THE NMR NEWSLETTER

No. 459

December 1996

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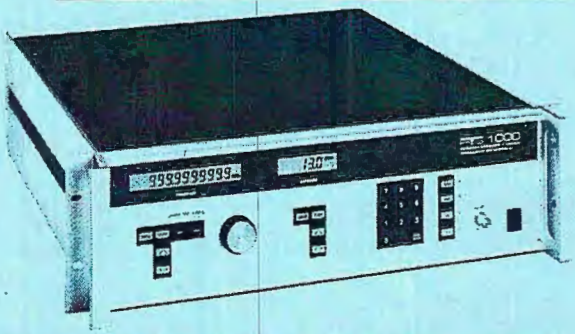
PTS 040	.1-40 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$5,330.00 (1 Hz resol., OCXO freq. std.)
PTS 120	90-120 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$5,330.00 (1 Hz resol., OCXO freq. std.)
PTS 160	.1-160 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$6,495.00 (1 Hz resol., OCXO freq. std.)
PTS 250	1-250 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$7,440.00 (1 Hz resol., OCXO freq. std.)
Type 1 **PTS 310** Type 2	.1-310 MHz	1 Hz	1-20μs	standard	3½"H×19"W	BCD (std) or GPIB (opt)	1 Hz resol., OCXO: $6,425.00 1 Hz resol., OCXO: $5,850.00
PTS 500	1-500 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$8,720.00 (1 Hz resol., OCXO freq. std.)
PTS 620	1-620 MHz	optional .1 Hz to 100 KHz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$9,625.00 (1 Hz resol., OCXO freq. std.)
PTS 1000	0.1-1000 MHz	optional .1 Hz to 100 KHz	5-10μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$11,830.00 (1 Hz resol., OCXO freq. std.)
PTS 3200	1-3200 MHz	1 Hz	1-20μs	optional	5¼"H×19"W	BCD (std) or GPIB (opt)	$14,850.00 (1 Hz resol., OCXO freq. std.)
PTS x10	user specified 10 MHz decade	1 Hz	1-5μs	standard	3½"H×19"W	BCD (std) or GPIB (opt)	$3,000.00 (1 Hz resol., OCXO freq. std.)
PTS D310	two channels .1-310 MHz	.1 Hz	1-20μs	standard	5¼"H×19"W	BCD (std) or GPIB (opt)	$8,560.00 (.1 Hz resol., OCXO freq. std.)
PTS D620	two channels 1-620 MHz	.1 Hz/.2 Hz	1-20 μs	standard	5¼"H×19"W	BCD (std) or GPIB (opt)	$13,240.00 (.1 Hz/.2 Hz resol., OCXO freq. std.)



1 Switching Time is dependent on digit (decade) switched; see detailed instrument specifications.

2 For applicable digits, see detailed instrument specifications.

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FORTHCOMING NMR MEETINGS

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.

International Society for Magnetic Resonance in Medicine, Fifth Scientific Meeting and Exhibition, Vancouver, BC, Canada, **April 12-18, 1997**; Contact: ISMRM, 2118 Milvia St., Suite 201, Berkeley, CA 94704, USA; (510) 841-1899; Fax (510) 841-2340; Email: info@ismrm.org.

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.

39th Rocky Mountain Conference on Analytical Chemistry, Denver, Colorado; NMR Symposium, **August 4-7, 1977**: Contact: J. P. Yesinowski, Code 6120, Naval Research Laboratory, Washington, DC 20375-5342; 202-767-0415; fax 202-767-0594; email yesinowski@nrl.navy.mil. See Newsletter 458, 8.

6th Meeting of AUREMN (NMR Users Association of Brazil), Rio de Janeiro, Brazil, **12 - 16 May, 1977**; Contact: Snia Maria C. de Menezes, Petrobás/Cenpes/Diquim/Radial 2, Quadra 07 - Ilha do Fundão, 21949-900 Rio de Janeiro, Brazil; Tel. +55 21 598-6171 and 598-6914; Fax. +55 21 598-6296; Email; sonia@cenpes.petrobas.gov.br.

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.

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Professor Alexander Pines
The President's Chair
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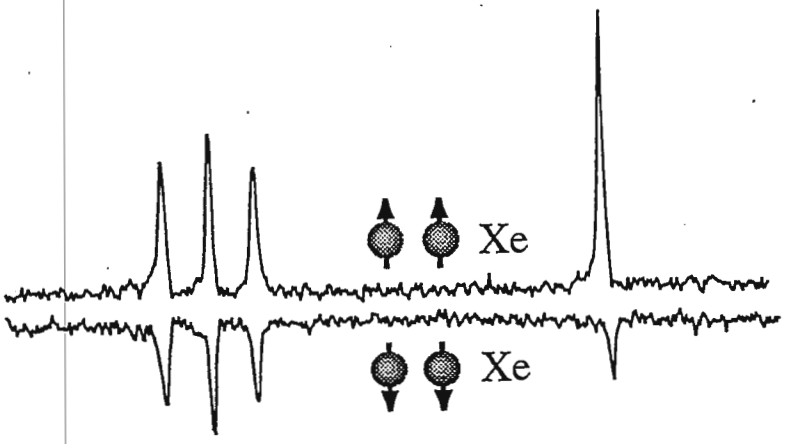
E-mail: Pines@cchem.berkeley.edu

November 13, 1996
(received 11/15/96)

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

Dear Barry:

We read with interest the recent letter reporting negative results on xenon-proton cross-polarization in solution (NMR Newsletter 457-33). We attempted the experiments described but, despite fervid efforts, we were unable to replicate their failure. On the contrary, we noted differential effects for the proton lines of p-nitrotoluene/benzene following the introduction of laser-polarized xenon into solution:



Proton NMR spectrum of p-nitrotoluene/benzene in benzene-d_6 after introduction into solution of laser-polarized xenon-129 with spins "up" or "down". The proton and xenon resonances were perturbed with π pulses in order to exhibit primarily the SPINOE effect over a period of two seconds. (Courtesy of Y.-Q. Song, B. M. Goodson, R. E. Taylor, G. Navon)

Although these experiments were disappointing, they do not by any means eliminate the possibility that insignificant enhancements may be observed with low polarization and reduced concentration.

Divertingly yours,

Alex Pines

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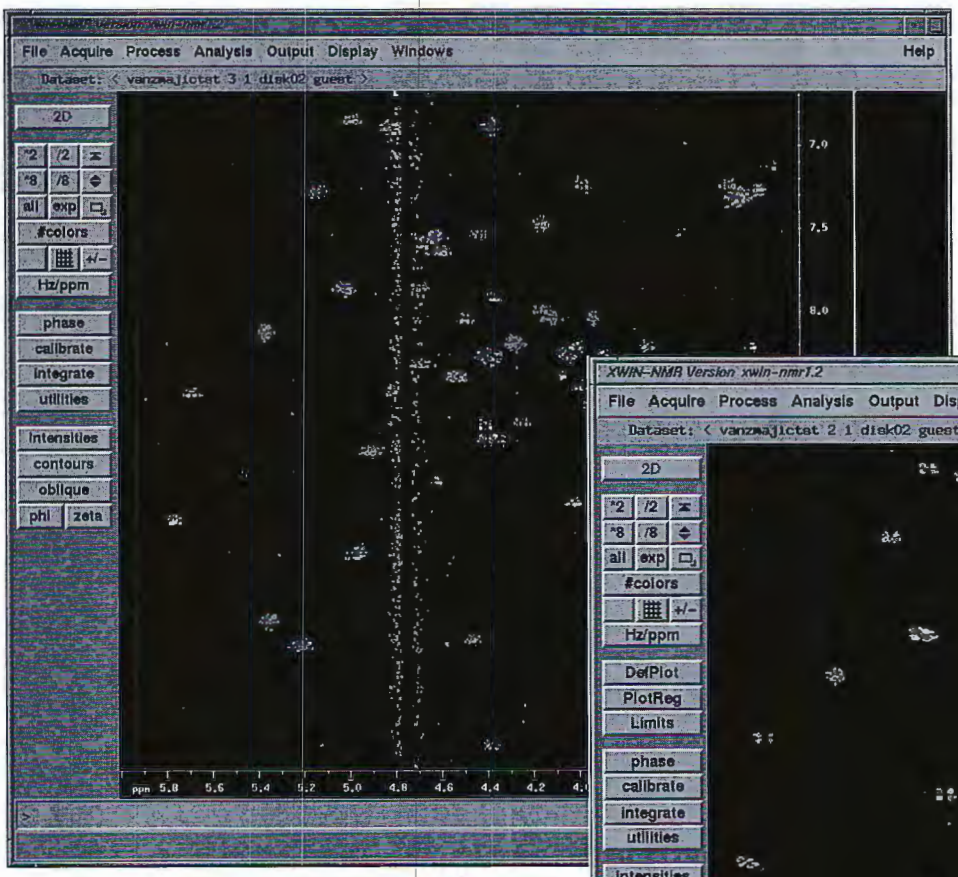

Results with the z-gradient only... residual water ridge is clearly visible and overlaps crosspeaks of interest.

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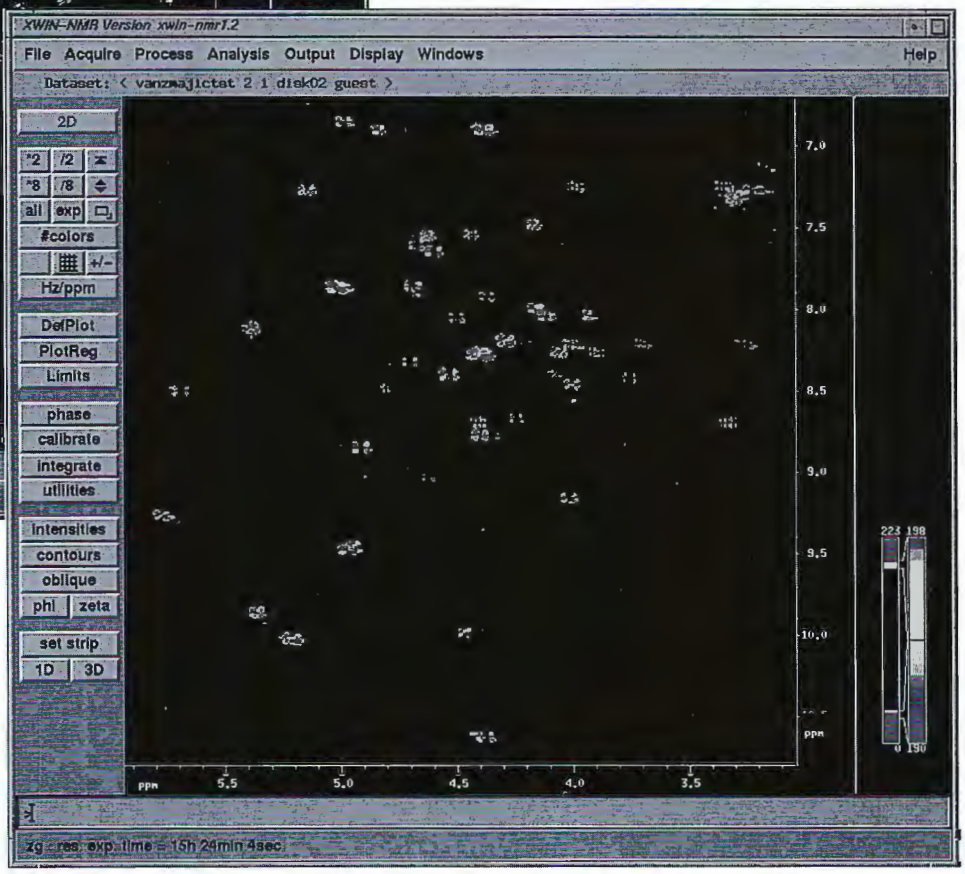
Both experiments were acquired on the Bruker AVANCE 500 equipped with a 5 mm inverse triple resonance (TXI) probe with GRASPTM-III. The sample is 1.5 mM BPTI in 90% H_2O/10% D_2O. The experiment is DQF-COSY.



Results with magic angle gradient... residual water ridge is eliminated! Crosspeaks previously overlapped by the water can be observed and used for correlation assignment. The elimination of the water signal is achieved by coherence selection. No presaturation is used in either experiment!

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November 12, 1996
(received 11/18/96)

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

Title: **CORVUS**, (**C**)omputations (**O**)n (**R**)andomly (**V**)italized (**U**)plifted (**S**)pins

Dear Barry,

To further my relentless investigations on the effects of motion on NMR signals, I have written several BASIC programs that use random numbers to choose molecular orientations and quadrupole coupling constants. Although they are computationally inefficient, the approach easily includes random parameters that are not included in the elegant simulations in programs such as John Waugh's ANTIOPE. In this contribution, I describe some parts of this approach (more will be presented in a future letter and publication). I have simulated a variety of types of NMR signals from spin-3/2 nuclei with a quadrupole splitting and, when a comparison is POSSIBLE, the results agree with ANTIOPE.

In CORVUS powder pattern models, we naturally assume a powder pattern distribution of the angles θ and ϕ that enter into the nuclear quadrupole interaction. Let RND denote a random number between 0 and 1. Note the average value $\langle \text{RND}^2 \rangle = 1/3$. Since $\langle 3\cos^2\theta - 1 \rangle = 0$, CORVUS chooses random θ values as given by $\cos\theta = \text{RND}$. Then, random values of ϕ are obtained by generating a new random number and defining $\phi = 2\pi$ RND. With this scheme, we can simulate powder pattern FID's for given values of QCC and η.

Sometimes we desire the FID's for a Gaussian distribution of ω_Q values. CORVUS generates a random Gaussian value $\omega_Q = \sigma$ GRND, where GRND is a Gaussian random number generated by $\text{GRND} = \{\sum_{i=1}^{12} \text{RND}_i\} - 6$ in which each RND_i is a new random number between 0 and 1 and the rms value of GRND is 1. Also, σ is the rms value of ω_Q.

Of course, a disordered sample can have a distribution of QCC values, or a distribution of η values, or both simultaneously. Since η ranges from 0 to 1, we can generate random η values by using $\eta = \text{RND}$. For a Gaussian distribution of QCC values, we can set $\text{QCC}_c = [1 + n \text{ GRND}]$, where n is the fractional rms value of the deviation of QCC from QCC_c.

We can versatilize the formula for GRND to form other distributions involving random numbers: $\text{VRND} = \{\sum_{i=1}^{V} \text{RND}_i\} - (V/2)$. Then, $\text{QCC}_V = \text{QCC}_c [1 + n \text{ VRND}]$. A square distribution is obtained when V = 1. As V is increased, the shape of the distribution becomes more peaked.

Figure 1, simulation

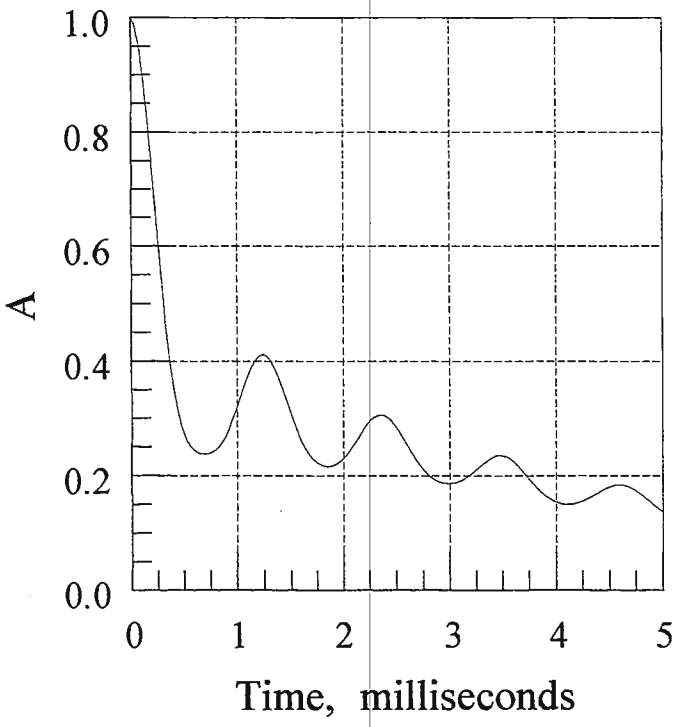


Figure 2, simulation

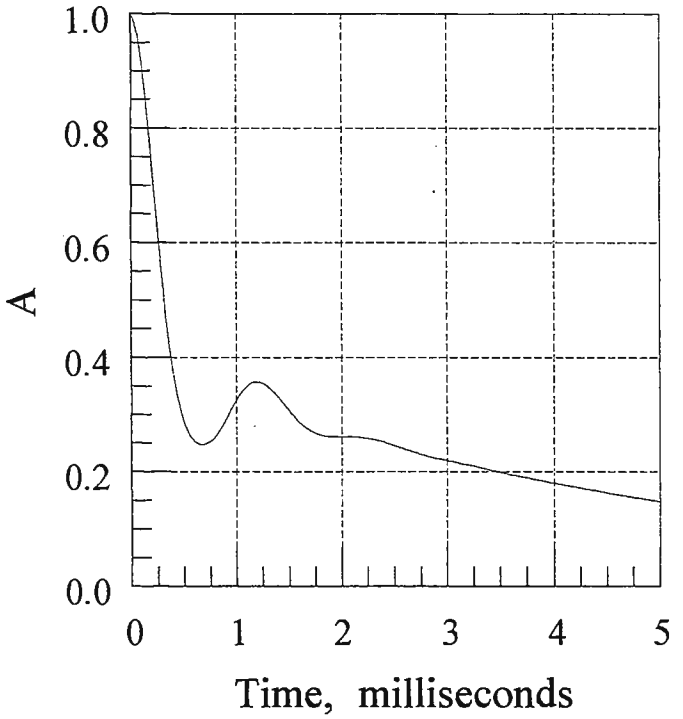


Figure 3, simulation

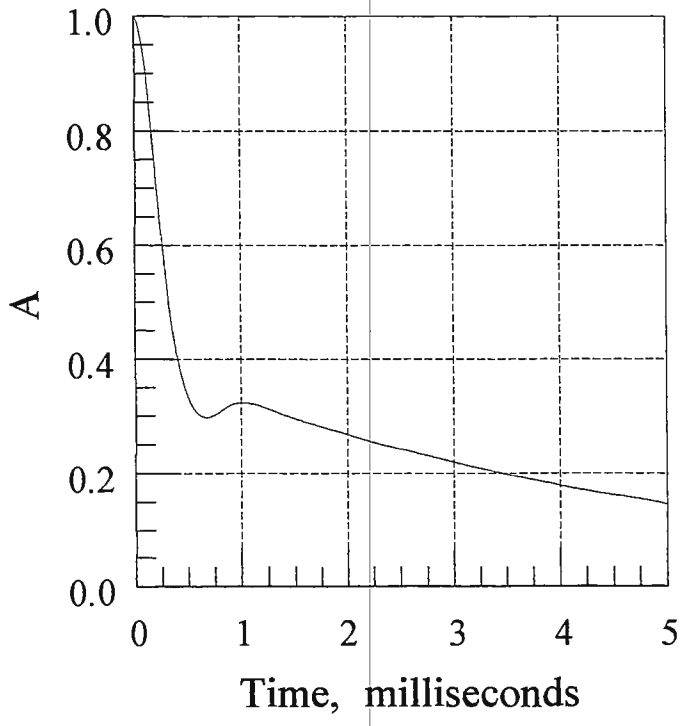
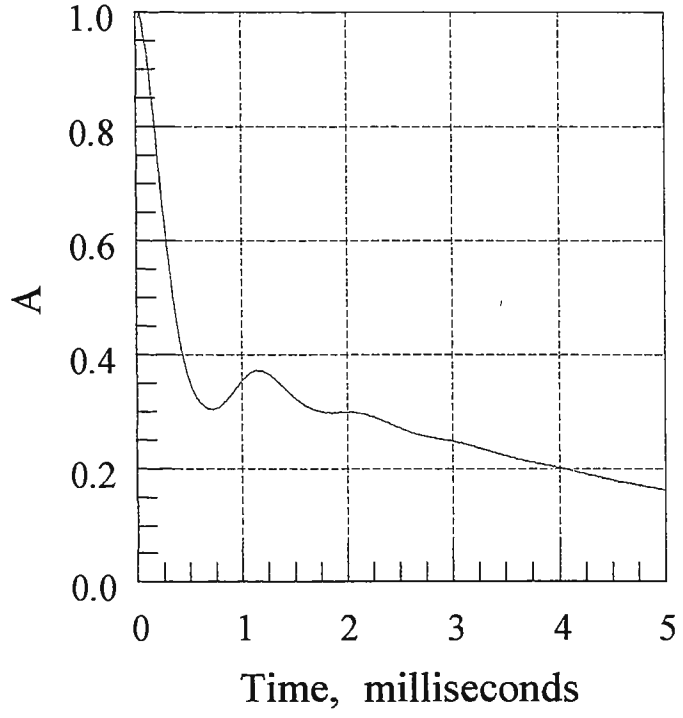


Figure 4, experimental



As you can see, CORVUS can simulate FID's for a wide variety of frequency distributions. CORVUS continually co-adds FID's and occasionally displays a the normalized, current sum of FID's computed with the random numbers. When the display is pleasing, the spectroscopist can hit "H" to halt the process and display on the screen and store as an ASCII file the final signal. To satisfy frequency domain freaks, CORVUS can do a DFT of the FID. However, the time domain signal is more informative.

Figures 1 - 3 show simulations of the on-resonance, real receiver channel FID following a 90° pulse for a powder sample of nuclei of spin 3/2 experiencing an average QCC of 3559 Hz with asymmetry parameter $\eta = 0$. Both the central and satellite signals have $T_2 = 5$ milliseconds. Figure 1 shows the FID for a single QCC value. Note the large oscillations on the "tail" of the signal. Figure 2 shows the FID with a Gaussian distribution of QCC values with $n = 1/6$. Note the rapid decay of the oscillations. Figure 3 shows the FID for the VRND distribution of QCC values for $V = 3$ and $n = 2/3$. Note the further damping of the oscillations.

Figure 4 shows the FID measured for ^{23}Na in a viscous aqueous biopolymer "solution." It is clear that simulations done with CORVUS can aid in the interpretation of spin 3/2 NMR signals from such aqueous systems. I have observed ^{23}Na NMR signals such as this in many different samples. The presentation of other types of ^{23}Na NMR signals from this sample, and the interpretation of the signals, is beyond the scope of this letter.

CORVUS is versatile and can simulate NMR signals from many different pulse sequences, and includes many more features in its physical model than presented here. Unlike ANTIOPE, however, CORVUS assumes infinitely strong rf pulses only. A paper is in preparation that describes the workings of CORVUS, its utility in interpreting signals, and a consistent physical picture of several aqueous systems that many of use almost daily.

If anyone finds this of interest, please let me know.

Sincerely,

Don

Donald E. Woessner
don.woessner@corvus.com

Merck & Co., Inc.
P.O. Box 2000
Rahway NJ 07065-0900

(received 10/31/96)
October 25, 1996

Prof. Bernard L. Shapiro
Editor, *The NMR Newsletter*
966 Elsinore Court
Palo Alto, CA 94303

MERCK
Research Laboratories

Combined 1D Z-Filtered Gradient TOCSY and Homonuclear Decoupling

In an attempt to determine the relative stereochemistry at C-17 of the triterpene derivative shown partially below, we used 1D Z-filtered gradient TOCSY with selective excitation of the 17-methyl protons in order to reveal the H15—H17 spin system. We were hoping to measure the coupling constants between H17 and its neighboring methylene protons but its signal was too complex to extract the J values by simple inspection (Figure 1b). It occurred to us to use homonuclear decoupling of 17-Me during acquisition of the 1D TOCSY (Figure 1c) which resulted in a much simplified signal (dd, $J = 13.0, 4.0$ Hz). The J values clearly indicate that H17 is axial.

Homonuclear decoupling and modern pulse sequences can thus be a handy combination.

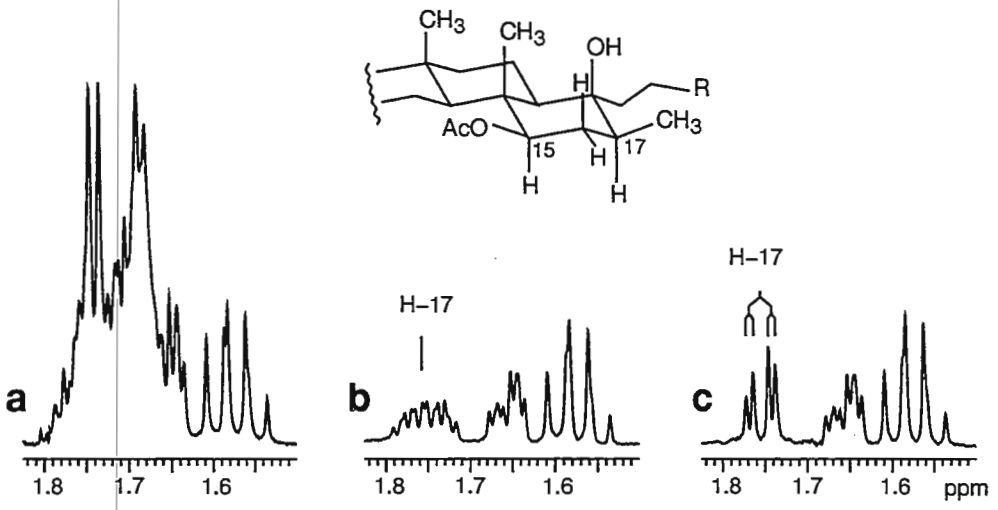


Figure 1. a) Part of the normal ^{1}H spectrum. b) Z-filtered gradient TOCSY from 17-Me. c) Same as b with homonuclear decoupling. The spectra were obtained on a Varian Unity-500.

George A. Doss
Merck Research Laboratories

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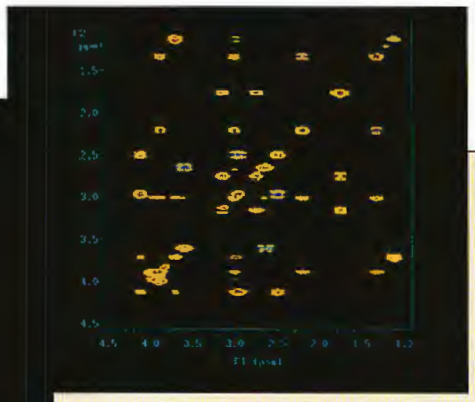
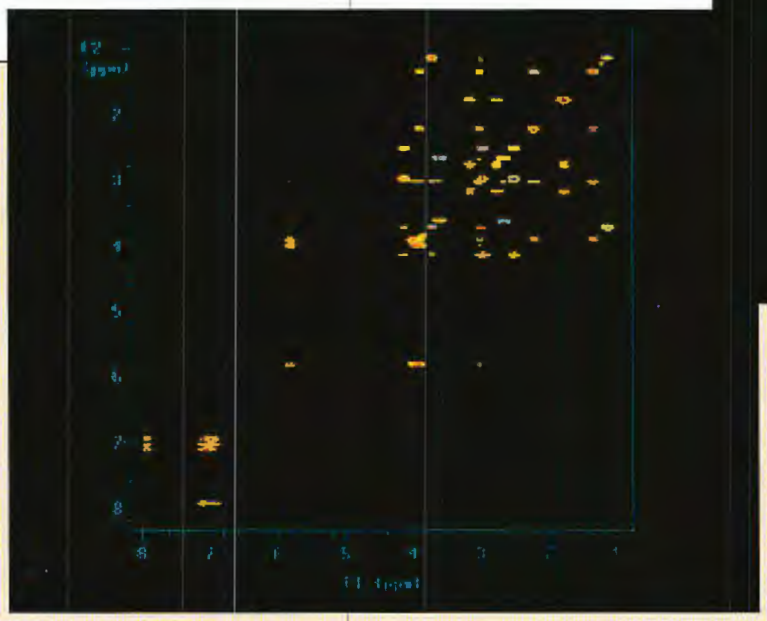


Expanded region of the TOCSY spectrum.



Spinlock experiments, such as this TOCSY spectrum of strychnine, are routinely performed with ***MERCURY***.

MERCURY features advanced experiment capability, as is demonstrated in this high-quality ROESY spectrum.

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November 15, 1996
(received 11/19/96)

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

X-Filtered 2D Spectroscopy Revisited

Dear Barry:

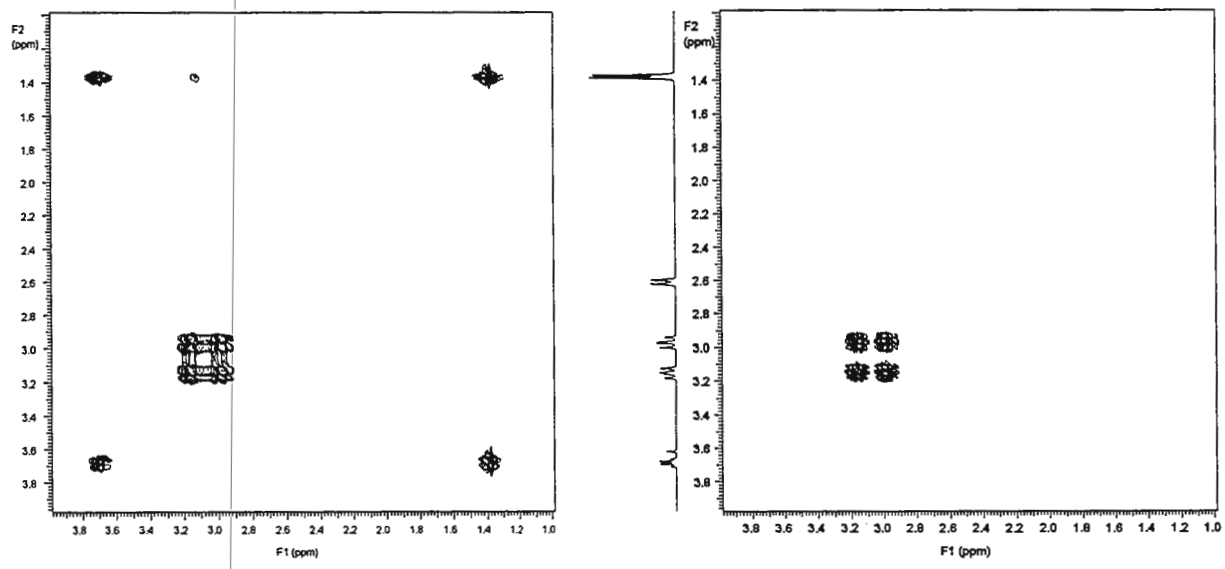
We recently had occasion to revisit X-nucleus filtered 2D spectra for one of our biochemists who is working with a cadmium bound enzyme. Worgotter, Wagner, and Wuthrich[1] demonstrated in 1986 that the addition of a 180° pulse on cadmium would cause cadmium coupled signal not to refocus, providing a mechanism to identify sites in the protein that are coupled to cadmium, even if they are obscured by a mass of other proton resonances. When we implemented this technique on our UnityPlus 500, we were faced with the problem of how best to take a difference between the normal 2D spectrum and the spectrum with the cadmium coupled signals suppressed. Rather than taking alternate scans with and without the 180° pulse and doing the subtraction 'on-the-fly' as the data is acquired, we opted to collect two sets of data , one with and one without the 180°pulse. Also, to aoid any subtle timing differences, the optional 180° pulse was implemented as two back-to-back 90° pulses which were either in phase to give the 180° pulse or 180° out of phase to give no net pulse. This ultimately resulted in very clean difference spectra of only the cadmium coupled signal.

This data was collected as hypercomplex phase sensitive data, so we already had a 2 element phase array, and we added a 2 element xpulse array to generate a total of 4 different data tables. The Varian wft2d command requires a list of 4 coefficients per data table in order to define how to process hypercomplex phase sensitive data. While it is fairly easy to define the 16 coefficients necessary to process this data, it it not especially convenient to have to enter that list all the time. Also, depending on the order in which the phase and xpulse arrays were originally defined, the order of the coefficients changes. Normally a user would simply use the macro which examines the data and choses a set of coefficients, but this macro could not take the extra array into account. Consequently, we wrote a modified version of the macro that examined both the order of the arrays and an ftmode parameter in order to properly calculate all 4 possible 2D spectra: the original 2D spectrum, the spectrum with the X-coupled lines removed, the sum of the two (root 2 S/N improvement), and the difference (X-coupled only). Once this wft2da macro was

[1] Simplification of Two-dimensional ^{1}H NMR Spectra Using an X-filter, Worgotter, Wagner, & Wuthrich. JACS **108**, 6162-6167 (1986)

complete, we extended it to the wft1da macro so that users could continue to use the interactive weighting and phase adjustment menus for interactive processing of their 2D data sets.

So far we have added the X-filter capability to the tndqcosy and tntocsy experiments, and will extend the technique to the tnnoesy sequence in the near future. The figures show the DQCOSY spectra obtained on a test sample of alanine and Cd-EDTA in D_2O. The first spectrum shows the normal DQCOSY, with the acetate methyl signals at 2.6δ suppressed by the double quantum filter. The methylenes of EDTA and the alanine give a normal COSY presentation. The second spectrum shows the difference spectrum with only the cadmium-coupled methylenes present. The alanine signals are completely suppressed. Unfortunately, I do not yet have any acual protein spectra to show. Perhaps the next samples will be better!



The code for the pulse sequence, sample parameter set, and setup macro, along with the modified wft1da and wft2da processing macros are all available to anyone who wants them. They are currently available by request by e-mail, but I anticipate getting everything posted on our Web page within the next several weeks. Please check the Web page, under software, before sending e-mail. This pulse sequence, and the supporting macros and parameters were all writtten and developed under VnmrX 5.1 on a UnityPlus console. There are currently no plans to move this to any of our other systems.

Sincerely,

Steven K. Silber
Senior Research
Instrumentation Specialist

s-silber@tamu.edu
http://wwwchem.tamu.edu/services/NMR

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Department of Biochemistry
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Dr. Bernard L. Shapiro
966 Elsinore Court
Palo Alto, CA 94303
USA

12 November 1996
(received 11/15/96)

^{13}C Relaxation Measurements in Cardiac N-Troponin C

Dear Dr. Shapiro,

One of the focal points of our group is the study of muscle protein structure and dynamics. Backbone dynamics measurements in proteins are usually performed with uniformly ^{15}N-labeled protein. Relaxation studies utilizing ^{13}C on the other hand, have relied on high concentration natural abundance or site-specifically labeled samples. Recently, Lewis Kay and co-workers designed pulse sequences for measurement of $^{13}C_\alpha$ relaxation in uniformly $^{13}C/^{15}N$-labeled proteins[1]. We have used these sequences for determining $^{13}C_\alpha$ T_1 and $T_{1\rho}$ at 500 MHz and T_1 at 600 MHz for cardiac N-Troponin C, an 89 residue Ca^{2+} -binding protein involved in the regulation of contraction in heart muscle (Figure 1).

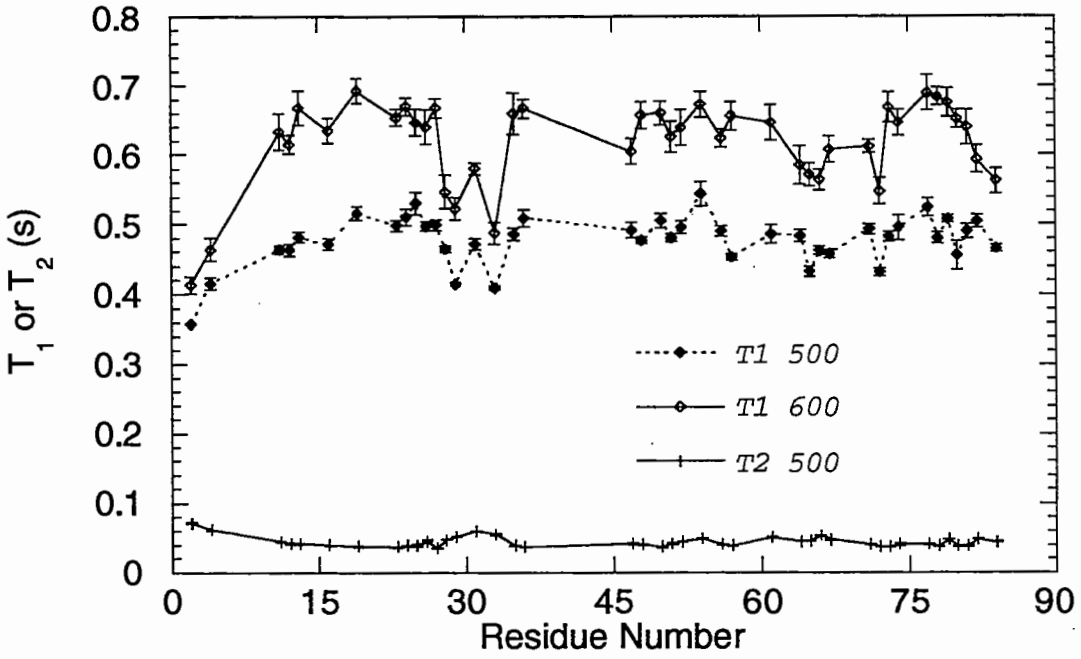

Figure1: $^{13}C_\alpha$ T_1 and $T_{1\rho}$ at 500 MHz and T_1 at 600 MHz for Ca^{2+} -free cardiac N-Troponin C dissolved in D_2O, 100 mM KCl, 15mM EDTA, 10 mM DTT, 10 mM imidazole, pH 6.7, 30 °C. The correlation time was calculated to be about 6 ns.

The results are an excellent complement to our structure determination of the protein. For example, the first Ca^{2+} binding loop (residues 29-34) is clearly more flexible than surrounding

helical regions, as evidenced by increased $T_{1\rho}$ and decreased T_1 values. We look forward to analyzing Ca^{2+}-induced effects on the dynamics and energetics of the protein, using a variety of heteronuclear-based relaxation measurements.

We thank Lewis Kay for providing pulse sequences.

References:

1. Yamazaki, T., Muhandiram, R., and Kay, L.E., *J. Am. Chem. Soc.* **116**: 8266-8278 (1994).

Sincerely,

Leo Spyracopoulos Stéphane Gagné Brian Sykes

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Institute on Aging
Gerontology Research Center
4940 Eastern Avenue
Baltimore, MD 21224

NIH POSTDOCTORAL POSITION AVAILABLE

A postdoctoral position is available in the NMR Unit of the National Institute on Aging of the National Institutes of Health, located in Baltimore, Maryland. Present research includes imaging studies of connective tissue biophysics (whole cartilage, chondrocytes in culture, and *in vivo* cartilage imaging), spectroscopic studies of muscle metabolism under a variety of pharmacologic and physiologic conditions, and methodology development in imaging and spectroscopy.

Instrumentation consists of a double-resonance Bruker ABX 1.9T/31 cm Biospec with shielded gradients, and a triple-resonance wide-bore Bruker AMX 400 with microimaging and solids capability. Upgrade to a DMX system will occur within the next few months.

A strong background in NMR spectroscopy or imaging is required. Experience with *in vivo* experiments and a background in biochemistry/physiology is preferred. The appointment will be as an IRTA Postdoctoral Fellow for US citizens, or as a Visiting Fellow for US non-citizens. Applicants must have fewer than five years of postdoctoral experience.

Interested individuals should send their CV and the names, telephone numbers, and e-mail addresses of three references to: Dr. Richard Spencer, NIH/NIA, GRC 4D-08, 4940 Eastern Avenue, Baltimore, MD 21224; Tel. 410-558-8226, e-mail: spencer@helix.nih.gov.

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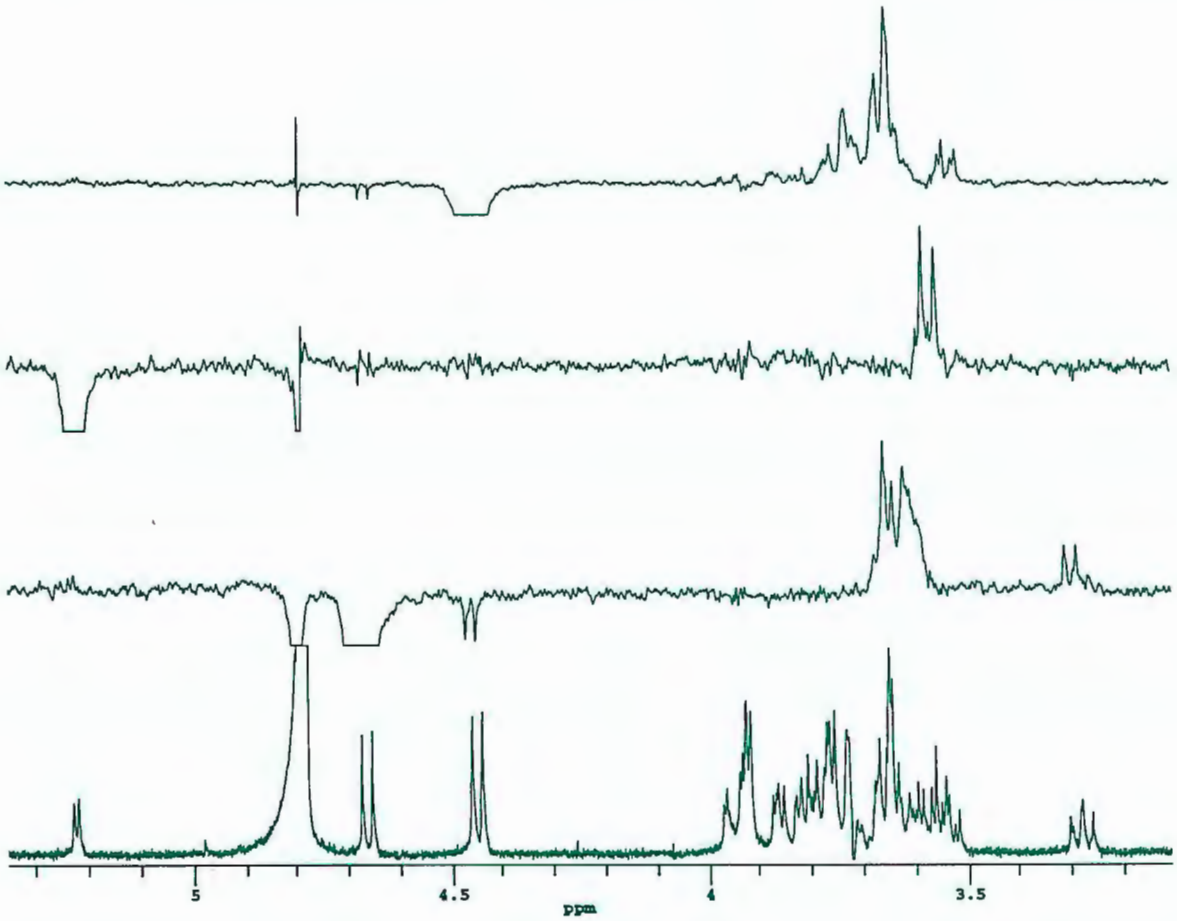

This spectrum was collected in 128 acquisitions

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NOe difference spectra of 1.0 mM lactose in D_2O are shown here. The bottom trace is the normal ^1H spectrum acquired in a single acquisition. Upper traces are the nOe spectra acquired with presaturation of different resonances. Note the excellent cancellation of unperturbed peaks.

Data were acquired on a CMX Infinity 400 MHz spectrometer equipped with a Nalorac™ 5 mm indirect detection triple resonance gradient probe. The nOe spectra were collected in 128 acquisitions.

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&
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"Double-WURST" Decoupling for ^{15}N- and ^{13}C-Double-Labeled Proteins in a High Magnetic Field

(received 11/12/96)

Dear Barry,

Recently, we constructed a "Double-WURST" sequence (1) for simultaneously decoupling the ^{13}CO and $^{13}C_\alpha$ regions in $^{15}N/^{13}C$ double-labeled proteins. It uses only one channel with a single waveform generator and a single power amplifier. The decoupling power can be reduced by two-thirds compared to the conventional WURST decoupling, which decouples the entire ^{13}C region including the unneeded region between ^{13}CO and $^{13}C_\alpha$, where there are no protein-backbone resonances. In our experiment, a WURST-20 sequence is used to decouple the ^{13}CO region as shown in Fig. 1a. The $^{13}C_\alpha$ region is decoupled with a frequency-shifted WURST-20 (Fig. 1b), which uses the same frequency offset as the ^{13}CO decoupling and a linear phase-increment in the pulse (2, 3). The amount of frequency shift is determined by $\Delta f = \Delta\varphi / 2\pi\Delta t$, where $\Delta\varphi$ and Δt are the phase and time increment, respectively. The two decoupling sequences are combined to a Double-WURST-20 sequence simply using a vector sum in the waveform generator together with a phase cycle of $(0^\circ, 150^\circ, 60^\circ, 150^\circ, 0^\circ)$. The result is shown in Fig. 1c and Fig. 2. This techniques is especially helpful at a high magnetic field, 750MHz for example, where one faces a ^{13}C decoupling range of ~27 kHz with single decoupling while only ~9 kHz with double decoupling.

(1) S. Zhang, J. Wu, and D. G. Gorenstein, *J. Magn. Reson.* A (in press).

(2). L. E. Kay, D. Marion, and A. Bax, *J. Magn. Reson.* **84**, 72 (1989).

(3) S. Zhang and D. G. Gorenstein, *J. Chem. Phys.* **105**, 5659 (1996).

Sincerely.

Shanmin Zhang

Shanmin Zhang David G. Gorenstein

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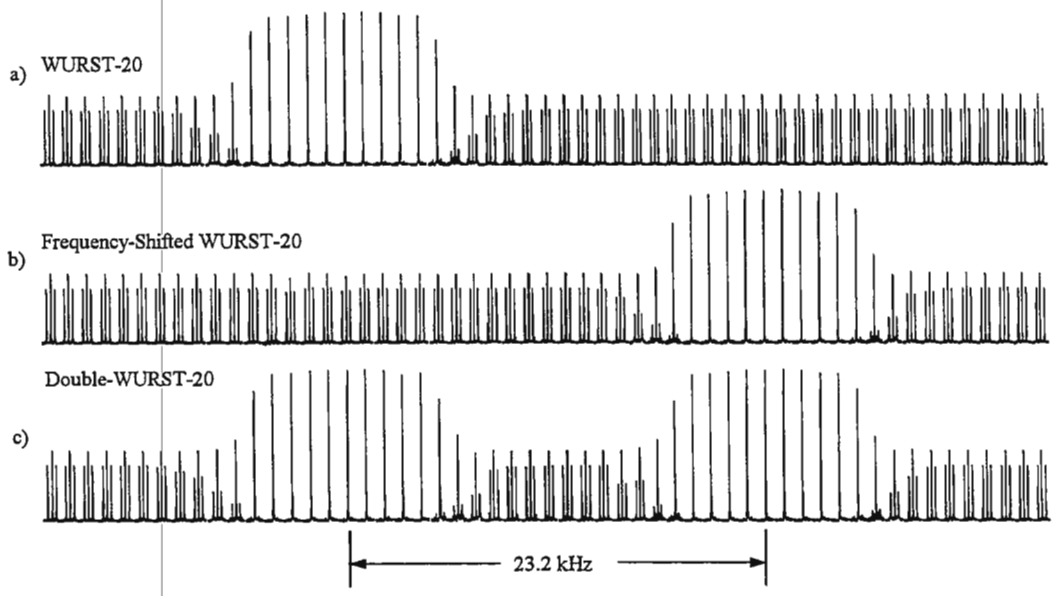


Fig. 1. ^{1}H spectra of ^{13}C (~64%) labeled CH_3I with WURST-20 decoupling (a), frequency-shifted WURST-20 decoupling (b), and the Double-WURST-20 decoupling (c).

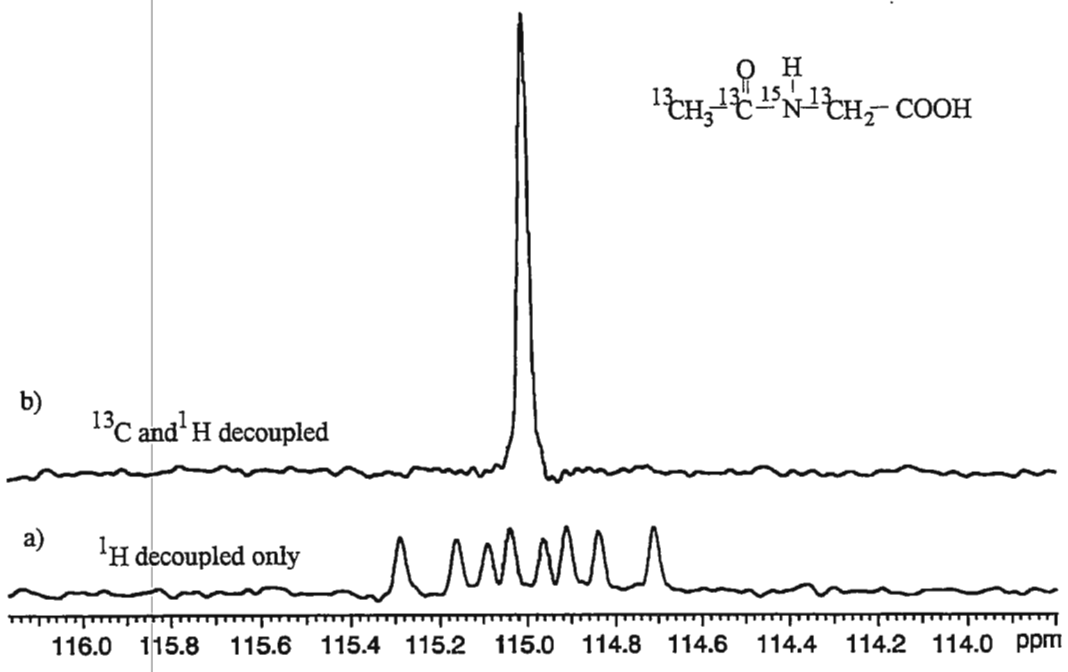


Fig. 2. ^{15}N spectra of a 85 mM ^{15}N- and ^{13}C-labeled (-COOH is unlabeled) N-acetyl-glycine without ^{13}C decoupling (a) and with Double-WURST decoupling (b).

DEPARTMENT OF THE NAVY
NAVAL RESEARCH LABORATORY
4555 OVERLOOK AVE SW
WASHINGTON DC 20375-5320

IN REPLY REFER TO:

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

13 November 1996
(received 11/21/96)

^{129}Xe NMR Studies of Polystyrene Microgels

Dear Barry:

Polystyrene microgels ("rubbery nanospheres") are an interesting class of materials exhibiting novel properties [1]. Synthesized by polymerization and crosslinking of styrene in microemulsion [2], microgels exist as globules, whose radii depend on their molecular weight. The polystyrene microgels that we have studied had low polydispersities, with molecular weights in the range of 4×10^4 to 3×10^6 g/mol, as measured by thermal field flow fractionation. All microgels have as many as one crosslink per every ten repeat units (i.e., "1/10"), distributed randomly.

We have recently obtained ^{129}Xe NMR spectra (B_0 = 7T, 83 MHz) of linear polystyrene and a variety of microgels, all of which were soaked in xenon gas at 25 psi overnight; the microgels varied both in molecular weight and cross link density (CLD). For example, Figure 1 shows ^{129}Xe NMR spectra of "powdered" samples of linear polystyrene "A" ($M_w = 0.1 \times 10^6$, CLD=0.0), microgel "B" ($M_w=7.2 \times 10^6$, CLD=1/10), microgel "C" ($M_w=1.9 \times 10^6$, CLD=1/20), and microgel "D" ($M_w=2.2 \times 10^6$, CLD=1/40).

Interestingly, the microgels exhibit chemical shifts (relative to ^{129}Xe gas @ 0.0 ppm) which lie 15-20 ppm *upfield* from linear polystyrene. These shifts do not appear to be a simple consequence of cross link density, since virtually no difference is observed between samples "C" and "D" (which have similar molecular weights); the effect of macroscopic particle size is as yet unknown. We intend to sort much of this out by obtaining ^{129}Xe spectra on neat and blended microgels, in both solution cast and melted forms. We'll keep the NMR community posted!

Please credit this contribution to the Naval Research Laboratory.

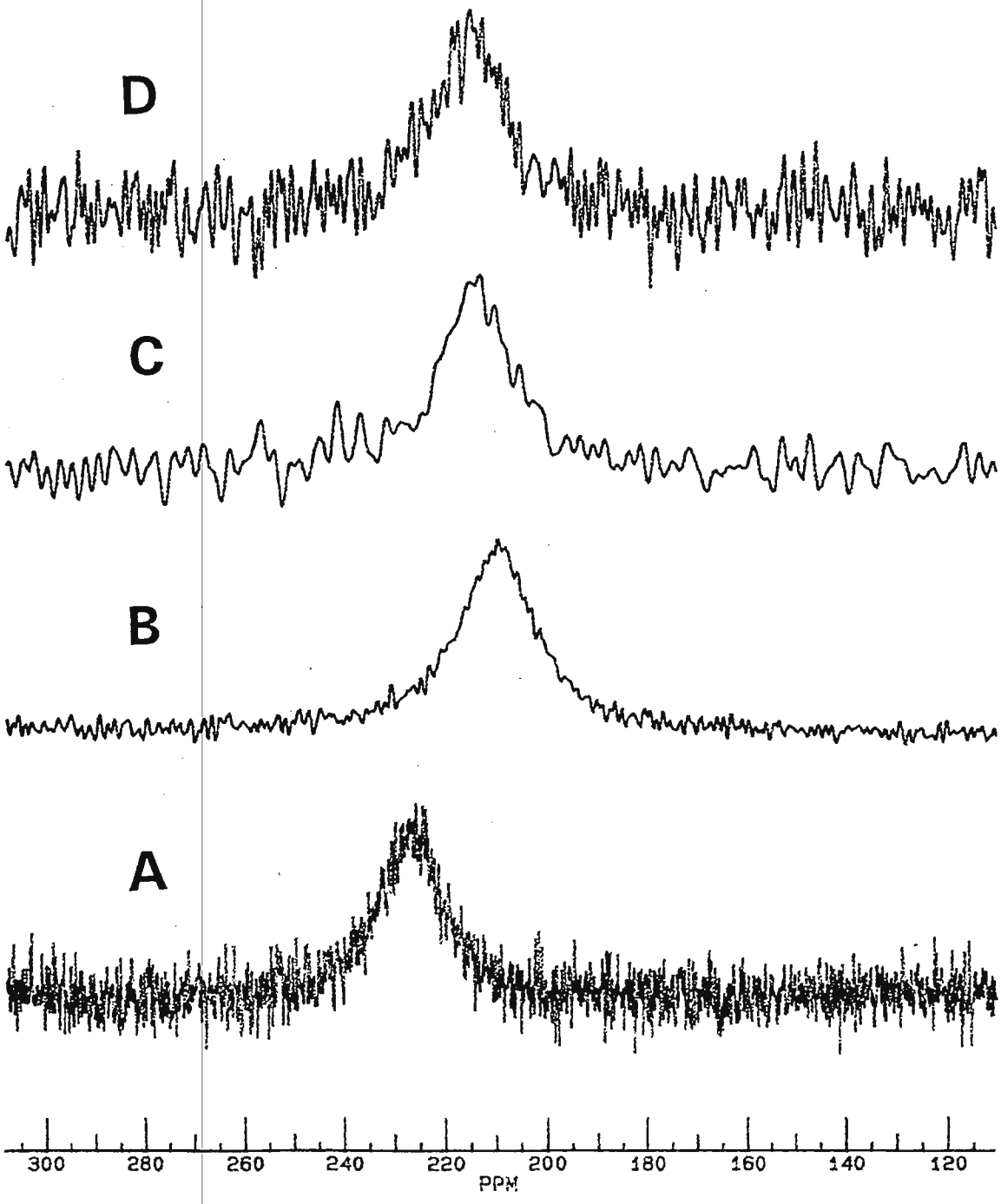
With best regards,

Ken McGrath

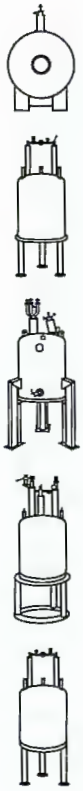
K.J. McGrath, Naval Research Lab
C.M. Roland, Naval Research Lab
M. Antonietti, Max-Planck-Institut
M. Neese, Max-Planck-Institut

References:
1. Roland, C.M.; Bero, C.A.; Ngai, K.L.; Antonietti, M. in *Electrically Based Microstructural Characterization, Mater.Res. Soc. Proc. Series*, Vol. 411, 1996.
2. Antonietti, M; Basten, R.; and Lohmann, S. *Macromol. Chem. Phys.* **1995**, *196*, 441.

FIGURE 1: ^{129}Xe NMR Spectra of Linear and Microgel Polystyrenes



Beware of imitations



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500	51	10	150	3.2
400	54	8	365	2.8
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
270	89	2.7	365	2.8
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Use of a 10mm probe for RNA Structure Determination

(received 11/19/96)

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

Dear Dr. Shapiro,

We recently have determined the structure of a flexible RNA molecule: a 17mer hairpin consisting of nucleotides 49-65 of the TΨ-loop derived from the T-arm of tRNA. This fragment of RNA is a substrate for the enzyme RUMT which methylates the U54 residue of tRNA. Homonuclear NMR methods in conjunction with restrained molecular dynamics (MD) methods were used to determine the solution structure of the 17mer T-arm fragment.

High salt buffers (50 mM NaCl or 5 mM $MgCl_2$) that on the one hand stabilized the hairpin thermodynamically (as evidenced by UV absorption melting curves) were not conducive to our NMR studies since, on the other hand, they promote formation of a second species. At 2.5 mM RNA (250 O.D.s in 250 µL, 5mm Shigemi tube), a complete second set of peaks for the pyrimidine H5-H6 protons is observed in the TOCSY spectrum (Figure 1, left). At 1.3 mM RNA (0.5 mL, regular 5 mm tube), growth of the second species is observed overnight. Gel filtration studies in various buffers and different annealing protocols indicated that this second species is a duplex. These observations are the typical, notorious behavior for RNA hairpins that are in equilibrium with duplex species.

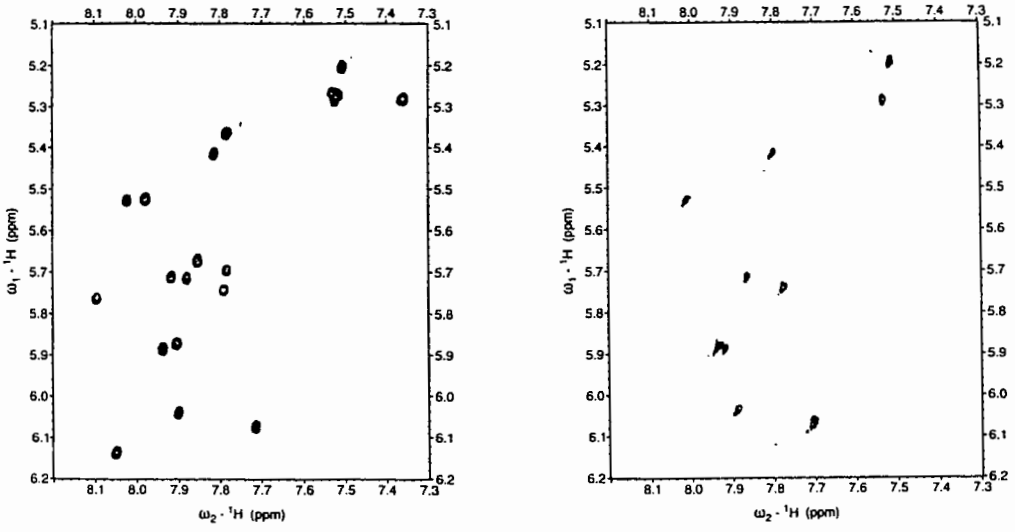


Figure 1. Portion of the TOCSY spectra of the 17mer RNA hairpin collected at 25 °C, 10 mM potassium phosphate, pH 6.4, 600 MHz showing the pyrimidine H5-H6 connectivities. (Left) 2.5 mM RNA; (Right) 0.3 mM RNA.

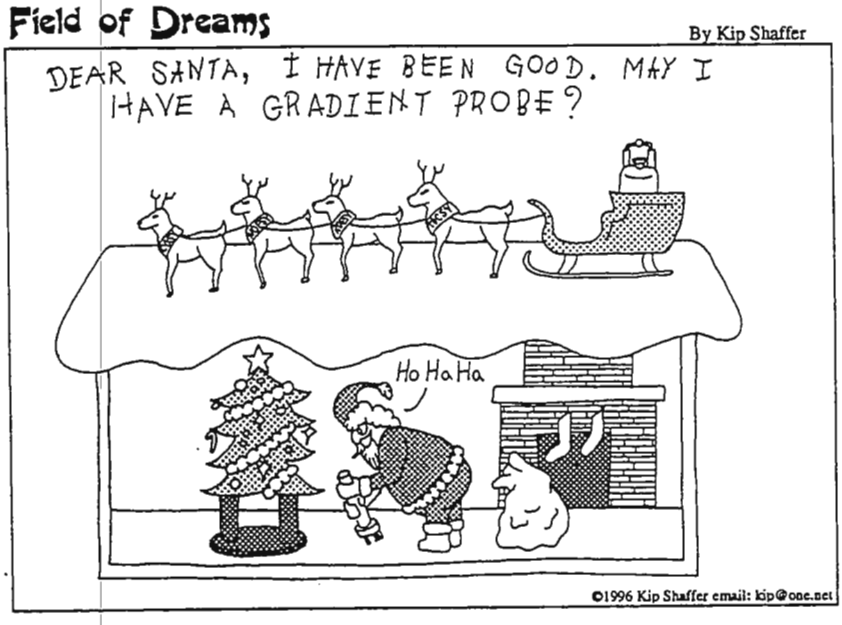
Eventually, dilution of the sample to 0.3 mM (3 mL) utilizing a 10 mm NMR probe (Varian Associates) practically eliminated formation of the second species, such that we were able to record high quality 2D NMR spectra on the single monomer species without interference by signals from the second species (Figure 1, right). All NMR experiments used for the structure determination were collected at this low concentration (25 °C or 10 °C, 10 mM potassium phosphate, pH 6.4). This strategy was also effective for the study of a 24mer RNA (Schmitz et al. *RNA* in press), where besides the problem of duplex formation we could also overcome RNA aggregation effects, notorious for high Mg^{2+}-concentrations. Even for the structural study of a Tat peptide (Mujeeb, et al. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 8248) lowering the concentration was very beneficial. Without the 10 mm probe, these studies would not have been possible.

Even at RNA concentrations around 0.2 mM, sensitivity was sufficient to perform the typical homonuclear experiments (COSY, TOCSY, NOESY) with 16-32 scans per t_1-increment. Our 10 mm probe is a proton-only probe and is not equipped with gradients. Water suppression was achieved using presaturation in D_2O samples. For observation of exchangeable protons, we used the SSNOESY sequence. In our experience with the Varian ultrashims, the shimming procedure is no more difficult or time-consuming than for our 5 mm probes. Furthermore, shimming appeared to be less sensitive to the positioning of the sample in the probe when Shigemi tubes were used that had a sample volume covering exactly the height of the coil. Subsequent projects involving RNA in our laboratory are being conducted using a triple resonance 8 mm probe.

We hope that this information will be useful to other researchers experiencing problems with sample aggregation and sample heterogeneity .

Sincerely,

Letitia Yao Uli Schmitz Tom James



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Professor J. C. Lindon

BIRKBECK COLLEGE
University of London

6 November 1996
(received 11/15/96)

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

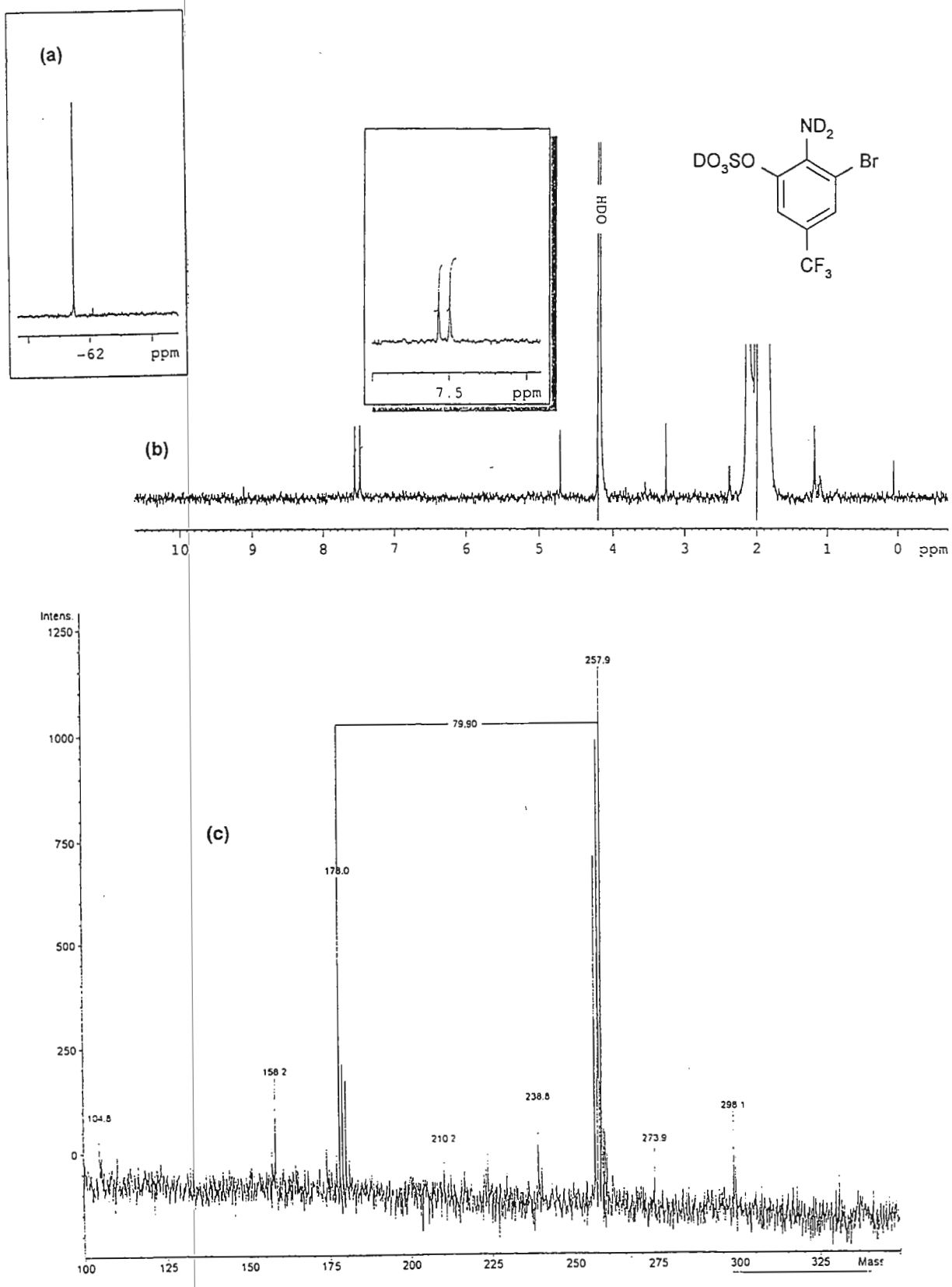
Dear Barry,
DIRECT COUPLING OF HPLC, NMR SPECTROSCOPY AND MASS SPECTROMETRY

Now that the direct coupling of HPLC and NMR spectroscopy (and also HPLC-MS) has become rather routine, attention has turned to an even more expensive detector for HPLC, the direct coupling of both NMR and MS concurrently. The first publication demonstrating this was by Pullen *et al*, Rapid Commun. Mass Spectrom. 9, 1003, 1995 and we have shown an application to a real sample, i.e. the separation and characterisation of endogenous and drug metabolites from human urine, (Shockcor *et al* Anal. Chem. Jan 1, 1997 issue). Although expensive, the approach is quite straightforward with the HPLC eluent being split about 19:1 after UV detection and the larger fraction going to the NMR flow probe. The usual HPLC-NMR approaches using continuous flow detection and stop-flow detection are possible. If the molecules of interest have distinctive NMR features such as fluorine resonances or marker ^{1}H signals this can be used to identify components for complementary MS examination. Alternatively if the molecules have good MS handles, such as bromine or chlorine atoms which give distinctive isotope patterns, then the MS can be used to direct attention to the relevant NMR data. The MS (and MS-MS etc) provides nicely complemenatary information to that given by NMR in cases where molecules are proton-sparse or where one needs to detect proton-free functional groups such as sulphate conjugates or N-oxide metabolites of drugs. The example shown here was obtained at Bruker's application laboratories in Germany with the help of Manfred Spraul, Martin Hofmann and Ulrich Braumann and shows the HPLC-NMR-MS of rat urine following dosing with 2-bromo-4-trifluoromethylaniline. This is part of a PhD project being undertaken by Graeme Scarfe in collaboration with Ian Wilson at Zeneca Pharmaceuticals. The ^{1}H NMR was at 500 MHz and the MS used electrospray ionisation on an ion-trap instrument. The advantages for mixture analysis by being able to conduct both NMR and MS structural studies on-line are obvious with the avoidance of clean-up stages and thus the potential loss of unstable materials together with the direct correlation of NMR and MS data on the same eluent fraction unambiguously.

Yours sincerely,

JEREMY NICHOLSON

JOHN LINDON



Directly-coupled HPLC-NMR-MS-MS study of metabolism of 2-bromo-4-trifluoromethylaniline in the rat. Injection of 50 μl of rat urine on to the C18 HPLC column, with UV detection at 254 nm. Acetonitrile/D_2O gradient elution at 0.4 ml/min. The figure shows data collected for a UV peak at a retention time of 52.3 min, provisionally identified as the sulphate conjugate of 2-bromo-4-trifluoromethyl-6-hydroxyaniline. (a) stop-flow ^{19}F NMR spectrum, (b) stop-flow ^{1}H NMR spectrum, (c) stopped-flow MS-MS spectrum of the fragment ions derived from the m/z = 340 ion (corresponding to the molecular ion plus 3 H→D substitutions from the D_2O). The ion at m/z = 258 corresponds to loss of Br which further fragments to give 178, i.e. loss of 80 (SO_3).

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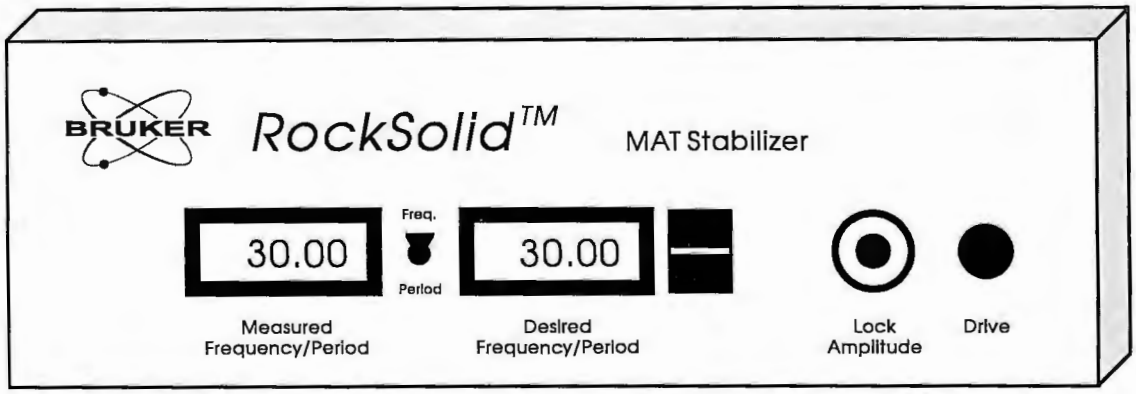
SPECIFICATIONS FOR THE MAT STABILIZER

Spinning Speed Range	20-200 Hz	^1H, $CHCl_3$ sample
Stability	0.1%	indefinite durations
Application	MAT	extracts CSA patterns from powders
Part Number	B1931	requires conventional MAS controller



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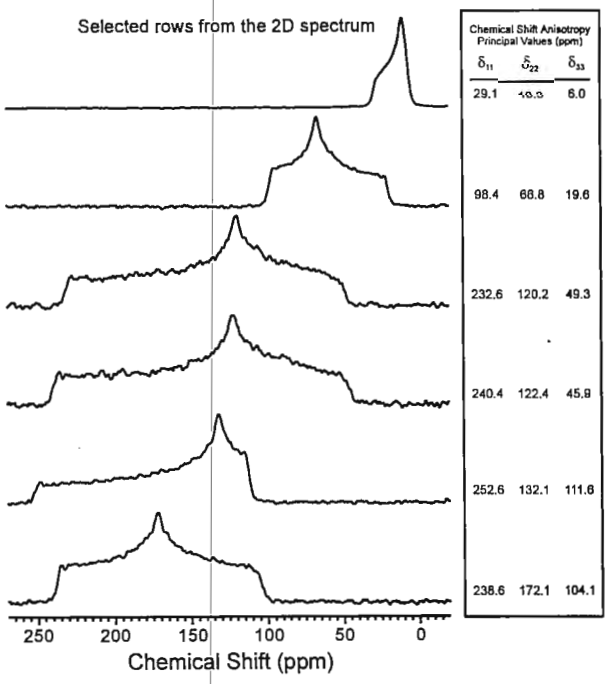
...The NMR Revolution advances

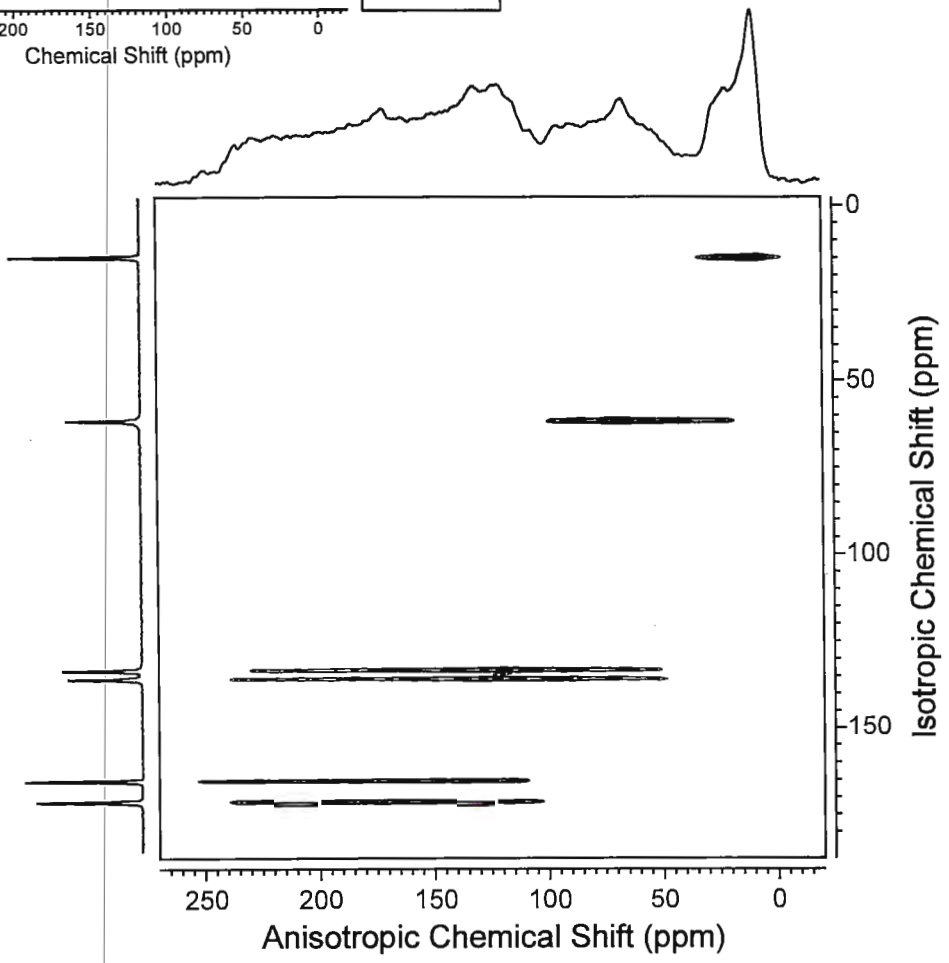
Magic Angle Turning 2D NMR

with the RockSolid™ MAT Stabilizer



Chemical Shift Anisotropy Principal Values (ppm)		
δ_{11}	δ_{22}	δ_{33}
29.1	[illegible]	6.0
98.4	66.8	19.6
232.6	120.2	49.3
240.4	122.4	45.8
252.6	132.1	111.6
238.6	172.1	104.1

Fumaric acid, monoethyl ester
33.33 Hz spinning speed
PHORMAT pulse sequence
No rotor synchronization needed



TLI THE LOVELACE INSTITUTES
Improving Human Health through Research and Technology

15 November 1996
(received 11/20/96)

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

Fluorinated Gases in Lungs are more like Solids than Liquids

Dear Dr. Shapiro:

We have been having some fun and frustration lately making images of rat lungs by having the rats breath a mixture of an inert fluorinated gas (C_2F_6 or SF_6) and O_2, and then imaging the fluorinated gas. Unlike our colleagues who image polarized noble gases, we are imaging the fluorine at its thermal equilibrium polarization. The trick that makes this seemingly preposterous method work is to take advantage of the short T_1s (1 to 3 ms) of these gases. We can signal average very rapidly, collecting up to 200 FIDs during the 40% of one breath cycle when the lungs are most inflated. As you might imagine there isn't much time for switching imaging gradients, so we don't. We do projection imaging, leaving a magnetic field gradient on in a given direction while we repeatedly excite with hard pulses and collect the ensuing FIDs.

With this imaging method and short T_2s, the desirable characteristics for the receiver chain are similar to those for imaging solids. In particular, we want to collect data soon after the hard rf pulses. A long dead time forces us to keep imaging gradient strengths weak so that the first k coordinates are not too large. Weak gradients in turn require long data collection times, which are incompatible with short T_2s. In short, the signal is decaying fast and the early k-space points are valuable. The analogy to solids NMR was especially impressed upon us when we discovered that in an early version of our probe, we were getting substantial interfering ^{19}F signal from the Teflon® in the variable capacitors. We moved the offending Teflon® further from the business part of the coil with great improvement. Subsequent progress came more slowly. Our imager was designed for imaging liquid-like stuff not this solid-like gas! Despite a long dead time (80 μs) we managed to obtain a good lung image, in which we could see small objects like the trachea, bronchi, aorta, vena cava, and esophagus. The hitch is that the data took 4.3 hours to collect.

During a discussion of our dead time problems with Irving Lowe, he kindly offered that I (DOK) make a trip to Pittsburgh to try imaging lungs with his system, which still contains the rf section he built nearly 25 years ago. Irv's graduate student, Michael Gach kindly put himself at my service for 2 weeks. I strongly recommend the eddy current pre-emphasis system that Irv and Arvind developed. Mike and I managed to precompensate the set of unshielded gradient coils we used to image lungs in three days! Having not learned what NMR was until 1987, I found Irv's rf section delightfully quaint, using rf trombones, cables, and knobs to adjust things I am used to adjusting with computer commands. The upshot was that the dead time was 10 μs, and we were able to make a 3D image of rat lungs (next page) with only 32 minutes of data collection.

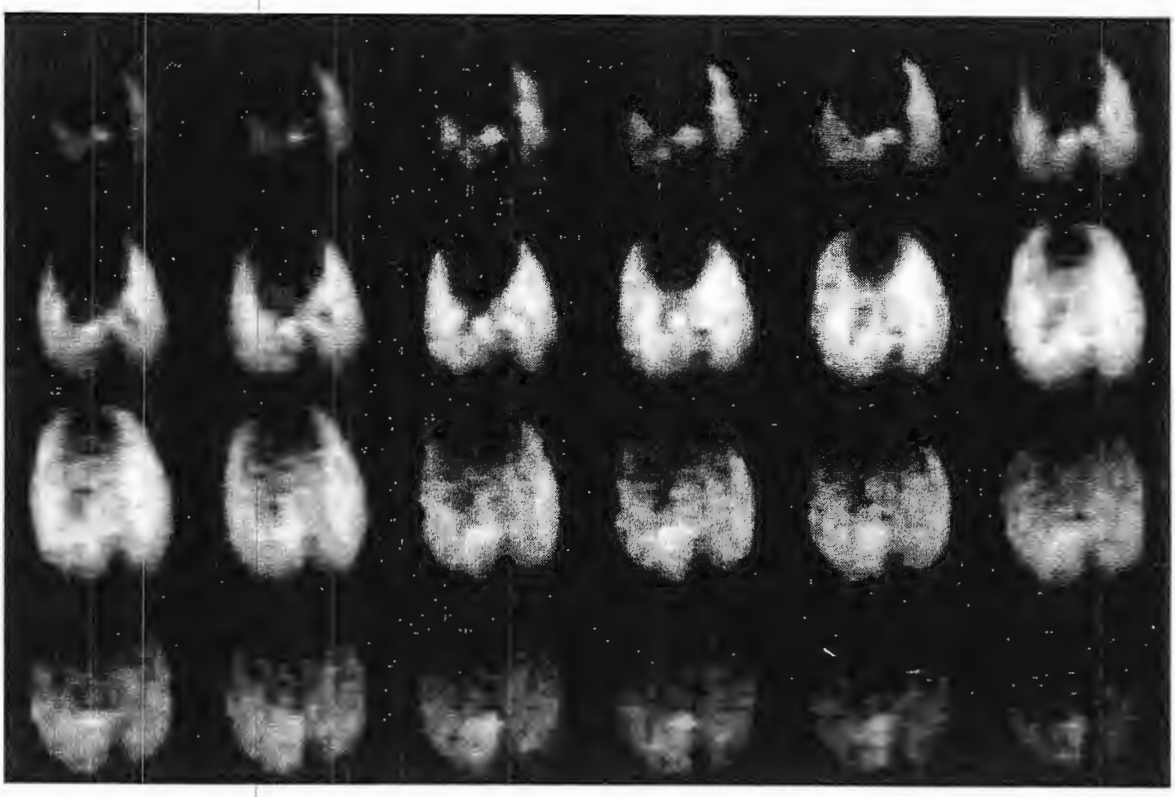


Figure. Twenty four consecutive axial planes of a three-dimensional image of SF6 in rat lungs from anterior (top left) to posterior (bottom right) made after 34 minutes of data collection. The central spot in first three frames is the trachea, which divides into two bronchi (frames 5 and 6). The dark spot in the third row is the vena cava. The heart and mediastinum are the dark area separating the smaller left lung from the larger right in the first and second rows. The broad dark area in the bottom two rows is the diaphragm and liver.

Happy winter solstice to all,

Dean O. Kuethe Arvind Caprihan Eiichi Fukushima H. Michael Gach Irving J. Lowe

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TEXAS A&M UNIVERSITY
Department of Chemistry
College Station, Texas 77843-3255
(409) 845-2011
FAX (409) 845-4719

November 15, 1996
(received 11/19/96)

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

UNIX Scripts

Dear Barry:

There are any number of interesting and useful things that can be done with fairly simple scripts on a Unix system. Current trends in computer programming seems to be toward using Perl for these scripts, but in fact much useful work can be done with a plain, old-fashioned bourne shell. One of the more intricate things we have done here is to modify the Varian vnmrplot script to enable plotting to remote X-servers. We are in the process of setting up '486 and Pentium based PCs as X-servers in various research group labs so that users can process their data off line from the spectrometer. One of the limitations of this approach has been the necessity of physically going down to the lab to pick up the hardcopy of the spectra. We have circumvented this problem by enabling the Unix workstations to plot directly to a laser jet printer connected to the parallel port of a PC without requiring the Unix machine to remote mount every printer we may want to use for printing.

Our solution to this has been to use FTP to transfer the plot file to the PC. In fact, we are able to specify LPT1: as the destination, so that the plot goes straight to the printer from the NMR software with no user intervention. This requires several different pieces of software to be in place, however. First, the vnmrplot script (bourne shell) is modified to recognize a device name 'remote_ljp_300R', and to use an inline ftp command to send this to the X-server. The name of the server is derived from the DISPLAY environment variable, so that it is impossible to send it to the wrong place. Next there must be an ftp server running in background on the PC in order to receive the file. A variety of network packages include an ftp server, as do some of the X-server packages. Finally, the printer is slow enough that the ftp server may time out before the file is vcompletely transferred. A print buffer, either hardware or software, eliminates this problem. We use a print buffer package that came from Hewlett Packard with one of our plotters.

Once these pieces are in place, a user simply specifies plotter='remote_ljp_300R', issues the desired plot commands, and get the plot on his local laserjet printer. A copy of our modified vnmrplot script, including changes that we made previously for aliasing plotter names, is available on our website under the software section.

We also have in place several other, simple scripts that make it easy for users to manage their Unix account space. We use 8mm tape drives, with a capacity of several Gigabytes, as the primary

archive storage medium, using the Unix tar facility to write the data. These tapes are relatively easy to use, except that there is no provision for maintaining a directory of the data stored, or keeping track of how much of the tape is used. Users will typically archive 30 - 50 Mbytes of data at a time, appending new tar archives to the end of the tape. We have a tape directory script that will read all of the tar files on a tape, generate a list of file names, file sizes, and modification dates. It will also maintain a running total of file size, both for the individual tar archives and for the entire tape. These totals are printed at the end of each archive and at the end of the tape respectively. This directory listing can be saved on disk for reference, and printed if necessary for permanent storage. This script is only 30 or 40 lines long, but it is more than you want to retype. See the software section of our webpage to download the script. Note too that this script is not customized in any way for our systems with Varian NMR data. It will work with any data tape.

The last script I'll mention is one that we use to characterize disk usage. We use quotas to avoid having a small group of users dominate the usage of disk space, and when a user has a quota problem they often have trouble identifying where the disk space is going. This is especially true since Varian data is stored in a directory structure, hiding the size of the fid file in a normal directory listing. The Unix 'du' command will give information about disk usage, but it gives either too much or too little, depending on the options chosen. We have a script du_summary that takes the full output of du and filters it, looking for only the first level subdirectories, and showing the usage in each of those subdirectories. This eliminates the overwhelming detail that hides where the disk space is actuall going. Once the offending directory is identified, you can decend to that subdirectory and repeat the process as necessary. The script for this is very short and concise:

```
#! /bin/sh
#   du_summary - display free disk space and space used by highest
#                level subdirectories only.
df -k .
echo "Summary of Usage, major directories:"
du -k . | grep -v '/[A-Za-z0-9_+.\-]*/'
# this prints directories that do NOT contain 2 / characters
```

Notice that the df and du commands are run on the current directory. You cannot run this script on an arbitrary directory. If it is modified to allow the user to specify the directory, the grep search pattern will no longer work properly. The advantage of short scripts like this is that it allows you to take an existing Unix tool and make it do what you would have done if you had written it!

Sincerely,

Steven K. Silber
Senior Research
Instrumentation Specialist

s-silber@tamu.edu
http://wwwchem.tamu.edu/services/NMR

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Aldrich offers all major deuterated NMR solvents in 100mL Sure/Seal bottles. This packaging option has quickly become popular because it minimizes both moisture contamination and the loss of TMS during transport and storage.

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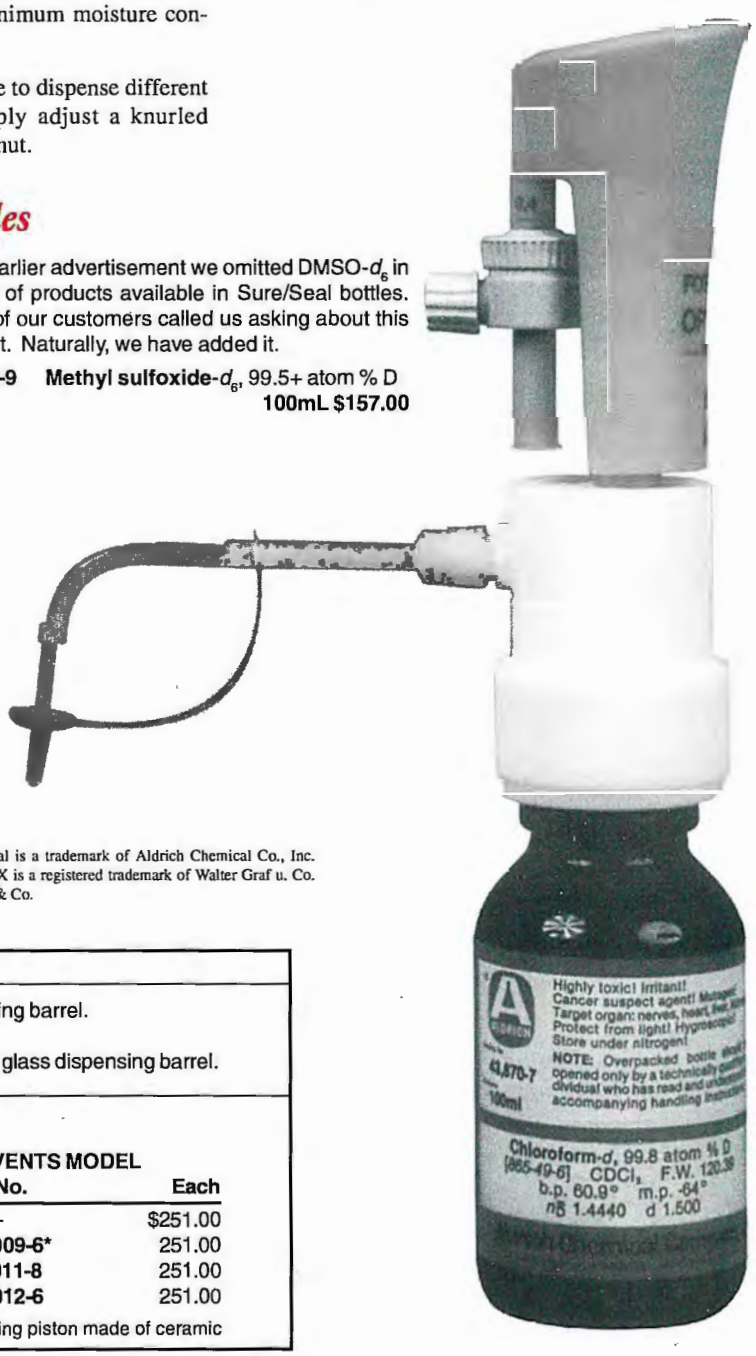
43,869-3	**Acetonitrile-d_3,** 99.6 atom % D	**100mL $205.00**
43,868-5	**Benzene-d_6,** 99.6 atom % D	**100mL $174.50**
43,870-7	**Chloroform-*d*,** 99.8 atom % D	**100mL $39.00**
43,872-3	**Chloroform-*d*,** 99.8 atom % D (contains 0.03% v/v TMS)	**100mL $39.00**
43,871-5	**Chloroform-*d*,** 99.8 atom % D (contains 1% v/v TMS)	**100mL $39.00**
15,182-3	**Chloroform-*d*,** 99.8 atom % D	**125g $32.50**
15,183-1	**Chloroform-*d*,** 99.8 atom % D (contains 1% v/v TMS)	**125g $32.50**
22,578-9	**Chloroform-*d*,** 99.8 atom % D (contains 0.03% v/v TMS)	**125g $32.50**
43,877-4	**Deuterium oxide,** 99.9 atom % D	**100g $57.50**
43,876-6	**Deuterium oxide,** 100.0 atom % D	**100g $151.50**
43,867-7	**Methyl-d_3 alcohol-*d*,** 99.8 atom % D	**100mL $398.50**

In an earlier advertisement we omitted DMSO-d_6 in the list of products available in Sure/Seal bottles. Many of our customers called us asking about this product. Naturally, we have added it.

45,052-9	**Methyl sulfoxide-d_6,** 99.5+ atom % D	**100mL $157.00**



OPTIFIX Dispensers

Dispenser Model	Piston Type	Features
BASIC	PTFE-encased glass	Has glass dispensing barrel.
SOLVENTS	Glass	Has safety-coated glass dispensing barrel.

Range (mL)	Increment (mL)	BASIC MODEL Cat. No.	SOLVENTS MODEL Cat. No.	Each
0.2-1.0	0.05	**Z25,998-5**	—	$251.00
0.4-2.0	0.1	**Z25,999-3**	**Z26,009-6***	251.00
1.0-5.0	0.1	**Z26,000-2**	**Z26,011-8**	251.00
2.0-10.0	0.2	**Z26,001-0**	**Z26,012-6**	251.00

* Dispensing piston made of ceramic

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41,127-2 **Acetone-d_6**, 99.5 atom % D (contains 1% v/v TMS, 1pkg = 10 x 1.0mL) **0.5pkg $12.00; 1pkg $20.50**

41,128-0 **Acetone-d_6**, 99.5 atom % D (contains 0.03% v/v TMS, 1pkg = 10 x 1.0mL) **0.5pkg $12.00; 1pkg $20.50**

42,366-1 **Chloroform-*d***, 99.8 atom % D (contains 0.03% v/v TMS, 1pkg = 10 x 1.0mL) **0.5pkg $6.60; 1pkg $10.25**

29,838-7 **Deuterium oxide**, 99.9 atom % D (contains 0.75% TSP) (1pkg = 10 x 1.0mL) **0.5pkg $12.90; 1pkg $18.00**

30,876-5 **Deuterium oxide**, 99.9 atom % D (contains 0.75% TSP) (1pkg = 5 x 0.5mL) **1pkg $8.40**

42,365-3 **(Methyl sulfoxide)-d_6**, 99.9 atom % D (contains 0.03% v/v TMS, 1pkg = 10 x 1.0mL) **0.5pkg $15.00; 1pkg $25.00**

In addition to 1% TMS and 0.03% TMS listings, solvents containing 0.1% TMS have been added at the request of some of our customers.

43,452-3 **Acetone-d_6**, 99.8 atom % D (contains 0.1% v/v TMS) **5g $13.70; 10g $22.50; 50g $98.10; 50 x 10g $690.00**

43,901-0 **Benzene-d_6**, 99.6 atom % D (contains 0.1% v/v TMS) **10g $24.50; 25g $54.60; 50g $97.00**

43,487-6 **Chloroform-*d***, 99.8 atom % D (contains 0.1% v/v TMS) **100g $23.30; 125g $32.50; 500g $96.30; 50 x 100g $710.00**

43,902-9 **Methyl-d_3 alcohol-*d***, 99.8 atom % D (contains 0.1% v/v TMS) **5g $35.00; 10g $57.95; 50g $248.85**

43,903-7 **(Methyl sulfoxide)-d_6**, 99.9 atom % D (contains 0.1% v/v TMS) **10g $17.35; 25g $40.70; 50g $78.15**

43,769-7 **(Methyl sulfoxide)-d_6**, 99.5+ atom % D (contains 0.1% v/v TMS) **10g $16.20; 50g $73.70**

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45,327-7 **Acetonitrile-d_3**, 99.6 atom % D (1pkg = 10 x 0.25mL) **0.5pkg $12.00; 1pkg $14.00**

45,330-7 **Benzene-d_6**, 99.95 atom % D (1pkg = 10 x 0.25mL) **0.5pkg $31.30; 1pkg $50.35**

45,328-5 **Chloroform-*d***, 100.0 atom % D (1pkg = 10 x 0.25mL) **0.5pkg $11.70; 1pkg $19.10**

45,335-8 **Deuterium oxide**, 100.00 atom % D (1pkg = 10 x 0.25mL) **0.5pkg $26.00; 1pkg $39.75**

45,333-1 **Deuterium oxide**, 100.0 atom % D (1pkg = 10 x 0.25mL) **0.5pkg $10.10; 1pkg $15.90**

45,329-3 **Dichloromethane-d_2**, 99.95 atom % D (1pkg = 10 x 0.25mL) **0.5pkg $68.90; 1pkg $116.60**

45,331-5 **Methyl-d_3 alcohol-*d***, 99.95 atom % D (1pkg = 10 x 0.25mL) **0.5pkg $61.50; 1pkg $75.80**

45,332-3 **(Methyl sulfoxide)-d_6**, 100.0 atom % D (1pkg = 10 x 0.25mL) **0.5pkg $30.75; 1pkg $51.45**

Solvents in 0.75mL Single-Use Ampules

44,471-5 **Acetone-d_6**, 100.0 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $55.00; 1pkg $91.00**

44,485-5 **Acetone-d_6**, 99.9 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $10.75; 1pkg $18.50**

44,129-5 **Acetone-d_6**, 99.8 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $10.50; 1pkg $18.00**

44,130-9 **Acetone-d_6**, 99.5 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $8.75; 1pkg $15.00**

44,472-3 **Acetonitrile-d_3**, 99.95 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $62.00; 1pkg $97.00**

44,131-7 **Acetonitrile-d_3**, 99.6 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $15.20; 1pkg $25.35**

44,132-5 **Benzene-d_6**, 99.6 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $11.05; 1pkg $18.25**

44,473-1 **Chloroform-*d***, 100.0 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $16.00; 1pkg $28.00**

44,133-3 **Chloroform-*d***, 99.8 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $6.20; 1pkg $7.50**

44,136-8 **Deuterium oxide**, 100.0 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $15.50; 1pkg $26.90**

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44,610-6 **Dichloromethane-d_2**, 99.8 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $57.70; 1pkg $96.10**

44,134-1 ***N,N*-Dimethylformamide-d_7**, 99.5 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $100.00; 1pkg $165.00**

44,475-8 **Methyl-d_3 alcohol-*d***, 99.95 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $66.00; 1pkg $118.00**

44,138-4 **Methyl-d_3 alcohol-*d***, 99.8 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $26.50; 1pkg $44.00**

44,476-6 **(Methyl sulfoxide)-d_6**, 100.0 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $51.00; 1pkg $85.00**

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44,140-6 **Tetrahydrofuran-d_8**, 99.5 atom % D (1pkg = 10 x 0.75mL) **0.5pkg $117.30; 1pkg $195.60**

44,141-4 **Toluene-d_8**, 99+ atom % D (1pkg = 10 x 0.75mL) **0.5pkg $24.50; 1pkg $41.00**

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Bethesda, Maryland 20892

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

November 17, 1995
(received 11/21/96)

Dear Barry:

Solids or liquids?

For many years many of us have lived with the notion that the solid phase is needed to study all the interesting interactions, such as quadrupole couplings, dipolar couplings, and chemical shift anisotropy. If we go to the liquid state, all these parameters average to zero, leaving us with a "featureless" spectrum, containing only the isotropic shifts and possibly some J couplings. Although the "solid-state" parameters are hidden in the liquid phase, this does not mean they can't be studied in a quantitative manner. For example, it is well known that relaxation of deuterium is dominated by its quadrupole coupling. Andy LiWang managed to measure ^{2}H relaxation rates for most of the backbone amide protons in the 76-residue protein ubiquitin.[1] Knowing all the rotational diffusion parameters of this protein from ^{15}N relaxation studies, he then was able to calculate the quadrupole coupling constant for each backbone amide. They range from 200 to 230 kHz and, as expected, correlate with the hydrogen bond length and angle observed in the crystal structure.

Chemical shift anisotropy represents another relaxation mechanism. Particularly with the ever increasing strength of magnetic fields, the effects of CSA are becoming an increasing nuisance to those of us longing for high resolution and high sensitivity. The flip side is, of course, that the high fields allow us to measure the magnitude of the CSA. Using experiments that exploit relaxation interference,[2] for example, we found that the peptide backbone ^{15}N CSA in ubiquitin, on average, is *ca* 170 ppm.[3] This is about 10 ppm larger than what is seen in ^{15}N solid state NMR powder patterns of model peptides. The difference is attributed to the small amount of fast small amplitude motions which remain present in the solid state and scale the CSA by an order parameter, S. The same applies to the ^{15}N-^{1}H dipolar coupling, which also yields a powder pattern width that is ~5% smaller than what is expected for a 1.02 Å N–H bond length. The parameters we measure in high resolution NMR have been scaled to account for the effect of such internal motions, whereas the solid state parameters typically are not.

Time dependence of dipolar couplings is the dominant relaxation mechanism in high resolution studies of macromolecules, and semi-quantitative measurement of these (cross-) relaxation rates forms the basis of protein structure determination as we know it. However, it is also well known from the work of Bothner-By, MacLean, and co-workers[4] that at high magnetic field one can sometimes observe a small degree of magnetic alignment of molecules in free solution. This occurs when the molecule's magnetic susceptibility, χ, is anisotropic. For example, aromatic ring systems, such as benzene, coronene, and porphyrins were shown to show small but measurable alignment with the magnetic field. More recently, Prestegard and co-workers noted that in the paramagnetic protein myoglobin the ^{15}N-^{1}H dipolar couplings are also measurable and correlate reasonably well with this protein's crystal structure.[5]

The ^{15}N-^{1}H dipolar couplings can be measured from the field dependence of the ^{15}N-^{1}H $^1J_{NH}$ splitting. The dipolar contribution increases with the square of the magnetic field strength, whereas the $^1J_{NH}$ coupling is constant. Relaxation interference between ^{15}N-^{1}H dipolar couplings and ^{15}N CSA results in a dynamic frequency shift (DFS) which is different for the two doublet components and which therefore also affects the apparent $^1J_{NH}$ splitting.[6] Fortunately, this effect is

relatively small and uniform.

Proteins are often packed with aromatic residues and we thought it therefore would be interesting to see if we could measure such dipolar couplings in diamagnetic proteins. A calculation for ubiquitin, adding the magnetic susceptibility tensors for all peptide bonds and aromatic rings, however, indicated that the net susceptibility anisotropy is disappointingly small, about twice that of a single benzene ring. Nevertheless, we thought it was interesting to see if we could measure the resulting minute dipolar contribution to $^1J_{NH}$. Fitting of the observed field dependent $^1J_{NH}$ splitting contribution to the orientation of the N–H bond vector, obtained from the ubiquitin's X-ray crystal structure, yielded remarkable agreement and indicated that the $^1J_{NH}$ splittings indeed could be measured with a precision of ±0.02 Hz.[6] The dipolar couplings themselves, in these original measurements, carried out at 360 and 600 MHz, were measured to fall in the ±0.15 Hz range (-0.3 to 0) when including the dynamic frequency shift contribution). The magnetic susceptibility tensor obtained from these data was in fair agreement with both the magnitude and orientation of the tensor calculated on the basis of the crystal structure. However, the experimentally derived tensor included a small rhombic component, $\Delta\chi_r = 0.7 \times 10^{-28}$ cm^3 per molecule, whereas the calculated rhombic component, although nearly parallel, was only 0.16×10^{-28}.

A few months ago, we finally received delivery of a long awaited 750 MHz spectrometer. As the dipolar couplings scale with the square of the field, and the change in 360 vs 750 MHz $^1J_{NH}$ splitting is expected to be 88% larger than that measured from the difference at 360 and 600 MHz. Thus, measurements at both 360 and 750 MHz were carried out, using a freshly prepared new sample,and the tensor calculated from these new data agrees even closer with the one calculated from the crystal structure than those in our earlier study. The rhombic component, $\Delta\chi_r = 0.46 \times 10^{-28}$, retains nearly the same direction but falls between the original value and the value calculated from the crystal structure.

Figure 1 shows the agreement between the observed change in J_{NH} splittings and the values calculated using the experimentally derived magnetic susceptibility tensor. Open circles mark our original 600/360 data, whereas the filled circles represent our new data. Clearly, even for a regular diamagnetic small protein these dipolar couplings are easily measurable. They contain potentially very important structural information as, in contrast to NOEs and J couplings, they provide constraints that are not strictly local in nature. In fact, the dipolar couplings drive the internuclear bond vectors to an orientation relative to the principal axis system of the susceptibility anisotropy tensor. This process can actually orient elements of a protein structure in the case of scarce NOE constraints. Preliminary applications to systems of hotter biological interest than the ubiquitin model protein indicate that the quality of the structure, as measured with standard evaluation procedures, also improves considerably upon addition of the dipolar coupling constraints.

Tremendous progress in solid state NMR over the past decade appears to make it possible to address many important questions in our quest to study the structure and folding of proteins. At the same time, many of the "solid-state" parameters are becoming increasingly accessible to everyday solution NMR studies. With increasingly stronger magnetic fields we expect that, in particular, measurement of dipolar couplings will become a standard tool for biomolecular structure determination.

Kindest regards,

Nico Tjandra

Ad Bax

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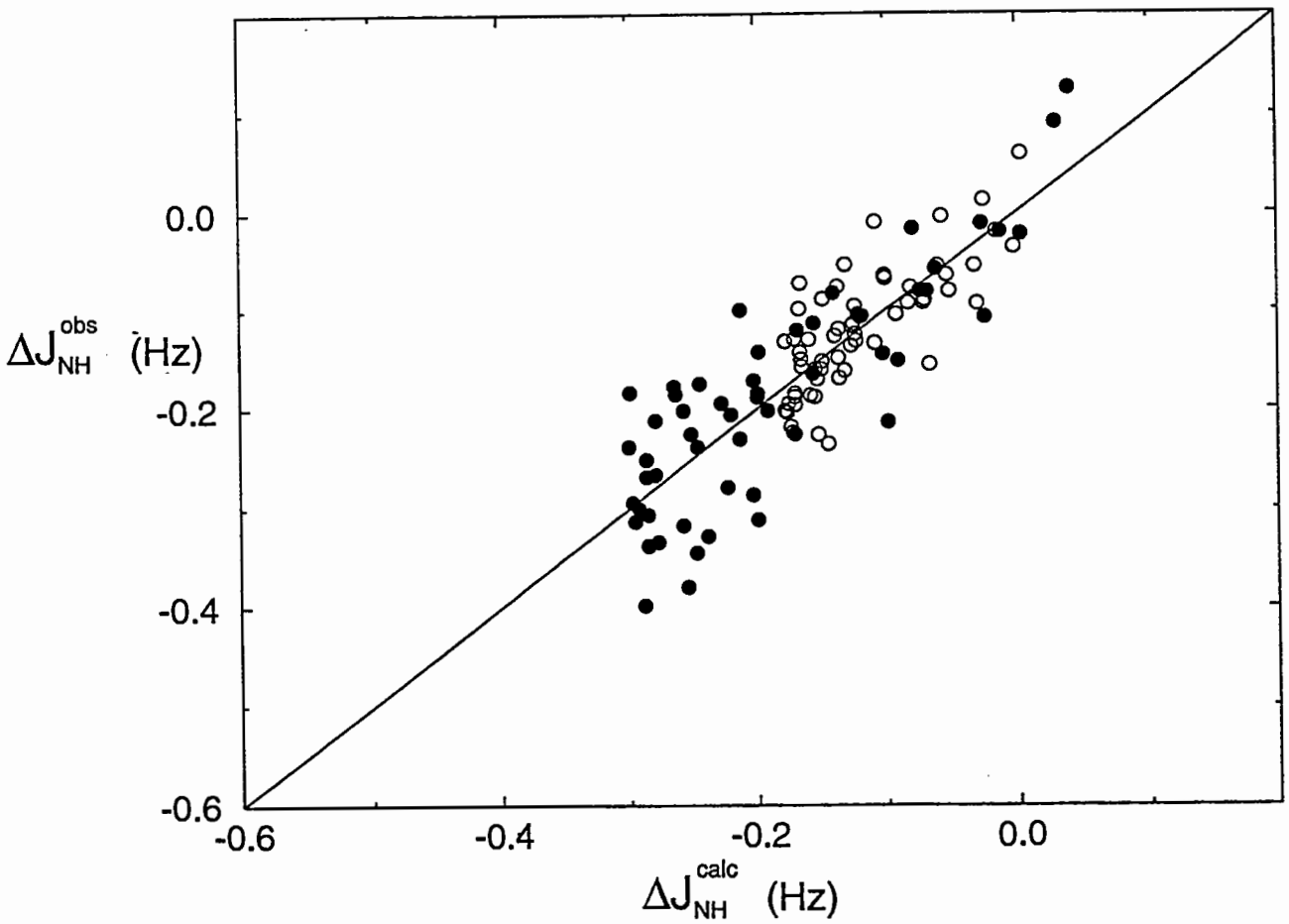

Figure 1. Correlation of the measured change in $^1J_{NH}$ versus the calculated change, including a uniform dynamic frequency shift contribution (-0.11 Hz for 600 vs 360 MHz; -0.15 for 750 vs 360 MHz). Open circles refer to measurements made at 360 and 600 MHz, solid circles refer to measurements on a different sample, taken at 360 and 750 MHz. The magnetic susceptibility tensor used for calculating the dipolar contribution to the $^1J_{NH}$ splitting can be decomposed into an axial component, $\Delta\chi_a = -2.35 \times 10^{-28}$ and a rhombic component $\Delta\chi_a = 0.46 \times 10^{-28}$ cm^3 per molecule. Orientation of the principal axes system in spherical coordinates (in the original X-ray structure coordinate frame): $\theta = 51^\circ$; $\phi = 118^\circ$; $\psi = -71^\circ$.

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Milano, 10/18/1996 (received 11/20/96)

Dr B. L. Shapiro
NMR Newsletter
996 Elsinore Court
Palo Alto, CA 94303

Primary and secondary isotope effects in perylenequinones

Dear Barry,

when we were involved in the study of the tautomerism in dihydroxyperylenequinones[1] we measured some deuterium isotope effects on ^{13}C and ^{1}H chemical shifts. In order to explain a few anomalies, we performed systematic measurements of primary $\Delta(^{1}H\ ^{2}H)$ and secondary $^{n}\Delta C(OD)$ isotope effects on these compounds and on other enol systems. However, not only our doubts were not clarified but the whole matter became even more complicated.

The primary isotope effect on the chemical shift of the intramolecularly hydrogen bonded OH protons is plotted in Figure 1 *vs* the proton shift of OH groups. The correlation previously found by Forsen et al.[2] still holds, when data from perylenequinones, anthraquinones and naphthoquinones are included. The results appear interesting, as perylenequinones actually fill up an empty area between the very strong hydrogen bonds of ß-diketones and bonds of medium strength such as those of ß-ketoesters and o-hydroxyacyl aromatics. Thus we interpreted that the strength of the intramolecular hydrogen bonds is increasing from naphthoquinones and anthraquinones to perylenequinones of type I and type II.

Encouraged by these results, we performed experiments at low temperature, down to -40°C in $CDCl_3$ and to -70° C in acetone. The primary effect for acetylacetone (ac.ac.) and benzoylacetone (benz.ac.) increases at low temperature., whereas hydroxyanthraquinones, naphthazarine and perylenequinones display an opposite trend. (see Fig. 2). Small changes with temperature in the tautomeric equilibrium might be possible for the latter compounds, as well as for benz. ac., which present an asymmetric double minimum potential curve. For instance phleichrome (type II) showed significant changes with temperature in the secondary effects at C-3 and C-4 sites, which clearly indicates an increase of tautomer A at low temperature. This increase however is less than 10%, as determined from coupling constant measurements.

In these systems it is difficult to know whether isotopic effects on chemical shifts are caused by intrinsic or equilibrium effects, whereas the symmetric situation for ac.ac. excludes any contribution from equilibrium effects.

Intrinsic isotope shifts normally do not change much with temperature, but in the case of a potential energy surface with shallow minima (which correspond to a positive primary effect as for ac.ac., benz.ac. and also perylenequinones) a temperature dependent intrinsic shift could be caused by changes in the vibrational energy levels[3].

Probably the equilibrium in benz.ac. does not change with temperature, whereas for perylenequinones the equilibrium contribution must be added to the intrinsic effect, and this induce the inversion of the trend observed in Fig. 2.

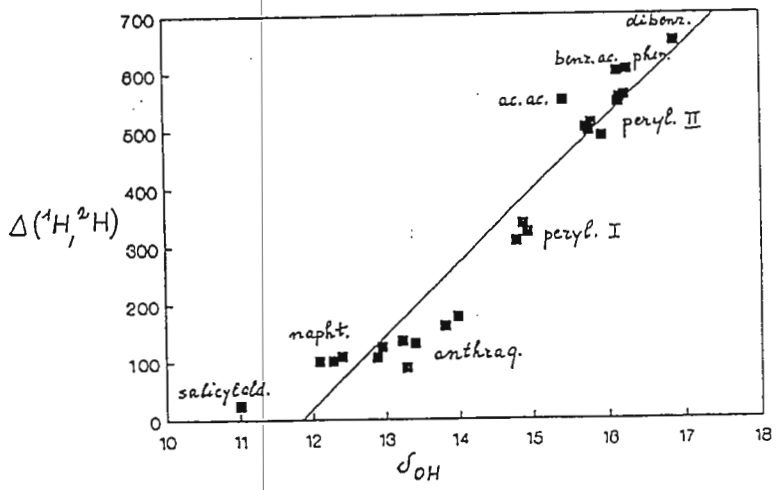
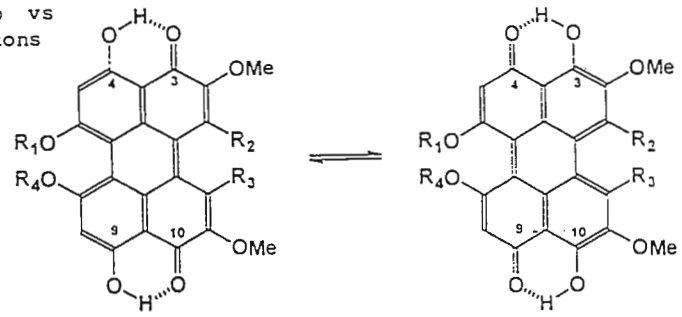


Fig.1 Primary isotope effect on the chemical shift of OH group vs ^1H chemical shift of OH protons



A B

$R_2 = R_3 = CH_2CHOHCH_3$
Type I $R_1 \cdots R_4 = CH_2$
Type II $R_2 = R_3 = CH_3$

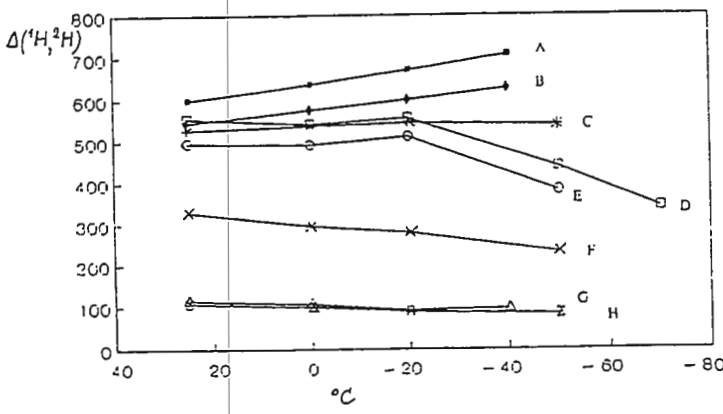


Fig.2

A benzoylacetone
B acetylacetone
D,E phleichrome (type II) in acetone or $CDCl_3$
C perylenequinone type II
F perylenequinone type I
G 9,10-dihydroxyanthraquinone
H naphthazarine

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With best greetings

Rosanna Mondelli
Leonardo Scaglioni

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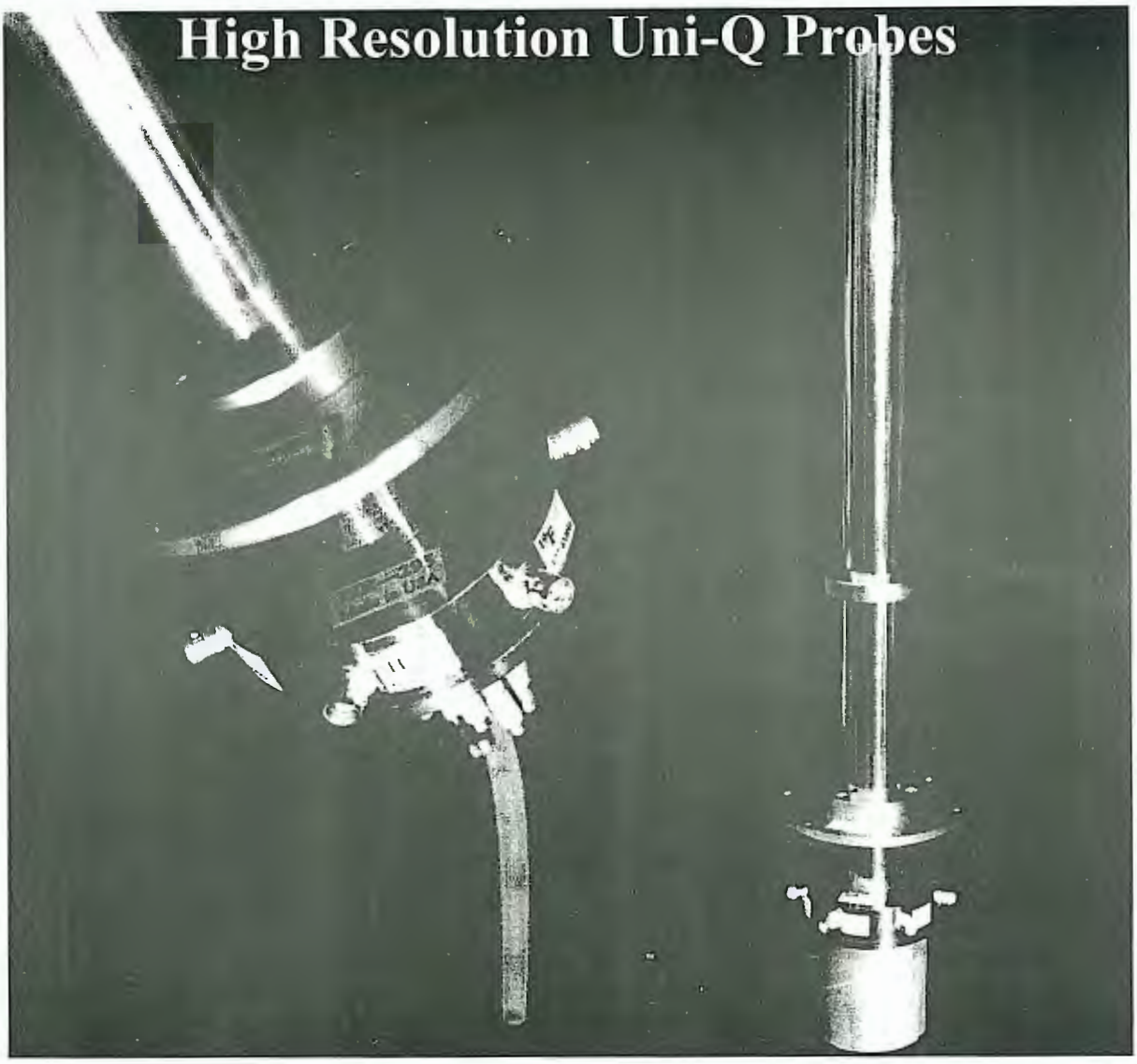

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(received 11/1/96)

Dear Barry

A Wish-list for Automation

I am sure that most readers of *The NMR Newsletter* will be taking advantage of the automation features which are built into any modern NMR spectrometer.

At Jealott's Hill we have built an automated system which has been running in excess of 20 0000 standard proton samples each year since it was installed in 1989. A second system, aimed at providing a range of the usual experiments which might be needed for routine structure elucidation (13C, DEPT, HETCOR, COSY etc), has been running for most of this year. Both instruments are very popular with the Jealott's Hill Chemists since the design philosophy of our system specifies minimum interaction from the user. All that is required is the entry of a lab. book reference, a solvent and a sample changer location and the spectrometer does the rest. The proton system typically runs for months on end without attention (except for replacing paper and pens!).

On the face of it we have a reliable system which keeps a track of all our work, bills our customers and archives all data without very much operator input (I estimate around 0.1 person per year, including software engineering).

Unbeknown to our happy Chemists, the whole system has grown into some kind of monster. Obtaining a spectrum involves:

- Generation of a work-list using a home built LIMS system running on a Vax (VMS).
- Transfer of the work-list to a JEOL GSX spectrometer (RSX/11) for data acquisition.

- Recovering the acquired data and transferring to Varian software (Unix) for processing.
- Saving the processed data in Bruker WinNMR format (PC) for further processing by Chemists.

Whilst doing all this was fun, I really would not want to go through the exercise again when our present equipment is due for replacement.

The point of this note is that I would like to use *The NMR Newsletter* as a vehicle for enquiring whether there is anyone else out there who can see an advantage to the manufacturers providing us with an "off the shelf" solution?

I have had preliminary discussions with the European offices of the major manufacturers and can report that I found them all most receptive. Understandably, they need confirmation that they are not merely listening to the ramblings of a tormented soul.

If anyone is interested in joining with me in discussing the matter further I would be glad to hear from them. If there is sufficient interest we could meet by asking to form a breakout session at ENC or the UK International Meeting.

Feel free to contact me at the above address or by e-mail at:

paul.p.d.stanley@gbjha.zeneca.com

US residents may prefer to contact my colleague Lydia Chang at:

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Please credit this contribution to Lydia Chang's account.

Yours sincerely

Paul Stanley

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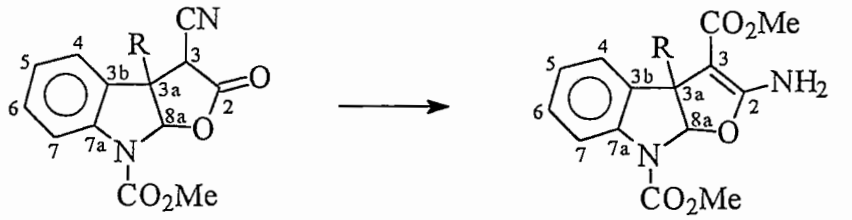
October 28, 1996
(received 11/7/96)

Dr. Bernard L. Shapiro
Editor/Publisher
TAMU NMR Newsletter
966 Elsinore Ct.
Palo Alto, CA 94303 USA

^{13}C NMR study of dihydrofuro[2,3-b]indoles containing a push-pull ethylene system

Dear Professor Shapiro:

We have reported recently the synthesis of compounds **1a-d**[1]. Alcoholysis of the nitrile group[2] of these compounds with MeOH and a catalytic amount of MeONa now gave compounds **2a-d**. These compounds contain a push-pull olefinic system having an electronic interaction between the electron-donor (NH_2) and the electron-acceptor (CO_2Me) groups *via* the C2,C3 double bond[3]. As a consequence of this push-pull effect, the ^{13}C NMR data (in DMSO-d_6) of **2a-d** show spectacular chemical shift differences of *ca.* 88 ppm between the C2 and C3 atoms, as is shown in the Table, since the chemical shifts of C3 are at very high fields for sp^2 carbon atoms.



a R = Me
b R = Et
c R = *i*-Pr
d R = *t*-Bu

The chemical shift values for C3 were secured from a long-range HETCOR measurement of **2a**.

Table. ^{13}C NMR Chemical Shifts (ppm) for **2a-d** in DMSO-d_6.

Comp.	C2	C3	C3a	C3b	C4	C5	C6
2a	166.2	79.5	53.0	138.0	124.1	123.8	127.8
2b	165.8	76.9	57.4	136.9	123.9	123.5	127.5
2c	165.7	77.4	61.4	136.7	123.6	123.5	127.6
2d	166.1	77.2	63.9	133.6	126.9	122.6	127.4

Comp.	C7	C7a	C8a	C=O ester	OMe	C=O carbamate	OMe
2a	114.2	139.2	99.7	166.2	49.9	152.9	53.3
2b	113.9	139.5	96.9	166.5	49.5	152.5	53.0
2c	113.8	139.5	93.8	166.2	49.6	152.4	53.1
2d	113.8	140.2	96.1	167.4	49.4	152.4	53.2

R substituent chemical shifts: **2a**, 24.2; **2b**, 27.8 and 8.8; **2c**, 30.1, 17.5 and 16.4; **2d**, 36.8 and 26.4.

The chemical shift difference between C2 and C3 depends on the donor capacity of the NH_2 group. In fact mono- or diacetylation of the amine group has a severe influence on the chemical shift of C3, which in **2a**, **3a**, and **4a** appear at 79.5, 92.0 and 108.2 ppm, respectively, as is shown in the figure.

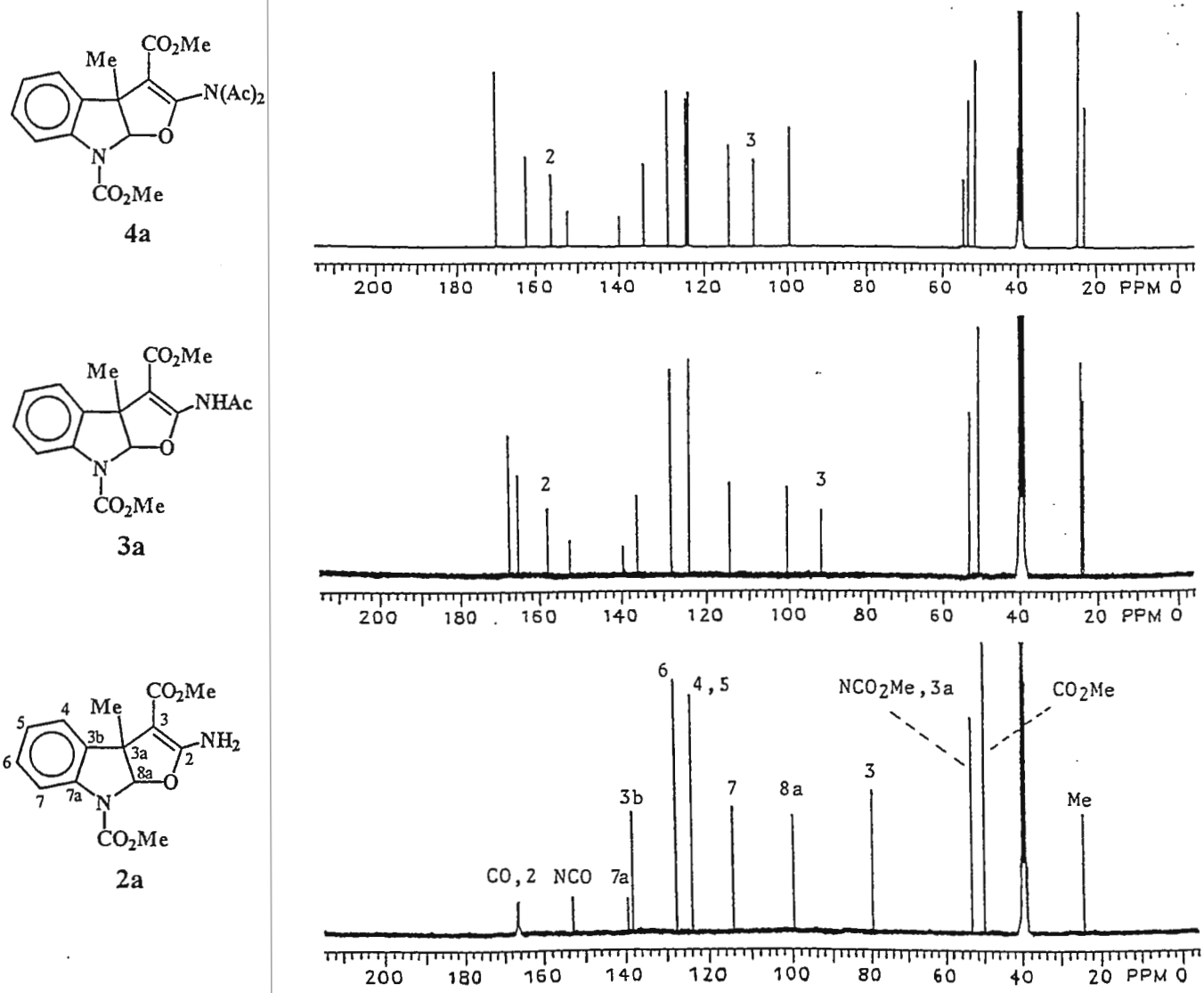


Figure. 75.4 MHz ^{13}C NMR spectra of **2a**, **3a**, and **4a** in DMSO-d_6.

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Sincerely yours,

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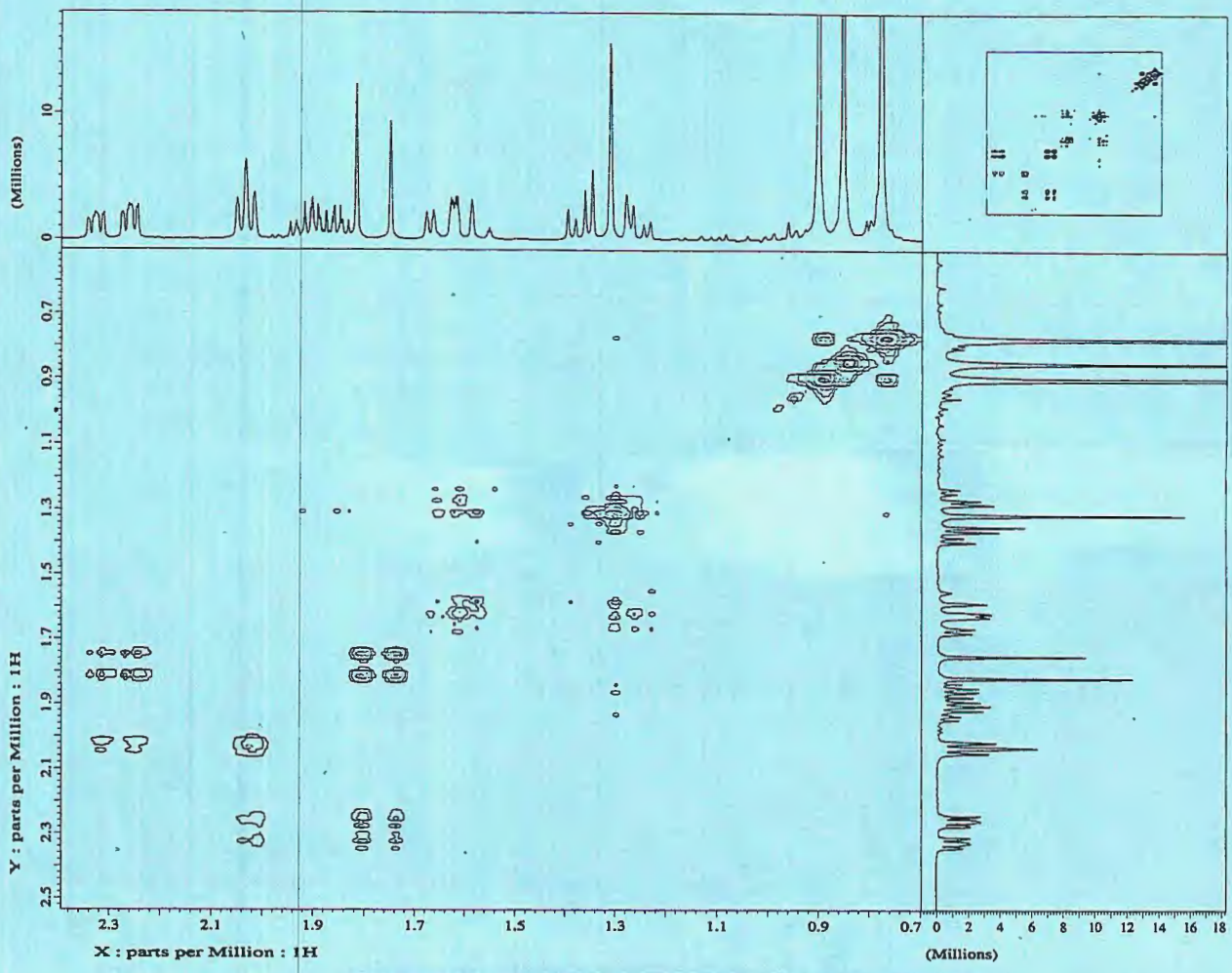

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