TEXAS A&M UNIVERSITY

NMR NEWSLETTER

No. 400

January 1992

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TEXAS A&M NMR NEWSLETTER NO. 400, JANUARY 1992 ADVERTISER INDEX

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FORTHCOMING NMR MEETINGS

Eighth Australian NMR Conference, Lorne, Victoria, Australia, **February 2-6, 1992**; See Newsletter 391, 38.

33rd ENC (Experimental NMR Conference), Asilomar Conference Center, Pacific Grove, California, **March 29 - April 2, 1992**; Contact: ENC, 750 Audubon, East Lansing, MI 48823; (517) 332-3667

Sixth Washington University-ENI/Emerson Electric Co. Symposium on NMR, St. Louis, Missouri, **May 18, 1992**; Contact: Karen Klein, Wash. Univ.; (314) 935-6405.

Eleventh Annual Scientific Meeting and Exhibition, Society of Magnetic Resonance in Medicine, Berlin, Germany, **August 8-14, 1992**; Contact: S.M.R.M., 1918 University Ave., Suite 3C, Berkeley, CA 94704; (415) 841-1899, FAX: (415) 841-2340.

XV International Conference on Magnetic Resonance in Biological Systems, Jerusalem, Israel, **August 16 - 21, 1992**; Contact: Prof. Gil Navon, XV ICMRBS, P. O. Box 3190, Tel Aviv 61031, Israel.; Tel. (972-3) 5271111, Fax: (972-3) 5239099.

High Resolution NMR Spectroscopy (a residential school), University of Sheffield, England, **April 1993**[*sic*]; Organizer: Dr. B. E. Mann (Sheffield); For information, contact Ms. L. Hart, The Royal Society of Chemistry, Burlington House, Piccadilly, London W1V 0BN, England; Tel.: 071-437-8656.

Additional listings of meetings, etc., are invited.

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* * * * *

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All Newsletter Correspondence

Should Be Addressed To:

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

(415) 493-5971

DEADLINE DATES

No. 402 (March)----------21 February 1992
No. 403 (April) ----------20 March 1992
No. 404 (May) ----------24 April 1992
No. 405 (June)----------22 May 1992

* * * * *

WESTERN RESEARCH INSTITUTE
The University of Wyoming Research Corporation

December 6, 1991 (received 12/12/91)

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

"Better Fly Fishing Through NMR"

Dear Barry,

We have a saying in Wyoming that, "Even a bad day of fishing is better than a good day at work", so even when we can't always be on the stream physically, at least we can be mentally. Maybe that's why we decided to use ^{13}C NMR to look at some of the composite materials used for making the modern fly rods. We also looked at the aesthetically pleasing and expensive bamboo. The results are summarized in the figure.

Modern fly rods are built from various types of "graphites". These are made by carbonizing polyacrylonitrile at high temperature. With newer techniques and processes, lighter and stiffer graphitic materials are always being introduced. These are usually characterized by the elastic modulus, which is the ratio of stress to deformation. Therefore, it is a measure of the stiffness of the fly rod.

In the figure, the modulus increases from about 33 million psi for the standard graphite to over 40 million psi for the GT40. Because we used CP/MAS, the lines in the spectra are probably not due to graphite, because the graphites should not have any hydrogens for cross polarization. Instead they are probably due to the epoxy resins used to bind and hold the alignment of the graphite fibers. The standard and IM6 materials have about the same NMR spectra, so we conclude that any differences in elastic moduli are due to processing and not to any differences in chemical composition. We are not sure about the GT40 material. The bamboo, without the pith, looks as it should-mostly cellulosic with a small lignitic contribution.

This is as far as we could proceed, because at the time of this study the mayflies and caddisflies were hatching so we went fishing. We also have another saying that, " fishing's not a matter of life and death, it's more important than that".

Please credit this to Dan Netzel's account.

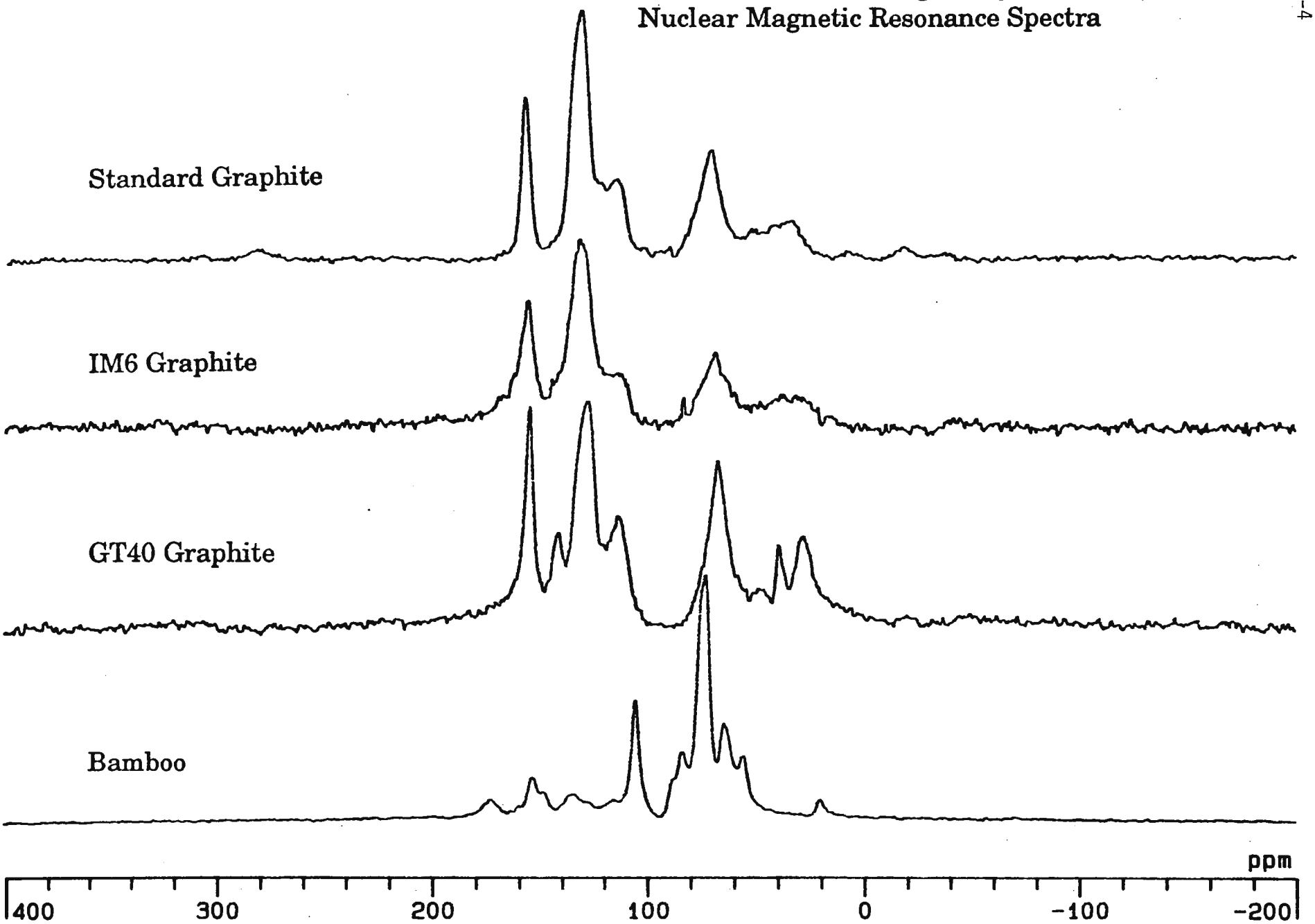
Best regards,

Dan Netzel

P.S. The spectra were recorded on a Chemagnetics CMX solids spectrometer. The materials were kindly provided by the Powell Rod Co., Chico, CA.

P.O. Box 3395, University Station, Laramie, WY 82071-3395 • Phone: (307) 721-2011 • Fax: (307) 721-2345

Cross Polarization Magic-Angle Spinning Carbon-13 Nuclear Magnetic Resonance Spectra



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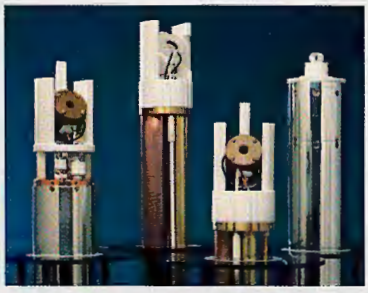

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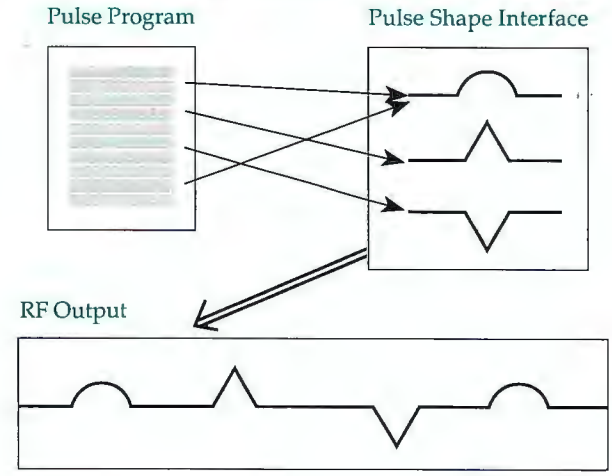

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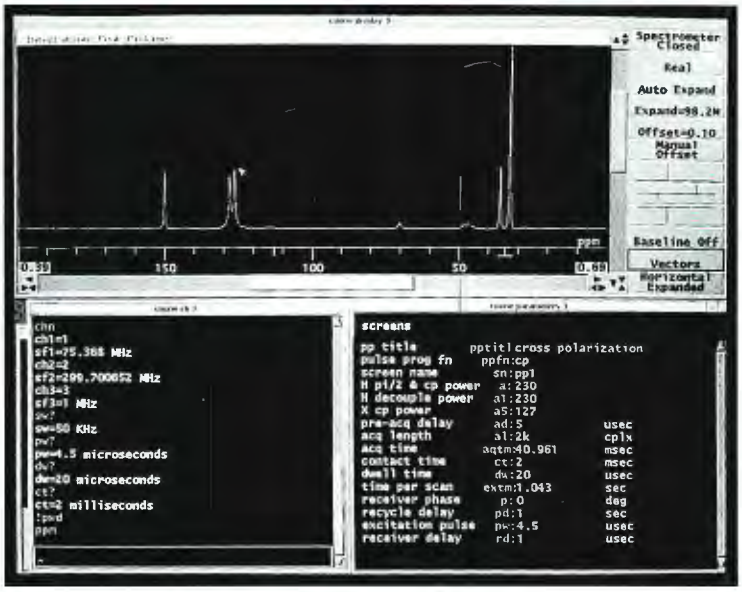

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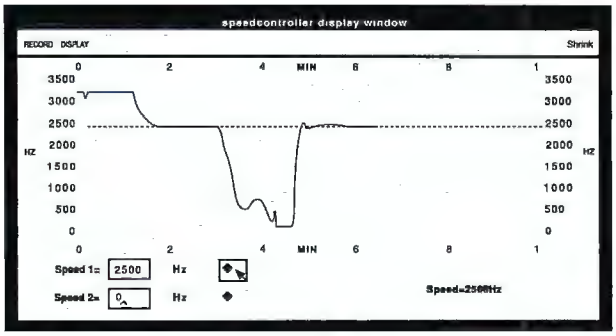

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20 September 1991
(received 11/23/91)

Dr B Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto
CA 94303
USA

Dear Barry

^{2}H-NMR of Headgroup-Deuterated Phosphatidylinositol (PI)

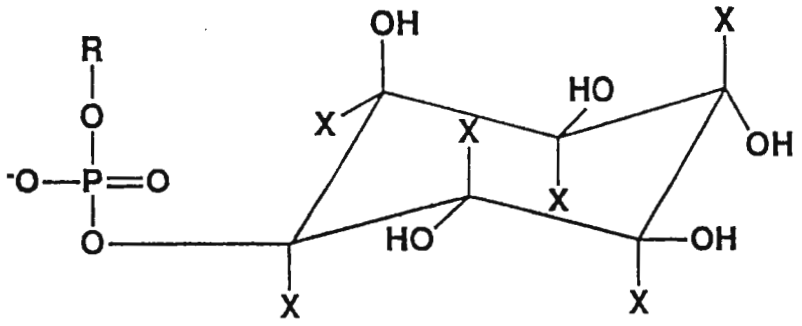
As part of a collaborative effort between SmithKline Beecham and Leeds University to understand the molecular mechanisms underlying the PI intracellular second messenger pathway, headgroup deuterated dimyristoyl PI (DMPI-d_6, I) has been synthesised. This feat was accomplished in Leeds by two graduate students, Steve Byard and Phil Hansbro, using a stereospecific synthesis based on one developed at SB (Young et al (1990) J Med Chem 33, 641-6). Figure 1 outlines the temperature dependence of the ^{2}H NMR spectrum (46MHz) of partially hydrated DMPI-d_6. At higher temperatures two quadrupolar powder doublets are discernible, corresponding respectively to the five orientationally equivalent axial deuterons, and the single orientationally unique equatorial deuteron of the myo-inositol ring. An analysis of the ^{2}H NMR properties of DMPI-d_6 in terms of its preferred membrane-bound conformations is currently being written up for submission to Biochim Biophys Acta.

Best wishes

Yours sincerely

David Reid DGR (for RJB)

David G Reid (SB) Richard J Bushby (Leeds University)

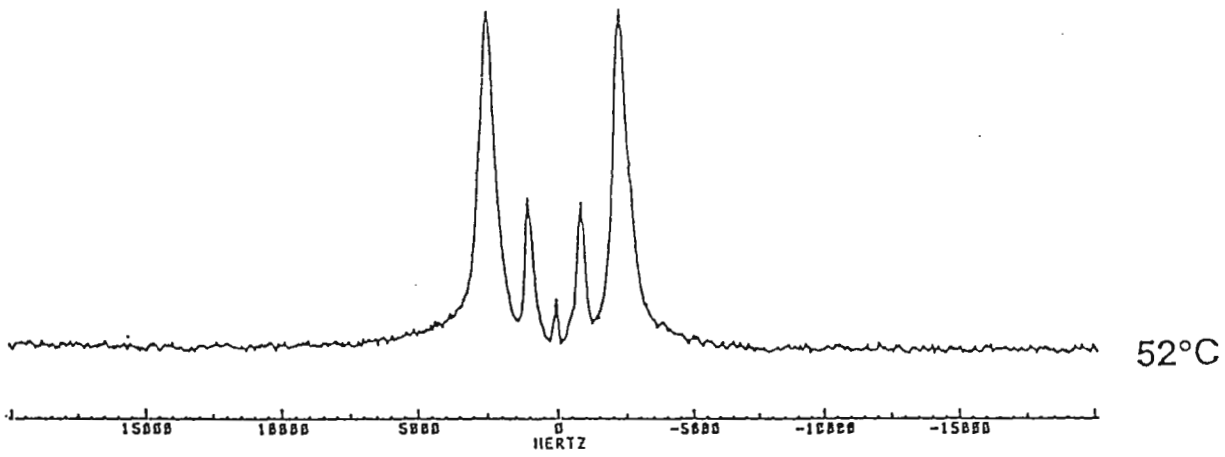


R = Dimyristoyl - *sn* - glycerol

X = ^2H

I

^2H NMR of DMPI-d_6



UNIVERSITY OF VIRGINIA
DEPARTMENT OF CHEMISTRY
McCORMICK ROAD
CHARLOTTESVILLE, VIRGINIA 22901

Prof. Bernard L. Shapiro
TAMU NMR Newsletter
966 Elsinore Ct.
Palo Alto, CA 94303

November 20, 1991
(received 11/23/91)

^{13}C relaxation measurements of acyl chain methylene segmental order in phospholipid bilayer vesicles

Dear Prof. Shapiro:

The effect of membrane curvature on the orientational order of phospholipid acyl chain methylene groups in bilayers has been the subject of many studies. Often these studies have involved quite different techniques for measurement of order in highly curved and relatively flat or planar bilayers. Typically, segmental order in multilamellar phospholipid dispersions (ie. planar bilayers) has been measured by including deuterated lipids in the membrane preparation and measuring the quadrupolar splitting, $\Delta\nu$, which is simply related to the order parameter parameter S,

$$\Delta\nu = (3 / 4)\ (e^2qQ / h)\ (S) \qquad (A)$$

where $e^2qQ/h = 170$ KHz for paraffinic C-D bonds. Bilayer preparations having a high degree of curvature (ie. sonicated bilayers) tumble at a rate which is rapid compared to typical quadrupolar splittings and therefore no splitting is observed. Acyl chain methylene segmental order in sonicated bilayers has usually been obtained by measuring spectral linewidths and using an expression similar to the following equation to obtain order parameters,

$$S^2 \sim (W / \text{constant})(\tau_r^{-1} + \tau_d^{-1}) \qquad (B)$$

where W is the linewidth, the constant depends on the nucleus observed, and τ_r^{-1} and τ_d^{-1} are correlation times for vesicle reorientation and phospholipid translational diffusion, respectively. One can see that in order to use (B), one must obtain values for τ_r^{-1} and τ_d^{-1}, in addition to obtaining W. Some previous studies have found that segmental order is considerably smaller in highly curved bilayers than in planar bilayers and others find little or no effect of bilayer curvature on segmental order.

We have reduced the uncertainty associated with the comparison of order parameters obtained with different techniques by making the same experimental measurements on bilayers having different degrees of curvature. ^{13}C spin lattice relaxation measurements were made at four resonance frequencies between 15 and 125 MHz on two palmitoyloleoylphosphatidylcholine vesicle populations; the average diameters of the two populations were 35 nm (highly curved) and 95 nm (low degree of curvature). The data was analysed in terms of the following spectral density function,

$$J(\omega) = ((1 - S_T^2)(S_j^2\,\tau_T) / (1 + (\omega\tau_T)^2)) + (1 - S_j^2)\,\tau_j \qquad (C)$$

where S_T is the order parameter for the long axis of the phospholipid molecule, S_j is the order parameter for methylene motions due to bond isomerization, libration, etc., $S = S_T S_j$, τ_T is the correlation time for long axis reorientation, and τ_j is the correlation time for bond isomerization, libration, etc. Previous work (1) showed that this function provides a suitable description of bilayer acyl chain methylene relaxation over the resonance frequency range used by us. We found that the maximum acyl chain segmental order difference between the two vesicle populations was 25% and the difference between most positions was < 10%. Therefore we conclude that bilayer curvature has little or no effect on acyl chain segmental order. A full account of this work will appear in the Biophysical Journal.

1. Pastor, R. W. et al. *J. Chem. Phys.* **89**, 1128 (1988).

Sincerely,

Leslie S. Lepore Jeff Ellena David S. Cafiso

ROCHE Hoffmann-La Roche

Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, New Jersey 07110-1199

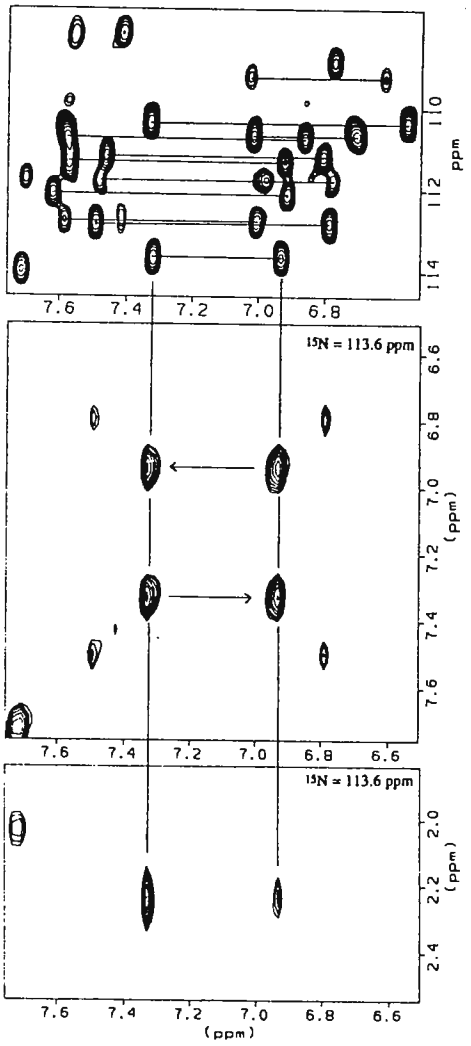
Direct Dial

November 15, 1991
(received 11/18/91)

DON'T NEGLECT THOSE NH_2's

Dear Barry:

We recently became interested in the side-chain NH_2 protons of a 152-residue protein which we have been studying, and wondered how readily they could be assigned. It was not nearly as bad as anticipated. The geminal primary amide pairs could be identified in the HMQC spectrum of a uniformly ^{15}N-labeled version of the protein (top of figure). Verification of linkage was provided by the strong cross-exchange peak in the appropriate slice of a 3-D NOESY-HMQC spectrum (middle of figure). The protons could usually be further assigned on the basis of intraresidual NOEs to CβH2 (asparagine) or CγH2 (glutamine) protons (bottom of figure).



Added Note: Early this year, we converted our VNMR software from version 2.2A to 3.2A. This allowed us to acquire 3-D data in a single experiment, rather than in a series of experiments controlled by macros, by implementing a double array of the two incremented time domains (d2 and d3). However, we neglected to read our manual thoroughly. The definition of the parameter "array" had been switched. In version 2.2A, array = 'x,y' indicated that x should take precedence in the double array; in version 3.2A, array = 'x,y' causes y to take precedence. This led to nice FIDs, but with out-of-order phases, and odd-looking 3-D spectra if processed as we thought they should be. After blaming our pulse sequence and FELIX, we finally tracked down the simple cause of our problem. Beware of redefined familiar parameters.

Sincerely yours,

Dave, Dave, Ross, Sudha

David Fry, David Greeley, Ross Pitcher,
and Sudha Narasimhan
Department of Physical Chemistry

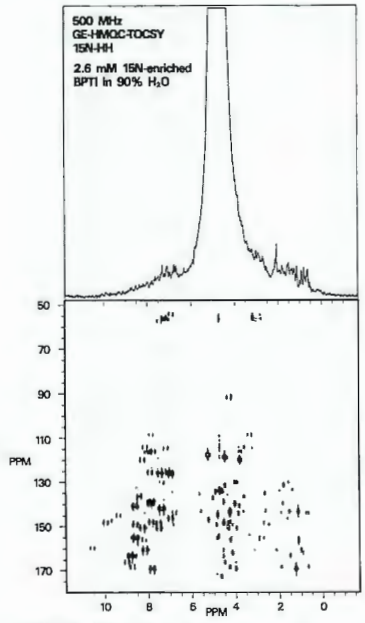
NMR UPDATE

Gradient Enhanced Spectroscopy: a new, practical answer

By Frank Huang, PhD, Paul Calderon, MS, and Boban John, PhD

Making Gradient Enhanced Spectroscopy (GES) a viable method for high resolution spectroscopy has long been of interest to researchers. The obvious benefits in speed and information content were too often overshadowed by the drawbacks of signal loss and distortion.

Technology developed at GE NMR Instruments has overcome these challenges. The S-17 Gradient Enhanced Spectroscopy Accessory with integrated inverse probehead makes GES practical for a broad range of applications in 1D, 2D, 3D and 4D experiments.



Gradient Enhanced Spectroscopy technique applied to an HMQC-TOCSY experiment demonstrates excellent water suppression without the need for presaturation or selective excitation.

Research Implications

- Speed without phase distortion or signal loss.
- Practical for 1D, 2D, 3D and 4D experiments.
- Accommodates proton and heteronuclear GES techniques.

A better design

The use of a three-axis, actively-shielded gradient set allows GE to overcome the inherent drawbacks of previous GES technology —most notably, phase distortion and signal loss:

Active shielding. Eddy current effects are the major source of phase distortion in GES spectra. Active shielding prevents interaction of strong gradients with magnet and shim components— the source of eddy current effects.

Fast, strong gradients. Short gradient pulses in excess of 20 G/cm minimize signal loss during pulse sequences.

Applications advantages

With its integral inverse probehead, the S-17 Accessory can accommodate proton as well as heteronuclear GES techniques.

Gradient fields in excess of 20 G/cm are able to suppress water in aqueous samples and to improve performance in heteronuclear experiments. Other applications advantages:

- Eliminates phase cycle requirements and subtraction error.
- Reduces T1 noise.
- Reduces collection times for 2D, 3D and 4D data sets.
- Provides lineshape independent water suppression in multiple quantum coherence selection experiments.
- Provides lineshape independent water suppression via diffusion differences for large molecular weight samples.
- Improves water suppression in experiments using selective time reversal RF pulses.
- Separates cross-correlation and exchange phenomena in NOESY experiments.
- Distinguishes chemicals inside the cell from those outside in whole cell applications.

For additional information on the S-17 GES Accessory, write to GE NMR Instruments, 255 Fourier Ave., Fremont, CA 94539. Or call toll free:

1-800-543-5934

GE NMR Instruments

Gradient-Enhanced ^{15}N HMQC

The pulse sequence and the coherence pathway diagram for a ^{15}N GE-HMQC are shown in Fig. 1. The pulse sequence was a standard ^{13}C GE-HMQC experiment (1) with different gradient amplitudes to account for the difference between the gyromagnetic ratios of ^{13}C and ^{15}N. The 90° proton pulse creates transverse magnetization which evolves into an anti-phase state with respect to J(NH) coupling at the end of the period Δ (where Δ = ½J (NH)). The antiphase components are converted into heteronuclear zero- and double-quantum coherence by the ^{15}N 90° pulse and the multiple quantum coherences are allowed to evolve during t_1. The 180° ^1H pulse in the center of the evolution period serves to eliminate the ^1H chemical shift evolution, yielding pure ^{15}N chemical shifts along that axis. The zero- and double-quantum signals are then *coherence-order labeled* by the gradient pulses G1 and G2. After conversion into antiphase proton magnetization by the last ^{15}N 90° pulse, the desired components are refocused by the gradient G3 and detected. The application of a gradient pulse results in a phase factor being applied to the magnetization which is dependent upon gradient strength, duration, the distance from the gradient isocenter, the gyromagnetic ratios of the coupled nuclei, and the desired coherence order. The relative amplitudes of the labeling and refocusing gradient pulses will determine the selection of a specific coherence pathway and are calculated to suppress magnetization components arising from the solvent and other protons not coupled to ^{15}N spins.

The fundamental principle of coherence selection using gradients is that for a pathway to be detected, the cumulative phase factor during the acquisition must be zero:

$$G_1p'_1 + G_2p'_2 + G_3p'_3 = 0. \quad [1]$$

The subscripts denote steps in the pulse sequence where p′ defines a composite coherence order for the heteronuclear case which includes the gyromagnetic ratios of the coupled nuclei:

$$p' = p^1H + (\gamma^{15}N/\gamma^1H)p^{15}N \quad [2$$

and p^1H and $p^{15}N$ are the coherence orders for the ^1H and ^{15}N spins respectively.

In the coherence pathway diagram, the relevant values of p' are given to the left and the relative gradient areas (gradient strength x duration) are given next to each gradient pulse. The following pathway (shown in *Fig. 1*):

$$H(+1) \rightarrow H(+1)N(0) \rightarrow H(+1)N(+1) \rightarrow H(-1)N(+1) \rightarrow H(-1)N(0)$$

is detected using a 5:5:1 ratio of gradient areas, since according to Equation 2:

$$5(1.1) + 5(-0.9) + 1(-1.0) = 0 \quad [3]$$

where the numbers in the parentheses refer to the composite coherence orders. Using these relative gradient areas, protons not coupled with ^{15}N spins may pass through an alternate pathway:

$$H(+1) \rightarrow H(+1) \rightarrow H(+1) \rightarrow H(-1) \rightarrow H(-1)$$

which results in a net phase factor:

$$5(1.0) - 5(-1.0) + 1(-1.0) = -1 \quad [4]$$

Thus, signals from this pathway remain defocused during the acquisition.

A 2D ^{15}N GE-HMQC spectrum of ^{15}N enriched BPTI is shown in *Fig. 2*. The spectrum was collected using a 5 mm inverse probe on an Omega™ PSG 500 spectrometer equipped with an S-17 gradient accessory. Half-sinusoid shaped gradient pulses were applied simultaneously along the X, Y, and Z axes with a maximum gradient strength of ≈20 Gauss/cm and a duration of 3.5 ms. A matrix size of 2048 × 128 resulted in 3.5 Hz resolution in the ω_2 dimension and 10 Hz in the ω_1 dimension. No decoupling was applied.

Gradient-enhanced experiments provide a viable alternative to traditional phase-cycling methods for the selection of coherence pathways. In cases where the sensitivity is adequate, gradient selection can substantially reduce the collection time in multi-dimensional experiments. The ^{15}N GE-HMQC data presented here has none of the t_1-noise from cancellation artifacts usually present in phase-cycled versions of the HMQC experiment. In addition, since the suppression of the single-quantum signals is done prior to acquisition, the receiver gain may be increased, which results in a substantial increase in signal-to-noise. For these reasons, gradient pulses should be the method of choice for coherence selection in HMQC experiments.

Reference

1. R.E. Hurd and B. K. John, *J. Magn. Reson.* **91,** 648 (1991).

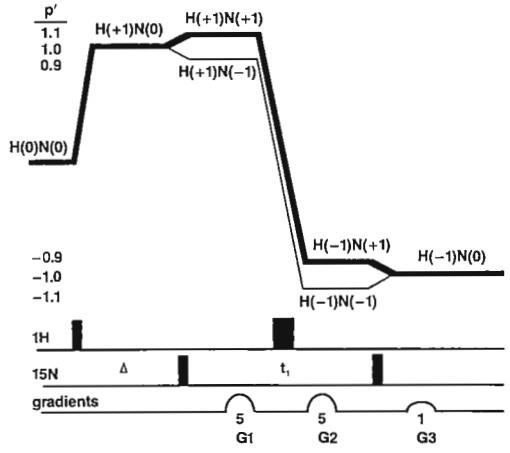


Fig. 1

Pulse Sequence and the coherence pathway diagram for a 15N GE-HMQC experiment.

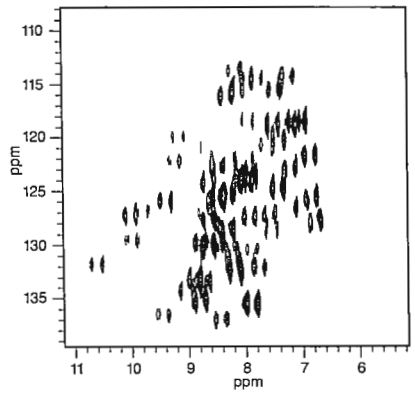


Fig. 2

A 2P Ge-HMQC spectrum of 15N enriched BPTI. The sample was 2.6mM in 90% H2). The data collection time was 2.6 hours.

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6 December, 1991
(received 12/14/91)

We enclose some recent efforts to obtain high resolution spectra (^{13}C and ^{1}H) of lightly crosslinked polymers (in this case poly(di-N-methylaminoethylmethacrylate - PDMAEM)) using the standard Bruker 4 and 7mm MAS probes and rotors. Spectra of solutions of these polymers in standard solution state probes are of poor quality. The material crosslinks during polymerisation, and therefore forms a partially insoluble gel in solvents such as chloroform. Apparently, the polymerisation can proceed to full conversion at 60°C since no double bonds are observed in the NMR or IR spectra. This is undoubtably due to the fact that T_g for the crosslinked polymer is less than 60°C, and therefore the so-called Tromsdorf gel effect has not been operative.

Figure 1A shows the ^{1}H spectrum of the fully reacted crosslinked polymer at ambient temperature. This was obtained with 10kHz MAS. Increasing the temperature (Figure 1B) and, more dramatically, swelling the polymer in a small amount of solvent (ω_r = 2.5kHz) such as chloroform (Figure 1C) dramatically improves the resolution of the spectra. Observations such as these have been made recently on lipids other crosslinked polymers [1, and references therein].

It is apparent that MAS spinning of 2.5kHz is sufficient to average the residual dipolar interactions in the swollen crosslinked polymer. Nonetheless, sufficient heteronuclear dipolar couplings exist in some parts of the sample to permit a degree of cross-polarisation to the ^{13}C nuclei, as seen by the spectrum in Figure 2A. It is likely that these strongly coupled protons are not observed in the ^{1}H spectrum 1C, or that the spectrum is too broad to be discerned in the baseline. It is envisaged that the cross-polarisation experiment may enable, in some systems, the detection of tightly constrained and crosslinked structures. The Bloch decay experiment is also shown in Figure 2B for comparison; the expanded region shows the tacticity information available from the peaks due to carbonyl carbons which were not resolvable by standard solution state techniques.

Finally, the spectrum of a closely related and partially polymerised polymer (Figure 1D) shows well resolved peaks due to protons on unreacted double bonds ay 5.5 and 6.1ppm. We have attempted to follow the disappearance of these signals at elevated temperatures and have found that the temperature of the probe must be calibrated for a particular spinning rate, rotor bearing and drive pressures, and pulse duty cycle otherwise errors in the indicated temperature of up to 10°C may be experienced. Full details of these experiments will be published elsewhere. Please credit this to the account of D.M. Doddrell.

Regards,

Andrew K. Whittaker

David Rogers

Reference
1. H.D.H. Stöver and J.M.J. Frechet, Macromolecules, 22, 1574-1576 (1989).

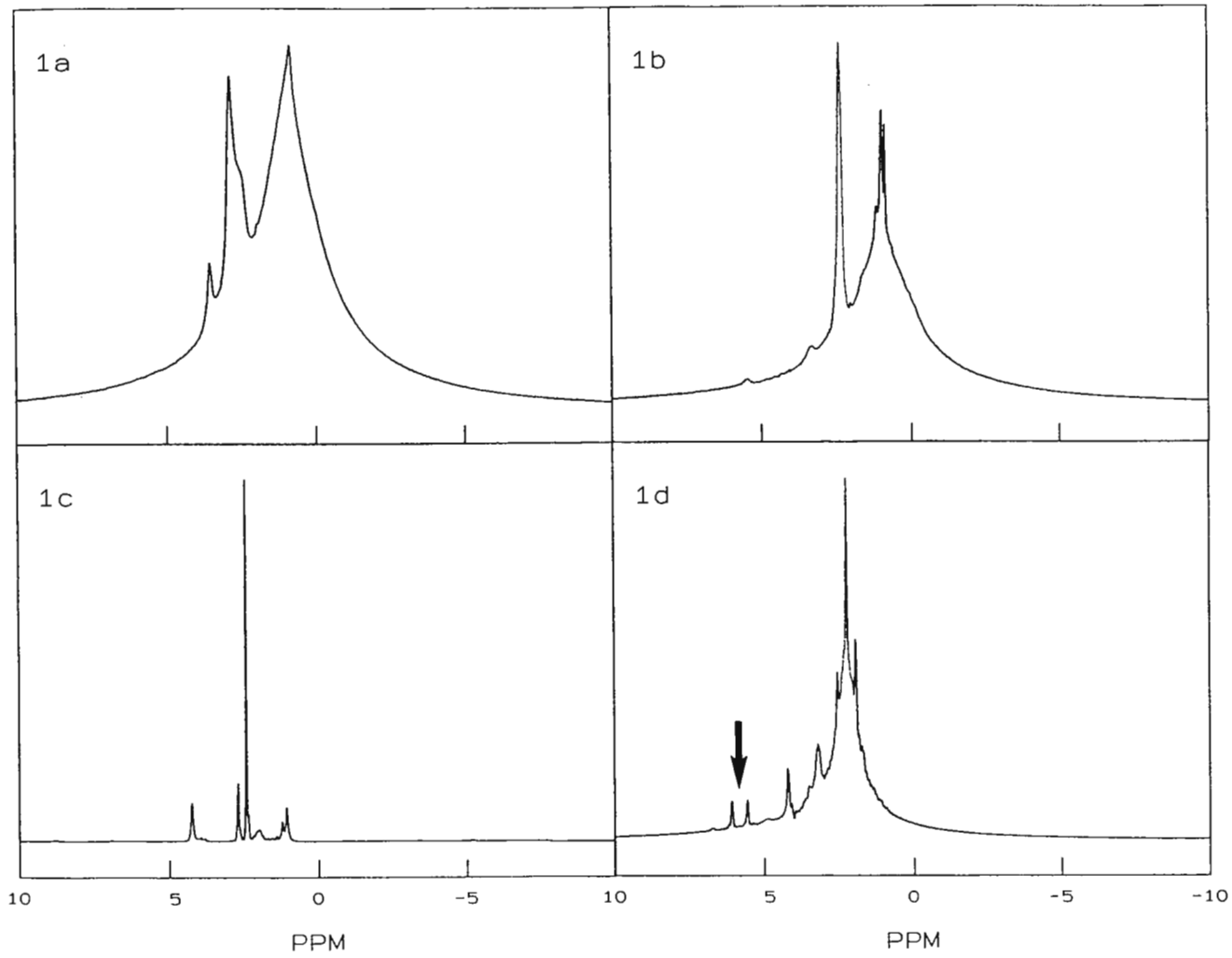

Figure 1. ^{1}H MAS spectra of PDMAEM a) 10kHz MAS at 298K, b) 10kHz MAS at 323K, c) 2.5kHz MAS at 298K swollen with $CDCL_3$, and d) of partially reacted poly(t-butylaminoethylmethacrylate) at 298K and 2.5kHz MAS.

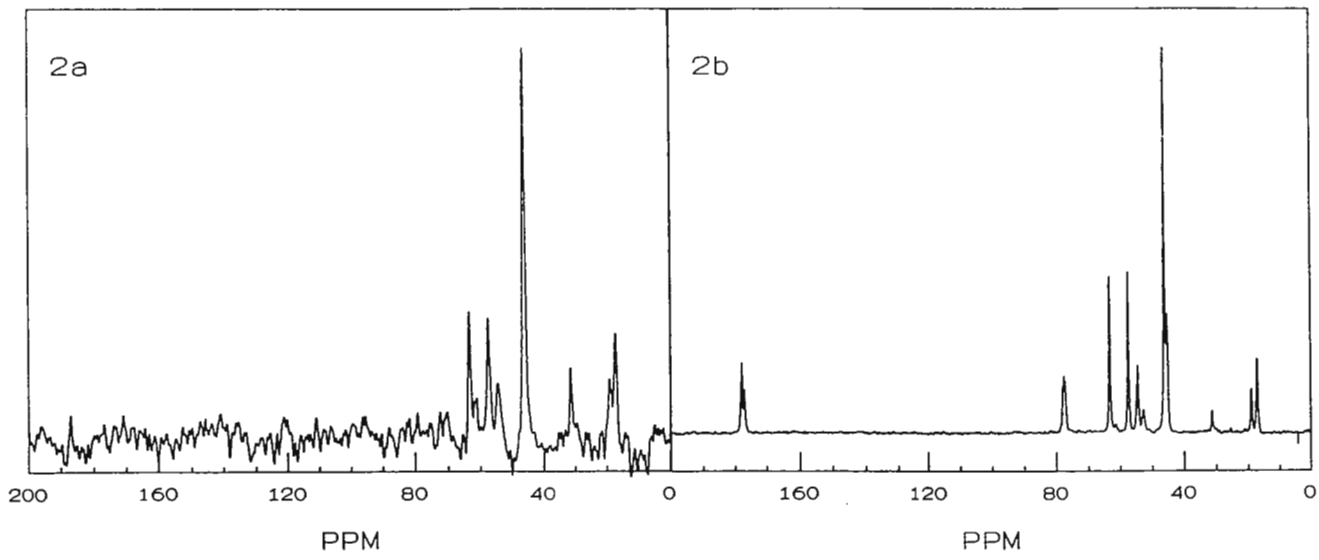

Figure 2. ^{13}C MAS spectra of PDMAEM swollen with $CDCL_3$ a) cross-polarisation experiment, 6ms CP contact and 2.5kHz MAS, and b) Bloch-decay experiment 2.5kHz MAS.

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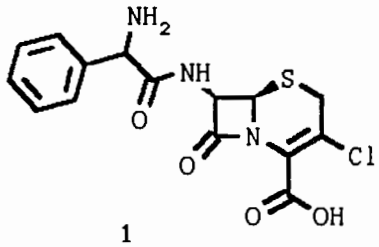
9 December, 1991
(received 12/13/91)

Dr. Barry Shapiro
Texas A&M Newsletter
966 Elsinore Court
Palo Alto, CA 94303

Degradation of Cefaclor

Cefaclor (**1**) is a β-lactam antibiotic that is taken orally and is therefore exposed to the acidic environment of the stomach. Hence it is of some importance to determine how cefaclor degrades in acid. In past studies it has been common to expose β-lactam antibiotics to fairly vigorous conditions, since this degrades the molecule to just a few products which could be isolated and identified. We decided to see if we couldn't use milder conditions and the enhanced separation capabilities of modern HPLC to obtain a more detailed picture of the mechanistic pathways through which cefaclor is degraded. So far we have proposed structures for about 10 predominant products, and shown that the pathways through which cefaclor degrade include some very interesting chemistry.

Some of the products are no surprise. For example, "Δ^2-cefaclor"



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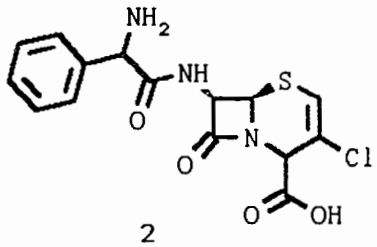

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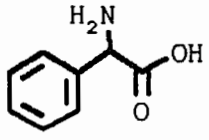

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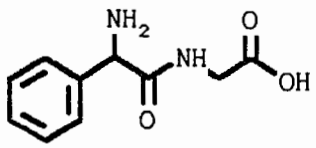
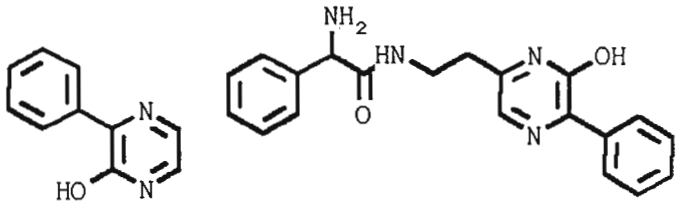


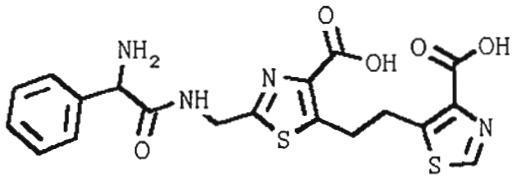
4

(**2**), phenylglycine (**3**), and phenylglycylglycine (**4**) account for a bit more than 15% of the degradation product. Two other compounds which have been isolated previously (**5**[1] and **6**[2]) make up a total of about one-quarter of the product. However, the major product (**7**,



5 6

25% of the degradation mixture) is apparently new, and the elucidation of this structure required a nice combination of all of our NMR tricks with mass spectrometry and other techniques. This structure also presents an amusing challenge to those who like to try to propose mechanisms. That this structure is correct, and that its mechanistic origin is indeed a major pathway for the degradation of cefaclor in acid, is supported by the isolation of other, minor products such as **8**.



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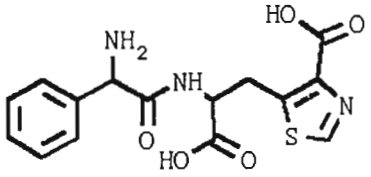

8

Space limitations here prevent us from detailing either our evidence for these structures or the mechnisms that we have proposed for the origins. Manuscripts are in preparation. In the meantime some of the readers of the Newsletter might enjoy speculating about the pathways leading to these products as much as we have. Suffice it to say that there is as much interesting chemistry hidden in the degradation of cefaclor as there is in its synthesis.

Steve Baertschi Doug Dorman

[1] Dinner, A. *J. Med. Chem.* **1977**, *20*, 963

[2] Masada, M. et al. *Chem. Parm. Bull.* **1981**, *29*, 1344.

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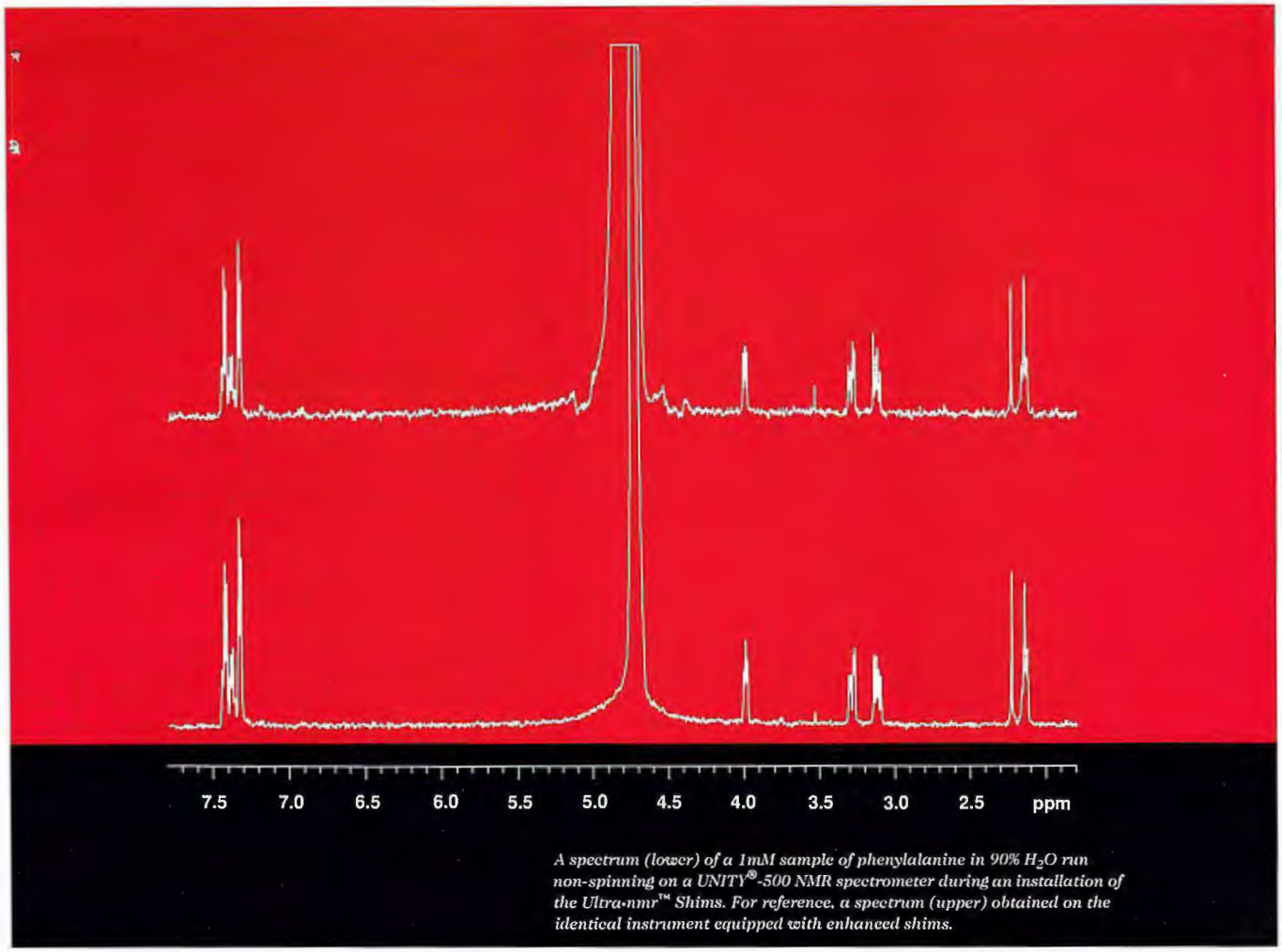

A spectrum (lower) of a 1mM sample of phenylalanine in 90% H_2O run non-spinning on a UNITY®-500 NMR spectrometer during an installation of the Ultra•nmr™ Shims. For reference, a spectrum (upper) obtained on the identical instrument equipped with enhanced shims.

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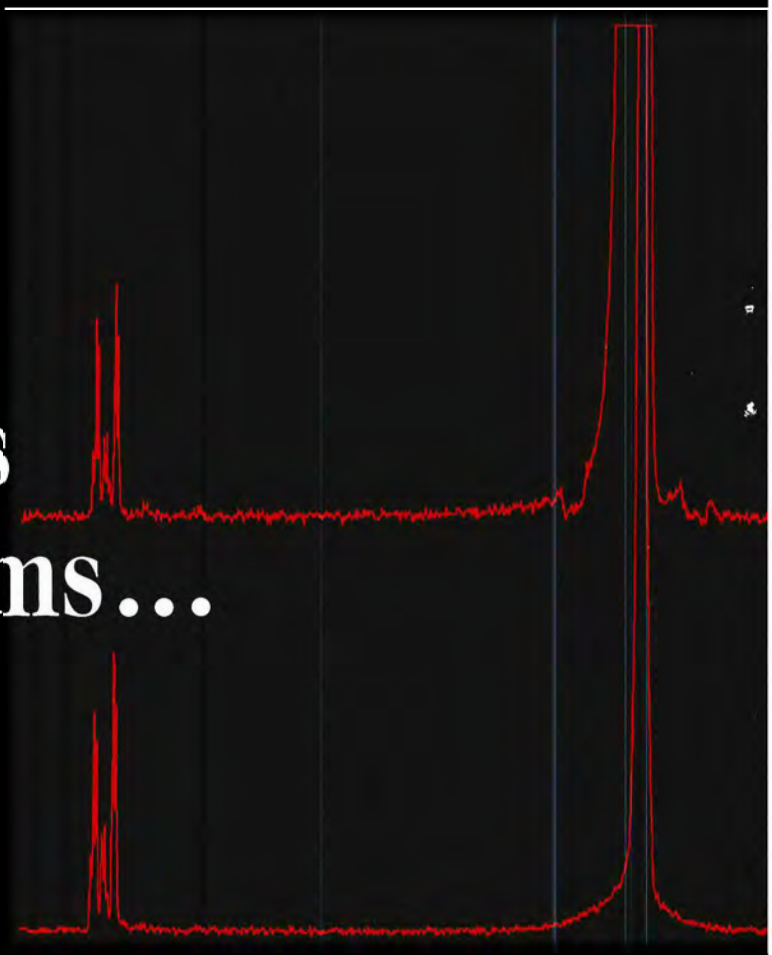

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Dr. Bernard L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

December 9, 1991
(received 12/13/91)

Re: Home-Built RF Channel

Dear Dr. Shapiro:

Triple resonance 3D/4D NMR techniques have become a powerful tool in the studies of large proteins. Such techniques require spectrometers equipped with three or more RF channels. Recently, we have built an additional RF channel on our Varian VXR-500S spectrometer. We found that adding an additional RF channel is quite easy and economical. Modification on other spectrometers should be similar.

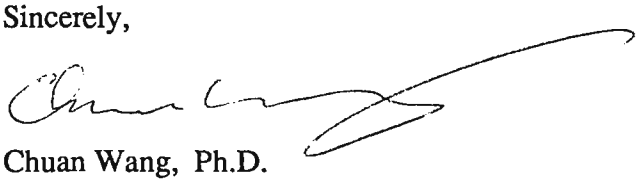
In order to control a home-built RF channel with required pulse width and phase selection (0°, 90°, 180° and 270°), at least three programmable TTL (transistor-transistor logic) lines should be available on the spectrometer. Additional hardware includes 1) a frequency synthesizer, 2) home-built interface circuit, and 3) an RF power amplifier. A continuous radio frequency with required phase is generated in the frequency synthesizer and then goes into the interface circuit. The radio frequency is gated on and off by a TTL line in the circuit to produce a pulse. The pulse phase is selected by two TTL lines in the circuit. The RF pulse is finally amplified by an amplifier. We purchased a PTS160 frequency synthesizer with digital phase modulation (160MJN1Y from Programmed Test Sources). This synthesizer can generates ^{15}N or ^{13}C frequency for spectrometers up to 600 MHz (for ^{1}H). Our total cost of home-built RF channel is about $6,300 since a spare pulse amplifier is available on our spectrometer.

There are four spare TTL lines available on XL-interface board of our VXR-500S spectrometer. Each TTL line has two states with the high and low voltages. The HIGH and

LOW states can be used to select a switch open or closed, as well as a 90°/180° phase shift true or false. Both states can be explicitly selected in Varian pulse sequence program. Therefore both pulse control and phase selection can be performed by selection of the high and low states of the TTL lines.

The interface circuit consists of two parts, the phase selection and the pulse width control. The phase selection is carried out by two TTL lines. One of the TTL lines is used to select 90° and connected to pin 5, 7, and 10 of the 15-pin connector of PTS160. Another TTL line is used to select 180° and connect to pin 9 and 11. Phase selection of 0°, 90°, 180° and 270° can be carried out by combination of high/low states of these two TTL lines. An RF pulse width is simply controlled by a TTL line which turns high speed RF switches on and off. We used two switches (PSW-111 from Mini-Circuits) in series to improve the isolation between the RF channel and the probe. Furthermore, one must insure that the circuit turns off the RF while the spectrometer is idle. In the case of VXR-500S, we need an inverter (7404) in the control line to invert the on/off states of the switches. As an important safety rule, we suggest to disconnect the pulse amplifier from the circuit during testing the interface circuit. This will avoid accidental damage to the pulse amplifier, the probe and the preamplifier. We use the fourth TTL line to control blanking of ENI-5100L amplifier on our instrument. But many amplifiers do not need to control blanking. The cost of the materials to build the interface circuit is less than $100. We spent a few hours to build the interface circuit and a day to tested it out.

Sincerely,



Chuan Wang, Ph.D.
Director of NMR Facility
Phone: (908) 932-3967
Fax: (908) 932-5312
E-mail: chuan@chemb.rutgers.edu

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A joint Institute of the University of Nijmegen and the Netherlands Foundation for Chemical Research

Prof. B.L. Shapiro
966 Elsinore Court
Palo Alto
CA 94303
USA

Nijmegen, december 1, 1991
(received 12/11/91)

Dear Dr. Shapiro,

On the Quantitative Evaluation of MLEV17-TOCSY Spectra.

We demonstrate here that J-coupling information can be extracted quantitatively from MLEV17-TOCSY spectra. For this purpose a circular DNA fragment cd(CpGp) is used for which J-coupling constants had been obtained from 1D spectra.

Although MLEV17-TOCSY is the most popular 2D experiment to assign J-coupled spins, quantitative evaluation of TOCSY cross peak intensities to extract J-coupling constants has not become widespread. Our computer program calculates TOCSY transfer quantitatively from first principles. It evaluates numerically the density matrix as it evolves under the influence of the MLEV17-mixing Hamiltonian, using relevant parameters, such as J-couplings, rf-field strength, and resonance offset values of the spins comprising the network. Fig. 1 illustrates the close correspondence between the experimental and calculated TOCSY data. This encouraged us to investigate the possibilities of using TOCSY to establish the sugar pucker. Instead of going via the intermediate of J-couplings and then assessing the pucker (P) via Karplus equations, we relate TOCSY transfers directly to P and/or the N/S ratio.

Generally, the sugar ring is a mixture of N- and S-puckers with $-18° < P < 18°$ and $140° < P < 180°$, respectively, and both a pucker-amplitude of $\Phi = 35 \pm 5$. We created a DataBase of TOCSY transfer versus mixing time of all relevant protons (H1' to H4') for P (see values above), and N/S ratios (with P=9° for N, and P=162° for S), all with $\Phi = 35$. This DataBase can be used in a number of ways. Fig.2 shows the considerable differences between the TOCSY cross peak intensities in case of an N-pucker and an S-pucker (e.g. the relay peak H1' to H4'). Fig.3 illustrates the dependence of TOCSY intensities on the N/S ratio. So, the type of pucker or the N/S ratio can directly be established. In order to use all TOCSY intensities, we use the Marquardt algorithm to fit (by optimizing P and/or N/S) TOCSY intensities to the experimental data. The N/S ratios of cd(CpGp) were determined in this way, using the transfers from H1'. The correspondence between experimental data and the model is quite good (Fig. 4). The N/S ratios thus obtained compare nicely with the values obtained via 1D J-coupling determination; G: N/S=73±4% (TOCSY), N/S=75% (1D); C: N/S=89±4% (TOCSY), N/S=86%(1D).

Fig.1

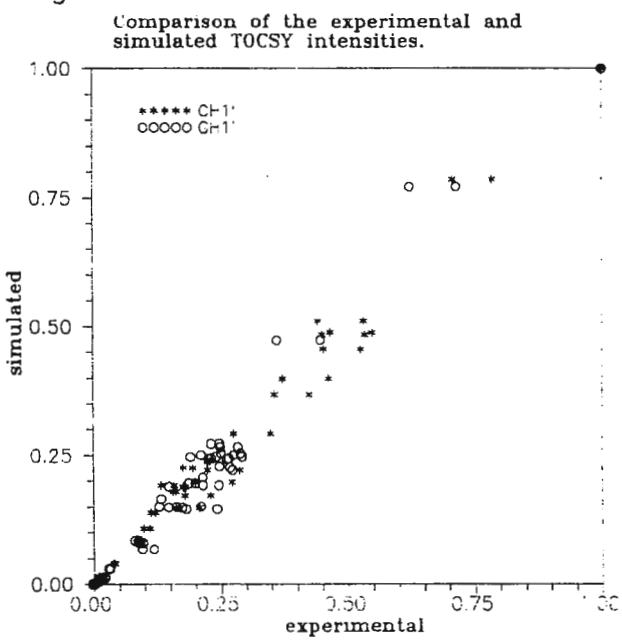


Fig.2

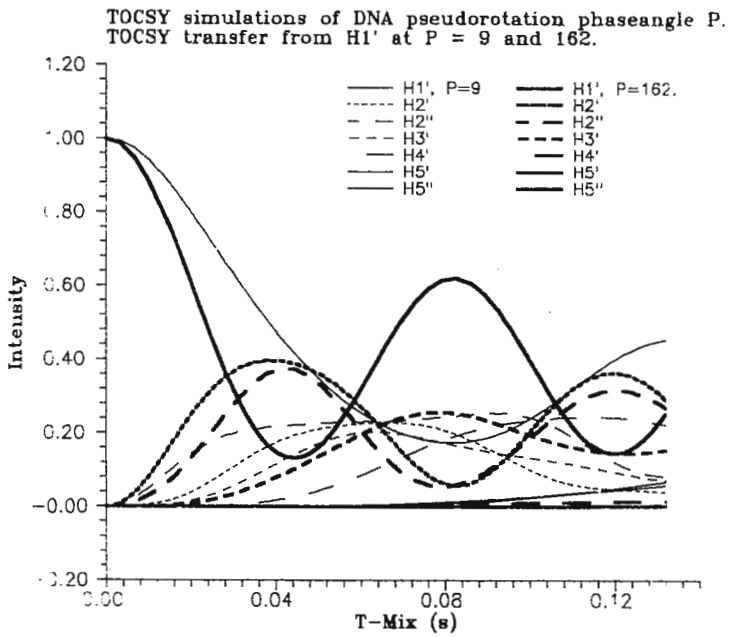
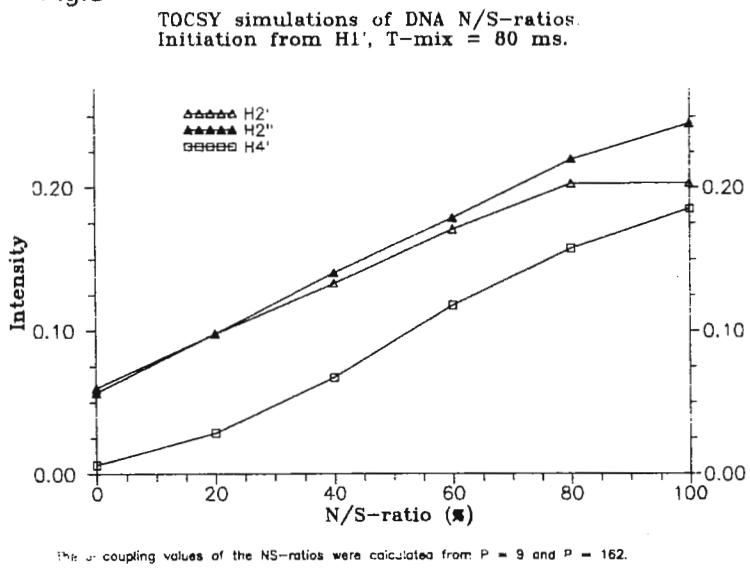
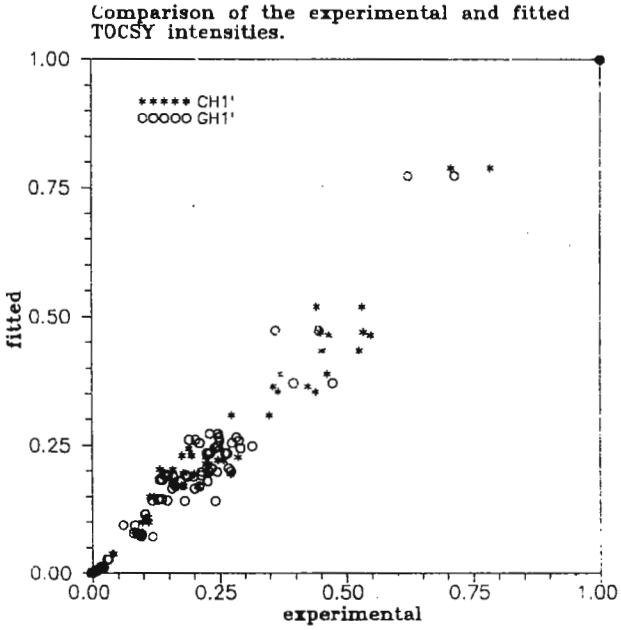


Fig.3



The J-coupling values of the NS-ratios were calculated from P = 9 and P = 162.

Fig.4.



In the TOCSY simulations of Fig.1 an rf-field of 10,869 Hz was used, and the actual chemical shifts of the protons. In the other cases an rf-field of 10,000 Hz was used, together with average values for chemical shifts.

Hanneke Goudriaan

John van Duynhoven

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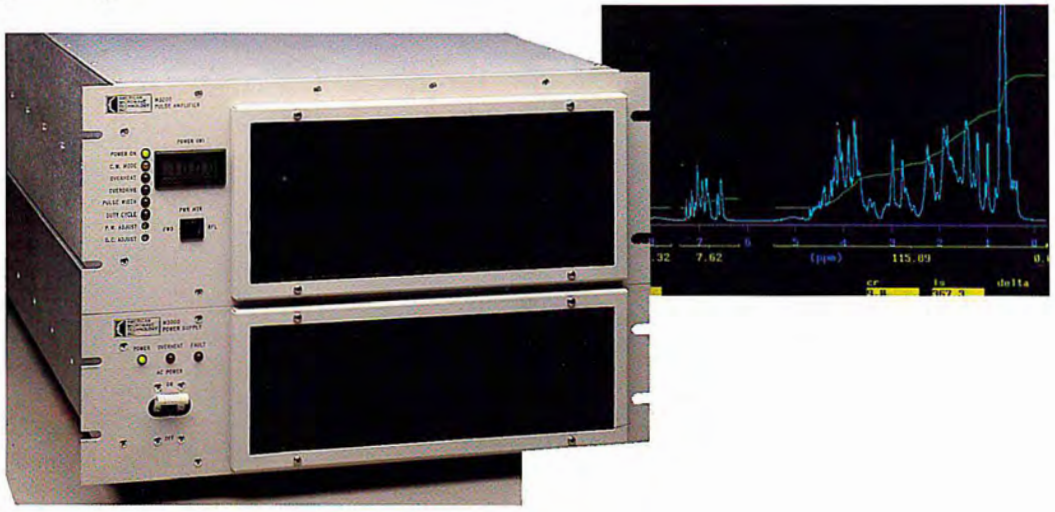

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Dr. B. L. Shapiro,
Editor,
TAMU NMR News letters,
966 Elsinore Court,
Palo Alto, CA 94303

December.9, 1991
(received 12/10/91)

Dear Barry:

" β-Hydrogens of the Phe^4 residue in cyclic enkephalin analogues"

The conformational properties of the highly potent delta-opioid receptor selective cyclic peptide [Tyr-D-Pen-Gly-Phe-D-Pen] enkephalin (DPDPE) in DMSO and aqueous solution have been studied in our laboratory by the use of one and two-dimensional nuclear magnetic resonance spectroscopy (DQF-COSY, ROESY etc.). The $^3J_{NH-\alpha H}$ and $^3J_{\alpha H-\beta H}$ coupling constants (determined with the help of computer simulations where necessary) were used to calculate all possible peptide angles, which along with distance constraints based upon NOEs provided the basis for molecular mechanics energy minimization calculation.

A critical question in the conformational structure-biological activity relationship is the importance of side chain rotamer populations to biological activity. The positioning of pro-R and pro-S β-hydrogens in Phe4 residue can determine the topographical properties of molecule which may effect peptide-receptor interactions. In order to obtain better understanding of the topography of the molecule we have made the complete ^1H chemical shift assignments for the (S,S), (S,R), (R,R) and (R,S) - [β Me -p- NO_2 Phe^4] DPDPE analogues (I - IV). A striking feature of the ^1H NMR spectra of these analogues is the very characteristics nature of $^3J_{\alpha H-\beta H}$ coupling constant value. In (S,S) and (R,R) analogues the $^3J_{\alpha H - \beta H}$ coupling constant was always larger than (S,R) and (R,S) analogues. (Table). We have found this in more than 24 analogues of DPDPE and now we are using the $^3J_{\alpha H-\beta H}$ coupling constant value as an NMR probe in deciding which of the β-hydrogens of a substituted of Phe^4 residue is pro-R or pro-S in these cyclic enkephalins.

Table: ^1H Chem. shift (ppm) and coupling constant (Hz) of β-Hydrogens of the Phe^4 residue in DPDPE analogues.

	Peptide	Chem. Shift. (ppm)	Coupling Constant (Hz)
I.	(S,S)-β Me-p-NO2 DPDPE	3.43	9.0
II	(S,R)-β Me-p-NO2 DPDPE	3.57	6.5
III	(R,R)-β-Me-p-NO2 DPDPE	3.48	9.0
IV	(R,S)-β-Me-p-NO2 DPDPE	3.56	7.0

Moreover these results in conjunction with extensive biological studies indicate that the β-Me Phe^4 side chain takes a particular orientation in those analogues that show high opioid receptor selectivity. Please credit this to the account of Professor Mike Barfield.

Sincerely,

Om Prakash Terry Matsunaga Victor J. Hruby

Cornell University

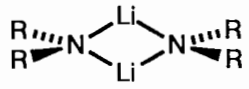
Department of Chemistry
Baker Laboratory
Ithaca, New York 14853-1301 USA

November 11, 1991
(received 11/25/91)

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

Dear Barry,

Despite mounting indirect evidence that solvated lithium dialkylamides exist as dimers (**1**) rather in donor solvent solutions and trimers (**2**) or higher oligomers in the absence of donor solvents, the symmetry of the cyclic oligomers has precluded a direct spectroscopic distinction. We report herein a simple NMR experiment in which indirectly detected homonuclear zero-quantum coherence unambiguously differentiates cyclic dimers from higher oligomers.



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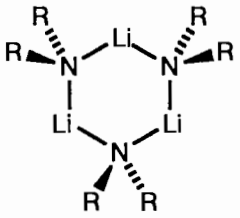

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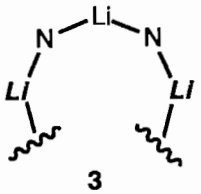

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We employed the pulse sequence developed by Müller (*J. Am. Chem. Soc.*, **1979**, *101*, 4481) and Bodenhausen and Ruben (*Chem. Phys. Lett.*, **1980**, **69**, 185) for heteronuclear shift correlations. For the phases shown, homonuclear ^{15}N two-spin coherence (a mixture of zero- and double-quantum coherence) is prepared from the two ^{15}N spins neighboring a ^{6}Li atom in a ^{6}Li-^{15}N doubly-labelled lithium dialkylamide cyclic oligomer (equation 1).

$$I_z \xrightarrow{90^{o}_{I,x}} -I_y \xrightarrow[(\tau = 1/4J)]{\pi J_{I,S1}\tau 2I_zS_{z1} + \pi J_{I,S2}\tau 2I_zS_{z2}} 4I_yS_{z1}S_{z2} \xrightarrow{90^{o}_{I,x},\ 90^{o}_{S,x}} 4I_zS_{y1}S_{y2} \qquad (1)$$

During the evolution period of the experiment, the zero-quantum coherence will evolve under scalar coupling only to ^{6}Li spins which are coupled to one, but not to both, ^{15}N spins. For a lithium amide dimer, all ^{6}Li spins coupled to ^{15}N spins involved in the two-spin coherence are coupled to *both* ^{15}N spins. As a consequence, the coupling pattern will be a singlet along the

f_1 dimension of the two-dimensional spectrum and a 1:-2:1 triplet along the f_2 dimension. In the case of higher cyclic oligomers, there exist two ^{6}Li spins (***Li*** in **3**) that are coupled to one, but not both, ^{15}N spins. The zero-quantum coherence will develop scalar coupling to the two non-shared ^{6}Li spins, resulting in a 1:2:3:2:1 pattern along the f_1 dimension and a 1:-2:1 pattern along the f_2 dimension.

The results of the experiment as applied to [^{6}Li,^{15}N]lithium 2,2,6,6-tetramethylpiperidide in tetrahydrofuran solution is illustrated in Figure 1A. The coupling patterns reveal a cyclic dimer rather than a higher oligomer. The complementary outcome is illustrated by the spectrum of the unsolvated amide recorded in benzene (Figure 2C). The ^{6}Li triplet shows coupling consistent with a higher oligomer rather than the dimer.

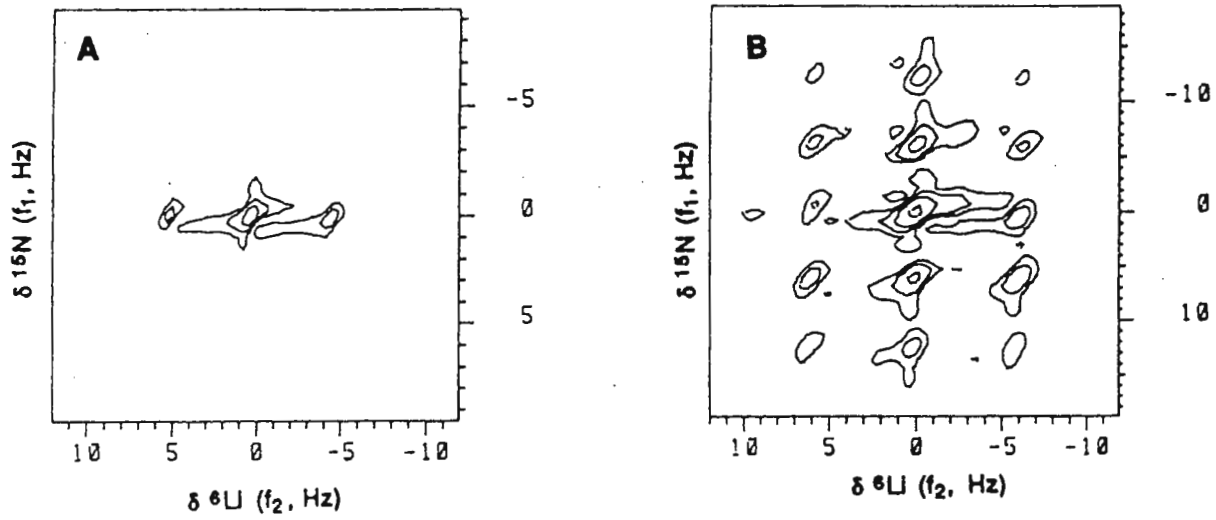

Figure 1. ^{6}Li-detected ^{15}N zero-quantum NMR spectra of: (A) 0.10 M [6Li,15N]LiTMP in 3:1 THF/pentane at -115 °C; (B) 0.25 M [^{6}Li,^{15}N]LiTMP in 3:1 benzene at 30 °C. Spectra were recorded on a Bruker AC 300 spectrometer operating at 44.17 MHz and 30.42 MHz for ^{6}Li and ^{15}N (respectively). Data were processed in phase sensitive mode. Digital resolution in f_1 prior to zero filling is1.0 Hz, and 2.4 Hz (respectively) for spectra (A) and (B).

We believe that determining aggregation state by detection of a multiple quantum coherence may see more wide-spread use for other NMR-active nuclei as well as other aggregation states and topologies.

Sincerely,

Aidan Harrison David Fuller James Gilchrist David Collum

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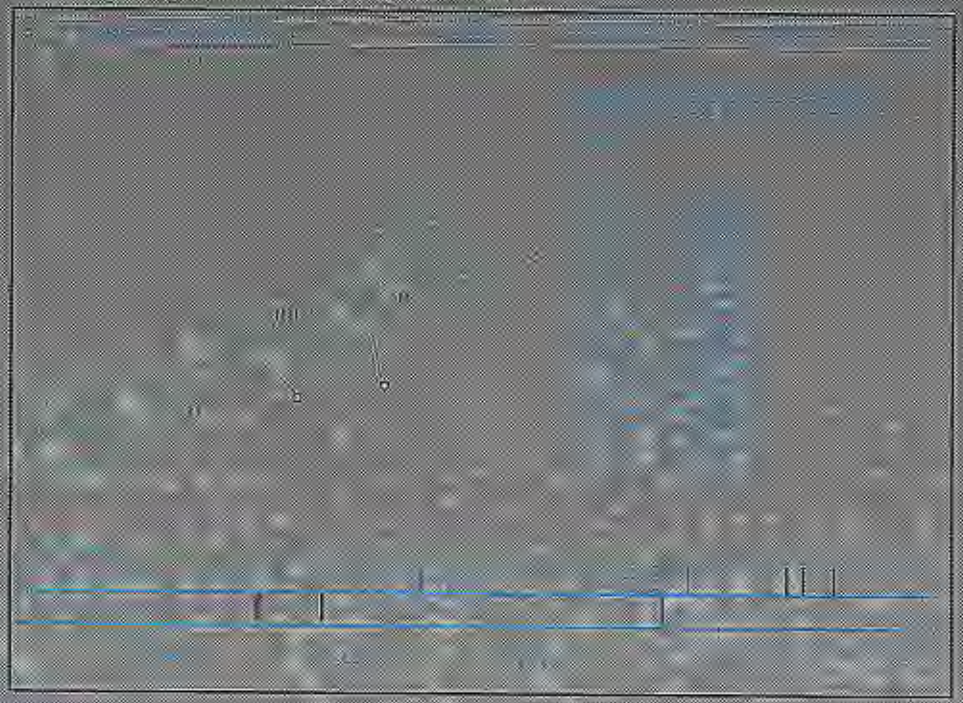

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THE UNIVERSITY OF MELBOURNE
School of Chemistry

27 November 1991
(received 12/5/91)

Dr Barry L. Shapiro
Editor, *TAMU NMR Newsletter*
966 Elsimore Court
PALO ALTO CA 94303
U.S.A.

Dear Barry,

1D-INADEQUATE of a Ketenimine

In connection with our work on the mechanism of termination in free radical polymerisation with Professor D.H. Solomon, we have been involved with the polymerisation of methacrylonitrile.[1]

It has been shown previously that a ketenimine is formed during thermal decomposition of α-^{13}C labelled azobisisobutyronitrile in the presence of styrene, but the assignments of two ^{13}C signals at 61.5 and 55.5 ppm to the quaternary carbons C2 and C5 were tentative.[2]

Two of our Honours students, Cindy Quach and Stephen Jones, have recently prepared relatively large and pure samples of the ketenimine in Figure 1 which allowed us to obtain a 1D-INADEQUATE spectrum on our JEOL GX-400. The spectrum allows unequivocal assignments: the nitrile carbon coupling of 60 Hz is reproduced in the multiplet at δ 55.5, which in turn shows a 40 Hz coupling to the signals at δ 27.3, thus establishing the connectivity C1 ---> C2 ---> C3 and providing the assignments as given in Figure 1. Thus the original tentative assignments should be reversed.

With best wishes.

Yours sincerely,

David

Dr D.P. KELLY

1. Danek S.K., Kelly D.P., Serelis A.K., *J. Org. Chem.*, **52**, 2911 (1987).

2. Moad G., Rizzardo E., Solomon D.H., Johns S.R., Willing R.I., *Makromol. Chem. Rapid Commun.*, **5**, 793 (1984).

The University of Melbourne, Parkville, Victoria 3052, Australia. Telephone $\frac{03}{+61\ 3}$ 344 6492 Fax $\frac{03}{+61\ 3}$ 347 5180

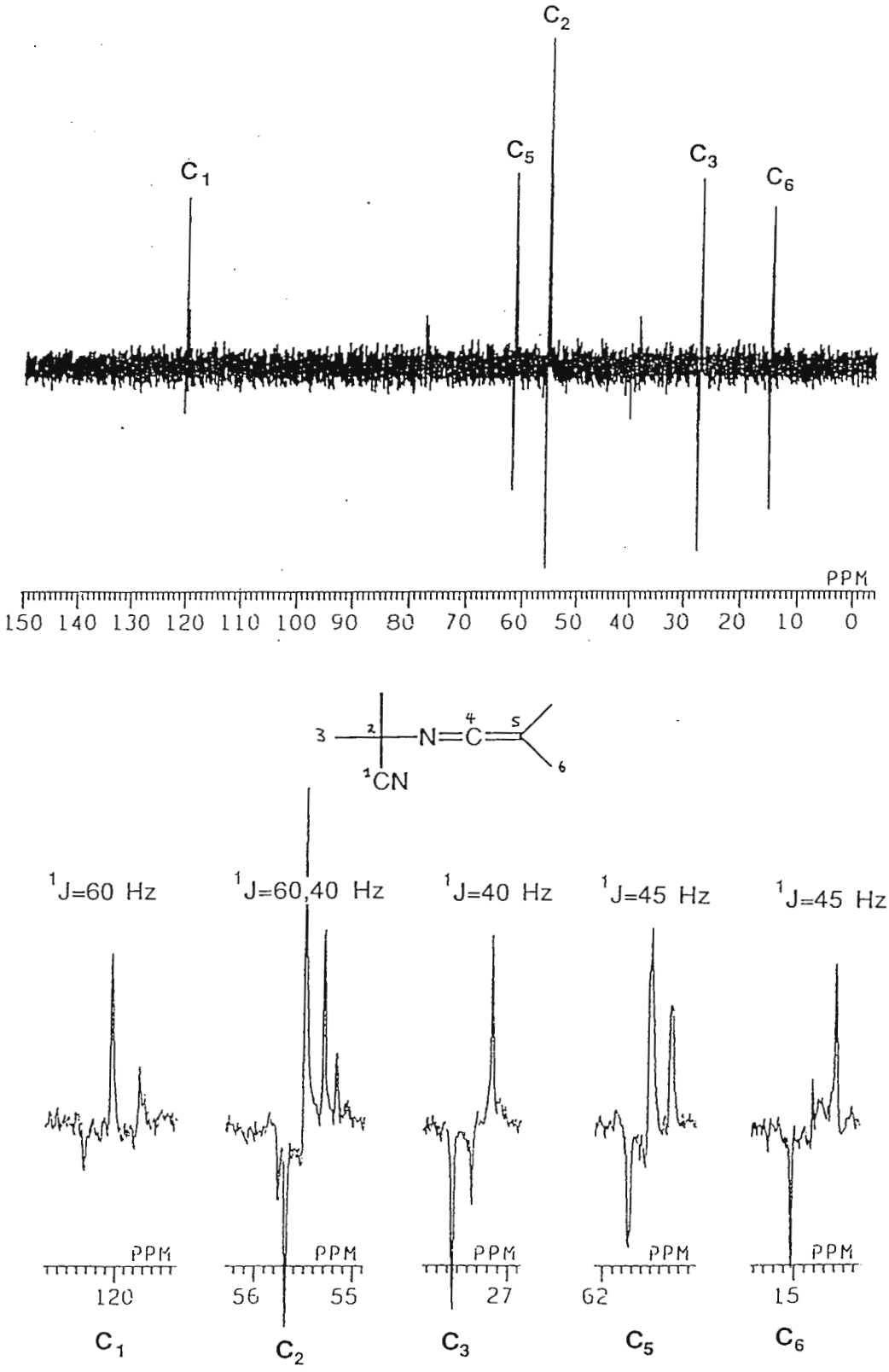
C2
C5
C3
C6
C1
PPM
150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
3
2
N=C
4
5
6
1CN
1J=60 Hz
1J=60,40 Hz
1J=40 Hz
1J=45 Hz
1J=45 Hz
PPM
120
56
55
27
62
15
C1
C2
C3
C5
C6

FIGURE 1

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Madison, WI 53706-1108

(608) 264-5407
E-Mail RALPHJ@VMS.MACC.WISC.EDU
November 18, 1991
(received 11/26/91)

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

Dear Dr Shapiro,

Fast TOCSY and COSY Acquisition

I have just returned from a couple of fabulous courses at Bruker, and as usual have come back with too many ideas to possibly try. One interesting concept that I don't think spectroscopists are generally aware of is how quickly it is possible to acquire TOCSY and COSY spectra. I have always been keen on fast acquisitions since noting the reactions of spectroscopists who routinely ran 2D C-H correlation spectra overnight, to the news that I was running them in as short as 6 minutes on a 200 MHz instrument (admittedly with at least 30 mg of relatively low MW material).

The neat aspects of the TOCSY experiment are that they produce very few artifacts and really don't need phase cycling, and that it really doesn't matter where the magnetization starts off. Consequently, a single scan per increment is tons for most normal samples, and you can pulse quite rapidly. But what does this have to do with COSY? Well, if you only use a short mixing time in the TOCSY experiment, you don't allow the magnetization to transfer very far down the system. A mixing time of around 10 ms produces a very nice COSY spectrum from a TOCSY experiment.

So, just how quickly can you run a phase-sensitive TOCSY? In anything from 2 to 5 minutes. In 2 minutes you can acquire 128 increments with a relaxation delay of 200 ms, and still have time for 32 dummy scans* (this assumes you have double buffering so that there is no delay between increments, and dummy scans are only run at the beginning of the experiment, such as is implemented in the Bruker AMX pulse programs). These spectra are remarkably good, but to be honest, two minutes is ridiculously short. We have settled on a more relaxed (!) 5 minute higher resolution experiment using 256 increments and a relaxation delay of 0.5 s for typical small molecules.

The spectra of rotenone on the next page were run in dmso-d_6 (concentration unknown, sample clearly a little degraded!) on an AMX-360 using Bruker's standard mlev17tp pulse program for phase-sensitive TOCSY. The true TOCSY was run with a mixing time of ca. 85 ms (L1 = 60 with the current pulse angles), while the 'COSY' used a 12 ms mixing time (L1 = 5). Each used 256 increments, a 0.5 s relaxation delay, and 32 dummy scans, giving a total acquisition time of just under 5 minutes. And yes! I know they could be phased a little better, but it gets embarrassing if it takes longer to process and plot than to acquire — it is a law in my lab that the instrument shall always be acquiring!!

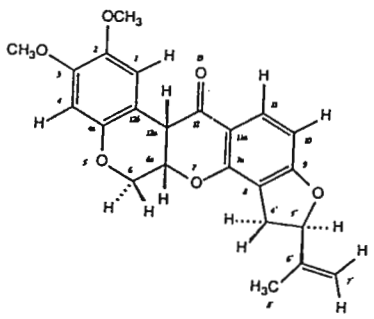
Sincerely,

John Ralph

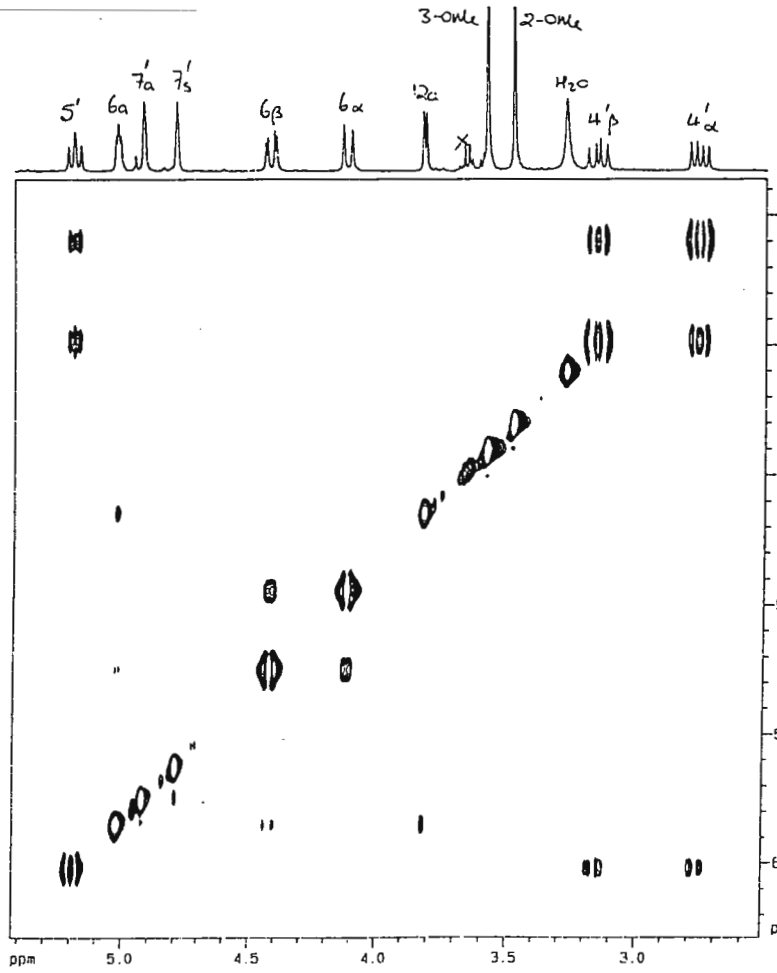
POSTDOC POSITION: I currently have funding for a further postdoc, in the field of plant chemistry. Any *American Citizen* who has a solid organic and/or plant background and would like to get further into natural products and plant chemsitry, please contact me. Please note that the US govt. is an equal opportunity employer and applications by minorities are strongly encouraged.

---

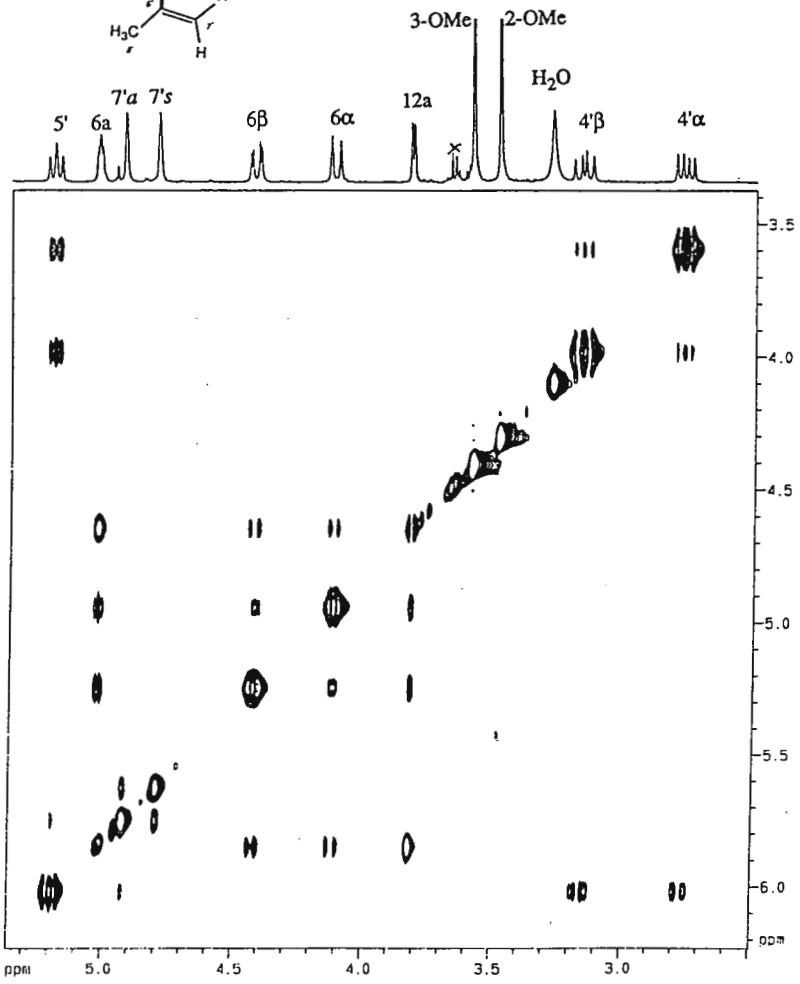
* Note: if the sample is in H_2O, it is advisable to do more dummy scans (e.g. 128) to allow equilibration because of the internal sample heating that is typical of these spin-locked experiments.



5 minute 'COSY'
mixing time: 12 ms



5 minute TOCSY
mixing time: 85 ms



Current Data Parameters
NAME quicktocsy
EXPNO 11
PROCNO 1

F2 - Acquisition Parameters
Date 911113
Time 18.44
PULPROG alevtp
NUCLEUS 1H
SOLVENT CDCl3
AQ 0.4079520 sec
FIDRES 1.225837 Hz
DW 199.0 usec
RG 64
NL1 1 dB
D1 0.5000000 sec
D12 0.0000200 sec
P1 10.7 usec
D13 0.0000030 sec
D0 0.0000030 sec
NL3 8 dB
P17 2500.0 usec
P6 20.6 usec
P7 41.2 usec
L1 80
P5 13.7 usec
RD 0.0000000 sec
PW 0.0 usec
DE 318.4 usec
SFO1 360.1373912 MHz
SW 2512.56 Hz
TD 2048
NS 1
DS 32

F1 - Acquisition parameters
NDE 2
SW 8.977 ppm
SFO1 360.1377 MHz
FIDRES 9.814698 Hz
TD 256

F2 - Processing parameters
SI 1024
SF 360.1357276 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0
PC 1.40

F1 - Processing parameters
MC2 TPPI
SI 512
SF 360.1357276 MHz
WDW QSINE
SSB 2
LB 0.00 Hz
GB 0

2D NMR plot parameters
CX2 15.00 cm
CY1 15.00 cm
F2PU1 5.355 ppm
F2U1 1928.55 Hz
F2PU2 2.494 ppm
F2U2 898.10 Hz
F1PU1 6.235 ppm
F1U1 2245.37 Hz
F1PU2 3.373 ppm
F1U2 1214.82 Hz
F2PPMCM 0.19077 ppm/cm
F2HZCM 68.70289 Hz/cm
F1PPMCM 0.19077 ppm/cm
F1HZCM 68.70289 Hz/cm

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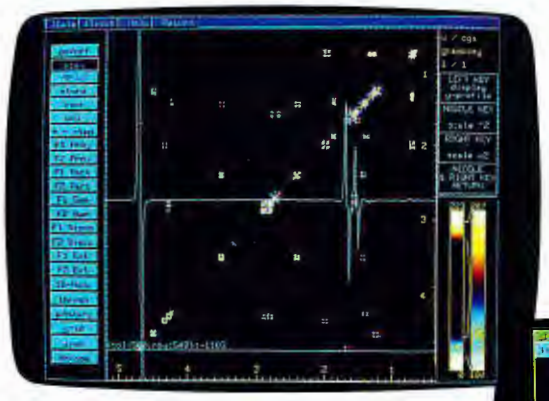

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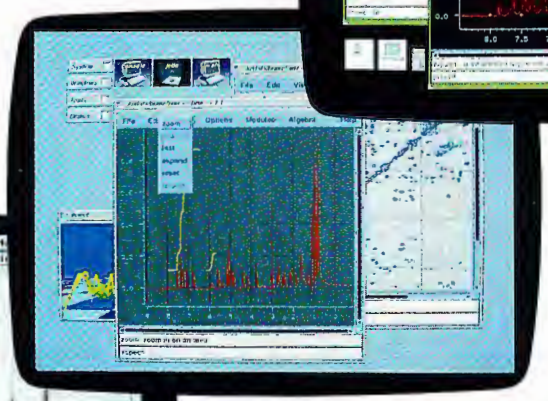
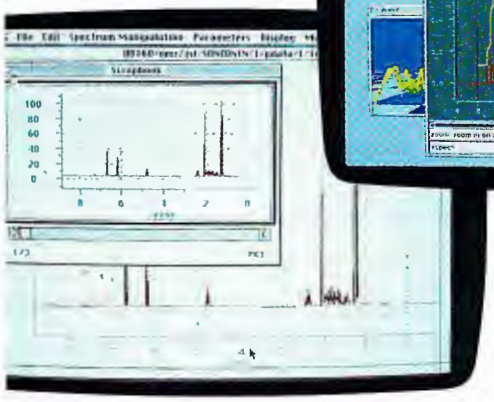


WINNMR on your Mac II or PC/PS system.



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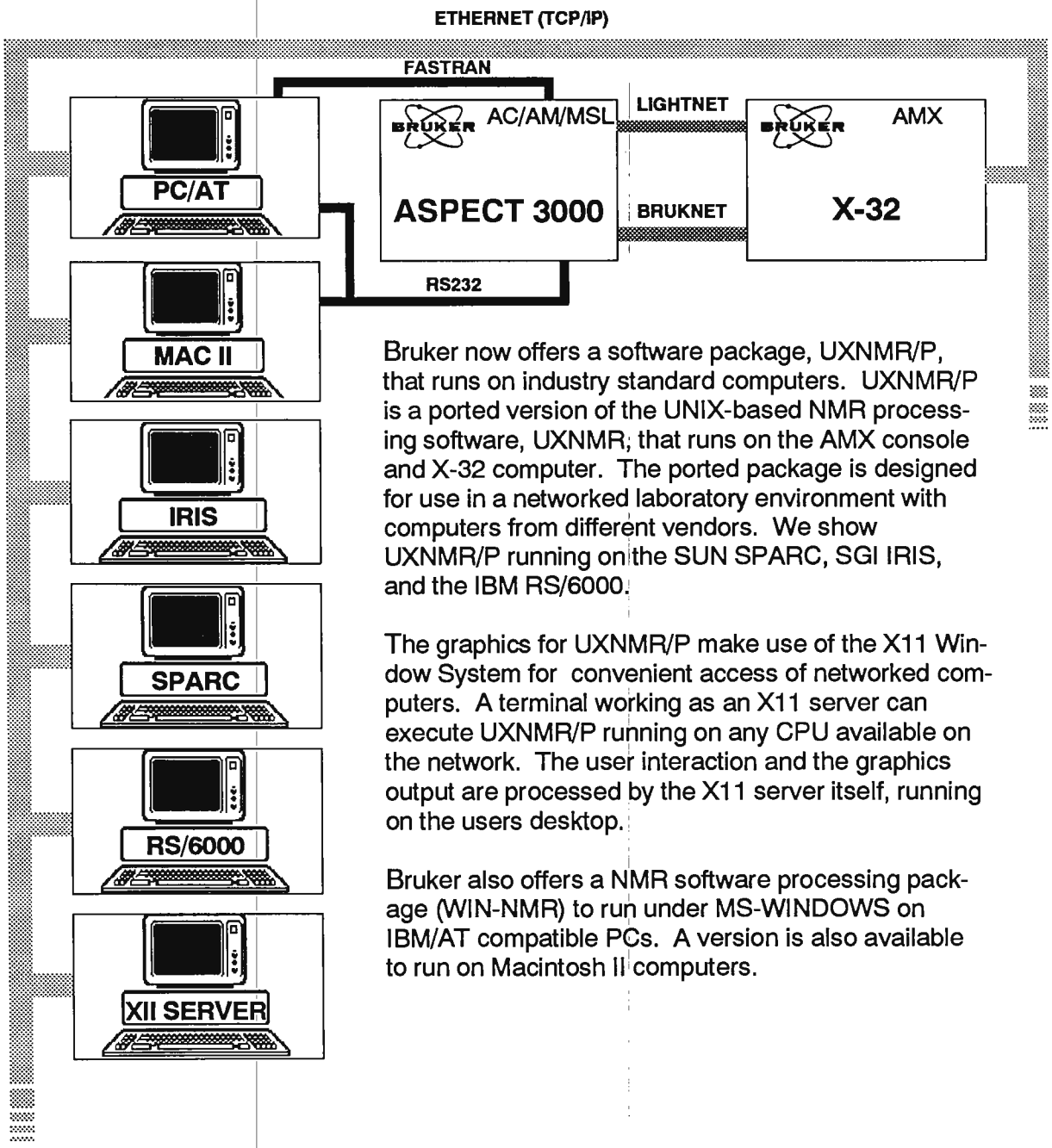
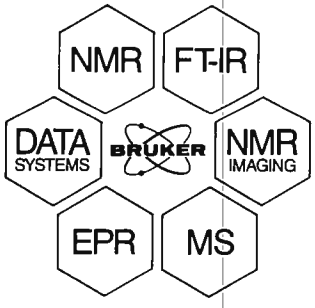


Bruker now offers a software package, UXNMR/P, that runs on industry standard computers. UXNMR/P is a ported version of the UNIX-based NMR processing software, UXNMR, that runs on the AMX console and X-32 computer. The ported package is designed for use in a networked laboratory environment with computers from different vendors. We show UXNMR/P running on the SUN SPARC, SGI IRIS, and the IBM RS/6000.

The graphics for UXNMR/P make use of the X11 Window System for convenient access of networked computers. A terminal working as an X11 server can execute UXNMR/P running on any CPU available on the network. The user interaction and the graphics output are processed by the X11 server itself, running on the users desktop.

Bruker also offers a NMR software processing package (WIN-NMR) to run under MS-WINDOWS on IBM/AT compatible PCs. A version is also available to run on Macintosh II computers.



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Department of Genetics

B.L.Shapiro
TAMU NMR newsletter
966 Elsinore Court
Palo Alto, CA 94303

20 November 1991
(received 11/25/91)

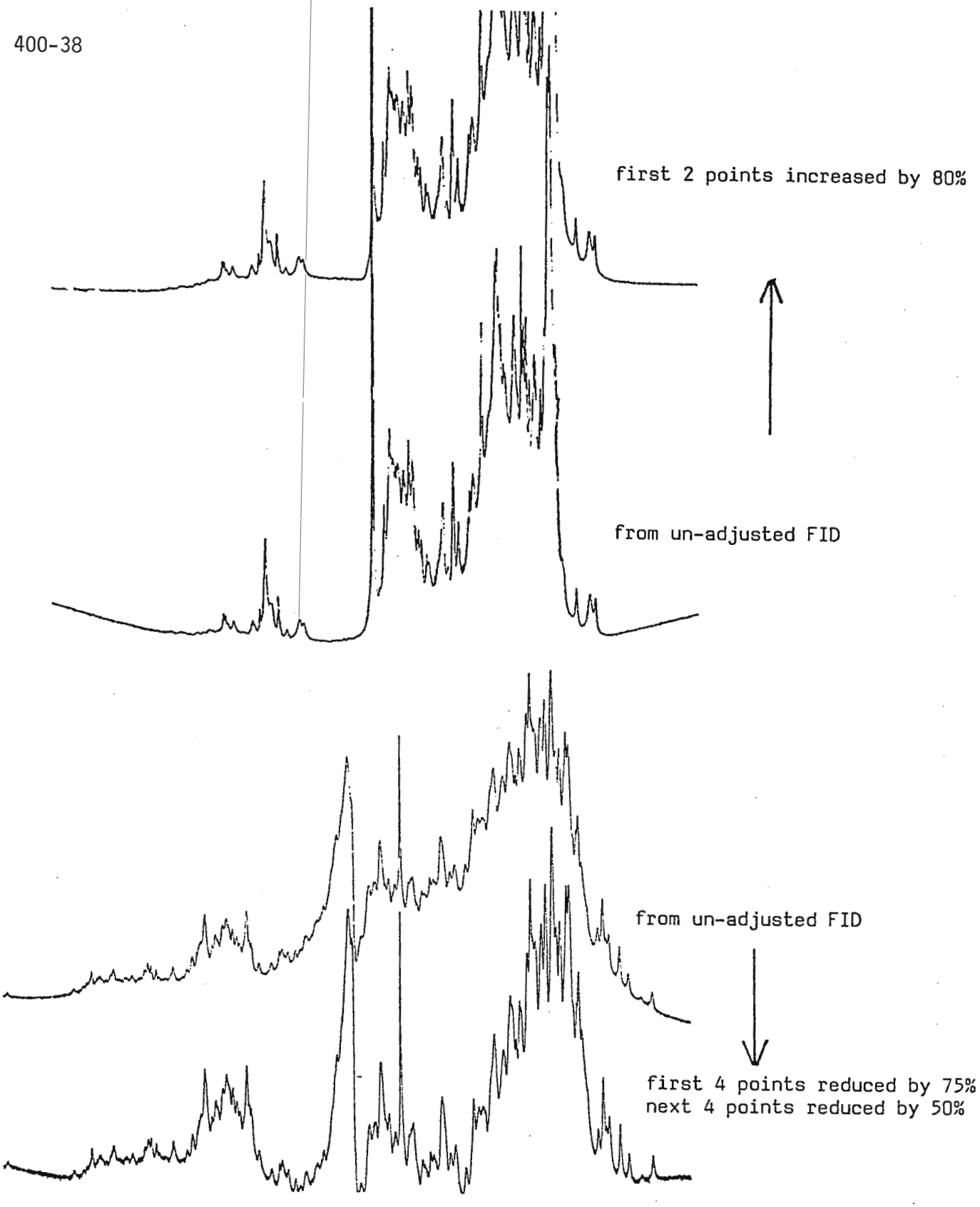
Epoch-making in NMR

Dear Prof. Shapiro,

Baseline distortion is all too common in NMR, and can arise from a variety of sources. In particular, inaccurate sampling of the early part of the FID is quite common. This can be circumvented by optimising the collection of the FID, which can give very rewarding results in 1 and 2D spectra. Another approach is to apply a polynomial or cubic spline function to correct the transformed spectrum. A third possibility lies between these two. We were considering how to improve the appearance of ^1H spectra that have not been recorded with any special precautions, using only the software of a Bruker AM console. Many spectra had baselines which rose or fell in the centre. It is found that for those which dip downwards, the early points of the FID need to be magnified, whilst baselines which curve upwards are corrected by reducing these points. Such adjustments were readily achieved by zeroing some early points (NZP, ZP commands), combined with additive transfer (DC and AT) with the original FID. The spectra here demonstrate this technique. Of course these improvements could be done in other ways, but this procedure is very simple. It also clearly demonstrates the importance of the weighting of the data at the start of collection, and how this can subsequently be optimised. Accordingly, it *could* be called Early POint Correction with Hindsight (EPOCH).
The spectra were recorded on the Bruker AM 500 at the Biological NMR Centre, Leicester, UK. The upper is DNA, the lower is a protein, both in D_2O with presaturation of residual solvent. No window functions have been used.

John R.P. Arnold

Please credit this contribution to the account of J.D.Kennedy, School of Chemistry, Leeds, UK.



SPECTRA WITH AND WITHOUT ADJUSTMENT OF INTENSITY OF EARLY FID POINTS
(both FIDs recorded in 16K)

University of Alberta
Edmonton
Canada T6G 2G2

Department of Chemistry
Faculty of Science
E3-44 Chemistry Bldg., Tel. (403) 492-3254 Fax (403) 492-8231

December 6, 1991
(received 12/13/91)

Dr. B.L. Shapiro
TAMU Newsletter
966 Elisnore Court
PALO ALTO, California
U.S.A. 94303

Re: SIMBA Sequence on a Unity 500

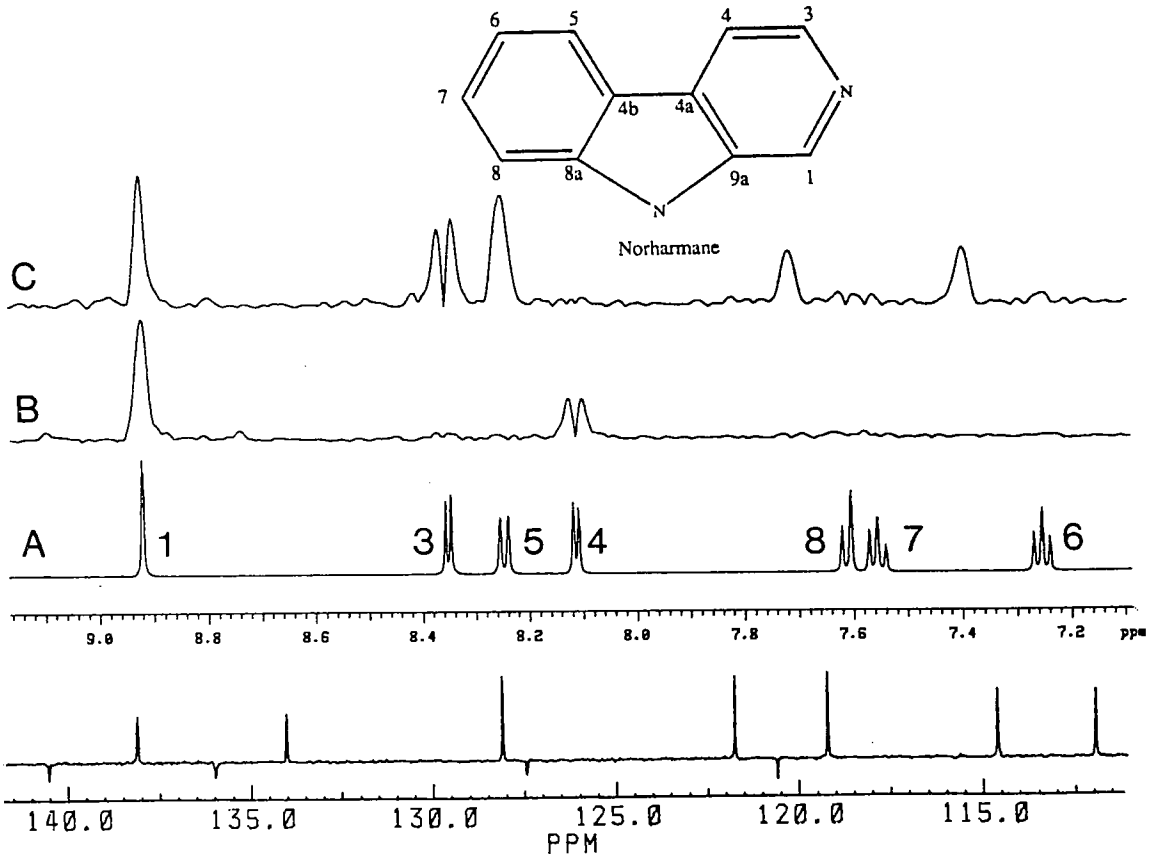
Dear Barry,

The selective 1D, three pulse version of the HMBC experiment described recently (1) has been implemented on our new Unity 500 spectrometer and tested on norharmane. The bottom spectrum is the C13 APT spectrum. Spectrum A is the normal H1 spectrum (4 scans) and B and C are the results of the selective HMBC experiment (64 scans) when carbons 9a and 4a are irradiated. All of the expected long range correlations show up (H1 and H4 when C9a is irradiated and H1, H3, and H5 for C4a) plus an unexpected directly bonded H7 correlation when C4a is irradiated. The reason for this 'artifact' is that both C7 (at 128 ppm) and C4a (at 127.5 ppm) are perturbed with the selective pulse (11 ms). To remove this artifact, a low pass J filter could be incorporated (2).

Sincerely,

Tom

Tom Nakashima



(1) M.A. Keniry and G.A. Poulton, Magn. Reson. in Chem., 29, 46 (1991).
(2) R.A. Couch and G.E. Martin, J. Magn. Reson., 92, 189 (1991).

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הפקולטה למדעים מדוייקים ע"ש רייموند ובברלי סאקלר בית הספר לכימיה

Dr. B. L. Shapiro
968 Elsinore Court
Palo Alto, CA 94303
USA

19 November, 1991
(received 11/30/91)

Dear Barry,

CAN RELAXATION OF NA-23 BE TRANSLATED INTO A SHIFT? AN OBSERVATION OF SINGLE AND MULTIPLE QUANTUM DYNAMIC SHIFTS IN SOLUTIONS.

One of the consequences of Redfield's relaxation theory is that the line broadening due to slow motion should be accompanied by a small shift. Its observation could be of a great help in the interpretation of the molecular motions leading to nuclear relaxation. However because of its smallness it was probably ignored in several works. One example where it can detected is in the case of triple- or double filtered spectra of Na-23 (Fig. 1). Here the sodium is tightly bound to the cryptate molecule KRYPTOFIX 221 dissolved in glycerol to slow down its motion. In contrary tothe first reaction of any experienced NMR spectroscopist, the phase is correct. It is the dynamic frequency shift (DFS) between the narrow and the broad component which make the spectrum to look so awkward.

It turns out that it is much easier to see DFS during the evolution of the triple quantum coherence (see our recent article in JMR **94,** 439 (1991)). After quadrature detection and Fourier transformation it looks like in Fig. 2. In the figure, the carrier frequency was zeroed on the single quantum transition. As expected, the DFS increases and the triple quantum coherence linewidth decreases as the motion slows down.

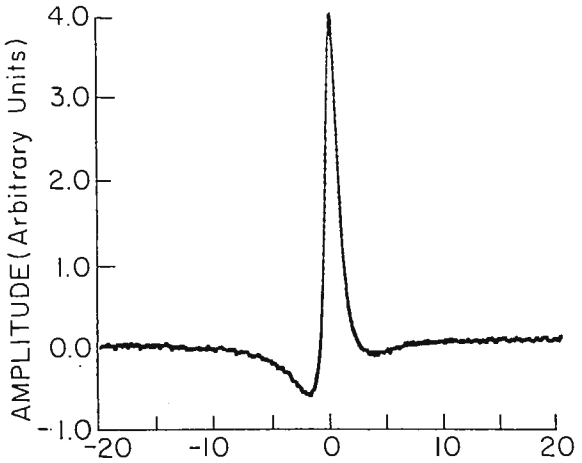

Fig. 1

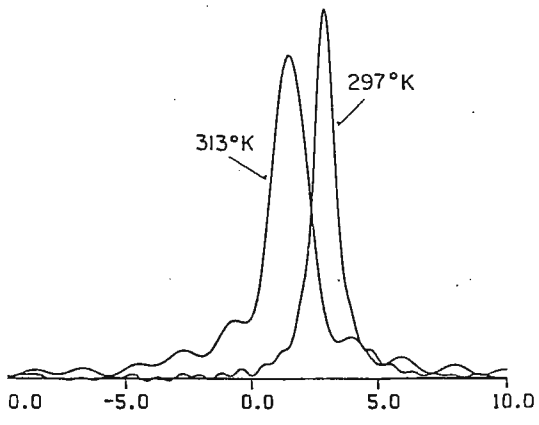

Fig. 2

Hoping to see you in the XV ICMRBS in Jerusalem, August 16, 1992.

With our best wishes,

Uzi Eliav Hadassah Shinar Gil Navon

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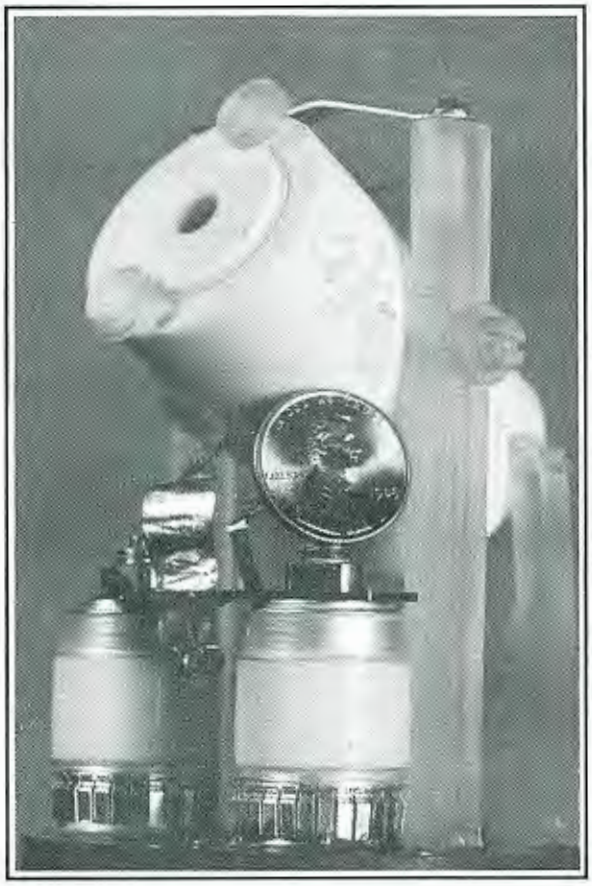

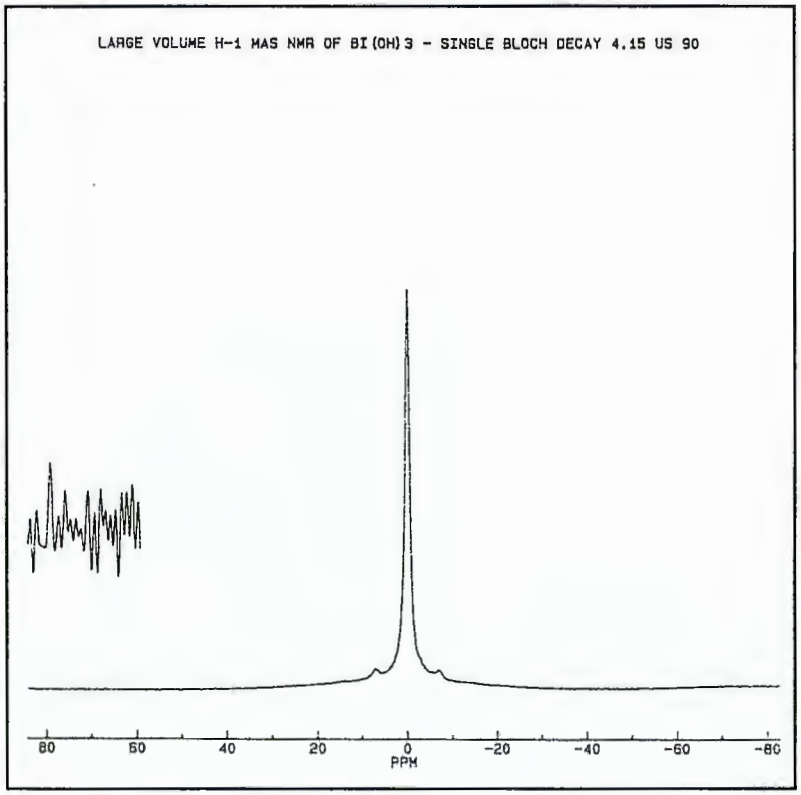

Spectra courtesy of Dr. Robert Honkonen, Proctor and Gamble.

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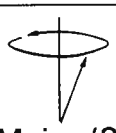

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Dr. B.L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, California 94303

(received 11/29/91)

Re: ^{13}C NMR Lineshape Analysis for Measuring the Erythrocyte Transmembrane Exchange of Urea

Dear Dr. Shapiro,

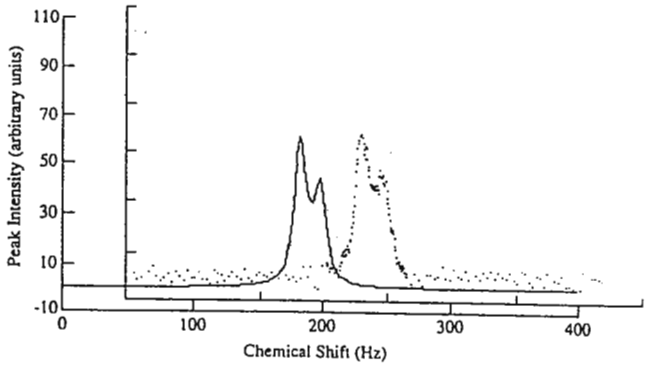
Urea exchanges rapidly across the human erythrocyte membrane, and previous studies in which the K_m and V_{max} of the exchange were measured produced a wide range of estimates of these parameters under equilibrium exchange conditions at ~25°C; we have developed a new method for measuring these parameters using NMR lineshape analysis. We observed that the ^{13}C NMR spectrum of ^{13}C-urea in a suspension of human red cells of reduced mean cell volume contained partially resolved resonances arising from the intra- and extracellular populations of the compound. At 25°C and a magnetic field strength of 9.4 T, the rate of transmembrane exchange of urea was 'intermediate' on the NMR timescale, i.e., the bandshape was sensitive to a reduction in the rate of exchange of ^{13}C-urea induced by the addition of ^{12}C-urea.

^{13}C NMR spectra of ^{13}C-urea in red cell suspensions containing different concentrations of ^{12}C-urea were acquired on a Varian 400 MHz VXR/XL NMR spectrometer. After phase-correction the digitised spectra were transferred (using a portable spooler) to an HP220 computer and a frequency (relative) axis was assigned. The spectrum was then 'weighted' by using a program that selected every sixth data point in the 'noise' but every point in the region of the spectra. After transfer of the data files to a Silicon Graphics IRIS 4D/20 workstation, we used nonlinear leastsquares regression to fit an equation describing the bandshape of two exchange-broadened resonances (Sandström, 1982) to the spectra and thus, obtained an estimate of the first-order rate constant for urea exchange in each sample. Figure 1 compares *acquired spectra* with the *calculated* lineshapes for samples with, (A) the lowest (0.029 M) and, (B) the highest (0.87 M) urea concentrations. A full account of this work has recently been submitted for publication (Potts *et al.*, 1991).

References:

Potts, J.R., Bulliman, B.T., and Kuchel, P.W. (1991) *Eur. Biophys. J.* (submitted)
Sandström, J. (1982) *Dynamic NMR Spectroscopy*, Academic Press, New York

A



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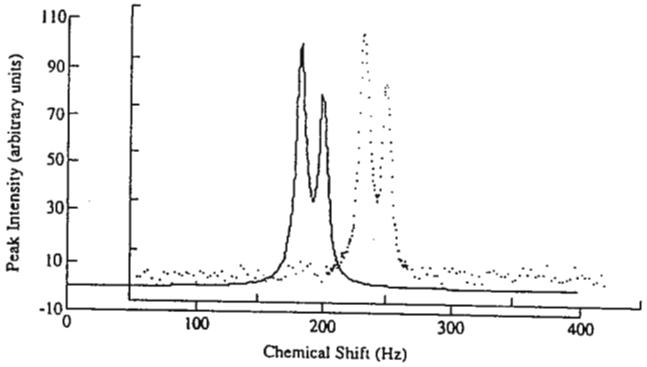


Figure 1

Yours sincerely,

Jennifer R. Potts

Philip W. Kuchel

Observation of Three-Bond Coupling in High Molecular Weight Polysaccharides

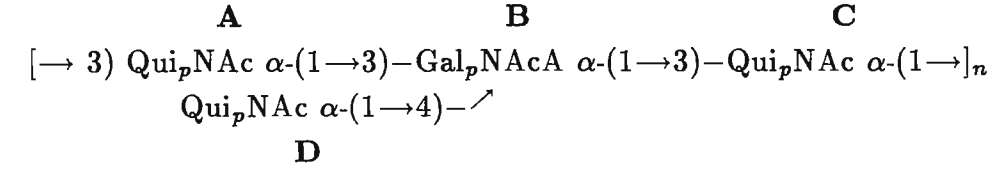
C. Abeygunawardana, G. P. Reddy and C. Allen Bush

Department of Chemistry and Biochemistry
University of Maryland Baltimore County
Baltimore, Maryland 21228

(received 11/23/91)

We have recently reported on a method for determination of the covalent structure of complex bacterial polysaccharides which relies almost exclusively on NMR spectroscopy. The ^{13}C and ^{1}H spectra are completely assigned with a combination of homonuclear and heteronuclear spectroscopy. Values of $^{3}J_{HH}$ are used to determine whether protons are axial or equatorial, thus providing the identity of each pyranoside as well as its anomeric configuration. $^{3}J_{C-H}$ correlations in the HMBC spectra are used to establish linkage positions between monosaccharides. The NMR data are supplemented by carbohydrate analysis by high pressure anion exchange chromatography and circular dichroism to identify absolute configurations of the sugars (Kaluarachchi and Bush, 1989).

We have applied the method to determine the structures of polysaccharides from several strains of oral streptococci which have 6 to 7 residues in the repeating subunit, (Abeygunawardana et al, 1990, 1991a, 1991b). Although the molecular weights of these polysaccharides are about 100 KD, the NMR lines are much narrower than those of a protein of comparable size, presumably due to internal motion of these polysaccharides which have a number of points of flexibility in their sequences which include furanosides, alditols, (1→6)−linkages and phosphodiester bonds (teichoic acid type). Our method would be especially useful if it could be more generally applied to polysaccharides whose glycosidic linkages are more constrained by steric interactions. The T_2 of a less flexible polysaccharide with too little internal motion, might be so short as to prevent observation of the three-bond correlations which are critical to the success of this method. $^{3}J_{HH}$ in pyranosides are 3-12 Hz and $^{3}J_{CH}$ across the glycosidic linkage range from 3 to 7 Hz. We have recently recorded spectra of a capsular polysaccharide from *Vibrio vulnificus* in collaboration with Dr. J. G. Morris of the U. of Maryland Medical School which offer a partial answer to this question. The structure of the polymer is:



This polysaccharide, with its sterically constrained α-(1→3)−glycosidic linkages in the main backbone is expected to be more stiff than those from the oral streptococci and as expected the ^{1}H NMR spectrum at 25° C shows fairly broad lines. But by raising the probe temperature to 60° C , the lines sharpen to 3-4 Hz in width and a very good quality DQF-COSY (Fig. 1) was recorded showing distinct cross peaks for all the vicinally coupled protons. Although it is not possible to extract accurate values of $^{3}J_{HH}$ from the cross peaks, one can easily distinguish those involving *trans* diaxial protons of the pyranosides

(J ≈ 7-11 Hz) from those involving equatorial protons (J ≈ 1-3 Hz). This spectrum in combination with TOCSY, NOESY and HMQC spectra allowed for complete assignment of all ^{1}H and ^{13}C signals. The HMBC spectrum (Fig. 2) showed good cross peaks within the residue which are characteristic of α--pyranosides and all the linkage positions between monosaccharides could be unambiguously determined from the cross peaks between each of the anomeric carbon resonances and those of the aglycone proton. Also some weaker cross peaks were observed between anomeric proton resonances and aglycone carbon atom resonances. We conclude that our three-bond correlation method for determination of structure appears to be applicable to wide range of bacterial polysaccharides.

REFERENCES

- C. Abeygunawardana, C. A. Bush and J.O. Cisar Biochemistry **29**, 234-248 (1990).
- C. Abeygunawardana, C. A. Bush and J.O. Cisar Biochemistry **30**, 6528-6540 (1991).
- C. Abeygunawardana, C. A. Bush and J.O. Cisar Biochemistry, **30**, 8568-8577, (1991).
- K. Kaluarachchi and C.A. Bush Analyt. Biochem. **179** 209-215 (1989).

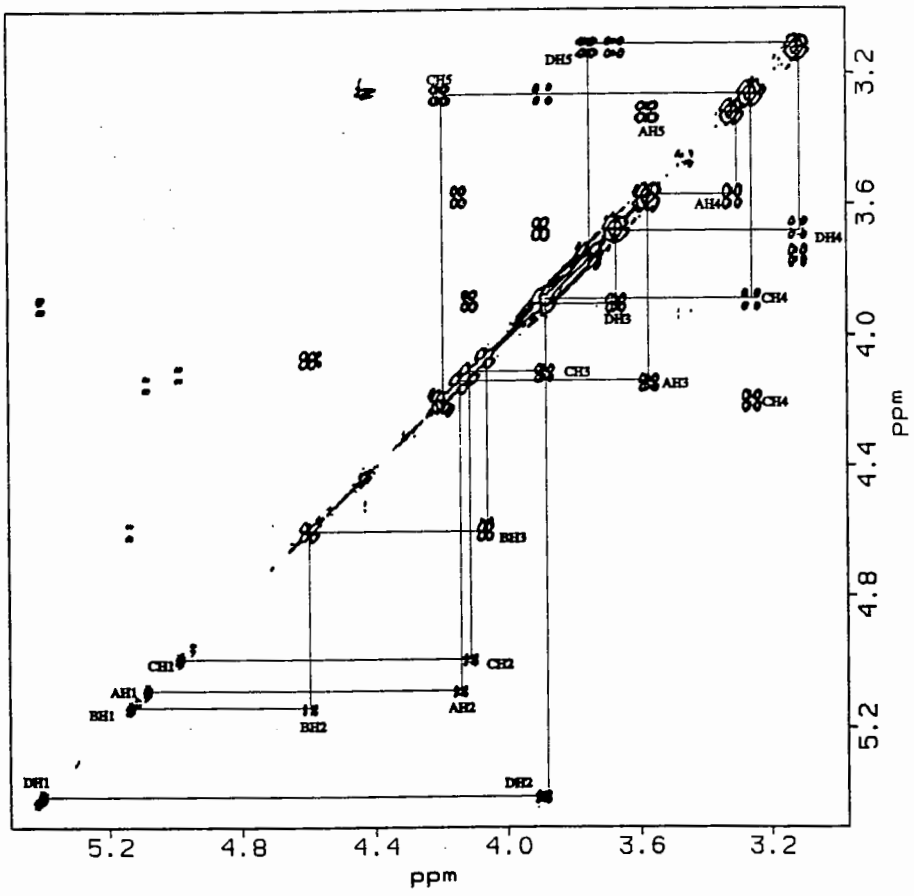


Figure 1. Phase-sensitive double quantum filtered (DQF) COSY spectrum of the polysaccharide from *V.vulnificus* at 500 MHz.

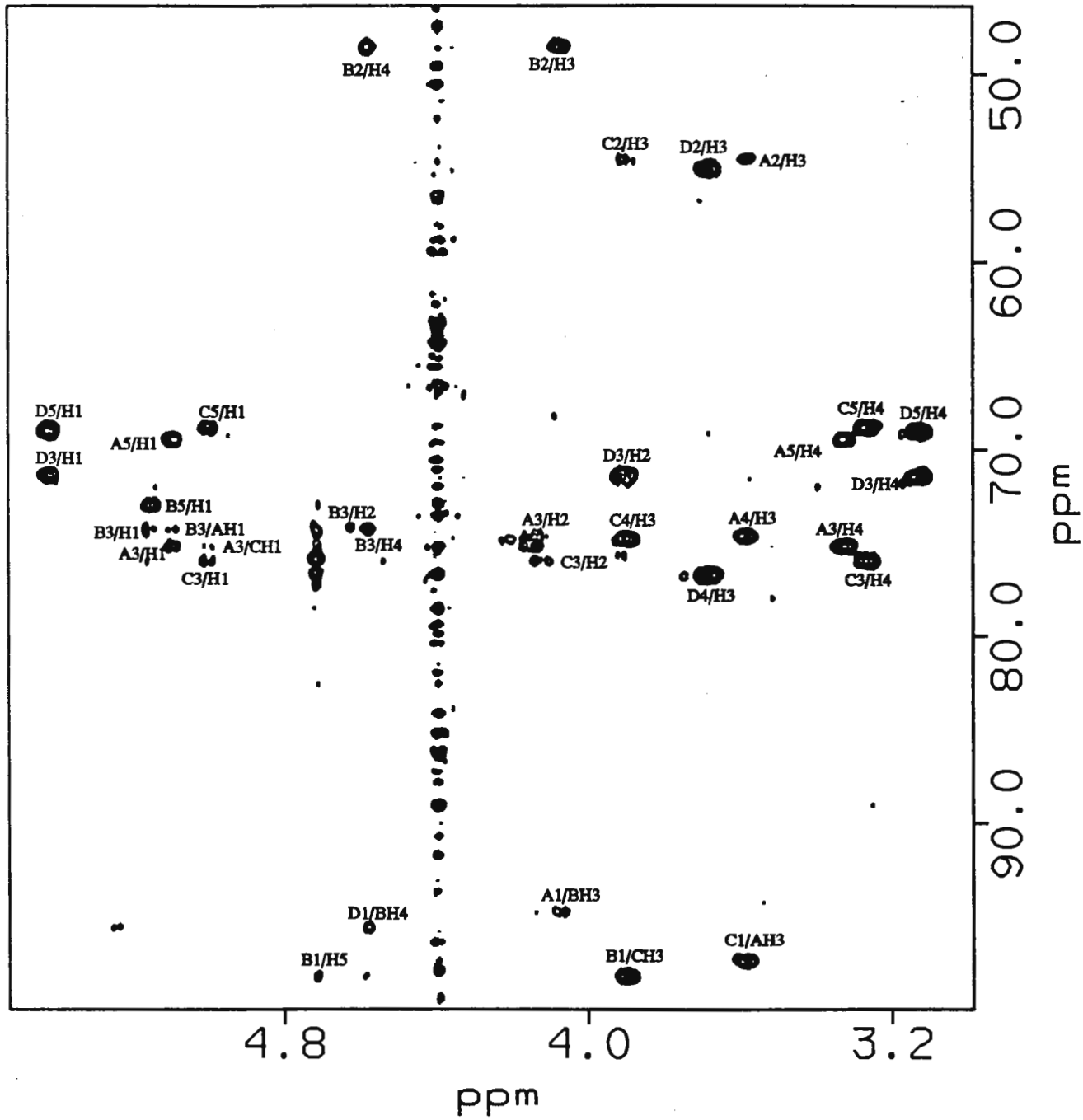


Figure 2 ^{1}H - ^{13}C multiple bond correlation (^{1}H [^{13}C]HMBC) spectrum of *V.vulnificus* polysaccharide at 500 MHz.

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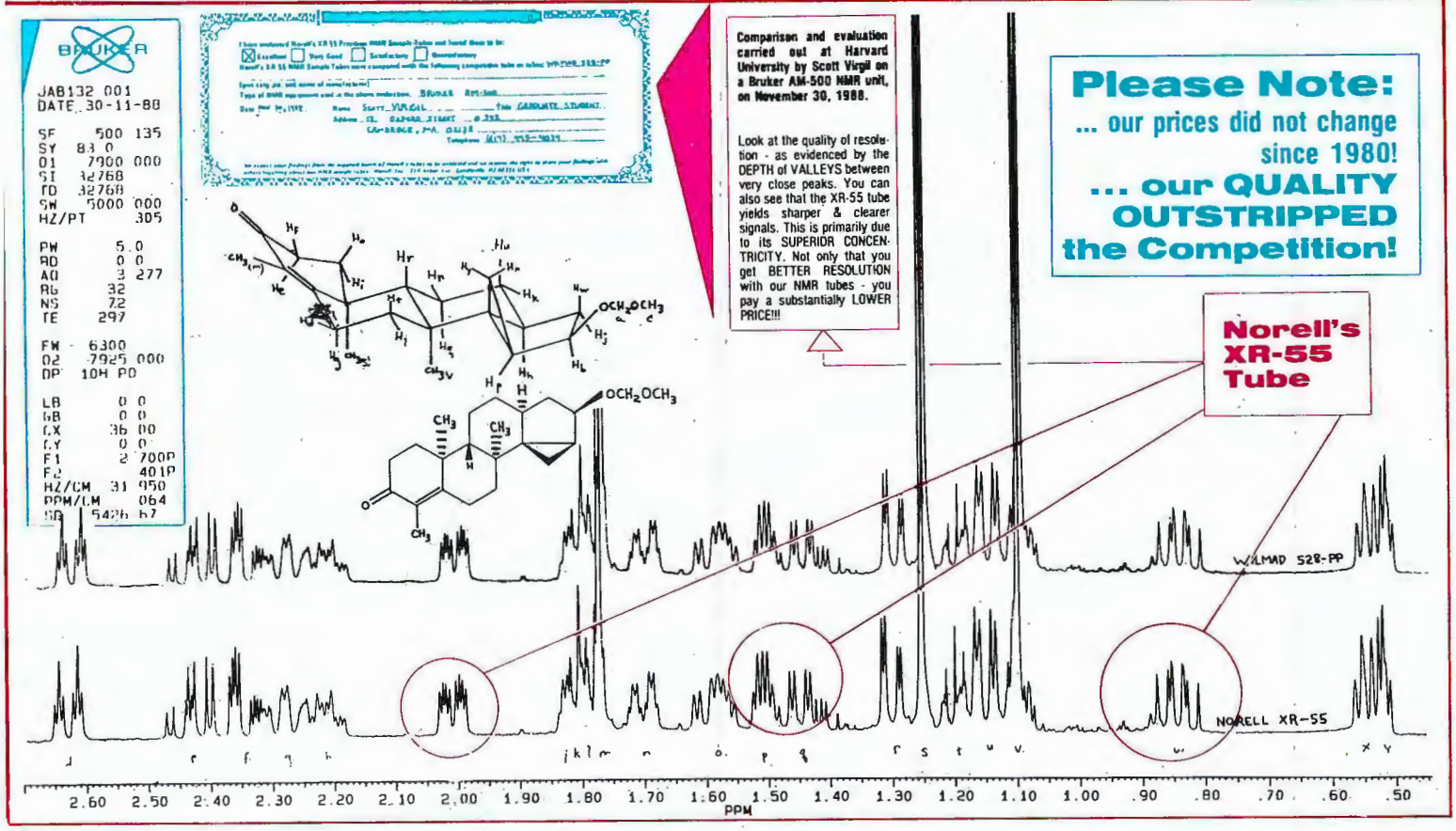

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XV INTERNATIONAL CONFERENCE ON MAGNETIC RESONANCE IN BIOLOGICAL SYSTEMS

August 16 - 21, 1992 Jerusalem, Israel

November 20, 1991
(received 11/30/91)

Dr. Bernard L. SHAPIRO
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto
CA 94303
USA

Dear Dr. SHAPIRO,

re: XV INTERNATIONAL CONFERENCE ON MAGNETIC RESONANCE IN BIOLOGICAL SYSTEMS

Further to our previous letter of August 27, 1991, we would like to bring the change of address of the secretariat to the attention of your readers.

I would appreciate it if you could include the following announcement in your journal:

NAME: XV International Conference
on Magnetic Resonance in Biological Systems

DATE: August 16 - 21, 1992, Jerusalem, Israel

DEADLINE FOR RECEIPT OF ABSTRACTS:

FOR FURTHER INFORMATION: Prof. Gil Navon
P.O. Box 50006
Tel Aviv 61500, Israel

Tel: 972 3 5174574; Fax: 972 3 655674

Yours sincerely,

Prof. Gil Navon
Conference Chairman

Professor Bernard L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto CA 94303
U. S. A.

20 November 1991
(received 12/7/91)

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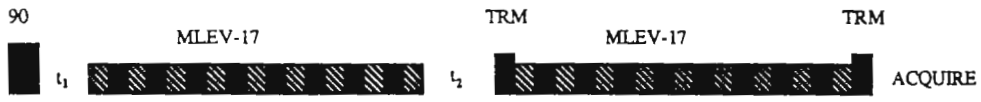
Department of Chemistry

Big Molecules on Small Instruments: Using the 3-D HOHAHA-HAHAHO to Delineate Spin Systems in Biomolecules on Mid-Field NMR Instruments

Dear Professor Shapiro:

We have developed what we are calling the "3-D HOHAHA-HAHAHO" experiment for delineating proton through bond connectivities. It essentially involves replacing the preparation pulse of a standard 2-D HOHAHA experiment with another 2-D HOHAHA pulse sequence as is shown below.



HOHAHA-HAHAHO

An analogous 3-D TOCSY-TOCSY pulse sequence has been reported by Cieslar et al (1). We have sucessfully implemented the 3-D HOHAHA-HAHAHO pulse sequence and wish to illustrate its utility with data collected on a 31-residue peptide on our GE OMEGA 300/PSG NMR. The experimental results nicely demonstrate the power that 3-D experiments can bring to mid-field NMR instruments for the purpose of improving spectral dispersion for biological macromolecules. In fact, we believe that 3-D homonuclear experiments will make it possible to unambiguously determine the structure of relatively large proteins and peptides from experimental data acquired on mid-field instruments—data which previously could only be obtained with 2-D experiments on high-field instruments.

The data set was acquired in DMSO on the 31-residue peptide (SD-DDWIPDIQTDPNGLSFNPISDFPDTTSPK) which constitutes the binding domain for prekallikrein in high molecular weight kininogen (2). This data set consisted of 96 t_1 and 96 t_2 points (9,216 total experiments). The spin lock periods for the two HOHAHA sequences were 40 and 45 ms respectively; spin lock field strengths were 8 kHz. Each (t_1,t_2) point consisted of 40 transients acquired over a 4 kHz sweep width with a 0.7 s repitition rate for a total acquisition time of 90 hrs. Quadrature was acheived in the F_1 and F_2 frequency domains using hypercomplex phase cycling (3). A homospoil pulse was inserted at the beginning of the pulse sequence to destroy x-y magnetization remaining after each acquisition. This allowed us to cut our total acquisition time by at least a factor of two. Data were processed with FELIX 2.0 (Hare

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Research, Bothell, WA) for phase sensitive display with cosine bell weighting functions and zero filling in the t_1 and t_2 dimensions to yield a 512 x 512 x 512 data matrix.
In Figure 1 we show a contour plot of the NH $C_\alpha H$ region from a standard 2D HOHAHA data set acquired at 300 MHz. From this figure it is clear that the resonance overlap is severe enough that it would be difficult, if not impossible , to obtain accurate chemical shifts for many of the structurally important $C_\alpha H$ resonances. The elongation of the highlighted cross peak in the F_1 dimension is indicative that there are two amino acid spin systems which have nearly degenerate chemical shifts for both their NH and $C_\alpha H$ resonances. This degeneracy would make it impossible to obtain unambiguous side-chain resonance assignments for these amino acids. In the 3-D data set, however, spectral dispersion is significantly improved. This is clearly indicated in the 2-D slice (Figure 2) extracted from our 3-D data set. This F2,F3 slice was taken through a single F1 point corresponding to the amide resonance marked by the box in Figure 1. Figure 2 shows that we can easily obtain accurate chemical shifts and unambiguous through-bond correlations for all of the resonances in these two amino acid spin systems. The autopeaks in this slice correspond to the NH–aliphatic crosspeaks in a conventional HOHAHA experiment and arise from magnetization transfers in the second incrementable delay which are coincident with those in the first incrementable delay, whereas the crosspeaks in this slice arise from magnetization transfers during the second incrementable delay which are distinct from those in the first. Much of the improvement in spectral dispersion arises from these additional off-diagonal crosspeaks in the 3-D spectral volume.

(1.) Cieslar, C.; Holak, T. & Oshkinat, H. *J. Magn. Reson.* **1990**, 89:184.

(2.) a) A 20 mg sample of the highly purified sequence homogeneous synthetic peptide was provided by Prof. Robert B. Harris, MCV-VCU;
b) Tate, J.F. & Fujikawa, K. *J. Biol. Chem.* **1987**, 262:11651.

(3.) States, D.; Haberkorn, R. & Reuben D. *J. Magn. Reson.* **1982**, 48:286.

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David Rovnyak
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University of Richmond

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Biochem. & Mol. Biophys
MCV-VCU, Richmond, VA

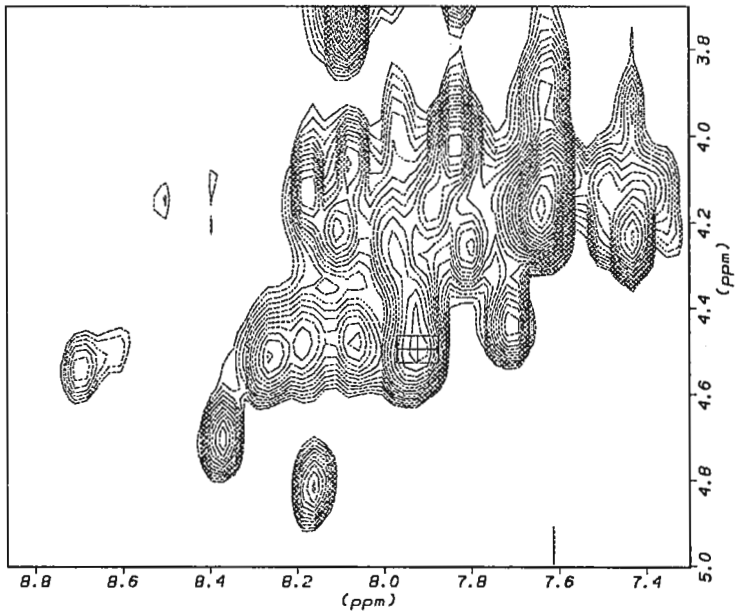

Figure 1: Contour Plot of the NH,C_αH region from a HOHAHA experiment on the 31-residue peptide. The severe resonance overlap would make unambiguous assignment of many of the structurally important C_αH,NH crosspeaks difficult if not impossible. In addition, this severe overlap would hinder delineation of amino acid spin systems by preventing unambiguous assignment of through-bond connectivities from these NH,C_αH crosspeaks to side chain resonances.

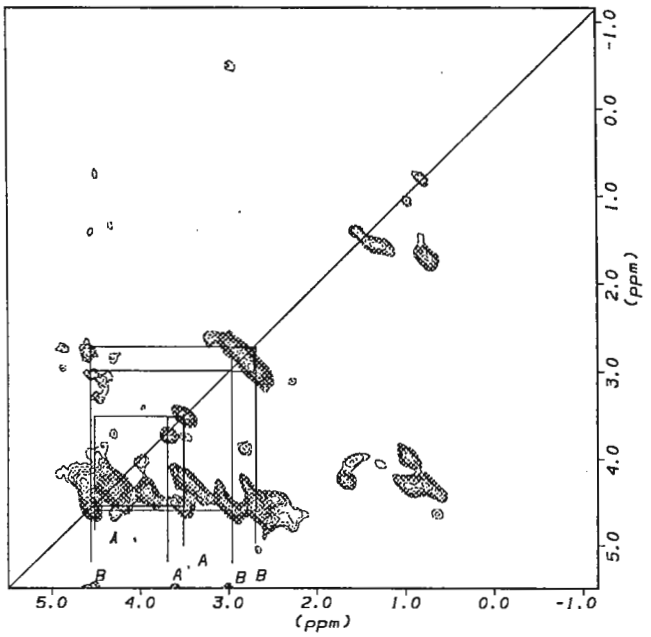

Figure 2: Contour plot of aliphatic,aliphatic region from an F2,F3 slice from the HOHAHA-HAHAHO data set. This slice was taken through a single F1 point corresponding to the highlighted amide resonance in Figure 1. From this slice, it is clear that two amino acids have nearly degenerate NH and C_αH chemical shifts. This degeneracy would have made delineation of the two amino acid spin systems impossible in the conventional 2D HOHAHA experiment. Even in this worst case scenario, the 3D HOHAHA-HAHAHO permits the determination of accurate chemical shifts for the NH and C_αH resonances from these two amino acids as well as the unambiguous deliniation of both spin systems which we have labeled **A** and **B**.

Gradient Enhanced Spectroscopy SWAT

GE introduces the use of Switched Acquisition Time (SWAT) gradients to achieve pure phase 2D spectra with quadrature detection in both the acquisition (ω_2) and evolution (ω_1) dimensions without any phase cycling and without an additional set of t_1 data.

One example of a pure phase gradient enhanced COSY spectrum of a solution of 2,3-dibromopropionic acid in benzene-d6 is shown in Fig. 1. SWAT gradients and a single acquisition per block were used. Data was collected on an Omega 300WB with Microstar actively-shielded gradients. A 5mm inverse probe was built for use within the gradient coils.

Digital resolution of 1.2 Hz in ω_1 and 2.4 Hz in ω_2 was achieved by collection of a 512 x 512 matrix with t_1 evolution time of 840ms and a t_2 acquisition time of 420ms. A single acquisition per t_1 evolution data block and an average recycle time of 1.84s resulted in a 15 minute total collection period.

Since the SWAT gradient method encodes the necessary information in a single t_2 acquisition time, it avoids the collection of additional data blocks required by traditional pure phase methods. This time efficiency is especially important for collection of large multidimensional data sets.

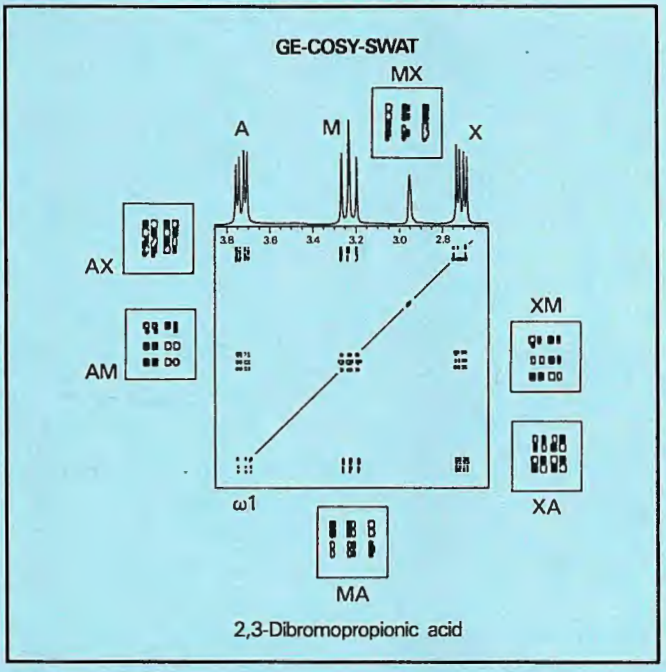

Fig. 1

Contour plot of a 300 MHz pure-phase COSY spectrum of a solution of 2,3-dibromopropionic acid in benzene-d6 acquired with only a single acquisition per t_1 evolution time increment using the GE-COSY-SWAT method. Cross peaks are shown in expanded insets with positive peaks as darkened contours and negative peaks as open contours. A one dimensional spectrum is plotted across the top of the 2D spectrum.

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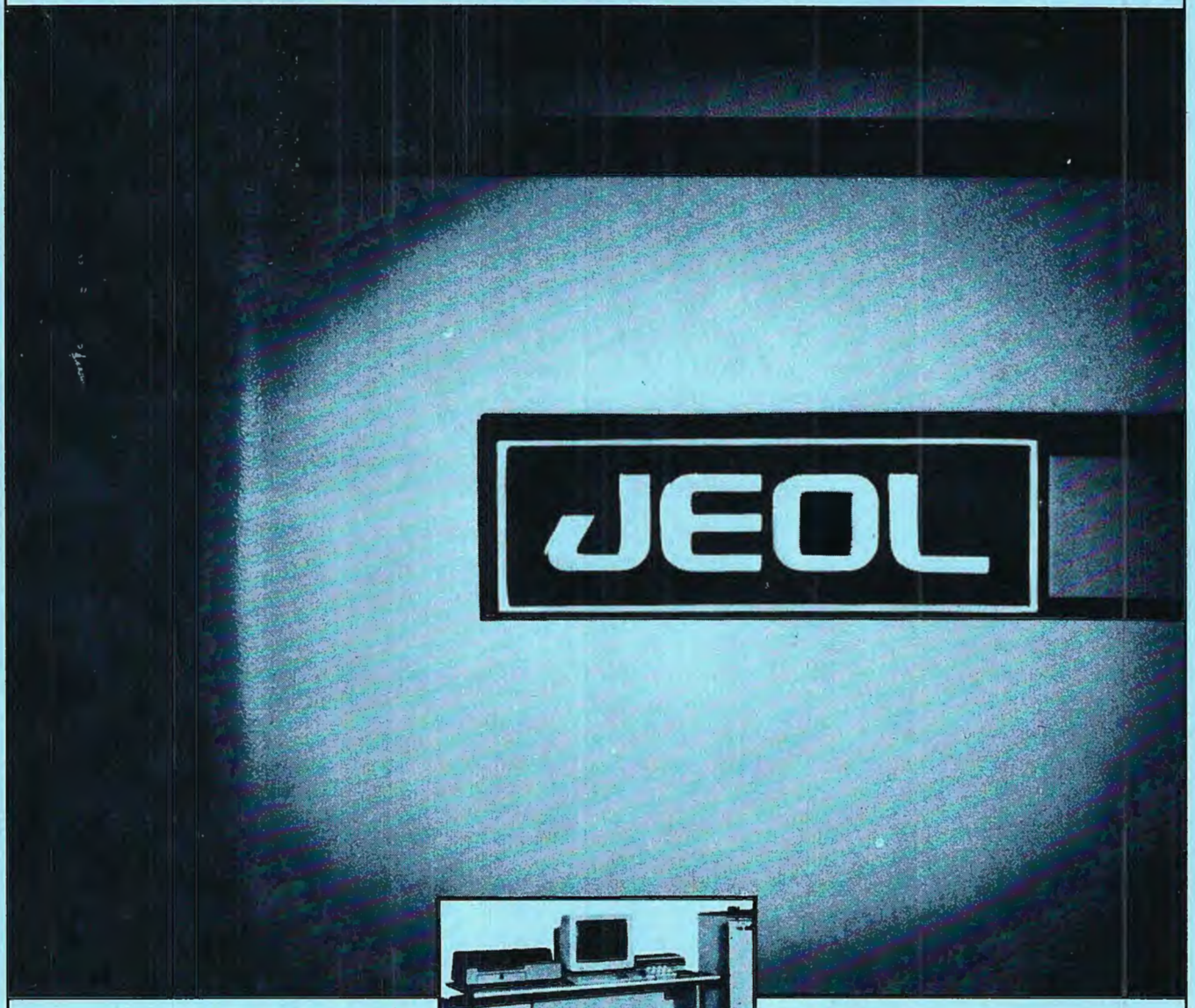
Of all the advanced technology on this research-level NMR, this may be the most important piece of hardware.



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