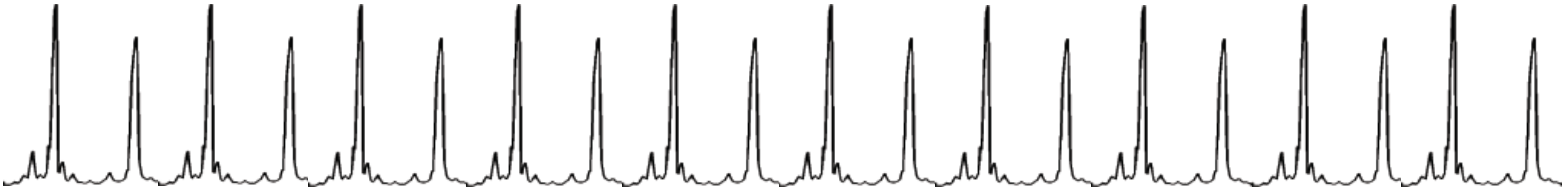
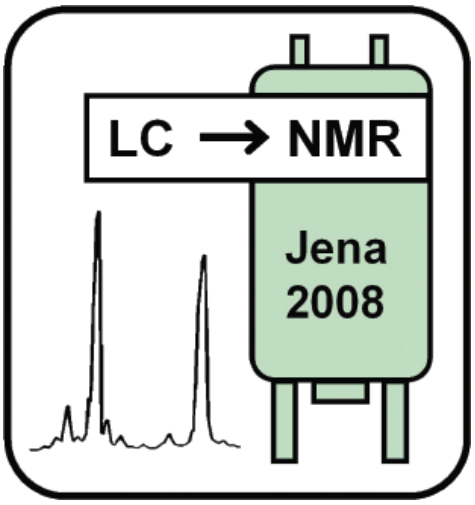
# International Conference on LC-NMR and Related Techniques:

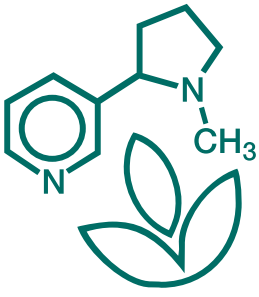
# **“Challenges in Biological Systems”**



27-29 August 2008
in Jena (Germany)

**Program • Abstracts • Information**




Max Planck Institute
for Chemical Ecology

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# 1. Organization

## Organizer

Max-Planck-Institute for Chemical Ecology
http://www.ice.mpg.de

## Scientific Committee

- Prof. Klaus Albert, Tübingen
- Dr. Cristina Daolio, Karslruhe
- Prof. Jerzy W. Jaroszewski, Copenhagen
- Dr. Bernd Schneider, Jena
- Prof. Jean-Luc Wolfender, Geneva

## Organization Committee

- Dr. Renate Ellinger
- Angela Overmeyer
- Diana Mewes
- Dr. Bernd Schneider

## Contact

LC-NMRJena2008 @ ice.mpg.de

## Conference Venue (lectures and poster session)

Beutenberg Campus, Abbe Centre (Central Building), Big Lecture Hall
Hans-Knöll-Str. 1, 07745 Jena

## Lunch

Beutenberg Campus, Abbe Centre (Central Building), Cafeteria
Hans-Knöll-Str. 1, 07745 Jena

## Social Events (welcome reception and farewell party)

Max-Planck-Institute for Chemical Ecology
Beutenberg Campus, Hans-Knöll-Str. 8, 07745 Jena

## Internet

Please contact the organizers.

# 2. Scientific Program

**WEDNESDAY, 27 AUGUST 2008**

| | |
|---|---|
| 08:30 - 10:00 | Arrival and Registration at Abbe Centre, Beutenberg Campus, Hans-Knoell-Str.1, D-07745 Jena |
| 10:00 - 12:00 | **Workshop „Introduction into basic principles of LC-NMR" and Discussion:** |
| | **Dr. Bernd Schneider**, Jena<br>LC-NMR in the Stopped-Flow Mode |
| | **Prof. Jerzy W. Jaroszewski**, Copenhagen<br>LC-SPE-NMR - Principle and Optimization |
| | **Prof. Jean-Luc Wolfender**, Geneva<br>Identification of Minor Constituents by Microflow LC-NMR Probes used at-Line |
| 12:00 - 14:00 | Lunch |
| 14:50 - 15:00 | **Opening** |
| 15:00 - 15:45 | **Klaus Albert**, Tübingen<br>On-line Coupling of Capillary Separation Techniques to Microcoil 1H Nuclear Magnetic Resonance Spectroscopy |
| 15:45 - 16:15 | **Jacques Vervoort**, Wageningen<br>Recent Developments in LC-(SPE)-NMR-(MS) |
| 16:15 - 16:30 | **Sofia Moco**, Wageningen<br>NMR- and LC-MS-Based Metabolite Correlations in Tomato |
| 16:30 - 17:00 | Coffee break |
| 17:00 - 17:45 | **Jean-Luc Wolfender**, Geneva<br>LC-MS Combined with Sensitive NMR Methods for Natural Product Dereplication and Biomarker Identification in Plant Metabolomics |
| 17:45 - 18:15 | **Sonja Sturm**, Innsbruck<br>HPLC-SPE-NMR - an Elegant Tool Aiding the Structure Characterization Process in Metabolite Profiling |
| 18:15 - 18:30 | **Tobias Mohn**, Basel<br>A Comprehensive Metabolite Profiling of *Isatis tinctoria* Leaf Extracts |
| 18:40 - 21:00 | Welcome Reception |

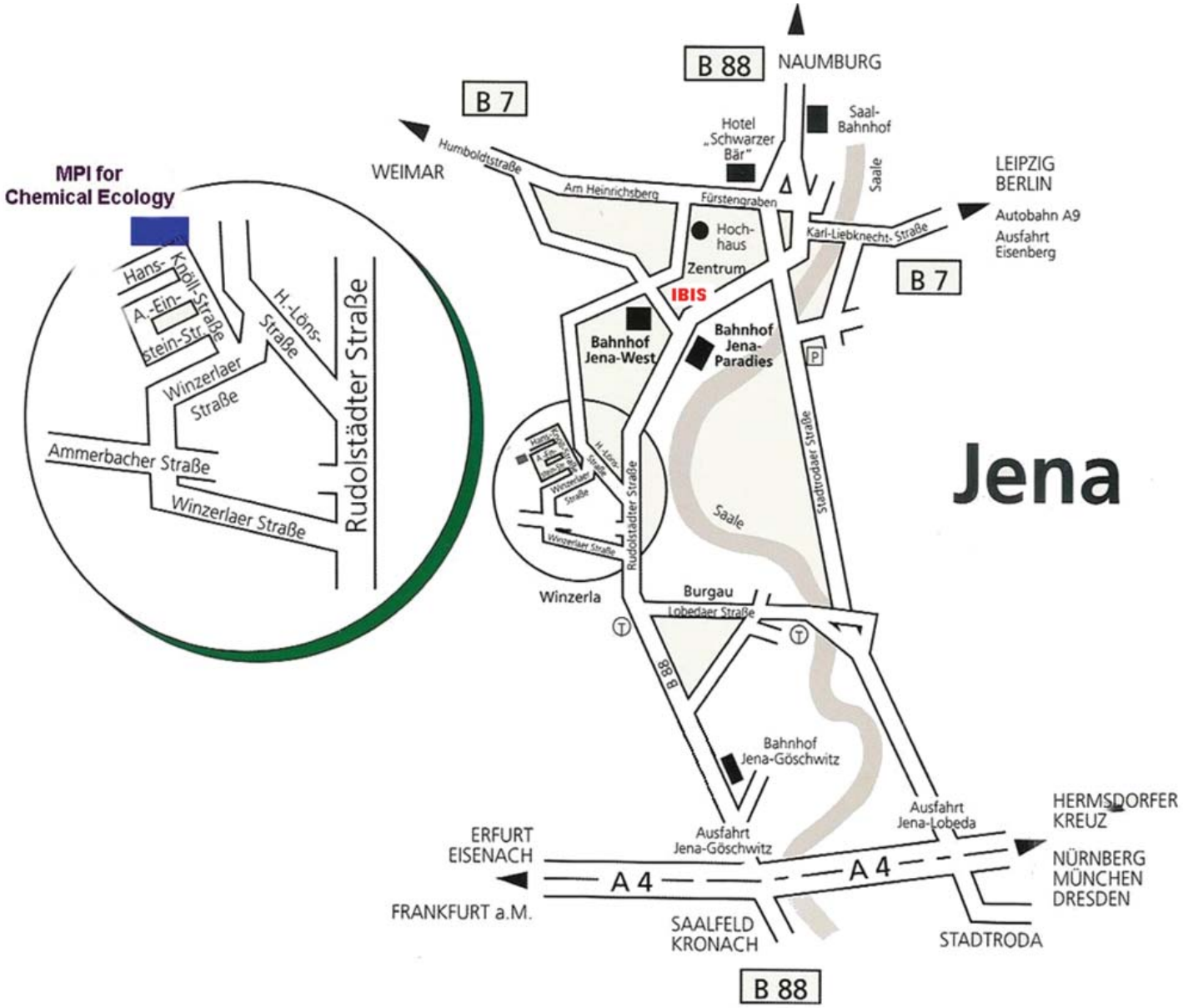
**THUESDAY, 28 AUGUST 2008**

| | |
|---|---|
| 08:30 - 09:15 | **Cynthia Larive**, Riverside<br>Pharmaceutical and Biochemical Microanalysis using Microcoil NMR |
| 09:15 - 09:45 | **Roger Kautz**, Boston<br>A High-Sensitivity LC-MS-NMR Approach Using 4 mm LC, nanospray MS, and Segmented-Flow Microcoil NMR Automation |
| 09:45 - 10:00 | **Jan Sykora**, Prague<br>Atropisomerism of 1,8-bis-(2-propynyl-phenyl)-naphthalene |
| 10:00 - 10:30 | Coffee break |
| 10:30 - 11:00 | **Andrew G. Webb**, Leiden<br>Hyphenated LC-NMR at the Microscale |
| 11:00 - 11:30 | **Alfred Preiss**, Hannover<br>LC-NMR and LC-MS Applications to Characterize Biotransformation Products of Pollutants in Environmental Samples |
| 11:30 - 11:45 | **Diana Meißner**, Halle/S.<br>Cd-induced structure of a small metallothionein determined by capillary HPLC-NMR |
| 11:45 - 12:00 | **Dmitry A. Bolibrukh**, Minsk and Jena<br>HPLC-SPE-NMR-analysis of 3-methoxy-14,17-etheno-16α-nitroestra-1,3,5(10)-trien-17β-yl acetate solvolysis products |
| 12:00 - 14:00 | Lunch |
| 14:00 - 14:30 | **Manfred Spraul**, Karlsruhe<br>Integrated Use of NMR, Mass Spectroscopy and Chromatography in Metabolomics |
| 14:30 - 15:00 | **Ulrich Braumann**, Karlsruhe<br>LC-(SPE)-(Cryo)NMR(/MS) as Routine Analysis Technique: Instrumentation and Software |
| 15:00 - 15:30 | **Maarten Honing**<br>Structural Characterization of „Unknowns“; Hyphenated LC-MSMS-NMR the Ultimate Tool! |
| 15:30 - 15:45 | **Peter W. A. Howe**, Blacknell<br>Practical aspects of LC-NMR with Cryogenically Cooled Probes |
| 15:45 - 16:15 | Coffee break |
| 16:15 - 18:00 | **Poster Session** |
| 18:30 - „open end“ | **Farewell Party** |

**FRIDAY, 29 AUGUST 2008**

| | |
|---|---|
| 08:30 - 09:15 | **Jerzy W. Jaroszewski**, Copenhagen<br>Hyphenated NMR Methods for Natural Product Research |
| 09:15 - 09:45 | **Bernd Schneider**, Jena<br>LC-(SPE)-NMR Applications in Chemical Ecology |
| 09:45 - 10:00 | **Christian Paetz**, Jena<br>Phenolics of Norway Spruce Colonized with *Heterobasidion annosum* |
| 10:00 - 10:30 | Coffee break |
| 10:30 - 11:00 | **Vassiliki Exarchou**, Ioannina<br>LC/SPE/NMR Hyphenation and its Application to Phytochemical Analysis |
| 11:00 - 11:15 | **Xin-Zhou Yang**, Basel<br>Profiling of two Chinese Medicinal Plants, *Sophora flavescens* and *Ligusticum chuangxiong*, by Off-Line LC-NMR and LC-MS |
| 11:15 - 11:45 | **Wolf Hiller**, Dortmund<br>Characterization of Copolymers by HPLC-NMR |
| 11:45 - 12:00 | **Milan Kurfuerst**, Prague<br>LC-29Si NMR analysis of Silicon Copolymer Mixtures |
| 12:00 - 12:15 | **Closing remarks** |
| 12:15 - 14:00 | Lunch and Departure |

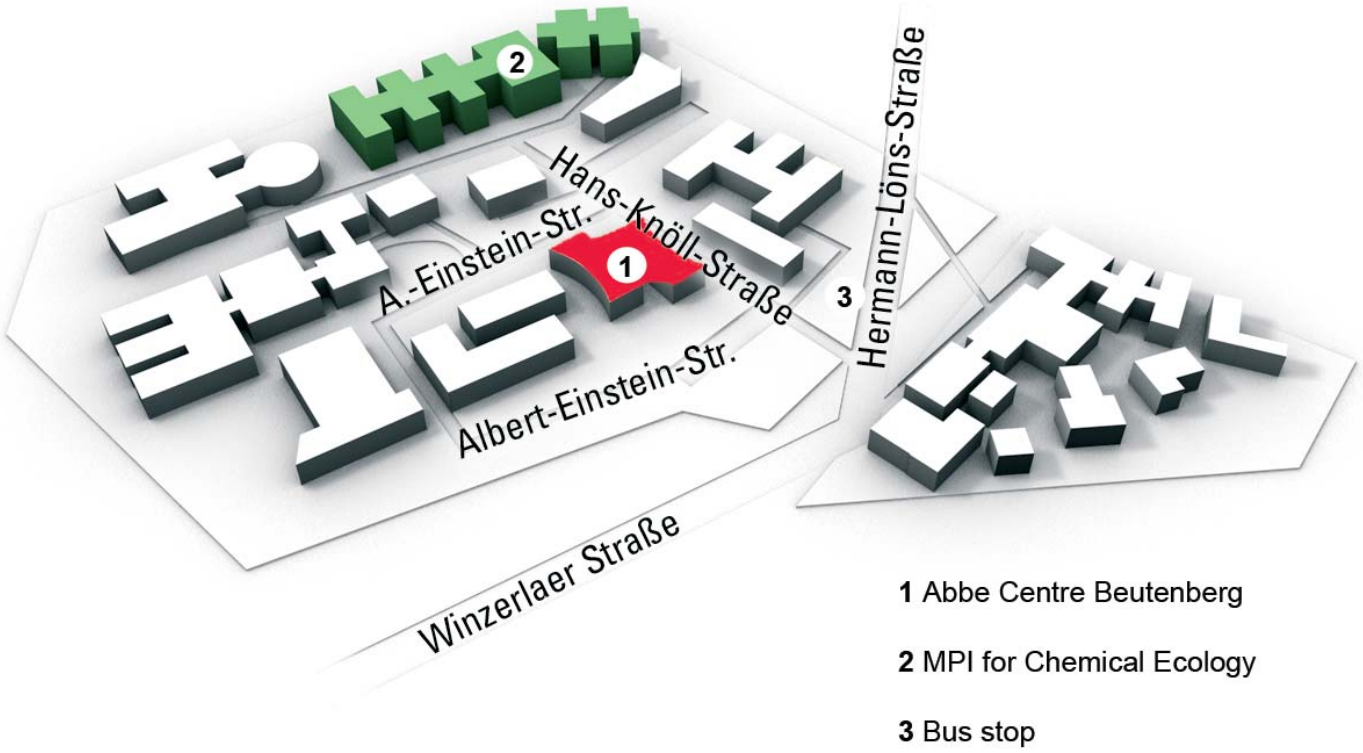
# 3. Conference Site




Abbe Centre Beutenberg
Lecture Hall
Hans-Knöll-Straße 1
D-07745 Jena
Germany

Max Planck Institute for Chemical Ecology
Hans-Knöll-Straße 8
D-07745 Jena
Germany
Fon: +49 (0)3641 - 57 0

## Beutenberg Campus




Bus lines from bus stop “Beutenberg Campus” to city centre (“Teichgraben”): 10, 13, and 40

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# On-line Coupling of Capillary Separation Techniques to Microcoil $^{1}$H Nuclear Magnetic Resonance Spectroscopy

Klaus Albert, Marc Bayer, Karsten Holtin, Max Kühnle, Walter Schaal, and Paul Schuler

Universität Tübingen, Institut für Organische Chemie,
Auf der Morgenstelle 18, D-72076 Tübingen, Germany

The technique of directly coupled analytical scale HPLC and NMR spectroscopy is a well established analytical tool for the analysis of complex mixtures. Recent advances to microscale analysis have opened up the fields of capillary High Performance Liquid Chromatography (cHPLC) and capillary Gas Chromatography (GC). The achieved progress was possible by the introduction of specially designed capillary microcoil NMR probes. Our design for the NMR registration of capillary separations employs homemade micro coils with detection volumes between 1.5 and 5.0 µl. The small flow rates used in liquid capillary separations enable the use of deuterated solvents. In capillary gas chromatography NMR spectra are recorded in the gaseous state. With both techniques, cHPLC-NMR and GC-NMR, continuous-flow as well as stopped-flow $^{1}$H NMR spectra can be recorded. Thus unequivocal structural assignment of unknown chromatographic peaks is possible by employing stopped-flow 2D NMR spectroscopy.

Practical examples from the analysis of food ingredients and volatile stereoisomers will demonstrate the high application power of combining capillary separation techniques together with microcoil $^{1}$H NMR spectroscopy.

# Recent Developments in LC-(SPE)-NMR-(MS)

Vervoort, Jacques

Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands

# NMR- and LC-MS-Based Metabolite Correlations in Tomato

S. Moco[1], J. Forshed[2], R.C.H. De Vos[3], R.J. Bino[3], J. Vervoort[1]

[1] Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands
[2] Karolinska Biomics Center, Karolinska Institutet, Stockholm, Sweden
[3] Plant Research International, Wageningen, The Netherlands

Nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LCMS) are frequently used as technological platforms for metabolomics applications. In this study, the metabolic profiles of ripe fruits from different tomato cultivars, including beef, cherry and round types, were recorded by both $^{1}$H NMR and accurate mass LC-quadrupole time-of-flight (QTOF) MS. Different analytical selectivities were found for both profiling techniques. In fact, NMR and LCMS provided complementary data, as the metabolites detected mostly belong to essentially different metabolic pathways.

Intra-method (NMR-NMR, LCMS-LCMS) and inter-method (NMR-LCMS) correlation analyses were performed, in order to identify metabolites from highly correlating signals. Many high correlations were found in the intra-method correlation analyses, revealing a large number of related signals belonging to the same metabolite and to chemically related metabolites. Inter-method correlation analysis produced highly informative and complementary information for the identification of metabolites, even in de case of low abundant NMR signals.

The applied approach appears to be a promising strategy in extending the analytical capacities of these metabolomics techniques with regard to the discovery and identification of biomarkers and yet unknown metabolites.

# LC-MS Combined with Sensitive NMR Methods for Natural Product Dereplication and Biomarker Identification in Plant Metabolomics

Jean-Luc Wolfender[1], Gaetan Glauser[1,2], Elia Grata[1,2],
Julien Boccard[2,3], Pierre-Alain Carrupt[3], Serge Rudaz[2]

[1]LPP, [2]LCAP, [3]LCT, School of Pharmaceutical Sciences, EPGL, University of Geneva,
University of Lausanne, 30, quai Ernest-Ansermet, CH-1211 Geneva, Switzerland.

The development of on-line LC-NMR and at-line methods (SPE-NMR and CapNMR) have open new exciting opportunities for the rapid identification of natural products (NP's) in the field of phytochemistry and plant metabolomics. The possibility to acquire rapidly $^{1}$H-NMR spectra of individual constituents in crude plant extracts in direct hyphenation with HPLC provides useful complementary information to LC-UV-MS profiling and give a strategic advantage in the dereplication process. On the other hand the use of at-line methods based on microflow NMR such as CapNMR [1] in combination with LC-MS triggered microfractionation methods provides high quality 1D and 2D NMR spectra for the analysis of NP's in the low microgram range. Such experiments are indeed essential for a complete *de novo* structural determination. The acquisition of NMR data on sample scales equivalent to biological screening amounts can thus considerably accelerated the lead finding process or the identification of biomarkers in metabolomic studies.

In this respect different examples of plant analyses will be discussed. In particular the results obtained with UPLC-TOF-MS and CapNMR in the frame of a plant metabolomic study of the wound response in *Arabidopsis thaliana* will be presented [2]. In this case a non-targeted high throughput metabolite fingerprinting of numerous specimens involving rapid UPLC-TOF-MS analysis and data mining enabled the detection of different stress biomarkers such as oxylipins [3]. Key wound-induced jasmonate derivatives were isolated at the microgram scale by a precise MS-directed fractionation procedure for their complete *de novo* CapNMR structural determination. Upscaling from UPLC to semi-prep LC-MS relied on efficient gradient transfer and computed optimised chromatographic conditions with Osiris® software. A special attention was paid to the separation of closely related isomers [4]. Thanks to this strategy, a broad survey of wound-biomarkers with various physicochemical properties was obtained in the leaf extracts and, besides known signalling molecules, original oxylipins and related products were identified. A careful interpretation of the UPLC-TOF-MS data provides an overview of the spatial and temporal induction of the jasmonates [3]. The approach enabled both a rapid estimation of the significant wound metabolome variations and the precise identification of biomarkers involved in these changes. The biological activity of these products in relation with their defence gene expression potential was evaluated based on DNA microarray experiments.

[1] Olson, D. L.; Norcross, J. A.; O'Neil-Johnson, M.; Molitor, P. F.; Detlefsen, D. J.; Wilson, A. G.; Peck, T. L. Anal. Chem. 2004, 76, 2966-2974.
[2] Grata, E.; Boccard, J.; Guillarme, D.; Glauser, G.; Carrupt, P. A.; Farmer, E.; Wolfender, J. L.; Rudaz, S. J. Chromatogr. B 2008, (in press) doi:10.1016/j.jchromb.2008.04.021.
[3] Glauser, G.; Grata, E.; Dubugnon, L.; Rudaz, S.; Farmer, E.; Wolfender, J. L. J. Biol. Chem. 2008, 283, 16400-16407.
[4] Glauser, G.; Guillarme, D.; Grata, E.; Boccard, J.; Thiocone, A.; Carrupt, P. A.; Veuthey, J. L.; Rudaz, S.; Wolfender, J. L. J Chromatogr A 2008, 1180, 90-98.

# HPLC-SPE-NMR - an Elegant Tool Aiding the Structure Characterization Process in Metabolite Profiling

S. Sturm, C. Seger, H. Stuppner

University of Innsbruck, Institute of Pharmacy / Pharmacognosy, Innsbruck, Austria

Plants and products made thereof serve mankind as foods and nutriceuticals, fragrances, medicines, or poisons since primeval times. The analysis of highly complex plant derived sample matrices is still one of the major challenges in analytical chemistry. It can be considered one of the most prominent driving forces for chromatographic and spectroscopic method development within the last decades.

At the present time, most secondary natural products analyses rely on fully validated quantitative assays. Within the first years of this millennium, biomarker discovery oriented metabolic profiling methods emerged as a novel analytical tool complementing conventional quantitative target analysis approaches [1,2]. Whenever the discovery of a chemical entity is the focal point of an analytical methodology, its identification is the logical and necessary next step. It is not only crucial for linking a monitored bioactivity with structural features – the basis of modern day phytopharmaceutical research but also the prerequisite for any target analysis approach confirming the discriminatory power of a discovered marker molecule.

Although modern mass spectrometry experiments provide a plenitude of structural information, only NMR does allow the unequivocal identification of an analyte in solution. However, being an inherently insensitive method, analyte amounts required for classical NMR analysis are often out of reach if working with plant materials.

HPLC-SPE-NMR is an elegant platform which helped to overcome this bottleneck in secondary metabolite identification. In contrast to the HPLC-NMR hyphenation, the separation of the HPLC and NMR process by the SPE interface allows using the routine HPLC-MS/MS mobile phase / stationary phase combinations for optimal analyte separation and well defined deuterated NMR solvents for spectra generation, which facilitates data comparison with reference materials. Due to the possibility of sample enrichment on the SPE trapping column by repeated analyte loading recording of heteronuclear shift correlations experiments can be considered a routine HPLC-SPE-NMR operation [3-5].

The benefits of HPLC-SPE-NMR in our daily routine phytochemical research processes will be demonstrated by recent application examples including the identification of novel biological active diterpene congeners from a Chinese Zingiberaceae, the characterization of putative biomarkers in a metabolic profiling approach of European *Corydalis* species as well as in a study of Asian *Leontopodium* representatives.

[1] C. Seger and S. Sturm, Journal of Proteome Research 6 (2007) 480-497
[2] E. Holmes, H. T ang, Y. Wang and C. Seger Planta Med 72 (2006) 771-785
[3] J.W. Jaroszewski, Planta Med. 71 (2005) 691-700
[4] V. Exarchou, M. Krucker, T.A. van Beek, J.Vervoort, I.P. Gerothanassis and Klaus Albert, Magn. Reson. Chem. 43 (2005) 681–687
[5] C. Seger and S. Sturm, LC-GC Europe 20 (2007), 587-597

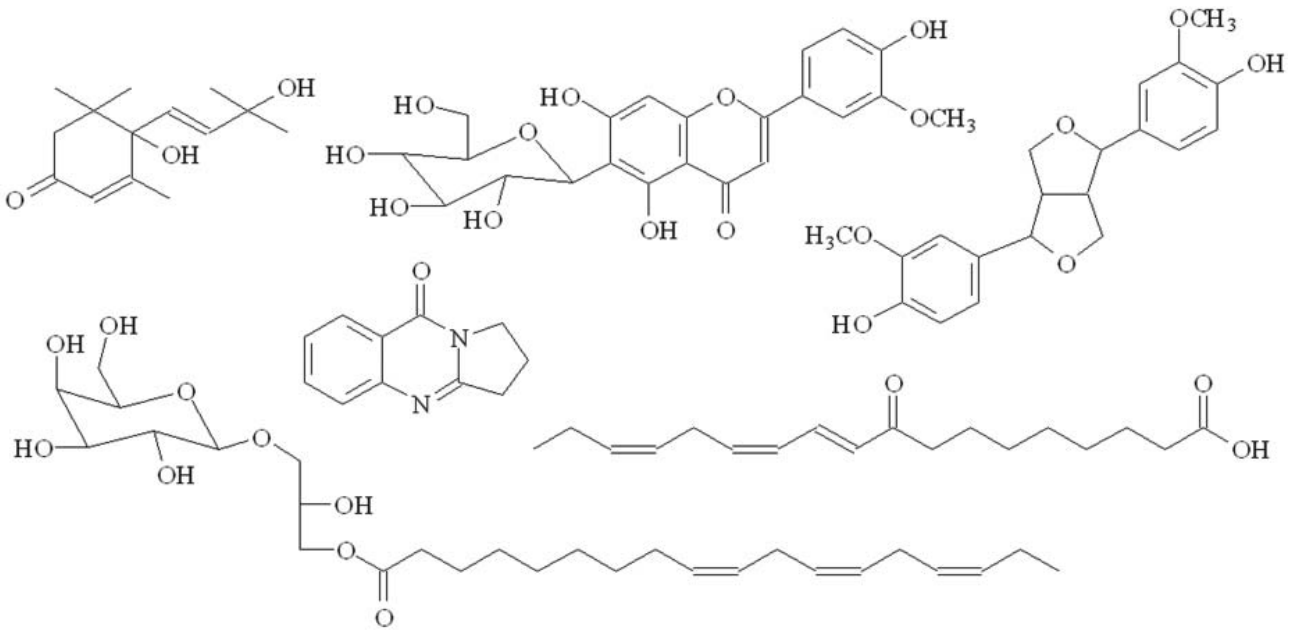
# A Comprehensive Metabolite Profiling of *Isatis tinctoria* Leaf Extracts

T. Mohn, I. Plitzko, M. Hamburger

Institute of Pharmaceutical Biology, University of Basel,
Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Woad (*Isatis tinctoria* L., Brassicaceae) is an ancient indigo dye and anti-inflammatory medicinal plant, which has been used and cultivated in Europe since antiquity. The anti-inflammatory potential of lipophilic leaf extracts was confirmed in a broad-based pharmacological profiling, in various animal models [1], and in a clinical pilot study [2]. Tryptanthrin, indirubin, an indolin-2-one derivative, and γ-linolenic acid were identified as pharmacologically active compounds inhibiting COX-2, 5-LOX, the expression of the inducible nitric oxide synthase, human neutrophil elastase, and the release of histamine from mast cells.

To further characterize the pharmacologically active extracts, we carried out a compre-hensive metabolite profiling with the aid of online spectroscopic measurements (HPLC coupled to PDA, ELSD, APCI- and ESI-MS, and HRESI-MS). Off-line semi-preparative HPLC-NMR analysis was used for structure elucidation of some constituents. For this purpose 15 mg of extract was injected onto a semi-preparative HPLC column (150 x 10 mm). Peak based collection was monitored at 210 nm. Mobile phase in fractions was removed by parallel evaporation, and samples were submitted to NMR analysis (Bruker Avance III 500 MHz, 1 mm TXI probehead, active volume 5 µl). So far, more than 60 compounds belonging to various structural classes such as alkaloids, flavonoids, fatty acids, porphyrins, lignans, carotenoids, glucosinolates and cyclohexenones have been unambiguously identified, and tentative structures proposed for additional compounds.



[1] M.C. Recio, M. Cerda-Nicolas, O. Potterat, M. Hamburger, J.L. Rios, Planta Med. 2006, 72, 539.
[2] C. Heinemann, S. Schliemann-Willers, C. Oberthür, M. Hamburger, P. Elsner, Planta Med. 2004, 70, 385.

# Finding the Needle in a Haystack: Pharmaceutical and Biochemical Microanalysis using Microcoil NMR

S. Eldridge, A. Korir, J. Limtiaco, C. Larive

University of California-Riverside, USA

Nuclear Magnetic Resonance Spectroscopy (NMR) is a powerful method for quantitative and qualitative chemical analysis because of the wealth of structural information this technique can provide. However, compared to other detection methods like mass spectrometry, NMR suffers from relatively poor sensitivity. NMR microcoils can improve sensitivity by lowering mass detection limits. These detection limits can be further enhanced by coupling to the online separation method capillary isotachophoresis (cITP). This technique utilizes electrophoretic separation with a discontinuous buffer system and can concentrate charged analytes by 2 to 3 orders of magnitude. Analysis with cITP-NMR requires only micrograms of material. Because NMR is a non-destructive analytical method, samples can be collected following cITP-NMR analysis for subsequent examination using mass spectrometry. Application of this technology for analysis of trace pharmaceutical impurities will be presented. In this application, we can take advantage of the ability of cITP to selectively focus charged analytes to remove the interference from neutral compounds present in large excess. Detection in cITP-NMR is accomplished using our home-built 25 nL microcoil NMR probe.

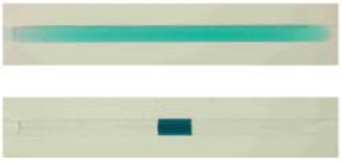
We are also using microcoil NMR techniques for the structural characterization of the glycosaminoglycans heparin and heparan sulfate (HS). This is a challenging analytical problem because of their high negative charge, polydispersity and sequence heterogeneity. Heparin and HS are important pharmaceutical targets as they bind a large number of proteins, including growth factors and cytokines, mediating biological processes such as cell adhesion, inflammation, formation of amyloid plaques, tumorigenesis and viral infection. Because of the heterogeneity of these biopolymers, determining the structural motifs of rare protein binding sites is an analytical challenge equivalent to finding a needle in a haystack. Our group is developing NMR, LC-MS and CE methods for analysis of heparin and HS oligosaccharides that have high specificity as well as high sensitivity. In this work, in addition to cITP-NMR using our home-built probes, we also make use of the CapNMR probe marketed by Protasis/MRM.

# A High-Sensitivity LC-MS-NMR Approach Using 4 mm LC, Nanospray MS, and Segmented-Flow Microcoil NMR Automation

Yiqing Lin[1], Susie Schiavo[1], Paul Vouros[1] , Jimmy Orjala[2], and Roger Kautz[1]

[1]Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, MA 02115 USA
[2]College of Pharmacy, University of Illinois, Chicago IL 60612 USA

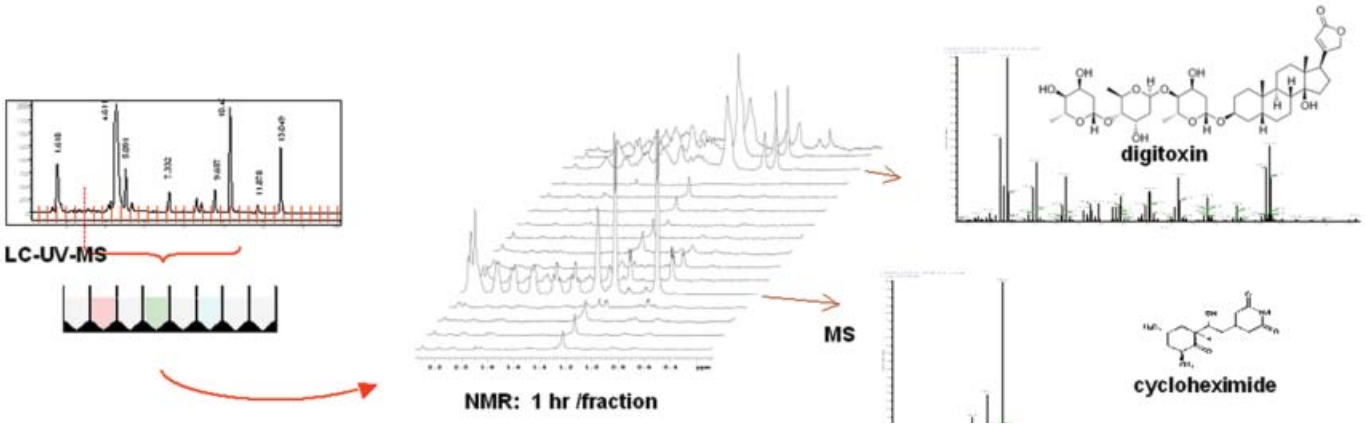
An LC-MS-NMR platform has been developed attempting to achieve the highest possible MS and NMR sensitivities, combining two recent innovations in these methods. For MS, a novel splitter can acquire nanoelectrospray MS from a 4 mm LC column and collect 99% of the eluant into fractions. For offline NMR analysis, an automated "segmented flow" microcoil NMR loading method (Microdroplet NMR) confines small samples to the NMR observed volume, providing several-fold higher sample efficiency than conventional flow-injection methods. For trace samples, microdroplet NMR at 500 MHz rivals the mass-sensitivity of a high-field cryoprobe. Performing NMR offline from LC-UV-MS accommodates the disparity between MS and NMR in their sample mass and time requirements. The simplicity of routinely collecting fractions into 96-well plates allows NMR data to be requested retrospectively, after review of LC-MS data. The approach may be used to obtain LC-NMR data to detect compounds with poor UV or MS signal, such as many glycans and lipids. Alternatively, NMR analysis time can be targeted to specific features of interest.



Loading of 1 µL dye sample into 10 µL microcoil NMR flowcell by segmented flow (bottom) avoids dilution seen in flow injection (top).

The limit of detection for LC-NMR analysis, where all collected fractions are analyzed using unattended automation at a throughput of 1 hr/well, was below 200 ng for 1D $^1$H spectra. In analysis of a single fraction overnight, good spectra could be obtained with 50 ng (500 Da) for 1D and 35 µg for HMBC, using a commercial microcoil probe. The system also showed excellent intra- and inter-detector reproducibility, with retention time RSD values less than 2%, and sample recovery on the order of 93 %.

When applied to dereplication of a cyanobacterial extract showing antibacterial activity, the platform was able to identify a number of previously-known metabolites, down to the 1% level, in a single 30 µg injection. Significantly, one metabolite could be established as previously unknown and worth further study.

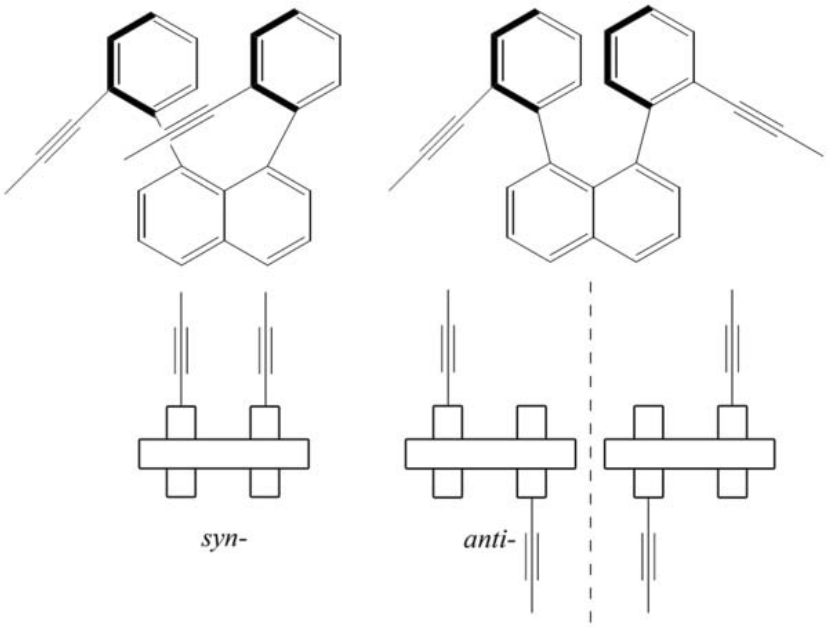


# Atropisomerism of 1,8-bis-(2-Propynyl-phenyl)-naphthalene

Jan Sýkora, Jan Storch, Jindřich Karban, Jan Čermák

Institute of Chemical Process Fundamentals of the ASCR, v. v. i.
Rozvojová 135, 165 02 Praha 6

1,8-Diiodonaphthalene (**1**) was coupled under Suzuki conditions to 2-propynyl-phenylboronic acid (**2**) to give 1,8-bis-(2-propynyl-phenyl)-naphthalene (**3**) in ~60% yield. The $^1$H NMR spectrum of **3** has revealed two set of signals in aproximate ratio 1:1 that can be atributed to *syn*- and *anti*- isomers of **3**. The restricted rotation around the single bond induces the chirality and give rise to two atropisomers at room temperature. Fortunately the partial separation is possible on the reversed phase HPLC even at ambient temperature and thus the individual $^1$H NMR spectra can be collected via on-flow LC-NMR technique. The stop-flow LC-NMR experiment has shown the slow *syn-anti* interconversion ($t_h$ = 24 h) in both cases.




Acknowledgements
Support from Czech Science Foundation (Grant no. 203/06/0738) and from the Ministry of Education, Youth and Sports of the Czech Republic (Grant no. LC06070) is gratefully acknowledged.

# Hyphenated LC-NMR at the Microscale

Andrew G. Webb

Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands

The small detection volumes associated with NMR microcoils make it a natural choice for coupling with many chemical microseparation techniques such as capillary liquid chromatography, capillary electrophoresis and capillary electrochromatography, and capillary isotachophoresis. In general, microseparation techniques enable faster analysis, higher concentration elution peaks and less chromatographic dilution than their larger scale counterparts. The use of small coils is ideally suited to small total sample amounts, present as relatively high concentrations in small volumes. The majority of hyphenated NMR detected studies carried out thus far have used cLC as the separation technique [1]. The mode of hyphenation consists of a long transfer capillary from the cLC separation column (which is usually magnetic), up through the magnet bore to the NMR probe. Typical transfer capillary inner diameters are narrow, between 50 and 100 μm, to minimize peak dispersion and broadening after the separation: flow rates are usually ~5 μL/min. cLC-NMR can be performed in either continuous-flow or stopped-flow mode. In order to increase the filling factor of the probes, and therefore the S/N of the NMR experiment, a "bubble cell" or flowcell is often used: the use of a tapered flowcell also helps considerably in the minimization of signal losses due to diffusion during data acquisition in stopped-flow mode.

Despite recent advances, the ultimate limiting factor remains signal-to-noise, which affects the trade-off between chromatographic resolution and NMR sensitivity, since the microcolumns may have to be overloaded. New directions in coil design, polymeric chromatographic materials and assessment of column integrity using MRI will be discussed.

[1] Albert K(Ed.), On-line LC-NMR and Related Techniques, Wiley, Chichester, NY, 2002.

# LC-NMR and LC-MS Applications to Characterize Microbial Transformation Products of Pollutants in Environmental Samples

A. Preiss[1], M. Elend[1], S. Gerling1, E. Berger-Preiss[1], A.-K. Reineke[2], and J. Hollender[2]

[1] Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany
[2] Institute of Hygiene and Environmental Medicine, RWTH Aachen, Germany

Over 300,000 sites which are contaminated by hazardous substances as a result of the high degree of industrialization have been registered in Germany alone, and these groundwater and soil contaminations can represent serious hazards for man and nature. Moreover, the parent pollutants originally released into the environment have undergone various chemical, photochemical, and microbiological transformation processes. As a result, a variety of new and unexpected compounds have been formed.

While the parent pollutants can be analyzed with optimized analytical methods (target analysis), transformation products and metabolites have often not been analyzed in the past, because they were unknown or not regulated and no effective analytical methods have been available for their determination. To improve risk assessment and insights into the environmental fate of pollutants, however, environmental samples should be characterized as comprehensively as possible, including identification of unknown compounds (non-target analysis). In this respect, the combined use of methods that provide a high separation efficiency and a maximum of structural information is most promising. For polar compounds these are hyphenated techniques such as LC-MS and LC-NMR.

Recent results obtained by using this analytical approach will be reported. Examples focus on polar transformation products of explosives and related compounds which are partly formed by microbial degradation. This type of compounds have been analyzed in ground water samples from various ammunition waste sites [1,2]. It will further be demonstrated how microbiological laboratory experiments and sophisticated analytical techniques can complement one another in investigating the environmental fate of pollutants: (i) Methyldihydroquinolinones, the formation of which was investigated in microcosm experiments with methylquinolines, could be detected for the first time in real ground water samples by using the LC-NMR technique [3]. (ii) The formation of unusual metabolites (derivatives of quinolinonyl acetic acid), which were first identified by LC-MS and LC-NMR in ground water samples from a mononitrotoluene waste site, could be verified later on by microcosm experiments.

References

[1] Alfred Preiss, Manfred Elend, Susanne Gerling, Simone Tränckner, Magn. Reson. Chem. 43, 736-746, 2005
[2] Alfred Preiss, Manfred Elend, Susanne Gerling, Edith Berger-Preiss, Klaus Steinbach, Anal Bioanal Chem 389, 1979-1988, 2007
[3] Anne-Kirsten Reineke, Alfred Preiss, Manfred Elend, Juliane Hollender, Chemosphere 70, 2118-2116 (2008)

# Cd-Induced Structure of a Small Metallothionein Determined by Capillary HPLC-NMR

D. Meißner[1], C. Mrestani-Klaus[1], M. P. Bayer[2], V. Friebolin[2], R. Golbik[1], D. Wesenberg[1], G.-J. Krauß[1], K. Albert[2]

[1]Martin-Luther-University Halle-Wittenberg, Institute of Biochemistry and Biotechnology, Kurt-Mothes-Str. 3, D-06120 Halle, Germany,
[2]Eberhard-Karls-University Tübingen, Institute of Organic Chemistry, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

A small fungal metallothionein MT1_NECLU is involved in heavy metal stress response of the aquatic hyphomycete *Heliscus lugdunensis* strain H4-2-4 [1]. This strain was isolated from a heavy metal polluted habitat [2]. MT1_NECLU (MT) contains 24 amino acids. All eight Cys as well as the C-terminal His might be involved in $Cd^{2+}$ complexation. On addition of $Cd^{2+}$ to MT, an induced structural change from random coil to a loop/turn was predicted for the protein by molecular modeling. As a result of these studies, the Cys residues coordinate two $Cd^{2+}$ in a tetrahedral manner. Additionally, far UV circular dichroism studies of the chemically synthesized protein were performed, indicating a loop/turn-like structure formation after $Cd^{2+}$ complexation. In order to obtain information on the conformation of the MT, some high-resolution $^1H$ NMR measurements at 400 MHz of the synthetic protein (apo-MT) were carried out. Under these conditions, the protein turned out to be unstructured.

Due to the low level of metallothionein induction and high sensitivity against oxygen, the hyphenation of capillary HPLC to microcoil NMR was used to further elucidate the structure of the MT. First investigations using capillary RP-HPLC showed the separation of apo-MT accompanied with glutathione and phytochelatin 2. In off-line microprobe 600 MHz NMR measurements of the synthetic MT1, differences in the 1D $^1H$ and 2D H,H-COSY spectra between the apo- and $Cd^{2+}$-MT could be detected. These differences clearly indicate a structural change of the protein conformation on addition of $Cd^{2+}$ and its complexation to the MT.

References

[1] Jaeckel, P., Krauss, G., Menge, S., Schierhorn, A., Rücknagel, P. & Krauss, G.-J. (2005). Cadmium induces a novel metallothionein and phytochelatin 2 in an aquatic fungus. Biochem. Biophys. Res. Commun. 333, 150-155.

[2] Sridhar, K. R., Krauss, G., Bärlocher, F., Wennrich, R. & Krauss, G.-J. (2000). Fungal Diversity in heavy metal polluted waters in Central Germany. Fungal Diversity 5, 119-129.

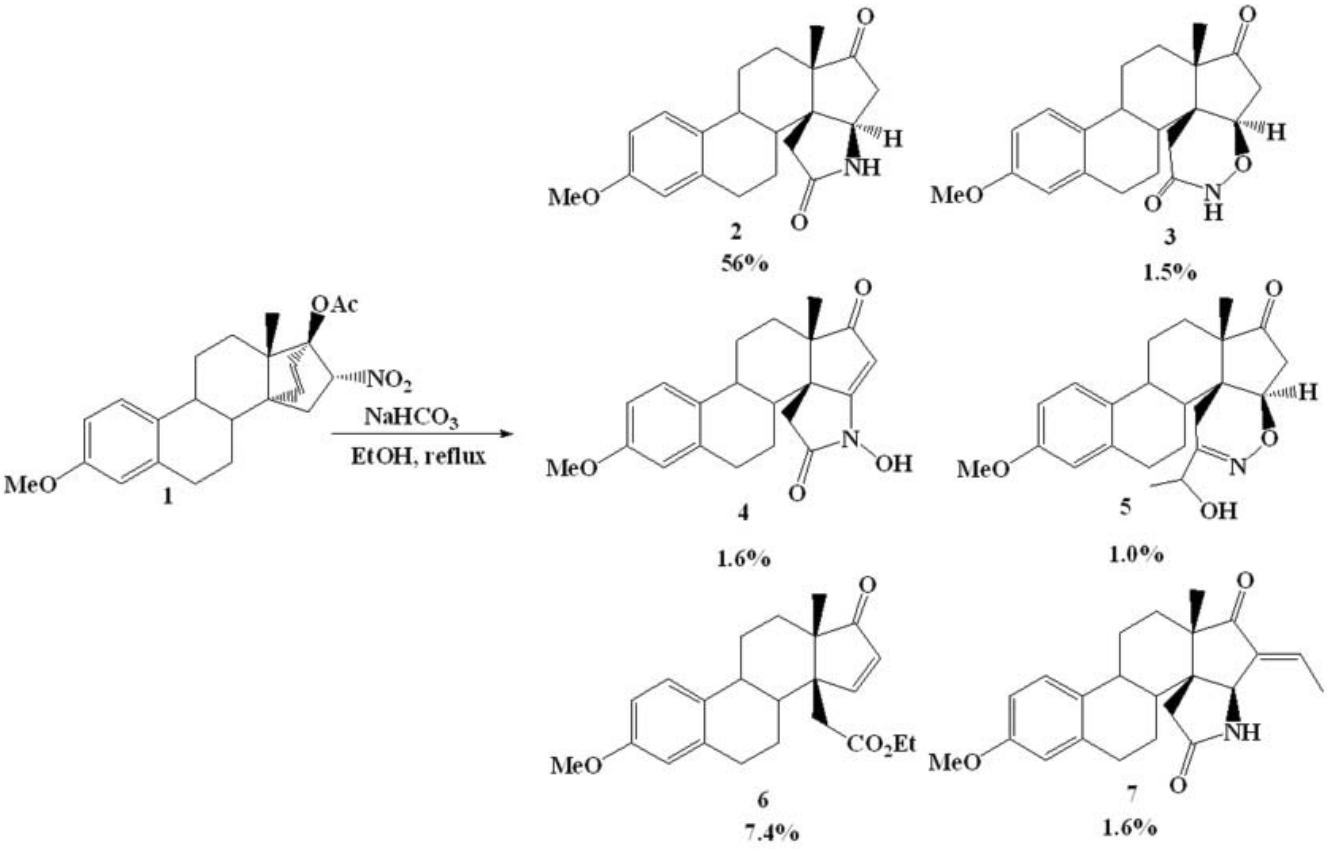
# HPLC-SPE-NMR Analysis of 3-Methoxy-14,17-etheno-16α-nitroestra-1,3,5(10)-trien-17β-yl Acetate Solvolysis Products

A. V. Baranovsky[1], D. A. Bolibrukh[1,2], V. A. Khripach[1], B. Schneider[2]

[1]Institute of Bioorganic Chemistry, Minsk, Belarus
[2]Max Plank Institute for Chemical Ecology, Jena, Germany

It was found that ethanol solvolysis of nitro steroid **1** in the presence of $NaHCO_3$ led to 3-methoxy-2'-oxopyrrolidino-[4',5':14β,15β]-estra-1,3,5(10)-trien-17-one **2** in 56% yield [1]. We undertook analysis of minor components in order to investigate mechanistic details of abnormal fragmentation pathways by use of LC-NMR techniques.

Due to the extraordinary sensitivity of the cryogenic NMR detector coupled with HPLC and SPE unit, we were able to isolate and characterize five compounds (**3-7**) beside the major in the reaction mixture.




The structures of all isolated compounds and a possible mechanism of transformation which includes formation of nitrile oxide as a first step of the reaction will be discussed.

[1] Steroids 2008; V 73; PP. 585–593.

# Integrated Use of NMR, Mass Spectroscopy and Chromatography in Metabolomics

M. Spraul

Bruker BioSpin GmbH

NMR and LC-MS are the main analytical tools in Metabolic Profiling and Fingerprinting. Integrated preparation, measurement and data-evaluation gives new possibilites for detection of metabolite networks, biomarker identification and structure elucidation, combining the strength of NMR (quantification, structural information, small molecule detection) with the advantages of MS (high sensitivity, sum formula generation, fragmentation). Examples for the use of such an integrated system are given and analysis methods discussed, using NMR and MS spectra simultaneously. Amongst those methods, heterospectroscopic covariance plays an important role, as it is possible to get information which NMR signals belong to a certain molecular ion of an LC-MS run without having to perform LC-NMR/MS separation.

It is also demonstrated, that using the NMR-MS covariance, NMR sensitivity can be enhanced substantially. All applications mentioned require high reporducability of the analytical methods used. Is shown how this is achieved, even using the integrated approach with NMr and LC-MS.

Having a combined high throughput-NMR and LC-MS systems also allows the expansion to LC-NMR/MS applications, especially in combination with a post column SPE unit. Such a combined system is especially useful to identify drug metabolites.

Examples on the usefulness of this approach are also presented.

# LC-(SPE)-(Cryo)NMR(/MS) as Routine Analysis Technique: Instrumentation and Software

Ullrich Braumann, Li-Hong Tseng, Manfred Spraul

Bruker Biospin, Rheinstetten, Germany

In LC-NMR a variety of possibilities to connect the chromatography system to the NMR spectrometer are available. The first and most straight forward is the direct connection of the LC system to a NMR flow probe by a stop-flow valve as it is available as BSFU (Bruker Stop Flow Unit). In a more complex working scheme, the peaks can be collected in storage loops without interrupting the chromatography and transferred from there independent from the chromatographic separation into a flow probe with a BPSU (Bruker Peak Sampling Unit).

As most advanced possibility the simple storage and transfer in loops can be replaced by a post column solid phase extraction process with the Bruker/Spark Prospekt2 LC-SPE system. The concentration effect on the SPE cartridges and the free selection of the NMR solvent makes it the method of choice in most cases. Besides the conventional transfer from the cartridges into a flow probe, the samples can be also measured in any standard NMR tube probe. The availability of NMR tubes in various diameters allows the adjustment of the tube volume to elution volume and the usage of LC-SPE-NMR as non-flow application. The collected peaks are transferred into NMR tubes of suitable size and measured in any NMR tube probe for tubes of 1.7 to 5mm, including CryoProbes.

The coupling of LC and NMR is accomplished with dedicated LC-NMR interfaces. Other instruments used in routine NMR spectroscopy and routine chromatography can be integrated into an LC-NMR setup as well. These are especially the mass spectrometry as a sensitive and specific detector for the LC and the CryoProbes with dramatically enhanced sensitivity for the NMR spectroscopy.

However the integration of these standard instruments into an LC-NMR setup must fulfil special requirements of the LC-NMR setup. For the connection of the MS an NMR-MS interface is required, that includes flow splitter, makeup pump and a valve for the connection. The conversion of a CryoProbe for flow applications is possible by the insertion of a CryoFit. CryoFits of different geometry allow the usage for LC-NMR, SPE-SPE-NMR or other flow applications.

The presentation will give an overview of the above mentioned possibilities of coupling LC and NMR in the different configurations. Application examples are given and the special advantages and limitations of the techniques are discussed.

# Structural Characterization of "Unknowns"; Hyphenated LC-MS/MS-NMR the Ultimate Tool!?

Maarten Honing and Martin Jaeger

Department of Medicinal Chemistry Oss, Schering Plough Research Institute,
P.O. Box 20, 5340 BH, Oss, The Netherlands

The characterization of molecular structures is one of the major responsibilities of analytical chemists within the Pharmaceutical R&D process. For the unambiguous assignment of the chemical structure, MS, NMR and other spectroscopic technologies are essential. The information needed is frequently "defined" in guidelines from regulatory agencies [1, 2] or scientific board of peer reviewed magazines. Nevertheless, they miss detailed information to what extend and in which manner (procedures to be followed) they need to be used. Hence, the "identification of "unknowns" at low concentration levels (typically ug/ml) in e.g. complex biological matrices can be rather troublesome.

Over the recent years, the hardware and software development within the so-called LC-MS/MS-NMR platforms has been tremendous, and its application has gained large interest from "routine" laboratories. Still, as has been documented in various reviews [3, 4], many parameters and practical obstacles need to be solved.

Here, after debating the "real" meaning of structural characterization [5, 6], some experiences with the application with on-line LC-NMR and "semi" on-line LC-MSMS and NMR are discussed, leading to some statements with regard to current status of the LC-MSMS-NMR art. Finally, a provocative answer on the postulated question is given.

[1] IUPAC Pure & Appl. Chem. 45, 11 (1976); IUPAC JOC 35, 2849 (1970).
[2] International Conference on Harmonization (ICH) guidelines
[3] M.V.S. Elipe, "Advantages and disadvantages of nuclear magnetic resonance spectroscopy as a hyphenated technique", Anal. Chim. Acta, 497 (2003) 1 – 25.
[4] Z. Yang, "Online hyphenated liquid chromatography – nuclear magnetic resonance spectroscopy mass spectrometry for drug metabolite and nature product analysis", J. Pharm. and Biomed. Analysis, 40 (2006) 516-527.
[5] H.D. Flack et al, absolute structure and absolute configuration, Acta Cryst., A55, 1999, 908
[6] T. Kuppens et al, Determination of the absolute configuration via VCD, DDT, 1 (2004), 269

# Practical Aspects of LC-NMR with Cryogenically Cooled Probes

Peter W.A. Howe

Bioscience, Syngenta, Jealott's Hill, Bracknell. RG42 6EY. U.K.

HPLC coupled NMR is an essential technique for the identification of pesticide metabolites and the 3-5-fold sensitivity gain offered by Cold Probe systems greatly extends the potential of the technique. We have identified three important practical considerations when using one.

First, failure of the compressed air supply to the probe will freeze LC-NMR samples in the flow-cell within 45 minutes, with potential for flow-cell breakage and leakage of samples into the probe. The risk posed by contamination should be considered when analysing radio labelled or toxic metabolites.

Second, decoupling during WET solvent suppression of organic solvents causes sample heating which shifts the frequencies of solvent resonances and reduces the effectiveness of suppression. Applying decoupling during calibration of the selective pulses used in WET avoids this problem and improves suppression.

Third, WET suppression can be further improved by applying volume selection. This is usually achieved using a 90° composite pulse, but this carries the risk of distorted integrals owing to incomplete relaxation. An alternative approach is to combine the FLIPSY method with an adiabatic 180º pulse. This not only provides volume selection and reduces the risk of incomplete relaxation, but further improves solvent suppression by exploiting the severe radiation damping observed with cold probes.

# Hyphenated NMR Methods for Natural Product Research

Jerzy W. Jaroszewski

Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences,
University of Copenhagen, Copenhagen, Denmark

Natural products continue to be a major source of chemical diversity for discovery of leads for new drugs. However, isolation and purification of natural products, the classical precondition for structure determination, is a major bottleneck in natural products research. The development of hyphenated NMR techniques has greatly increased the analytical capabilities for studies of extracts of natural origin. However, direct HPLC-NMR methods, employing NMR data accumulation from HPLC effluent, suffer from a number of limitations, often restricting their use to major extract constituents.

On the other hand, HPLC-SPE-NMR circumvents most of the problems associated with direct HPLC-NMR methods. The combined effect of analysis focusing and accumulation, use of deuterated solvents, and use of optimized detection cells enable acquisition of full sets of high-quality 2D NMR spectra with multiple chromatographic peaks, including minor peaks. This enables structure elucidation of complex natural products directly from crude extracts as rigorously as with milligram quantities of analytes in classical NMR tubes. Although the reversed-phase SPE adsorption mechanism is incompatible with very polar analytes, the technique has now been shown to work well with all major classes of secondary plant metabolites.

The productivity enhancements bought about by HPLC-SPE-NMR represents a paradigm shift with respect to how natural products research can and should be approached regarding structure elucidation. While isolation and purification of natural products remains to be a fundamental requirement in many areas, prior knowledge of structures of extract constituents will enable focusing of the isolation efforts on truly novel and interesting entities, leading to very significant productivity gains. In many situations, the exact knowledge of structures of extract constituents will be sufficient by itself, making isolation and purification with the aim of structure determination superfluous.

This lecture will focus on the performance of HPLC-SPE-NMR in natural product discovery, including optimization of SPE trapping and elution efficiency and sorbent screens. Examples of threshold-triggered and time-sliced SPE trapping as an aid in plant metabolomics will be demonstrated. Recent combination of HPLC-DAD/MS-SPE-NMR with CD spectroscopy afforded the solution to one of the major limitations of NMR hyphenation, i.e., the absence of chirality information.

# LC-(SPE)-NMR Applications in Chemical Ecology

B. Schneider

Max Planck Institute for Chemial Eckology, Beutenberg-Campus,
Hans-Knöll-Str. 8, 07745 Jena, Germany

LC-NMR in the stopped flow mode and LC-SPE-NMR have been used to identify ecologically relevant natural products. Phenolics and other natural products of various structural types were identified without isolation from crude extracts or prepurified fractions of different plant species. The target compounds are involved in plant defense against herbivores and pathogens or play a role in ecological interactions between different plant species.

In addition to identification and elucidation of stereochemical aspects, accumulation of ecologically interesting natural product in specific plant tissue and their formation by biosynthetic processes are in the focus of our interest. LC-NMR coupling methods were used to demonstrate the occurrence of specific natural products in extracts of special root and leaf tissue. Biosynthetic products formed from isotopically labelled precursors ($^{2}H$, $^{13}C$) were analyzed by LC-(SPE)-$^{1}H$ NMR, precursor – product relationships were established in the sub-µg scale, and the isotopomer composition of the products were determined.

Natural products formed by enzymes catalyzing individual steps of various plant biosynthetic pathways have been identified by LC-(SPE)-NMR using a Cryoprobe equipped with a CryoProbe Flow Conversion System (CryoFit) device.

[1] B. Schneider, C. Paetz, S. Opitz, D. Hölscher, Magn. Reson. Chem. 43, 724-728 (2005)
[2] B. Schneider, J. Gershenzon, G. Graser, D. Hölscher, B. Schmitt, Phytochemistry Reviews 2, 31-43 (2003)

# Phenolics of Norway spruce Colonized with *Heterobasidion annosum*

C. Paetz, B. Schneider

Max-Planck-Institute for Chemical Ecology, Jena, Germany

*Heterobasidion annosum*, a common pathogenic fungus in the Northern hemisphere, causes severe economical damage infesting timber forests. Colonizing the Norway spruce, the fungus induces a cascade of defense mechanisms in the living bark that are not understood in detail. Together with terpenes [1] and resin acids [2], phenolic compounds like stilbenes and lignans [3,4] were shown to be involved in the chemical response, but also higher condensed phenolics seem to play an important role [5]. In order to clarify the complex reactions induced upon infestation, a metabolic profile of infected wood zones was created. Hyphenated analytical techniques like HPLC-SPE-NMR and HPLC-MS were utilized. Structures of isolated compounds are introduced and interpreted regarding their possible role in plant defense.

[1] L. Zamponi, M. Michelozzi, P. Capretti; For. Path. 37 (2002), 243-250.
[2] M. Lindberg, L. Lundgren, R. Gref, M. Johansson; Eur. J. For. Path. 22 (1992), 95-106.
[3] J. Stenlid, M. Johansson; Eur. J. For. Path. 17 (1987), 217-226.
[4] S. Willföhr, J. Hemming, M. Reunanen, C. Eckemann, B. Holmbom; Holzforschung 57 (2003) 27-36.
[5] M. Johansson, L. Lundgren, F. Asiegbu; For. Path. 34 (2004) 197-210.

# LC-SPE-NMR Hyphenation and its Application to Phytochemical Analysis

Vassiliki Exarchou

NMR Center, University of Ioannina, Ioannina GR-45110, Greece

Natural environment and especially plants continue to provide a rich source of therapeutic agents. Characteristically, 67% of established drugs may be traced to natural origin and approximately half of 155 active small molecules identified from around the 1940s to date are either natural products or directly derived therefrom1. Phytochemical analysis is a challenge for scientists and the development of analytical methodologies for rapid screening of plant extracts and extensive analysis of each one of the components is essential. LC-SPE-NMR hyphenation is an analytical tool that is frequently used in phytochemical analysis, proving adequate data for structure elucidation2.

LC-SPE-NMR applications in analyzing the phytochemical content of several extracts of plants growing in Greece (*Origamun vulgare*, *Olea Europaea*, *Teucrium polium*, *Sideritis syriaca*, etc.) are going to be presented herein. Optimization procedures such as the effect of the stationary phase of cartridges, adequate SPE cartridge elution and the multiple trapping process in terms of a signal- to- noise ratio study in phenolic acids and flavonoids will also be discussed3. The use of cryogenic technology increases the overall system efficiency, and the implementation of mass spectrometry enhances the potency of LC-SPE-NMR. This powerful technology coverts the phytochemical analysis of natural products to a less time consuming task speeding up identification.

References

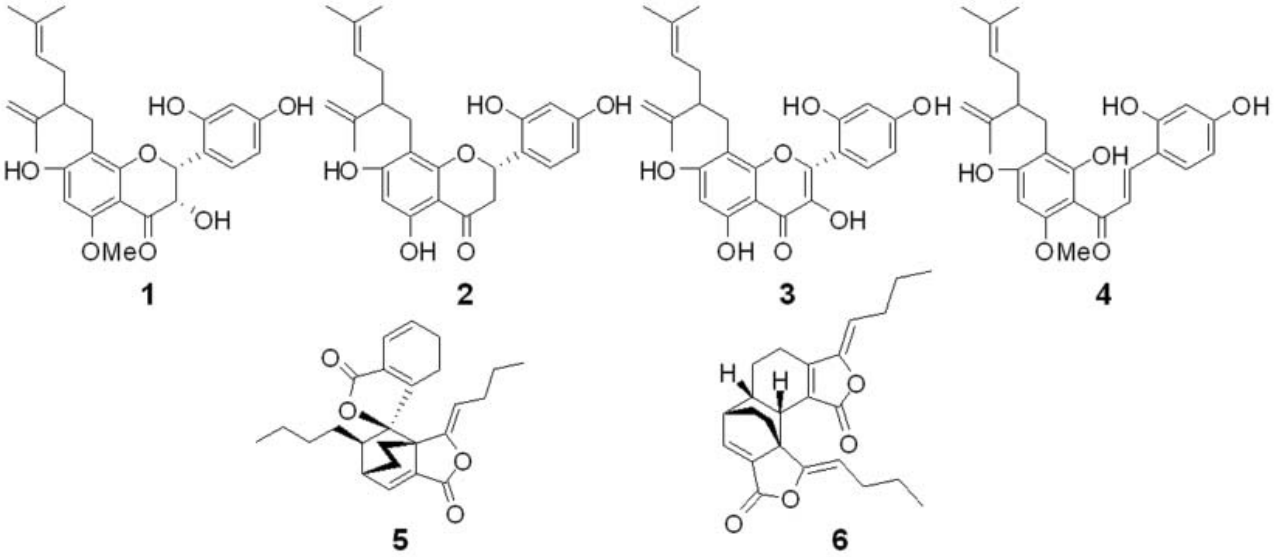
[1] Newman, D. J.; Cragg, G. M. J. Nat. Prod., 2007, 70, 461-477.
[2] Jaroszewski, J.W., Planta Med. 2005, 71, 795-802.
[3] Exarchou, V., Godejohann, M., van Beek, T.A., Gerothanassis, I.P., Vervoort, J. Anal. Chem., 2003, 75, 6288-6294.

# Profiling of two Chinese medicinal plants, *Sophora flavescens* and *Ligusticum chuangxiong*, by off-line LC-NMR and LC-MS

X.Z. Yang, I. Plitzko, M. Hamburger

University of Basel, Basel, Switzerland

For the identification of new natural product based lead compounds, we combine initial screening of extract libraries in a range of functional assays with HPLC-based micro-fractionation for activity profiling and chemical profiling by LC-MS and off-line NMR [1]. A 1-mm microprobe with z-gradient was used to measure one and two dimensional NMR spectra [2], and fractions were obtained by peak-based fractionation of a single injection of 40 mg of extract on a semipreparative (10 x 250 mm i.d.) HPLC column. The protocol was applied to two plants used in Traditional Chinese Medicine, *Sophora flavescens* and *Ligusticum chuanxiong*, to identify 32 compounds including **1-4**, and **5-6**, respectively, as structures with promising activity in a CNS-related target.




[1] O. Potterat, Chimia 2006, 60, 19.
[2] G. Schlotterbeck, A.Ross, R. Hochstrasser, H. Senn, Anal. Chem. 2002, 74, 4464.

# HPLC-NMR of Copolymers

Wolf Hiller[1], Harald Pasch[2]

[1]TU Dortmund, Faculty of Chemistry, Otto-Hahn-Str. 6, 44221 Dortmund, Germany
[2]German Institute for Poylmers, Schlossgartenstr. 6, 64289 Darmstadt,Germany

HPLC-NMR is a very powerful tool for the structure elucidation of complex mixtures. However, it is rarely used for polymers. Most papers are related to GPC-NMR. In these cases slow flow rates as well as deuterated solvents are often used. A comprehensive analysis of copolymers requests the separation according to different distributions. LAC (liquid adsorption chromatography) and GPC (gel permeation chromatography) are the most common used methods for separating polymers. Nevertheless, LCCC (liquid chromatography at critical conditions (or critical point of adsorption)) is one of the most powerful and sophisticated separation methods for polymers due to the capability of separating polymers according to their chemical heterogeneities. It will eliminate the influence of the molar mass.

The lecture will focus on different applications of HPLC-NMR of copolymers. The studies are related to the determination of the molar mass distribution (MMD) and the chemical composition distribution (CCD) of copolymers.

GPC-NMR is mainly applied to study the MMD. However, this method is usually only applicable to the investigation of polymers at ambient temperature. The new development of high temperature on-line GPC-NMR [1] allows the study at temperatures above 100°C. New applications for polyolefins will be presented. Especially, copolymers containing ethylene will be investigated.

Copolymers can also be analysed by LAC-NMR. In this case the copolymers are separated according to their chemical heterogeneity. Gradient chromatography is applied for the separation of random ploy[(styrene)-co-(ethyl acrylate)]s [2]. This method provides quantification for the chemical composition distribution without calibrations.

Block copolymers will be analysed by using LC-NMR at the critical point of adsorption. The power of LCCC-NMR will be demonstrated for the differentiation of mixtures of homo- and copolymers. In particular, the possibility to determine the true molar mass of block copolymers will be shown. Critical conditions for both monomer units of PS-*b*-PMMA block copolymers [3] allow a comprehensive analysis of the molar masses and chemical composition of the monomer units.

[1] W.Hiller, H.Pasch, T.Macko, J.Ganz, M.Hofmann, M.Spraul, U.Braumann, R.Streck, J.Mason, F. Van Damme: J. Magn. Res. 2006, 183, 309-321.
[2] I.Krämer, W.Hiller and H.Pasch: Macromol.Chem.Phys., 2000, 201, 1662-1666.
[3] W.Hiller, P.Sinha, H.Pasch: Macromol.Chem.Phys. 2007,208,1965-1978

# LC-29Si NMR Analysis of Silicon Copolymer Mixtures

Milan Kurfürst, Jan Schraml

Institute of Chemical Process Fundamentals of the ASCR, v. v. i.
Rozvojová 136, 16502 Praha 6, Czech Republic

Industrially important organosilicons, as they are often polymer mixtures, are usually characterized by gel permeation chromatography. NMR spectroscopy of whole mixtures provides structural information only at statistical level; only isolated compounds can be fully indentified by NMR. In that case, $^{1}$H NMR spectroscopy brings only limited information about silicon functional groups, while $^{29}$Si NMR has been recognized as one of the most effective tools for their analysis. Combination of both methods into one analytical technique, LC-{$^{29}$Si}$^{1}$H NMR has been proven suitable for silicon polymer analysis.

We have already shown [1] that LC-{$^{29}$Si}$^{1}$H NMR is applicable for analysis of mixture of homologous oligomers, that can be separated by chain length into individual components, each being fully characterized by $^{1}$H and $^{29}$Si NMR.

In analysis of copolymer mixtures, both chain length and functional group distribution is in focus of interest. In presented case of poly(dimethyl/methylphenyl)siloxanes, chemical shift of $^{29}$Si nuclei is dependent on substitution. Thus, it is possible to acquire stop-flow $^{29}$Si NMR spectra of individual fractions and then obtain statistical distribution of siloxane blocks within the fractions by pentad analysis. In addition, full Si-Si connectivity of low molecular weight compounds can be established by utilization of the $^{29}$Si,$^{29}$Si-INADEQUATE experiment.

Acknowledgement
Financial support from Czech Science Foundation (grant no. 203/08/P412, 203/06/0738 and 203/04/0120) is gratefully acknowledged.

[1] Blechta V., Kurfurst M., Sýkora J., Schraml J.: J. Chromatogr. A 1145, 175 (2007).

# 5. Abstracts of Posters

| No. | Author | Title |
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| 6 | Yang, Xinzhou | Profiling of Two Chinese Medicinal Plants, *Sophora flavescens* and *Ligusticum chuangxiong*, by Off-Line LC-NMR and LC-MS |
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# 1
# Practical Aspects of LC-NMR with Cryogenically Cooled Probes

Peter W.A. Howe

Bioscience, Syngenta, Jealott's Hill, Bracknell. RG42 6EY. U.K.

HPLC coupled NMR is an essential technique for the identification of pesticide metabolites and the 3-5-fold sensitivity gain offered by Cold Probe systems greatly extends the potential of the technique. We have identified three important practical considerations when using one.

First, failure of the compressed air supply to the probe will freeze LC-NMR samples in the flow-cell within 45 minutes, with potential for flow-cell breakage and leakage of samples into the probe. The risk posed by contamination should be considered when analysing radio labelled or toxic metabolites.

Second, decoupling during WET solvent suppression of organic solvents causes sample heating which shifts the frequencies of solvent resonances and reduces the effectiveness of suppression. Applying decoupling during calibration of the selective pulses used in WET avoids this problem and improves suppression.

Third, WET suppression can be further improved by applying volume selection. This is usually achieved using a 90° composite pulse, but this carries the risk of distorted integrals owing to incomplete relaxation. An alternative approach is to combine the FLIPSY method with an adiabatic 180º pulse. This not only provides volume selection and reduces the risk of incomplete relaxation, but further improves solvent suppression by exploiting the severe radiation damping observed with cold probes.

## 2
# On-line Coupling of Gas Chromatography to Nuclear Magnetic Resonance Spectroscopy: A New Method for the Analysis of Volatile Isomers

Holtin K., Kühnle M., Kreidler D., Schuler P., Schaal W., Schurig V. and K. Albert

University of Tübingen, Institute of Organic Chemistry,
Auf der Morgenstelle 18, D-72076 Tübingen, Germany

Nuclear magnetic resonance spectroscopy is the most powerful analytical method for identification of organic compounds and elucidation of their structure. Organic compounds usually are not obtainable in the pure state but they often appear as complex mixtures with other compounds. The combination of NMR detection and a separation technique avoids the problem of signal overlapping in the NMR spectra and enables the analysis of complex systems. The hyphenation of high-performance liquid chromatography and nuclear magnetic resonance spectroscopy has evolved into a versatile tool in the analysis of complex mixtures. Several applications of this technique are outlined in numerous articles which demonstrate the usefulness combining a separation method together with a structure elucidation technique. However, little is known about the coupling of GC with nuclear magnetic resonance spectroscopy. Whereas it would be promising to combine the high separation performance and the great selection of stationary phases of GC with the high information content of NMR. Here, we present the use of a custom-built solenoidal NMR microprobe with an active volume of 2 µl for a GC separation with a subsequent NMR detection of several compounds at 400 MHz in continuous and stopped-flow experiments.

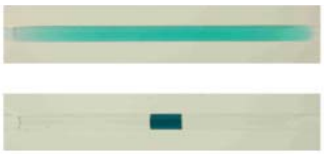
[1] Marc David Grynbaum, Diana Kreidler, Jens Rehbein, Armin Purea, Paul Schuler, Walter Schaal, Harri Czesla, Andrew Webb, Volker Schurig, and Klaus Albert, Anal Chem. 2007, 79, 2708-2713

# 3
# A High-Sensitivity LC-MS-NMR Approach Using 4 mm LC, Nanospray MS, and Segmented-Flow Microcoil NMR Automation

Yiqing Lin[1], Susie Schiavo[1], Paul Vouros[1] , Jimmy Orjala[2], and Roger Kautz[1]

[1]Barnett Institute of Chemical and Biological Analysis,
Northeastern University, Boston, MA 02115 USA
[2]College of Pharmacy, University of Illinois, Chicago IL 60612 USA

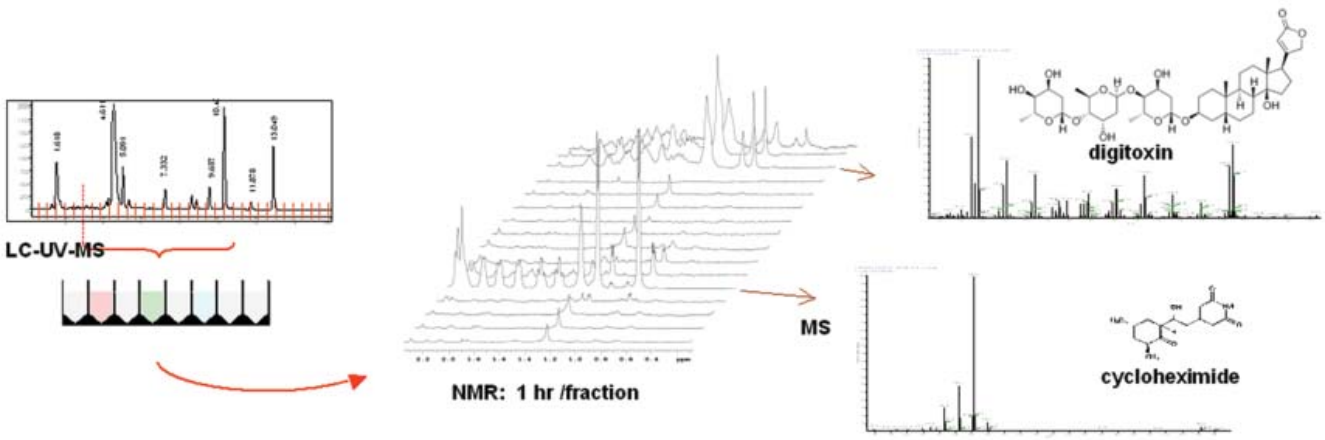
An LC-MS-NMR platform has been developed attempting to achieve the highest possible MS and NMR sensitivities, combining two recent innovations in these methods. For MS, a novel splitter can acquire nanoelectrospray MS from a 4 mm LC column and collect 99% of the eluant into fractions. For offline NMR analysis, an automated "segmented flow" microcoil NMR loading method (Microdroplet NMR) confines small samples to the NMR observed volume, providing several-fold higher sample efficiency than conventional flow-injection methods. For trace samples, microdroplet NMR at 500 MHz rivals the mass-sensitivity of a high-field cryoprobe. Performing NMR offline from LC-UV-MS accommodates the disparity between MS and NMR in their sample mass and time requirements. The simplicity of routinely collecting fractions into 96-well plates allows NMR data to be requested retrospectively, after review of LC-MS data. The approach may be used to obtain LC-NMR data to detect compounds with poor UV or MS signal, such as many glycans and lipids. Alternatively, NMR analysis time can be targeted to specific features of interest.



Loading of 1 µL dye sample into 10 µL microcoil NMR flowcell by segmented flow (bottom) avoids dilution seen in flow injection (top).

The limit of detection for LC-NMR analysis, where all collected fractions are analyzed using unattended automation at a throughput of 1 hr/well, was below 200 ng for 1D $^{1}$H spectra. In analysis of a single fraction overnight, good spectra could be obtained with 50 ng (500 Da) for 1D and 35 µg for HMBC, using a commercial microcoil probe. The system also showed excellent intra- and inter-detector reproducibility, with retention time RSD values less than 2%, and sample recovery on the order of 93 %.

When applied to dereplication of a cyanobacterial extract showing antibacterial activity, the platform was able to identify a number of previously-known metabolites, down to the 1% level, in a single 30 µg injection. Significantly, one metabolite could be established as previously unknown and worth further study.



# 4
# Metabolite Identification: Combining High Resolution UPLC-FTMS and LC-SPE-NMR in Phytonutrients Metabolomics

J.J.J. van der Hooft[1,2,3], S. Moco[1], R.C.H. de Vos[2,3,4], R.D. Hall[2,3,4], R.J. Bino[2,3,4,5], J. Vervoort[1,3]

[1]Laboratory of Biochemistry, Wageningen University, The Netherlands
[2]Plant Research International, Wageningen, The Netherlands
[3]Netherlands Metabolomics Centre, The Netherlands
[4]Centre for Biosystems Genomics, Wageningen, The Netherlands
[5]Laboratory for Plant Physiology, Wageningen University, The Netherlands

In biological research, there is an increasing demand for the unambiguous identification of metabolites. This work therefore aims at the development and optimization of protocols for rapid *de novo* identification of metabolites present in biological samples. LC-MS and NMR is a powerful combination in the unambiguous assignment of spectrometric and spectroscopic signals to unique metabolites. In our research phytonutrients will be measured before and after human consumption.

High resolution UPLC-FTMS will be used for obtaining accurate masses, isotopic patterns and $MS^n$ fragmentation trees. In this way, a partial identification can be obtained for each metabolite. For a selected number of chromatographic signals, LC-SPE-NMR will be used to capture and concentrate these metabolites for NMR analysis. In this procedure, UV-oriented trapping can be used for metabolites containing chromophores, or MS-based trapping for any ionizing metabolite. For each selected metabolite, the experimental data (LC, MS, NMR) will be combined with data present in available databases to achieve full identification. In addition, we aim to implement metabolite databases for automated or semi-automated identification, using standard compounds and newly identified metabolites.

Within this work, we will focus on the identification of low-abundant metabolites, such as secondary metabolites, present in both plants and human biofluids such as urine. In crop plants, phytonutrients can be present in the mM or µM range, while in urine the metabolites derived thereof are usually present in lower concentration ranges than in the original plant material. The unambiguous identification of these plant-derived metabolites in human body fluids is an important analytical challenge.

In summary, this work aims at creating protocols that can bridge the gap between putative and unambiguous identification of metabolites.

# 5
# NMR- and LC-MS-Based Metabolite Correlations in Tomato

S. Moco[1], J. Forshed[2], R.C.H. De Vos[3], R.J. Bino[3], J. Vervoort[1]

[1] Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands
[2] Karolinska Biomics Center, Karolinska Institutet, Stockholm, Sweden
[3] Plant Research International, Wageningen, The Netherlands

Nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LCMS) are frequently used as technological platforms for metabolomics applications. In this study, the metabolic profiles of ripe fruits from different tomato cultivars, including beef, cherry and round types, were recorded by both $^{1}$H NMR and accurate mass LC-quadrupole time-of-flight (QTOF) MS. Different analytical selectivities were found for both profiling techniques. In fact, NMR and LCMS provided complementary data, as the metabolites detected mostly belong to essentially different metabolic pathways.

Intra-method (NMR-NMR, LCMS-LCMS) and inter-method (NMR-LCMS) correlation analyses were performed, in order to identify metabolites from highly correlating signals. Many high correlations were found in the intra-method correlation analyses, revealing a large number of related signals belonging to the same metabolite and to chemically related metabolites. Inter-method correlation analysis produced highly informative and complementary information for the identification of metabolites, even in de case of low abundant NMR signals.

The applied approach appears to be a promising strategy in extending the analytical capacities of these metabolomics techniques with regard to the discovery and identification of biomarkers and yet unknown metabolites.

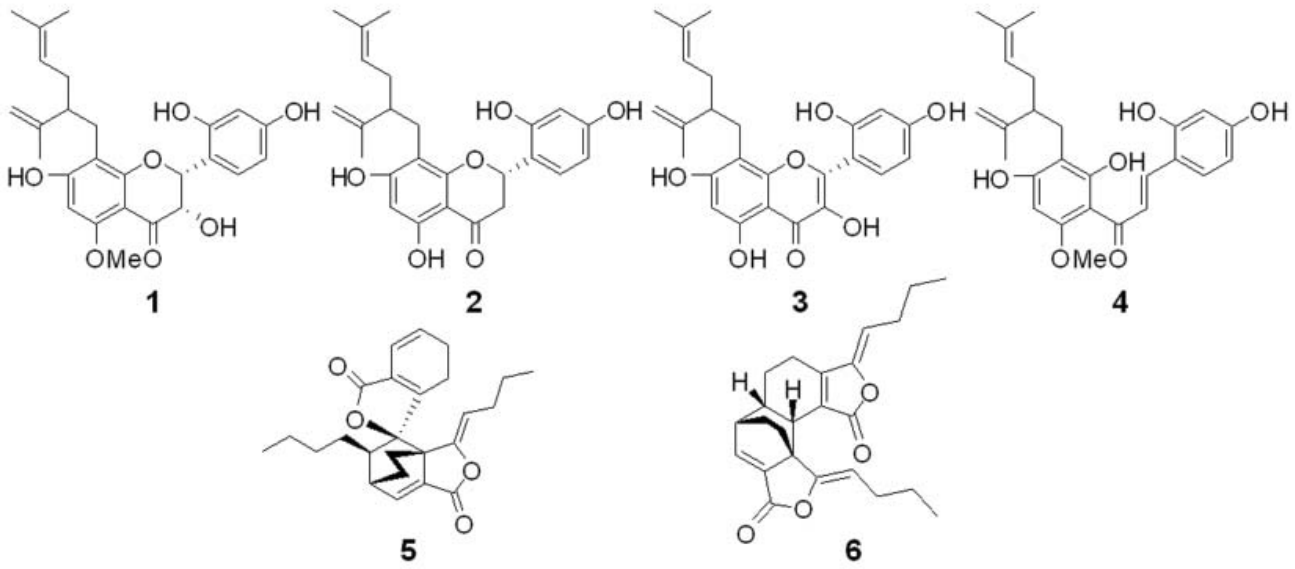
**6**

# Profiling of two Chinese medicinal plants, *Sophora flavescens* and *Ligusticum chuangxiong*, by off-line LC-NMR and LC-MS

X.Z. Yang, I. Plitzko, M. Hamburger

University of Basel, Basel, Switzerland

For the identification of new natural product based lead compounds, we combine initial screening of extract libraries in a range of functional assays with HPLC-based micro-fractionation for activity profiling and chemical profiling by LC-MS and off-line NMR [1]. A 1-mm microprobe with z-gradient was used to measure one and two dimensional NMR spectra [2], and fractions were obtained by peak-based fractionation of a single injection of 40 mg of extract on a semipreparative (10 x 250 mm i.d.) HPLC column. The protocol was applied to two plants used in Traditional Chinese Medicine, *Sophora flavescens* and *Ligusticum chuanxiong*, to identify 32 compounds including **1-4**, and **5-6**, respectively, as structures with promising activity in a CNS-related target.




[1] O. Potterat, Chimia 2006, 60, 19.
[2] G. Schlotterbeck, A.Ross, R. Hochstrasser, H. Senn, Anal. Chem. 2002, 74, 4464.

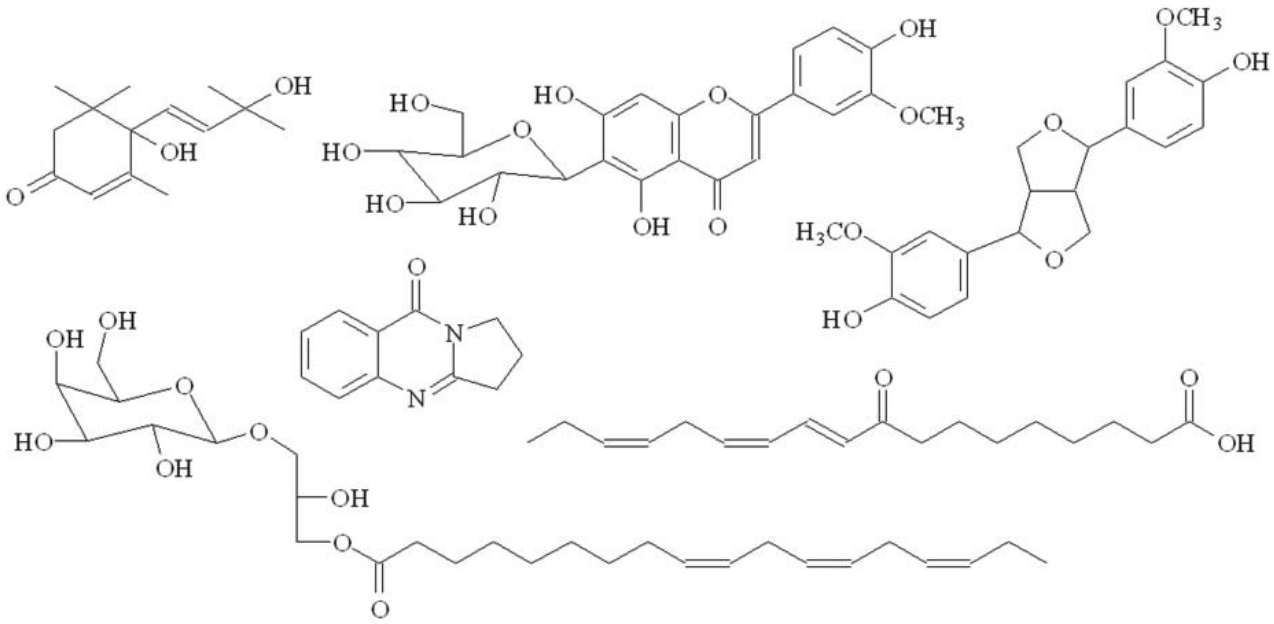
# 7
# A Comprehensive Metabolite Profiling of *Isatis tinctoria* Leaf Extracts

T. Mohn, I. Plitzko, M. Hamburger

Institute of Pharmaceutical Biology, University of Basel,
Klingelbergstrasse 50, CH-4056 Basel, Switzerland

Woad (*Isatis tinctoria* L., Brassicaceae) is an ancient indigo dye and anti-inflammatory medicinal plant, which has been used and cultivated in Europe since antiquity. The anti-inflammatory potential of lipophilic leaf extracts was confirmed in a broad-based pharmacological profiling, in various animal models [1], and in a clinical pilot study [2]. Tryptanthrin, indirubin, an indolin-2-one derivative, and γ-linolenic acid were identified as pharmacologically active compounds inhibiting COX-2, 5-LOX, the expression of the inducible nitric oxide synthase, human neutrophil elastase, and the release of histamine from mast cells.

To further characterize the pharmacologically active extracts, we carried out a compre-hensive metabolite profiling with the aid of online spectroscopic measurements (HPLC coupled to PDA, ELSD, APCI- and ESI-MS, and HRESI-MS). Off-line semi-preparative HPLC-NMR analysis was used for structure elucidation of some constituents. For this purpose 15 mg of extract was injected onto a semi-preparative HPLC column (150 x 10 mm). Peak based collection was monitored at 210 nm. Mobile phase in fractions was removed by parallel evaporation, and samples were submitted to NMR analysis (Bruker Avance III 500 MHz, 1 mm TXI probehead, active volume 5 µl). So far, more than 60 compounds belonging to various structural classes such as alkaloids, flavonoids, fatty acids, porphyrins, lignans, carotenoids, glucosinolates and cyclohexenones have been unambiguously identified, and tentative structures proposed for additional compounds.



[1] M.C. Recio, M. Cerda-Nicolas, O. Potterat, M. Hamburger, J.L. Rios, Planta Med. 2006, 72, 539.
[2] C. Heinemann, S. Schliemann-Willers, C. Oberthür, M. Hamburger, P. Elsner, Planta Med. 2004, 70, 385.

# 8
# LC-NMR/LC-MS Analysis of Berberine Alkaloid Metabolites in Cell Cultures

Dong-Ung Lee[1], Kinuko Iwasa[2]

[1]Division of Bioscience, Dongguk University, Gyeongju, Republic of Korea
[2]Kobe Pharmaceutical University, Kobe, Japan

Protoberberine alkaloids differ from each other in the number and placement of various oxygen functions on the aromatic rings. The two oxygenation patterns most frequently are oxygen atoms at carbons 2,3,9,10 and 2,3,10,11. The former is the most commonly occurring type, while the latter has been labeled "pseudoprotoberberine", and is not as widespread in its occurrence [1]. Some representatives of the 2,3,10,11-oxygenated alkaloids display higher activity in some biological testa than the corresponding 2,3,9,10-substituted alkaloids [2]. While the biosynthetic conversion of the 2,3,9,10-oxygenated protpberberines into other alkaloidal types, such as the protopines, benzophenanthridines, rhoeadines, benzindanoazepines, and spirobenzylisoqyinolines has been demonstrated [3,4], no studies on the biosynthesis of 2,3,10,11-oxygenated protoberberines have been presented, in spite of their occurrence in some plant species including Corydalis species [1]. Now the metabolism of 2,3,10,11-oxygenated protoberberine alkaloids was studied in cell cultures of Corydalis species. Without prior isolation, the structures of the matabolites were determined by LC-MS and LC-NMR snalysis [5]. Tetrahydropseudocoptisine a-N-metho salt, pseudoprotopine, and pseudomuramine were identified for the first time, and preliminary evidence for matabolic pathways to the formation of these alkaloids were obtained.

References

[1] Preninger, V. et al. (1986) The Alkaloids, Chemistry and Pharmacology, Vol. 29, Academic Press, New York, pp. 1-98.
[2] Iwasa, K. et al, (1999) Eur. J. Med. Chem., 34, 1077-1083.
[3] Zenk, M.H. (1994) Pure Appl. Chem., 66, 2023-2028.
[4] Iwasa, K. et al., The Alkaloids, Chemistry and Biology, Vol. 46, Academic Press, San Diego, pp. 273-346.
[5] Wolfender, J.L. et al., Phytochem. Anal. 12, 2-22.

# 9
# Identification of Indole/Flavonoid Alkaloids from Flowers of Papaver Species by the Combined Use of Chromatographic and Spectroscopic Techniques

Evangelos C. Tatsis, Bernd Schneider

Max Planck Institute for Chemical Ecology, Jena, Germany

Plants are an inexhaustible resource of natural products with biological and medical interest. Although most natural product studies are still about drug discovery, increasing information about the role of natural products in chemoecological interaction between different organisms and with their environment and involvement in regulation of physiological processes is available. Plants usually produce so-called "secondary" compounds in response to their interactions with the environment or their needs to protect themselves against herbivores and pathogens.

Yellow colour of Iceland poppy's (*Papaver nudicaule*) flower petals is attributed mainly to the occurrence of nudicaulins. Those alakoloids from *P. nudicaule* contain an indolic substructure together with a $C_6$-$C_3$-$C_6$ substructure similar those of flavonoids and chalcones. Although nudicaulin aglycons represent a unique structure, they can be classified into the wider category of indole alkaloids.

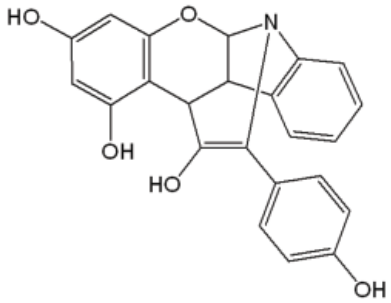



Figure 1. Aglycon structure of nudicaulins

Yellow coloured flower plants the Papaveraceae family are investigated for the occurrence of nudicaulins. A dereplication strategy based on LC-SPE-NMR technique employed to find out new nudicaulin-type compounds in extracts from flower petals. In extract from yellow flower petals of *Meconopsis cambrica* (Welsh poppy) six compounds with nudicaulin skeleton structure were identified, with different glycosidic substitution.

References

[1] Schliemann W., Schneider B., Wray V., Schmidt J., Nimtz M., Porzel A., Böhm H. Phytochemistry 2006, 61, 191-201.

# 10
# Profiling of a *Piper nigrum* Extract by LC-MS and Semipreparative HPLC Offline NMR

J. Zaugg, I. Plitzko, M. Hamburger

Institute of Pharmaceutical Biology, University of Basel,
Klingelbergstrasse 50, CH-4053 Basel, Switzerland

HPLC based activity profiling is an effective approach to find new lead structures among natural products [1]. Micro-fractionation is usually supported by sensitive on-line detectors (PDA and MS), but structure elucidation typically requires NMR data which in this scale are difficult to obtain. We present here a convenient procedure for rapid structure elucidation of extract constituents by means of Microprobe-NMR.

In a screening of our in-house liquid library, an ethyl acetate extract from *Piper nigrum* fruit showed promising activity. Activity profiling revealed an active fraction, which according to on-line spectroscopic data (UV-vis, (HR)-MS) contained piperine as main and other amides as minor constituents. Off-line semi-preparative HPLC–NMR analysis was carried out for structure elucidation of extract constituents. For this purpose 5 mg of extract were fractionated by HPLC in semi-preparative scale. Mobile phase was removed by parallel evaporation, samples were resolved in 10-20 µl deuterated solvent and Microprobe-NMR analysis was performed. (Bruker Avance III™, 500MHz, 1mm TXI probe with z-gradient, active volume 5 µl). The structures of piperine and a series of other compounds were elucidated by $^{1}$H-, COSY- and HSQC-NMR experiments.

The limit of detection for a qualitative off-line semi-preparative HPLC-NMR analysis was assessed via dilution series of piperine. $^{1}$H-NMR experiments of 500 to 0.5 µg piperine showed the minimum amount of a small molecule to be identified by this approach is approx. 5 µg, and 10-50 µg are required for recording homonuclear 2D-NMR spectra within a reasonable time.

[1] M. Hamburger and O. Potterat, Current Organic Chemistry, 2006, 10, 899 920.

# 11
# Application of Modern Hyphenated Techniques for Structure Elucidation of Carotenoids in Pepper

Marc Bayer, Volker Friebolin, Karsten Holtin, Klaus Albert

University of Tuebingen, Institut of Organic Chemistry,
Auf der Morgenstelle 18, D-72076 Tuebingen, Germany

Carotenoids are responsible for the common orange color of carrot roots as well as the bright orange, red, and yellow colors of plant organs such as fruits and flowers in other species. They represent an important class of widely distributed natural pigments and play an important role in protecting the photosynthetic systems in plant tissue against photo-oxidative processes.

Numerous epidemiologic studies have shown that individuals consuming a relatively large quantity of carotenoid-rich fruits and vegatables have a decreased risk of degenerative diseases like certain kinds of cancer, coronary heart disease and cataracts. There are several dozen carotenoids available in food and most of these carotenoids have antioxidant activity.

Due to the fact that carotenoids are light-, heat and oxygen-sensitve, their processing has to be gentle and rapid to avoid degradation and isomerisation prior to their analysis. Therefore, the carotenoids and their esterified analoga were extracted by Matrix-Solid-Phase-Dispersion (MSPD) and analyzed by using the combination of the enhanced shape selectivity of C30 phases with RP-HPLC (DAD) and atmospheric pressure chemical ionisation mass spectrometric detection (RP HPLC-APCI-MS). Esterified fatty acids of the hydroxylated carotenoids (Xanthophylls) were determined after basic catalyzed transesterification via gas chromatography-mass spectrometry (GC-MS).

HPLC-MS and GC-MS are useful for the identification of different carotenoids, but only the results of HPLC coupled to NMR (HPLC-NMR) allows the unequivocal structure determination of carotenoids.

To prove the utilization of those hyphenated techniques to those biological systems, capsanthin extracted from red pepper was used as model compound for stucture elucidation of the different carotenoid isomer

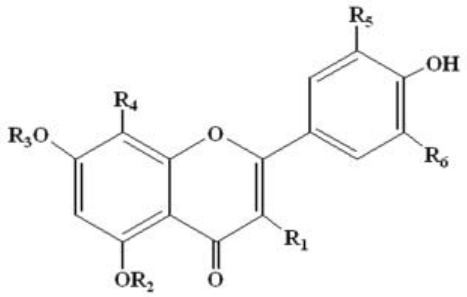
# 12
# Flavonoids from some Mongolian Medicinal Plants

Odontuya Gendaram.[1], Sukhkhuu B.[1], Ryu Sh Y.[2], Kim Y S.[2]

[1]`Natural Product Chemistry Laboratory, ICCT, MAS 4th building,13330 Peace ave., Ulaanbaatar, Mongolia
[2]Medicinal Plant Phytochemistry Laboratory, KRICT, Daejeon, 305-600, Korea

A total of 107 crude ethanol extracts of 95 medicinal plants from the Mongolian flora have been screened for their biological activities as acetylcholinesterase, poly(ADP-ribose) polymerase, malondialdehyde inhibitory effects and *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Micrococcus luteus* antimicrobes activity. From all screened plants *Paeonia anomala* L, *Myricaria alopecuroides* Schrenk, *Dasiphora fruticosa* Rydb, *Sedum hybridum* L, *Sedum aizoon* L, *Cotoneaster mongolica* Fisch, *Cimicifuga foetida* Maxim, *Dracocephalum grandiflorum* Bunge, *Schizonepeta annua* Schischk, *Pyrola incarnata* Freyn, *Chryzanthemum zawadskii* Herb and *Comarum salesovianum* Aschers exhibited potential activities.

Preliminary phytochemical analysis indicated phenolics and flavonoids are contained mainly in these plant species. In particular, they concentrated in polar fractions as ethylacetate and n-butanol, in turn these fractions possessed the best biological activities. Biological activity guided purification of the ethylacetate fractions of *Sedum hybridum*, *Myricaria alopecoroides*, *Dasiphora fruticosa* and *Cotoneaster mongolica* by column chromatography with different kinds of absorbents as Sephadex LH-20, MCI gel CHP 20P and Sepra C18-E eluting with 100% $H_2O$ → 100% MeOH obtained 13 flavonol derivatives, one flavone glycoside, one isoflavonoid and 3 aromatic substances. Their molecular structures were elucidated by UV, $^1H$, $^{13}C$ NMR and MS analysis as quercetin (**1**), kaempferol (**2**), herbacetin-8-O-xylopyranoside (**3**), gossypetin-8-O-xylopyranoside (**4**), myricetin-3-O-α-L-arabinofuranoside (**5**), quercetin-3-O-α-L-arabinofuranoside (**6**), quercetin-3-O-α-L-arabinopyranoside (**7**), quercetin-3-O-β-L-galactopyranoside (**8**), quercetin-3-O-(6''-galloyl)-β-L-galactopyranoside (**9**), quercetin-5-O-α-L-arabinopyranosi-de (**10**), kaempferol-5-O-β-D-glucopyranoside (**11**), kaempferol-3-O-α-L-rhamnopyranosi-de (**12**), kaempferol-3-O-β-D-(6"-p-coumaroyl)-glucopyranoside (**13**), apigenin-7-O-β-D-(6"-p-coumaroyl)-glucopyranoside (**14**), 5-hydroxy-4'-methoxyisoflavone-7-O-β-glucopyra-noside (**15**), gallic acid (**16**), 6-O-galloylarbutin (**17**) and ethylgallate (**18**), respectively.




**1.** $R_2$=$R_3$=$R_4$=$R_6$=H; $R_1$=$R_5$=OH;
**2.** $R_2$=$R_3$=$R_4$=$R_6$=H; $R_1$=OH;
**3.** $R_2$=$R_3$=$R_4$=$R_5$=H; $R_1$=OH; $R_4$=O-xylopyranose
**4.** $R_2$=$R_3$=$R_6$=H; $R_1$=$R_5$=OH; $R_4$=O-xylopyranose
**5.** $R_2$=$R_3$=$R_4$=H; $R_5$=$R_6$=OH; $R_1$=O–α-L-arabinofuranose
**6.** $R_2$=$R_3$=$R_4$=$R_6$=H; $R_5$=OH; $R_1$=O–α-L-arabinofuranose
**7.** $R_2$=$R_3$=$R_4$=$R_6$=H; $R_5$=OH; $R_1$=O–α-L-arabinopyranose
**8.** $R_2$=$R_3$=$R_4$=$R_6$=H; $R_5$=OH; $R_1$=O–β-L-galactopyranose
**9.** $R_2$=$R_3$=$R_4$=$R_6$=H; $R_5$=OH; $R_1$=O-(6"-galloyl)-β-L-galactopyranose
**10.** $R_3$=$R_4$=$R_6$=H; $R_1$=$R_5$=OH; $R_2$=α-L-arabinopyranose
**11.** $R_3$=$R_4$=$R_5$=$R_6$=H; $R_1$=OH; $R_2$=β-D-glucopyranose
**12.** $R_2$=$R_3$=$R_4$=$R_5$=$R_6$=H; $R_1$=O–α-L-rhamnopyranose
**13.** $R_2$=$R_3$=$R_4$=$R_5$=$R_6$=H; $R_1$=O–(6"-*p*-coumaroyl)-β-D-glucopyranose
**14.** $R_1$=$R_2$=$R_4$=$R_5$=$R_6$=H; $R_3$=(6"-*p*-coumaroyl)-β-D-glucopyranose

# 13
# LC-DAD-SPE-NMR and LC/MS Hyphenated Techniques for the Identification of the Major Constituents of *Ligustrum lucidum*

A. Nerantzaki[1], V.G. Kontogianni[1], V. Exarchou2, A. Troganis[3] and I.P. Gerothanassis[1]

1Department of Chemistry, University of Ioannina, Ioannina GR-45110, Greece.
[2]NMR Center, University of Ioannina, Ioannina GR-45110, Greece.
[3]Department of Biological Applications & Technology, University of Ioannina, Ioannina GR-45110, Greece

Over the last two decades there has been an increasing interest in the investigation of natural products in order to unveil new pharmacologically active substances. *Ligustrum lucidum* Ait., a member of the *Oleaceae* family, is widely distributed providing an easily available natural material of low cost. In traditional medicine, extracts from different plant parts (leaves, fruits, and flowers) of *Ligustrum* spp. was considered to serve functions of nourishing liver, kidney and brightening eyes. Studies in modern medicine [1] showed that its constituents are effective immunostimulants and excellent biological antioxidants. As a part of our search on bioactive compounds derived from plants we report here the chemical characterization of Greek *Ligustrum lucidum* leaves extracts using hyphenated techniques [2].

Fresh leaves of *Ligustrum lucidum* grown in Greece were extracted with solvents of increasing polarity and the respected fractions were collected. The nine (9) extracts under study were initially screened for their antioxidant activity, using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH●) scavenging assay and their total phenol and flavonoid content was measured colorimetrically. In a second step the extracts were further analysed by HPLC-ESI-MS to identify their major components. Based on the results of antioxidant activity and phenolic content and chromatographic profile, the ethyl acetate extract was selected for HPLC-DAD-SPE-NMR subsequent analysis. The results showed that *Ligustrum lucidum* extracts are rich in antioxidants such as secoiridoids and flavonoids in both free and glucosidic form.

References

[1] A. Romani et al., J. Agric. Food Chem. 2000, 48, 4091-4096
[2] V. Exarchou et al., J. of Chromatography A 2006, 1112, 293-302

# 14
# *Arrabidaea chica* Verlot Red Dye Extraction by Biotechnological Techniques Monitored by ESI-MS(/MS)

M. A. Foglio[1,2] , C , D. Taffarello [1,2]*, E. C. Cabral [3], C.L.Queiroga.[2,3], R. R. Catharino[3], P. M. Magalhães[2], G. M. Figueira2, M.C.T.Duarte[2],A. N. Santos[2], A. Alves[2], I. M. O. Sousa[2], R.A. F. Rodrigues[2], M. Jorge[2], A. Ruiz2, J. E. Carvalho[2], M. N. Eberlin[3]

[1] Biotechnology Department, ICB, USP, São Paulo-SP, Brazil
[2] CPQBA, UNICAMP Campinas-SP, Brazil
[3] Thomson Mass Spectrometry Laboratory/IQ - UNICAMP Campinas-SP, Brazil

*Arrabidaea chica* (H&B) Verlot, popularly known as Pariri, is a native tropical American vine, with pink flowers. Amazon Indians use a red pigment extracted from this species, carajurin (6,7-dihydroxy-5,4'-dimethoxy-flavilium), as tattooing agent, adstringent and for treatment of various diseases. Recently, dyes derived from natural sources have emerged as an important alternative to synthetic ones. This genus is a source of anthocyanins, flavonoids and tannins. Other studies undertaken by our group revealed that *A. chica* produces a high content of glycosylated anthocyanins. Kim *et al.* (2005) proposed a method of enzymatic pigment production based on the introduction of hydrolytic enzymes prior to the usual extraction process, improving pigment extractable yields. Therefore in accordance to this author we proposed to study the influence of the red color dye extraction with enzymatic incubation of plant material prior to extraction procedures.

Climate and regional factors have shown to significantly affect the production of chemical compounds. The red color extracts chemical composition among plant species obtained throughout Brazil (North, South and West of Brazil) and introduced at CPQBA-experimental field under the same agronomic and climate conditions without and with enzyme treatment prior to extraction process were evaluated monitored by screening for anthocyanidins content by ESI-MS/MS. The dry leaves were extracted with MeOH/citric acid 0.3% solution (3X) during periods of 1,5 hours. These extracts were dissolved in MeOH/$H_2O$ (1:1) + 7% formic acid and infused in (Q-Tof Micromass). The variety that produced the best color and healing profile was variety 06 with high Carajurin (*m/z* 299), carajurone (6, 7, 3'-trihydroxy-5-metoxy-flavinium) and 6, 7, 3', 4'-tetrahydroxy-5-metoxy-flavinium, m/z 285 and 301 respectively intensities. In extracts of different geographic regions (variety 03 and 05) Carajurin (*m/z* 299) ions were not detected in significant amounts without a red color profile extract. Despite the final extraction yield without enzyme treatment were higher (24.28%) compared to the enzyme treated material (19.03%), the Carajurin (*m/z* 299), carajurone (6, 7, 3'-trihydroxy-5-metoxy-flavinium) and 6, 7, 3', 4'-tetrahydroxy-5-metoxy-flavinium ratio was enhanced in the latter case as determined by electrospray ionization mass spectrometry with tandem mass spectrometry by direct infusion.

Key words
*Arrabidaea chica*, dye extraction, biotechnology, ESI-MS(/MS)

[1] KIM, D.H. et al. Journal of Agricultural and Food Chemistry. 53 (2005).

# 15
# Phenolic Fraction of Olive Oil: Studied over Decades but Still Quite Unknown. New Platforms to Face the Characterization of this Family of Compounds: HPLC-NMR and 2D HPLC-CE-MS

A. Carrasco-Pancorbo[1], R. García-Villalba[1], A. M. Gómez-Caravaca[1], A. Bezerra Schefer[2], M. Spraul[2], T. Parella[3], A. Segura-Carretero[1], A. Fernández-Gutiérrez[1]

[1]Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Granada, Spain
2Bruker BioSpin GmbH, Rheinstetten, Germany
[3]Servei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona, Barcelona, Spain

The importance of virgin olive oil is attributed, among other factors, to its richness in phenolic compounds, which act as natural antioxidants and may contribute to the prevention of several human diseases. They have been determined over decades by HPLC and GC, and more recently by CE as well, being classified in simple phenols, phenolic acids, lignans, secoiridoids and flavonoids. Although excellent progress has already been made, it is expected that the use of different methodologies of potent techniques coupled to reliable and sophisticated detectors will become more common in the near future; there are still many "unknown" compounds present in the polar fraction of olive oil and their determination could be very important to understand better some characteristics of olive oil (antioxidant activity, anticancer activity, etc).

A possible approach to face this problem could involve the combination of the advantages of NMR spectroscopy with those of HPLC. The separation was carried out by using a 25 cm x 3 mm i.d., 5 µm, Phenomenex C-18 reverse phase column with water + 0.1% acetic acid and ACN + 0.1 % acetic acid the mobile phases. In this case, we tried to increase the sensitivity by adding a postcolumn SPE system to replace loop collection (Prospekt II peak-trapping unit) equipped with 10 x 2 mm Hysphere trap cartridges. UV detection controlled the collection of the different fractions in the SPE cartridges, they were then dried with $N_2$ and transferred to the NMR with ACN-d.

The previously isolated fractions (SPE Prospekt II peak-trapping unit) were analyzed by CE-ESI-TOF MS afterwards. It has been demonstrated that in instances in which an HPLC method does not provide enough resolution, CE with its flexible experimental conditions should be assayed as a complementary second choice technique. With the CE analyses we added a new dimension of separation (based on completely different principles) after HPLC separation; moreover, MS will separate the analytes depending on the mass/charge ratio.

Electrophoretic separation was carried out using an aqueous CE buffer system consisting of 40 mM ammonium acetate at pH 9.5, and a sheath liquid containing 2-propanol/water 50:50 (v/v) in a capillary of 85 cm and 50 µm of i.d..

# 16
# An HPLC-SPE-NMR Optimization Study and its Application to *Sideritis syriaca* (Greek Mountain Tea) Extracts

V. Goulas[1], V. Exarchou[2], P.Charisiadis[1], I. P. Gerothanassis[1]

[1]Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, Ioannina GR-45110, Greece,
[2]NMR Center, University of Ioannina, Ioannina GR-45110, Greece

*Sideritis*, also known as "mountain tea", is considered to be the most popular decoction in Greece. Over the years the phytochemistry of the genus *Sideritis* has been studied and various phenolic and, especially, flavonoid aglycones and glycosides have been identified [1,2]. Aqueous preparations of Sideritis are known to possess antioxidant, antimicrobial, anti-ulcer and anti-inflammatory activity, which are mainly attributed to their flavonoid content [3]. The genus numbers more than 150 species, which in combination to their biological activity and daily use make *Sideritis* a challenging raw material. HPLC-SPE-NMR is unambiguously the most powerful analytical tool in phytochemical analysis; however, the overall system performance is greatly influenced by a list of parameters that need to be evaluated.

In this work the effect of (a) the stationary phase of cartridges, (b) two different deuterated solvents for adequate SPE cartridge elution, (c) the multiple trapping process and (d) the drying time, was investigated in terms of a signal-to- noise ratio study. The experimental procedure was carried out using standard phenolic acids of different polarity due to their abundance in Sideritis species. The higher signal-to-noise ratio values on the $^1$H-NMR spectra of phenolic acids were obtained using the GP cartridges eluted with acetonitrile-$d_3$. The effect of multiple trapping was determined on chlorogenic acid and the results demonstrated a linear increase of the S/N in the NMR spectrum in seven repeated trappings. The optimized parameters were then applied to the analysis of *Sideritis syriaca* extracts. The extracts under study were further evaluated for their total phenol content, and antiradical scavenging activity.

References

[1] Janeska, B. Stefova, M. and Alipieva, K. Acta Pharm. 2007, 57, 371–377.
[2] Fiamegos, Y.C., Nanos, C.G., Vervoort, J., Stalikas, C.D. J. Chromatogr. A. 2004, 1041, 11-18.
[3] Gabrieli, C.N., Kefalas, P.G., Kokkalou, E.L. J. Ethnopharmacol. 2005, 96, 423-42

# 17
# HPLC-SPE-NMR Use in Screening *Teucrium polium* Extracts with Antiradical Activity

V. Goulas[1], A. M. Gomez-Caravaca[2], V. Exarchou[3], A. Segura-Carattero[2], I. P. Gerothanassis[1]

[1]Section of Organic Chemistry and Biochemistry, Department of Chemistry, University of Ioannina, Ioannina GR-45110, Greece,
[2]Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Granada, Spain,
[3]NMR Center, University of Ioannina, Ioannina GR-45110, Greece

*Teucrium polium* (*Lamiaceae*) is well known as a medicinal plant for more than 2000 years and its extracts has been long used as diuretic, antipyretic, diaphoretic, tonic, stimulant, antispasmodic, anti-inflammatory, anorexic analgesic and antibacterial agent [1]. Capsules containing powder of *Teucrium* plants were marketed in France, as adjuvants to weight control, in the U.S.A *Teucrium* is used as a nature flavouring substance in beverages and in Mediterranean resin *Teucrium* decoctions are very popular. Although some benefits of *T. polium* have been claimed by researchers, there are reports demonstrating a strong hepatotoxicity capacity and some products of *Teucrium* were prohibited from sale [2]. It is essential, thus, to develop analytical methodologies that can be used for a rapid screening of phytochemicals in *Teucrium* extracts.

In this work different extracts of the aerial parts of *Teucrium polium* grown in Greece were prepared and analysed using HPLC-SPE-NMR in order to provide insight of the major and minor constituents present. The screening of the extracts revealed the presence of phenylpropanoid glycosides that can be used as marker compounds, and flavonoids. The extracts were further evaluated for their total phenol and total flavonoid content as well as their antiradical scavenging activity. The results showed that the methanol extract had the higher scavenging activity and phenolic content.

References

[1] Hasani, P., Yasa, N., Vosough-Ghanbari, S., Dehghan, G., Abdollahi, M., Acta Pharm., 2007, 57, 123-129.
[2] Sundaresan, P.R, Slavoff, S.A., Grundel, E., White, K.D, Mazzola, E., Koblenz, D., Rader, J.I. Phytochem. Anal. 2006, 17, 243-250

# 18
# LC-NMR Analysis of the Bulbs of *Scilla nervosa*

M. Bezabih

University of Botswana, Department of Chemistry, Private Bag 704, Gaborone, Botswana

The genus *Scilla* globally represents about 80 species. Four species occur in southern Africa of which *Scilla nervosa* is the most widely spread. It is also the only member of the genus known to occur in Botswana. *S. nervosa* has long been recognized as a poisonous species particularly to stock but nonetheless holds an important place in local pharmacopoeias. In Zulu medicinal practice it is used to treat pains associated with rheumatic fever and as purges for children. Phytochemical reports on *S. nervosa* reveal the isolation and characterization of homoisovalvonoids and stilbinoides.

*S. nervosa* is an important plant in the traditional medicinal practice of the people of Botswana. The decoctions of the bulbs are believed to enhance female fertility and are also used to treat infections. It is sold in open markets at bus stops and other public places. Thirteen homoisoflavonoids and three stilbinoids are reported from bulbs purchased in Botswana.

HPLC-NMR provides a powerful tool that can provide important information in the structural determination of natural products on-line. The importance of *S. nervosa* in the local medicinal practice as well as availability of authentic samples prompted us to develop HPLC-NMR method for the analysis of this plant. Fresh bulbs of *S. nervosa* were collected from our experimental garden. The leaves of the bulbs (dry weight 5 g) were pilled and deepen in acetone for 5 seconds. The solvent was removed under reduced pressure to yield acetone extract. The extract is subjected to HPLC-NMR analysis using an Agillent quaternary pump HPLC system with a photo diod array detector that is coupled to a 600 MHz NMR spectrometer. The results of on-flow and stop-flow HPLC-NMR analysis of the acetone extract will be presented.

# 19
# Cd-Induced Structure of a Small Metallothionein Determined by Capillary HPLC-NMR

D. Meißner[1], C. Mrestani-Klaus[1], M. P. Bayer[2], V. Friebolin[2], R. Golbik[1], D. Wesenberg[1], G.-J. Krauß[1], K. Albert[2]

[1]Martin-Luther-University Halle-Wittenberg, Institute of Biochemistry and Biotechnology, Kurt-Mothes-Str. 3, D-06120 Halle, Germany,
[2]Eberhard-Karls-University Tübingen, Institute of Organic Chemistry, Auf der Morgenstelle 18, D-72076 Tübingen, Germany

A small fungal metallothionein MT1_NECLU is involved in heavy metal stress response of the aquatic hyphomycete *Heliscus lugdunensis* strain H4-2-4 [1]. This strain was isolated from a heavy metal polluted habitat [2]. MT1_NECLU (MT) contains 24 amino acids. All eight Cys as well as the C-terminal His might be involved in $Cd^{2+}$ complexation. On addition of $Cd^{2+}$ to MT, an induced structural change from random coil to a loop/turn was predicted for the protein by molecular modeling. As a result of these studies, the Cys residues coordinate two $Cd^{2+}$ in a tetrahedral manner. Additionally, far UV circular dichroism studies of the chemically synthesized protein were performed, indicating a loop/turn-like structure formation after $Cd^{2+}$ complexation. In order to obtain information on the conformation of the MT, some high-resolution $^1H$ NMR measurements at 400 MHz of the synthetic protein (apo-MT) were carried out. Under these conditions, the protein turned out to be unstructured.

Due to the low level of metallothionein induction and high sensitivity against oxygen, the hyphenation of capillary HPLC to microcoil NMR was used to further elucidate the structure of the MT. First investigations using capillary RP-HPLC showed the separation of apo-MT accompanied with glutathione and phytochelatin 2. In off-line microprobe 600 MHz NMR measurements of the synthetic MT1, differences in the 1D $^1H$ and 2D H,H-COSY spectra between the apo- and $Cd^{2+}$-MT could be detected. These differences clearly indicate a structural change of the protein conformation on addition of $Cd^{2+}$ and its complexation to the MT.

References

[1] Jaeckel, P., Krauss, G., Menge, S., Schierhorn, A., Rücknagel, P. & Krauss, G.-J. (2005). Cadmium induces a novel metallothionein and phytochelatin 2 in an aquatic fungus. Biochem. Biophys. Res. Commun. 333, 150-155.

[2] Sridhar, K. R., Krauss, G., Bärlocher, F., Wennrich, R. & Krauss, G.-J. (2000). Fungal diversity in heavy metal polluted waters in Central Germany. Fungal Diversity 5, 119-129.

# 20
# Saponins Evaluation of the *Pfaffia glomerata* Spreng. Pedersen (Amaranthaceae)

Carmen Lucia Queiroga[1], Simone de Almeida Eira[1], Ilio Montanari Jr.[2], Elaine Cristina Cabral[3], Rodrigo Catharino[3], Marcos Nogueira Eberlin[3]

[1] Division of Phytochemistry, Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA), State University of Campinas (UNICAMP), Campinas, SP, Brazil
[2] Division of Agrotechnology, CPQBA, State University of Campinas (UNICAMP), Campinas, SP, Brazil
[3] Thomson Mass Spectrometry Laboratory, Institute of Chemistry, State University of Campinas (UNICAMP), Campinas, SP, Brazil

*Pfaffia glomerata*, known as Brazilian ginseng, is a shrub distributed in the southeast and south of Brazil. The roots have been used by folk medicine as tonic aphrodisiac and for pharmacological properties. Our goal was to evaluate the presence of saponin in *P. glomerata* root extracts. For these saponins, there is no UV absorption, the chemical composition profile of different extracts was analyzed by Electrospray Ionization Mass Spectrometry (ESI-MS).

Crude extract of the *P. glomerata* roots was obtained with EtOH:$H_2O$ (7:3) at room temperature. Partial extracts were prepared by extraction of *P. glomerata* roots with the following solvents: $CHCl_3$, MeOH, and EtOH:$H_2O$ (7:3). A QTof MS spectrometer (Micromass, Manchester, UK) was used for ESI-MS analysis, in the positive ion mode.

The ESI-MS spectrum fingerprint of the crude ethanol extract of *P. glomerata* roots was characterized by the ion at m/z 285, 381, 481, 543, 705, 867, 1029, 1191 e 1353. The β-ecdysone major concentration was in $CHCl_3$ extract, whose ESI-MS spectrum showed ion fragments at m/z 481 $[M+H]^+$ and 961 [β-Ecdysone dimer + H] $^+$. The highest saponin concentration was found in the MeOH extract. The ESI-QTof-MS provide accurate molecular formulae. Presently APCI-MS (Atmospheric Pressure Chemical Ionization - Mass Spectrometry) is being optimized to investigate further structure information.

# 21
# Phenolics of Norway spruce Colonized with *Heterobasidion annosum*

C. Paetz, B. Schneider

Max-Planck-Institute for Chemical Ecology,
Hans-Knöll-Str. 8, 07745 Jena, Germany

*Heterobasidion annosum*, a common pathogenic fungus in the Northern hemisphere, causes severe economical damage infesting timber forests. Colonizing the Norway spruce, the fungus induces a cascade of defense mechanisms in the living bark that are not understood in detail. Together with terpenes [1] and resin acids [2], phenolic compounds like stilbenes and lignans [3,4] were shown to be involved in the chemical response, but also higher condensed phenolics seem to play an important role [5]. In order to clarify the complex reactions induced upon infestation, a metabolic profile of infected wood zones was created. Hyphenated analytical techniques like HPLC-SPE-NMR and HPLC-MS were utilized. Structures of isolated compounds are introduced and interpreted regarding their possible role in plant defense.

[1] L. Zamponi, M. Michelozzi, P. Capretti; For. Path. 37 (2002), 243-250.
[2] M. Lindberg, L. Lundgren, R. Gref, M. Johansson; Eur. J. For. Path. 22 (1992), 95-106.
[3] J. Stenlid, M. Johansson; Eur. J. For. Path. 17 (1987), 217-226.
[4] S. Willföhr, J. Hemming, M. Reunanen, C. Eckemann, B. Holmbom; Holzforschung 57 (2003) 27-36.
[5] M. Johansson, L. Lundgren, F. Asiegbu; For. Path. 34 (2004) 197-210.

# 22
# Formation of Phenylphenalenones in Musaceae after *Radopholus similis* Attack

D. Hölscher, R. Ellinger, B. Schneider, D. Heckel

Max-Planck-Institute for Chemical Ecology,
Hans-Knöll-Str. 8, 07745 Jena, Germany

A series of phenylphenalenone-type compounds have been described in plants of the Haemodoraceae, Strelitziaceae, Pontederiaceae, and Musaceae families. Substances of this type have been identified from anthracnose-infected banana fruits (*Musa acuminata*) [1], from banana plants infected with *Fusarium oxysporum* (causing Panama disease) [2], from *M. sp.* Plants infected with *Mycosphaerella fijiensis* (causal agent of Black Sigatoka disease) [3], or from *M. acuminata* infected with the burrowing nematode *Radopholus similis* [4]. The increase of the biosynthesis of certain secondary metabolites after pathogen attack can be a promising starting point for pest control. Due to their intense red-orange colour these secondary compounds are visually detectable in stems, roots and rhizomes of some species of the plant families cited above and have been isolated and structurally identified from a small number of secretory cavities of *Dilatris pillansii* (Haemodoraceae) using a combination of laser microdissection microscopy and cryogenic NMR techniques [5]. In the case of members of the Musaceae plant family the detection of high concentrations of these banana phytoalexins seems to be connected to the "hot-spot"-activity-areas of longer-lasting pathogen activities.

Especially the burrowing nematodes (*R. similis*) migrate in the corm and the roots of banana cultivars. Interestingly the nectrotic lesion areas are bluish-crimson red coloured. We achieved successfully the detection of the secondary metabolites by using an alliance of hyphenated analytical techniques. Laser microdissection microscopy was used to get the necrotic lesions isolated from the healthy part of the plant. After extraction of microdissected material with ethanol compounds were separated and structurally identified by HPLC-SPE-NMR. The newly developed application of MALDI MSI allowed the proof of the direct localization of phenylphenalenones from cyrosections of nemtatode-contaminated banana material. Resarch on *R. similis* as one of the major constraints to banana production includes further investigations of the ingestion and the metabolism of the phenylphenalenone-enriched plant material to understand the plant-nematode interactions.

[1] N. Hirai et al.; Phytochem. 37 (1994), 383.
[2] J.G. Luis et al.; Phytochem. 41 (1996), 753.
[3] J.G. Luis et al.; J. Org. Chem. 58 (1993), 4306.
[4] R.H. Binks et al.; Phytochem. 45 (1997), 45.
[5] D. Hölscher & B. Schneider; Planta 225 (2007) 763.

# 23
# Effects of Plant Density and Sowing Date on Yield and Yield Components of *Hibiscus subdariffa* Var. Subdariffa in Zabol Region

Behrooz Mir[1], Ahmad Ghanbari[2]

[1] MSc of Agronomy
[2] Assistant Professor of Zabol University

This study was carried out to evaluate the effects of plant density and sowing date on yield and yield components of *Hibiscus subdariffa* Var. Subdariffa during 2004-5 in Agricultural Research Center of Zabol University. A split plot design based randomized complete block design with four replications were used. Three plant densities including 3, 4, 5, and 8 plants per meter square in main plot and 3 sowing dates including 10 and 26 March, 10 and 25 April 2005 in sub plot was employed. The results showed that plant density and sowing date had significant effects ($p< 0.01$) on calyx weight, capsules weight, dry matter yield, Number of capsules per plant, weight of an individual capsule, and number of capsules per surface area, as well as morphological traits, first phillocron, and stem diameter. Plant density and sowing date interaction had significant effects on most factors ($p< 0.01$) and dry matter yield ($p< 0.05$). Calyx yield, capsules number, number of capsules per surface area and calyx weight to capsule weight ratio increased with increasing plant density. An increase in these parameters attributed to faster rate of increment in leaf area index and CGR. Highest calyx yield was attained at first sowing date and 8 plants per meter square. In addition to dry matter yield, number of capsules per surface area, number of capsules per plant, plant height and first phillocron was highest at first planting date. Correlation between calyx yield and weight of an individual capsule, plant height, stem diameter, number of capsules per surface area and dry weight was significant at 1 percent level. Moreover capsules weight had positive correlation with calyx weight to capsules weight ratio, plant height, dry weight, and number of capsules per surface area and negative correlation ($p< 0.01$) with yield of an individual capsules and stem diameter. It can be concluded that first sowing date (i.e. 10 March) and highest planting density (i.e. 8 plants per meter square) was suggested.

# 24
# Structure-based Design, Parallel Synthesis, and Anticonvulsant Screening of some N-(2,6-Dimethylphenyl) Substituted Benzamidines

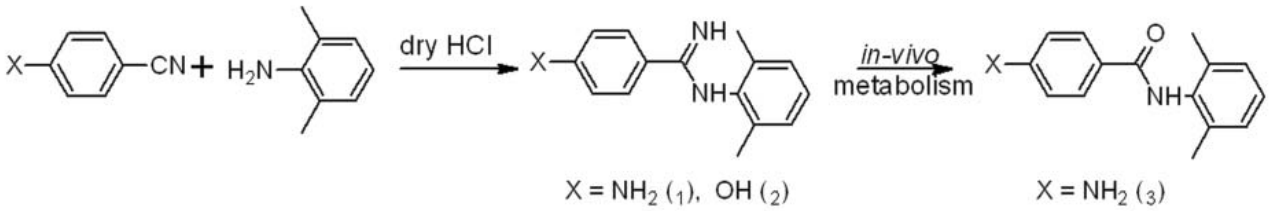
E. O. Afolabi[1], L. O. Amah[2], O. G. Enwereuzo[2], and V. Okolie[2]

[1,2] Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Jos, Jos. Nigeria.

The anticonvulsant activities of some analogues of N-substituted benzamidines were determined at three dose levels in accordance with the Antiepileptic Drug Development Program (ADD) of National Institute of Health (NIH), USA. These compounds are analogues of 4-amino-N-(2,6-dimethyl-phenyl)benzamide (Ameltolide®, LY201116) which is the most potent benzamide anticonvulsant studied to date. The benzamidines were designed as pro-drugs of Ameltolide by isosteric replacement of the carbonyl oxygen (=O) with amidine (=NH) group.

Two benzamidines (1 and 2) were prepared by reacting 2,6-dimethylaniline in parallel synthesis with saturated solutions of 4-aminobenzonitriles and 4-hydroxybenzonitrile with HCl(g) in dichloromethane. After evaporation of solvent, CHN elemental analysis of the sublimated products were determined and found within 0.04% of the calculated masses. The *in-vivo* effect of these compounds on white albino rats were compared with previously available ameltolide (3), cabamaze-pine (+C) and pentylenetetrazol(-C). Compound 1 offered a better profile of onset of seizure while compound 2 is less toxic even at a high dose of 100mg/Kg. The metabolic product of benzamidine is believed to give the corresponding benzamide and Nitric oxide.

Synthetic Scheme



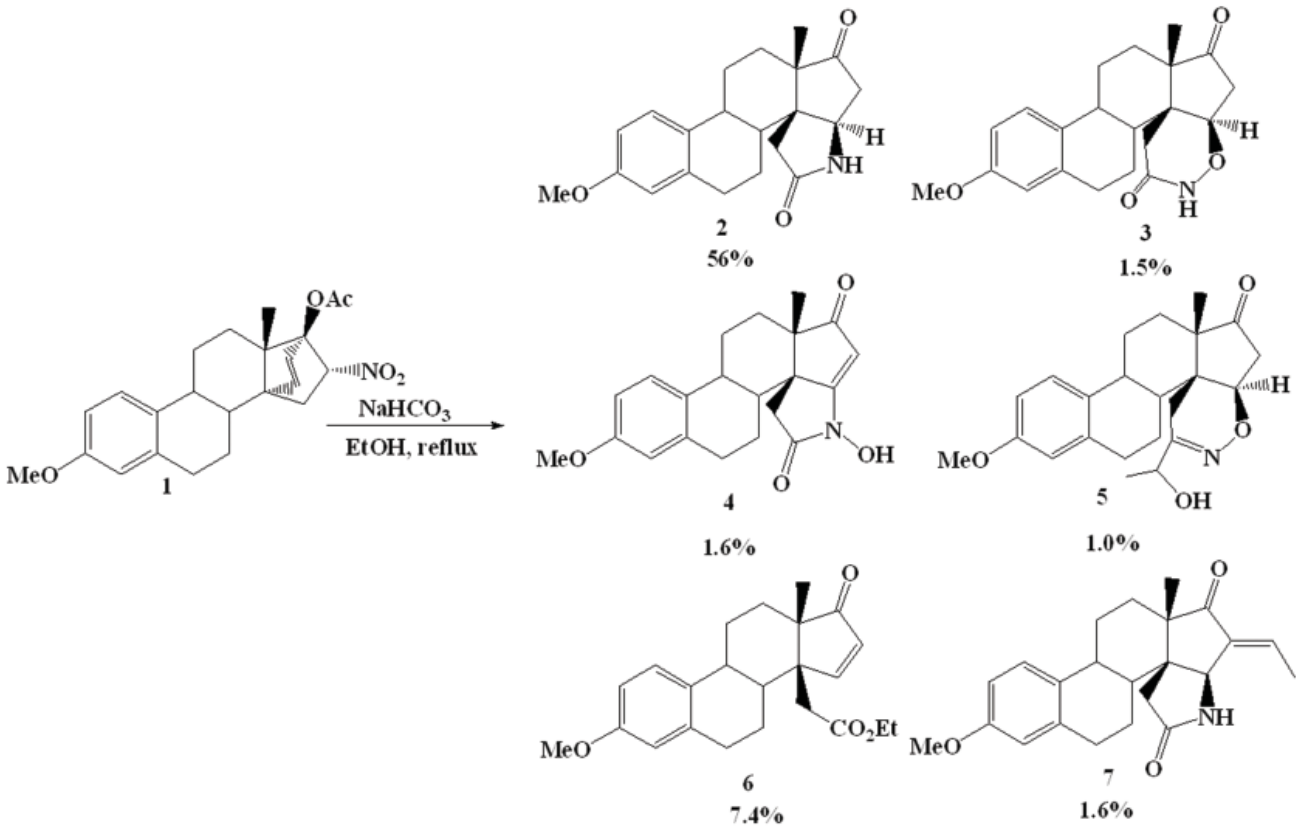
# 25
# HPLC-SPE-NMR Analysis of 3-Methoxy-14,17-etheno-16α-nitroestra-1,3,5(10)-trien-17β-yl Acetate Solvolysis Products

A. V. Baranovsky[1], D. A. Bolibrukh[1,2], V. A. Khripach[1], B. Schneider[2]

[1]Institute of Bioorganic Chemistry, Minsk, Belarus
[2]Max Plank Institute for Chemical Ecology , Jena, Germany

It was found that ethanol solvolysis of nitro steroid **1** in the presence of $NaHCO_3$ led to 3-methoxy-2'-oxopyrrolidino-[4',5':14β,15β]-estra-1,3,5(10)-trien-17-one **2** in 56% yield [1]. We undertook analysis of minor components in order to investigate mechanistic details of abnormal fragmentation pathways by use of LC-NMR techniques.

Due to the extraordinary sensitivity of the cryogenic NMR detector coupled with HPLC and SPE unit, we were able to isolate and characterize five compounds (**3-7**) beside the major in the reaction mixture.




The structures of all isolated compounds and a possible mechanism of transformation which includes formation of nitrile oxide as a first step of the reaction will be discussed.

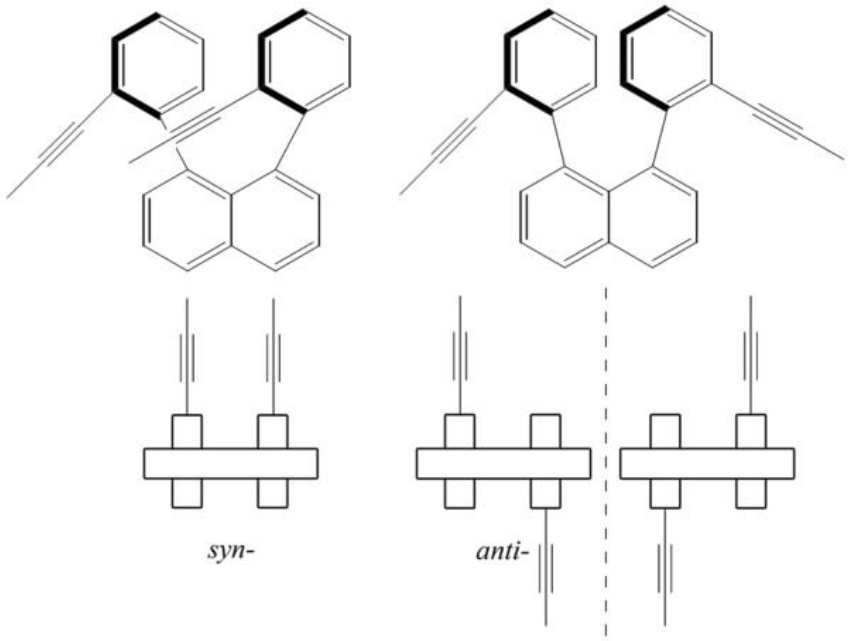
[1] Steroids 2008; V 73; PP. 585-593.

# 26
# Atropisomerism of 1,8-bis-(2-Propynyl-phenyl)-naphthalene

Jan Sýkora, Jan Storch, Jindřich Karban, Jan Čermák

Institute of Chemical Process Fundamentals of the ASCR, v. v. i.
Rozvojová 135, 165 02 Praha 6

1,8-Diiodonaphthalene (**1**) was coupled under Suzuki conditions to 2-propynyl-phenylboronic acid (**2**) to give 1,8-bis-(2-propynyl-phenyl)-naphthalene (**3**) in ~60% yield. The $^1$H NMR spectrum of **3** has revealed two set of signals in aproximate ratio 1:1 that can be atributed to *syn*- and *anti*- isomers of **3**. The restricted rotation around the single bond induces the chirality and give rise to two atropisomers at room temperature. Fortunately the partial separation is possible on the reversed phase HPLC even at ambient temperature and thus the individual $^1$H NMR spectra can be collected via on-flow LC-NMR technique. The stop-flow LC-NMR experiment has shown the slow *syn-anti* interconversion ($t_h$ = 24 h) in both cases.




Acknowledgements
Support from Czech Science Foundation (Grant no. 203/06/0738) and from the Ministry of Education, Youth and Sports of the Czech Republic (Grant no. LC06070) is gratefully acknowledged.

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# Identification of some new Phenolic Compounds in *Hasseltia floribunda* by LC/NMR

E. Dagvadorj[1], B. Schneider[2], R. Ellinger[2], W. Boland[2]

[1] Chemistry & Biochemistry Department of HSUM,Ulaanbaatar, Mongolia
[2] Max Planck Institute for Chemical Ecology, Jena, Germany

*Hasseltia floribunda* (K) is a medium sized tree that is branched near the ground. It is found mostly in tropical and subtropical areas. The chemical composition of the plant has not been established yet. The association between *Hasseltia floribunda* and Lepidoptera had been reported in a study (1). In general, phenolic compounds tend to be eaten by specialized herbivores therefore, in this study *Hasseltia floribunda*`s phenolic compound has been studied in particular.

Biological activity test of methanol extracts *H. floribunda* showed an inhibiting effect on β-glucosidase activity.

Air dried leaves (3 g) of *H. floribunda* from Panama were transferred to 15 ml of 80% aqueous methanol, grounded for 3 min with an Ultra-turrax T25, and were allowed to be shaken continuously for 60 min. The homogenate was centrifuged, and the pellet was re-extracted twice with 10 ml of 80% methanol. The combined extracts were evaporated to thickness mass.

The Methanolic extract was diluted with water until 50 mg/ml, and afterwards 2 ml from this solution was applied through Octadecyl micro columns and was eluted with various percentages of methanol solution.

The fractions were analyzed by LC/NMR using acetonitrile\water as a mobile phase containing TFA acid. In the fraction, which was eluted with 5% methanol, Salicin was found, Salicortin with 30% methanol, and 6'-Acetyl-salicortin with 50% and some new Coumaroylic acid-derivatives of the above compounds as well as Apigenin-β-glucuronid, Tricetin-β-glucocuronid were identified.

Coumaroylic acid-derivatives such as ***2'-trans;Coumaroyl-salicin, 2'-cis;Coumaroyl-salicin, 2'-trans-Coumaroyl-salicortin, 2'-cis-Coumaroyl-salicortin, 2'-trans-Coumaroyl-6'-acetyl-salicortin, 2'-cis-Coumaroyl-6'-acetyl-salicortin*** were determined for the first time.

References

[1] Nielsen, Vanessa. Priscilla Hurtado, et al. (2004) Rev. Biol. Trop 52(1): 119-132

# 6. Participants

| | |
|---|---|
| Afolabi, Ezekiel | University of Jos, Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Chemistry, Bauchi Road, 930001 Jos, Nigeria, e-mail: afolabie@unijos.edu.ng |
| Albert, Klaus | University of Tuebingen, Institute of Organic Chemistry, Auf der Morgenstelle 18, 72076 Tuebingen, Germany, e-mail: klaus.albert@uni-tuebingen.de |
| Bayer, Marc | University of Tuebingen, Institute of Organic Chemistry, AK Prof. Dr. Albert, Auf der Morgenstelle 18, D 72076 Tuebingen, Germany, e-mail: marc.bayer@uni-tuebingen.de |
| Bezabih, Merhatibeb | University of Botswana, Department of Chemistry, P. Bag 00704, Gaborone, Botswana, e-mail: bezabihm@mopipi.ub.bw |
| Bolibrukh, Dmitry | MPI for Chemical Ecology, Biosynthesis / NMR Group, Hans-Knöll-Straße 8, D-07745 Jena, Germany, e-mail: balibrukh@yahoo.com |
| Braumann, Ulrich | Bruker Biospin, Flow NMR Application, Silberstreifen, 07287 Karlsruhe, Germany, e-mail: ulrich.braumann@bruker-biospin.de |
| Brindle, Rainer | Bischoff Analysentechnik und -geräte GmbH, R&D Stationary Phases, Böblinger Straße 23, 71229 Leonberg, Germany, e-mail: brindle@bischoff-chrom.de |
| Charisiadis, Pantelis | University of Ioannina, Chemistry, Ioannina University Campus, GR 45110 Ioannina, Greece, e-mail: pcharisiadis@gmail.com |
| Crawford, Alexander | Katholieke Universiteit Leuven, Department of Pharmaceutical Sciences, Campus Gasthuisberg, O&N2 08.4213, Herestraat 49, PB824, BE-3000 Leuven, Belgium, e-mail: alexander.crawford@pharm.kuleuven.be |
| Daolio, Cristina | Bruker Biospin, Flow NMR Application, Silberstreifen, 76287 Karlsruhe, Germany, e-mail: cristina.daolio@bruker-biospin.de |
| Dong-Ung, Lee | Dongguk University, Bioscience, 707 Seokjang-dong, 780-714 Gyeongju, Republic of Korea, e-mail: dulee@dongguk.ac.kr |
| Dötzer, Reinhard | BASF SE, APR/DA, Carl-Bosch-Str. 64, Geb. Li 444, Zi 228, 67117 Limburgerhof, Germany, e-mail: reinhard.doetzer@basf.com |
| Dubat, Hassan Abdi | Centre for Economy and Liberitarian Affairs, Biochemistry, P.O. BOX 16005-00100, 254-20 Nairobi, Kenya, e-mail: ausaadi_cela@yahoo.ie |
| Ellinger, Renate | MPI for Chemical Ecology, NMR, Hans-Knoell-Strasse 8, 07743 Jena, Germany, e-mail: ellinger@ice.mpg.de |
| Enkhmaa, Dagvadorj | Health Sciences University of Mongolia, Chemistry and Biochemistry, Choidog Gudamj 3, 49 Ulaanbaatar, Mongolia, e-mail: edagvado@yahoo.de |
| Exarchou, Vassiliki | University of Ioannina, NMR Center, University of Ioannina, NMR Center, GR-45110 Ioannina, Greece, e-mail: vexarc@cc.uoi.gr |
| Foglio, Mary | Unicamp, CPQBA, CP 6171, 13081-970 Campinas, Brazil, e-mail: foglioma@cpqba.unicamp.br |

| | |
|---|---|
| Gaye, Abdoukarim | Olurass Enterprises, Sales /Technician, 5 Jang Jang Road, 00220 Serrekunda, The Gambia, e-mail: andyleaf66@yahoo.com |
| Girard, Frederic | Spinnovation Analytical BV, Management, Toernooiveld 100, P.O.Box 31070, 6503CB Nijmegen, The Netherlands, e-mail: F.Girard@spinnovation.eu |
| Gómez-Caravaca, Ana María | University of Granada, Analytical Chemistry, Avda.Fuentenueva s/n, 18071 Granada, Spain, e-mail: anagomez@ugr.es |
| Goulas, Vlasios | University of Ioannina, Chemistry, Ioannina University Campus, GR 45110 Ioannina, Greece, e-mail: me01723@cc.uoi.gr |
| Haiber, Stephan | Givaudan, Natural Products Research, Huizerstraatweg 28, 1411 GP Naarden, The Netherlands, e-mail: stephan.haiber@givaudan.com |
| Hartmann, Beate | symrise, FNEAME-FI-QR-AS-NMR/IR, Mühlenfeldstraße 1, 37603 Holzminden, Germany, e-mail: beate.hartmann@symrise.com |
| Hellriegel, Christine | Sigma-Aldrich, R&D, Industriestr. 15, 9471 Buchs, Switzerland, e-mail: christine.hellriegel@sial.com |
| Hiller, Wolf | TU Dortmund, Faculty of Chemistry, Otto-Hahn-str. 6, 44221 Dortmund, Germany, e-mail: wolf.hiller@tu-dortmund.de |
| Hölscher, Dirk | MPI for Chemical Ecology, Entomology, Hans-Knöll-Str. 8, 07745 Jena, Germany, e-mail: hoelscher@ice.mpg.de |
| Holtin, Karsten | University of Tuebingen, Institute of Organic Chemistry, AK Albert, Auf der Morgenstelle 18, 72076 Tuebingen, Germany, e-mail: karsten.holtin@uni-tuebingen.de |
| Honing, Maarten | Schering Plough Research Institute, Department of Medicinal Chemistry Oss, P.O. Box 20, 5340 BH, Oss, The Netherlands, e-mail: maarten.honing@spcorp.com |
| Howe, Peter | Syngenta, Analytical Sciences, Jealott‘s Hill, RG42 6EY Bracknell, United Kingdom, e-mail: peter.howe@syngenta.com |
| Jaroszewski, Jerzy W. | University of Copenhagen, Department of Medicinal Chemistry, Universitetsparken 2, DK-2100 Copenhagen, Denmark, e-mail: jj@farma.ku.dk |
| Josch, Jan | TU Clausthal, Institut für Thermische Verfahrens- und Prozesstechnik, Leibnizstraße 15, 38678 Clausthal-Zellerfeld, Germany, e-mail: josch@itv.tu-clausthal.de |
| Kautz, Roger | Northeastern University, Barnett Institute, 341 Mugar Hall, 360 Huntington Avenue, 02115 Boston, USA, e-mail: rkautz2007@neu.edu |
| Kontogianni, Vasiliki | University of Ioannina, Chemistry, Ioannina University Campus, GR 45110 Ioannina, Greece, e-mail: me01375@cc.uoi.gr |
| Kurfürst, Milan | ICPF, Department of Analytical Chemistry, Rozvojova135, 16502 Prague, Czech Republic, e-mail: kurfurst@icpf.cas.cz |

| | |
|---|---|
| Larive, Cynthia | University of California-Riverside, Department of Chemistry, 500 Big Springs Rd., 92507 Riverside, California, USA, e-mail: clarive@ucr.edu |
| Le Grand, Flore | Spinnovation, R&D, Toernooiveld 100, P.O. Box 31070, 6503 CB Nijmegen, The Netherlands, e-mail: f.legrand@spinnovation.eu |
| Lopes da Fonseca, Luís | Instituto de Tecnologia Química e Biológica-UNL, Cell Physiology and NMR, Rua da Quinta Grande, 6, Apartado 127, 2780-156 Oeiras, Portugal, e-mail: llf@itqb.unl.pt |
| Meißner, Diana | MLU Halle-Wittenberg, Institut für Biochemie/Biotechnologie, AG Prof. Krauß, Kurt-Mothes-Str. 3, 06120 Halle, Germany, e-mail: diana.meissner@biochemtech.uni-halle.de |
| Mir, Behrooz | University of Zabol, Agriculture, Mofattah Street, 98615 Zabol, Iran, e-mail: moas@uoz.ac.ir |
| Moco, Sofia | Wageningen University, Laboratory of Biochemistry, Dreijenlaan 3, 6703HA Wageningen, The Netherlands, e-mail: sofia.moco@wur.nl |
| Mohamed, Ali Abdirahaman | Green Planet International, Chemical Ecology, P.O.Box 16005-00100, 254-20 Nairobi, Kenya, e-mail: gplint@yahoo.com |
| Mohn, Tobias | University of Basel, Institute of Pharmaceutical Biology, Klingelbergstrasse 50, CH-4056 Basel, Switzerland, e-mail: tobias.mohn@unibas.ch |
| Naghibi, Farzaneh | Shaheed Beheshti University of Medical Sciences, Traditional Medicine & Materia Medica Research Center, No.10, Shams str., Vali assr ve., 14155-6354 Tehran, Iran, e-mail: fnaghibi@itmrc.org |
| Nerantzaki, Alexandra | University of Ioannina, Chemistry, Ioannina University Campus, GR 45110 Ioannina, Greece, e-mail: me01633@cc.uoi.gr |
| Ngunyi, Leah Wambui | International Children and Health Development Centre, Biochemicals, Mama Ngina, 254-20 Nairobi, Kenya, e-mail: ccwb2000ke@yahoo.com |
| Odontuya, Gendaram | Institute of Chemistry and Chemical Technology, MAS, Natural Product Chemistry Laboratory, Peace ave, the 4th Building, MAS, Bayanzurkh district, 976-11 Ulaanbaatar, Mongolia, e-mail: odontuyag@hotmail.com |
| Paetz, Christian | MPI for Chemical Ecology, NMR/Biochemistry, Hans-Knöll-Strasse 8, 07745 Jena, Germany, e-mail: cpaetz@ice.mpg.de |
| Plitzko, Inken | University of Basel, Institut für Pharmazeutische Biologie, Klingelbergstrasse 50, 4056 Basel, Schweiz, e-mail: inken.plitzko@unibas.ch |
| Preiss, Alfred | FhG-ITEM, Analytical Chemistry, Nikolai-Fuchs-Str. 1, D-30625 Hannover, Germany, e-mail: alfred.preiss@item.fraunhofer.de |
| Queiroga, Carmen Lucia | CPQBA - Unicamp, Phytochemistry Division, CPQBA-Unicamp, P.O. BOX 6171, 13083-970 Campinas - Sao Paulo, Brazil, e-mail: queiroga@cpqba.unicamp.br |
| Said, Ataa | National Research Center, Pharmacognosy, El Tahreer St-Dokki Giza Egypt, 84 Shehab st.El-Mohandeseen-Egypt, 12311 Giza, Egypt, e-mail: ataasaid@yahoo.com |

| | |
|---|---|
| Sandvoss, Martin | Sanofi-Aventis Deutschland GmbH, ICMS (Isotope, Chemistry and Metabolite Synthesis), Industriepark Hoechst, building G876, 65926 Frankfurt, Germany, e-mail: martin.sandvoss@sanofi-aventis.com |
| Santos, Helena | Instituto de Tecnologia Química e Biológica - UNL, Cell Physiology and NMR, Rua da Quinta Grande, 6, Apartado 127, 2780-156 Oeiras, Portugal, e-mail: santos@itqb.unl.pt |
| Schmidt, Jochen | Solvay Pharmaceuticals, Preclinical Candidate Selection / Metabolism NMR, Hans-Böckler-Allee 20, 30173 Hannover, Germany, e-mail: jochen.schmidt@solvay.com |
| Schneider, Bernd | MPI for Chemical Ecology, NMR, Hans-Knöll-Str. 8, Beutenberg Campus, 07745 Jena, Germany, e-mail: schneider@ice.mpg.de |
| Seger, Christoph | University of Innsbruck, Pharmacy / Pharmacognosy, Innrain 52, 6020 Innsbruck, Austria, e-mail: christoph.seger@uibk.ac.at |
| Sicker, Dieter | Universität Leipzig, Institut für Organische Chemie, Johannisallee 29, 04103 Leipzig, Germany, e-mail: sicker@chemie.uni-leipzig.de |
| Sohretoglu, Didem | Hacettepe University, Pharmacognosy, H.U. Eczacilik Fak. Sihhiye, 06100 Ankara, Turkey, e-mail: didems@hacettepe.edu.tr |
| Sokolowski, Tobias | Beiersdorf AG, Strukturanalytik, Unnastraße 48, 20245 Hamburg, Germany, e-mail: tobias.sokolowski@beiersdorf.com |
| Sommer, Horst | Firmenich SA, Corporate Research, Route des Jeunes 1, 1211 Geneva, Switzerland, e-mail: horst.sommer@firmenich.com |
| Spraul, Manfred | Bruker Biospin, Flow NMR Application, Silberstreifen, 76287 Karlsruhe, Germany, e-mail: manfred.spraul@bruker-biospin.de |
| Sturm, Sonja | University of Innsbruck, Pharmacy / Pharmakognosie, Innrain 52, A-6020 Innsbruck, Austria, e-mail: Sonja.sturm@uibk.ac.at |
| Svatos, Ales | MPI for Chemical Ecology, MS, Hans-Knoell-Str. 8, 07745 Jena, Germany, e-mail: svatos@ice.mpg.de |
| Sykora, Jan | Institute of Chemical Process Fundamentals of the ASCR, v. v. i., Department of Analytical Chemistry, Rozvojova 135, 16502 Prague, Czech Republic, e-mail: sykora@icpf.cas.cz |
| Tatsis, Evangelos | MPI for Chemical Ecology, Biosynthesis/NMR, Hans Knoll Straße 8, D-07745 Jena, Germany, e-mail: etatsis@ice.mpg.de |
| Thomas, Steve | GlaxoSmithKline, TKB, Park Road, SG12 0DP Ware, United Kingdom, e-mail: steven.r.thomas@gsk.com |
| Tóth, Gábor | TU Budapest, Lehrstuhl fuer Anal. Chem., Gellért tér 4, H-1111 Budapest, Hungary, e-mail: drgabortoth@t-online.hu |
| Um, Byung Hun | Korea Institute of Science and Technology (KIST), Gangneung Institute, Gangneung, 210-340 Gangneung, South Korea, e-mail: albertum@kist.re.kr |
| van der Hooft, Justin | Wageningen University, Biochemistry / Biosciences (PRI), Dreijenlaan 3, 6703 HA Wageningen, The Netherlands, e-mail: Justin.vanderHooft@wur.nl |

| | |
|---|---|
| Vervoort, Jacques | Wageningen University, Biochemistry, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands, e-mail: jacques.vervoort@wur.nl |
| Webb, Andrew | Leiden University Medical Center, Radiology C3Q, Albinusdreef 2, 2333 ZA Leiden, The Netherlands, e-mail: a.webb@lumc.nl |
| Weber, Michael | Sigma-Aldrich, R&D, Industriestrasse 15, 9471 Buchs, Switzerland, e-mail: michael.weber@sial.com |
| Wolfender, Jean-Luc | University of Geneva, Lab of Pharmacognosy and Phytochemistry, Quai E. Ansermet 30, 1211 Geneva, Switzerland, e-mail: jean-luc.wolfender@pharm.unige.ch |
| Yalcin, Funda Nuray | Hacettepe University, Pharmacognosy, H.U. Eczacilik Fak. Sihhiye, 06100 Ankara, Turkey, e-mail: funyal@hacettepe.edu.tr |
| Yang, Xinzhou | University of Basel, Pharmaceutical Sciences, Klingelbergstr. 50, 4056 Basel, Switzerland, e-mail: Xinzhou.Yang@unibas.ch |

# 7. Acknowledgements

The organizers appreciate the generous support of the LC-NMR conference by the following companies and orgnaizations:

- **Bischoff Analysentechnik und -geräte GmbH**
- **Bruker-Biospin GmbH**
- **Deutero GmbH**
- **Deutsche Forschungsgemeinschaft**
- **Euriso-Top SA**
- **ScienceServe GmbH**
- **Spinnovation Holding BV**

## Notes