PL01 Trends and challenges for Phytomedicine - Research in the new millenium

Hildebert Wagner
Department Pharmacy, Center of Pharma Research, University of Munich, Butenandtstr. 5, 81377 Munich, Germany.

The development of new, effective and standardized plant-based drugs for a causal and rational therapy of diseases is both a need and a challenge for the phytomedicinal research at the beginning of the new millenium. To reach this goal, extensive efforts in the following three research areas are necessary:

1. A comprehensive and more thorough chemical analysis of established and new medicinal plants for their major bioactive compounds, with the aims of improving the quality of HPLC-fingerprinting and developing standardization methods for monoextracts and multi-component phytopharmaceuticals according to TCH- and FDA guidelines.
2. The inclusion of new molecular biological models for screening and evaluating the overall pharmacological mechanism of action and therapeutic potency of herbal drug preparations. One goal must be to elucidate the polyvalent action of isolated plant constituents and to find a rationale for the overadditive (potentiated) pharmacological and therapeutic effects of multicomponent herbal drug mixtures. These investigations should be focused on approved herbal drugs as well as on phytopharmaceuticals of traditional medicine (e.g., TCM or Ayurvedic-fixed herbal drug combinations) which could replace synthetic drugs.
3. More efforts must also be made to intensify the efficacy proof of standardized phytopreparations, using controlled double-blind studies against placebo and, if possible, against synthetic drugs. These studies should be paralleled by pharmacokinetic and bioavailability studies using modern, high-tech methods.

The strategies and performance of such studies in the described research areas, along with progress made, are demonstrated by several examples from recent and ongoing research projects.

Prof. Dr. Hildebert Wagner

Professor Wagner was born in 1929 in Laufen on Salzach (Germany). He finished his studies on Pharmacy in 1953 and his Doctoral Thesis in 1956. He did the Habilitation in 1960. Since 1965 he has been Full Professor of Pharmacognosy and Director of the Institute of Pharmaceutical Biology, Munich, till 1999. He has also been: Distinguished Visiting Professor (Columbus, Ohio, USA, 1970/71), Dean of the Faculty of Chemistry/Pharmacy (Munich, 1981-83), Ph.D. *honoris causae* of the Universities of Budapest and Debrecen (Hungary) in 1989, Dijon (France) and Helsinki (Finland) in 1997.

He is Member of Editorial/Advisory Boards of several international journals: *Phytochemistry*, *Journal of Ethnopharmacology*, *Journal of Natural Products* (*Lloydia*); and Editor of *International Journal of Phytomedicine*.

His main research areas covered different topics: isolation, structure determination, synthesis and analysis of biologically and pharmacologically active compounds of medicinal plants, particularly in the fields of alkaloids, heart-glycosides, flavonoids and lignans; drugs with antiviral, antiasthmatic, antiinflammatory, immunostimulating and adaptogenic activity, standardisation of Chinese drugs.

He has published more than 800 original papers and 30 review articles, as well as several books.

PL02 Mechanisms of action of new natural antiinflammatory drugs

María José Alcaraz
Departamento de Farmacología, Universidad de Valencia, Facultad de Farmacia, Av. Vicent Andrés Estellés s/n, 46100 Burjasot, Valencia, Spain.

A wide range of mediators participate in the recruitment and activation of cells during inflammation, suggesting multiple therapeutic strategies for inflammatory conditions. Increased expression of cyclooxygenase-2 and high levels of prostaglandins can be detected in rheumatoid synovial tissues, where they mediate erosion of cartilage and bone, as well as angiogenesis. These effects would be prevented by drugs able to inhibit enzyme activity or expression. As cytokines play a critical role in this response, the control of their synthesis, release or effects are important targets for new antiinflammatory drugs. Thus, inhibition of kinases involved in intracellular signaling would lead to transcriptional and posttranscriptional regulation of cytokines, whereas inhibition of some proteolytic enzymes would prevent cytokine activation. Transcription factors play a crucial role in the expression of proinflammatory mediators. Inhibition of nuclear factor-κB-mediated transcription can suppress the production of a large number of proinflammatory proteins including cytokines, chemokines, receptors for these mediators, cell adhesion molecules, nitric oxide synthase, cyclooxygenase-2 and other inducible enzymes. Several mechanisms may account for this effect. We have studied new antiinflammatory agents such as cacospongionolide B and petrosaspongiolide M, which inhibit the nuclear factor-κB pathway through an interference with the phosphorylation and degradation of inhibitory proteins. Apart from direct antioxidant properties, some natural phenolic derivatives and synthetic analogs can induce antioxidant genes in mammalian cells. The resulting enzyme activity can contribute to the modulation of inflammatory responses. In addition, apoptotic death of inflammatory cells contribute to immunosuppressive and antiinflammatory drug effects in chronic conditions.

Prof. Dr. María José Alcaraz

She is professor in Pharmacology at the Department of Pharmacology, University of Valencia, Spain. She got the Ph D. in 1981 at the University of Valencia with Extraordinary Award, followed by a postdoctoral training at the Department of Pharmacology, King's College of London (1983-1984). After the positions of assistant and lecturer, she got the professorship in 1991, when she was elected Head of the Department of Pharmacology, University of Valencia. In 1998 she worked as a visiting researcher at the Unité 348 INSERM, Paris. Her research has always been focused on inflammation and antiinflammatory agents, mainly natural products, with ca. 100 international publications in the field and a large number of funded projects. She has recently received the Dr. Esteve Foundation Award to the best scientific publication in Pharmacology. Referee of different international journals and expert for scientific projects evaluation in several countries and international organizations, such as the International Foundation for Science or the European Commission, she has played an active role in international cooperation, mainly with Latin America. At present, she coordinates an international project on antiinflammatory natural products within the CYTED program.

PL03 Biological activities of capsaicinoids

Eduardo Muñoz
Departamento de Biología Celular, Fisiología e Inmunología, Facultad de Medicina, Universidad de Córdoba, Av. de Menéndez Pidal, s/n, Córdoba 14004. Spain.

The hot taste of chili pepper (*Capsicum annuum* L.) has generated an intense research activity aimed at the characterization and exploitation of the pungent compounds responsible for the sensory properties of this spice. The active principle, named capsaicin (*E*-8-methyl-*N*-vanillyl-6-nonenamide, CPS), was first isolated in 1876, and an enormous body of literature has been accumulated on its chemistry and pharmacology. The outgrowth of this attention was the discovery and cloning of a cell surface receptor (VR1) that binds CPS in a specific way, and a structurally heterogeneous group of pungent compounds named vanilloids. VR1 belongs to the family of putative store-operated calcium channels, and is expressed mainly in peripheral pain-sensing neurons. The generation of *knock out* mice for the VR1 gene supported a correlation between the pungency of CPS and the activation of this receptor *in vivo*, while several investigations have highlighted the therapeutic value of vanilloids to treat painful disorders such as peripheral neuropathies and rheumatoid arthritis, considerable amounts of CPS-like compounds have also been reported in sweet pepper, but the lack of pungency of these CPS analogues has long made them unattractive targets for research. Recent studies have shown that hot pepper and sweet pepper contain similar compounds, whose structural hallmark is the presence of a vanillyl core bound to a branched fatty acid. The remarkable difference between the sensory properties of these plants is solely due to the way the vanillyl and the acyl moieties of this basic structural motif are linked, via amide bond in hot pepper (capsaicin-type compounds) and via ester bond in sweet pepper (capsiate-type compounds). Despite the growing interests in secondary metabolites from edible plants, and the presence of sizeable amounts of capsiates in sweet pepper, no biological property apart from the lack of pungency has been reported for these compounds. We will show that capsainoids have anti-inflammatory properties by targeting the NF-κB pathway independently of the VR1 and that this type of compounds could serve as lead compounds for the development of novel, potent anti-inflammatory drugs for the treatment of inflammatory disorders.

Prof. Dr. Eduardo Muñoz

Prof. Muñoz was born in 1958 in Córdoba (Spain). He studied Medicine at the University of Córdoba, where he got his PhD degree in 1986. He has been a postdoctoral in Tufts University with Prof. Brigitte T. Huber (Boston, USA) from 1987-1990 working in Th2-type cell signaling and cytokine gene expression. He continued working in the field of gene transcription with Prof. Alain Israël during two years at the Institute Pasteur (Paris, France). Since 1992, he is Associated Professor of Immunology at the University of Córdoba. He is interested in two overlapping areas of research centered in the identification of new anti-inflammatory and anti-AIDS natural compounds. His group is aimed to identify novel health food nutraceuticals and plant based medicines (phytomedicines) with anti-inflammatory activities, specially in the field of non pungent capsaicinoids and synthetic AEA-capsaicin "hybrids" molecules such is Arvanil and other N-AVAMs (vanillylamides of fatty acids), which have been recently developed and represent lead compounds amenable for pharmaceutical development as anti-inflammatory and analgesic compounds. The second area of his research deals with the identification of cellular genes that are regulated by the HIV-1 Tat protein, specifically upregulated cellular genes by viral proteins may represent new molecular targets of interest in the development of therapeutic strategies anti-HIV-1. In addition to the validation of new anti-HIV molecular targets, we are isolating new natural phytochemical compounds targeting Tat-LTR-HIV-1 interaction.

PL04 Quality of herbal medicinal products (HMP) as the requirement for succesful phytopharmaceuticals

Gerhard Franz
Department of Pharmaceutical Sciences, University of Regensburg, Regensburg , Germany.

Increased use of herbal medicinal products (HMP) in Europe requires a through review of appropriate guidelines to evaluate them for therapeutic benefits. The accepted quality management is laid down in the European Pharmacopoeia, where an increasing number of monographs for HMP is documented. Quality of HMP defines the optimal specifications for each compound by specifying one or more analytical procedures, which define qualitative (identification) and quantitative (limits for impurities and content of active ingredients) characteristics of the respective HMP. As a consequence of adulterations, specific identification methods have to be developed for each individual HMP. Contamination with organic compounds such as pesticides, heavy metals or microorganisms demonstrate the need for highly sensitive analytical procedures. Actually new methods are developed for the detection and limitation of mycotoxins (Aflatoxins, Ochratoxins) since HMP's from tropical areas are often shown to be contaminated with these toxic compounds. Due to the often demonstrated presence of heavy metals in many examples of HMP's, a new general method for heavy metal determination was established as a further important guideline. Finally, the quality of herbal extracts, which actually represents a large proportion of herbal medicines on the European market, was laid down in a frame monograph specifying the different types of extracts (quantified, standardised and other extracts) which are the basis for all future individual extract monographs. However, it is often difficult to attribute a specific extract to the newly proposed types, since the arguments for therapeutically active ingredients are often a matter of the current scientific discussions.

Prof. Dr. Gerhard Franz

Prof. Gerhard Franz (1937) received his Diploma in Pharmacy by the University of Karlsruhe in 1963. He got his PhD at the Université de Fribourg (Switzerland) in 1966. In 1967 he did a postdoctoral training at the Department of Biochemistry of the University of California (Berkeley) to do his Post doc. After this, he has occupied different positions in several Universities: Université de Fribourg (Switzerland) Dept. of Biology (1970–1977); Full Professor of Pharmacognosy in the University of Regensburg (Germany, since 1977); Guest Professor University of Basel (Switzerland) and Grenoble (France).

Extra University activities: member of the Commission and chairman of the group of experts on Pharmacognosy of the German Pharmacopoeia, chairman of the group of experts 13B of the European Pharmacopoeia, member of Commission E of the BfArM (Bonn, Germany), member of the Scientific Council of the BfArM. Former President of the International Society for Medicinal Plant Research, he is co-editor of the journals Planta Medica and Pharmazie.

He has received the Egon Stahl Award (1985), Sebastian Kneipp Award (1994), Order of Merit of the FRG (1998), and he is corresponding member of the Royal Belgian Medicinal Society (1990) and the Academie Francaise de Pharmacie (1998), as well as honory advisor of the Department of Health, Executive Yuan ROC, Taiwan (1999).

PL05 Clinical trials in the safety and efficacy evaluation of phytopharmaceuticals – A scientific challenge

Bruno M. Giannetti
Clinical Research Management, Oberdorf 59, 53347 Bonn, Germany.

The presentation provides an overview on recent clinical results on safety and efficacy of a number of phytopharmaceuticals.

A set of around 100 clinical study publications of the last 5 years is critically highlighted. Key drugs evaluated include cone flower (*Echinacea* sp.), horse chestnut (*Aesculus hyppocastanum*), willow bark (*Salix* sp.), ginkgo (*Ginkgo biloba*) and Saint John's wort (*Hypericum perforatum*).

Like any other drug, efficacy of phytopharmaceuticals has to be shown in controlled, double-blind clinical studies performed according to GCP (1). Whilst most of the rather formal requests related to GCP could be fulfilled in a fairly easy way, the real challenge lies in the clinical and pharmacological methodology, which very often does not address the particular profile of the phytopharmaceutical drugs under evaluation. Hence, a number of analysed studies in this presentation - especially those of the "early days" of clinical research in this field - fails to show efficacy.

At the same time single case reports and analogies have often been used to question safety of phytopharmaceuticals (2). In the light of lack of proven efficacy the risk/benefit ratio was then considered to be negative.

Recent data on cone flower and horse chestnut are presented as examples showing the above mentioned methodological difficulties but also solutions in proving efficacy of phytotherapeuticals.

As a matter of fact, the number of clinical trials conducted according to GCP with relevant positive information concerning efficacy has increased.

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Dr. Bruno Massimo Giannetti

Dr. Bruno Gianetti was born in Lyon (France) in 1952. After his studies in chemistry, he did his Doctoral Thesis in the University of Bonn under the title: “New antibiotic acting agents from basidiomycetes” (1977-1981). In 1981 he became PhD in Organic Chemistry and Pharmacology. Registered as a physician (1983), he also did a Doctoral Thesis in medicine (University of Bonn) under the title: "Characterisation of CEA and NCA by monoclonal anti-CEA antibodies in combination with Gel-Permeation-Chromatography" (1983-1985).

He has occupied different positions in several companies, such as Madaus AG (Cologne, Germany), Immuno AG (Vienna, Austria), Coopers & Lybrand (Basle, Switzerland), CRM GmbH (Rheinbach, Germany), α Care GmbH (Celle, Germany), VTS Denmark, CRMB GmbH (Rheinbach, Germany), Verigen Inc. (USA), VTSI AG (Leverkusen, Germany), He has 40 scientific papers published in several journals and 8 patents.

PL06 Treatment of menopausal patients with herbal drugs: what is proven?

Wolfgang Wuttke
Division of Clinical and Experimental Endocrinology, Department of Obstetrics and Gynecology, University of Göttingen, Robert-Koch-Strasse 40, 37075 Göttingen, Germany.

Soy and red clover-derived phytoestrogens (primarily isoflavones such as genistein and daidzein, and the coumestrane compound coumestrol) are currently being promoted as ideal substitutes for hormone replacement therapy for estradiol-17ß. In addition, yet unidentified substances present in the rhizome of *Cimicifuga racemosa* have selective estrogen receptor modulator (SERM) activities in postmenopausal women, i.e. they have been shown to have no estrogenic effects in the uterus and in the mammary gland but they do reduce climacteric complaints and have beneficial estrogen-like effects in the bone. Animal experimental results give evidence for (desired) estrogenic effects also in the urinary bladder and in the cardiovascular system. Soy isoflavones have also been investigated concerning their effects on climacteric complaints. In most studies the number of hot flushes remained unaffected under this isoflavone treatment. Evidence for an antiosteoporotic effect of soy isoflavones is increasing, though still scarce. Undoubtedly, soy isoflavones increase serum HDL and decrease LDL levels which should have theoretically a beneficial effect on the development of arteriosclerosis. Such effects are also inherent to estradiol-17ß and yet the American Heart Association claimed recently that E2 should not be used in the primary or secondary treatment of arteriosclerosis because for unknown reasons the number of fatal incidences increased under E2-treatment. In conclusion, the most convincing evidence for anticlimacteric effects have been given for *Cimicifuga racemosa* extracts, particularly for the extract BNO 1055. Much less convincing are the data obtained with soy or red clover-derived isoflavones: Their effectiveness to reduce climacteric complaints is not high, if present at all.

Prof. Dr. Wolfgang Wuttke

Prof. Wuttke was born in Berlin (1942). He studied in the Medical School of the Free University of Berlin where he did the internship and residency. From 1969 till 1971 he had a Postdoctoral fellowship (Neuroendocrinology) in the Michigan State University (U.S.A.). Since 1971 he has been the head of several departments in Göttingen. At present he is the Head of Division of Clinical and Experimental Endocrinology, Dept. of OB/GYN in the University of Göttingen. In 1972 he did his habilitation and in 1976 he became Professor.

He has been Board Member of the German Endocrine Society, Board Member and Congress President of the German Endocrine Society, Vice President of the International Society of Neuroendocrinology, Board Member of the European Neuroendocrine Association and President of the the German Endocrine Society. Since 1979 he has been in the Editorial Board of several international journals related to the field of Endocrinology: *Journal of Endocrinology*, *Neuroendocrinology*, *Clinical Endocrinology*, *Experimental and Clinical Endocrinology and Diabetes*, etc. He has given his Scientific Services as Reviewer to the German Research Society; to the Ministry for Science; to the Ministry for the Environment; Nature Conservation and Nuclear Safety; and to the National Science Foundation (USA).

Dr. Wuttke is the recipient of the Schoeller-Junkmann Award of the German Endocrine Society (1972), of the Carlo Erba Award of the German Society for Cancer Research (1988) and of the Rudolf-Fritz-Weiß Award of the Society of Phytotherapy (1990). Since 1991 he is a Corresponding Member of The Argentine Society of Endocrinology and Metabolism.

PL07 Marine actinomycetes: new, genetically novel resources for drug discovery

William Fenical, Paul R. Jensen, Tracy Mincer, Gregory Buchanan and Robert H. Feling
Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0204. USA.

Catalyzed by the discovery and widespread utility of Penicillin in the 1940's, the pharmaceutical industry began explorations of the metabolites produced by a wide variety of soil-derived microorganisms. In the same period, Selman Waxman discovered a new antibiotic, which he called Actinomycin, from a poorly known group of fungi-like bacteria called actinomycetes. These discoveries spurred enormous interest in the actinomycetes as an abundant and readily exploited source for new drug candidates. Over the past 50 years, this successful activity resulted in the discovery of over 120 antibiotics and drugs for many other applications from the actinomycetes, and it supported the development of the commercial fermentation industry as the preferred method for drug manufacture. However, within the past 10 years the continual discovery of "old compounds" has discouraged many from continuing in this pursuit. The diversity of readily cultured actinomycetes in the soil has apparently been reached, thus many pharmaceutical industries are looking to synthetic approaches for chemical diversity. After significant investment in new approaches, it has become clear that synthetic compounds will not reach the levels of inherent bioactivity found in natural products. What is difficult to understand is why the pharmaceutical industries never considered the oceans as a source of novel actinomycetes. In our recent work, we have observed actinomycetes in almost every sample retrieved. Actinomycetes are common in marine sediments, even in the deepest parts of the oceans. Recently, we described a new genus of marine actinomycetes, the *Salinospora*. Members of this newly discovered group are chemically rich and produce secondary metabolites of unique structures and bioactivities. From other deep ocean sediments, we have isolated bacteria from another new genus, the *Marinomyces*, a group related to the abundant soil organism *Streptomyces*. Overall, our investigations show that the oceans can provide a diversity of new actinomycetes likely to be of significant utility in the discovery of new drugs.

Prof. Dr. William Fenical

William Fenical received his college education in California, all in the field of organic chemistry. After his Ph.D. at UC-Riverside, Dr. Fenical was employed for one year at the Shell Development Company in Emeryville, CA. After this industrial experience, his interests turned to the development of an academic program in the field of marine natural products chemistry and ecology, a new direction accommodating his long term interest in the ocean. In 1973, he joined the Scripps Institution of Oceanography (SIO), University of California, San Diego, where he has resided ever since. Dr. Fenical is currently Professor of Oceanography, and Director of SIO's Center for Marine Biotechnology and Biomedicine, and is very involved in his personal research program in marine natural products chemistry. Dr. Fenical's past interests have involved the chemistry of marine plants and tropical soft-corals, a topic in which he gained a strong appreciation of the roles secondary metabolites play in the chemical defenses of soft-bodied marine plants and animals. More recently, his interests have focused on the field of marine microbiology and the utilization of marine microorganisms as a source for new drug discovery. As in the past, the ecological roles of marine microorganisms as symbionts and in interspecies interactions continues to be a topic of strong interest. Dr. Fenical has co-authored more than 300 papers in these fields and is the recipient of the Paul Scheuer Award in Marine Natural Products Chemistry, (1996) and the International Society of Chemical Ecology's Silver Medal Award (1997).

PL08 Marine derived anticancer compounds, a journey from the seaside to clinical trials

J. Jimeno, G. Faircloth, J.A. López-Martín and I. Manzanares
PharmaMar R&D, Madrid, Spain & Boston, Massachusetts, USA.

Nature has been an instrumental tool in the acquisition of new therapeutics. Terrestrial derived natural drugs are essential components in the anticancer armamentarium both in the palliative and in the curative setting; the complexity and the policlonal nature of the cancer cell anticipate the need to design rationally based combinations including cytotoxics and "targeted/selective" entities. The sea covers more than 70% of the surface of our planet and represents 95% of the biosphere; evolutionary speaking marine based organisms have had to deal with the survival challenge during more than 600 million years anticipating a highly functional enzymatic capability to produce complex chemical entities. A demonstrative example of the therapeutic potential of the marine ecosystem stands in the discovery, development and regulatory approval 25 years ago of citarabine, a synthetic derivative of a family of nucleotides analogs identified in the Caribbean sponge *Cryptothethya*; ARA-C is an essential component in the curative treatment of acute myeloid leukemia. In line with the relevance of biodiversity our expedition programs have and are covering most of the seas of the planet such program has resulted in a continuous growing library that currently includes more than 28.000 taxonomically classified organisms and more than 100 chemical entities discovered. Our criteria to select compounds for clinical development includes: new chemical entity, innovative mechanism of action, positive therapeutic index in experimental models and feasibiliy for supply. **Ecteinascidin-743** (ET-743), an alkaloid discovered in the Caribbean tunicate *Ecteinascidia turbinata*, is in advanced phase II trials, the available data demonstrates that the compound is feasible in adult and pediatric patients and evidence of antitumor activity in patients with advanced solid tumors resistant to conventional therapy has been generated. **Aplidine** is a new cyclopdepsipeptide discovered in the Mediterranean tunicate *Aplidium albicans*; the phase I program has just been completed with data supporting a positive therapeutic index in adult patients with advanced pretreated solid tumors and lymphoma. Phase II studies are underway. Both compounds share innovative mode of actions including DNA binding/interaction with nuclear transcription factors and targeting of the VEGF pathway for ET-743 and Aplidine respectively. **Kahalalide F (KF)** a peptide based entity is completing the phase I program in patients with advanced prostate cancer with evidence of feasibility in such critical setting; KF appears to target lysosomes and mechanistic work proposes that the erb-2 pathway might be one of the putative targets for this compound. ES-285 is a new chemical entity suggested to interact with Rho related pathways. **ES-285** displays *in vivo* activity against hepatoma and prostatic cancer xenografts and a phase I program is under implementation. In a seven years journey in the planet of clinical development we have learned the need to make strong-interdisciplinary focus in pharmacological development (supply, formulation, analytical methods) as well as in the identification of xenotoxicities, a potential limiting factor for clinical development. Our program has generated the availability of innovative preclinical models to better characterize the therapeutic index of a given candidate thus maximizing the feasibility in the clinical scenario. To implement these actions a continuous collaboration including marine biologists, medicinal chemists, preclinical scientists-toxicologists, pharmacologists and medical oncologists is essential.

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Dr. José Jimeno
Dr. Jimeno graduated as an MD Cum Laude at the University of Zaragoza (Spain) in 1981. He did his fellowship in Medical Oncology at the National Cancer Institute in Bari, Italy. Furthermore, he joined the pharmaceutical industry and has had relevant positions in the R&D area at Farmitalia Carlo Erba (Milan, Italy) and at US Bioscience (Watford, UK): He has published over 50 papers in the field of cancer research, holds the European Certification of Medical Oncology (Copenhagen, 1990) and is an active member of the ESMO, AACR and ASCO. Dr. Jimeno joined PharmaMar in late 1994.

PL09 Bioactive natural products from marine dinoflagellates

Jun'ichi Kobayashi
Graduate School of Phamaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Marine dinoflagellates have proven to be a good source of compounds with intriguing structures and interesting biological acivity.

A series of cytotoxic macrolides, amphidinolides A~H and J~W, have been isolated from marine dinoflagellates of genus *Amphidinium*, which were separated from the marine flatworms *Amphiscolops* sp. Amphidinolides B, C, G, H, and N were potent cytotoxic, among which amphidinolides C and H showed antitumor activity *in vivo*. The absolute stereochemistries of amphidinolides B, C, E, G, H, J, T, and W have been established by combination of syntheses of the degradation products and NMR analysis. On the basis of stable isotope incorporation experiment, the backbones of some amphidinolides were shown to be derived from non-successive mixed polyketide chains.

On the other hand, colopsinols and luteophanols, long chain polyhydroxyl compounds, have been isolated from dinoflagellates of the genus *Amphidinium* and the structures were elucidated by 2D NMR techniques and FABMS/MS data and chemical means. Colopsinol A exhibits potent inhibitory activity against DNA polymerases α and β.

In this congress, the isolation, structure elucidation, biosynthesis, and bioactivity of these macrolides and polyketides will be described.

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Prof. Dr. Jun'ichi Kobayashi

Prof. Jun'ichi Kobayashi (1949) studied in Hokkaido University, Sapporo (Japan) where he became PhD in Pharmaceutical Sciences (1979). Since 1975 he has occupied several positions: Researcher in the Mitsubishi-Kasei Institute of Life Sciences (1975-1986), Research Associate in the University of Illinois (with Prof. Kenneth Rinehart) (1982-1984), and Senior Researcher in the Mitsubishi-Kasei Institute of Life Sciences (1986-1989). Since then he is Professor of the Graduate School of Pharmaceutical Sciences (Hokkaido University). His main research interests have been in the fields of bioactive metabolites from marine micro- and macro-organisms, stereochemistry and biogenesis of unique marine products, bioactive taxoids from yew trees, *Nocardia* metabolites as drug leads.

PL10 ^{13}C-NMR as a tool for identification of individual components in natural mixtures

Joseph Casanova
Université de Corse-CNRS, Equipe Chimie-Biomasse, Route des Sanguinaires, 20000 Ajaccio, France.

The structural and quantitative analysis of complex mixtures such as essential oils or extracts, is commonly carried out by GC/KI, GC/MS, GC/IRFT, HPLC/MS or a combination of these techniques. In a different approach, ^{13}C-NMR spectroscopy could be used for the non separative and non destructive identification of the individual components of complex mixtures (essential oils, petroleum distillates, vegetable oils, biomass pyrolysis liquids).
Following the pioneering work of Formácek and Kubeczka, we applied this methodology to identify the main components of essential oils and extracts. We developed an experimental procedure, based on computer-aided analysis of the ^{13}C-NMR spectrum of the mixture. We compared the chemical shift of each carbon in the experimental spectrum with the spectra of pure compounds listed in our spectral library. In order to obtain reproducible chemical shift values we began simultaneously: i) to define the best experimental and acquisition conditions to record NMR spectra (solvent, concentration, pulse width, …) and ii) to create our ^{13}C-NMR spectral library. First, we checked the experimental procedure and the software with synthetic mixtures of terpenic derivatives. Then we analysed a large number of essential oils and solvent extracts. In all cases, this method allowed a good identification of sesquiterpenes as well as diterpenes present in essential oils (up to 24 compounds, lower limit: 0.5%) including structurally close molecules (such as stereoisomers) and compounds which exhibit insufficiently resolved mass spectral patterns or co-eluate on GC or thermolabile compounds.
In this presentation we will describe the interest of this methodology for the unambiguous identification of terpenes (including unusual sesquiterpenes and diterpenes) present in essential oils as well as diterpene acids and triterpenic derivatives present in oleoresins and in solvent extracts.
Further work is in progress to carry out: i) quantitative analysis of the components with or without internal reference, ii) complete and unambiguous analysis of some complex essential oils by GC-MS and Carbon-13 NMR spectroscopy, iii) enantiomeric differentiation of the major components using chiral shift reagents.

Prof. Dr. Joseph Casanova
He is Professor of Organic Chemistry and Spectroscopy at the University of Corsica. He has been researcher of the CNRS during 10 years (1971-1981) and received a grant of the NATO-NIH to stay at the Rice University (Houston, Texas) (1974-1975). Since 1992 he is *Professeur de 1ère classe* of the University of Corsica, where he has also occupied several positions, such as: Dean of the Faculty of Sciences and President of the Scientific Council, Responsible of the International Relationships, etc.
At present, he is Head of the Research Centre on "Biodiversity in Islands and in the Mediterranean". His main research topics are: methodology of NMR analysis, analysis of natural complex mixtures, aromatic plants, essential oils, extracts, bio-oils. He has more than 70 papers published in international journals and has participated in many international congresses with conferences and communications.
His research group has had active cooperation with other universities: Marseille, Montpellier, Sassari, Cagliari et Pise (Interreg), Barcelona (Picasso), Coimbra, Eindhovein, Abidjan, Conakry, Cotonou and Hanoï.

PL11 The potential of HPLC coupled with UV, MS and NMR in the discovery of new bioactive plant constituents

Kurt Hostettmann
Institut de Pharmacognosie et Phytochimie, Université de Lausanne, BEP, CH-1015 Lausanne, Switzerland

Plants represent an extraordinary reservoir of novel molecules and there is currently a resurgence of interest in the vegetable kingdom as a possible source of new lead compounds for introduction into therapeutical screening programs. Plant constituents of interest are usually isolated following a bioactivity guided fractionation procedure. In order to render this approach more rapid and efficient, the dereplication of crude plant extracts with LC-hyphenated techniques represents a strategic element to avoid finding known constituents and to target the isolation of new bioactive compounds. Spectroscopic information can be obtained on-line, directly from crude plant extracts, with hyphenated techniques such as high performance liquid chromatography (HPLC) coupled to UV photodiode array detection (LC-DAD/UV), to mass spectrometry (LC/MS) and to nuclear magnetic resonance (LC/NMR) (1,2). In addition, LC-bioassay based on microfractionation of the LC-peaks followed by TLC bioautography allows a rapid and precise estimation of the bioactivity of a given peak. LC/UV/MS and LC/NMR combined with LC-bioassays have permitted the rapid identification or localisation of compounds presenting antifungal or antioxidant activities in various plant species, as in the on-line identification of various potent antifungal prenylated isoflavonoids from a Leguminosae, *Erythrina vogelii.* LC/MS and LC/NMR were also used for the study of compounds which are difficult to isolate on a preparative scale; they were particularly useful, for example, for the investigation of unstable iridoids from *Jamesbrittenia fodina* (Scrophulariaceae). In this case, light induced *cis/trans* isomerisation as well as transesterification of cinnamic acid moieties could be shown.
LC/NMR and LC/MS are powerful tools for solving phytochemical problems. They allow a rapid estimation of the interest of a given compound in a complex extract and are also very useful for the detection of toxic compounds in phytopharmaceuticals. These techniques do not replace the activity-guided fractionation of the extracts, but provide a strategic complement to standard isolation procedures. Furthermore, they permit the recording of the spectroscopic data of labile constituents which cannot be recorded by other means.

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Prof. Kurt Hostettmann

Prof. Hostettmann studied chemistry at the University of Neuchâtel (Switzerland). After a postdoctoral stay at Columbia University, New York, he joined the Department of Pharmacy of ETH Zürich as senior research associate. At the same time, he had teaching duties as privat-docent at the University of Neuchâtel and at the University of Fribourg as lecturer. Since 1981, he is Professor at the University of Lausanne and Director of the Institute of Pharmacognosy and Phytochemistry. Since 1997, he is also in charge of the pharmacognosy teaching at the University of Geneva.
He is involved in the phytochemical investigation of plants used in traditional medicine. The aim of his research is to find new lead compounds from Nature which could become drugs. He is also interested in the development of new separation techniques for natural products. He is the author of more than 450 publications, of 60 chapters in books and of 10 books. Among them, one has been translated in Japanese, in Chinese, in Spanish and Indonesian language. He obtained several distinctions, for example he is Dr. *honoris causa* of the University of Medicine and Pharmacy of Iasi, Romania and is Honorary Professor at Nanjing University, China.

PL12 Exploring natural products from Mexican biodiversity as a source of potential medicinal agents

Rachel Mata
Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Coyoacán 04510, México DF, México.

Regardless of its richness and variety, only a small percentage of the Mexican medicinal flora has been investigated from the phytochemical or pharmacological point of view. Thus, the potential of Mexican medicinal plants as a source of bioactive compounds remains largely unexplored; mostly in view that many drugs or pesticide agents have come to us from the use of plants by indigenous cultures. In this scenario we have established a multidisciplinary research program to obtain phytochemicals of medicinal or agrochemical interest from Mexican plants used in folk medicine. In the medicinal area, our efforts have been mainly focused on the discovery of active principles useful for the treatment of gastrointestinal disorders which are a major health problem in Mexico. In this presentation the potential of Mexican medicinal flora as a source of useful biologically active products will be illustrated through selected examples stemming from our research program. Brief considerations of the botanical sources, and of the isolation, the structure elucidation, and the biological properties of the active phytochemicals are presented.

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Prof. Dr. Rachel Mata

Prof. Rachel Mata was born in Caracas (Venezuela) and holds Mexican citizenship. She received her undergraduate degree in Pharmacy at the Universidad Central de Venezuela (1972), and her M.S. (1976) and PhD (1979) degrees in Pharmacognosy from Purdue University, U.S.A. In 1984 she joined the faculty of the Universidad Nacional Autónoma de México (UNAM), where she is Professor of Pharmacognosy. She received a Distinguished Alumna Award (1998) from the School of Pharmacy and Pharmacal Sciences, Purdue University, the National University Prize in Natural Sciences (2000) from UNAM and more recently the Martin de la Cruz Prize awarded by the Mexican government. Her *curriculum vitae* lists more than 120 peer reviewed scientific publications, several chapters, numerous abstracts and one book. She served as President of the Phytochemical Society of North America (1996-1997) and currently she is on the editorial board of the *Journal of Natural Products*.