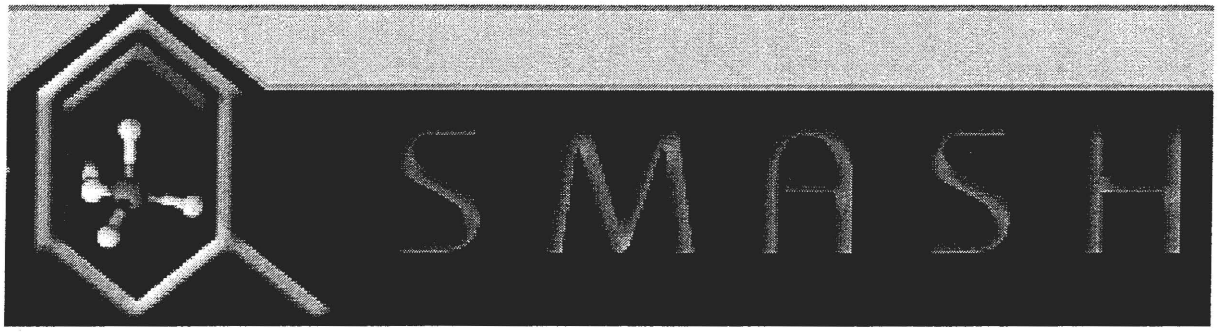
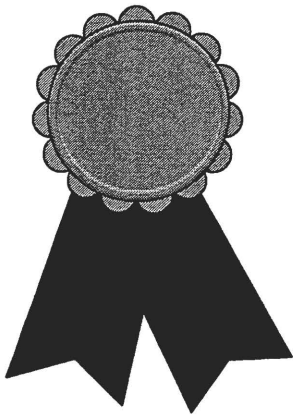


SMASH: Small Molecule NMR Conference
(Small Molecules Are Still Hot)

August 15 - August 18, 1999
Argonne Guest House,
Argonne National Laboratory
Argonne, Illinois

Thank you!



The SMASH organizing committee would like to graciously thank our corporate sponsors. Without their support, this event could not have occurred. Thanks to all of you.

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Charter Meeting of SMASH
(Small **M**olecules **A**re **S**till **H**ot)
Sunday, August 15 – Wednesday, August 18, 1999
Argonne Guest House,
Argonne National Laboratory
Argonne, Illinois

Sunday

Noon – 5:30 pm	Arrival and registration at the Argonne House
5:45 – 7:15 pm	Introductory Plenary Lecture by Dr. James Shoolery "From Neat Liquids to Nanograms" Sponsored by Varian NMR Instruments
7:15 – 9:00 pm	Social Mixer

Monday

7 – 8:30 am	Breakfast at the Argonne House
8:30 – 10:30 am	**Session 1: Natural Products** Chair: Gary Martin

"^{1}H, ^{13}C, and ^{15}N NMR Study of Benzo[c]phenanthridine and Other Isoquinoline Alkaloids," R. Marek, J. Tousek*, R. Dommisse*, and V. Sklenar, Masaryk University, Brno, Czech Republic and *University of Antwerp, Antwerp, Belgium.

"Teaching Young Dogs Old Tricks," W.F. Reynolds, W. W. Harding*. and H. Jacobs*. University of Toronto, Toronto, Canada and *University of the West Indies, Kingston, Jamaica.

"The Structure Elucidation of Natural Products --- An Industrial Approach," P.J. Sidebottom, GlaxoWellcome, UK.

"New Gradient-Enhanced HSQMBC Experiments for the Determination of Heteronuclear Coupling Constants and their Application to the Structure Elucidation of Natural Products," R.T. Williamson, B. Marquez, K. E. Kover*, and W.H. Gerwick, Oregon State University and *L. Kossuth University, Debrecen, Hungary.

10:30 – 11 am	Morning Break

Monday

11 – 12:30 pm	**Session 2: Pulse Sequences** Chair: Krish Krishnamurthy "Methods to Obtain Clean NMR Spectra and Rapidly Extract Useful Information from Them," A.J. Shaka, University of California Irvine, Irvine, CA. "Impeach and Beyond," K. Krishnamurthy, G.E. Martin*, C.E. Hadden*, Varian Inc., Palo Alto, CA, *Pharmacia & UpJohn, Kalamazoo, MI. "Liquid State NMR Approaches for the Study of Molecular Interactions," J.W. Peng, Vertex Pharmaceuticals Inc., Cambridge, MA
12:30 – 2 pm	Lunch at Bldg 401
2 – 4 :00 pm	Free Afternoon
4 – 6:30 pm	**Session 3: New Probe and Magnet Development** Chair: Albert Zens "NMR Magnet Technology at Ultra High Fields," R. Teodorescu, Bruker Magnetics, USA. "Cold Probe Developments," B. Adams and H. Hill, Varian Inc., Palo Alto, California. "Recent Developments in High Resolution NMR Using Micro Coils," A. Webb, University of Illinois, Urbana, Illinois. "Looking Ahead……High Performance Micro Cold Probes," A. Zens, Nalorac Corporation, Martinez, California.
7 – 8:30pm	Dinner at the Argonne House Dinner Lecture: Ray Freeman "The Tale of One Small Molecule" Sponsored by Advanced Chemistry Development

Tuesday

7 – 8:30 am	Breakfast at the Argonne House
8:30 – 10:30 am	**Session 4: Applications of Flow NMR** Chair: Alistair Swanson
	"Recent Advances in LC-NMR and LC- MS-NMR," A. Kaerner, Eli Lilly and Company, Indianapolis, Indiana.
	"From Total LC-NMR Automation to LC-NMR-MS," Ulrich Braumann, Bruker, Karlsruhe.
	"Flow NMR, Flow-Injection-Analysis NMR and Direct-Injection NMR Techniques," Paul Keifer, Varian Inc., Palo Alto, California.
10:30 – 11 am	Morning Break
11 – 12:30 pm	**Session 5: Metabolism** Chair: John Shockcor
	"Characterization of Novel Glutatione Adducts of the HIV-1 Non-Nulceoside Reverse Transcriptase Inhibitor DPC 961," J. R. Espina*, A.E. Mutlib, H. Chen and J.P. Shockcor, DuPont Pharmaceuticals Company, Drug Metabolism & Pharmacokinetics, Stine-Haskell Research Center, Newark, Delaware
	"Structure Elucidation of the Primary Metabolite of the Neuromuscular Blocker GW280430A," T. Spitzer, J. Walsh, M. Reese, C. Stafford, K. Facchine, D. Burinsky, and S. Sides, Glaxo Wellcome, Inc,. Research Triangle Park, NC.
	"Structures of Metabolites of [14C]Celecoxib in Healthy Male Volunteers," R. Bible Jr, E. Hajdu, S.K. Paulson*, J. Hribar, N. Liu, and A. Karim[#], Chemical Sciences Analytical, *Pharmacokinetics, Bioanalytical & Radiochemistry, [#]Clinical Pharmacokinetics and Bioavailabilty, Searle, Skokie, Illinois.
	"NMR Spectroscopy and Toxicology," J. K. Nicholson, Biological Chemistry, Biomedical Sciences Division, Imperial College, University of London.
12:30 – 2 pm	Lunch

Tuesday

2 – 4 pm	Free Afternoon
4 – 6:30 pm	**Session 6: Applications of NMR to High Throughput Screening and Combinatorial Chemistry** Chair: Michael Shapiro "Novel Applications of SHAPES NMR in Drug Discovery", J. Moore, Vertex Pharmaceuticals, Inc., Cambridge, Massachusetts. "AutoDrop: A New Approach to Automated Structure Verification in Combinatorial Chemistry", P. Neidig and H. Schroeder*, Bruker Instruments, Ettlingen, Germany, and *Hoffmann-LaRoche AG. "Applications of Flow NMR to Monitoring Binding of Small Molecules to Proteins", K. Farley, Pharmacia and UpJohn, Kalamazoo, Michigan. "High Throughput Diffusion NMR for the Evaluation of Ligand-Receptor Binding", D. Detlefson and F. Xu, Bristol-Meyers Squibb Pharmaceutical Research Institute, Wallingford, Connecticut. "TBA," A. Kline, Eli Lilly and Company, Indianapolis, Indiana
7 – 8:30 pm	Dinner at the Argonne House Dinner Lecture: Tom Farrar "Antediluvian Anecdotes of Sensitivity Enhancement Advances in NMR Spectrosopy" Sponsored by Advanced Chemistry Development
8:30 – 10:30 pm	**Session 7: Posters** Refreshments sponsored by Bruker Instruments

Wednesday

7 – 8:30 am	Breakfast at the Argonne House
8:30 – 11 am	**Session 8: Post acquisition data processing, databasing and spectral prediction** Chair: C. Andy Evans "The Advantages and Limitations of Linear Prediction," W. F. Reynolds, University of Toronto, Toronto, Canada. "Automated Structure Eludication from 1D C-13 Spectral Data," A. J. Williams, ACD Laboratories. "Data to Information, Information to Knowledge," D. E. Dorman, Eli Lilly and Company, Indianapolis, Indiana. "Modeling Time-Domain NMR Data with Bayesian Statistics: An Alternative Method for Spectral Analysis," W. C. Hutton, Monsanto, St. Louis, Missouri.
11 – 11:30 am	Conference Wrap-Up and box lunch

Introductory Plenary Lecture Sunday Evening

From Neat Liquids to Nanograms

James Shoolery

The developments in high resolution NMR from 1952 to 1999, that resulted in many orders of magnitude increase in sensitivity and corresponding reduction of sample requirement, will be examined in the light of their effect on structure elucidation of natural products.

During the period from 1952 to 1962, high resolution NMR became widely accepted by organic chemists as a valuable tool for small molecules. Although large samples were needed, proton NMR spectra of that period proved to be very useful in elucidating various aspects of molecular structure and in providing quantitative analytical data for multicomponent mixtures. Substantial progress was made in improving sensitivity, but chemists were still not satisfied.

During the 1960s, many limitations imposed by sensitivity, resolution and stability were overcome, and pulsed Fourier transform excitation and detection largely replaced CW operation. Techniques for obtaining useful C-13 NMR spectra were developed and applied to solving problems. However, the low sensitivity of C-13 required large samples to give good spectra in a short time.

From 1970 to the present, the explosive evolution of families of powerful two-diminensional NMR experiments, along with software to set-up, adjust, and operate the spectrometer and display the data in interpretable form, has increased the number and complexity of chemical problems that can be addressed. However, the price for this information is time, which translates into sensitivity. In short, the complaint that we heard in the 1950s--The sample's too small-- had returned.

Nearly fifty years of innovation and product improvement will be reviewed to illustrate the efforts that have gone into maximizing signals and minimizing noise. Special attention will be paid to the evolution of microcells and microprobes. At Varian, the path has led from the first commercial insturment, which required a neat liquid or highly concentrated solution, to the latest spectrometer systems, which offer a variety of small sample "nanoprobes."

The ability to obtain useful proton NMR spectra with sub-microgram quantitites of small molecules, or C-13 spectra from sub-milligram quantities opens the way for the use of 2-D correlation experiments dependent on the scalar coupling between C-13 nuclei joined by a chemical bond, or H/C couplings propagated through two or three bonds, even with samples of a few milligrams. In conjuction with the molecular formula from MS, and suitable NOE data, the over-determined set of bonding pathways can be interpreted to give the full structure, complete with stereochemistry. Strategies will be described and illustrated with suitable examples.

Session 1. Natural Products

^{1}H, ^{13}C and ^{15}N NMR STUDY OF BENZO[*C*]PHENANTHRIDINE AND OTHER ISOQUINOLINE ALKALOIDS

Radek MAREK , Jaromír TOUŠEK, Roger DOMMISSE[†], and Vladimír SKLENÁŘ

Laboratory of Biomolecular Structure & Dynamics, Faculty of Science,
Masaryk University, Kotlářská 2, CZ – 611 37, Brno, Czech Republic
[†] Research Group for Applied NMR Spectroscopy, Department of Chemistry,
University of Antverp, Groenenborgerlaan 171, B – 2020, Antwerpen, Belgium

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Benzo[*c*]phenanthridine alkaloids are widely distributed in the plant families *Papaveraceae*, *Fumariaceae*, and *Rutaceae*. These biologically active alkaloids are anti-microbial, anti-inflammatory, anti-mycotic, specifically inhibit particular enzymes and the interaction of quaternary benzophenanthridine alkaloids with DNA is also of interest.

Structural, NMR and theoretical study of quaternary benzophenanthridine alkaloids and their free bases was carried out [e.g. see ref.[1-3]]. Results obtained for pseudobases and both diastereomers of some benzophenanthridine dimeric structures will be discussed[4,5]. The chemical shifts, calculated by quantum chemical methods, were compared with experimental results in order to differentiate between both diastereomers.

The systematic ^{15}N NMR study of other selected alkaloids of isoquinoline type[6] was carried out using GHMBC[7] and GSQMBC[8] techniques in order to evaluate influence of constitutional type, alkaloid form (salt *vs*. free base) and substitution on ^{15}N parameters and the results obtained will be presented.

References:

1. R. Marek, J. Toušek, J. Dostál, J. Slavík, R. Dommisse, and V. Sklenář: *Magn. Reson. Chem.* submitted.
2. J. Dostál, R. Marek, J. Slavík, E. Táborská, M. Potáček, and V. Sklenář: *Magn. Reson. Chem.* **36**, 869 (1998).
3. R. Marek, J. Toušek, L. Králík, J. Dostál, and V. Sklenář: *Chem. Lett.* **1997**, 369.
4. R. Marek, V. Sklenář, J. Dostál, and J. Slavík: *Tetrahedron Lett.* **37**, 1655 (1996).
5. J. Dostál, J. Slavík, M. Potáček, R. Marek, O. Humpa, V. Sklenář, J. Toušek, E. de Hoffmann, and R. Rozenberg: *Collect. Czech. Chem. Commun.* **63**, 1045 (1998).
6. R. Marek, O. Humpa, J. Dostál, J. Slavík, and V. Sklenář: *Magn. Reson. Chem.* **37**, 195 (1999).
7. W. Willker, D. Leibfritz, R. Kerssebaum, and W. Bermel: *Magn. Reson. Chem.* **31**, 287 (1993).
8. R. Marek, L. Králík, and V. Sklenář: *Tetrahedron Lett.* **38**, 665 (1997).

Session 1. Natural Products

^{13}C and ^{1}H Spectral Assignments for Polyprenol-12 Using High Resolution ^{13}C-Detected ^{13}C-^{1}H Shift Correlation Sequences – or – Teaching Young Dogs Old Tricks

W.W. Harding[1], H. Jacobs[1], and W. F. Reynolds[2]
[1]Department of Chemistry, University of the West Indies, Mona Campus
[2]Department of Chemistry, University of Toronto

There seems to be a widespread assumption that ^{1}H-detected ^{13}C-^{1}H shift correlation sequences are always superiod to the older ^{13}C detected sequences because of the higher sensitivity of the newer techniques. However, this must be balanced against their lower ^{13}C resolution and, in cases where high ^{13}C resolution is critical, the olde sequences may give results unattainable with the newer sequences. This is illustrated with polyprenol-12, a natural product containing a chain of 12 isoprene units.

Session 1. Natural Products

The Structure Elucidation of Natural Products - An Industrial Approach.

Philip J. Sidebottom
Physical Sciences Unit, GlaxoWellcome Medicines Research Centre, Stevenage, Herts.
SG1 2NY
UK.

This talk will cover the following topics:

1) The purpose of structure elucidation.
2) The use of databases to aid the rapid identification of "known" natural products. The focus will be on Chapman & Hall's Dictionary of Natural Products.
3) Our approach to solving the structures of "unknowns". Details of the NMR equipment used together with an outline of the main experiments employed will be given. These include 1D proton spectra and the following 2D experiments, HMQC, HMBC, GAHMBC and GAPSDQFCOSY. An idea of the amount of material needed and the time requirements for each of these experiments will be presented.

10 Hz for HMBC Long range correlations ^{13}C-^{1}H

New Gradient-Enhanced HSQMBC Experiments for Determination of Heteronuclear Coupling Constants and their Application to the Structure Elucidation of Natural Products

R.Thomas Williamson, B. Márquez, K. E. Kövér*, and W.H. Gerwick,
Oregon State University and *L. Kossuth University, Debrecen, Hungary

Secondary metabolites isolated from natural sources often have very complicated structures. The challenge that presents itself, once the planar structure is defined, is the determination of stereochemical configuration. Typically, in constrained systems, the use of ^{1}H-^{1}H coupling constants and ^{1}H-^{1}H dipolar couplings are used to determine the configuration about a particular bond. However, in situations where there are no unambiguous homonuclear couplings or NOE correlations, as in acyclic systems, the stereochemical assignments become difficult. Under these circumstances, the use of $^2J_{H,C}$ and $^3J_{H,C}$ heteronuclear coupling constants becomes the only NMR data to provide decisive information regarding stereochemistry. Until now, a sensitive and convenient method for obtaining this spin-coupling information has not been available.

In the past, the most often used pulse sequences for determination of these coupling constants has been the hetero half-filtered TOCSY (HETLOC) in combination with the phase sensitive HMBC (psHMBC). One of the disadvantages in using HETLOC is that the sensitivity is dependent on the magnitude of the $^3J_{H,H}$ couplings through which the TOCSY transfer takes place. Another disadvantage is that this sequence is only useful for continuous ^{1}H-^{1}H spin-systems. The limitations to the psHMBC are caused by the evolution of ^{1}H-^{1}H homonuclear couplings during the long delay necessary for the evolution of the long range heteronuclear couplings of interest. Aside from the loss in sensitivity due to an additional refocusing period required for the acquisition of phase sensitive data, line shape distortion causes problems in data interpretation. We will discuss several new pulse sequences, named HSQMBC experiments, which provide pure absorption data in both F_1 and F_2 and eliminate the disadvantages associated with these previously described methods. These pulse sequences have proven to be very reliable and extremely useful for many structural problems in our laboratory.

As an example, these new HSQMBC experiments have been demonstrated on the complete relative stereochemical determination of phormidolide. Phormidolide, isolated from a cyanobacterium, is a macrolide with a molecular weight of >1,000 that contains 11 chiral centers. The relative stereochemistry was determined exclusively through a combination of ROESY, E-COSY, and HSQMBC experiments with no semi-synthetic derivitization. This approach along with subsequent work for the absolute stereochemical determination will be discussed.

Session 2. Pulse Sequences

Methods to Obtain Clean NMR Spectra and Rapidly Extract Useful Information From Them

A. J. Shaka
University of California
Irvine, Irvine, CA

Methodological improvements in pulsed field gradient (PFG) experiments and methods to simplify and speed up data analysis will be described. Using "excitation sculpting" we can obtain improved detection of cross peaks in 2D spectra, suppress large unwanted peaks and measure very small nuclear Overhauser enhancements (NOEs). By using the recently developed Filter Diagonalization Method (FDM) we can obtain heteronuclear 2D spectra with improved resolution, and can decouple various spectra so that singlets are obtained for all the peaks.

Session 2. Pulse Sequences

IMPEACH and Beyond

G.E. Martin, C.E. Hadden and V.V. Krishnamurthy.

Accordion optimization of the long-range coupling delay in HMBC experiment, as in the ACCORD-HMBC experiment proposed by Wagner and Berger[1], can be highly beneficial in terms of increasing the number of long-range responses observed relative to "standard" HMBC experiment[2]. The IMPEACH-MBC [Improved Performance long-range Accordion Heteronuclear Multiple Bond Correlation spectroscopy] experiment[3] employs a "constant time variable delay" element to suppress homonuclear coupling modulation of the cross peaks in ACCORD-HMBC. Factors influencing the intensity and shape of the cross peaks in ACCORD-HMBC and IMPEACH-MBC, as well as further refinement of IMPEACH-MBC experiment will be discussed.

References:
1. R. Wagner and S. Berger, Magn. Reson. Chem. 36, 544 (1998).
2. G.E. Martin, C.E. Hadden, R.C. Crouch and V.V. Krishnamurthy, Magn. Reson. Chem., 37, 517 (1999).
3. G.E. Martin, C.E. Hadden and V.V. Krishnamurthy, J. Magn. Chem., in press.

Session 2. Pulse Sequences

Liquid State NMR Approaches for the Study of Molecular Interactions

Jeffrey W. Peng
Vertex Pharmaceuticals, Inc.
130 Waverly Street
Cambridge, MA 02139

An understanding of ligand-receptor interactions at the atomic level is of central importance in the optimization of drug candidates using structure-based approaches. This understanding can be greatly enhanced by the elucidation of the bound-state properties of ligands. Properties of interest include a description of the bound-state conformations and residual flexibility, as well as estimates of the bound fraction and lifetime. Liquid state NMR pulse sequences that involve isotope-filtering or isotope-editing can provide a powerful means for elucidating these bound-state properties. Accordingly, this presentation will focus on both recent developments and practical considerations in the use of such isotope-filtered and isotope-edited pulse sequences.

Monday Dinner Lecture

The Tale of One Small Molecule

Ray Freeman
Cambridge University

For someone interested in NMR methodology, a nice, small, well-behaved molecule is all that is necessary. We have our particular favorite, and it has served us well over many many years. It has been used to illustrate several interesting NMR manipulations and even some original physics. We first acquired this minimalist paragon for the purpose of proving that one of Karplus' two theories of coupling constants must be erroneous -- a contentious topic in the early 1960's. Since then, one only hears about *vicinal* coupling constant curves.

While the "INADEQUATE" experiment for establishing the carbon framework of an organic molecule is well-known, its proton analogue is not often mentioned. This is because proton systems do not provide nice, neat, isolated pairs of spins like carbon-13. Nevertheless, by the trick of employing a 135° flip angle for observation, these double-quantum filtered proton spectra can be simplified, and then they resemble INADEQUATE spectra from carbon-13.

Multi-dimensional spectroscopy is now endemic in the NMR community, but just occasionally, certain kinds of information are best extracted by the alternative of selective pulse excitation. Good results can be obtained by pulse-width modulated DANTE sequences, and it is demonstrated here that simultaneous excitation at several different frequencies can also be achieved by interleaving such sequences.

Forbidden transitions can be excited with selective pulses, and this technique can be used to observe the spectrum of triple-quantum and combination lines in a three-spin system.

The most recent developments (still with our "mystery" molecule) involve attempts to use NMR as a quantum computer, specifically to implement the Deutsch-Jozsa algorithm to distinguish constant and balanced binary functions. Building a viable NMR quantum computer is a very tough challenge and we may have to wait for some technical breakthroughs before real progress is possible, but the problem can be turned around to exploit these new ideas of quantum physics to design useful new manipulations in traditional NMR spectroscopy.

Session 4. Applications of Flow NMR

Recent Advances in LC-NMR and LC-NMR-MS

Andreas Kaerner, Steven R. Maple
Anthony G. Borel, and David A. Jackson
Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, IN 46285

HPLC coupled to NMR has seen tremendous growth in recent years, especially in the pharmaceutical industry. A wealth of information can be obtained without the need for tedious off-line sample isolation. In a stopped-flow mode, an array of 2-D experiments can be performed to aid in structure elucidation. This methodology has been successfully used for the identification of drug metabolites, process contaminants, and a host of other analytes. The acquisition of 2D experiments usually requires increased loading on the HPLC column, which often leads to chromatographic peak broadening. Since the active volume of the flow-probe is 60 □L it would be advantageous to decrease the width of the chromatographic peak so as to reduce the NMR acquisition time. This can be accomplished by trapping peaks of interest on secondary columns and eluting off using a high percentage of organic mobile phase. Some 2D homonuclear and heteronuclear data sets acquired using column trapping will be demonstrated. The advantages and disadvantages of column trapping will also be illustrated. Often LC-NMR only tells part of the picture, typically mass spectrometry is also used for unambiguous structure identification. This leads to the in-line coupling of LC-NMR-MS to obtain all of the necessary information on one sample. The coupling of an ion cyclotron resonance MS to an LC-NMR will be demonstrated. This configuration allows for the on-line determination of the analyte exact mass, leading to the molecular formula, and affords additional valuable exact mass fragmentation information.

Session 5. Metabolism

Characterization of Novel Glutatione Adducts of the HIV-1 Non-Nulceoside Reverse Transcriptase Inhibitor DPC 961.

J. Robert Espina*, Abdul E. Mutlib, Hao Chen and John P. Shockcor
DuPont Pharmaceuticals Company
Drug Metabolism & Pharmacokinetics
Stine-Haskell Research Center
Newark, Delaware

The metabolism of DPC 961, a non-nucleoside reverse transcriptase inhibitor, has been studied by NMR and LC/MS. A novel reactive α, β-unsaturated cyclobutenylketone and subsequent glutathione adduct is a major metabolic product. This metabolite suggests the possible formation of an oxirene intermediate. In order to elucidate the mechanism of formation for this novel structure the alkyne carbons of DPC 961 were ^{13}C labeled, rats dosed and bile collected. Using ^{13}C LC-NMR we confirmed the proposed mechanism of formation.

Session 5. Metabolism

Structure Elucidation of the Primary Metabolite of the Neuromuscular Blocker GW280430A

Timothy Spitzer*, John Walsh, Mindy Reese, Caroline Stafford, Kevin Facchine, David Burinsky, and Scott Sides
Glaxo Wellcome, Inc., Research
Triangle Park, NC, USA.

Compound GW280430A is a neuromuscular blocker that has the highly desirable properties of quick onset and quick recovery. Since the short-acting nature of GW280430A is due to its rapid metabolism, we sought to determine the structure of the primary metabolite. LC/MS studies revealed that the primary transformation of GW280430A *in vivo* is its conversion to a cysteine adduct. Upon investigation, it was found that the cysteine adduct could be generated *in vitro* by allowing GW280430A to react with cysteine in an aqueous buffer. The synthetic material was found to be identical to the metabolite by LC/MS. Sufficient amounts of the cysteine adduct were produced to permit analysis by NMR.

Formation of the cysteine adduct of GW280430A involves loss of a vinyl chlorine and gain of a cysteine moiety. It was expected that the metabolite would have a structure analogous to that of the parent compound, with the cysteine attached at the vinyl position through displacement of the chlorine with sulfur. Both the regiochemistry and the stereochemistry of the double bond would have to be established, a difficult task given that neither NOE nor chemical shift arguments could establish the structure of the trisubstituted double bond. Fortunately, we were spared the effort; it soon became clear that the cysteine, obviously ignoring our expectations, had found a different way to react with GW280430A. This talk describes the spectroscopic evidence that led to the determination of the structure of the primary metabolite of GW280430A.

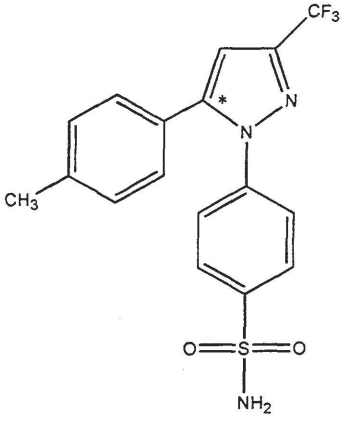
Session 5. Metabolism

Structures of Metabolites of [14C]Celecoxib in Healthy Male Volunteers.

Roy H. Bible, Jr.*(a), Elisabeth Hajdu (a), Susan K. Paulson (b),
Jeremy Hribar (a), Norman Liu (a), and Aziz Karim (c)
Departments of: Chemical Sciences Analytical(a)
Pharmacokinetics, Bioanalytical & Radiochemistry (b)
Clinical Pharmacokinetics and Bioavailability (c)
Searle, Skokie, Illinois

Celecoxib (Structure I), a cyclooxygenase-2 inhibitor, has recently been approved in the United States for relief of the signs and symptoms of osteoarthritis and rheumatoid arthritis in adults.

It has been shown that celecoxib is metabolized to the hydroxyl compound II, and that this compound is further oxidized to the carboxylic acid, compound III. Compound III is further converted to a glucuronide. In this talk we will discuss the NMR spectra of these metabolites, and will show how the position of attachment of the glucuronide was established.



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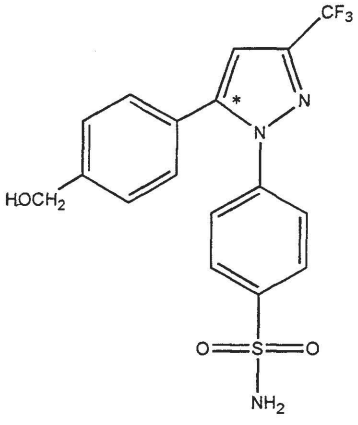

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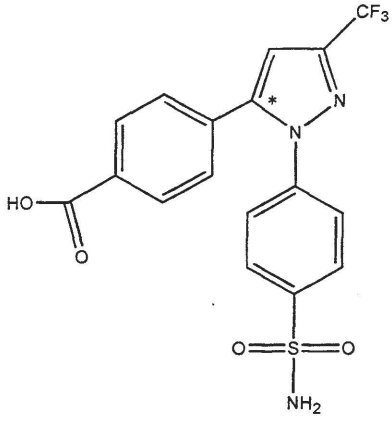

III

Session 5. Metabolism

NMR Spectroscopy and Toxicology

Professor Jeremy K. Nicholson,
Biological Chemistry, Biomedical Sciences Division,
Imperial College, University of London.

High frequency 1H NMR spectroscopy provides a rapid method of characterizing and quantifying a wide range of metabolites in untreated biological fluids and a powerful means of exploring the biochemical effects of drug-induced toxicological processes (1). 1H NMR spectra of biofluids such as urine and plasma are highly complex, containing signals from hundreds of metabolites that represent many key biochemical pathways. The 1H NMR-generated biofluid metabolite profiles are characteristically changed in different toxicological conditions according to the exact site and mechanism of the lesion. Thus interrogation of the biofluid NMR data can give direct diagnostic information and aid the detection of novel biomarkers of toxic effect. Recent advances in MAS-NMR spectroscopy also allow high resolution 1H data to be collected on small intact tissue samples, thus giving metabolic information that is complementary to those obtained from biofluid NMR. By use of computer pattern recognition (PR) methods, complex biofluid/tissue NMR data can be reduced and analyzed quantitatively to provide PR maps showing the physiological and toxicological effects of test xenobiotics in pre-development drug toxicity studies (2). Neural network, rule induction and other expert system approaches can also be implemented to give direct diagnostic outputs on toxicity type based on NMR input data. NMR-PR based diagnostics can be extremely sensitive for the detection of low level damage in a variety of organ systems and is potentially a powerful new adjunct to conventional toxicological procedures for lead compound selection in drug development programmes. With the recent advent of flow injection NMR technology, it is now possible to analyse hundreds of biofluid samples per day using one NMR spectrometer and to use NMR as a novel tool for toxicological screening. The NMR-PR and expert system approach to rapid in vivo toxicological assessment of drugs will be illustrated for a wide range of drug and toxin-induced lesions in both experimental animals and man and the relevance to the pharmaceutical industry discussed.

1. Nicholson, J.K. and Wilson, I.D. (1989) Progress in NMR Spectroscopy 21 449-501.

2. Holmes, E. Connor, S.C. Nicholls, A. Polly, S. Nicholson, J.K. Lindon, J.C. and Connelly, J. (1998) Chemometrics and Intelligent Laboratory Systems 44 251-261.

Session 6. Applications of NMR to High Throughput Screening and Combinatorial Chemistry

Jonathan M. Moore,
Senior Research Fellow
Vertex Pharmaceuticals
Incorporated

The SHAPES strategy is a method for lead generation in drug discovery that uses simple NMR techniques to detect binding of a limited, but diverse library of low molecular weight, soluble compounds to a potential drug target. SHAPES library compounds are derived largely from molecular frameworks most commonly found in known therapeutic agents. The NMR experiments used in these protocols are based on the well known techniques of differential line broadening and the transferred nuclear Overhauser enhancement (tNOE), and may be applied to targets with no limitation on molecular weight and no requirement for isotope labeling. SHAPES screening may be easily integrated into a discovery effort based on numerous biophysical and computational approaches. Following screening, weak binding (Kd ~ uM - mM) hits may be used to guide virtual screening of in-house or available compound databases, guide synthesis of combinatorial libraries, and bias the first compounds which undergo high throughput screening. Data derived from several in-house drug discovery programs indicate that high throughput screening hit rates for follow-up compounds chosen on the basis of an initial SHAPES screen are up to ten-fold higher than hit rates for compounds chosen randomly. Recent results from SHAPES screening with several enzyme targets as will be presented.

Session 6. Applications of NMR to High Throughput Screening and Combinatorial Chemistry

AutoDROP: a New Approach to Automated Structure Verification in Combinatorial Chemistry

P. Neidig & H. Schroeder

Multicomponent reactions are used to generate large numbers of compounds starting from a small number of building blocks which are combined in a systematic way. In contrary, compounds can be decomposed into molecular fragments and 2D HSQC spectra of the compounds can be regarded as the sum of individual sub-spectra of individual fragments. Sub-spectra are described as spectral patterns. Spectral patterns can automatically be defined by performing spectral algebra combined with peak picking and cluster analysis. They can be tested for existence in a spectrum by integration techniques. By testing spectral patterns it is possible to classify spectra of compounds (and thus underlying reactions) into three classes (true, false , unclear). Various result obtained with this new method are presented.

Session 6. Applications of NMR to High Throughput Screening and Combinatorial Chemistry

Applications of Flow NMR Spectroscopy to Monitor Binding of Small Molecules to Proteins

Kathleen Farley, Christine Schering, Edwin Steinbrecher,
Casey Zhou, and Brian Stockman

Numerous NMR spectroscopic techniques are currently available that detect binding of small molecules to protein targets, including targets identified using genomics techniques that lack a functional assay. Ligands with weak binding affinities, which might be overlooked in a traditional functional assay but yet might serve as templates for subsequent synthetic chemistry efforts, can potentially be identified. Flow NMR techniques can reduce the amount of time and effort required to evaluate small molecules for binding to a given target. Our experiences applying these methods to several proteins of interest will be described.

Session 6. Applications of NMR to High Throughput Screening and Combinatorial Chemistry

High Throughput Diffusion NMR for the Evaluation of Ligand-Receptor Binding

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The Pharmaceutical Research and Development process has changed significantly over the last few years. Perhaps most characteristic is the emerging trend that centers on the introduction of screening and high throughput analysis to as many R&D areas as possible. This model challenges the traditional application of NMR: a detail oriented technique that is time consuming, expertise intensive and therefore not suitable for high throughput studies. We have been working to evolve NMR into a higher throughput tool by 1) developing NMR Profile Methods that can provide information consistent with compressed discovery and development timelines, 2) tracking and managing the NMR data from these methods and 3) integrating these methods with other high throughput protocols. One NMR Profile Method that shows promise is diffusion coefficient measurements for the identification of lead candidates binding to target receptors. Key advantages of such an approach are the receptor does not need to be isotopically labeled (and assigned) and the size of the receptor is no longer a limiting factor. Furthermore, diffusion measurements are exquisitely sensitive to weaker binding (mM to sub uM) which may be critical when searching for initial leads. However, the application of high throughput diffusion measurements for drug binding presents some challenges. First, an understanding of conditions where these experiments succeed and fail is critical. Second, appropriate sample delivery systems (flow probes) along with high throughput diffusion coefficient measurements are required. Finally, the development of automated data reduction methods to identify hits coupled with procedures to capture and store information is key. Here we describe our efforts to develop and apply a high throughput diffusion NMR method to identify compounds that bind to a target receptor. Approaches such as this, along with the traditional detailed NMR studies bracket a continuum (less detailed and fast to more detailed and slow) that helps position NMR for increased flexibility allowing it to continue to play a critical role in Pharmaceutical R&D.

Tuesday Evening Dinner Lecture

Antediluvian Anecdotes of Sensitivity Enhancement Advances in NMR Spectroscopy

Tom Farrar
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Since the early days of NMR spectroscopy, sensitivity improvement has beena major concern. The Varian A-60 was the first commercial instrument used in routine, proton high resolution NMR measurements. For the A-60 a minimum of about 4.0 mg of sample in 250 microliters of solvent was required. Today, for proton NMR, samples as small as xxx ng in 50 microliters are run routinely. For carbon-13 NMR yyy ng samples in 50 microliters are now routine. A brief history is presented of the hardware and software work done to achieve enormous progress in sensitivity enhancement. Prognostications of further developments in this area are also given.

Session 8. Post Acquisition Data Processing, Databasing, and Spectral Prediction

Advantages and Limitations of Forward Linear Prediction

W.F. Reynolds (University of Toronto) and R.G. Enriquez (Instituto de Quimica, Mexico City)

Following up on our earlier report [Magn. Reson. In Chemistry, 35, 505 (1997)], we have further explored the advantages and limitations of F1 forward linear prediction for processing 2D data sets. We find no significant limitations for processing phase sensitive spectra with four-fold linear prediction being consistently reliable and more extensive linear prediction (up to 32 fold) often possible. This allows significant time saving. There are more problems and fewer advantages for processing absolute value spectra, due to more extreme weighting functions [sine bell or (sine bell)**2] which is usually used. However, four-fold linear prediction is possible, at a price in resolution, using a cosine function.

Session 8. Post Acquisition Data Processing, Databasing, and Spectral Prediction

Automated Structure Elucidation from 1D C-13 Spectral Data

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There have been a number of approaches to structure elucidation using NMR spectroscopy as the foundation spectroscopic technique. Recent approaches have generally used crosspeak responses from multi-dimensional NMR experiments together with classical structure generation from a given molecular formula. We report our recent approach for structure elucidation that can be initiated simply by using a basic 1D spectrum as the input set.

Our Structure Elucidator identifies the structure of an organic compound based on a 1D C-13 NMR spectrum. Suggested structures are generated from fragment overlap using the unique fragment-based rules system which is part of the our NMR prediction packages which have been successfully applied to the predictions of H1, C13, F19 and P31 spectra. Since any particular fragment has associated with it a set of C13 chemical shifts, and any spectrum is simply a superposition of spectral subsets with associated fragments, theoretically it is possible to extract suggested fragments with associated chemical shifts and build the full spectrum from appropriate overlap of these fragments. Using additional data including multiplicity data, quantitative intensities, H1 NMR spectral data, infra-red spectral data, mass spectroscopic data and the molecular formula the probability of extracting appropriate fragments and generating the final structure is greatly increased.

During the structure elucidation process the user can participate directly in the process of structure elucidation. The software will display a number of possible structures, with comparison of on-screen experimental and fragment spectra, or, in the case of failure, a set of structural fragments corresponding to portions of the spectrum which can then be used to help assemble the structure of the unknown compound. Using lists of included fragments and excluded fragments, assuming knowledge of the basic synthetic starting materials or partial identification of functional groups and fragments from the spectral data, the structure elucidation maybe biased and simplified.

The Structure Elucidator software also includes a self-training system: if the accuracy of spectral calculations for a new class of compounds is poor, then a user data base can be populated experimental chemical shifts to provide a fragment training set. Subsequently the data base can be used to make calculations for any new and related compound with the program automatically using the information from both the user data base and theinternal data base. This power alone enables this particular toolset to be focused on structure elucidation within particular structure classes, especially for those that are not yet reported within the public domain, a common occurrence in todays world of pharmaceutical and chemical research secrecy.

The structure elucidator tool offers the opportunity to process and manipulate NMR, IR and MS data directly from spectrometers using direct vendor formats. Using the processed spectra as the foundation for the structure elucidation the analysis itself can be performed without the provision of any structural information (known substructures or fragments). Under these conditions full elucidation can take from a few minutes to a number of hours depending on the complexity of the task. Using additional constraints such as known fragments and molecular formula elucidation can be performed much quicker.

Session 8. Post Acquisition Data Processing, Databasing, and Spectral Prediction

Data to Information, Information to Knowledge

D.E. Dorman
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Indianapolis, IN

Automation in the laboratory and on our instruments has revolutionized the way we collect spectra. The goal of this presentation is to show ways in which we can make other aspects of our research life more efficient.

Once the data are collected there are two remaining steps to the process of molecular structure elucidation. In the first step the raw spectral data are analyzed and resulting "information" tabulated and/or graphed. To a large extent this step involves "peak picking," a process which in the past involved the measurement of a peak position from a printed spectrum or a computer monitor, transfer of that information to handwritten reports or laboratory notebooks, often followed in turn by a keyboarding step in preparation of a final report. Each of these steps takes time and provides opportunity for error. We will show some ways to expedite this process and make it more error free at the same time.

In the second step this information is turned into "knowledge." For a molecular structure chemist, the simplest form of knowledge is a chemical structure. At present the most efficient way to do this is to use human experience to interpret the information resulting from the first step. This leads to a proposed structure, but there are sometimes occasions when this structure may not be the only one that fits the data. In such occasions it is useful to interact with a Computer Assisted Structure Elucidation (CASE) program, which can provide alternative structures or simply assure the human spectroscopist that he has adequately explored the structure space available. We will report some of our experiences with such programs over the past 25 years.

Session 8. Post Acquisition Data Processing, Databasing, and Spectral Prediction

Modeling Time-Domain NMR Data with Bayesian Statistics: An Alternative Method for Spectral Analysis

W. C. Hutton,
'Monsanto, St. Louis MO

For almost thirty years, the discrete Fourier transform (DFT) has been the standard tool for NMR data analysis. The DFT converts time-domain NMR data into frequency-domain spectra from which spectral parameters (i.e. frequencies, amplitudes, and decay-rates) can be subsequently extracted. Its computational efficiency has been crucial to the rapid growth and success of modern pulsed NMR spectroscopy. Because of the DFT's success, its inherent limitations, including sensitivity to baseline distortions and difficulties in resolving overlapped signals, particularly in low signal-to-noise spectra, are largely overlooked. However, these difficulties limit the accuracy of the parameters derived from NMR spectra and drive the recent development of alternate methods for analyzing NMR data.

Bayesian probability theory (BPT), is a fundamentally new method for the estimation of NMR spectral parameters. It is a statistical method, using probability theory to directly model time-domain NMR data. BPT gives significantly improved amplitude and frequency estimates on one-dimensional data, particularly under conditions of low signal-to-noise, broad peaks, and resonance overlap. Unlike other methods available for data analysis, BPT
provides error limits from a single measurement on all estimated spectral parameters . In this talk, the success and practicality of BPT in analyzing a variety of NMR data is demonstrated. When conventional data analysis (DFT) yields unsatisfactory parameter estimates, BPT (often combined with post-acquisition digital filtering) becomes the method of choice .

POSTER 1

Simulation of Exchange Lineshapes

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Many molecules studied by NMR show the effects of dynamics. These range from simple two-site exchange processes, readily described by the Gutowsky-Holm equations, to very complex coupled systems with multiple processes. In order to analyze these spectra, software is needed to simulate the systems as a function of rates.

Several recent developments have made this problem easier. Simpler theoretical formulations, more powerful computers, better computer languages (C), and more sophisticated numerical methods, have all meant that much more complex systems are now tractable. This poster describes some of the background behind the program MEXICO, and some applications to both liquids and solids.

POSTER 2

NMR Prediction Software and Tubeless NMR - an Analytical Tool for Screening of Combinatorial Libraries

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Tubeless NMR is quickly becoming the method of choice for the application of the Nuclear Magnetic Resonance technique to the analysis of combinatorial libraries. Coupling automation with flow NMR technology now allows NMR spectra to be acquired on materials populating a combinatorial plate in only a few hours. This routine acquisition of large amounts of spectral data can indeed increase the rate of throughput for such analyses but the technology can lead to an inordinate amount of data with no appropriate manner to track and database the information in a facile manner. Since the chemist can often offer suggestions for the structures expected for each vial on the plate it would be appropriate to attempt to relate the experimental spectra to those predicted for the structure. The development of software to allow the databasing of NMR spectral curves associated with molecular structures, and the application of NMR prediction algorithms to allow comparison of experimental and predicted spectra has been addressed. We will present a software suite, which allows the user to access and process NMR data directly from the spectrometer and display in a 96 well plate format. The spectral curves generated can be stored directly in a database and associated with chemical structures and user definable textual data fields. H1 NMR prediction algorithms allow predicted spectra to be generated for each of the suggested structures and displayed on screen for direct visual comparison with the experimental spectra. An analysis for matching experimental and predicted spectra can be performed based on the differences in shifts between the spectra. The obtained values are then displayed using color coding to display ranges for the match factors. The software allows the user to access time domain or processed NMR data directly from the spectrometer and bulk process a plate of experimental data using a group macro processing feature. Following processing the spectra automatically populate a database of proprietary or industry standard SQL format. In the database window spectra can be associated with chemical structures and up to 16,000 user definable text fields. A database built in such a manner can contain ca. 500,000 spectra, with or without associated structures. Such databases can be searched individually using search capabilities for structure, substructure, full spectrum, single or multi subspectra, and via textual fields describing spectral parameters and user data fields. Spectral, subspectral or multi-subspectral searching can be performed across the combinatorial plate data contained within the database. This method can be used for the searching of starting material spectra for example, as well as spectral responses associated with particular "functional groups" that can be detected by NMR. We will provide an overview of the toolset in its entirety.

POSTER 3

Multi-Layered RF Microcoils: Signal-to-Noise and Spectral Distortion Analysis

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The application of microcoil technology to mass-limited NMR spectroscopy recently has been shown to increase coil sensitivity (B_1/i) dramatically [1,2]. The tradeoffs between sensitivity and spectral resolution in microcoils also have been well-documented [3]. Furthermore, microcoils have been applied to multidimensional spectroscopy with less than 100 nanomoles of sample [4]. To this point, however, microcoil research involving high-resolution spectroscopy has been limited to a simple solenoidal geometry, mainly because of the superior homogeneity of the radio frequency (RF) field generated by this type of coil and the ease of fabrication. Further gains in coil sensitivity may be possible by improving upon this cylindrical geometry while maintaining its field uniformity. By adding multiple sensing layers to the solenoid, the sensitivity of the RF microcoil can be increased beyond the gains achieved solely by reducing its diameter. In addition, microfabrication techniques can be applied readily to specific multi-layered coils, decreasing the cost and difficulty of microcoil construction. In this presentation, the theory and limitations of the multi-layered microcoil design are developed. Numerical and analytical simulations of this design predict both the improved coil sensitivity and spectral distortion arising from magnetic susceptibility mismatches. To examine fabrication feasibility and provide experimental evidence of increased sensitivity, a series of similarly dimensioned microcoils (equal lengths and diameters of 850 μm) was constructed and tested at 500 MHz. This series compares the performance of simple wire solenoids, multi-layered wire solenoids and scroll coils, a novel solenoid-based geometry. The wire coils were fabricated with rectangular copper wire while the scroll coils were constructed from copper-clad polyimide patterned with micro-photolithography. Mirroring the predicted theoretical increase, the experimental data demonstrates a factor of 1.5 to 2 improvement in the signal-to-noise ratio (SNR) for multi-layered RF microcoils. This SNR improvement translates into lower limits of detection (LODs) or reduced acquisition times for small samples. Therefore, this new approach may be of particular use to the study of mass- or volume-limited samples, with special application to the burgeoning fields of combinatorial chemistry and in vitro biochemistry.

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POSTER 4

4-TOSYL-3,4,5,6-TETRAHYDRO-2*H*-PYRIDAZINO[2,3-a][1,3,6]TRIAZOCINES: An NMR-study of the dipolar properties and conformational behavior.

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Over the years chemists synthesised several bicyclic compounds containing a 3-aminopyridazine moiety fused via the N2-C3 bond of the pyridazinamine ring. Surprisingly a six-membered ring fused to the 3-aminopyridazine skeleton via the N2 and C3 ring atoms is the largest existing system. Although there is evidence in the literature that some research groups tried to synthesise larger systems, no positive results are published yet. We used a method based on a one step dialkylation reaction of 6-substituted 3-aminopyridazine in HMPA in order to obtain such hitherto unknown compounds. The use of HMPA as a solvent has already been described to be excellent for ringforming dialkylation of anilines[(1)]. Using this methodology we were able to prepare a series of 9-substituted 4-tosyl-3,4,5,6-tetrahydro-2*H*-pyridazino[2,3-a][1,3,6]triazocines.

9-Methoxy-4-tosyl-3,4,5,6-tetrahydro-2*H*-pyridazino[2,3-a][1,3,6]triazocine was chosen as a model compound. The structures of the free base as well as the hydrochloride are confirmed by ^{1}H-NMR, ^{13}C-NMR and DCI-MS. A full assignment of ^{1}H- and ^{13}C-NMR signals, shift values, relative integration values, $^{3}J_{H\text{-}H}$ - constants, COSY-, HETCOR- and long range HETCOR-spectra, will be shown. ^{1}H-NMR spectra of this 9-methoxy and other derivatives reveal that the hydrochlorides of these compounds have to be considered as aromatic 9-substituted-1,2,3,4,5,6hexahydropyridazino[2,3-a][1,3,6]triazocin-7-ium chlorides whereas the free bases are resonance hybrids of an analogous aromatic dipolar form and a neutral non aromatic form. The relative contribution of these resonance forms depends on the 9-substituent, and can be determined comparing the spectra of the hydrochloride and the free base. A dynamic NMR study of the mobility of the eight-membered [1,3,6]-triazocine ring, in the range of -50°C up to 140°C, will be discussed.

POSTER 5

Practical Aspects of a New Pulse Sequence for the Determination of Long Range Heteronuclear Coupling Constants, the geHSQMBC.

R. Thomas Williamson, Brian Márquez, and William H. Gerwick
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Long range heteronuclear coupling constants are invaluable to the stereochemical and conformational analysis of biologically active natural products. However, until now, a sensitive and convenient method for their measurement has not been generally available. As a result, application of $^{2,3}J_{CH}$ coupling constants to the configurational and conformational assignment of organic molecules has often been overlooked. In this presentation, we describe two new, gradient enhanced HSQC-based methods for the accurate determination of $^{2,3}J_{CH}$ coupling constants These experiments possess excellent sensitivity and are easily performed in a routine manner. The 1D and 2D geHSQMBC pulse sequences provide pure absorption lineshapes in *F2* for accurate measurement of long range heteronuclear coupling constants. In addition, this presentation will discuss practical aspects of the experiments including considerations for data acquisition, processing and analysis. Considering that the 2D geHSQMBC experiment is accumulated in time periods comparable to those required for the traditional HMBC experiment, but has the added advantage of yielding numerical values for heteronuclear coupling constants including their relative signs, suggests that it will find broad applicability in natural products structure elucidation.

POSTER 6

GRADIENT ENHANCED ID STRUCTURE ELUCIDATION METHODS

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The development of NMR paradigms, using indirect detection 2D methods, e.g., 1D proton and carbon spectra combined with 2D COSY, gHMQC/ HSQC, gHMBC spectra and computer assisted analysis programs specifically designed for novel structure building/searching (such as NMRSAMS), have dramatically streamlined the structure elucidation of natural and synthetic drug Asmall@ molecules (3-2 kD). Such methods, possibly including INADEQUATE or ROESY spectroscopy, work well if sufficient spectrometer and analysis time is available and overlapping resonances do not create ambiguities in data analysis. To approach the latter cases, there has been ongoing interest in 1D methods that are analogs of 2D or 3D spectra. These methods generally incorporate gradient techniques to improve selectivity and reduce artifacts. Recently we have investigated the practical implementation of several of these promising 1D methods which include gradient optimization, such as DPFGSE NOESY (A.J. Shaka) and 3D TOCSY or COSY type experiments (D. Uhrin), especially TOCSY-TOCSY. We are interested in fast 1D methods which not only allow connectivity determination but also fine structure analysis despite overlap of all peaks involved in a connectivity. Examples of these methods from practical work will be shown for cases in which the assignments are unambiguous and for cases in which overlap obscures both resonances in the proton 1D.

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POSTER 7

AROMATIC COMPOUNDS FROM *AGLAIA LAXIFLORA* (MELEACEAE)

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Cytotoxicity guided phytochemical analysis of the leaf extract of Malaysian plant *Aglaia laxiflora* lead to the isolation of known cyclopentatetrahydrobenzofuran derivative rocaglaol (**1**) and the aminopyrrolidine odorine (**2**), dehydroodorin (**3**) and odorinol (**4**) together with 5 new compounds: recaglamide glycoside (**5**), aglalaxiflorins A (**6**), B(**7**), C (**8**) and D (**9**). Compounds **6-9** posses a cyclopentatetrahydrobenzofuran skeleton linked to an odorinol type moiety. All the structures were elucidated notably by 2D NMR spectroscopy. In addition the structure of aglalaxiflorin A was established by X-Ray crystallographic analysis. The strong cytotoxic activity against P388 and Molt4 cell lines of the plant was corresponded to recaglaol (**1**) (IC_{50}<0.05) and its glycoderivative **5** (IC_{50}<0.05).

POSTER 8

Synthesis of Novel Pyrindine Derivatives from a Facile Reaction of 2,5-Bis-Arylidenecyclopentanone and Malononitrile

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Starting from 2,5-bis-arylidenecyclopentanone and malononitrile (1:1) five novel pyrindine derivatives were synthesized by a facile and convenient one-pot reaction using sodium ethoxide in absolute ethanol at room temperature. The structure of the reaction products was unambiguously deduced from their infrared, ^{1}H-nuclear magnetic resonance spectroscopy, elemental analysis and single crystal X-ray crystallography. Because of our continued interest in the condensation reaction of a,b-unsaturated Michael acceptors with active methylene compounds we expanded this study to cover the reaction of 2,5-bis-arylidenecyclopentanone with malononitrile using sodium ethoxide in absolute ethanol. The two starting materials were mixed and stirred at room temperature. As a result of this reaction, 2-ethoxy-6,7-dihydro-4-phenyl-7-(phenylmethylene)-5H-1-pyrindine-3-carbonitriles were obtained. Although we have not undertaken a mechanistic investigation of the reaction, a possible mechanism is suggested. Using this simple reaction, five novel pyrindine derivatives were prepared, isolated, purified, and characterized. The structure elucidation was based on the single crystal x-ray crystallography as well as on analytical and IR and NMR spectral data .

POSTER 9

Long-range Coupling: "W" Conformation in Check

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The well known W rule, which establishes that nuclei in a planar W arrangement exhibit significant four bond coupling constants, has been a useful tool in molecular structure determination since early times of the use of NMR spectra for this purpose. Many configurations and conformations have been decided based on this rule.

The continuous evolution of the NMR equipment, however, produces modifications in quality and number of available experimental data, thus forcing the chemists to frequent revisions of their points of view about the relative importance of the data that can be obtained from NMR spectra. The more recent equipment has a higher resolution and several additional features that throw some shadow over early concepts: on one hand, the high power of modern techniques such as NOESY and other multi-dimensional methods reduce the importance of coupling constants; on the other hand, it is now possible to determine many more coupling constants, due mainly to the higher resolution. A natural consequence is that the chemist can now exploit the use of subtle splittings in conformational analysis.

As part of our research work on the synthesis of natural products, we have prepared a number of cyclopentane derivatives. Our attention was strongly attracted to the long-range ($^4J_{HH}$) coupling constants that occurred in some of these compounds, as no planar W conformation seems to be possible in cyclopentanes. We have thus decided to start a more detailed study of the NMR spectra of these compounds, seeking for a more clear interpretation of the data.

We have first assigned all hydrogens, including the stereochemistry of each hydrogen, and measured all J values for the compounds; for this task we have used NMR spectra both of ^1H (300 MHz) and ^{13}C (75 MHz), NOE measurements, etc.

Bond angles and dihedral (torsion) angles were *calculated* with molecular mechanics programs; several different programs and methods were used to improve reliability.

The first result obtained is a confirmation that a $^4J_{HH} \neq 0$ occurs even in cases where a planar W conformation is not possible. More important, however, is the conclusion that there is a correlation between the $^4J_{HH}$ values and the involved dihedral angles. There are two dihedral angles in the path through the bonds between two hydrogens which show $^4J_{HH}$ coupling. When $^4J_{HH}$ values are plotted against ($\cos^2 \theta_1 \times \cos^2 \theta_2$) (a simple extension of Karplus equation) the points are not aligned over a continuous curve, but they show a clear tendency: $^4J_{HH}$ values become higher as the angles θ_1 and θ_2 vary from 90 to 180°.

POSTER 10

APPLICATIONS OF NMR IN FORENSIC DRUG ANALYSIS

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Abstract: DEA's primary responsibility is to enforce the Controlled Substances Act (CSA). DEA forensic chemists are tasked with the analysis of samples associated with this enforcement, and are normally required to perform all of the analyses on the exhibit assigned to them. This requires knowledge in the use of several instruments (microscope, FTIR, GC, GC-MS, HPLC, CE) to identify and quantitate the various components in a sample. NMR is one of the instruments available to the chemist. This presentation will give an overview of the applications of NMR in the analysis of illicit drugs.

POSTER 11

High Throughput Drug Binding Analysis by NMR Spectroscopy

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The introduction of flow-NMR systems presents industrial spectroscopists with the opportunity to develop high throughput methods. This is especially valuable in Pharmaceutical Research and Development where speed of analysis is emerging as a dominant consideration. However, in order to take advantage of fast sample delivery systems, it is necessary to develop NMR methods that can provide relevant information quickly. The traditionally slow turnaround time, as a consequence of extended data acquisition, processing and analysis prevents NMR from becoming a front line player in pharmaceutical screening efforts. We have been developing rapid NMR Profile Methods that can provide information compatible with the time constraints of discovery and development efforts. These methods, when coupled with rapid sample delivery systems, present an opportunity for NMR to participate in more front-line efforts. Here we describe our efforts to address rapid drug screening by NMR spectroscopy with a new algorithm based on diffusion coefficient and binding affinity correlation. Some exemplary applications will be presented along with the automation scheme.

POSTER 12

STRUCTURAL STUDIES USING GRADIENT INVERSE ^{15}N NMR SPECTROSCOPY

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Application of ^{1}H and ^{13}C NMR spectroscopy (including 2D) to the structure elucidation of organic compounds is well established. However, the study of nitrogen containing compounds can be significantly facilitated by ^{15}N NMR spectroscopy as a result of ^{15}N sensitivity to changes of structure and electronic arrangements. Direct measurements of ^{15}N NMR parameters (chemical shifts and coupling constants) are hampered by very low NMR sensitivity of the ^{15}N isotope. This limitation can be partially eliminated by application of inverse-detected NMR experiments, which have been lately improved by the introduction of magnetic field gradients.

Examples of applications of recently developed ^{1}H-^{15}N gradient techniques (GHMBC [1, 2], GSQMBC [3], IDR-HSQC-TOCSY [4]) for structural studies of isoquinoline alkaloids [5], purine derivatives [6], selenoureas [7], hydrazones [8] and other nitrogen containing compounds [e.g. 9] will be presented.

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POSTER 13

Identification and Quantitation of GHB by Nuclear Magnetic Resonance Spectroscopy

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This presentation addresses the technique of proton and carbon nuclear magnetic resonance spectroscopy (IH and I 3C NMR) for the identification of gamma-hydroxybutyrate (GHB) and its lactone, gamma-butyro-lactone (GBL). The application of 1H NMR for GHB quantitation is also discussed.

GHB is a central nervous system depressant and has been used as an anesthetic adjunct originally in Europe over thirty years ago. It is known to promote the release of growth hormones and was used for muscle growth and as a sleep aid among bodybuilders during the 1980s. In 1989, the FDA recalled L-tryptophan and GHB began to surface and was marketed as the replacement supplement. In the early 1990s, hospital emergency rooms across the United States reported numerous cases of GHB overdose and related poisoning episodes. As a result, this prompted the FDA ban on over-the-counter sales of GHB.

Ever since the ban, GHB has been manufactured clandestinely. GHB is currently popular for its hypnotic and euphoric effects and is frequently associated with the "rave party" atmosphere. Reported GHB-related deaths and sexual assault cases continue to soar.

Traditional means of identification of GHB involve using Fourier transform infrared spectroscopy (FTIR) or gas chromatography-mass spectrometry (GC-MS) employing derivatization techniques. Samples containing mixtures of both compounds and other impurities require cleanup procedures such as acid/base extraction followed by evaporation of excess water prior to FTIR determination. Analysis via these methods becomes potentially difficult due to the possible shifts in chemical equilibrium. It is forensically important to identify GHB as being present in the original sample since only GHB and not GBL, is controlled.

More recently, 1H and 13C NMR have been used successfully for GHB and GBL identification and to demonstrate sharp characteristic peaks that allow for definitive compound identification without altering the chemistry of the existing sample. The lH spectra of GHB and GBL each exhibit three principal resonance signals with very different chemical shifts. 13C NMR of GHB and GBL may also be used alone for spectroscopic identification. Each spectrum displays four carbon resonance peaks, which are unique and specific.

Other methodologies available for the quantitation of GHB could be potentially problematic since GHB is extremely hygroscopic and can absorb enough moisture just from the air to convert it from a powder into a slush-like material.

This presentation will address GHB quantitation using NMR spectroscopy. This technique does not require the use of a GHB standard. A 1H NMR spectrum of the compound sample in D20 is acquired with an internal standard such as 1,4-dioxane. This method provides a fast, simple and accurate quantitation without derivatization, extraction or the use of a GHB standard. NMR techniques, therefore, may be ideally suited for compound identification and quantitation of GHB in the forensic environment.

POSTER 14

APPLICATIONS OF CONTEMPORARY NMR TECHNIQUES TO THE IDENTIFICATION AND QUANTITATION OF SUBSTANCES OF ABUSE

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The Drug Enforcement Administration has eight laboratories that are charged with analyzing controlled substances in evidentiary samples. These samples can range from pure drugs to mixtures containing a variety of drug and non-drug additives. This work describes three areas where contemporary techniques have been used to solve analytical problems. The first is the identification of controlled substances in mixtures. For example, cocaine is often adulterated with a wide variety of substances. The use of 1H and 13C NMR provides easy identification. Secondly, the controlled substances present must be quantitated. This can readily be done with NMR. Lastly, NMR has been used to unambiguously determine the structure of controlled substance analogues. Examples of each area will be presented.

POSTER 15

Identification of Methamphetamine Synthesis By-Product Formed in Birch Reduction

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In recent years, clandestine laboratories have manufactured illicit d-methamphetamine via the Birch reduction of l-ephedrine and d-pseudoephedrine. Frequently, analyses of these illicit methamphetamine preparations reveal several by-products that differ slightly from methamphetamine in both GC retention times and mass spectra. These by-products, generally accepted to be the result of ring reduction, serve as chemical markers for this surprisingly elegant illicit synthesis. The major by-product typically found is the 1,4-cyclohexadienyl analogue of methamphetamine, which was synthesized, purified, and identified by NMR and other instrumental techniques in this work.

The "Birch reaction" is general terminology used to describe any of a number of dissolving metal reductions involving the action of active metals (usually group I) and anhydrous ammonia on a reducible substrate. In this particular incarnation of the procedure, which has become quite popular in clandestine laboratories, the intended product is not reduction of the ring, but rather of the benzyl alcohol. When the reaction is carried out with an additional proton source (usually hydroxylic), reduction into the benzene ring produces this "1,4-cyclohexadienyl" by-product. In controlled studies, ring reduction is only observed when a suitable proton source is present in the reaction pot. Since many clandestine laboratory operators extract the precursor from legitimate over-the-counter tablets with an easily obtained alcohol such as methanol or even water, it is believed that residual methanol or water in the precursor causes ring reduction. Given that these illicit laboratories do not operate under carefully controlled conditions, even atmospheric moisture can cause ring reduction. An interesting caveat to this work has been the lack of by-product formation when hydrochloride salts of l-ephedrine or d-pseudoephedrine are used in the absence of any additional proton source. These results strongly suggest that the suitability of the proton source with respect to ring reduction is a function of pKa. Studies are currently in progress to explore this hypothesis.

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POSTER 16

On-Line NMR Coupling Of A Commercial Microbore HPLC System With Microcoil NMR

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While NMR offers a wealth of chemical information, it normally requires fairly pure compounds to assay. Hence, the hyphenation of HPLC separations with on-line NMR detection is a powerful tool to analyze complex mixtures, drug metabolites, and impurities. LC-NMR experiments performed with microbore and capillary columns have improved separation characteristics and mass detection limits compared to LC using standard HPLC columns. The use of deuterated mobile phases becomes economical with the sub-millimeter columns and reduces the requirement for elaborate solvent-suppression techniques. Microcoil NMR probes with 100 nL to 1 μL volumes offer improved mass sensitivity compared to larger volume probes, and so offer advantages for the coupling of NMR with microseparations. We have integrated a commercial microbore HPLC system using a 0.5 mm × 150 mm C_{18} column with a 500 MHz proton solenoidal microcoil NMR probe that has an observe volume of 1 μL. Careful attention to capillary connections and NMR flow cell design prevents significant degradation of LC band shape. The on-line LC-NMR has been performed using mixtures of amino acids and small peptides with analyte injection amounts as small as 800 ng being detectable. By observing the beginning of an analyte band, the chromatographic flow can be stopped while the band remains in the detection cell, allowing longer NMR acquisitions. Stopped flow COSY spectra of the dipeptide Phe-Ala has been acquired in 3 hours with 5 μg injected amount and no more than 1 μg in the observe volume. With further improvements in low-volume interfaces, capillary scale LC-NMR will be possible for improved chromatographic resolution and mass sensitivities.

POSTER 17

Imaging Diffusion of Small Molecules in a Toroid Cavity NMR Probe.

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The inverse-distance dependence of the radiofrequency magnetic field (B1) in a toroid cavity detector has been used to image one-dimensional profiles of magnetization along the radial direction. The rotating-frame imaging technique introduced by Hoult in 1979 was the basis for recording these radial images. The application of a single radiofrequency pulse prior to the imaging sequence modulates the alpha and beta nuclear spin states, thus spin-labeling the molecules across the radial dimension. Stochastic and coherent molecular displacements scramble this labeling, and phenomenological or finite-difference analyses of the data give diffusion[1] and other transport numbers.[2] In this presentation, optimization of the toroid cavity detector, methods for data analysis and applications that involve small molecules will be discussed.

This work was supported by the U.S. Department of Energy, Division of Chemical Sciences, Office of Basic Energy Sciences, under Contract W-31-109-Eng-38.

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POSTER 18

The conformational study of N-acetylneuramic acid by Hetero 2D-NOE and relaxation time(T1)

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The hetero 2D-NOE is known as a measuring method which obtains proton and spatial range information between hetero atom. Authors synthesized the sample which 13C-enriched the N-acetylcarbonyl carbon of N-acetylneuramic acid(sialic acid) and measured 2D 1H-13C NOE spectra. This result shows that 7-OH proton and carbonyl carbon have done the hydrogen bond. And relaxation time (T1) of 7-OH was 0.29s. That about the half value was in comparison with other hydroxyl protons. The Proton T1 was measured on some sialyllsaccarides,and the structural calculation was carried out using distance geometry.

POSTER 19

Neighbouring Group Participation of the Indole Nucleus – An Unusual DAST Mediated Rearrangement Reaction Monitored by Low Temperature NMR Spectroscopy

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Treatment of (3*S*,4*S*)-3-hydroxy-4-(2-phenyl-1*H*-indol-3-yl)-piperidine-1-carboxylic acid benzyl ester (>90% e.e.) with diethylaminosulfur trifluoride gave (3*S*,4*S*)-4-fluoro-3-(2-phenyl-1*H*-indol-3-yl)-piperidine-1-carboxylic acid benzyl ester (>90% e.e.).

The stereochemical outcome of the reaction (assigned by X-ray crystallography) together with low temperature ^{1}H NMR experiments indicate the involvement of the spirocyclopropylindolenine species. This reaction intermediate was characterized *in situ* by DQFCOSY and HMQC experiments at –50 °C.

POSTER 20

LONG-RANGE DEUTERIUM ISOTOPE EFFECTS IN ^{13}C NMR SPECTRA OF SMALL AROMATIC MOLECULES

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In aromatic molecules the long-range deuterium isotope effects (LRDIE) on ^{13}C NMR chemical shifts can be observed. The magnitude and the sign of these effects alternate, depending on the distance between C and D atoms and position of D atom in aromatic ring. The pattern of the sign alternation of LRDIE parallels that of π-polarization.

Usually, heavy isotope substitution induces upfield shifts, but in the case of D both upfield and downfield shifts are observed. In *trans*- and *cis*-stilbenes and in 1,4-diphenylbutadiene LRDIE even through 10-12 bonds, as small as 0.1 Hz, were detected.[1]

Isotope effects on chemical shifts are interpreted in terms of vibrational and rotational averaging of nuclear shielding. The subtle changes of average bond lengths and average bond angles could be connected with deuteration. This is because of different zero-point vibrational motion and anharmonicity of C-H and C-D bonds.

We postulated that LRDIE arise from shorter C-D than C-H mean bond length at the site of deuteration and that this perturbations is transmitted to the remote site. Although bond reduction is small there are enough experimental evidences for it. We modeled the C-D bond by reduction of the corresponding C-H bond in the range 0.003-0.018 Å. For a series of aromatic molecules fully optimized geometry, nuclear shieldings (GIAO / TEXAS) and atomic charges (Löwdin / GAMESS) were calculated using different *ab initio* basis sets. Differences in shieldings and charges between protonated and deuterated molecules correlate very good with experimental LRDIE. On the basis of this model one can reproduce a majority of experimental LRDIE, even those through 10 and 12 bonds.[2]

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POSTER 21

Characterization of Biflavonoids and Flavonoids from *Leycesteria formosa* by NMR Spectroscopy

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The usefulness of NMR spectroscopy for the structure elucidation of flavonoids is today well established. 13C like 1H NMR measurements are decisive to determine not only the flavonoid moiety but also the position of substituents and of the interflavonoid linkage, in the case of dimeric compounds.

We report here an example of structural elucidation of monomeric and dimeric flavonoids, isolated from the leaves of *Leycesteria formosa* (Caprifoliaceae), Himalayan honeysuckle : luteolin and 4'-methoxyluteolin, podocarpusflavon A and amentoflavone. Complete 1D and 2D NMR spectra proved to be sufficient to identify less than 5 mg of pure natural products, without the help of derivatization nor ultraviolet and mass spectroscopy.

POSTER 22

Atranones, Novel 11-Membered Ring Secondary Mold Metabolites

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Abstract: Ten unique metabolites, atranones A to J, have been isolated from the toxigenic fungus Stachybotrys atra. These compounds contain several unusual features including an enol-lactone bridging a novel 11-membered ring. In addition, two new dolabellanes were isolated, suggesting a diterpenoid origin for the C-24 atranones. Biosynthetic pathways are also suggested for these atranones and certain eramantholide analogs.

POSTER 23

Proton NMR Predictions and Database Building

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Sample and time limitations for LC-NMR experiments often prevent the acquisition of carbon data necessitating the assignment of structural information from proton data alone. Proton NMR prediction software offers a convenient method to quickly assign the resonances for a structure, and to predict the chemical shifts of protons in proposed structures. Predicted spectra are generated by using a combination of algorithms and chemical shift information from a database of experimental data. Therefore, the accuracy of the prediction for each chemical shift is dependent upon the similarity of the fragment found in the database, and the acquisition conditions used to collect the spectrum for the assignments of that fragment.

In our work with ^{13}C NMR prediction software (ACD/CNMR™ and Chemical Concepts SpecInfo) we learned that the accuracy of predicted carbon chemical shifts is greatly improved by the creation of a small local database containing assigned chemical shifts for molecules similar in structure to the one of interest[1]. The same strategy is applied to our investigation of proton spectrum predictors. For this study we have limited our investigation to the benzene protons of substituted indole and benzothiophene structures whose resonances have been observed to be dependent upon substituent effects and solvent systems. We present data that demonstrate the utility of supplementing commercially supplied structure databases with locally determined information and the impact of alternate solvent systems.

[1] McCune et al, 39th ENC Pacific Grove, CA.

POSTER 24

Effect of Probe Tuning with PulseTune™

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Probe tuning is dramatically effected by changes in temperature and solvent. Although this phenomenon is well known, the process of tuning a probe is often neglected since the signal to noise ratio achieved for most spectra, particularly those for proton spectra, is sufficient without optimal tuning. However, when sample concentrations are limited or 2D experiments are being conducted sensitivity must be maximized and artifacts minimized for each sample. In order to efficiently achieve this goal, we recently purchased an automated probe tuning unit, Nalorac's Z·SPEC® PulseTune™. The Z·SPEC® PulseTune™ automatically adjusts the probe tuning and/or the observation nucleus in response to information it gathers from the rf pulse applied by the NMR console to the probe. It does this automatically, without user interference, and without compromising the applied rf pulses.

We have installed Z·SPEC® PulseTune™, on the proton channel of a Nalorac 3mm triple resonance PFG-ID probe on a Varian Unity Inova 500 system. Here we present initial experimental improvements obtained on samples in different solvents. The utility of PulseTune™ for experimental optimization is also shown for cases where sample heating occured.

POSTER 25

Application of LC-NMR and chemometrics for the identification of dietary induced changes in the urinary metabolite profile in the rat.

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1H NMR spectroscopic and pattern recognition (NMR-PR) analysis of biofluids has been demonstrated unequivocally to be an invaluable method for toxicological profiling, capable of identifying the site and/or mechanism of toxicity in experimental animals treated with tissue-specific toxins [1]. However, NMR-PR analysis of control rat urine has revealed the presence of significant physiological variance within a "normal" population of animals. This variability is attributable to factors such as genetic drift, age and diet. In the current work, two sub-populations of control animals were identified and further investigated using NMR-PR techniques. Principal components analysis of the NMR data was used to locate the spectral regions that differed between the two sub-populations and LC-NMR was used to identify the metabolites within these selected regions. Metabolites of chlorogenic acid (e.g. m-(hydroxyphenyl) propanoic acid were observed in only one of the sub-populations. These metabolites have been previously observed in rat urine following a change in diet affecting the gut microflora [2]. The combination of LC-NMR and NMR-PR enabled the identification of urinary metabolites of chlorogenic acid and may aid the understanding of the influence of gut microflora on the metabolism of xenobiotics.

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POSTER 26

High resolution magic angle spinning proton NMR (MAS NMR) spectroscopy of intact biological tissues.

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Characteristic spectral profiles for intact hepatic and renal tissue obtained from control rats were established using 1D (CPMG and NOESYPR1D) and 2D (TOCSY, JRES, HMBC and HMQC) 1H MAS NMR experiments. The effects of physical parameters such as rotor volume, rotor spinning speed and temperature were optimised. 1H MAS NMR spectroscopy was subsequently applied to the investigation of toxicological episodes.

2-bromoethanamine hydrobromide (BEA), a papillary toxin, was administered to rats and hepatic and renal tissues removed at 2, 4, 6 and 24 hours post dose (p.d.). 1H MAS NMR spectra were acquired for all samples and the metabolic consequences of BEA were evaluated. A decrease in the renal osmolytes GPC, myo-inositol and betaine were observed together with an increase in creatine concentration (24 h p.d.). Additionally, glutaric acid (GTA) was observed in hepatic and renal papillary tissue between 4 and 6 hours p.d. indicating the occurrence of a mitochondrial defect.

MAS NMR analysis of renal, hepatic and brain tissue following the administration of hydrazine, a hepatotoxin known to induce steatosis, showed marked changes to the biochemical composition of all tissues. In particular, alterations in the lipid profile of liver and the presence of 2-amino-adipate in the kidney cortex were noted. Data analysis and interpretation was augmented by the application of multivariate statistical analysis of the NMR spectral data.

1H MAS NMR spectroscopy of intact tissues offers a direct insight into the mobility and compartmentalisation of metabolites in biomaterials. In addition, the techniques ability to give an insight into the biochemical characterisation of chemically induced damage in tissues may lend insight in mechanism of drug toxicity.

POSTER 27

IDENTIFICATION OF CYANIDE TRAPPED METABOLIC INTERMEDIATES OF N-CYCLOPROPYLANILINES

Christopher L. Shaffer, Martha D. Morton, and Robert P. Hanzlik, Department of Medicinal Chemistry and NMR Laboratory, University of Kansas, Lawrence, KS 66045. This work supported by NIH predoctoral fellowship [GM07775].

The cyclopropyl amine substructure, found in numerous drugs and drug candidates, undergoes cytochrome P450-catalyzed oxidative N-dealkylation with the loss of the cyclopropyl group. Although some cyclopropylamines are suicide substrates for P450 enzymes, little is known about the fate of the cyclopropyl group lost and the reactive intermediates involved in P450 destruction. Much discussion and speculation has centered on radical generation via cyclopropyl ring opening subsequent to one-electron oxidation at the amine nitrogen. As a model for this one-electron oxidation process, we have investigated the oxidation of N-cyclopropyl-N-methylaniline (1) and its 1'-methylcyclopropyl analog (2) by horseradish peroxidase (HRP), a known one-electron hemoprotein oxidant. Oxidation of 1 and 2 bye HRP leads exclusively to N-methylaniline (NMA). When cyanide is present (1-2 mM) during incubation as a radical scavenger, 1 and 2 each give a new unique acid-stable product, whose mass corresponds to [parent - H + CN]. NMR studies revealed the cyanide trapped product from the HRP oxidation of 1 to be of N-methyl-2-cyan0-1,2,3,4-tetrahydroquinoline [3].

POSTER 28

Application of Accordion-Optimization to 1H-15N Long-Range Heteronuclear Shift Correlation Experiments at Natural Abundance

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Accordion excitation provides the means of sampling a broad range of potential long-range heteronuclear couplings in a single experiment. Two long-range heteronuclear experiments, ACCORD-HMBC and IMPEACH-MBC, have been developed thus far that offer accordion excitation. The results obtained with these experiments for 1H-15N heteronuclear shift correlation at natural abundance are compared. Low digitization in the second (F1) frequency domain of 1H-15N of ACCORD-HMBC potentially hampers this use of the experiment since it is prone to coupling modulation in the second frequency domain as a function of the accordion excitation delay. The constant time variable delay used in lieu of a simple accordion delay in the IMPEACH-MBC experiment circumvents homonuclear coupling modulation in F1, thereby improving resolution relative to the ACCORD-HMBC experiment for the same number of increments of the evolution period. Minor differences are found in the responses that are observed in each experiment. Finally, results obtained using accordion-optimized experiments are contrasted to the conventional GHMBC experiment, which can only be statically optimized. Accordion-optimized experiments offer a potential advantage for 1H-15N long-range heteronuclear shift correlation experiments due to the wide variability of long-range 1H-15N couplings.

POSTER 29

New Accordion-Optimized Long-Range Heteronuclear Shift Correlation Techniques:IMPEACH-MBC

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ACCORD-HMBC advantageously allows a broad range of potential long-range heteronuclear couplings to be sampled by a single experiment. The variable duration delay used to accomplish "accordion"-optimization serves as a third, pseudo evolution time for homonuclear coupling processes. The evolution of homonuclear couplings during the variable duration delay leads to "skewing" of responses in the second frequency domain, F1. This inherent disadvantage of accordion-optimization is suppressed by the utilization of a constant time variable delay in the IMPEACH-MBC (IMproved PErformance ACcordion Heteronuclear Multiple Bond Correlation) experiment. Details of the constant time variable delay and the IMPEACH-MBC pulse sequence will be presented. The ability of this novel pulse sequence element to suppress the F1 skew of long-range responses characteristic of the ACCORD-HMBC experiment is demonstrated using the alkaloid strychnine as a model compound.

POSTER 30

Varian $^{1}H\{^{13}C/^{15}N\}$ Flow-Probe Performance at 600 MHz

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Coupling of HPLC to NMR has only been practical in the past several years due in part to advances in probe technology. During the earlier years of LC-NMR, the overloading of chromatographic columns was usually necessary to obtain sufficient signal from the analyte of interest in a reasonable amount of time. Current probe configurations have substantially improved signal to noise values such that column overloading is less prevalent. In fact, the newer designs allow for the acquisition of 2D homonuclear data in less than 6 hours for 44-60 nmoles (11-15 μg) of sample in the active coil volume (60 μL). Indirectly detected heteronuclear data such as ^{1}H-^{13}C HSQC can be acquired in about 19 hours. Some data acquired at 600 MHz using the newest Varian $^{1}H\{^{13}C/^{15}N\}$ flow probe will be demonstrated along with some of the standard specifications obtained.

POSTER 31

HETERONUCLEAR NMR STUDIES ON AQUEOUS HEPES BUFFER SOLUTIONS

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We wish to report the pH dependence of the 35Cl NMR of aqueous sodium chloride-HEPES solutions. This study, an outgrowth of our earlier work on the pH dependence of the 33S and 17O NMR of aqueous HEPES solutions, provides independent evidence that both titrateable protons from HEPES come from ammonium ions. Even in concentrated HCl, the 33S NMR of HEPES showed none of the broadening which would have been expected if the sulfonate was even partially protonated. We will discuss the general utility of quadrupolar NMR in such studies.

POSTER 32

Further Prenylated Benzophenones from Caribbean Clusia species (Guttiferae).

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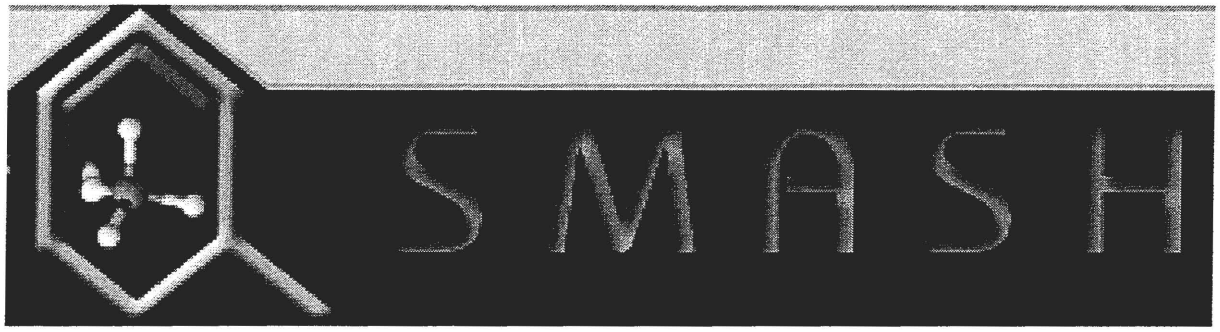
We have recently reported the structure elucidation of 9 prenylated benzophenones. These have unusual structures, including one with an adamantyl core structure. Compounds of this class are of considerable interest in view of their biological activity. Here we report 4 further compounds of this class which we have characterized by 2D N.M.R.



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