THE NMR NEWSLETTER

No. 456

September 1996

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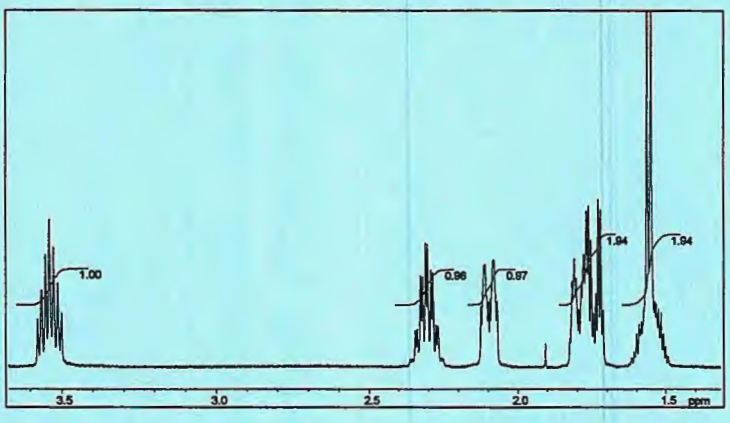

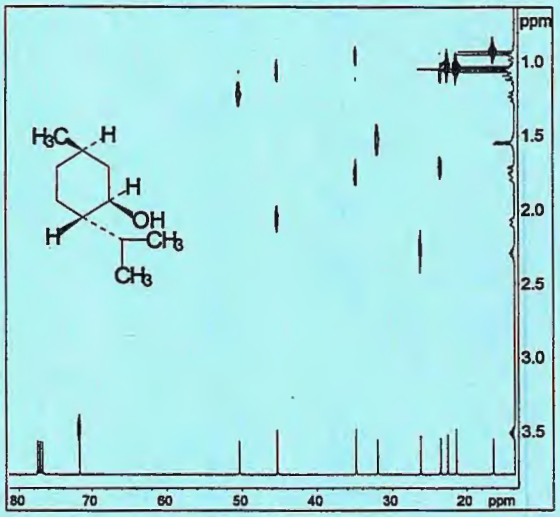

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FORTHCOMING NMR MEETINGS

Missouri Magnetic Resonance Symposium (MMRS) and FACSS Meeting, Kansas City, MO, **Sept. 29 - Oct. 4, 1996**; Contact: (MMRS) Frank D. Blum, Dept. of Chemistry, Univ. of Missouri-Rolla, Rolla, MO 65409-0010; 573-341-4451 fblum@umr.edu. (FACSS) 198 Thomas Johnson Dr., S-2, Frederick, MD 21702-4317.

50 Years of NMR At Stanford, An International Symposium, Stanford, CA, **October 4, 1996**; Contact: Robin Holbrook: Tel: 415/723-6270; Fax: 415/723-2253; Email: holbrook@camis.stanford.edu; http://cmgm.stanford.edu/SMRL/50.html. See Newsletter 455, 38.

38th ENC (Experimental NMR Conference), Orlando, FL, **March 23 - 27, 1997**; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87505; (505) 989-4573; Fax: (505) 989-1073.

Symposium on NMR Spectroscopy of Synthetic Macromolcules, ACS National Meeting, San Francisco, **April 13-17,** 1997; Contact: H. N. Cheng or English, A. D. See Newsletter 456, 20.

4th International Conference on Magnetic Resonance Microscopy "Heidelberg Conference in Albuquerque", **Sept. 21-*25, 1997**: Contact: E. Fukushima, The Lovelace Institutes, 2425 Ridgecrest Drive SE, Albuquerque, NM 87108-5127; (505) 262-7155; Fax: (505) 262-7043. See Newsletter 449, 37.

Additional listings of meetings, etc., are invited.

****Please note corrected date!***

CENTRE FOR NUCLEAR MAGNETIC RESONANCE

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PROF. RAY DUPREE

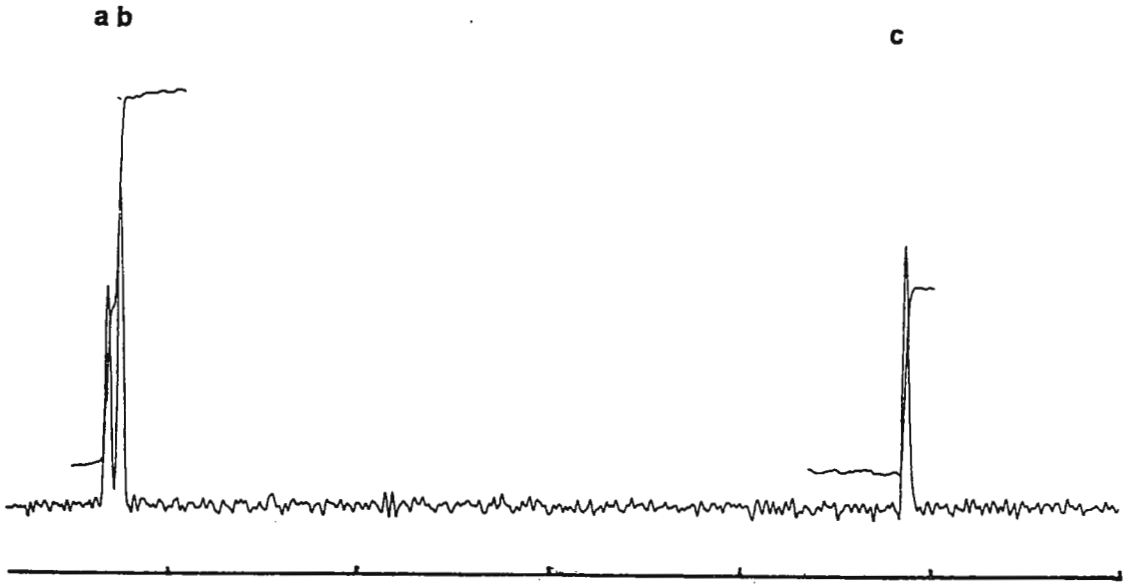
19th August 1996

(received 8/24/96)

Abnormal Deuteriation Shifts and a Better Mousetrap?*

Dear Barry,

In our efforts to apply NMR to natural fats and oils, we have been looking at chirally deuteriated triacylglycerols, in collaboration with Ian Chandler and Professor David Crout. We have made trilaurin from glyceraldehyde that has been selectively monodeuteriated, to 83%, at the *sn*-3 methylene only. We were anticipating a detectable shift at the *sn*-3 ester CO, but we did not expect it to be to high frequency. Deuterium usually has the same shift effect as lower temperature, i.e. a tightening of the electron cloud in the CH/D bond, and hence more shielding. But in this case the shrinkage of the bond reduces the γ-*gauche* shieldings at the CO bonds, thus increasing δ_{CO}.



^{13}C spectrum (CO region only) of selectively deuteriated trilaurin.
Axis markers at 0.1 ppm (10 Hz) intervals.
a 3-CHDOCO (84% D) **b** (1+3)-CH_2OCO **c** 2-CHOCO

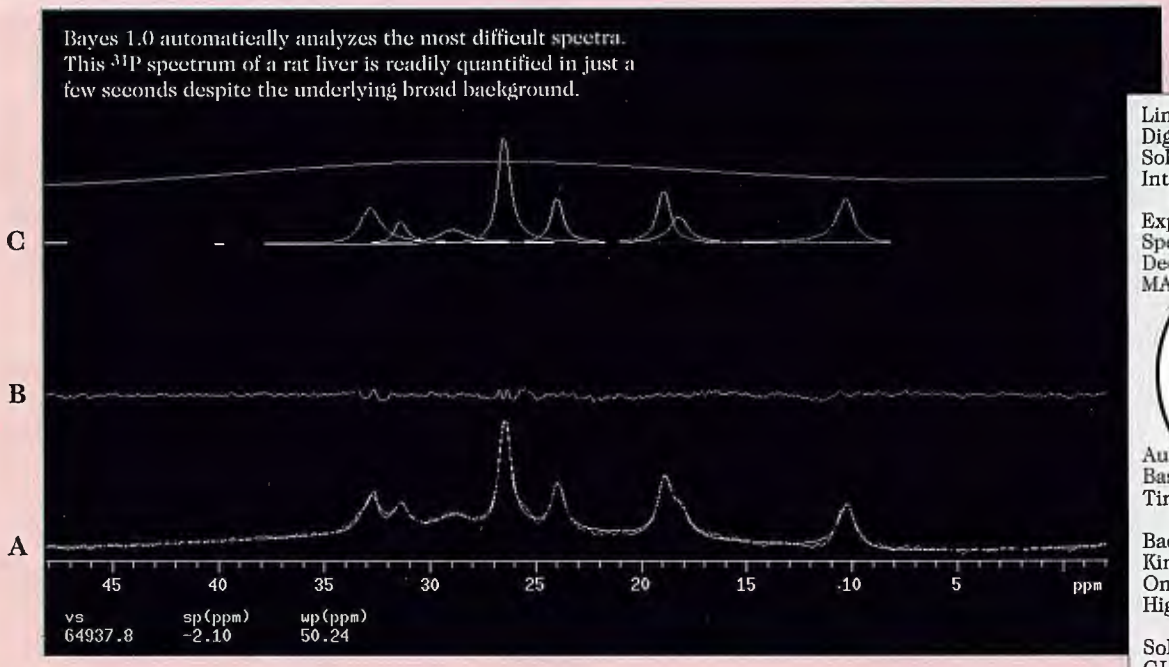
Needless to say, we are doing all this for a reason. You may remember that in an earlier letter I showed how the ester CO shifts are also sensitive to which acyl chain is attached at that position. If we combine this with our selective labelling, we have a new and widely applicable method for investigating the stereospecificity of any lipase enzyme, either as an agent for hydrolysis or for transesterification. This in turn should lead to better product control in the food industry, for the properties of a fat depend on its chirality.

Best wishes,

Oliver

*for the benefit of readers unaware of the minor privations of World War 2, 'Mousetrap' was our generic term for the weekly rations of unbranded hard cheese. Their size and taste were better suited to *mus musculus*.

Results You Can Believe In



A Experimental and calculated spectra
B Difference
C Individual resonances calculated by Bayes 1.0

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New Bayes 1.0 Software Automates 1D Analysis

Bayes 1.0 software, the result of a collaborative effort between Washington University, Monsanto, and Varian, revolutionizes NMR spectral analysis. Bayes 1.0 is a unique tool which quantifies analysis of time domain data quickly and easily, ensuring reliable results. The first software to implement Bayesian analysis for 1D FIDs, Bayes 1.0 will take a postulated model, fit it to the experimental data, and show the correlation between observed and calculated results — all at the click of a mouse!

Bayes 1.0 is ideal for robust quantitation of noisy or overlapped peaks, for automatic analysis of large data sets, or for determining how well your model fits the data. Available as an add-in to Varian's VNMR™ software, Bayes enhances signal amplitude, frequency, and line width analyses.

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The advantages are clear:

- Believable results
- Quantifies analysis of time domain data
- Enhances signal amplitude, frequency, and linewidth analysis
- Available as an add-in to VNMR software

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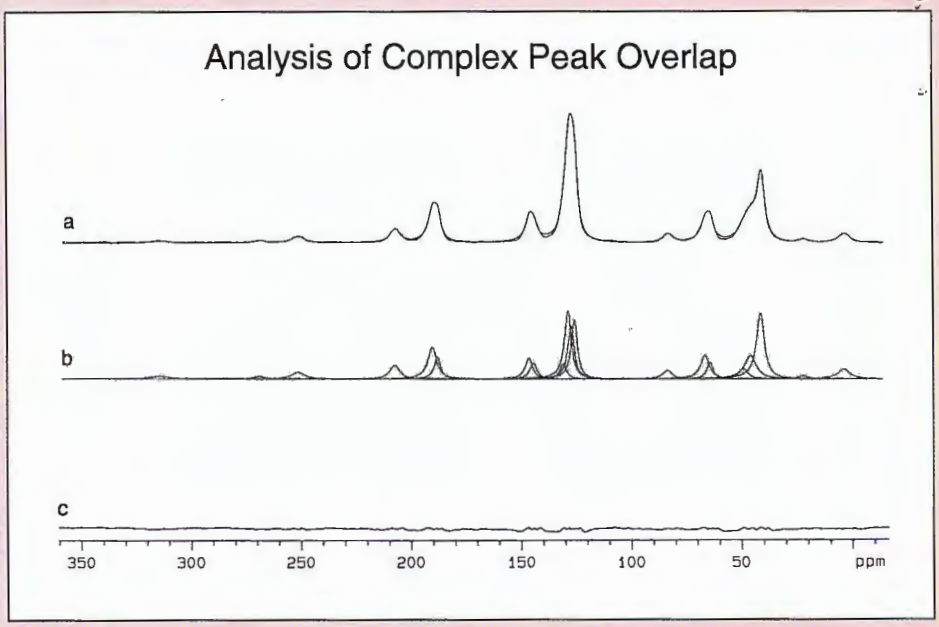
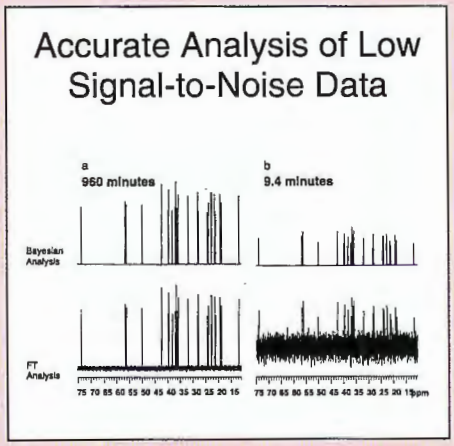
Bayes 1.0 Applications

I.
a. Overlay of Bayesian-modeled and experimental ^{13}C CP/MAS spectra of polystyrene, b. Bayesian analysis of the FID of polystyrene, showing individual modeled resonances in the frequency domain, c. Difference spectrum from subtraction of FT spectrum from FT of Bayesian-modeled spectrum.



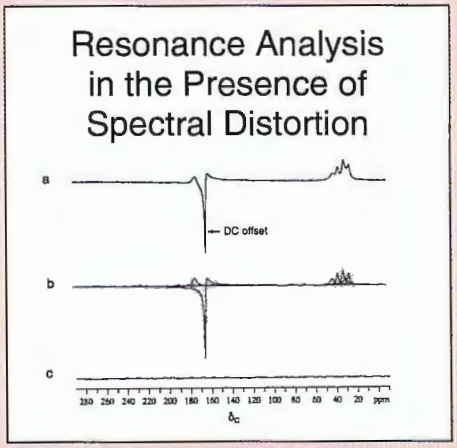
I

II.
a. ^{13}C FT spectrum of cholesterol, 8192 transients, total experiment time 960 minutes (bottom) and Bayesian-modeled spectrum (top), b. ^{13}C FT spectrum of cholesterol, 80 transients, total experiment time 9.4 minutes (bottom) and Bayesian-modeled spectrum (top), demonstrating the accuracy of Bayesian analysis even under low signal-to-noise conditions.



II

III.
a. Overlay of Bayesian-modeled and experimental 32 MHz ^{13}C CP/MAS spectra of Nylon 6,6, b. Bayesian analysis of the FID of Nylon 6,6, showing individual modeled resonances in the frequency domain, and demonstrating accurate modeling of resonances in the presence of a strong DC offset, c. Difference spectrum from subtraction of FT spectrum from FT of Bayesian-modeled spectrum.



III

Spectra provided courtesy of Dr. W. C. Hutton, Monsanto, Co.

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Cornell University

Department of Chemistry
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Cathy C. Lester
August 1, 1996
(received 8/8/96)

Dr. Barry Shapiro, Editor
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

Water Suppression for TR-NOESY with the DPFGSE Technique

Dear Professor Shapiro,

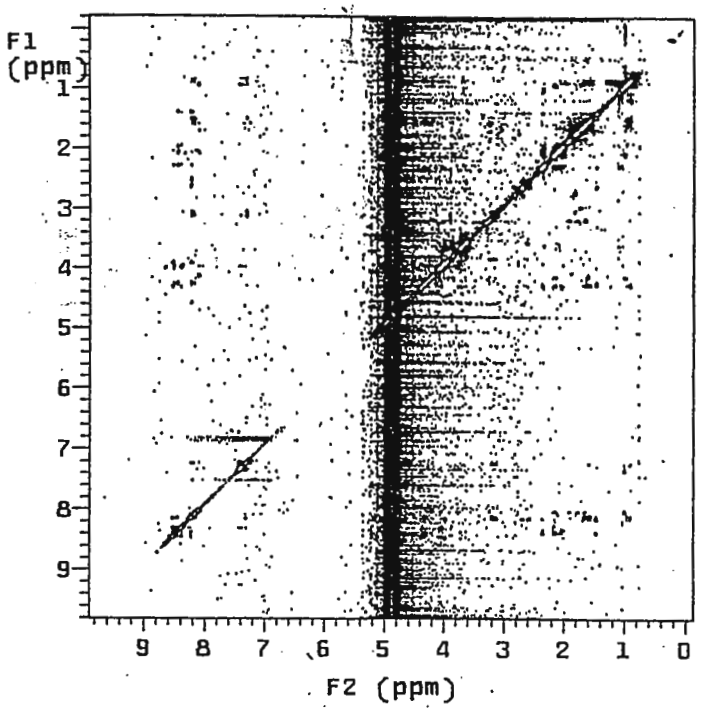
We utilize the transferred NOESY(TR-NOESY) experiment for studying the conformations adopted by fibrinogen-like peptides when bound to native and recombinant-mutant thrombin. These experiments typically are applied to systems containing a low concentration of enzyme in the presence of a large excess of ligand. The experiment can be a convenient tool for examining solution structures of bound ligands when the target enzyme is either too large for standard multidimensional NMR methods or too difficult to obtain in large quantities; however, the procedure does require the peptide to exchange rapidly between the free and bound states. During this exchange process, information about the spatial proximity of protons in the bound state is transferred to the resonances in the free state. The experiment is carried out with the standard NOESY pulse sequence, and crosspeak intensity is approximately equal to a population-weighted average of the cross-relaxation rate for resonances in the bound state. In the presence of a large excess of ligand, crosspeaks can be relatively weak, particularly in solutions containing 90% H_2O/10% D_2O.

Recently, we have applied the double pulsed-field gradient spin echo(DPFGSE) technique developed by Shaka and coworkers(1) to suppress the water signal in TR-NOESY spectra of the fibrinogen Aα-like peptide (^{1}ADSGEGDFLAEGGGVRGPRV^{20}) in the presence of native bovine thrombin. The pulse sequence consists of application of the WATERGATE(2) water-suppression scheme twice before acquisition of the spectrum. We present two NOESY spectra below that demonstrate the improvement in water suppression obtained with the DPFGSE technique relative to suppression by selectively saturating the H_2O resonance during the 1.5 second preacquisition delay and during the mixing time. The significant improvement in water suppression permits us to use the dynamic range of the digitizer more efficiently and to increase the receiver gain by about 30 dB.

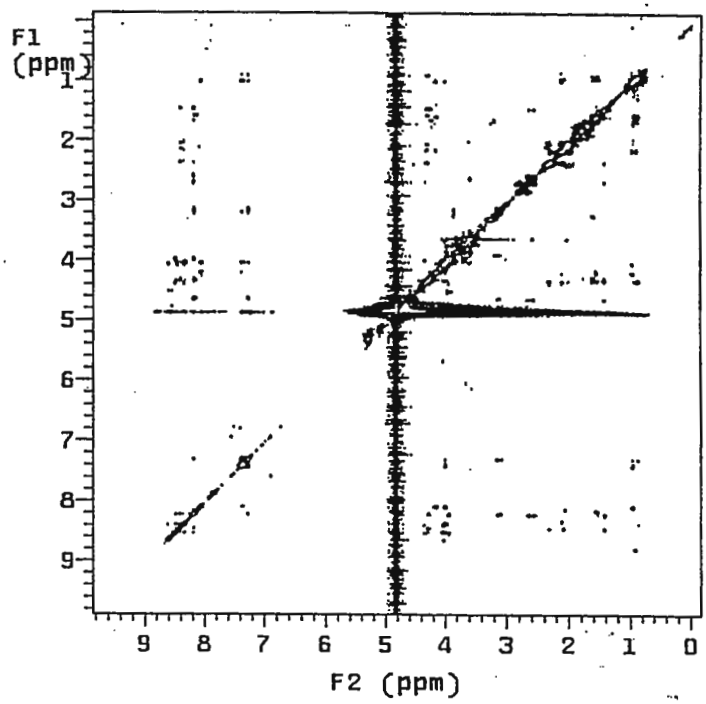
(1) Hwang, T.-L. and Shaka, A.J. (1995) *J. Magn. Reson. Ser A* **112**, 275-279.
(2) Piotto, M., Saudek, V. and Sklenar, V. (1992) *J. Biol. NMR* **2**, 661-666.

Spectra A and B: Phase-sensitive transferred NOESY spectra of human fibrinogen Aα-like peptide (^{1}ADSGEGDFLAEGGGVRGPRV^{20}) in the presence of native bovine thrombin acquired on a Varian Unity 500 spectrometer. The concentration of peptide was 1.4 mM and enzyme was 0.14 mM in a 90% H_2O/10% D_2O aqueous solution containing 25 mM Phosphate buffer, 150 mM NaCl and 0.2 mM EDTA at pH 5.5. Acquisition parameters were as follows: 2048 complex points in t_2 and 512 increments in t_1, a spectral width of 5000 Hz, and a mixing time of 400 ms. Water suppression for spectrum A was achieved by selectively saturating the H_2O resonance for 1.5 seconds during the preacquisition delay and during the mixing time. Water suppression for spectrum B was achieved with the DPFGE technique using the following sequence in place of the last pulse in the standard NOESY sequence: 90_x - G_1 - $90_{sel(-x)}$ - 180_x - $90_{sel(-x)}$ - G_1 - G_2 - $90_{sel(-x)}$ - 180_x - $90_{sel(-x)}$ - G_2 - acquire. The 90_{sel} pulses were 2.8 ms applied at the H_2O resonance frequency. G_1 had an amplitude of 11 G/cm applied for 2 ms and G_2 was 5.5 G/cm with a duration of 1 ms.

Spectrum A



Spectrum B



Sincerely,

Cathy C. Lester

Muriel C Maurer

Harold A. Scheraga

Cathy C. Lester

Muriel C. Maurer

Harold A. Scheraga

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Laboratorium für Physikalische Chemie
Prof. Richard R. Ernst

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Fax ++0041 1 632 10 21
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CH-8092 Zürich
E-mail: ernst@nmr.phys.chem.ethz.ch

Zürich, August 20, 1996
(received 8/24/96)
2515

Prof. Dr. Bernard L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303
U.S.A.

High-Pressure NMR of Proteins

Dear Barry:

We are currently investigating partial unfolding and refolding of proteins under the influence of pressure. For this purpose, we constructed for a wide-bore 300 MHz magnet a high pressure NMR proton probe operating in the range between 1 and 5000 bar which is connected to a Bruker MSL console. The probe design follows loosely blueprints kindly provided by Prof. Jiri Jonas.

In most high-pressure NMR probes the capacitors of the resonance circuit are placed outside the high-pressure vessel. This requires relatively long rf transmission lines of highly unmatched impedance leading to a decrease of the probe sensitivity. The problem can be circumvented by incorporation of a capacitor and an additional tuning coil inside of the high-pressure vessel. We found that a ceramic chip capacitor (type ATC 100B) is not affected by our pressure transmission liquid CS_2 even after two years of operation. This device allowed us to lower the impedance and to minimize losses in the rf feedthroughs (see Figure 1). While in the traditional design inductive tuning is needed outside of the vessel, the new design allows for capacitive tuning. The signal-to-noise ratio for a 1 mM ubiquitin sample at 5 kbar is thereby improved by more than a factor of two.

At present, we are studying in collaboration with Dr. Bernhard Brutscher and Prof. Joshua Wand (University of Buffalo) partially unfolded states of ubiquitin under various pressure, temperature, pH, and solvent conditions. In Figure 2, the H^N-$H^{\alpha,\beta}$ region of a 2D TOCSY experiment of a partially folded form of ubiquitin in 80% H_2O and 20% methanol at 4 kbar is shown. It displays a characteristic collapse of chemical shift dispersion of the H^N resonances.

Best wishes,

M. Schick R. Brüschweiler R. R. Ernst

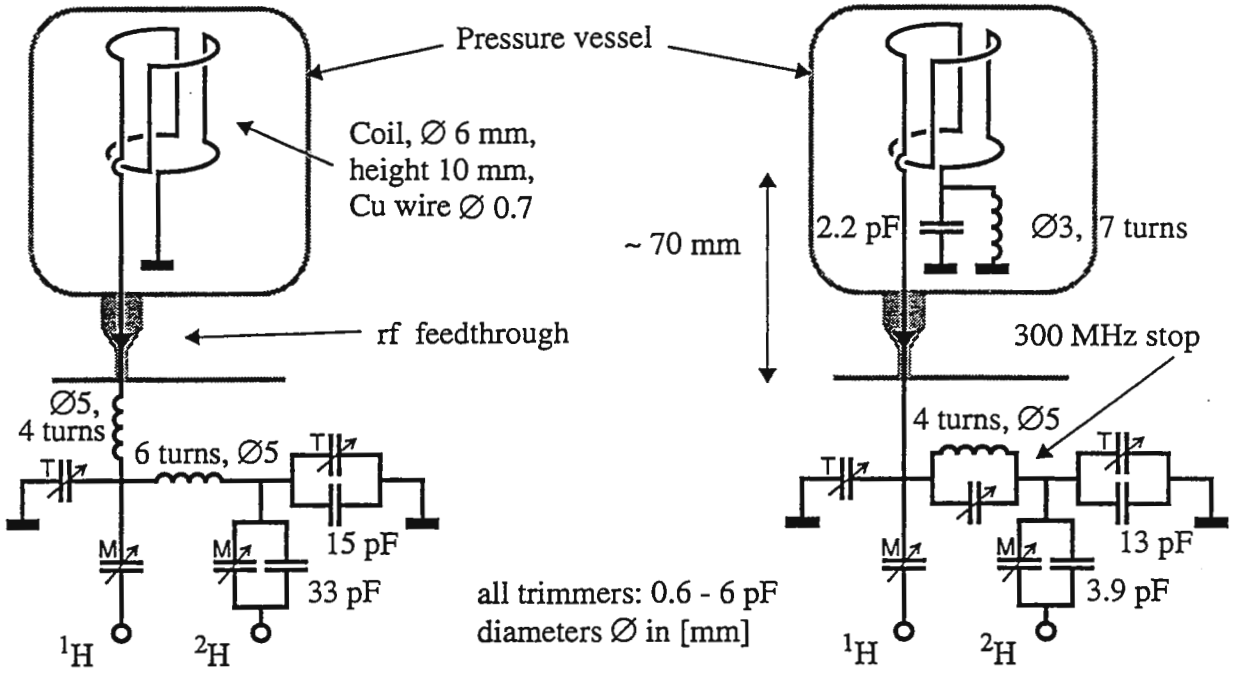

Figure 1: (a) Tuning and matching network with all elements outside of the pressure vessel. (b) Improved version with a ceramic chip capacitor and a tuning coil inside of the vessel.

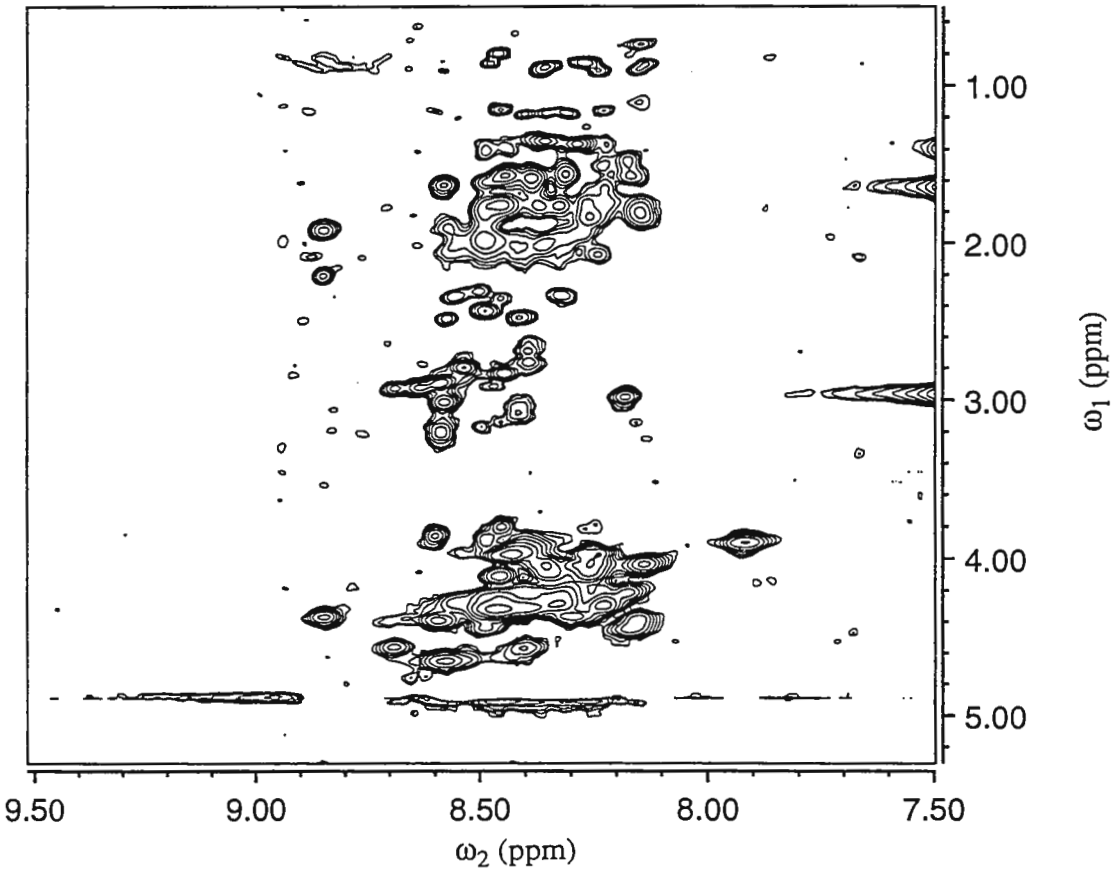

Figure 2: H^N-$H^{\alpha,\beta}$ section of 2D TOCSY of 10 mM ubiquitin in 80% H_2O and 20% methanol-d_3 at pH 2 at a pressure of 4000 bar and a temperature of 300 K. DIPSI-2 was used as mixing sequence with a mixing time of 50 ms and water suppression was achieved by presaturation.

The NMR Revolution advances...

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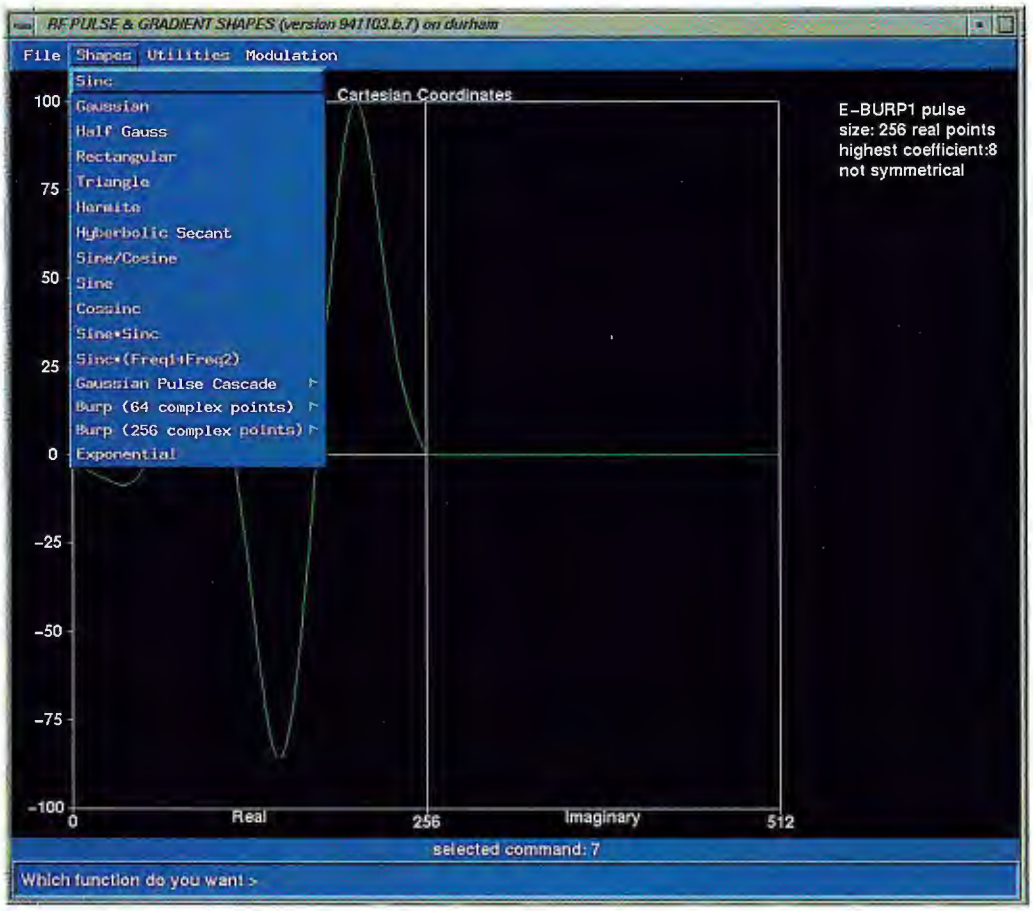

Figure 1: Select a shape and specify the parameters

Bruker's new NMR software package, *XWIN-NMR*™, offers a windows-based utility for creating RF pulse shapes, called *xShape*.

- Enter *xShape* and select a shape from a list of predefined options.
- The program prompts you to enter appropriate parameters to completely define the shape.
- Write the shape to a file for use by the *XWIN-NMR*™ software.

- Finish by inserting the shape into your pulse sequence using the file that was written in *xShape*. This is easy and convenient for both routine and advanced applications.

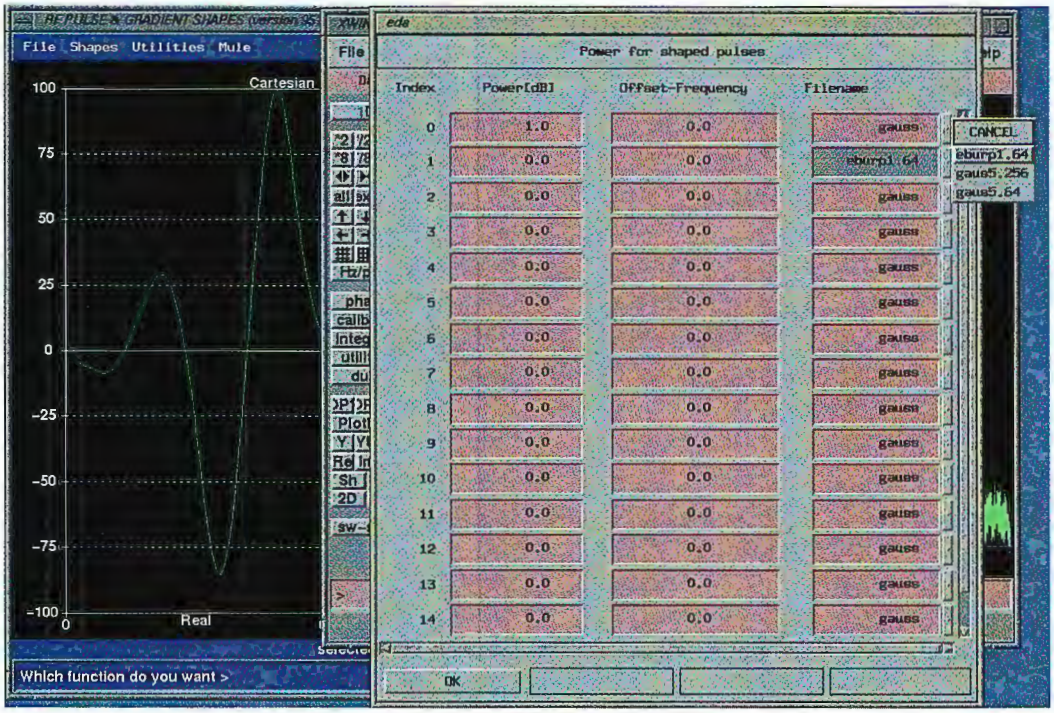

Figure 2: Enter the optimized shape into pulse sequence

The *xShape* routine can also read ASCII text files for shapes created in other software programs, and save them in the *XWIN-NMR*^{TM} format. This makes any pulse shape available for pulse sequences. Pulse shapes are only limited by your imagination, not by the software!

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(received 8/23/96)

Prof. B.L. SHAPIRO
The NMR News Letter
966 Elsinore Court
Palo Alto, CA 94303

How to fit a broad NMR resonance line with a strongly distorted baseline?

Dear Dr. Shapiro,

A main problem in the quantitative analysis of NMR spectra of quadrupolar nuclei such as ^{17}O or ^{14}N is the rolling baseline coming from, for example, truncation of the first data points of the FID. To get linewidths and integrals with high accuracy the baseline is normally corrected by choosing manually some points on the baseline and fitting a polynomial to these points (Fig. 1). The corrected spectrum is obtained by subtraction of the baseline from the experimental data points. In the next step a Lorentzian line is fitted to the corrected data (Fig. 2).

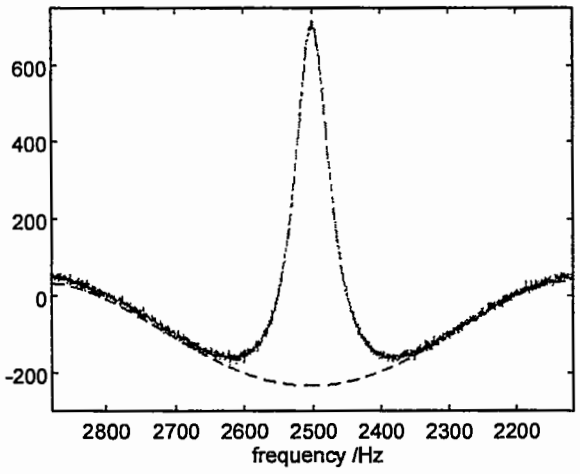

Fig. 1: *"Experimental" NMR spectrum with baseline distortion and fitted 4th order polynomial (- -). The "experimental" spectrum was generated by a FT of a calculated FID (with 2 % random noise) whose first 4 points were set to zero (parameters see Table)*

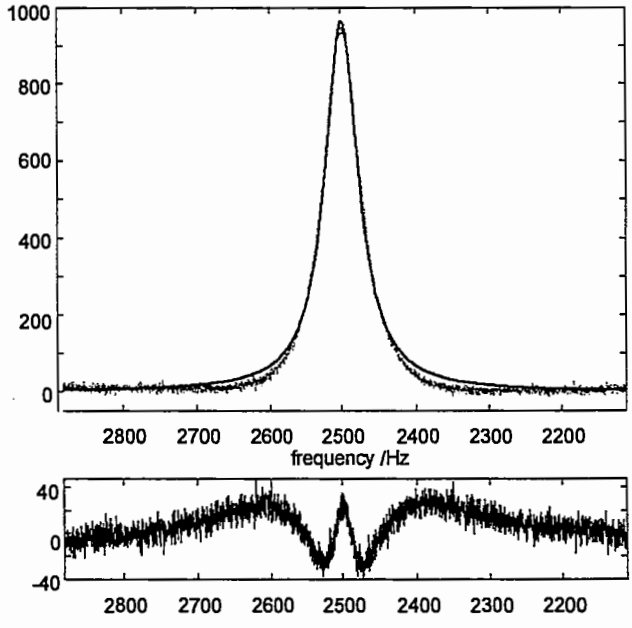

Fig. 2. *Lorentzian line fitted to the experimental spectrum with 4th order polynomial (from Fig. 1) subtracted*

	ν_0 /Hz	$1/T_2$ /s^{-1}	Height /a.u.
from separate fit:	2'500 ± 0.05	171.1 ± 0.5	959 ± 1.9
from global fit:	2'500 ± 0.02	196.2 ± 0.3	990 ± 0.7
original parameters:	2'500	200	1000

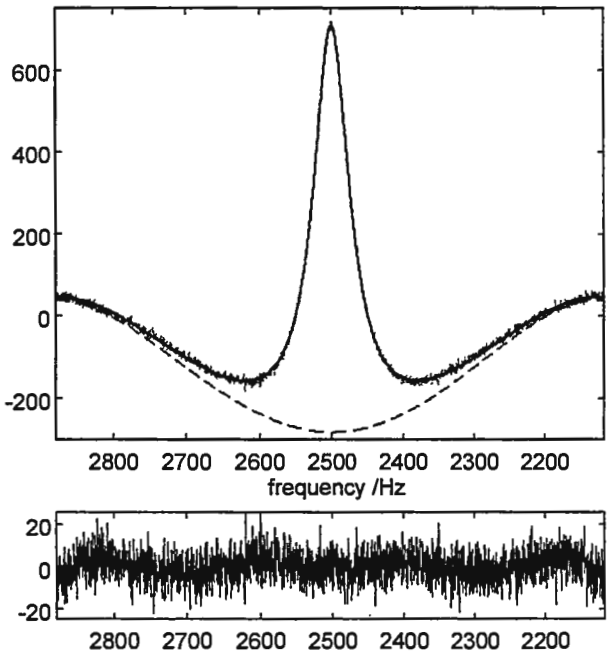

Fig. 3. *Lorentzian line fitted and 4th order polynomial fitted together to experimental spectrum*

The difference plot (Fig. 2 lower part) clearly shows that there is some systematic error. Even a careful choice of baseline points does not prevent from these systematic errors. The obtained linewidth and height are substantially different from the values used to generate the "experimental" data (see Table).

One possibility to improve the data evaluation is the use of linear prediction methods to reconstitute the truncated first FID data-points. Very good results can be obtained in a much easier way by simply fitting the baseline parameters (5 for a polynomial of 4th degree) together with the parameters defining the resonance line (height, width, frequency). Figure 3 shows that systematic errors are very much reduced and the parameters obtained from the fit are in much better agreement with the "true" values (Table).

With best regards,

Lothar Helm

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Amplitude droop	5% to 20 ms typ.	5% to 20 ms typ.
Harmonics	Second: -25 dBc max. Third: -24 dBc max.	
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August 19, 1996 (received 8/20/96)

Eliminating Convection Effects from Gradient Experiments using X-Axis Gradients

Dear Barry,

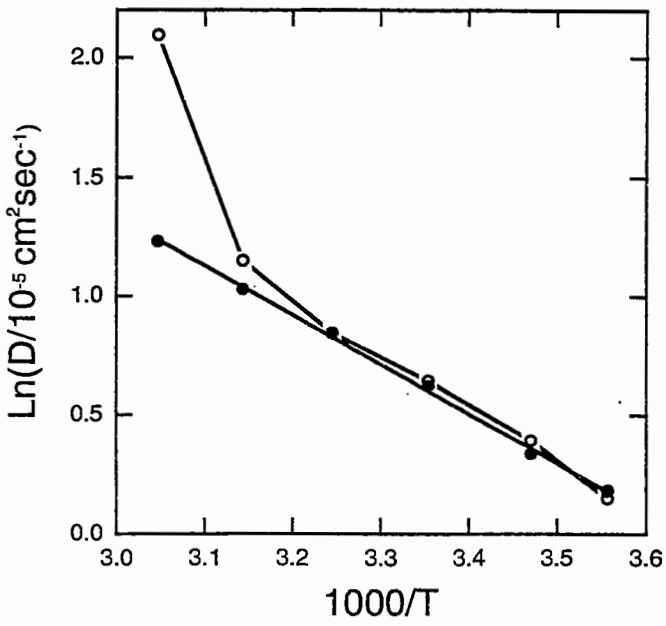
We recently encountered complications from convection while carrying out gradient-based experiments above 35° C on our GE Omega 500 spectrometer. We happened to be measuring diffusion coefficients (D), but convection can undermine any gradient-based experiment if material moves significantly between gradient pulses. For the diffusion experiment, the symptoms included oscillatory or non-linear semi-log plots used for extracting D, poor reproducibility, and faster than expected values of D. These symptoms are consistent with convection, which can move material coherently and rapidly. We tried several tricks to remove the effect, such as waiting hours for thermal equilibration, or using restricted-volume sample tubes. The trick that worked best was to apply the gradient pulses at right angles to convection by using the X gradient instead of the Z gradient.

The figure shows a plot of the $\log_e$ of the apparent diffusion coefficient of water vs. 1/T, determined using the X gradient (filled circles) and the Z gradient (open circles). The sample contained approximately 1% H_2O in D_2O, doped with $GdCl_3$. To give a worst-case temperature gradient, data were collected as soon as the instrument indicated the sample was at the desired temperature. Using the Z gradient, the D values deviate from linearity starting somewhere above 35° C. The data for the X gradient are linear over the entire range of temperatures studied (from 8° to 55° C), as expected for a well-behaved sample. Apparently, convective transport along the Z axis doesn't interfere with diffusive transport along the X axis, so that diffusion measurements and other gradient experiments can be performed successfully using X gradients, even in the presence of convection. This probably requires laminar convective flow and a long sample so that the change of direction at the ends of the tube doesn't cause lateral movement of the material within the detected region of the sample. The temperature at which convection becomes faster than diffusion and starts degrading experiment performance will depend on the specifics of the probe and lab. It's probably worth checking since, at least in our lab, it occurred well within the usual operating temperature range.

Best Regards,

Charles D. Eads

Charles D. Eads



The NMR Newsletter - Book Reviews

Book Review Editor: **William B. Smith**, Texas Christian University, Fort Worth, TX 76129

Nuclear Magnetic Resonance: Concepts and Methods

by

Daniel Canet

John Wiley & Sons, Ltd., Baffins Lane, Chichester,West Sussex PO19 1UD, England.
1996. ISBN 0-471-96145-0 (paperback), 260 pages, $ 49.95.

Originally published as "La RMN: Concepts et Méthodes" in 1991, this book purports to examine the physical and mathematical features of liquid state NMR spectroscopy and then to explore the applications of this technique in depth. There are five chapters (and an index):

1. Structure of Nuclear Magnetic Resonance Spectra
2. Basic Mathematics and Physics of NMR
3. Fourier Transform NMR and Data Processing
4. Dynamic Phenomena in NMR
5. Multipulse and Multidimensional NMR

The writing style is somewhat stilted but acceptable, and there are no particularly glaring typographical errors. This book is difficult to evaluate since its intended audience is not apparent. The summary on the back cover describes the book as a text. Yet, with the exceptions of a few isolated sections of chapter 1, the material appears much too advanced for undergraduates and most graduate students to grasp. On the other hand, the book falls well short of the several comprehensive reference books on the theory and application of NMR that the serious spectroscopist will prefer. The subject matter is hit hard and heavy with little or no development, as one might expect of a book that attempts to accomplish so much in so few pages.

On the positive side, there may be a niche for this book among those who simply need a succinct advanced overview for refreshment purposes. In that regard, the discussions of data processing in chapter 3 and polarization transfer in chapter 5 are among the best I have read in any publication. These items may be worth the price of the book, but do not expect to learn NMR by reading it. This is not a teaching tool.

David E. Minter
Department of Chemistry
Texas Christian University
Fort Worth, Texas

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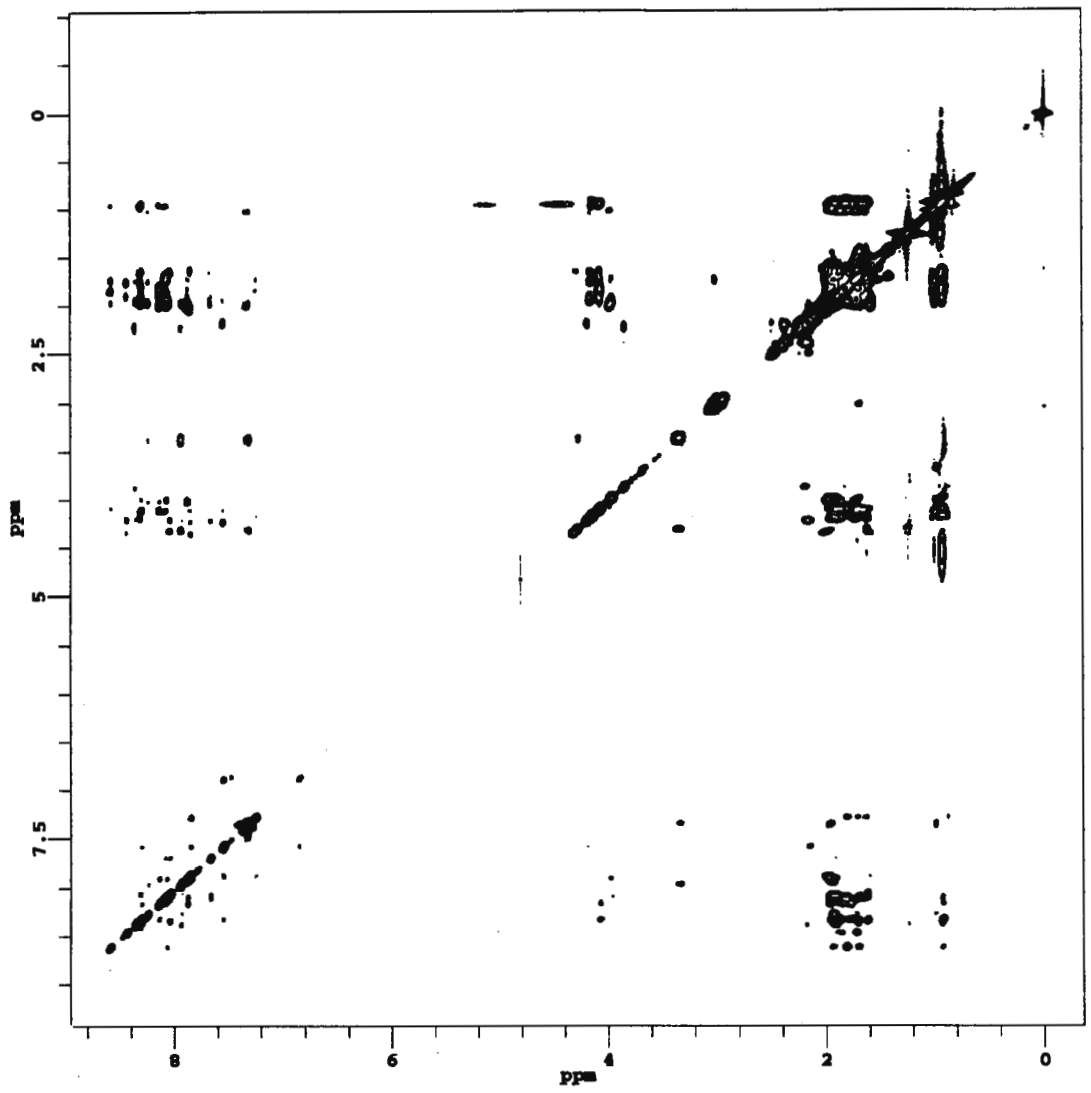

This spectrum was acquired as 2048 rows of 512 hypercomplex points each. Thirty-two scans per row were used to achieve the excellent signal-to-noise seen here.

NOESY SPECTRUM WITH FLIPBACK WATER SUPPRESSION OF A DILUTE PEPTIDE IN MICELLES

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Shown Here is a Noesy Spectrum with Flipback Water Suppression of a Dilute Peptide in Micelles Collected on the *Chemagnetics*™ 400 MHz CMX Infinity Spectrometer.

Noesy spectra are particularly informative for structure elucidation of large molecules. Biological systems usually exist in normal water with no more than 10% D_2O added for locking purposes. In order to observe the weak nOe signals, the water signal must therefore be suppressed. One method for doing this is called the "flipback" or "jump and return" method. This can be combined with Watergate gradient solvent suppression to virtually eliminate all traces of the water signal.

The spectrum shown here is of 2mM PIF in sodium dodecylsulfate-d_{25}. PIF is a peptide of eighteen residues, and sodium dodecylsulfate-d_{25} is a micelle surfactant. The solvent mixture is 90% H_2O, 10% D_2O.

Absolutely no t_1 ridges nor any other artifacts are present. The impressive resolution of the fine structure shown here attests to the CMX Infinity's ability to deal with systems with difficult dynamic range requirements. This, combined with the superb water suppression as demonstrated here, makes the CMX Infinity Spectrometer an excellent choice for biological applications.

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Newark, Delaware 19716-2522

Cecil Dybowski
Professor

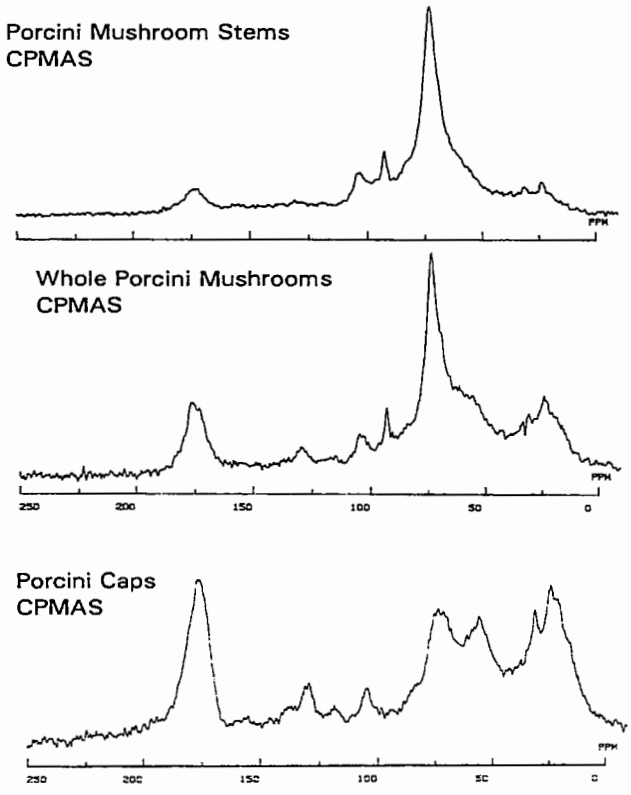
(302) 831-2726
FAX: (302) 831-6335
Internet: dybowski@udel.edu

July 31, 1996
(received 8/7/96)
Dr. Barry Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

Pasteurian NMR of Mushroom

Dear Barry:

In my last missive, I mentioned that ^{13}C CPMAS NMR of whole mushrooms may be useful for identifying components of, or for fingerprinting, specific whole dried mushrooms. We have recently taken to analyzing mushroom stems and caps separately by removing the caps from the stems before desiccation. Not surprisingly, the spectroscopy shows that caps and stems are different; what is surprising is the extent to which they are different. The figure shows three spectra [contact time = 1 ms] of roughly the same amounts of powdered stems, whole mushrooms, and caps from a sample of dried Porcini mushrooms. The figure shows very clearly that different components exist in different parts of the mushroom. For example, the region between 80 and 100 ppm, where the major cellulose resonances occur, demonstrates that a large portion of the cellulose in mushrooms lies in the stems, not the caps.



As we all learned, in 1848 Louis Pasteur separated sodium ammonium tartrate crystals by hand on the basis of crystalline features and then went on to demonstrate that the two parts of the original material were each separately optically active. Perhaps we should call NMR of physically separated components Pasteurian NMR.

Good eating,

Cecil

Cecil Dybowski

August 2, 1996

Dr. Bernard L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

Dear Dr. Shapiro:

Symposium on
NMR Spectroscopy of Synthetic Macromolecules
ACS National Meeting, San Francisco, April 13-17, 1997

At the 1997 Spring ACS National Meeting, we are organizing a symposium featuring the latest developments in the NMR studies of polymers and bringing together representative researchers from universities, government labs, and industry. Equal emphasis will be placed on fundamental research and on industrially important problems. The symposium will consist of a tutorial session, 5 technical sessions, and a poster session. The symposium is sponsored by the ACS Division of Polymer Chemistry.

The following is a partial list of speakers:

K. Adamsons, DuPont
P. Berger, Monsanto
K. Beshah, Rohm and Haas
F. Blum, U. of Missouri, Rolla
R. A. Byrd, NCI, Frederick
H. N. Cheng, Hercules
T. T. P. Cheung, Phillips Petroleum
P. P. Chu, National Central U., Taiwan
D. G. Cory, MIT
S. Curran, Allied-Signal
J. Ebdon, U. of Lancaster
A. D. English, DuPont
M. F. Grenier-Loustalot, CNRS
C. Wutz, Hamburg Univ.
A. A. Jones, Clark U.
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A. Natansohn, Queen's U.
T. G. Neiss, Hercules
P. Rinaldi, U. of Akron
L. Resconi, Montell
F. C. Schilling, AT&T Bell Labs
A. L. Segre, Inst. of Struc. Chem., Rome
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J. L. White, Exxon
G. v.d. Velden, DSM, The Netherlands
H. J. Yue, Dow Corning
R. C. Zeigler, Montell
N. Zumbulyadis, Kodak

If you are interested in presenting a poster, please respond to one of the symposium organizers prior to October 20, 1996. The deadline for abstracts and preprints is November 1, 1996.

Organizers

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500	51	10	150	3.2
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360	54	8	365	2.8
300	54	3	365	2.8
270	54	2.7	365	2.8
200	54	2	365	2.8
100	54	1	365	2.8
500	89	15	120	3.4
400	89	10	180	2.8
360	89	10	365	2.8
300	89	3	365	2.8
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LONG ISLAND UNIVERSITY BROOKLYN CAMPUS UNIVERSITY PLAZA, BROOKLYN, NEW YORK 11201

August 21, 1996 (received 8/24/96)

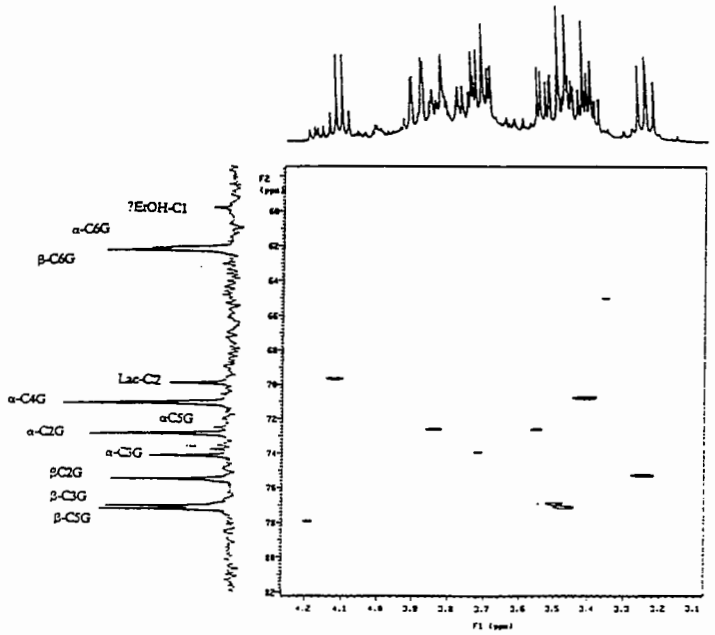
Dr. B. L. Shapiro
The NMR News Letter
966 Elsinore Court
Palo Alto, CA 94303

High Resolution NMR Studies of Human Cerebrospinal Fluid

Dear Barry,

As you will note from the new letter head, I have relocated to Long Island University. Along with my new teaching commitments, and interests in the micro-imaging of transgenic mice, I am also interested in pursuing research in the area of high resolution NMR of biological fluids using 1D and multi-D NMR. (Commodari *et al.*, "^{1}H NMR Characterization of Normal Human CSF and the Detection of Methylmalonic Acid in a Vit B_{12} Deficient Patient", *NMR In Biomedicine* **4** 192-200, 1991). ^{1}H NMR spectra of human cerebrospinal fluid (CSF) have been analyzed. All of the CSF samples showed peaks for lactate, acetate, glutamine, citrate, creatine/ creatinine, and sugar resonances. The metabolite concentrations, as determined by NMR, agreed with those obtained using conventional chemical methods. Methylmalonic acid (about 154 µM) was present exclusively in CSF from a vitamin B_{12} deficient patient.

NMR is unique as an analytical tool since it is non-invasive, non-destructive and not dependent on specific preparative methods limiting it to detecting only certain compounds. The true potential of high resolution NMR in a biochemical laboratory remains to be realized. NMR can be used to provide information on the underlying metabolism by probing a wide range of toxicological and biochemical problems.



^{13}C - {^{1}H} HETCOR of human cerebrospinal fluid acquired at 9.4 T.

The use of heterocorrelated ^{13}C-{^{1}H }, as shown in the preceding figure, or indirect ^{1}H - {^{13}C} NMR spectroscopy allows characterization of the ^{1}H carbohydrate region of human CSF. Although the long spectral acquisition times would not make this practical for routine assignments, new indirect detection approaches such as HMQC[1] and higher magnetic field strengths offer interesting possibilities and applications for more routine use of heterocorrelation spectroscopy in the assessment of disease. The goals of these studies on CSF will be to continue to develop novel methods of analysis of human CSF and to try and determine whether correlations between diseased states and the presence of metabolic markers in CSF exist, as seems apparent from preliminary studies. New tools for pattern recognition and multivariate analysis will be developed and used for the automated analysis of the NMR spectra generated.

Sincerely,

Fernando Commodari, Ph.D.
Assistant Professor
Chemistry Department

********Postdoctoral Position********

I am looking for a postdoctoral fellow to carry out high resolution NMR studies of human cerebrospinal fluid (Commodari *et al.*, "^{1}H NMR Characterization of Normal Human CSF and the Detection of Methylmalonic Acid in a Vit B_{12} Deficient Patient", *NMR In Biomedicine* **4** 192-200, 1991) and/or studies on the study of 30-40 amino-acid neuropeptides using 1D,2D, and multi-D NMR methods. The latter project being an ongoing project in collaboration. (Y. Boulanger, Yanmin Chen, F. Commodari *et al.*, "Structural Determinations of Neuropeptide Tyrosine (NPY) and of It's Agonist Analog, [Ahx $^{5-17}$] NPY by NMR and Molecular Modeling.", *Int. J. Pep. & Protein Res.* **45**, 86-95, 1995.). The appointment will be at the Adjunct Assistant Professor level involving teaching of a class and the associated laboratory. This is a unique opportunity for an individual to gain teaching experience while carrying out post-doctoral research in New York city (www.brooklyn.net/ashkenaz/brooklyn.html). **Contact:**

Fernando Commodari, Ph.D., Assistant Professor,Chemistry Department,
Long Island University, University Plaza, Brooklyn, NY 11201,Tel:(718)488-1664,
fax:(718)488-1465; e-mail: fern@hornet.liunet.edu ; http://www.liunet.edu.

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[1] - M.F. Summers, *et al.*, *J.A.C.S.* **108**, 4285 (1986).

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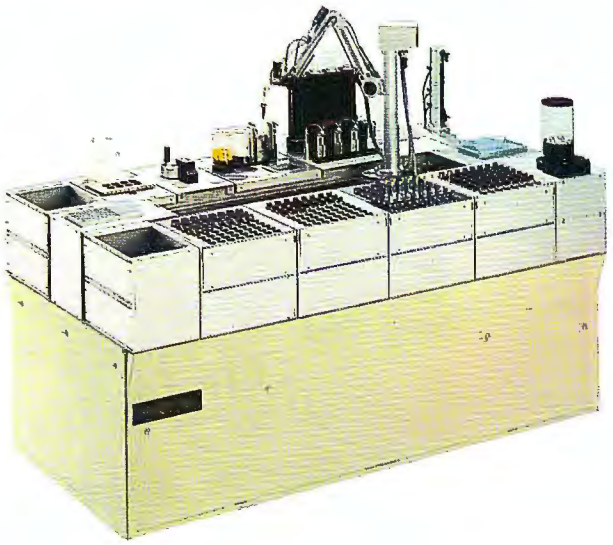

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Dr. Barry Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

(received 8/22/96)
August 20, 1996

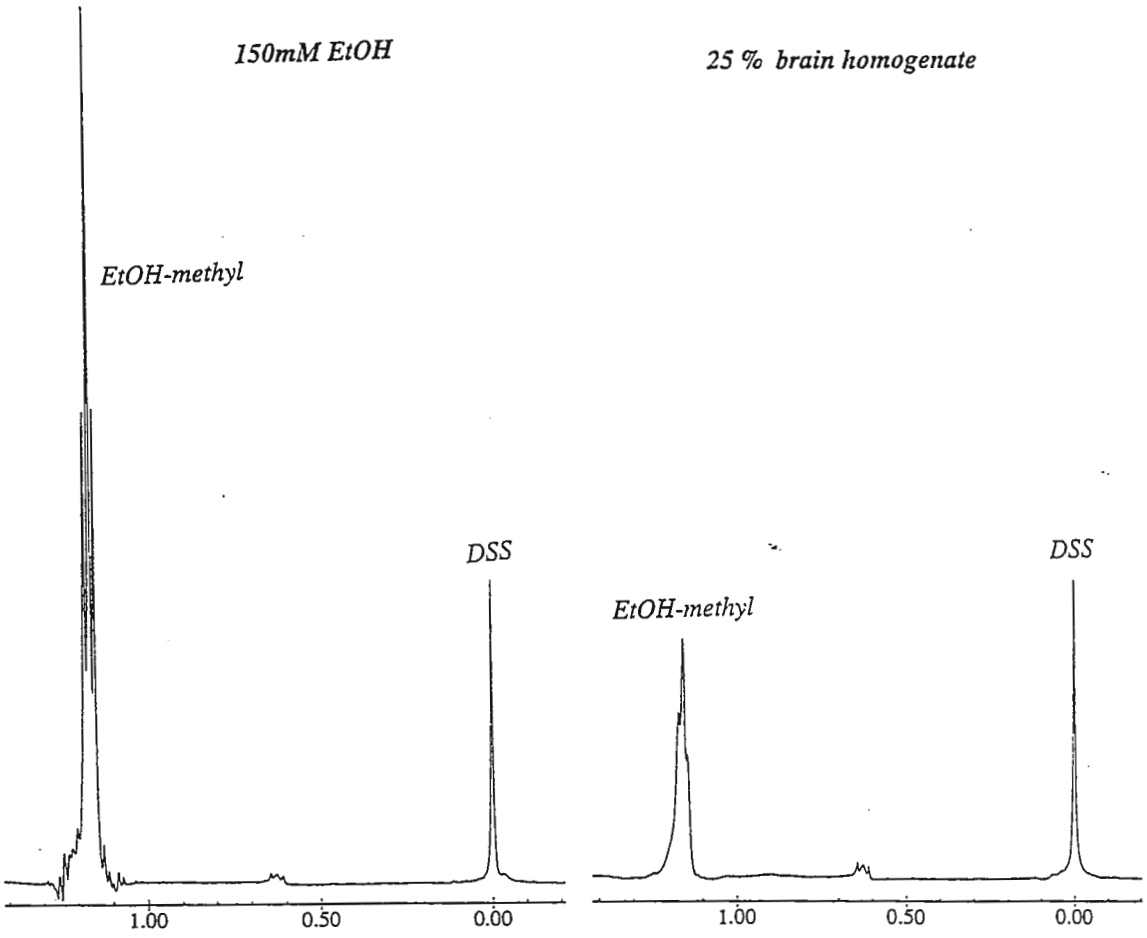
Proton MRS 'visibility' of ethanol in brain tissue homogenates

Dear Dr. Shapiro,

Recently, we have become interested in the proton MRS 'visibility' of ethanol (EtOH) in brain (Meyerhoff et al., Alc. Clin. Exp. Res., in press (1996)). In-vivo proton MRS detects EtOH in the brain following EtOH administration in animals and humans. However, the detected level accounts for less than 40% of blood alcohol levels, and it has been postulated that alcohol associated with membranes may be invisible to in-vivo MRS. Also, previous studies have shown that the visibility of alcohol is higher in alcohol-tolerant than in alcohol-naive animals and humans, adding some clinical importance to the question of MRS visibility of brain alcohol. The goals of this *in vitro* study were (i) to determine the MR visibility of EtOH in brain homogenates, and (ii) to determine if a magnetization transfer (MT) effect can be observed for EtOH in brain homogenates, giving indirect evidence for the existence of an immobilized pool of EtOH.

Fresh brains of decapitated Sprague-Dawley rats were homogenized in saline and centrifuged. The supernatant containing soluble metabolites was discarded, and the pellet containing membranes dried. This homogenized dry tissue was used to prepare 25 % (w/v) brain membrane solution in 150mM EtOH (in saline). All proton NMR spectra including phantom spectra from 150mM EtOH (in saline) were recorded in 5mm tubes using a 500 MHz spectrometer at 20° C. A capillary coaxial insert tube containing 50mM DSS [3-(trimethylsilyl) propane sulfonic acid, sodium salt] in D_2O was used as chemical shift reference and frequency-to-field lock. All data were processed using *NMR1* software. The DSS (singlet at 0.0 ppm) and the EtOH-methyl signal (triplet at 1.18 ppm) were fitted to Lorentzian lines, and integrated to calculate the ratio ETOH/DSS.

The representative spectra shown below were obtained using a spin-echo sequence with TE = 1ms, and with 6s water presaturation pulse. The EtOH/DSS ratios were 5.84 for the phantom containing no membranes and 1.83 ± 0.68 (SD, n = 6) for the 25% brain homogenate. The partial volume increase that was measured after adding brain membranes was less than 20%. Accounting for this volume effect alone one expects an EtOH/DSS ratio of 4.67 for the brain homogenate; the measured value, however, was lower, and corresponded to only 39% ^{1}H MRS 'visibility' of EtOH in brain homogenate. Therefore, the small EtOH signal in brain homogenates can not be explained by simply accounting for partial volume effects. It lends support for the hypothesis that there may be a motionally restricted EtOH pool in membranes not detected by these ^{1}H MRS experiments.



To investigate further whether this invisibility was due to a magnetization transfer (MT) effects, MT experiments were performed. The MT experiment used a 1331 water suppression pulse and a 6s presaturation pulse for MT. We measured the EtOH-methyl signal intensity change after applying an MT pulse at at least 29 offset values between -50kHz and +40kHz around the methyl resonance. This frequency-selective saturation resulted in signal intensity change from the highly visible pool due to MT. The maximum MT effect of 36 % without direct signal saturation was achieved at 1.8kHz. A possible explanation for this result is the presence of a weak and broad EtOH resonance, corresponding to motionally restricted EtOH pool in brain membranes that rapidly undergoes chemical exchange with the highly mobile MRS-visible EtOH pool.

These preliminary experiments show that (i) the EtOH signal decreases in the presence of brain tissue homogenates, and (ii) that off-resonance saturation results in reduced EtOH signal intensity giving clear evidence for an MT effect on the EtOH methyl resonance in rat brain homogenates. These experiments suggest the presence of a ^{1}H MRS invisible EtOH pool in brain homogenates, possibly due to partitioning of EtOH into membranes.

Sincerely,

Michael W. Weiner V. Govindaraju. Martina Vermathen Andrew A. Maudsley Dieter J. Meyerhoff

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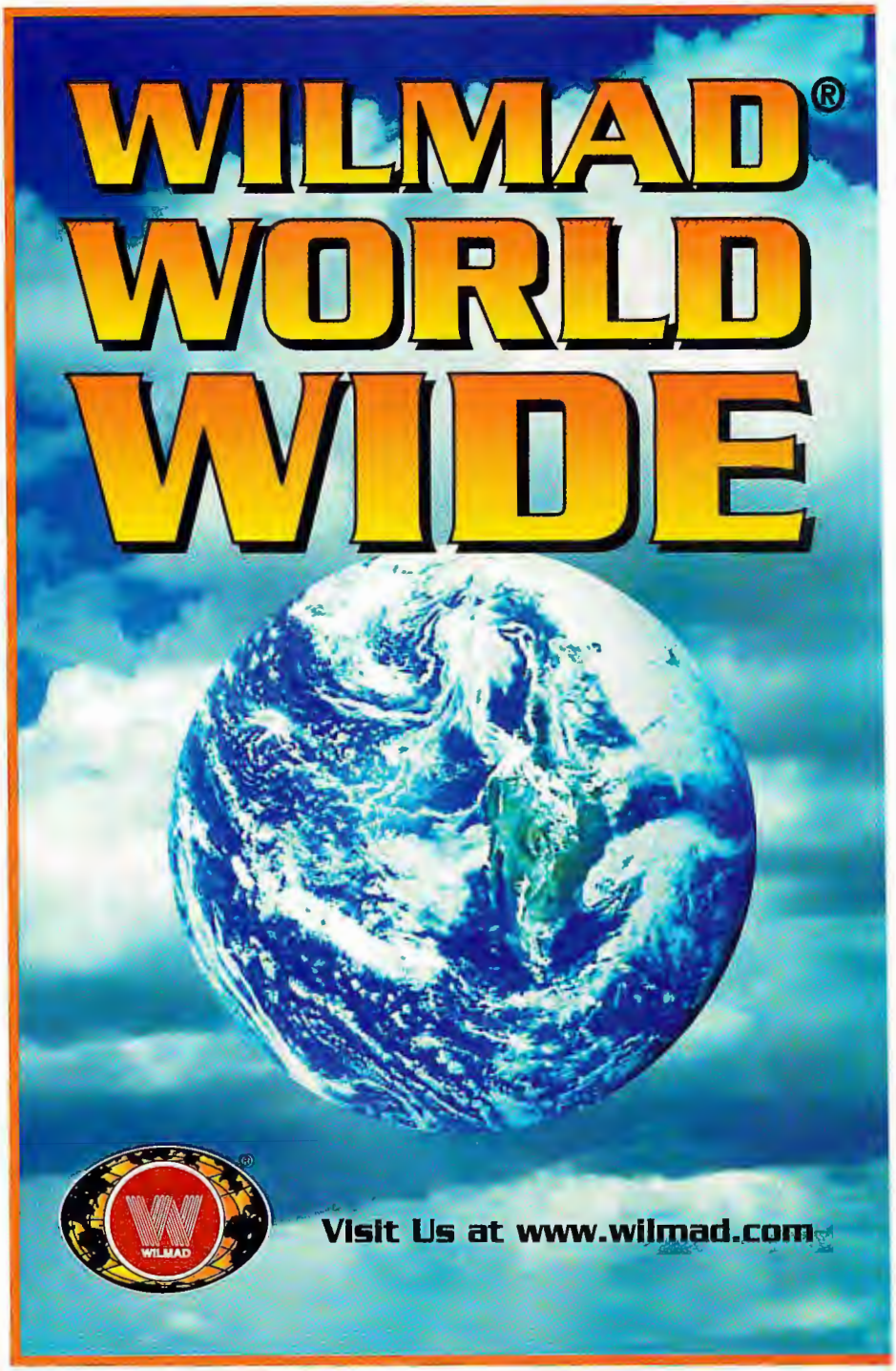

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Billerica, 08/05/96
(received 8/12/96)

Gradient High Resolution Magic Angle Spinning Spectroscopy

Dear Barry,

The enclosed spectra are the first spectroscopic applications of a new probe that combines magic angle spinning and magnetic field gradients.

Magic angle spinning is increasingly used to improve the resolution of spectra from non-solid samples such as polymer gels, lipids, tissue samples and the swollen resins used as supports in combinatorial chemistry. For these samples MAS is useful for averaging both residual dipolar interactions and variations in the bulk magnetic susceptibility.

The addition of a magnetic field gradient leads to a significant reduction of artifacts and t_1-noise, and improved coherence pathway selection; the same advantages that have led to the popularity of gradients in high resolution spectroscopy.

We have designed a new gradient set for these high resolution MAS experiments, that generates a magnetic field gradient oriented along the magic angle spinning axis ($\partial B_z / \partial \theta_m$). The z-component of the magnetic field increases along the axis of the spinner and is uniform in the planes perpendicular to the spinner axis. Consequently spins rotating at the magic angle experience a time-independent gradient field.

Although a gradient at the magic angle is in itself not new, our design is however more convenient than most since the gradient set is tied to the magic angle stator and:

- there is no need to synchronize the gradient pulses with the spinner rotation;
- the gradient is perfectly aligned with the spinner axis;
- the gradient is linear over the full sample;
- the design combined with our high resolution MAS probe does not interfere with our automatic sample insertion and ejection capability.

The standard-bore g-HR-MAS probe for 4 mm samples is now available for 400 and 500 MHz AVANCE™ systems, and requires an ACUSTAR 10 A gradient power supply, and the GCU.

A few examples of gradient high resolution MAS experiments are shown below, which demonstrate excellent spectral quality and a high degree of artifact suppression. We anticipate

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BRUKER

that gradient methods will shortly become as common in high resolution MAS spectroscopy as they are in high resolution liquid state experiments. In fact, one can argue that the need is more pronounced in the hr-MAS case since the probe geometry and sample spinning builds into the measurement an unavoidable modulation of the coupling of the spins to the receiver and hence pronounced artifacts.

Sincerely,

Werner Maas

David Cory

Joel Lewandowski

Frank Laukien

Martin Rindlisbacher

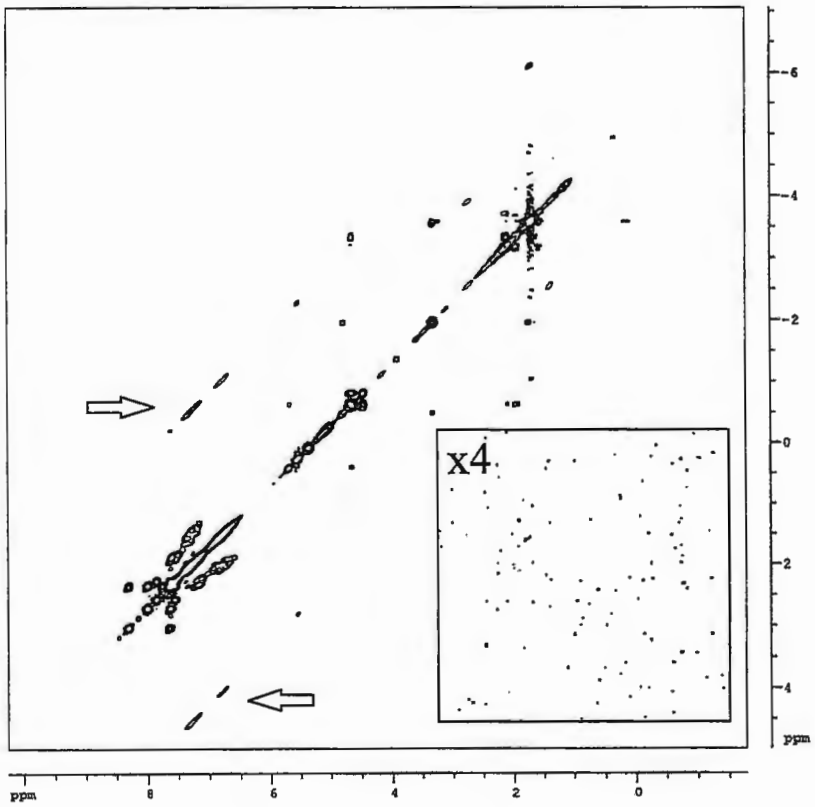

Figure 1: ***A 500 MHz gradient ^1H-^1H COSY spectrum of an N-FMOC-N-Boc-L-Lysine derivatized Wang resin swollen with $CDCl_3$. The sample is spinning at 5 kHz. Two, 1 ms sine shaped gradient pulses (10 G/cm) were used to select the N-type coherence pathway. The arrows indicate spinning sidebands that are folded in the F_1 dimension. The insert shows the noise floor and demonstrates the excellent suppression of t_1-noise.***

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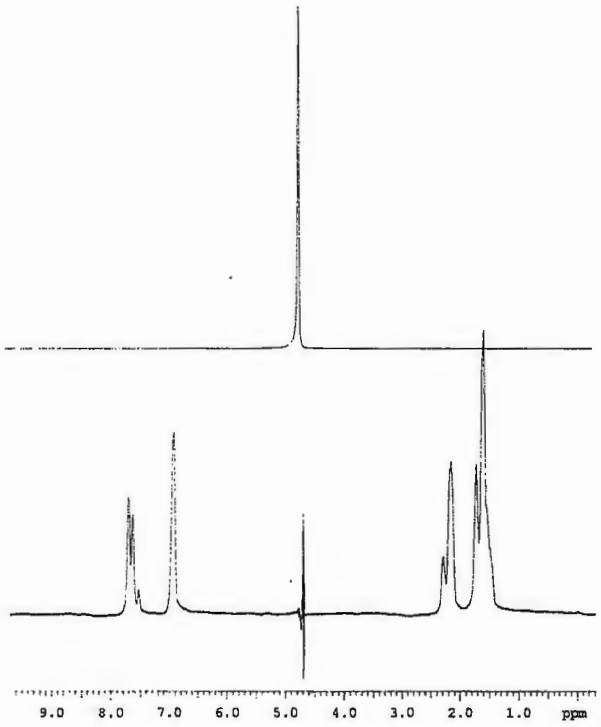
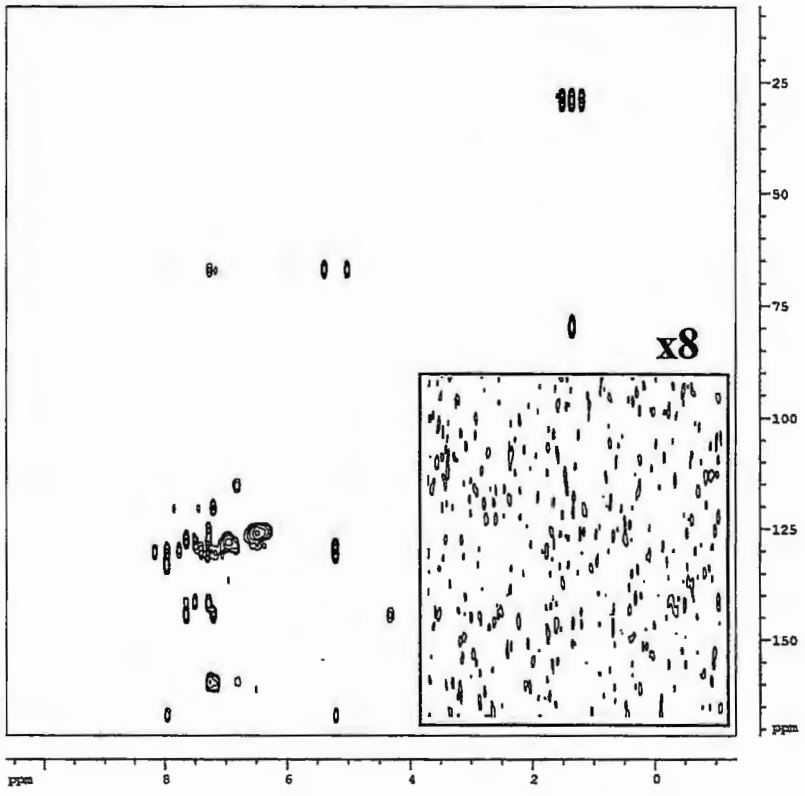


Figure 2: *500 MHz single pulse proton spectrum of an acrylamide hydrogel (top). The lower spectrum was acquired with Watergate using 1 ms sine-shaped gradient pulses with a strength of 30 G/cm.*

Figure 3: *^{1}H-^{13}C HMBC spectrum of an N-FMOC-N-Boc-L-Lysine derivatized Wang resin swollen with $CDCl_3$, obtained at a ^{1}H frequency of 400 MHz and at a spinner frequency of 5 kHz. 1 ms pulsed field gradients were used with strengths of 10, 10 and 5 G/cm. A J-evolution time of 50 ms is used to reveal long-range proton-carbon correlations. No ^{13}C decoupling is applied during the acquisition. The expansion shows the noise floor.*



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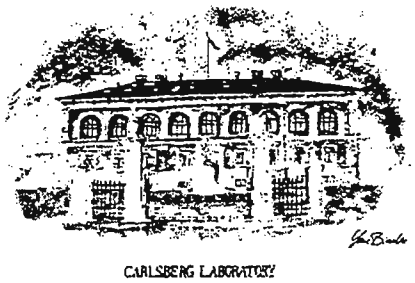
CARLSBERG LABORATORY

Department of Chemistry

Professor Klaus Bock



Senior NMR Scientist

At the Carlsberg Laboratory a National Instrument Centre for NMR Spectroscopy of Biological Macromolecules will be opened in 1997 with 800 and 500 MHz Varian UNITY INOVA equipment. The Chemistry Department of the Carlsberg Laboratory has Bruker 600 AMX and 250 DRX instruments also available for research activities. Two research groups are active in the structural determination of biological macromolecules by NMR spectroscopy at the Carlsberg Laboratory, i) the Protein Structure and NMR group and ii) the Carbohydrate NMR group.

In order to strengthen the research at the National Centre a 5 years position for a senior research scientist is open. The senior scientist position will be filled with a NMR spectroscopist, who can complement the present structure research at the Carlsberg Laboratory by developing NMR methods for NMR spectroscopy of biological molecules. The successful candidate will have the opportunity to develop an independent research project, but will be committed to collaborate with the existing research groups at the Carlsberg Laboratory and with external users of the instrument centre. For the same period a junior scientific position at the Instrument Centre will be available at the Laboratory.

The candidate for the senior research position should have a Ph.D. and substantial post-doctoral training and research accomplishments and should have demonstrated expertise in NMR spectroscopy.

A 5 years contract will be offered to the successful candidate by a grant from the Carlsberg Foundation and with a salary level as a research professor.

An appointment committee will be established by the Carlsberg Foundation and applications and CV should be sent before **October 1,** 1996 to Professor Klaus Bock.

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NMR Analysis & Consulting

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August 8, 1996 (received 8/12/96)

Dr. Barry Shapiro
The NMR Newsletter
966 Elsinore Ct.
Palo Alto, CA 94303

An Observation and Conclusion Will Depend on the Time Scale

Dear Dr. Shapiro:

Thank you for your reminder. This gives me an opportunity to announce the opening of my new business, *NMR Analysis and Consulting*. I purchased a VXR 4000 from A. E. Staley, by whom I am no longer employed , and should be running samples by August 23, 1996. Anyone should feel free to call for a brochure.

In the past, I had been doing PFG "diffusion" experiments with the above instrument. I used the LED method of Charles Johnson.* In any of the PFG experiments there is a time delay used to allow the nuclei to move before sampling. When dealing with highly entangled polymers, e.g. starch "solutions," varying the time delay, Δ, over a large range leads to some interesting and informative data.

The technique for PFG experiments is to hold Δ constant and vary the gradient strength, g. The signal attenuation is plotted *vs* g or g^2, which leads to an exponential decay in the case of simple self diffusion†. For the starch "solutions" we found the following trend: At short Δ's, <20msec, the PFG curves are exponential appearing to be simple diffusion, but the apparent diffusion coefficients D_{app} decrease as Δ increases. As Δ increases still further 50msec to 300msec (depending on concentration) the curves become completely non-exponential, convex rather than concave, indicating some reciprocating motion. (See Kaerger's review for the explanation.) Surprisingly, when Δ was increased still further, >400msec, the curves became exponential again. The D_{app} decrease again as Δ increases. Here we see the polymer breaking free from the entangled state for a short period of time only to get entangled again. We found that D_{app} could be extrapolated to infinite Δ, leading to a long range diffusion coefficient. I have worked out a more detailed and ever so slightly more rigorous explanation.

Gary Juneau 

---

* Gibbs, Johnson, *J. Magn. Reson.*, **93**, p395-402, (1991)

† Kaerger et al., *Advances in Magnetic Resonance*, **12**, p1-89, (1988)

UNIVERSITY OF MISSOURI-ROLLA
Missouri's Technological University

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(573)-341-4451 (or 4420)
fblum@umr.edu

Dr. Bernard L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

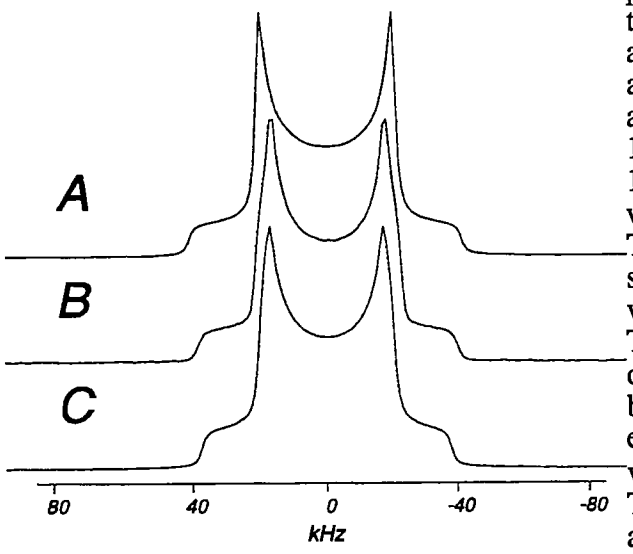
July 25, 1996
(received 7/29/96)

Dynamics of Poly(vinyl acetate)-d_3

Dear Barry:

The experimental deuterium quadrupole-echo spectrum of PVAc-d_3 in bulk at 49 °C is shown in Figure B along with two simulations. The spectrum resembled a Pake powder pattern with a reduced splitting. To first order, this was similar to that expected for a methyl group undergoing fast motions about its symmetry axis. However, upon closer examination, this spectrum had features not normally seen in deuterated methyl groups. However, there is a definite inward curvature in the spectrum in the "horns". The effect is formally similar to that due to a non-zero asymmetry parameter. A similar effect has been observed before for other samples including thymine-d_3[1] and [1,1,1,3,5,6-d_6]*n*-butyl 2,4,6-octatrienylideneimine.[2] For thymine-d_3, it was suggested that the interaction of the methyl deuterons with the carbonyl oxygen was anisotropic resulting in to differences in quadrupole coupling constants and asymmetry parameters of as much as 6 kHz and 7%, respectively. An alternative explanation for the compound with the long name above, was that the interaction caused a distortion from tetrahedral geometry[2] without a change in the axial symmetry of the electric field gradient. We also know that other methyl-labelled polymers without the acetate group do not show this effect.

Based on our spectrum alone, it is probably not possible to distinguish between the explanations proposed in the preceding paragraph. However, the bond angle distortion was easiest to simulate, and consistent with knowledge on the structure of acetic acid. A neutron diffraction study of acetic acid[3] has yielded bond angles for CCH of 112.3°, 107.7°, and 108.9° and HCH angles of 111.3°, 108.6°, and 108.1°. The two simulated spectra were obtained by the method of Wittebort *et al.* [4] The first (Fig. A) was for threefold jumps between sites with tetrahedral geometry (*i.e.* each C-D bond was taken to be 70.5° off of the symmetry axis). The second (Fig. C) was for threefold jumps with one C-D bond distorted from tetrahedral geometry by 5° (*i.e.* 65.5° off of the symmetry axis). The exchange rate and quadrupole coupling constant were taken to be 10^{12}/s and 165 kHz, respectively. The simulated spectrum in C is in excellent agreement with the experimental spectrum B.



I thank Drs. Harbison and Torchia who were very helpful in this endeavor.

1. Hiyama, Y.; Roy, S.; Guo, K.; Butler, L.G.; Torchia, D.A. *J. Am. Chem. Soc.* **1987**, *109*, 2525.
2. Wann, M.-H.; Harbison, G. *J Chem. Phys.* **1994**, *101*, 231.
3. Jöhnsson, P.-G. *Acta Crystallogr. B*, **1971**, 27, 893.
4. Wittebort, R.J.; Olejniczak, E.T.; Griffin, R.G. *J. Chem. Phys.* **1987**, *86*, 5411.

Sincerely,

Frank D. Blum
Curators' Professor of Chemistry

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Department of Neurology

Professor B.L. Shapiro,
The NMR Newsletter,
966 Elsinore Court,
Palo Alto, CA 94303

August 5, 1996
(received 8/15/96)

QUANTITATIVE AUTOMATED BRAIN SPECTROSCOPY

Dear Professor Shapiro,

Over the last few years there has been increasing interest in techniques for quantifying *in vivo* proton spectra of the human brain. One of the more straightforward methods involves the use of the cerebral water signal as an internal intensity reference [1, 2]. General Electric has developed an automated single-voxel, proton spectroscopy package (called "PROBE"), which sequentially records a localized water signal and then the water-suppressed metabolite spectrum. The results of a multi-site trial of this methodology, analyzed in terms of metabolite ratios, were published in 1994 [3]. Recently we re-analyzed these data in terms of absolute metabolite concentrations using the water referencing method. Average metabolite concentrations (100 spectra from 8 different institutions) were found to be 2.00 ± 0.50, 8.43 ± 1.26 and 12.55 ± 1.76 μmol/gm wet weight respectively. These values are in good general agreement with previously published values [4]. Metabolite concentrations for NAA, Cr and Cho across all sites had standard deviations of 14.1%, 14.9%, and 25.1% respectively. While most sites reported consistent values, there were a few inter-site differences which reached statistical significance (Figure 1). The most likely explanation of these differences is the partial saturation of the water resonance due to RF transmitter leakage, which varies from machine to machine. This problem has been fixed in the newest versions of the pulse sequences. In summary, quantitation of "PROBE" spectra is routinely possible using the water signal. The method, of course, relies on an accurate estimate of the cerebral water content. Software for the analysis and quantitation of PROBE spectra is available from me on request,

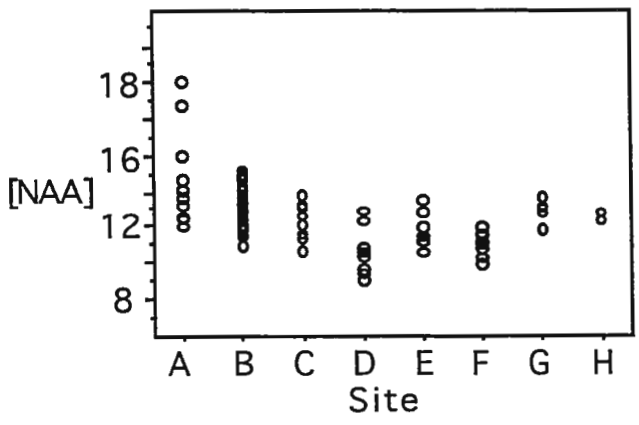
Sincerely Yours,

P.B. Barker.

Peter B. Barker.

1. PB Barker et al., *NMR in Biomedicine* **6,** 89-94 (1993).
2. P Christiansen et al., *Magn. Reson. Imaging* **11,** 107-118 (1993).
3. PG Webb et al., *Magn. Reson. Med.* **31,** 365-373 (1994).
4. P Barker et al., *Magn. Reson. Med.* **32,** 157-163 (1994).

Figure 1



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Dr. B.L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, California 94303

NMR ADVENTURES WITH [Ru(II) (BIPYRIDYL)$_2$ X,X] OCTAHEDRAL COMPLEXES

August 22, 1996

(received 8/24/96)

Dear Barry,

We have been engaged for some time now in molecular recognition studies of biological molecules. One aspect is the use of metal complexes to probe DNA specific chiral recognition and binding. Many workers are active in this research area not only for the opportunities for fundamental studies and their appropriateness for teaching and student theses, but for the potential design of another generation of anti-cancer drugs to augment presently used ones which have side effects and drug resistance. Of course, the role of metal ions in medicine and the life sciences is increasingly understood from the remarkable number and systematization of structures determined by methods of both NMR and X-ray crystallography, plus advances in computational capabilities. Observed functions of such metals and their complexes, aside from mere electrostatics, include using the available rigid geometries to organize and stabilize local structures, for oxidation/reductions and other catalytic aspects, and to use other unique properties of the metallic centers modulated by biomolecules and water.

Specifically, one complex family we have studied involves cis-[Ru(II)(bpy)2-DHAQ] (PF6), where bpy is 2,2'-bipyridine, and DHAQ is either 1,8- 1,4- or 1,5-Dihydroxyanthraquinone. We, and our colleagues have done not only high-resolution 1-D, and 2-D (500-MHz) NMR (typical COSY, TOCSY, and ROESY) studies of the solution structures of these chiral octahedral complexes in acetone-d6, obtaining chemical shifts, coupling constants, multiplicities, and ROE's, and the solutions structures, but also the syntheses, purifications, chiral resolution, and DNA binding studies. Don't panic---These results are in press(1) or being submitted, and we will not burden you with that data (now). Spectral analysis used the ZFD program and spin simulation using FTNMR LAOCN/VAX. Thus, we did much of what we had intended to do, obtained and investigated the three-bladed propeller-type ruthenium(II) complexes wherein two blades are bipyridines and the third is related to anthracycline drugs. We

also have dimeric forms of these complexes. Diamagnetic Ru(II) was used for this NMR study, but para-magmetic Ru(III) and mixed valence species can be prepared.

The real subject of this letter is cis-RU(II)$(bpy)_2$ Cl_2 • n(H2O) a parent for our complexes and many other related, interesting ones under study by various workers. Two chlorides replace the coordinated oxygens (one phenolate and one quinoidal) in Figure 1. The molecule is neutral. The synthesis for the cis form was standard(2) and avoided the trans form ; we routinely obtained 500-MHz spectra NMR spectra and parameters for this and all the quinone species needed for our syntheses. Literature spectra were done at 60- or 90-MHz, without the benefits of more modern instrumentation. We needed the key fragment peaks for spectral analysis. The Ru-Dichloro complex NMR was run in both (deuterated) CH3CN and DMSO because our DNA binding studies required 5-10% organic solvent to solubilize the Ru-DHAQ complexes in aqueous media with calf thymus DNA or synthetic oligonucleotides even though the complexes are plus-one cations. It was thus no surprise when after rather detailed sorting out of the somewhat overlapping aromatic regions 16 major peaks for 16 aromatic protons were observed. This, of course, implies the general case, that the coordination octahedron is not symmetric, and two sets of bipyridal NMR peaks result. The Ru-DHAQ samples also showed this. Chiral propeller species of this sort lead to delta and lambda enantiomeric forms (part of their charm) with the same NMR spectrum unless the environment is chiral as in a DNA complex or a chiral solvent.

Meanwhile, we began to find in the synthetic literature that some workers noted only eight NMR peaks for the Dichloro species. Chemical reactions, heating (refluxing DMSO), hydrolysis or other solvolysis can exchange chloride anions by neutral solvent ligands, but we had not heated our samples. We had tried to exclude water--stored samples in dessicators (in the dark), lyophilized to avoid water(HDO) peaks, made up samples in a dry box, dried the NMR tubes, and used good quality solvents. Often the case, the X-ray structure of Ru(II) Dichloro complex with 3.5 waters is highly informative(2). The chlorine atoms are involved in an elaborate, well-defined crystallographically, hydrogen bonding network with each Cl forming two hydrogen bonds to waters which form additional H-bonds. The molecule has a twofold axis with two waters sitting on it (hence 8 peaks with full hydration and H-bonding optimized with a twofold symmetry) and 16 peaks when the symmetrical solution form of a H-bonded network is disrupted by heating, drying, or

possible solvolysis including Cl substitution. Interesting aromatic pi-pi stacking interactions were also reported.

So what's the point? In summary, we have several:

(1) We have determined the chemical shifts, coupling constants and multiplicities for the sixteen-peak structure if anyone needs such data in their own analyses. The spectra are even better resolved than in the 8-peak case. The J-values (d,d,d) make it easy to identify the bipyridyl proton types even in complex spectra. (There is a smaller well defined but not unequivocally identified 17th peak at about 9.3 ppm).

(2) Water, although often a nuisance, deserves careful tracking. The enhancement of hydrogen bonding structure in the presence of aromatic moieties has implications for biological structures such as proteins, nucleic acids and their interactions.

(3) Even "small" metal complexes can be interesting. Trans-$RuCl_2(DMSO)_4$ upon conversion to the tri-aqua form, with three waters replacing three DMSO molecules, octahedrally cis-coordinates to DNA analogs at guanine-N7's (as does cisplatin(square-planar) and shows antitumor activity(3). Several other related Ruthenium complexes are showing anticancer activity, or provide mechanistic insights (4-6 and references therein). NMR is a salient feature of much of this work.

Look us up if you have any comments on these structures. Some data is still in thesis form (our student coworkers). We are especially interested in NMR evidence for H-bonding waters.

Sincerely,

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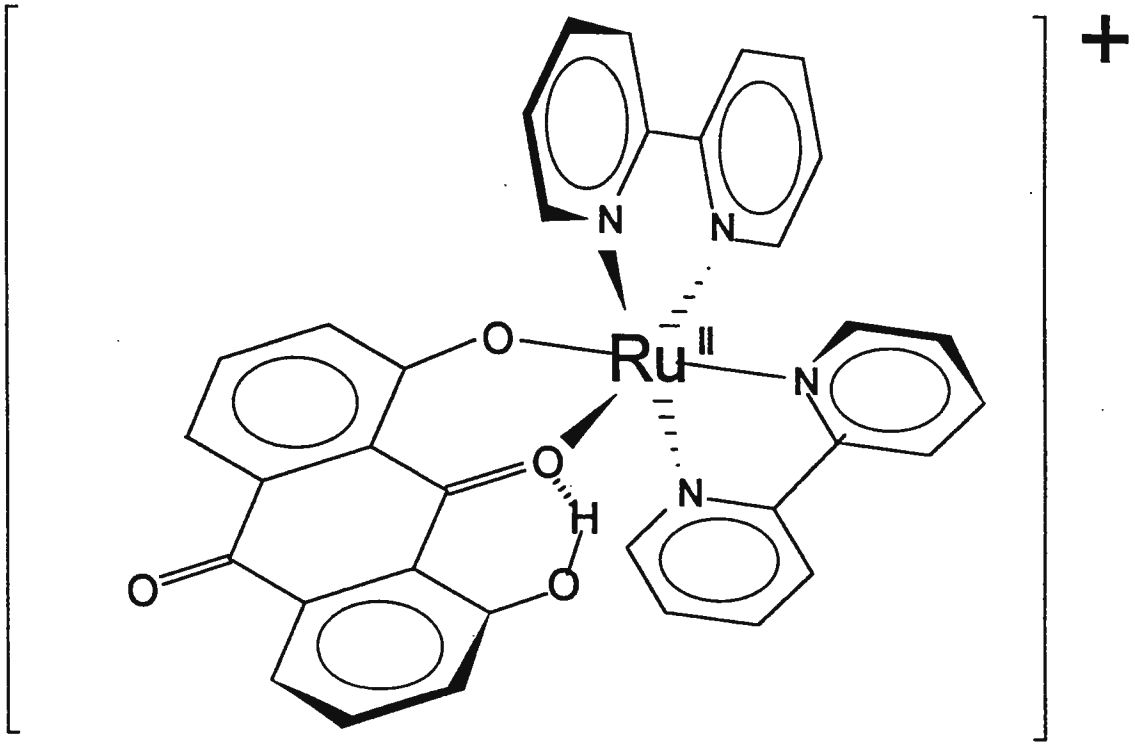
SELECTED REFERENCES

1. V. M. Gooden, H. Cai, T.P. Dasgupta, N. Rowen Gordon, , L. J. Hughes, and G. Sadler, *Inorganica Chimica Acta*, Accepted for publication. The NMR paper being separately submitted as part II.
2. D. S. Eagleston, K. A. Goldsby, D.K. Hodgson, and T. J. Meyer, *Inorg. Chem.* **1985**, 24,45734580.
3. G. Esposito, S Cauci, Fogolari, E. Asessio, M. Scocchi, F. Quadrifoglio, and P. Viglino, *Biochemistry* ***1992***, 31, 7094-7103.
4. R. Hage, J. G. Haasnoot, J. Reedjijk, R Wang, and J. G. Vos, *Inorg., Chem.* **1991**, 30, 3263-3369.
5. P M. van Vliet, J. G. Haasnoot, and J. Reedijk, *Inorg. Chem.* **1994**, 33, 1934-1939.
6. P. M. van Vliet, M.S. Toekimin, J. G. Haasnoot, J Reekijk, O. Vrana, and V. Brabec, *Inorganica Chimica Acta* **1995,** 231, 57-64.

Figure 1. Structure of the Lambda, *Cis*-[Ru(II) (Bipyridy)2 -1,8-Dihydroxy Anthraquinone] Cation:



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1 September 1996

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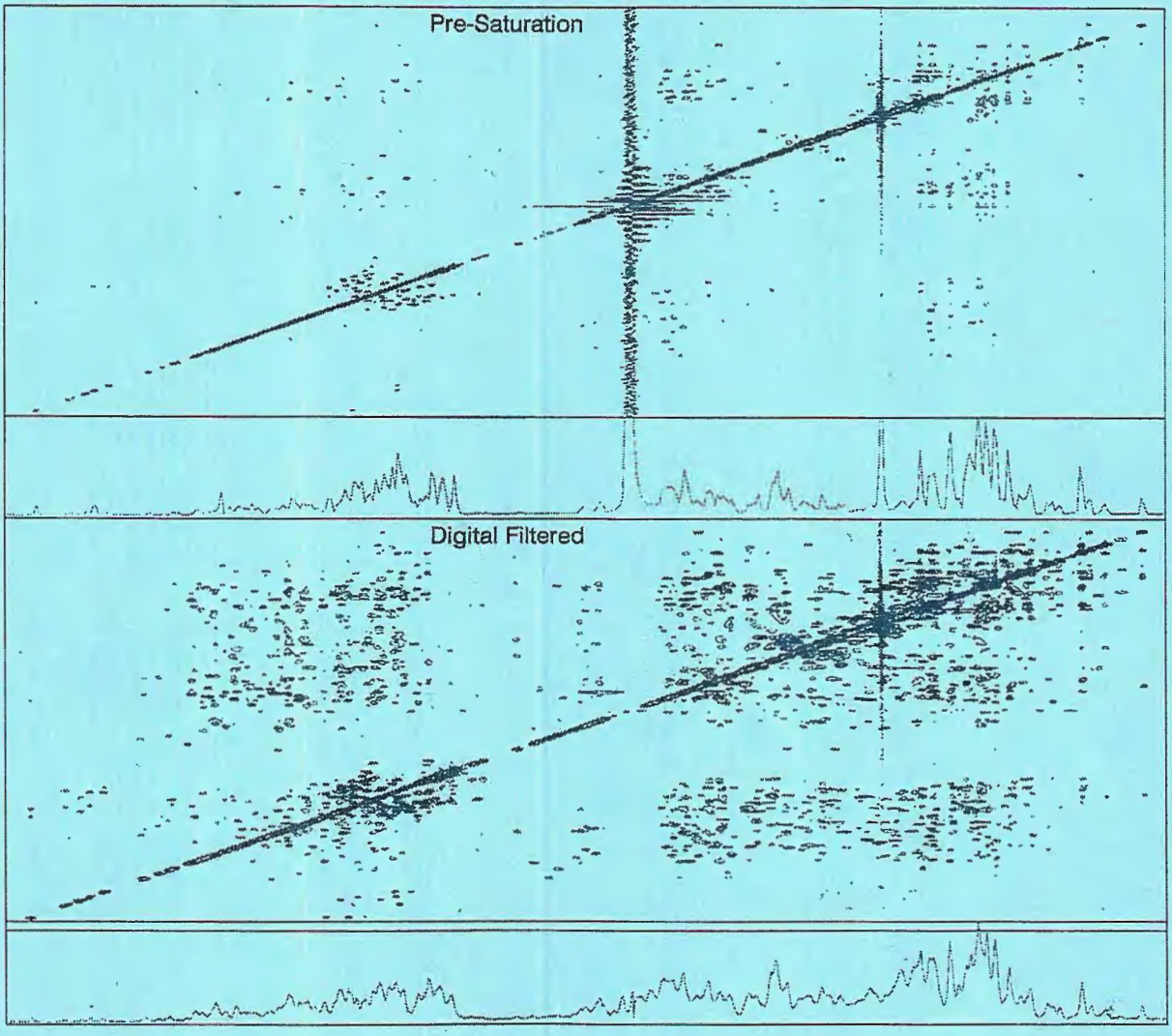

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