. Box 490, Sault Ste. Marie, Ontario, Canada P6A 5M7

Acer rubrum L. (red maple) and *A. saccharum* L. are two of the 6 species of *Acer* (family *Aceraceae*) endemic to eastern Canada. Red maple is not a preferred host of forest tent caterpillar and is rarely consumed. In order to identify chemical resistance factors in red maple and/or feeding stimulants in sugar maple, leaves (1 kg. fr. wt.) of both species were collected in May 1992, from mature trees in the city of Sault Ste. Marie, Ontario and extracted with 70% EtOH. The ethanolic extracts were freeze-dried and fractionated using a Buchner funnel packed with 200 g polyvinylpyrrolidone (PVPP) with water followed by increasing concentrations of ethanol. Final purification was achieved on Sephadex LH-20 column using MeOH. Pure compounds were subjected to chemical and physical investigations (UV, ¹H-NMR, ¹³C-NMR and FAB-MS). Seven phenolics and acylated flavonol glycosides were isolated and identified in red maple. Crude extracts of red maple incorporated into artificial diet at concentrations of 1 and 5 mg/g were evaluated for their effect on growth and mortality of forest tent caterpillar. Both concentrations significantly reduced growth of larvae after only 10 days of feeding.

Poster 31

POLYACETYLENE INDUCTION IN SAFFLOWER

Lauralyn Beaverson and K.R. Downum, Dept. of Biol. Sci., Florida Int'l Univ., Miami, FL 33199

Safflower (*Carthamus tinctorius* L.; Asteraceae) provides an ideal model system for investigating the roles of endogenous photosensitizers within plants. Early studies identified two acetylenic phytoalexins, safynol and dehydrosafynol, which were induced following inoculation with *Phytophthora drechsleri* (*Phytopath.* 6,1107-1109). In the current investigation, safynol (3,11-tridecadiene-5,7,9-triyn-1,2-diol) was isolated from healthy leaf tissue of safflower and found to be a potent phytotoxin in antimicrobial bioassays against *Bacillus cereus* and *Saccharomyces cerevisiae*. A second phototoxic acetylene was also isolated from healthy leaf tissues. Studies are underway to confirm the structure of this second derivative; preliminary spectral data suggests the chromophore is a diene-diyne-ene. Dehydrosafynol has not been found to date. Data on the quantitative significance and phototoxic potential of these constituents in UV-treated and control leaf tissues will be presented.

Poster 32

EFFECTS OF OZONE ON PHENYLPROPANOID METABOLISM IN SOYBEAN

Fitzgerald L. Booker, Air Quality Research Program, Dept. of Botany, North Carolina State University, Raleigh, NC 27695

In many plants, phenolic pigments accumulate in leaves following exposure to the air pollutant ozone. Studies have found that phenylalanine ammonia-lyase (PAL), cinnamyl alcohol dehydrogenase (CAD) and enzymes of the flavonoid biosynthetic pathway are induced in conifers by ozone (Galliano et al., 1993, *Phytochem.* 32: 557-63, and refs. cited therein).

I have found increased activity of phenylpropanoid enzymes in leaf tissue from soybean (*Glycine max*) plants treated 6 h daily with 0.1 ppmv ozone. Increased activity of PAL and 4-coumarate:CoA ligase was measured after only 3 h of treatment with ozone. Foliar injury was apparent the next day, along with increased activities of CAD and peroxidase. On the following day, increased levels of soluble esters of caffeic, 4-coumaric and ferulic acids were measured in EtOH extracts of ozone-treated tissue. The level of caffeic acid in alkaline hydrolysates of extractive-free cell wall material as well as alkali-insensitive thioglycolic acid derivatives also increased in the ozone treatment after 3 d. Increased autofluorescence of palisade parenchyma cell walls was observed in ozone-treated plants, but the phloroglucinol-HCl test was negative. These results suggest that ozone promoted the biosynthesis of esterified phenolic compounds and the deposition of non-core lignin.

Poster 33

ISOLATION, IDENTIFICATION, AND QUANTITATION OF C₁₆-, C₁₈-, AND C₂₀-ESTERS OF P-COUMARIC AND FERULIC ACIDS IN THE LATEX OF SWEETPOTATO CULTIVARS.

Maurice C. Snook, USDA-ARS, P.O. Box 5677, Athens, GA 30613; Emma S. Data, Visayas State College of Agriculture, Baybay, Philippines; Stanley J. Kays, Dept. of Hort., Univ. of Georgia, Athens, GA 30602.

Vine latex from sweetpotatoes with different susceptibilities to the sweetpotato weevil [*Cylas formicarius* Summer], has been analyzed for its chemical phenolic composition by reverse-phase HPLC. Late-eluting phenolics were identified as *cis*- and *trans*-C₁₆-, C₁₈-, and C₂₀-p-coumarates and ferulates by UV spectra, hydrolysis data, syntheses, and GC/MS of their trimethylsilyl-derivatives. The mass spectral data will be presented. The most abundant ester was the C₁₈-p-coumarate, ranging from 0.7% to 2% of fresh weight. No correlation was apparent between levels of the esters and sweetpotato weevil resistance.

Poster 34

NEW FLAVONOL-C-GLYCOSIDES FROM CORN (*Zea mays* L.) FOR THE CONTROL OF THE CORN EARWORM (*Helicoverpa Zea*).

Maurice C. Snook, USDA-ARS, P.O. Box 5677, Athens, GA 30613; Billy R. Wiseman and Neil W. Widstrom, USDA-ARS, P.O. Box 748, Tifton, GA 31793, and Richard L. Wilson, USDA-ARS, Univ. of Iowa, Ames, Iowa 50011.

The resistance of certain comsilks to the corn earworm, *Helicoverpa Zea* (Boddie), has been attributed to the presence of maysin (a luteolin-C-glycoside). Recent HPLC screening of large numbers of corn germplasm for maysin contents has resulted in the discovery of several inbreds and populations with high levels of flavonol-C-glycosides other than maysin in their silks. These include 3'-methoxymaysin, apimaysin (the apigenin-analogue of maysin), and galactosyl-C-luteolin. Laboratory bioassays were used to determine the antibiosis activities of these flavonols as compared to maysin. The structures of other, new flavonols will be presented.

Poster 35

CHARACTERIZATION OF THE PEA MITOCHONDRIAL PHOSPHATE TRANSPORTER

Cecilia A. McIntosh and David J. Oliver, Dept. Biochemistry, University of Idaho, Moscow, ID 83843 U.S.A.

The Pi transporter facilitates a Pi/hydroxyl and Pi/Pi exchange across mitochondrial membranes. This is essential for rapid rates of oxidative phosphorylation and coupling substrate transport to the pH gradient generated by electron transport. This transporter was solubilized from mitochondrial membranes, purified 500-fold, and reconstituted into liposomes loaded with 10 mM KPi. Transport was initiated by the addition of [32]Pi and terminated with 32 mM diethylpyrocarbonate (DEPC). Unincorporated isotope was removed by Dowex chromatography. Uptake was linear for 2 minutes but continued for up to 40 minutes. Transport had a V_{max} of 701 nmol/mg/min and a K_s of 1.6 mM. Pi transport was insensitive to N-ethylmaleimide (unlike mammalian Pi transport), carboxyatractyloside (inhibits adenylate transport), and N-butylmalonate (an inhibitor of the dicarboxylate transporter). DEPC, PLP, p-OH-phenylglyoxal, and dansyl-Cl were inhibitors.

Poster 36

ISOLATION OF LIMONOATE DEHYDROGENASE (LDH) *ARTHROBACTER GLOBIFORMIS*

Charles Suhayda, Shin Hasegawa, Terres Ronneberg and Chi Fong, USDA, Fruit & Vegetable Chemistry Lab, Pasadena, CA 91106.

Bitter limonoids in citrus juice diminish the quality and value of commercial juices. LDH converts the precursor of bitter limonin, limonoate A-ring lactone, to nonbitter 17-dehydrolimonoate A-ring lactone. This enzyme was isolated from *A. globiformis* cells by a combination of (NH₄)₂SO₄ fractionation, Cibacron Blue affinity chromatography, and DEAE ion exchange HPLC. Gel filtration HPLC indicated a molecular mass of 118 kD for the native enzyme. SDS-PAGE indicated an individual subunit molecular mass of 30 kD. LDH activity in citrus is very low. Cloning the gene for this bacterial enzyme into citrus trees should enhance the natural debittering mechanism in citrus fruit.

Poster 37

FERULIC ACID 5-HYDROXYLASE

Lloyd Yu, The Center For Engineering Plants For Resistance Against Pathogens, The University of California, 1930 Fifth Street, Davis, CA 95616

The demonstration of ferulic acid 5-hydroxylase activity (Grand, FEBS, 1984) has strengthened the position of ferulic acid as the physiologically relevant precursor to sinapyl alcohol. This enzyme system is apparently comprised of a cytochrome P-450 and an associated reductase which uses NADPH, and molecular oxygen to hydroxylate ferulic acid. My overall goal is to study the regulation of this enzyme activity during plant development and defense, particularly with regard to the deposition and compartmentation of sinapyl-rich lignin. Current efforts are focused on the isolation of 5-hydroxyferulic acid from corn and the development of an enzyme assay for the hydroxylase.

Poster 38

THE LATE BIOSYNTHETIC STEPS OF THE 6 α -HYDROXY PTEROCARPAN PISATIN

Greg DiCenzo and Hans VanEtten, Department of Plant Pathology, University of Arizona, Tucson, AZ 85721, USA.

While most legumes studies synthesize (-) phytoalexins, pea is unusual among those which are agronomically important by the ability to make and accumulate the (+) pterocarpin, pisatin. We have particular interest in the formation of 6 α -hydroxymaackiain (HMK), which is directly converted to (+) pisatin by HMK methyl transferase. Previous results suggested that the 6 α -oxygen appears not to derive from a monooxygenase-catalyzed reaction, ruling out a direct hydroxylation of the pterocarpin maackiain. Although pea makes the (-) isoflavanone sophorol, pea cotyledon can incorporate both (-) and (+) sophorol into pisatin and can 6 α -hydroxylate (+) maackiain (but not the (-) isomer). Using tritiated (-) sophorol as a substrate, we are attempting to accumulate labelled intermediates for identification and subsequent ordering of the biosynthetic pathway. Ultimately, we want to purify the HMK-forming enzyme in order to clone the gene(s) which are required to form (+) 6 α -OH pterocarpan in pea.

Poster 39

TEMPORAL AND SPATIAL EXPRESSION OF AMYGDALIN HYDROLASE IN ROSACEOUS STONE FRUITS (*PRUNUS SEROTINA* AND *P. DOMESTICA*)

Chun Ping Li and Jonathan E. Poulton, Department of Biological Sciences, The University of Iowa, Iowa City, Iowa 52242, U.S.A.

In seeds of rosaceous stone fruits, the catabolism of (*R*)-amygdalin to HCN is initiated by the β -glucosidase, amygdalin hydrolase (AH). A black cherry cDNA library was screened using monospecific polyclonal antibodies raised against deglycosylated AH. This yielded two partial length AH cDNA clones whose inserts showed high sequence homology with white clover linamarase. Northern analysis showed that AH transcript levels were maximal in mid-maturation seeds but were undetectable in 3 week-old cherry seedlings. AH transcripts and protein were localized in the procambium of developing seeds by tissue printing and immunocytochemistry, respectively. In mature plum seeds, premature cyanogenesis is apparently prevented by tissue level compartmentalization. Amygdalin is confined to cotyledonary parenchyma cells, while AH, as in black cherries, is restricted to the procambium.

Poster 40

TISSUE AND SUBCELLULAR LOCALIZATION OF PRUNASIN HYDROLASE IN YOUNG STEMS AND LEAVES OF BLACK CHERRY (*PRUNUS SEROTINA* EHRH.)

Elisabeth Swain and Jonathan E. Poulton, Department of Biological Sciences, The University of Iowa, Iowa City, Iowa 52242, U.S.A.

The leaves and stems of black cherry seedlings contain the cyanogenic monoglucoside (*R*)-prunasin, which, upon tissue disruption, is rapidly degraded to HCN, benzaldehyde and glucose by the sequential action of prunasin hydrolase and mandelonitrile lyase. Using immunocytochemical techniques, prunasin hydrolase was localized in the vacuoles of phloem parenchyma cells (possibly companion cells) which lie adjacent to sieve tube members. The implications of this localization pattern for the developmental fate of (*R*)-amygdalin, the corresponding cyanogenic diglucoside found in black cherry cotyledons, were also investigated.

Poster 41

PUTATIVE FAD-BINDING SITE OF *PRUNUS SEROTINA* (*R*)-(+)-MANDELONITRILE LYASE AS DEDUCED FROM THE NUCLEOTIDE SEQUENCE OF A FULL-LENGTH cDNA CLONE

I-Ping Cheng and Jonathan E. Poulton, Department of Biological Sciences, The University of Iowa, Iowa City, Iowa 52242, U.S.A.

The flavoprotein (*R*)-(+)-mandelonitrile lyase (MDL, EC 4.1.2.10) catalyzes the final step in amygdalin catabolism to HCN in disrupted black cherry seeds. Screening a *Prunus serotina* λ gt11 cDNA library using monospecific polyclonal antibodies raised against deglycosylated MDL yielded a full-length cDNA clone. Its insert (MDL1) was subcloned into the pBluescript vector for double strand sequencing. The deduced amino acid sequence displayed an open reading frame of 563 amino acids which included the known N-terminus and four internal peptides of MDL. Consistent with MDL's protein body location, a 27-residue signal peptide preceded the start of the mature protein. Analysis of the entire MDL sequence according to rules established by Wierenga *et al.* (J. Mol. Biol. (1986) 187:101) revealed a putative FAD-binding site near the N-terminus of this enzyme.

Poster 42

ANTHOCYANIN CONTENT AND ANTHOCYANOPLAST FORMATION IN RED CABBAGE SEEDLINGS IN RESPONSE TO MINERAL NUTRIENT DEFICIENCIES

C. Nozzolillo and M. Hodges, Department of Biology, University of Ottawa, Ottawa, Canada, K1H 5G6

Red cabbage seedlings, *Brassica oleracea* var *capitata* cv April Red, were grown hydroponically on mineral nutrient solutions complete in all respects (control) or lacking N, P or K. Anthocyanin content increased in -N and -P plants over that in control and -K plants. Anthocyanoplast numbers were highest in -N plants and reduced in -P and -K plants in comparison to control plants. Total phenolics were not significantly different but -N plants contained higher amounts of a luteolin-like flavone.

VOLATILE COMPOUNDS OF THE LIPOXYGENASE PATHWAY INVOLVED IN *ASPERGILLUS FLAVUS*/COTTON PLANT INTERACTIONS

H.J. Zeringue, Jr., SRRC, ARS, USDA, 1100 Robert E. Lee Blvd., P.O. Box 19687, New Orleans, LA 70179-0687

C₆-C₁₀ alkenals, derived from fatty acids via activation of the lipoxygenase pathway, may function both as "volatile elicitors" and as "gaseous phytoalexins" in the cotton plant. C₆-C₁₀ alkenals accumulating on damaged cotton plant tissues (e.g., damaged by fungal attack) were found to inhibit *Aspergillus flavus* and reduce aflatoxin contamination. Airborne signals consisting of individual C₆, C₇, C₈, C₉, and C₁₀ alkenals in enclosed systems with developing cotton balls were found to elicit the sesquiterpenoid naphthol phytoalexins, 2,7-dihydroxycadalene, and 2-hydroxy-7-methoxycadalene, and their oxidation products lacinilene C and lacinilene C 7-methyl ether, together with the coumarin phytoalexin, scopoletin. These diverse alkenals may coordinate defense responses within the same cotton plant and even among neighboring cotton plants.

MEETINGS AND PROGRAMS OF INTEREST

PLANT GROWTH REGULATOR SOCIETY 20TH ANNUAL MEETING: Clarion Hotel, St. Louis, MO, August 6-9, 1993. The meeting will feature research reports on a variety of topics related to plant growth regulation and two symposia: Double the Power of Sunlight: Methanol - Is It a Plant Growth Regulator or a Foliar Nutrient? Speakers: Dr. Arthur Nonomura (organizer), Dr. Hans Bohnert; **The Role of Plant Growth Regulators in the Development and Reproduction of Cereal Plants.** Speakers: Dr. David Ho (organizer), Dr. Paige Morgan, Dr. Stewart Rood, and Dr. Suzanne Abrams. Research reports are invited in all areas of plant growth regulation and will be published in the Society's proceedings. Prizes of \$300 and \$100 will be awarded for the two best student papers. For further information, contact Dr. Louise Ferguson, Program Chair, Univ. California, Kearney Agricultural Research Center, 9240 S. Riverbend Ave., Parlier, CA 93648. (Tel. 209/891-2500).

XV INTERNATIONAL BOTANICAL CONGRESS: Tokyo, Japan, August 28-September 3, 1993. The scientific program will include about 240 symposia and more than 1,000 posters in the following divisions: 1. Systematics and Evolution, 2. Structure and its Dynamics, 3. Phytochemistry and Natural Products, 4. Metabolism and Bioenergetics, 5. Developmental Botany, 6. Ecology and Environmental Botany, 7. Genetics, 8. Biotechnology and Breeding. For further information, contact the Congress Secretariat, XV International Botanical Congress Tokyo, c/o Department of Botany, Faculty of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

FOURTH INTERNATIONAL CONGRESS ON PLANT MOLECULAR BIOLOGY: Amsterdam, The Netherlands. June 19-24, 1994. The Congress will be composed of Plenary Sessions, Concurrent Symposia, Poster Sessions and Interactive Workshops. The first program announcement will be mailed in the fall of 1992. For further information, contact the Congress Secretariat, RAI Organisatie Bureau Amsterdam by Europaplein 12, 1078 GZ Amsterdam, The Netherlands. (Tel. 31-0-20-549-12-12; FAX 31-0-20-646-44-69).

POSITION AVAILABLE

THE UNIVERSITY OF IOWA, IOWA CITY. A postdoctoral position is available immediately to investigate molecular aspects of cyanogenesis (HCN production) during maturation of rosaceous stone fruits. The research will follow up that described in *Plant Physiology*, 100: 282-300 (1992) on temporal and spatial regulation of cyanogenesis in *Prunus serotina*. Candidates should be trained in molecular biology and have documented research experience with techniques such as construction and screening of cDNA and genomic libraries, DNA sequencing, RFLP analysis, and Southern and Northern blot analyses. Experience with immunocytochemical protein localization and/or *in situ* hybridization techniques is desirable. Position involves limited supervision of graduate and

undergraduate students. Preference will be given to applicants with background in plant biochemistry, physiology or molecular biology. Appointment is for 2 years with possible renewal for a third year. Annual salary is \$23,000 plus benefits. The University of Iowa is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are encouraged to apply. Applications will be accepted until a suitable candidate is found. Send curriculum vitae, three letters of recommendation, relevant reprints, and a statement of research interests to Dr. Jonathan E. Poulton, Department of Biological Sciences, 312 CB, The University of Iowa, Iowa City, Iowa 52242, FAX 319/335-3620, telephone 319/335-1322.



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N. SAITO & J. B. HARBORNE (UK), Correlations between anthocyanin type, pollinator and flower colour in the Labiatae.

E. VILLARREAL-ROSALES, P. METZGER & E. CASADEVALL (France), Ether lipid production in relation to growth in *Botryococcus braunii*.

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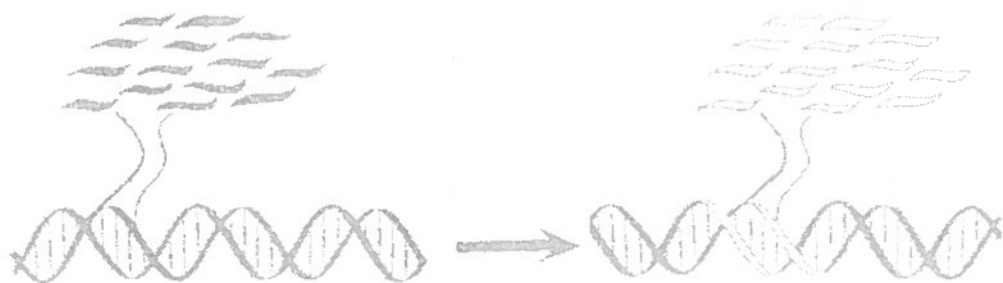
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Newsletter

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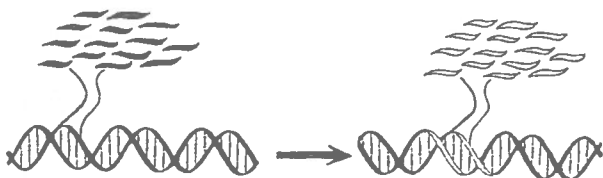
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PSNA Newsletter

Editor: Dr. Alicja M. Zobel



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, the role of plant substances, and related fields. Annual membership dues are U.S. \$20.00 for regular members and \$10.00 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. 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 to the Society Secretary. Annual dues and changes in addresses should be sent to the Society Treasurer.



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From the Editor

Since the last issue, with the Society's election of a new secretary and editor, the Newsletter has undergone a change of venue, as our legal brethren would say, from Goucher College, Towson, Maryland to Trent University, Peterborough, Ontario. You will see a few changes in its appearance. Most noticeably, the front cover has been redesigned with the competent aid of Trent's graphics department, and we have now gone from two columns of text to three in the interests of easier reading. Every effort has been made to perpetuate the high standards of my predecessor, Helen Habermann, and of the earlier editors going back over 30 years to the early days of the Society. I shall have some help with this task from my colleague Stew Brown.

This fall issue, as usual, contains material originating from the annual meeting, at Asilomar in June, including the minutes of the annual business meeting and the Executive Committee meeting, and the Treasurer's interim financial report presented at the annual meeting. As well you will find biographical mate-

rial on our newest Life Member and the student award winners, and a selection of photographs taken at Asilomar by Connie Nozzolillo, who has contributed so many to past issues, and by Mamdouh Abou-Zaid.

Our Newsletter can be no better than the material in it, and with this in mind I'll always welcome items of interest to the members for inclusion. Notices of upcoming meetings, and of positions vacant or wanted will continue to be published and are solicited. Photographs of general interest to members will be published as space permits.

A new feature that is being introduced is a Letters column. I believe that it is desirable to provide a medium through which PSNA members can easily communicate with other members as a group, and we have lacked such a medium until now. If you have an idea that you'd like to publicize, comments on any relevant subject, a request for information you'd like to broadcast, brief reports on meetings attended, etc., please address your Letter to my attention, mark it as being for publication, and it will be published

(subject to editing, of course) in the next available issue.

A vitally important function of the Newsletter is, I think, to keep members informed about current research in the field. This has been done mainly through notices and reports of our annual meetings, and of course will continue to be one of our most important functions. But in future, as has been done occasionally in the past, I'd like to publish, regularly if possible, brief summaries of some of the research under way in the various laboratories of the Society's members, items of the order of 500 words. If you would like to inform others of what you regard as particularly significant aspects of your work, feel free to phone or write me with your suggestion. I will publish as many as space permits, so get into the queue!

Suggestions for further improvements would be greatly welcomed and will be carefully considered.

The Editor

Life Membership in the PSNA



Constance Nozzolillo's earliest interest in biology developed during her childhood on a dairy farm not far from Ottawa. Later, at Queen's University in Kingston, Ontario,

she was inspired by the late Gleb Krotkov to become a plant physiologist. After receiving her MA she took a position with Agriculture Canada at Ottawa, working first on the effect of herbicidal oils on photosynthetic activity. Later, after marrying Louie Nozzolillo in 1952, Connie, working under J.H. Craigie, studied the axenic culture of rust fungi for four years, succeeding to the extent of inducing the fungus to grow in tissue cultures of the host. She then transferred to the Microbiology Research Institute of Agriculture Canada.

After two years there she enrolled in the PhD program of the University of Ottawa under protein biochemist Claude Godin, and upon completion of her PhD in 1963 she joined the faculty of that institution. She spent the remainder of her career "teaching a gamut of courses"

in the Biology department until her retirement in 1991. Her earlier research involved phenylalanine as a protein building block, but about 1968 she switched her interest to anthocyanins. About this time, too, she first joined the PSNA, finding the juxtaposition of chemists and botanists in our Society "a refreshing change" from the narrower interests of the botanical societies of which she was already a member. Post-retirement, she is continuing research in the laboratory of her colleague at Ottawa, our President-Elect John Arnason.

Connie became Secretary of our Society in 1975, and served until 1979, but her respite from executive duties was brief, as she was elected President in 1981. She organized and hosted the annual meeting at the University of Ottawa in 1982.

Student Award Winners, Asilomar, 1993

*** BEST ORAL PAPER: Albena Dinkova-Kostova



Albena Dinkova-Kostova received her MSc degree in biochemistry from Sofia University, Bulgaria in 1991, in the Institute of General and Comparative Physiology, Bulgarian Academy of

Sciences, under Dr. Rajna Tosheva. Her research focussed on characterization of the changes in the dolichol pathway occurring during carcinogenesis in Zajdela hepatoma in rats. Since that year she has been in the biochemistry program at Washington State University under Dr. Norman Lewis of the Institute of Biochemistry, doing thesis research on lignan biosynthesis in *Forsythia intermedia*. Her major focus has been the purification and characterization of the enzyme pinoresinol/lariciresinol reductase, which permits entry into the furano, dibenzylbutane, dibenzylbutyrolactone and aryltetrahydronaphthalene subfamilies. The next phases of her research will involve cloning the gene(s) encoding the enzyme and establishing a system where it can be

expressed, in order to obtain sufficient quantities to investigate the mechanism at the enzyme active site.

*** BEST POSTER: Kui Shin Voo



Kui Shin Voo is a native of Sabah, Malaysia, but his university education

Student Award Winners, Asilomar, 1993

has been in the United States, where he took a BS in agronomy from Ohio State University, and an MS in plant molecular biology at that university under Dr. Joseph Kamalay. He then moved to North Carolina State University, where his PhD thesis research under Drs. David O'Malley and Ronald Sederoff has involved a study of hydroxycinnamate: CoA ligase in loblolly pine xylem. His hobbies are music and badminton, and his laudable ambition is to make a positive contribution to human society.

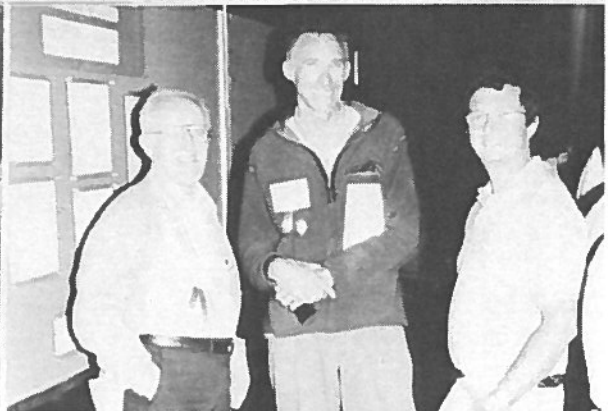
Student Travel Award Winners, Asilomar, 1993

- **Sirinart Ananvoranich**
Biology Department
Concordia University
Montreal, PQ H3G 1M8
- **Lauralyn Beaverson**
Department of Biological Science
Florida International Univ.
Miami, FL 33199
- **Ronald J. Burnett**
Department of Biology
Texas A&M University
College Station, TX 77843-3258
- **D. Palitha Dharmawardhana**
Biotechnology Laboratory

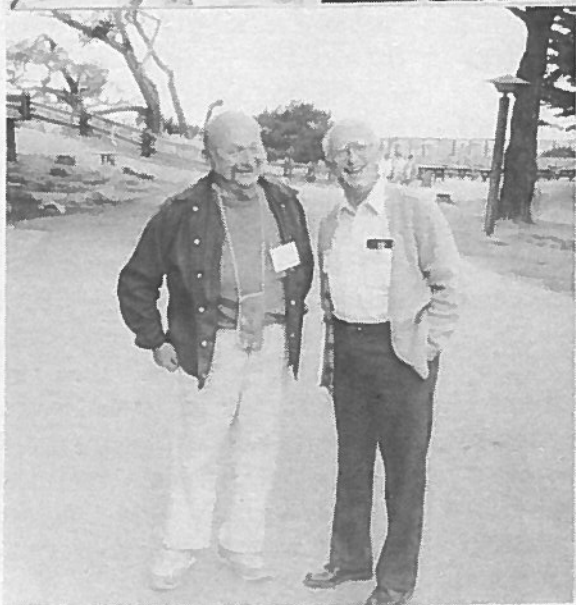
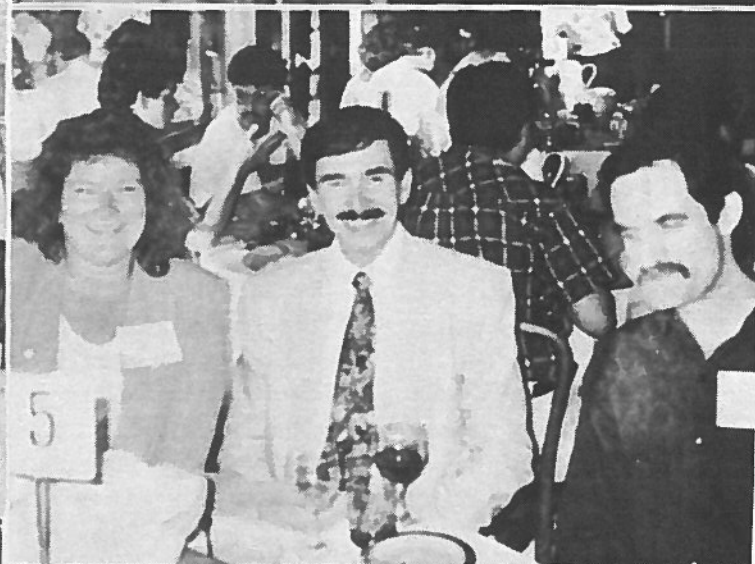
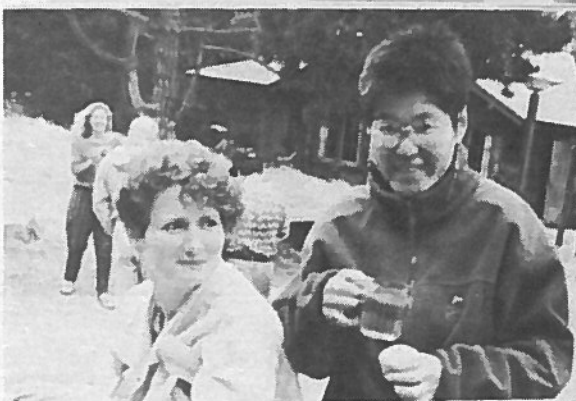
- **University of British Columbia**
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- **Albena Dinkova**
Institute of Biological Chemistry
Washington State University
Pullman, WA 99164-6340
- **Shona Ellis**
Department of Botany
University of British Columbia
Vancouver, BC V6T 1Z4
- **Seong Hwan Kim**
Department of Plant Science
University of British Columbia
Vancouver, BC V6T 1Z4
- **Monica Lam**
Department of Plant Science
University of British Columbia
Vancouver, BC V6T 1Z4
- **Azhar Lodhi**
School of Life Sciences
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Campden Hill Road
London W8 7AH
U.K.
- **Fabricio Medina-Bolivar**
Department of Plant Pathology/
Biotechnology Institute
Pennsylvania State University
University Park, PA 16802
- **Paula Moreno**
Division of Pharmacognosy
Leiden/Amsterdam Center for Drug
Research
Gorlaeus Laboratories
2300 RA Leiden
The Netherlands
- **Timothy C. Morton**
Department of Ecology and
Evolution

- **SUNY at Stony Brook**
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Vancouver, BC V6T 1Z4

PSNA ANNUAL MEETING ASILOMAR, CALIFORNIA, JUNE 1993



PSNA ANNUAL MEETING ASILOMAR, CALIFORNIA, JUNE 1993



Minutes of the Annual Business Meeting, Asilomar, 29 June 1993

The meeting was called to order by president Jim Saunders at 4:35 PM.

Gary Kuroki, local organizer for the Asilomar meeting, reported that 126 individuals had registered. Seventy-five to 80% were members of the PSNA, and 25 to 30% were students. The cost of the meeting to the society included \$7000 to \$8000 for facilities, refreshments during breaks, banquet, and fees for the aquarium visit. Accommodations and transportation for speakers cost approximately \$10,000 and other conference facility charges, \$4000. Corporate support was obtained from Pioneer Hybrid, *Transgenic Research*, Blackwell Scientific Publications, Inc., the Nobel Foundation and DNA Plant Technology Corp. Companies seemed to have no money this year and some of those listed above provided assistance by helping with speakers' expenses, free advertising or supplies.

Jim Saunders thanked Gary Kuroki and the others involved in the organization of the meeting and symposium: Brian Ellis, Vincenzo De Luca and Eric Conn.

Thor Arnason reported on plans for the 1994 annual meeting which will be held August 15-19 in Mexico City. A poster was distributed at the Asilomar meeting. The location was selected after consultation with Mexican members of the organizing committee and the PSNA executive and advisory committees. Mexico City has a number of advantages over more remote sites in Mexico. It is the least expensive, with low air fares from the U.S. and Canada, modest hotel costs and is expected to draw greater numbers of Mexican attendees (especially students) than other possible locations.

The headquarters hotel has excellent meeting facilities, its own purified water supply and good security.

Accommodations at other nearby hotels (with even more modest costs) will also be available. Thor Arnason emphasized that Mexico City's air quality is best in mid to late August.

The 1995 PSNA meeting will be held in Sault Ste. Marie, Ontario, Canada. Dates for the meeting have not yet been set. The Local Organizer will be Mamdouh Abou-Zaid and other members of the organizing committee will be appointed. The symposium topic will be "Plant/Insect Interactions." Mamdouh stated that the symposium will emphasize the plant chemistry aspects of these interactions. Accommodations (at very reasonable rates) will be available at a Holiday Inn on Lake Superior. The meeting will be held at the Forest Pest Management Institute.

Jim Saunders asked PSNA members to propose sites for future meetings by contacting any member of the executive or advisory committees (listed on the inside cover of the newsletter). A possible site for the 1996 meeting is New Orleans with Klaus Fischer as local organizer. Another joint meeting with the PSE has been proposed, possibly in 1997.

Treasurer Susan McCormick reported that the number of PSNA members is currently at a record high (447). Forty-six new members resulted from the 1992 meeting registrations. The society's total assets as of June 21, 1993 were \$25,643.11.

Almost \$8000 in payment for page charges and secretarial expenses were soon to be received from Plenum Press (A check for \$7332.00 was mailed to Susan on June 30, 1993. Still outstand-

ing was \$500 for secretarial expenses for *Recent Advances in Phytochemistry*, volume 26 but this was to be paid within a week). Also, Gary Kuroki expected to return \$6000 to \$7000 from the Asilomar meeting to the treasurer.

Total income from dues and royalties increased in the past year. Certificates of deposit earnings of 6 to 7% will continue. (Rates start lower but increase over time).

Jim Saunders noted the serious depletion of the PSNA treasury in the past year which resulted from reduced corporate sponsorship, high cost of the Miami meeting (including grants to the PSE) and a drop in the number of members. He challenged meeting organizers to make do with less. Meeting registration fees may have to increase. Jim Wallace questioned whether meeting at University sites would be less expensive. Thor Arnason said that he had negotiated with the Mexico City headquarters hotel to obtain use of meeting rooms at no cost. Ragai Ibrahim proposed that the society raise membership fees to \$40 or \$50 (pointing out low cost compared to the Plant Physiology Society). Helen Stafford noted the problem of constantly changing society membership (often new members added as a result of annual meeting registration fees stay in the PSNA for only one year). Thor Arnason urged that costs of the annual meeting be related to meeting expenses and that raising registration fees be justified. Possibly meetings could be held in free facilities. Klaus Fischer suggested that the PSNA has been too generous in funding foreign symposium speakers and that levels of reimbursement could be negotiated. Jim Saunders pointed out that Thor Arnason is already doing this. Brian Ellis suggested that guidelines be set up for reimburs-

Minutes of the Annual Business Meeting, Asilomar, 29 June 1993

ing speakers. Jim Saunders said he would be updating guidelines for meeting organizers and that financial guidelines would be included.

Editor-in-chief Helen Stafford reported that *Recent Advances in Phytochemistry*, Volume 26, was on display at the back of the meeting hall. She will edit the symposium volume resulting from the Asilomar meeting (Volume 28) and John Romeo will take over with Volume 29. Helen asked that PSNA members help advertise these volumes, urging their libraries and graduate students to buy them. Jim Saunders thanked Helen Stafford for her hard work.

Secretary Helen Habermann reported that she had nothing to report. She thanked the members for their support during her six years as secretary. Jim Saunders thanked Helen for her excellent job and getting the newsletters out on time.

There has been no progress in establishing the Phytochemical repository discussed at length at past PSNA meetings. Jim Saunders noted that the committee working on the repository still exists. He also reported that the Sigma Chemical Company will accept chemicals for deposit and storage. Helen Habermann reminded members that there have been at least two notices in the newsletter concerning chemical companies and government agencies willing to accept new chemicals or collections.

Jim Saunders announced the results of the 1993 PSNA elections. Thor Arnason is President-Elect and Alicia Zobel is our new secretary.

There was no old business or new business to discuss. The meeting was adjourned at 5:13 PM.

Summary of the Executive Committee Meeting, Asilomar 29 June 1993

The meeting began at 9:30 AM with Jim Saunders, Kelsey Downum, Susan McCormick, Helen Habermann and Helen Stafford present. John Romeo, the next editor-in-chief, attended to participate in discussions of the new contract negotiations with Plenum. Murray Isman was not present.

Editor-in-chief Helen Stafford reported that negotiations with Plenum Press on the *Recent Advances in Phytochemistry* contract are in progress. An increase in the page charge from \$4.00 to \$6.00 is being requested and the \$500 per volume for "secretarial" costs will be dropped. The changes are a consequence of changes in the technology of processing manuscripts (formatting and editing manuscripts submitted on disks vs. typing manuscripts on blue sheets). The secretarial and page charges for *Recent Advances in Phytochemistry* Volumes 24-27 have not been paid because Plenum expected specific requests and verification of secretarial costs. Helen also noted difficulties in dealing with the hierarchy at Plenum on problems associated with publication.

Jim Saunders reported that Connie Nozzolillo would be honored with a life membership award at the Asilomar meeting. The award certificate had been prepared by the secretary and was signed by the PSNA officers at the executive committee meeting. It was presented to Connie at the annual banquet.

Jim Saunders reminded members of the executive committee that the PSNA paid for an extra night at Asilomar for each of them in order to make possible their Sunday morning meeting.

Treasurer Susan McCormick reported a balance of \$25,643.11 in the PSNA

accounts. Plenum has promised to reimburse the society for page charges at \$4.00 per page for *Recent Advances in Phytochemistry* volumes 24-27 and also for secretarial grants of \$500 per volume for these volumes. (A check for \$7,332.00 was forwarded to the treasurer on June 30. An additional check for \$500 to cover the secretarial grant for volume 26 was to be forwarded within a week). Funds were also to be returned from the Asilomar meeting. PSNA assets thus were greater than at this time last year but well below the historic highs of several years ago. Jim Saunders wondered how the society can avoid going bankrupt. Helen Stafford commented that the annual symposium volume produces income. Members tend to come to annual meetings sporadically and attendance seems to be related to expense for participants. Meeting at less expensive locations may persuade more people to come. Kelsey Downum commented that royalty amounts and percent seemed to be less in recent years with fewer volumes sold. Jim Saunders stated that the society would either have to increase revenues or cut costs and wondered whether it would be possible to limit costs for each meeting. Kelsey Downum wondered whether continuity was lacking in the executive committee and whether the advisory committee could provide this. He proposed that a committee of past meeting organizers advise the organizer and treasurer. Hotels differ from conference centers in expecting gratuities. Universities tend to be the least expensive sites and often support meetings organized by their faculty.

Jim Saunders reported that the Mexico City meeting is expected to be relatively inexpensive even though it will be held in a very nice hotel. Of the 14 speakers who have accepted invitations to participate in the symposium, five are Mexican and nine come from elsewhere. Speakers have been informed that money is a problem and limited support has been offered. Guidelines for meeting organizers will be revised with emphasis on monetary restraint.

Support for travel of students attending meetings was discussed. Should travel for graduate student PSNA members from Europe be supported? Should there be a maximum amount granted for travel to the Mexico City meeting? Kelsey Downum proposed that \$2000 be budgeted again next year and that preference be given to students who are already members of the society. John Romeo noted that the Chemical Ecology society has a committee to make student awards and that their abstracts are due three months in advance. Kelsey urged that we keep the present system for student travel awards in which all students receive some support.

Jim Saunders reported receiving a letter from Dr. E. Caldwell, director of the Forest Pest Management Institute in Sault Ste. Marie, Ontario, Canada, confirming support for the 1995 PSNA meeting and promising that bus service will be organized to the nearest airport. The

symposium topic, as proposed by Mamdouh Abou-Zaid, will be "Plant/Insect Interactions." The executive committee moved to accept the invitation to meet in Sault Ste. Marie in 1995 and suggested that there be a program co-chair selected by the PSNA. Rick Kelsey (Forest Service, Corvallis OR) or Pedro Barbosa (University of Maryland, College Park) were proposed as possible participants in program planning. It was also suggested that funds to support speakers be limited to \$6000. The organizer will be free to increase registration fees and the PSNA treasurer will monitor costs of the meeting.

Kelsey Downum proposed that a future joint meeting with the PSE might be planned for 1997 (five years after the joint meeting in Miami). No location has been proposed but it would presumably be in Europe. The question of travel support was raised. Would the NSF approve a grant submitted by the society for travel support? If there is a *Recent Advances in Phytochemistry* volume based on the symposium at the joint meeting, would royalties be split between the two societies? It would have to be published by Plenum because our contract with Plenum specifies a volume each year.

Jim Saunders asked for suggestions for location of the 1996 meeting which should probably be in the eastern U.S. He suggested that the advisory committee be asked for suggestions and also

members through the newsletter.

Helen Habermann reported that the request for nominations for the Pergamon Prize (an award of \$5,000 and a medal given annually since 1991 to an outstanding phytochemist) arrived too late for an announcement to appear in the newsletter (see items in the Spring 1991 and Spring, 1992 newsletters for details about the prize and information needed for nominations). Announcement of the award also appears in *Phytochemistry*. Helen proposed that the executive committee select a nominee at each annual meeting and ask the secretary to prepare the required documentation to be submitted the following spring. Members of the executive and advisory committee met at lunch on June 28 and selected a nominee for next year's award.

Jim Saunders reported no progress in establishing a Phytochemical Repository. However, Sigma Chemical Company has distributed an advertisement indicating their willingness to accept chemicals for deposit and storage. [This advertisement is reproduced on page 12 from *Aldrichemia Acta.*]

Jim Saunders reported the results of the 1993 PSNA election: Thor Arnason is the new president-elect and Alicja Zobel the new secretary.

Jim's last recommendation to incoming President Downum and Treasurer Susan McCormick was to watch the society's finances. The meeting was



PSNA - INTERIM FINANCIAL REPORT JANUARY 1, 1993 TO JUNE 21, 1993

RECEIPTS

Membership Dues	\$ 5408.66
Royalties RAP	4558.22
Mailing List Rental	100.00
Interest on Checking	31.39
Transfer from CD	10,000.00
Transfer from Coral Gables Account	3101.23
Best Poster Award Refund	200.00
Total Receipts	\$ 23,399.50

EXPENDITURES

1993 Meeting Advance	\$ 12,000.00
1993 Speaker Travel	652.55
Secretary Expenses	2000.00
Treasurer Expenses	1880.68
Checking Service Charges	57.90
Total Expenditures	\$ 16,591.13

CHECKING SUMMARY

Total Receipts	\$ 23,399.50
Total Expenditures	16,591.13
Net Change	\$ 6,808.37

SAVINGS ACTIVITY

Interest year-to-date	776.20
Transfer to Checking	10,000.00

ASSETS

Checking	\$ 7,397.15
Savings	18,205.96
Total	\$ 25,643.11

UPCOMING MEETINGS

PHYTOCHEMICAL SOCIETY OF NORTH AMERICA:

The 1994 annual meeting will be held in Mexico City, D.F., 15-19 August. The symposium topic is to be announced (*see p. 11*). The 1995 annual meeting will be in Sault Ste. Marie, Ontario on dates to be announced. The symposium topic will be Plant-Insect Interactions. Further details of these meetings will be forthcoming in subsequent issues of the Newsletter.

Other Meetings of Interest

ANNUAL CITRUS RESEARCH CONFERENCE:

Hilton Hotel, Pasadena, California, 1993 December 9. This meeting features reports of research in progress at the USDA Fruit and Vegetable Chemistry Laboratory in Pasadena, along with some presentations from outside institutions. Topics include biochemistry of citrus terpenoid and flavonoid constituents, bioregulation of plant growth, and iron uptake and translocation. There is no registration fee.

For further information contact Raymond Bennett, Fruit and Vegetable Chemistry Laboratory, Pasadena, CA 91106. (Telephone 818 796-0239)

19TH IUPAC SYPOSIUM ON THE CHEMISTRY OF NATURAL PRODUCTS:

Karachi, Pakistan, 1994 January 16-20. The key aspects at the frontiers of natural product chemistry such as stereoselective synthesis, modern spectroscopic techniques for structure elucidation, bioactive natural products and bioorganic chemistry will be covered. The aim is to increase self-reliance in the developing Third World countries via effective utilization of their natural resources.

GROUPE POLYPHÉNOLS XVIIIth INTERNATIONAL CONFERENCE ON POLYPHENOLS (JIEP 94):

Palma de Majorca, Spain, 1994 May 23-27. Themes to be included are polyphenols in the plant and plant products, polyphenol biosynthesis, molecular biology and genetics of polyphenols, biological and pharmaceutical activities of polyphenols, polyphenols and metal ions, and new analytical techniques for polyphenol studies. A simultaneous translation service, Spanish / English / French will be available. For further information contact Clara Diez de Bethencourt, Instituto de Estudios Avanzados, Universitat de les Illes Balears, Cra. Valldemossa Km 7,5, 07071 Palma de Mallorca, Spain. (Telephone 34-71-17 34 50; FAX 34-71-17 32 48 or 34-71-17 31 84)

FOURTH INTERNATIONAL CONGRESS ON PLANT MOLECULAR BIOLOGY:

Amsterdam, The Netherlands, 1994 June 19-24. The Congress will be composed of plenary sessions, concurrent symposia, poster sessions and interactive workshops. For further information contact the Congress Secretariat, RAI Organisatie Bureau Amsterdam by Europaplein 12, 1078 GZ Amsterdam, The Netherlands. (Telephone 31-0-20-549-12-12; FAX 31-0-20-646-44-69)

PLANT PHENOLICS:

Ghent, Belgium, 1994 August/September. This meeting is being co-organized by the Groupe Polyphénols and the Phytochemical Society of Europe. For further information contact Prof. P.J. Lea, Division of Biological Sciences, Institute of Environmental and Biological Sciences, Lancaster University, Lancaster LA1 4YO, United Kingdom.



Xochiquetzal

1994 ANNUAL MEETING - MEXICO CITY
August 15-19, Calinda Geneve Hotel
symposium on
PHYTOCHEMISTRY OF MEDICINAL PLANTS



Ix Chel

Invited speakers

J. BEUTLER, N.C.I.: Antiviral and antitumor plant metabolites

R. BYE, U.N.A.M.: Medicinal plants of Mexico: their biodiversity and phytochemistry

K. HOSTETTMANN, U. Lausanne: Applications of liquid chromatography/mass spectroscopy to the investigation of medicinal plants

T. JOHNS, McGill U.: Anticholesteremic agents from African plants

V. LOYOLA-VARGAS, C.I.C. Yucatan: Root cultures in biosynthesis of medicinal compounds

R. MARLES, Brandon U.: Sesquiterpene lactones revisited: Recent developments in the assessment of biological activities and structure relationships

J.L. McLAUGHLIN, Purdue University: Annonaceous acetogenins: potent mitochondrial inhibitors with diverse applications

H. NIEMEYER, U. de Chile: Biologically active compounds from the Chilean flora

R. PEREDA-MIRANDA, U.N.A.M.: Biologically active natural products from Mexican medicinal plants

J. PEZZUTO, U. of Chicago: Natural product cancer chemopreventative agents

L. RODRIGUEZ-HAHN, U.N.A.M.: Neoclerodan diterpenoids from American *Salvia* species

A.J. VLIETINCK, U. Antwerpen: Bioassay guided isolation and structure elucidation

H. WAGNER, U. München: Plant immunostimulants and adaptogens medicinal plants

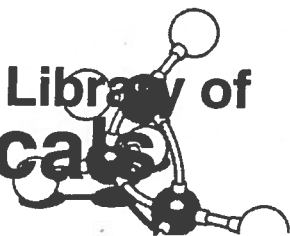
Ix Chel, the Maya goddess of medicine and Xochiquetzal, the Aztec moon goddess of the flowered quetzal bird, look forward to receiving you in Mexico. The program will include plenary lectures on the conference theme, and contributed papers and posters on all aspects of phytochemistry. Because of the conference location in Mexico, a series of conference activities related to the ancient culture and botanical tradition of Mesoamerica is planned. Climate is pleasant at this time of year in Mexico City and direct flights are available from most North American centers.

Organizing Committee: Mexico: X. Lozoya, Instituto Mexicano de Seguro Social; R. Mata, U.N.A.M.; J. Calderon, U.N.A.M.; J.A. Serratos, I.N.I.F.A.B. **Canada:** J.T. Arnason, University of Ottawa; D.V.C. Awang, Profile Botanicals

For more information FAX: 613 564-9295 (Arnason), 525 761-0952 (Lozoya), 525 622 5329 (Mata).



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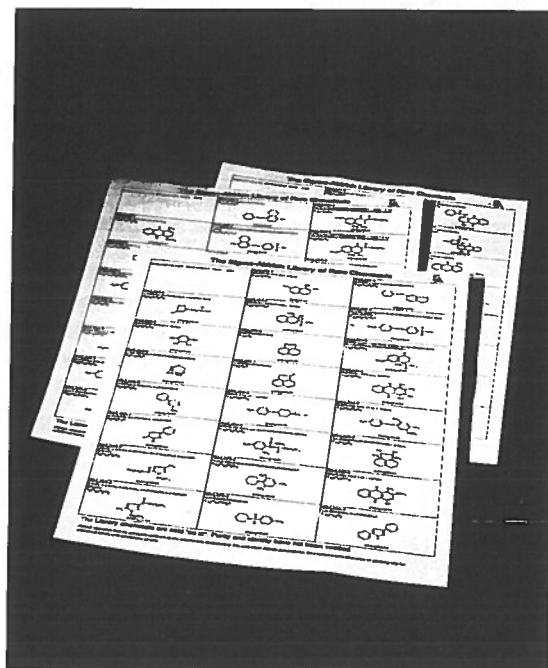
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rings systems	elements
molecular weight range	

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