## Contents

### MOLECULES OF INTEREST

**Vitamin B6: Killing two birds with one stone?**

Sutton Mooney, Hanjo Hellmann*

Pyridoxal 5'-phosphate is one of six common B6 vitamers that broadly functions as an enzymatic co-factor in amino acid, carbohydrate, and fatty acid metabolism.

### PROTEIN BIOCHEMISTRY AND PROTEOMICS

**Misincorporation of the proline homologue Aze (azetidine-2-carboxylic acid) into recombinant myelin basic protein**

Kyrylo Bessonov, Vladimir V. Bamm, George Harauz*

Misincorporation of the proline homologue Aze (azetidine-2-carboxylic acid) into proteins results in severe distortion of the polypeptide chain at every site of substitution.

**Flavone synthase II (CYP93B16) from soybean (Glycine max L.)**

Judith Fliegmann, Katarina Furtwängler, Georg Malterer, Corrado Cantarello, Göde Schüler, Jürgen Ebel, Axel Mithöfer*

The enzyme responsible for the inducible accumulation of 7,4'-dihydroxyflavone in soybean cell cultures was identified as a flavone synthase of type II, and classified as CYP93B16. The biochemical characterization of the recombinant protein indicated a direct and stereoselective formation of the flavone from the flavanone substrate.
Structure–function characterization of the recombinant aspartic proteinase A1 from *Arabidopsis thaliana*

Miguel A. Mazorra-Manzano, Takuji Tanaka, Derek R. Dee, Rickey Y. Yada*

Interactions of the plant specific insert and histidine residues may explain the broad pH stability (pH 3–8) of recombinant aspartic proteinase A1 from *Arabidopsis thaliana*.

Biochemical comparison of two proteolytic enzymes from *Carica candamarcensis*:
Structural motifs underlying resistance to cystatin inhibition

Marco Túlio R. Gomes, Henrique A. Ribeiro, Miriam T.P. Lopes, Fanny Guzman, Carlos E. Salas*

While CMS1MS2 displays the highest enzyme activity, the mitogenic proteinase CMS2MS2 is barely inhibited by cystatin. It is proposed that Arg180 is responsible for cystatin resistance.

**MOLECULAR GENETICS AND GENOMICS**

Consequences of antisense down-regulation of a lignification-specific peroxidase on leaf and vascular tissue in tobacco lines demonstrating enhanced enzymic saccharification

Bahram Kavousi, Arsalan Daudi, Charis M. Cook, Jean-Paul Joseleau, Katia Ruel, Alessandra Devoto, G. Paul Bolwell, Kristopher A. Blee*

Peroxidase down-regulated tobacco plants were profiled for effects on cell walls, photosynthetic capacity and improved saccharification efficiency. Growth and potential industrial utility were not compromised by morphological changes.

The C-terminal zinc finger domain of *Arabidopsis* cold shock domain proteins is important for RNA chaperone activity during cold adaptation

Su Jung Park, Kyung Jin Kwak, Hyun Ju Jung, Hwa Jung Lee, Hunseung Kang*

Domain swapping between the cold shock domain proteins (CSDPs) 1 and 2 from *Arabidopsis thaliana* demonstrates that a specific modular arrangement of the cold shock domain and the zinc finger domain establishes both RNA chaperone activity and nucleic acid-binding property of CSDPs; this, in turn, contributes to enhanced chilling tolerance in plants as well as in bacteria.
Cytosolic APx knockdown indicates an ambiguous redox responses in rice

Silvia B. Rosa, Andréia Caverzan, Felipe K. Teixeira, Fernanda Lazzarotto, Joaquim A.G. Silveira, Sérgio Luiz Ferreira-Silva, João Abreu-Neto, Rogério Margis, Márcia Margis-Pinheiro*

Ascorbate peroxidases convert \( \text{H}_2\text{O}_2 \) into \( \text{H}_2\text{O} \), using ascorbate as an electron donor. Rice transgenic plants silenced for cytosolic Apxl and Apx2 genes were obtained. The double silencing of cytosolic OsApx genes induced compensatory antioxidant mechanisms while single knockdown of these genes resulted in the impairment of normal plant development.

METABOLISM

Distribution and biosynthesis of flavan-3-ols in *Camellia sinensis* seedlings and expression of genes encoding biosynthetic enzymes

Hiroshi Ashihara, Wei-Wei Deng, William Mullen, Alan Crozier*

The distribution of flavan-3-ols in various parts of *Camellia sinensis* seedlings was investigated along with the expression of genes encoding enzymes associated with the biosynthesis of flavan-3-ols and related phenolic compounds. The main biosynthetic pathways leading to (−)-epigallocatechin-3-O-gallate and (−)-epicatechin-3-O-gallate biosynthetic pathways in tea leaves are proposed.

Biological variation of *Vanilla planifolia* leaf metabolome

Tony Lionel Palama*, Isabelle Fock, Young Hae Choi, Robert Verpoorte, Hippolyte Kodja

The effects of the leaf age, day-time, season and accession on the metabolome was carried out on *Vanilla planifolia* leaves using \(^1\text{H} \) NMR spectrometry and multivariate data analysis techniques.

Survey of volatile oxylipins and their biosynthetic precursors in bryophytes

Emmanuel Croisier, Martin Rempt, Georg Pohnert*

A screening of 23 moss species revealed that these bryophytes release a surprising diversity of C5, C6, C8 and C9 volatile oxylipins upon tissue disruption. Precursors of these oxylipins are both, C18 and C20 fatty acids.
Metabolic responses of *Thellungiella halophila/salsuginea* to biotic and abiotic stresses: Metabolite profiles and quantitative analyses

M. Soledade C. Pedras*, Qing-An Zheng

Analyses of non-polar and polar metabolites from *Thellungiella salsuginea* subjected to various forms of stress led to elucidation of the chemical structures of five constitutive metabolites and identification of a broad range of polar metabolites identical to those of other crucifers. The phytoalexins wasalexins A and B were consistently elicited by biotic and abiotic stresses.

Dual metabolomics: A novel approach to understanding plant–pathogen interactions

J. William Allwood, Andrew Clarke, Royston Goodacre, Luis A.J. Mur*

A co-culture method is proposed for the dual metabolomic profiling of both *Arabidopsis* and bacterial pathogens which will facilitate the modelling of the interaction of both partners.

Constituents and secondary metabolite natural products in fresh and deteriorated cassava roots

Soad A.L. Bayoumi, Michael G. Rowan, John R. Beeching, Ian S. Blagbrough*

Hydroxycoumarins from cassava roots: scopoletin, scopolin, esculetin, and esculin.

Synergetic effects of nitrogen depletion, temperature, and light on the content of phenolic compounds and gene expression in leaves of tomato

Trond Løvdal, Kristine M. Olsen, Rune Slimestad, Michel Verheul, Cathrine Lillo*

Phenolics are valued for improving pathogen-resistance and other quality properties in plants. We show how their accumulation may be enhanced in tomato by non-transgenic means, and how flavonoid-associated genes are affected by various abiotic factors.
ECOLOGICAL BIOCHEMISTRY

Cadmium activates *Arabidopsis* MPK3 and MPK6 via accumulation of reactive oxygen species

Xiao-Min Liu, Kyung Eun Kim, Kang-Chang Kim, Xuan Canh Nguyen, Hay Ju Han, Mi Soon Jung, Ho Soo Kim, Sun Ho Kim, Hyeong Cheol Park, Dae-Jin Yun, Woo Sik Chung*

Cadmium activates the mitogen-activated protein kinases, MPK3 and MPK6, in *Arabidopsis*, with the Cd sensing pathway using build-up of ROS to trigger activation of the MPKs.

Induction of acid phosphatase transcripts, protein and enzymatic activity by simulated herbivory of hybrid poplar

Vasko Veljanovski, Ian T. Major, Joseph J. Patton, Eric Bol, Stephanie Louvet, Barbara J. Hawkins, C. Peter Constabel*

The enzymatic activity and protein levels of a pest defense-related enzyme of hybrid poplar, acid phosphatase, are shown to be inducible by tissue damage.

CHEMOTAXONOMY

Chemotaxonomy of *Gonospermum* and related genera

Jorge Triana, José Luis Eiroa, Juan José Ortega, Francisco León, Ignacio Brouard, Juan Carlos Hernández, Francisco Estévez, Jaime Bermejo*

Sesquiterpenes lactones isolated from *Gonospermum* and *Lugoa*, as well as endemic Canarian species of *Tanacetum* show a close relationship, and feature highly oxidized eudesmane or germacrane lactones. The presence of these types of lactones supports the proposal to group them in a monophyletic group in the Canary Islands.

BIOACTIVE PRODUCTS

Bioactivity-guided isolation of cytotoxic sesquiterpenes of *Rolandra fruticosa*

Li Pan, Daniel D. Lantvit, Soedarsono Riswan, Leonardus B.S. Kardono, Hee-Byung Chai, Esperanza J. Carcache de Blanco, Norman R. Farnsworth, Djaja Doel Soejarto, Steven M. Swanson, A. Douglas Kinghorn*

Bioassay-guided fractionation of a methanol extract of *Rolandra fruticosa* led to the isolation of seven germacrane-type derived sesquiterpenes, including two previously unknown compounds, 13-methoxyisorolandrolide (1), and 2α,13-diacetoxy-4α-hydroxy-8α-isobutyloxybourbonen-12,6α-olide (2). All isolates were evaluated for their cytotoxicity using the HT-29 cell line. Compounds 3, 5, 6 were also tested in an enzyme-based ELISA NF-κB (p65) inhibition assay. 13-Acetoxyrolandrolide (3) was investigated in an in vivo hollow fiber assay.
Isolation and evaluation of kaempferol glycosides from the fern *Neocheiropteris palmatopedata*

Jian-Hong Yang, Tamara P. Kondratyuk, Laura E. Marler, Xi Qiu, Yongsoo Choi, Hongmei Cao, Rui Yu, Megan Sturdy, Scott Pegan, Ying Liu, Li-Qin Wang, Andrew D. Mesecar, Richard B. Van Breemen, John M. Pezzuto, Harry H.S. Fong, Ye-Gao Chen*, Hong-Jie Zhang*

Six kaempferol glycosides including three hitherto unknown compounds (1–3) were isolated from the roots of the fern *Neocheiropteris palmatopedata*. Compounds 1 and 2 were determined to possess an unusual sugar moiety containing a 4,4-dimethyl-3-oxo-butoxy substituent group, respectively. The isolates were evaluated for their cancer chemopreventive potential based on their ability to inhibit tumor necrosis factor-α-induced induced NF-κB, nitric oxide (NO) production, aromatase, quinone reductase 2 (QR2) and COX-1/2 activities.

Toxic isolectins from the mushroom *Boletus venenatus*

Masashi Horibe, Yuka Kobayashi*, Hideo Dohra, Tatsuya Morita, Takeomi Murata, Taichi Usui, Sachiko Nakamura-Tsuruta, Masugu Kamei, Jun Hirabayashi, Masanori Matsuura, Mina Yamada, Yoko Saikawa, Kimiko Hashimoto, Masaya Nakata, Hirokazu Kawagishi*

Ingestion of the mushroom *Boletus venenatus* causes a gastrointestinal syndrome, such as diarrhea. A family of isolectins (*B. venenatus* lectins, BVLs) was isolated as the toxic principles from the mushroom. BVL ingestion resulted in fetal toxicity to mice and also caused diarrhea.

**CHEMISTRY**

Triterpenoid saponins from *Astragalus wiedemannianus* Fischer

Emre Polat, Erdal Bedir*, Angela Perrone, Sonia Piacente, Ozgen Alankus-Caliskan*

Three cycloartane type glycosides, together with eight known compounds were isolated from the roots of *Astragalus wiedemannianus*. The presence of an arabinose moiety on the acyclic side chain of cycloartanes is reported for the first time.

Triterpene glycosides from *Agrostemma gracilis*

Omer Koz, Erdal Bedir, Milena Masullo, Ozgen Alankus-Caliskan*, Sonia Piacente*

Four triterpene saponins, agrostemmoside A–D were isolated from the methanol extract of *Agrostemma gracilis*. Their structures were determined by a combination of one- and two-dimensional NMR techniques, and mass spectrometry.
Euphane triterpenoids of *Cassipourea lanceolata* from the Madagascar rainforest pp 669–674


A Madagascar collection of the leaves and fruit of *Cassipourea lanceolata* Tul. led to the isolation of three euphane triterpenoids 1–3. The three compounds showed weak antiproliferative activities against the A2780 human ovarian cancer cell line, with IC50 values of 25, 25 and 32 µM, respectively.

Microtropiosides A–F: ent-Labdane diterpenoid glucosides from the leaves of *Microtropis japonica* (Celastraceae) pp 675–681

Yuka Koyama, Katsuyoshi Matsunami, Hideaki Otsuka*, Takakazu Shinzato, Yoshio Takeda

From the leaves of *Microtropis japonica*, collected in the Okinawa islands, six ent-labdane glucosides, named microtropiosides A–F, were isolated. The structures of the compounds were elucidated by a combination of spectroscopic analyses, application of the β-β-glucopyranosylation-induced shift-trend rule in the 13C NMR spectroscopy and the modified Mosher’s method.

Chemical constituents from *Saussurea cordifolia* pp 682–687

Xu-Wen Li, Zi-Tao Guo, Yun Zhao, Zheng Zhao, Jin-Feng Hu*

C10-Acetylenic glycosides (1–4), and a lignan (5), together with 31 known compounds, were isolated from whole plants of *Saussurea cordifolia*. Selected compounds were evaluated for their cytotoxicity against MCF-7 human breast cancer cells using the MTT assay.

A C14-polyacetylenic glucoside with an α-pyrene moiety and four C10-polyacetylenic glucosides from *Mediasia macrophylla* pp 688–692

Shin-ichiro Kurimoto, Mamoru Okasaka, Yoshiki Kashiwada*, Olimjon K. Kodzhimatov, Yoshihisa Takaishi

Polyacetylenic glucosides (1–5) were isolated from the MeOH extract of *Mediasia macrophylla*. Their structures were established by spectroscopic analyses. Compounds 2–4 were the first examples of C10-polyacetylenic glucosides found in the family Umbelliferae, while compound 1 was a unique polyacetylenic glucoside possessing an α-pyrene moiety.
Gynostemosides A–E, megastigmane glycosides from *Gynostemma pentaphyllum* pp 693–700

Zhen Zhang, Wei Zhang, Yan-Ping Ji, Yun Zhao, Chuan-Gui Wang, Jin-Feng Hu*

Megastigmane glycosides (1–5) were isolated from the whole plants of *Gynostemma pentaphyllum*. Their absolute structures were elucidated by means of spectroscopic methods including 2D NMR, HR-ESIMS, and circular dichroism (CD), as well as by chemical transformations.