TEXAS A&M UNIVERSITY

NMR NEWSLETTER

No. 294

March, 1983

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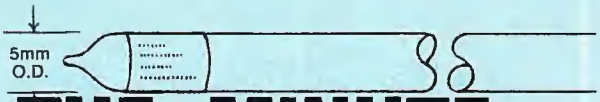

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All Newsletter Correspondence, Etc., Should be Addressed To:

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February 7/1983

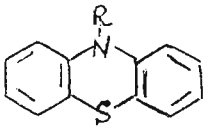
Professor B.L. Shapiro
Department of Chemistry
Texas A&M University
College Station, Texas 77843

Dynamic Aspects of Phenothiazines in solution.

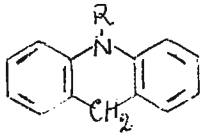
Dear Barry,

Although quite a number of papers have been devoted to studies of phenothiazines in the solid state, and the importance of mobility in drug/receptor interactions is now acknowledged, the dynamic features of phenothiazines in solution have not been systematically investigated. The librational motion originating from N-inversion and ring-inversion processes in tricyclic molecules with "butterfly" conformation, like phenothiazine I, was previously found by some of us to be fast at temperature higher than 60°C. However the population of the extra isomer increases significantly when the ligand at nitrogen is heavier than proton (i.e. R = methyl or alkyl chain).

We have now studied the capacity of the nitrogen lone pair to delocalize into the tricyclic system related to phenothiazine and N-acetylphenothiazine Ia,b, i.e. acridane II, phenoxazine III and 2,3-diazaphenothiazine IV, by using as a probe the ^{13}C chemical shifts and anilines as models. The preferred conformation of the N-acetyl group has been found to be extra for all the tricyclic systems with "butterfly" shape. Thus the nitrogen lone pair delocalization of the N-acetyl group into the aromatic rings is strongly hindered for all tricyclic compounds, and greatly reduced also for N-acetyldiphenylamine.



Ia R = H
Ib R = $COCH_3$



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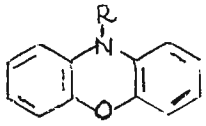

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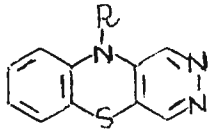
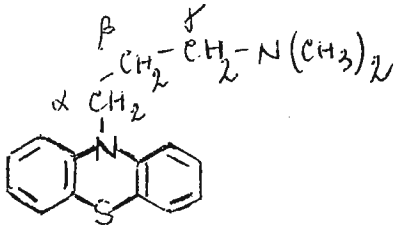
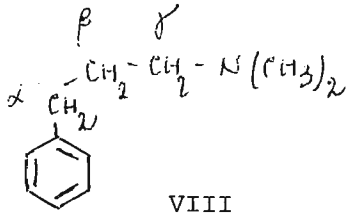


IV

We have then studied the conformation and the mobility of the chain at the thiazine nitrogen for some promazines with antipsychotic activity V-VII and for the model compound N,N-dimethyl-3-phenylpropylamine VIII.



V promazine
VI chloropromazine
VII acepromazine



VIII

^{13}C T_1 relaxation times have shown that the mobility of the dimethylaminopropyl chain in $CDCl_3$ solution varies depending on whether the chain is protonated or unprotonated, and whether is attached to a phenyl or to a tricyclic system. Segmental motion was only detected in unprotonated promazines and in the model VIII hydrochloride. The results indicate that the motion is reduced by the tricyclic ring as much as by the ionic site.

The conformation of the side-chain has been deduced for different solvents. Protonated promazines show as preferred conformation the gauche forms for the $C_\alpha - C_\beta$ fragment and the trans for the $C_\beta - C_\gamma$, in $CDCl_3$ as well as in water solution; whereas the model compound VIII exists preferentially in the fully extended trans-trans conformation. The unprotonated promazines instead do not show any conformational preference.

These results differ from those reported in the literature for the solid state and also for the solution they disagree with the theoretical predictions. The most interesting result is the preference for the α,β -gauche forms of the three salts. The presence and the nature of the 2-substituent, which is so important for the biological activity, does not appear to have any effect, whereas the tricyclic system is determinant for the stabilization of the α,β --gauche form. These conformation seems more stable when the chain is attached to the phenothiazine than to the dihydrodibenzazepine system, as it appears from the results in D_2O solution[1].

The stabilization of the α,β -gauche form can be related to the ability of promazine salts to aggregate in water as well as in $CDCl_3$ solution with a vertical stacking-type association. In this structure the arrangement of the chain in α,β -gauche conformations allows a better distribution of the polar heads, thus decreasing the electrostatic interactions. This is proved by the changes in N values observed for DMSO and dioxane solution. These hydrophylic solvents can be involved in H-bonding with NH^+ and are expected to have a disaggregating effect. The interaction between solvent molecules and the alkyl chain, disturbing the vertical-stacking aggregation to form more complicated equilibria, results in a destabilization of the α,β -gauche forms.

Example of T_1-relaxation times values of the side-chain carbon atoms[a]

	CH_2-α	CH_2-β	CH_2-γ	$N(CH_3)_2$
V	1.06 ± 0.02	1.21 ± 0.02	1.54 ± 0.03	1.62 ± 0.05
V,HCl	0.34 ± 0.01	0.41 ± 0.06	0.46 ± 0.04	1.03 ± 0.02
VIII	3.59 ± 0.05	3.54 ± 0.07	3.37 ± 0.06	2.44 ± 0.03
VIII,HCl	1.09 ± 0.02	1.00 ± 0.02	0.79 ± 0.01	1.15 ± 0.03

Example of the conformational analysis for the side-chain in promazines[b]

		$CDCl_3$			D_2O			Dioxane			DMSO		
		N	ΔE	trans %	N	ΔE	trans %	N	ΔE	trans %	N	ΔE	trans %
VI	C_α-C_β	12.2	-0.97	9	12.2	-0.97	9	13.0	-0.30	23	13.9	0.15	39
VI,HCl	C_β-C_γ	16.0	1.13	76	16.2	1.25	79	15.5	0.86	67	15.5	0.86	67

a) 0.5 M $CDCl_3$ solution. The correlation coeff. are in the range 0.997-0.999.
b) The analysis was carried out following the approach of Abraham et al.[1]

1) R.J.Abraham, L.J.Kricka, A.Ledwith, J. Chem. Soc. Perkin II, 1974, 1648.

Our papers shall appear in J. Chem. Soc. Perkin II.

Sincerely yours

G.Fronza E. Ragg R.Mondelli

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Prof. B.L.Shapiro
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January 31, 1983

TITLE: NOE and X-Pro
Peptide Bond

Dear Barry,

In the course of NMR conformational study of the 35-membered polypeptide insectotoxin I_5A, isolated from *Buthus eupeus* Caucasian scorpion venom, it became important to evaluate the configuration of the X^{i}-Pro^{i+1} amide bond. There are three prolyl residues in the molecule, that are in the fragments Met^{3}-Pro^{4}, Asp^{9}-Pro^{10} and Gly^{28}-Pro^{29}.

By direct structural consideration the following interproton distances are estimated depending on the ψ angle rotation in the i-th residue:

X-Pro	$H^{\alpha}_{i} \cdots H^{\alpha}_{i+1}$	$H^{\alpha}_{i} \cdots H^{\delta}_{i+1}$
trans	43-48nm	29-39nm
cis	22-38nm	43-50nm

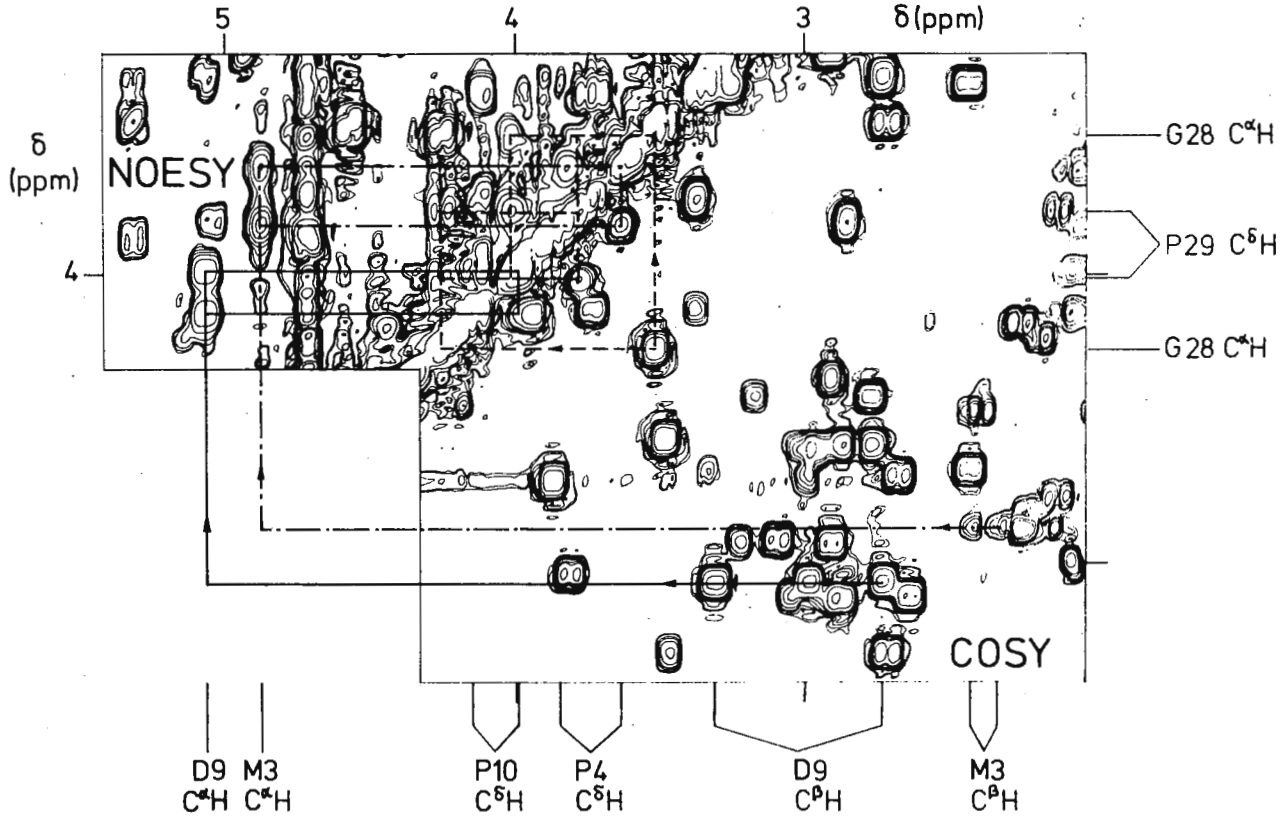
Under employed 2D-NMR NOESY experimental condition (mixing time 100 ms) we have observed NOE cross peaks for protons separated by less than 30-35nm. For instance, low intensity peaks were observed for the H-N-C^{α}-H fragments where the maximal interproton distance is 30nm.

As shown in the Figure only NOE cross peaks for the $C^{\alpha}_{i}H$, and $C^{\delta}_{i+1}H_2$ protons were observed for all three prolyl residues, which is compatible with the *trans* configuration of all three X-Pro bonds in the insectotoxin.

The Fugure represents combined Bruker WM-500 MHz COSY-NOESY diagram (ω_1 3.3-5.4ppm, ω_2 2.0-5.4ppm) for 0.011 M solution in D_2O at pH 2.9 and 30°C. Connections indicate diagonal and cross peaks assigned to the $C^{\alpha}_{i}H$ and $C^{\delta}_{i+1}H_2$ protons in the Met^{3}-Pro^{4} (—·—), Asp^{9}-Pro^{10} (———) and Gly^{28}-Pro^{29}

(- - -) fragments. Their chemical shifts are shown on the diagram margins. The assignment as indicated by arrows was started from the J-peaks in the COSY diagram for $C^{\alpha}H$ and $C^{\beta}H_2$ of the i-th residue in case of Met^{3} and Asp^{9}.

Vladimir Bystrov Alexander Arseniev Vladimir Kondakov



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23 February 1983

Professor B. L. Shapiro,
Texas A and M NMR Newsletter,
College Station, Texas 77843,
U. S. A.

Dear Barry:

UNRAVELLING MINOR COMPONENTS FROM DEUTERIUM POWDER SPECTRA

The ^2H NMR spectra of powders are made up of quadrupole doublets due to the various angles between the magnetic field and the principal axis of the quadrupole splitting tensor, see the left side of the Figure. Their overlapping can obscure, or make difficult the measurement of, patterns due to minor components in the system. Myer Bloom and his coworkers have developed a cute method to reduce the spectrum to the components due to one particular angle (1), which they have dubbed "de-Pake-ing". We have found it to be extremely useful in our studies of biological membranes, and cite a particularly impressive example below.

The Figure shows the powder (left) and "de-Pake-ed" (right) spectra of dispersions of the membrane lipids of the microorganism Acholeplasma laidlawii. The lipids are enriched at the sn-2 position of glycerol with a cyclopropane-containing fatty acid, dihydrosterculic acid, labelled at C-2 with two deuterons. The upper left spectrum shows some fine structure, but the "de-Pake-ed" spectrum in the upper right shows dramatically the presence of at least four different quadrupole splittings. Two splittings are expected due to the magnetic inequivalence of the two deuterons (2); the extra splittings of lower intensity are possibly due to minor lipid components of the membrane with the same fatty acid content but different headgroup. Substantiating evidence for this view comes from the lower intact and "de-Pake-ed" spectra of the isolated, major lipids of the membranes. The minor components are absent. We have found this behaviour in membranes enriched in other fatty acids as well, but so far only for the C-2 position.

The "de-Pake-ing" method is extremely useful, but must be used with some care. It assumes an axially symmetric tensor whose principal interaction scales as $P_2(\cos\theta)$. We are very grateful to Myer Bloom, Jim Davis, and Alex Mackay for showing us how to do this, and for a copy of their computer programme. More details of the present example will appear in print soon (3).

Yours sincerely,

Ian

Ian C. P. Smith

Harold

Harold C. Jarrell

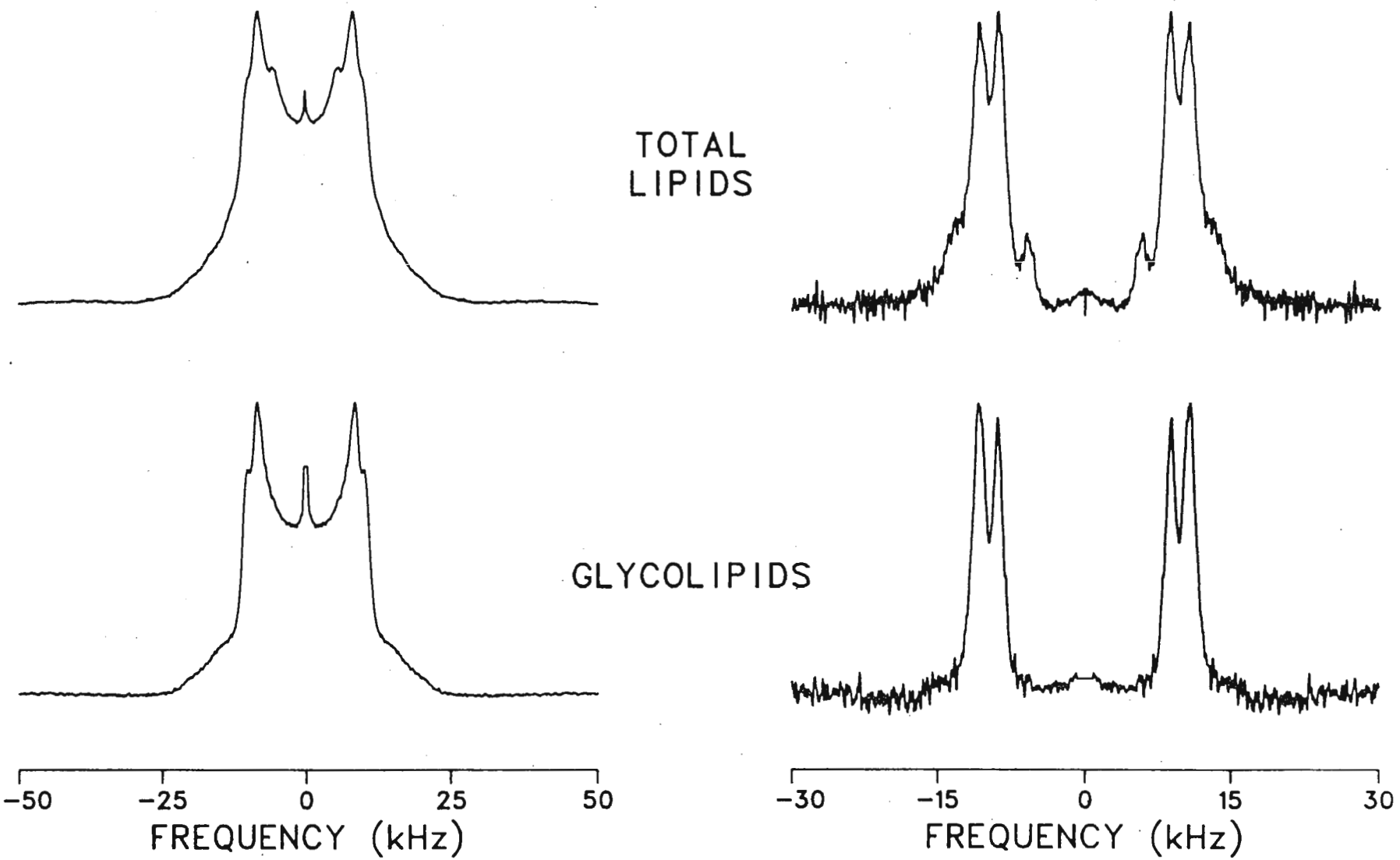
Mark Rance

1. M. Bloom, J.H. Davis, and A.L. Mackay, Chem. Phys. Lett. 80, 198 (1981).

2. A.K. Engel and D. Cowburn, FEBS Lett. 126, 169 (1981).

3. M. Rance, I.C.P. Smith, and H.C. Jarrell, Chem. Phys. Lipids (1983, in press).

Figure legend. Powder and "de-Pake-ed" ^2H NMR spectra (30.698 MHz), 25°C, of aqueous dispersions of the total extracted lipids, and of the glycolipids, from A. laidlawii enriched in [2-$^2H_2$] dihydrosterculic acid.


TOTAL
LIPIDS
GLYCOLIPIDS
−50
−25
0
25
50
FREQUENCY (kHz)
−30
−15
0
15
30
FREQUENCY (kHz)

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Until recently, NMR spectral throughput was dependent upon the sensitivity limits of the NMR spectrometer hardware. For example, ten years ago a simple carbon spectrum could take hours to collect. Nowadays, that same experiment takes only several minutes (See Figure 1). Hence, the profusion of routine and complex data now being generated by modern, commercially available NMR spectrometers has placed an ever increasing demand for high quality spectral throughput.

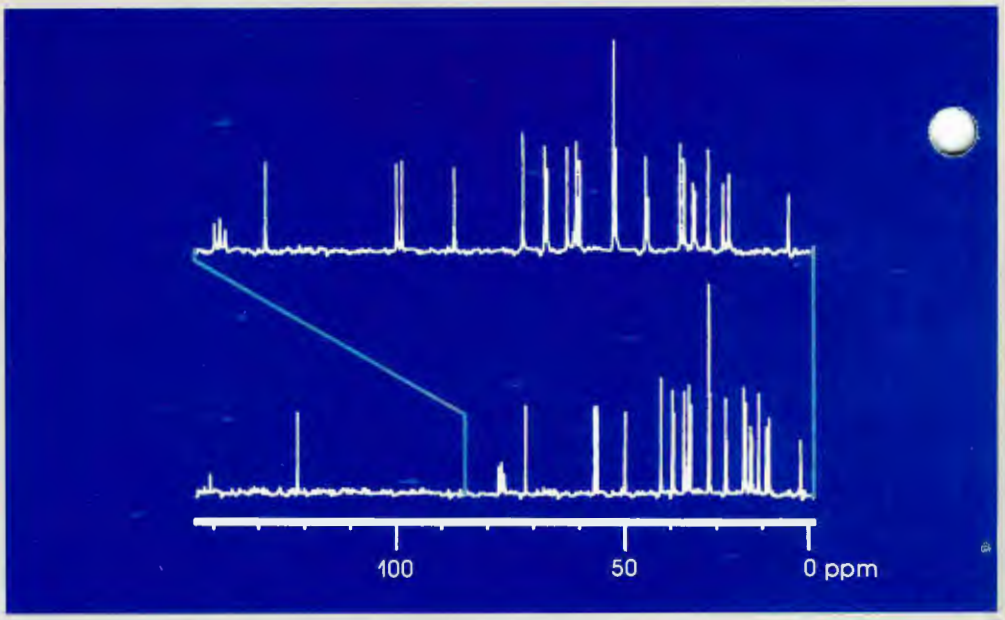


Figure 1. Carbon-13 spectrum of 5 mg. of cholesterol, acquired in five minutes at 50.0 MHz using the *MICRO-TILT PROBE* especially designed for high S/N when sample amounts are limited.

The Throughput Dilemma

In many cases, a spectroscopist can collect spectral data at the same rate or faster than the data can be processed and output. Continuing advances in NMR signal detection and high sensitivity probe design have created a "throughput dilemma."

The typical single operator/single terminal data system available in most NMR spectrometer systems cannot efficiently handle such fast rates of data acquisition.

In a typical single access scenario, operators must wait in line in order to collect data from that single terminal. One by one, each operator takes his or her turn at the instrument, places a sample in the probe, collects data and finally works up the results for hardcopy output.

Any operation (such as FFT, integration, printing and plotting of spectra) which takes place after spectral accumulation is data system intensive. However, they all cause unnecessary and costly dead time on the system. The spectrometer cannot be utilized in any matter until the operator currently using it completes his or her work and allows the next operator in line to have physical access to the instrument.

This scenario, of a typical single terminal instrument, effects a tremendous waste of valuable time. The waste is magnified when the cost of commercially available NMR systems is considered.

To solve the throughput dilemma, the most efficient and logical recourse would be to increase operator access to the spectrometer thereby eliminating costly dead time. In other words, the answer is the PLEXUS solution.

The PLEXUS Solution

The GX Series of FT NMR spectrometers with the PLEXUS data system, offers multiple user access to maximize operator interaction and data throughput. JEOL has achieved the unique position of offering multi-terminal spectrometer systems by incorporating the unsurpassed expertise of DEC hardware. The GX multiple-user access systems include a DEC LSI 11/23 microprocessor with the RSX multi-terminal/multi-tasking operating system, and a 32-bit word, high speed "NMR processor." This data system package yields the uncompromising speed and efficiency demanded by today's sophisticated NMR market.

The advantages of a multi-terminal/multi-tasking NMR spectrometer are numerous as well as obvious. When an operator is actively collecting data and maintaining privileged control of the spectrometer, it is possible for other operators to manipulate previously accumulated data (e.g., process two-dimensional spectra, write pulse programs or request FFT operations on two or more different sets of data). A typical three-terminal PLEXUS system is illustrated in Figure 2 below.

Here Terminal One is being used to collect and display a Free Induction Decay (FID). This implies temporary spectrometer control by Terminal One. Concurrently, Terminal Two is involved with data manipulation of a previously accumulated data set. The data set could have been accumulated minutes before on Terminal Two or weeks before and read from a disc. Terminal Three is being used for pulse program editing.

Consequently, the often time-consuming job of writing novel pulse sequences or experimental menus can be achieved without

Figure 2. Two JEOL graphics terminals and one DEC terminal being used simultaneously, with the RSX-11M Multi-terminal/Multi-tasking operating system.

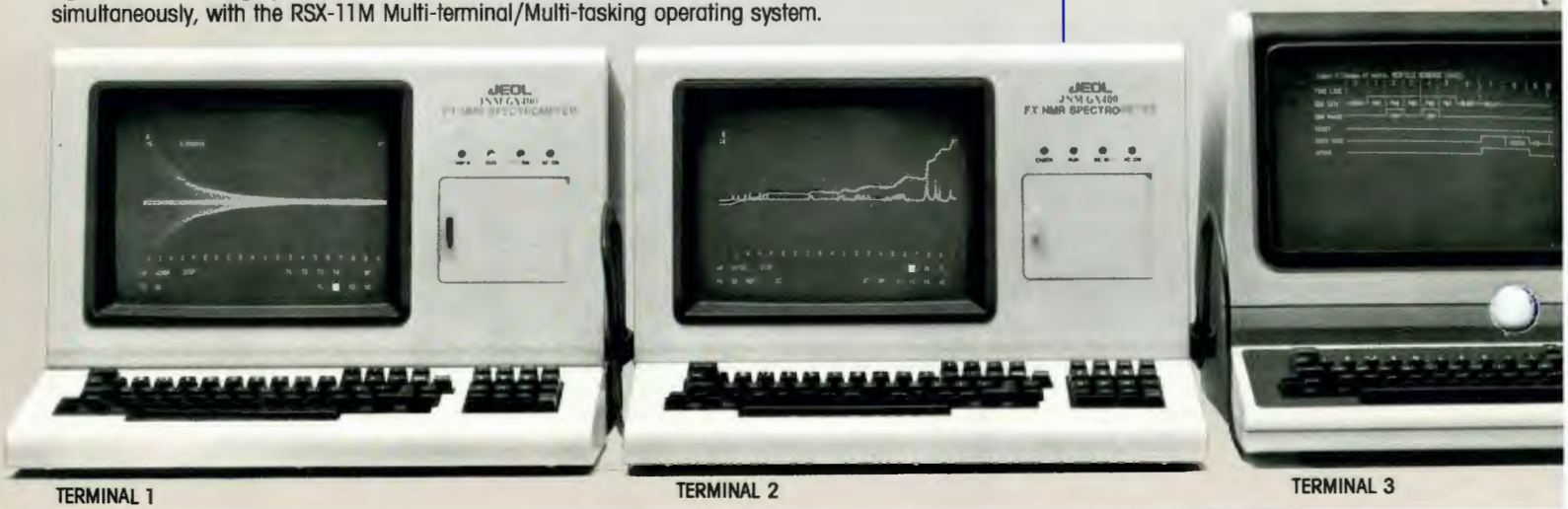


Figure 3. Redfield water suppression pulse program generated with PEGS (Pulse Editing Graphic Software).

taking up valuable instrument time. In short, the activities of Terminal One, Two, and Three can be accomplished simultaneously with only one spectrometer and data system.

Advantages Over Conventional NMR Systems

The operational configuration of a multi-terminal system has distinct logistical benefits over non-instrument interactive satellite stations. First of all, in a multi-terminal system, there is no time wasted by the transfer of data through a RS-232 interface or by physically moving a disc from the spectrometer to a work station. Secondly, there is no difference in software from one terminal to another; since the entire data system uses a one multi-terminal monitor program.

Thirdly, and most importantly, each JEOL graphics terminal has potential control of spectrometer operation. This fact alone eliminates the bottleneck of single-user interaction as shown in the maximized throughput scheme of Figures 4a and 4b. The throughput of a multi-terminal system is double that of a single terminal spectrometer without a satellite station. When compared to a single terminal system with a satellite station, the throughput of a multi-terminal station is still faster. Under the best of circumstances, transfer time is usually several minutes for each spectrum. When large data sets, such as 2-D data, are relocated, transfer time may approach one-half hour.

A further benefit (and one that shouldn't be overlooked), is a psychological benefit. In a multi-terminal set-up, each operator at the spectrometer remains at one terminal for the full duration of the experiment. This continuity allows for greater concentration on each job without the distraction of moving from the spectrometer to a satellite station for final data processing.

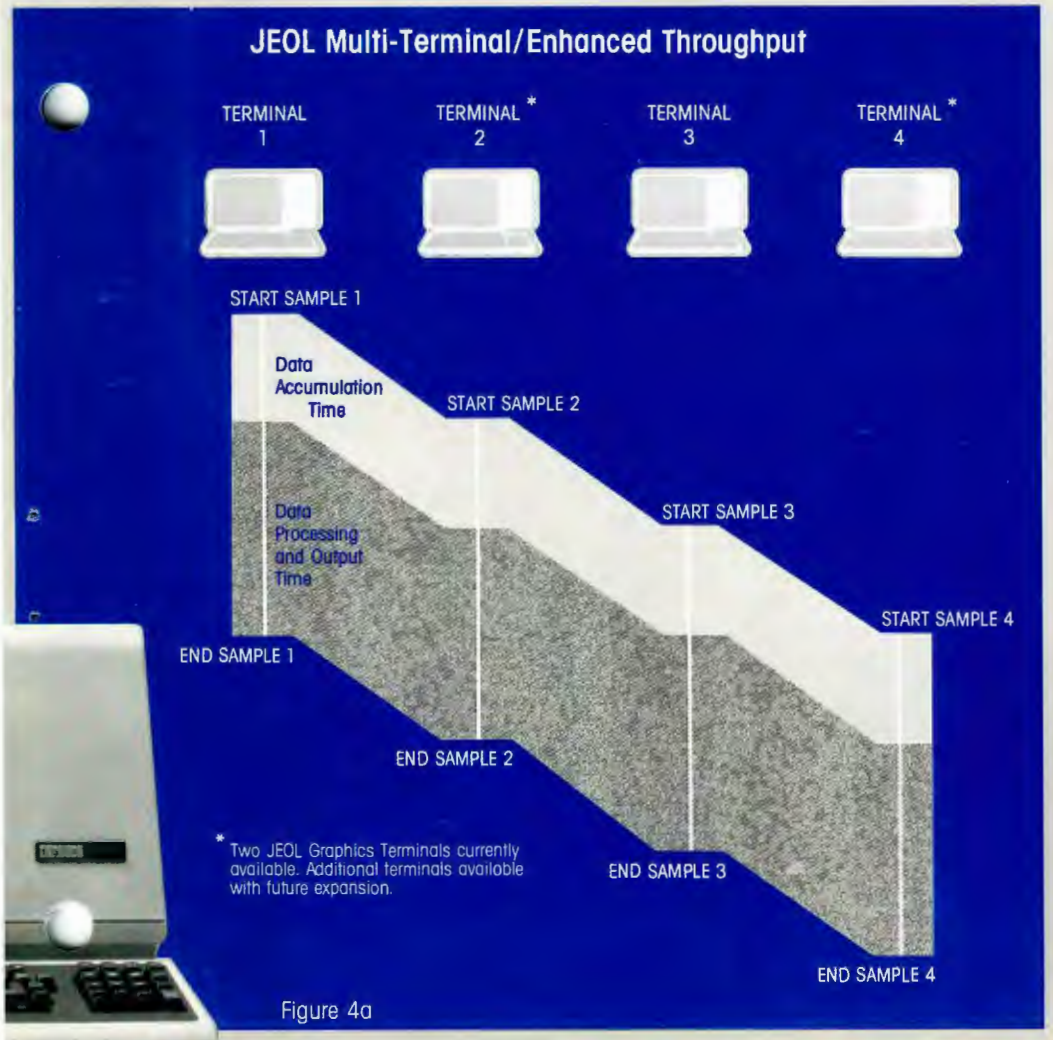
RSX Operating Systems

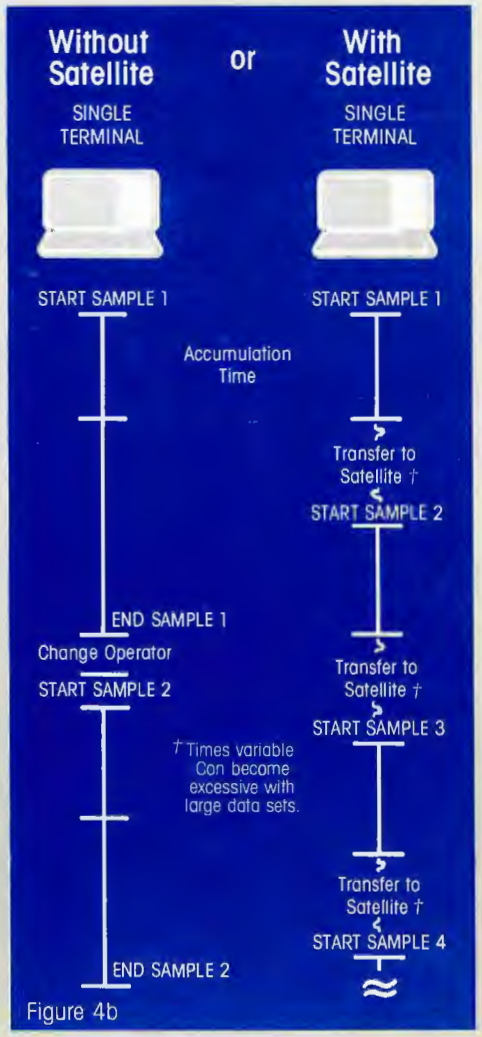
RSX systems allow realtime activities to execute concurrently with less-time-critical activities. Through priority-based scheduling, the assigned priority and activities of a task determine the level of service it needs. With an RSX system, each terminal can operate independently of others in the system. That way, each terminal can run a different task and each can run more than one task.

In addition, RSX systems are highly reliable. They feature data integrity and increased system availability. For example, in a multi-user/multi-programming environment, the LSI-11 micro-computer processor provides protection as well as multiple access.

Multi-Programming

Multi-programming is the simultaneous execution of two or more tasks that reside in memory. Since task execution usually involves more than the central processor unit (CPU), multi-programming is feasible. For example, a realtime task that initiates a procedure and then waits for the completion of the procedure, may not need access to the CPU while it is waiting. Therefore, with multi-programming





Figures 4a and 4b. Time and motion schematic of enhanced throughput of Multi-terminal/Multi-tasking NMR system vs. conventional configurations.

PLEXUS data system equipped with two JEOL graphics terminals and one DEC terminal.

capability, while one task waits for an event to finish, the control of the CPU can be given to another task. Tasks are multi-programmed by logically dividing available memory into a number of named positions. The transition of task control occurs so rapidly, it appears that many individual users have control of the CPU at the same time.

The System for Today and Tomorrow

Throughput is just one of the many problems confronting today's spectroscopists. The growing field of NMR spectroscopy has created new demands and the GX series meets those demands . . . head on.

Pulse Programming

Advances in pulse programming techniques have opened up new areas in NMR research. JEOL has developed the PGX-300 multi-pulse programmer and incorporated it into the GX series to give NMR users the ultimate in spectrometer control and programming flexibility. A few of the PGX-300's specifications are: 2048 programming steps, 16 loop counters, 4096 counts each, 27 dynamically programmable output channels, and 5 statically programmable output channels.

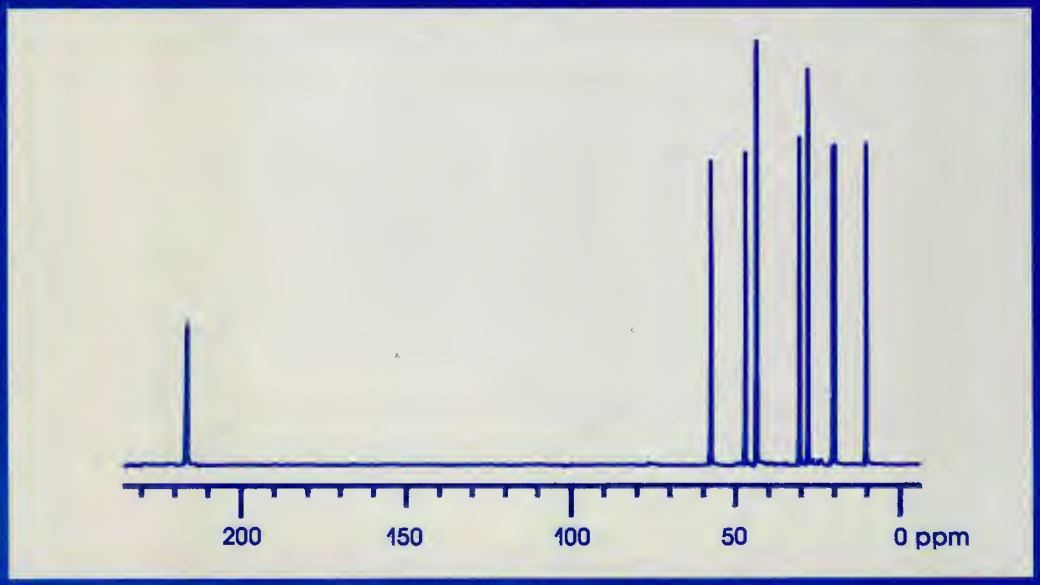
To intensify the spectrometer's ability to create novel pulse programs with the PGX-300, JEOL has also developed PEGS (Pulse Editing Graphic Software). This software package enables the user to enter and edit pulse programs in the same fashion as the sequences are reported in the literature (e.g., simple timing diagrams for pulses, decoupling and other hardware triggers). PEGS eliminates the need for abstract codes and mnemonics in generating pulse programs. The visual representation of the pulse program makes sequence generation straightforward and easy to understand. An example of this programming technique, a Redfield water suppression sequence, is shown in Figure 3.

Liquids and Solids Probes

In addition to a comprehensive repertoire of high performance liquids observation probes, the GX series also offers a variety of solids sample probes. For example, cross polarization/magic angle spinning solids probes are available for specific nuclei (e.g., C-13, Si-29 and Al-27), and nuclear ranges at both ambient and variable temperatures.

Optional Magnets

The magnetic field strength options for the GX series allow for proton observation at 270, 400, 500 and 600 MHz. The 270 and 400 MHz solenoids are also available as wide bore (89mm) systems.



Carbon-13 spectrum of solid camphor at 67.5 MHz. Total accumulation time was 8.5 minutes.

For further discussion on multi-terminal operation, please contact us at

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Telefonzentrale 01 / 256 22 11

February 3/1983

0430

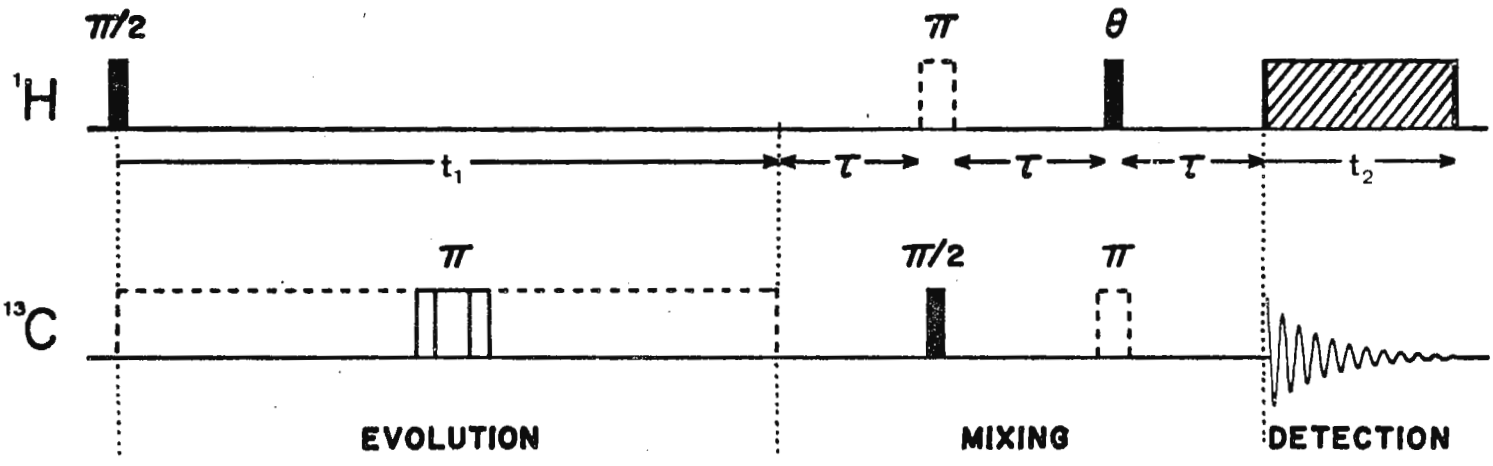
Prof. B.L. SHAPIRO
Department of Chemistry
Texas A & M University
COLLEGE STATION
Texas 77843 U S A

Multiplet-Separated Heteronuclear 2D NMR Spectroscopy

Dear Barry,

We are presently exploring improvements of conventional 2D heteronuclear correlation spectroscopy which allow one to obtain separate 2D shift maps for each multiplicity class, e.g. CH, CH_2 and CH_3 groups. Several possibilities for achieving this goal will be described in a paper recently submitted to Chem. Phys. Letters. Here we would like to mention only one of them.

In the experimental scheme,



the traditional sequence for coherence transfer is replaced by a DEPT-like sequence consisting of a ^{13}C $\pi/2$-pulse and a ^{1}H θ-pulse with variable flip

angle. In the course of the 2D sequence, the flip angle θ is incremented in synchrony with the evolution period t_1 so that $\theta = \omega_\theta t_1$, in analogy to the time-proportional phase increment method used in multiple quantum spectroscopy and in the "accordion" method. The effect is to give the transferred ^{13}C signals a second modulation. Not only do the responses oscillate according to the chemical shift evolution of the directly-bonded protons, but they are also modulated according to the functional dependencies $\sin \omega_\theta t_1$, $\sin 2\omega_\theta t_1$, and $\sin \omega_\theta t_1 + \sin 3\omega_\theta t_1$ for CH, CH_2 and CH_3 groups respectively. Hence each peak in the conventional correlation map is split into sidebands in ω_1, two for CH and CH_2, and four for CH_3. By arranging that the minimum sideband separation ω_θ exceeds the estimated spread in proton chemical shifts, the 2D signals from the three different molecular units are separated from each other. The result is distinct heteronuclear shift correlation maps for CH, CH_2 and CH_3 groups, all obtained in one experiment. The only residual cause of confusion is that CH_3 group responses also appear in the CH region, but this is not serious since the methyl and methine responses are usually well separated and the methyl signals are duplicated in the outermost spectral regions.

A multiplet-separated 2D spectrum of menthol obtained in this way is shown in the figure. The spectrum demonstrates the predicted decomposition of the shift correlation map into seven sections, of which three pairs contain duplicate correlation maps from the three types of molecular subunit. The CH_3 groups also appear in the CH region: However, these could be removed by a subtraction of the two regions before the absolute value is taken.

Best regards.

Sincerely yours,

MH Levitt

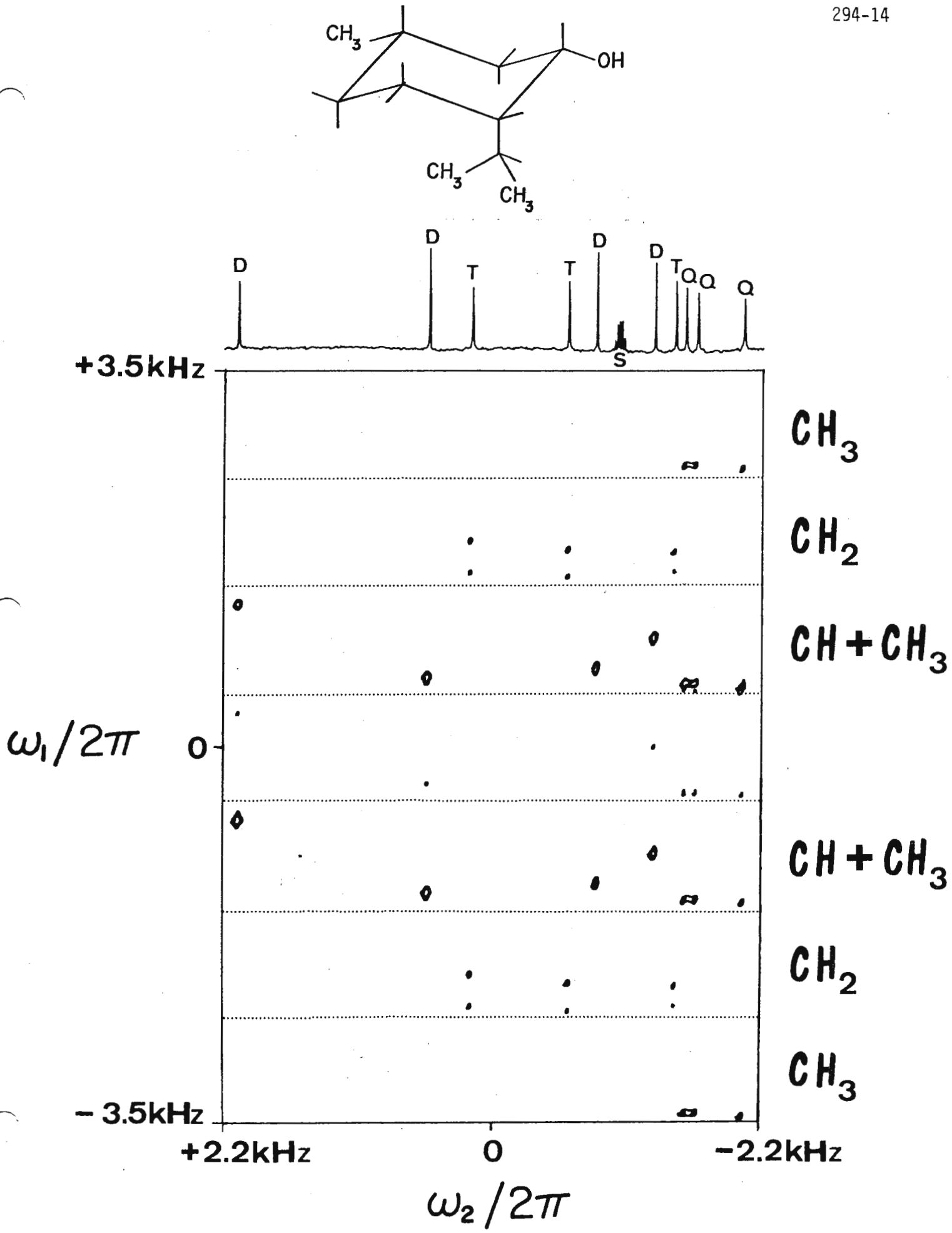
Malcolm H. Levitt

Ole W. Sørensen

Ole W. Sørensen

R.R. Ernst

Richard R. Ernst


CH3
OH
CH3
CH3
D
D
T
T
D
D
T
Q
Q
Q
S
+3.5kHz
CH3
CH2
CH+CH3
ω1/2π
0
CH+CH3
CH2
CH3
-3.5kHz
+2.2kHz
0
-2.2kHz
ω2/2π

294-15

GRIFFITH UNIVERSITY

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School of Science

Ref: PFB:kw
Please Contact:
Telephone: 275 7636

1st February, 1983

Dr. B.L. Shapiro,
Department of Chemistry,
Texas A & M University,
College Station,
TEXAS. 77843.
U.S.A.

Dear Dr. Shapiro,

Solid State ^{29}Si Spin-Lattice Relaxation

Please accept our apology for the late contribution. As David Doddrell is on study leave, the ultimatum was delayed in reaching me.

High resolution, solid state ^{29}Si NMR has really taken off in the last year or so and is providing very useful information in a number of applications. One pleasant surprise has been the ability to obtain spectra without cross-polarization as ^{29}Si T_1's have been found to be much shorter than is generally the case for solid state ^{13}C T_1's. Hence, quantitatively interpretable spectra have been obtainable without the cross-polarization uncertainties as with ^{13}C. To my knowledge, none of the published papers on solid state ^{29}Si NMR have commented on why this is so or have looked into whether this is a general phenomenon.

We have been examining a wide range of aluminosilicate minerals and have found that ^{29}Si T_1's vary widely and, not surprisingly, can be extremely long. For example, in a series of highly crystalline kaolins ('1:1' layer aluminosilicates) from different sources, we have measured ^{29}Si T_1's ranging from 4 s to ~5000 s, i.e. three orders of magnitude variation. Kaolins cross-polarize very well, facilitating measurement of such long T_1's. The ^{1}H T_1's also varied widely from 0.1 to 6.0 s. Kaolins can contain up to a few percent Fe^{3+} but in these cases contained less than 0.25%. It is known that Fe^{3+} can substitute for octahedral aluminium in the lattice, although it is difficult to determine to what extent it is present as such, rather than in separate phases. Hence, it seems likely that small amounts of Fe^{3+} present in the aluminosilicate lattice can, via spin-diffusion, result in extremely efficient ^{29}Si relaxation.

Another point which may be of interest is that on occasion we have either observed weak spinning sidebands in 59 MHz ^{29}Si MAS spectra of clay fractions or not been able to obtain usable spectra. This would

appear to be due to significant Fe-contamination as pointed out by Oldfield for ^{27}Al. We are able to detect when this is the problem by observing the ^{79}Br resonance of some added KBr, as used for angle setting, through broadening of the $+\frac{1}{2} \leftrightarrow -\frac{1}{2}$ transition resulting in a dramatic increase in sideband intensity. The effect is often detectable observing single scan FID's.

Yours sincerely,

Peter Barron

Dr. Peter Barron
Brisbane NMR Centre

DEPARTMENT OF HEALTH & HUMAN SERVICES — **Public Health Service**

National Institutes of Health
National Institute of
Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, N.C. 27709

February 8, 1983

Dr. Barry L. Shapiro
Department of Chemistry
Texas A&M University
College Station, Texas 77843

Dear Dr. Shapiro:

The Laboratory of Environmental Chemistry of the NIEHS has a permanent full-time position available for a chemist/NMR operator at a starting salary of $24,508 per annum.

DUTIES: The incumbent will assist in conducting research in nuclear magnetic resonance spectroscopy. The primary function of the incumbent will be to independently operate and maintain high resolution nuclear magnetic resonance (NMR) spectrometers and associated data systems by: (1) assuring the proper preparation of biological/environmental/organic samples; (2) determining adequacy of samples; (3) improving procedures for sample preparation and analysis; (4) establishing the instrumental parameters for equipment which will achieve satisfactory performance; (5) monitoring or performing instrument maintenance; (6) developing and performing analyses based upon sophisticated methods of NMR such as relaxation spectroscopy, 2D-NMR, etc.; and (7) presenting justifications to ensure that projects receive commitments with regard to available resources.

If interested in the position contact Emily Farrior, Personnel Office, P.O. Box 12233, Research Triangle Park, NC 27709 for application forms or additional information. Ph: (919) 541-7810.

Sincerely,

James D. McKinney, Ph.D.
Chief
Laboratory of Environmental Chemistry

294-17

ROCHE

10 February 1983

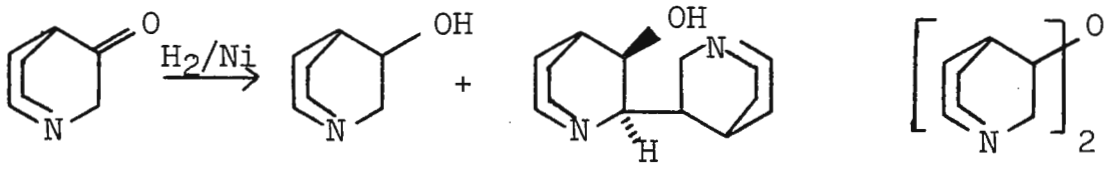
Professor B L Shapiro
Department of Chemistry
Texas A and M University
College Station
Texas 77843
U S A

Roche Products Limited · PO Box 8 · Welwyn Garden City · Hertfordshire AL7 3AY
Telephone Welwyn Garden 28128 *Telex 262098 ROCHEW*

Dear Barry

Quinuclidinol Impurity - Post Doctoral Fellowship

In reply to your threat of excommunication, we have found our WM-300 over the past year to be of immense benefit to our general operations in n.m.r. spectroscopy, enabling us to solve many long-standing problems. A typical case was the structural identification of an impurity (III) from the synthesis of quinuclidinol (II) from the corresponding ketone (I), itself obtained by an acid catalysed decarboxylation.



(I) (II) (III) (IV)

Mass spectrometry suggested the empirical formula $C_{14}H_{24}O$ for (III). The existence of 14 non equivalent carbons ruled out the symmetrical ether (IV). A combination of DEPT, extensive decoupling and chemical intuition led to the structure shown with the stereochemistry indicated, obtained presumably through acid-catalysed self condensation of (I) and consequent reduction. Sorting out the ^1H spectrum with 24 non-equivalent spins would not have been possible at lower field strnegths. Had our 2-D software been operational at the time, we may have solved the structure even quicker, but I doubt it!

Finally we are in the process of setting up as soon as possible in 1983, a Post Doctoral Fellowship for 1 or 2 years duration, involved in particular with n.m.r. studies of enzyme/inhibitor interactions. I would be interested to hear directly from prospective candidates with experience in this kind of work using high field spectrometers.

Sincerely

Tony

Dr W A Thomas
Head of Physical Methods Department

Efficient ^{15}N studies by NMR

Low gamma nuclei are often difficult to detect directly because of their low sensitivity, long T_1's, and unfavorable NOE's. However, using the INEPT technique, signal intensity can be borrowed from the abundant coupled proton spins through a process called "magnetization transfer," allowing their direct observation.

^{15}N is an example of such a nucleus, found in all important biochemical systems. The spectrum shown illustrates a natural abundance ^{15}N INEPT experiment on adenosine in DMSO. Only a few hundred scans are necessary to allow the observation of all five nitrogens and their long-range couplings. Reference: G.A. Morris, *J. Am. Chem. Soc., 102, 428* (1980).

Q.E.D. The above INEPT experiment was performed on a routine NMR spectrometer at the Bruker Applications Laboratory. The new AM Series of high-field NMR spectrometer systems comes with an extensive software system, including programs for INEPT processing, display and plotting. A new 8-color graphic display processor further facilitates speed of analysis and clarity of data presentation.

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New Literature Available from BRUKER

NMR-Tomography

– A simple introduction into a fascinating NMR technique –

The "NMR-Imaging" technique is without any doubt a revolutionary new method for obtaining pictorial information about internal structures e.g. of the human body. The evolution of this method has now reached the state were non-specialists have recognized the extraordinary power of this technique and consequently BRUKER has now available an introductory six-page brochure for those not familiar with this new method. In order to facilitate the understanding of the physical background to this method the basic principles are given in a simplified manner and are illustrated by a large number of figures.

In a short survey it is shown that for the last twenty years the instrumental development in the pulsed NMR field has been synonymous with the name of BRUKER and it is pointed out that the first commercially available Fourier Transformation (FT) spectrometers were developed by BRUKER in 1969. Since NMR tomography is based on both "pulsed" and "FT"-NMR, the unique experience of BRUKER in these fields represents the ideal basis for the recently developed imaging systems.

After a short introduction, the principles of NMR are described in the brochure followed by a short representation of the "Projection-Reconstruction-Technique". Due to the expected extraordinary importance of NMR tomography in the field of diagnostic medicine a comparison of the average X-ray tissue contrast with NMR data is given as well as some remarks about theoretically possible risks for patients. At the end of this brochure an "outlook" is given into new applications and of the expected development of NMR tomography.

The three new BRUKER brochures.

With the general title "BRUKER Info", periodically illustrations of BRUKER's latest results are added to the NMR Tomography brochure.

If you wish to obtain the new brochure containing two "BRUKER Info" illustrations please return the reply card.

Two-Dimensional NMR *aspect 2000*

A practical introduction into this new technique by an experienced spectroscopist.

The common 2-D experiments are described, measuring conditions and microprograms are given. Application examples on various spectrometers demonstrate the capabilities of the method and naturally the outstanding performance of BRUKER spectrometers in 2-D spectroscopy.

DEPT

Distortionless Enhancement by Polarization Transfer

A new method with significant advantages over other polarization transfer techniques is described in a new brochure.

This method developed at the Griffith University by Drs. Bendall, Doddrell and Pegg can be performed on any BRUKER Spectrometer equiped with a CXP or high speed pulse programmer. Using this sequence the sensitivity in coupled spectra can be significantly increased or the multiplicity selection in ^{13}C spectra can be performed without the critical adjustments required for other polarization transfer pulse sequences.

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Professor B L Shapiro
Texas A & M University
Chemistry Department
College Station
Texas
U S A

11 February 1983

Dear Barry,

Since I am becoming more and more convinced that I am an nmr spectroscopist first and organic chemist second, rather than the other way round, I take my plunge into your newsletter with this first contribution. This change is best illustrated by the following account.

In collaboration with Peter Brophy, one of our biochemists, and my student Jit Hayer, we have been trying ^{39}K nmr as a means of investigating concentration of K^+ inside living cells. The Dy^{3+} tripolyphosphate shift reagent developed by Gupta and Gupta can also be used to shift potassium, although the maximum shifts obtainable are not so large (6-7 ppm vs ca 25 ppm). These shifts are large enough for us to distinguish signals from $^{39}K^+$ inside and outside red blood cells and have led to reliable quantitation of $[K^+]$ in Peter's blood. (It was, he was relieved to find, quite normal.)

Although ^{39}K is a much less receptive nucleus than ^{23}Na, it is typically present in concentrations an order of magnitude greater than ^{23}Na making the low receptivity less of a problem. ^{39}K nmr therefore can be used to examine $[K^+]$ inside and outside cells, and therefore transport processes across cell walls may be studied.

This work was done on the SERC WH360 instrument in Edinburgh.

Best wishes,

Frank

Dr F G Riddell

Ref: R K Gupta and P Gupta, J.Mag.Res., 1982, 47, 344.

February 9, 1983

Dr. Bernard L. Shapiro
Department of Chemistry
Texas A & M University
College Station, TX 77843

SIGNALS FROM DEUTERATED POLY(METHYL METHACRYLATE)
MAGIC-ANGLE SPINNERS

Dear Barry:

Bruker Instruments offers sample containers for magic-angle spinning made of deuterated poly(methyl methacrylate) (PMMA). The PMMA does not generally give significant carbon signals upon cross polarization because of the scarcity of protons. We have found, however, that with very careful matching of the carbon and proton rf powers, substantial peaks from the PMMA still result, even with averaging of only 1000 transients (see Fig. 1).

Even at high isotopic purity, there are some residual protons in the deuterated PMMA. These protons are coupled both to nearby carbons and to distant carbons. Because the size of the coupling is proportional to $1/r^3$, the distant couplings can be quite weak. Cross polarization is nevertheless possible with very precise matching of carbon and proton irradiation power.

The moral may be that sometimes it may be best not to do an experiment too well. For most organic materials, efficient cross polarization results when the power match is not nearly so good as that required to give signals from the deuterated PMMA.

We are currently doing experiments on polymer blends of deuterated and protonated components. Again, precise rf power matching is required for cross polarization of carbons in the deuterated material from protons in the protonated material. Under these conditions it is

Dr. Bernard L. Shapiro
February 9, 1983

necessary to use a sample container such as one made from boron nitride if interfering signals are to be avoided.

/slk

Sincerely yours,

P. Mark Henrichs
Chemistry Division
Research Laboratories

J. Michael Hewitt
Chemistry Division
Research Laboratories

Max Linder
Chemistry Division
Research Laboratories

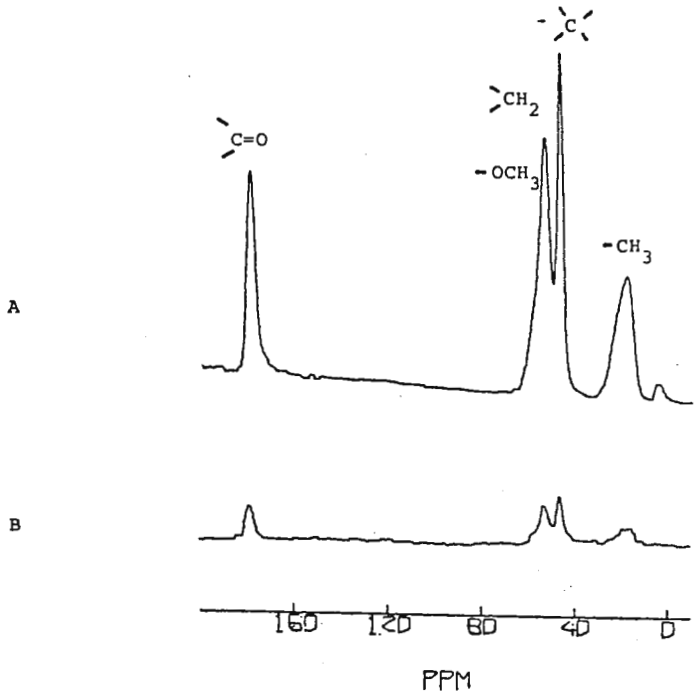


Figure 1

25 MHz proton decoupled magic-angle spinning spectra of ^{13}C in deuterated PMMA (1000 scans each).

A. Single ^{13}C 90° pulses, 40 sec recycle time

B. Cross polarization spectrum, 10 msec contact time, 4 sec recycle time

HARVARD MEDICAL SCHOOL

JEAN L. DELAYRE, Ph.D.
Research Associate in Biophysics

25 Shattuck Street
Boston, Massachusetts 02115
617 · 732-[redacted]
1893

February 9, 1983

Professor B. L. Shapiro
Department of Chemistry
Texas A-M University
COLLEGE STATION, TX 77843

1) STEREO NMR SPECTROSCOPY

2) NIC-293A' WANTED

Dear Barry,

Recently, we have started looking at the intra- and extra-cellular sodium contents of various specimen (hearts, blood). Done in collaboration with Charles Springer (Stony Brook), this work requires the use of shift reagents in order to differentiate between the intra- and extra-cellular signals. It is also desirable to observe not only the sodium spectrum, but also the phosphorus spectrum, in order to monitor the metabolic activity of the sample. It seemed to us that the easiest and best way to do this was to observe both signals simultaneously. We used an approach similar to the one mentionned by Peter Styles et al. (J. Mag. Res. 1979, 329-336). In our case, however, we have excited the two spin systems <u>at the same time.</u>

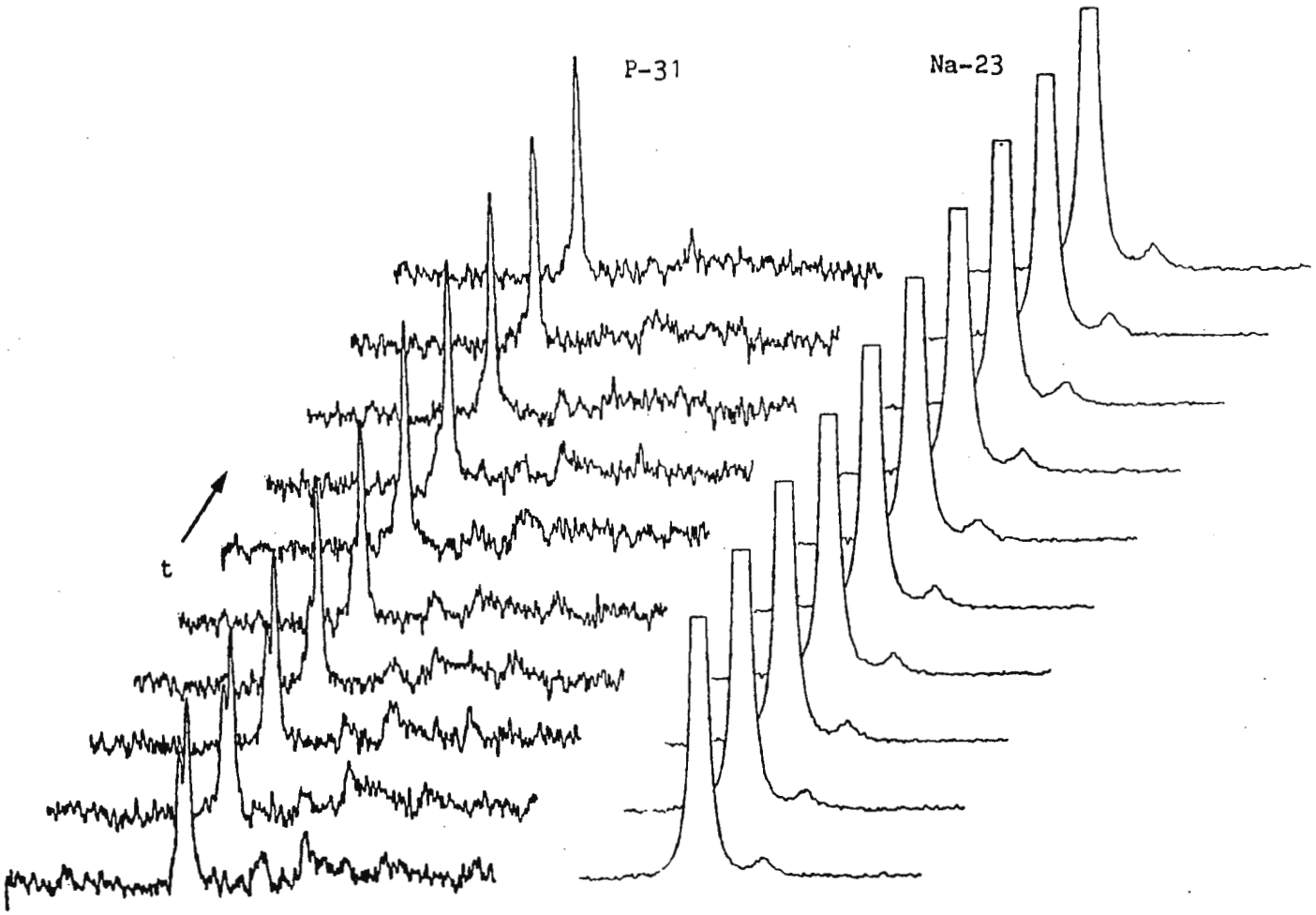
John Baldo (Nicolet Magnetics) designed a dual coil probe tuned to P-31 and Na-23/H-2, while we were building a second spectrometer around our NIC-1280 data station. In fact, this spectrometer has just one observe channel, since all the other functions (lock and variable temperature) can be performed with our NMC-360/WB spectrometer. The pulse programmer on the Nicolet spectrometer was modified so that the pulse sequence could be started by a TTL pulse coming from the pulse programmer on our spectrometer. We could not use the "external trigger" feature, since it is software controlled and exhibits a 100 usec to 150 usec delay between the time it gets the signal till the time it actually starts the pulse sequence.

The spectra shown here, represent the first results obtained with the two observe channels combined together (hence the word stereo). The P-31 and Na-23 spectra were recorded before and after the addition of Dy(TTHA) to a solution of red blood cells. We were thus able to follow the depletion of the high energy phosphate and the uptake of intra-cellular sodium. Complete results will be presented at a later date.

Incidentally, we would like to buy a used Nicolet 293A' pulse programmer for the second spectrometer. I would be pleased to hear of anyone who has such a device for sale. Thanks.

Yours sincerely,

Jean L. Delayre Dye Jensen Martin Pike



Red blood cells without glucose.

294-25

Lilly

Lilly Research Laboratories
A Division of Eli Lilly and Company

307 East McCarty Street
Indianapolis, Indiana 46285
(317) 261-2000

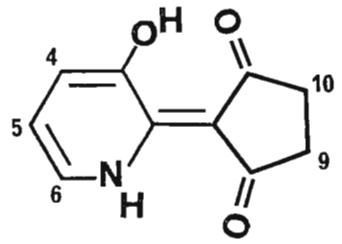
February 23, 1983

Professor B. L. Shapiro
Department of Chemistry
Texas A and M University
College Station, TX 77843

Dear Professor Shapiro:

Unusual Couplings to an Exchangeable Proton

Recently an unknown fluorescent material was isolated and submitted to the Lilly molecular structure group for characterization. The compound has well resolved NMR spectra (both ^{1}H and ^{13}C) in $CDCl_3$, and during a series of carbon accumulations I had the good fortune of growing crystals in the NMR tube. The structure of the principal tautomer (from x-ray crystallography) is shown below, along with proton NMR parameters obtained at 308°K. The 16.62 ppm peak is very broad and extremely temperature dependent; for such a proton to be coupled to two ring protons was a surprise. I would be glad to hear from TAMU Newsletter readers who have other examples of such couplings.



Proton	^{1}H Chemical Shift	Structure
1 (NH)	15.60	broadened singlet
3 (OH)	16.62	very broad; chem. shift is variable
4	7.54	broadened triplet; $J_{34} \simeq 6$ Hz, $J_{45} = 5.9$ Hz, $J_{46} = 1.5$ Hz
5	7.16	broadened doublet of doublets; $J_{35} < 1$ Hz, $J_{45} = 5.9$ Hz $J_{56} = 8.1$ Hz
6	7.38	doublet of doublets; $J_{46} = 1.5$ Hz, $J_{56} = 8.1$ Hz
9 or 10	2.72	hextet; coupled to 2.57
9 or 10	2.57	hextet; coupled to 2.72

Sincerely,

LILLY RESEARCH LABORATORIES

Ann Hunt

Ann H. Hunt, Research Scientist
Physical Chemistry Research

AHH:JCW

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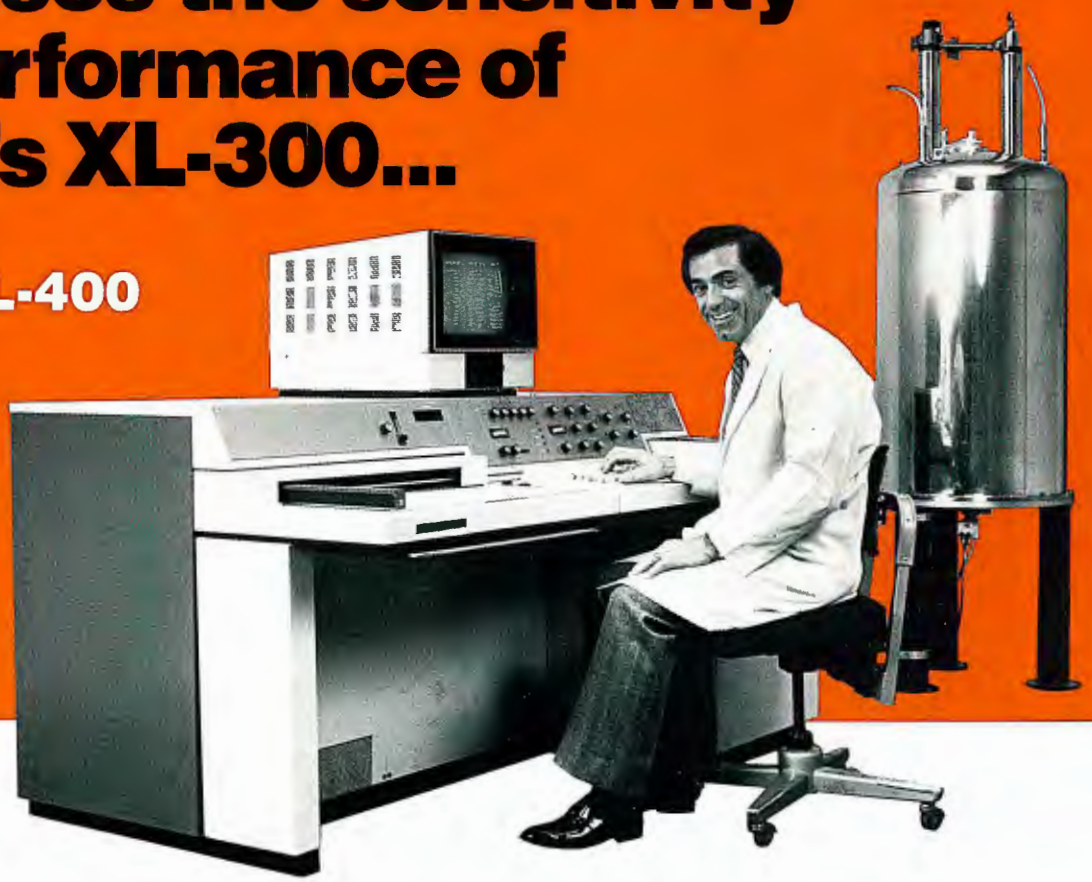

Varian's new ADVANCE XL-400 Superconducting FT NMR Spectrometer gives you:

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See more information—faster—on the 16-color CRT. The ADVANCE XL-400's operator interface, with its large display screen and wide spectrum of colors, allows you to make quicker and more accurate decisions based on CRT visuals.

More memory than any other NMR system. The new ADVANCE Data System features a multi-computer design with 464K of memory and dual processors, each further expandable to 16 megabytes: big enough to handle the largest 2D data tables.

Flexible and easily expandable. This system lets you add commercially available peripherals to meet specific application requirements. This capability and our expandable Pascal-based software keeps your instrument "state-of-the-art" in the rapidly evolving field of NMR research.

Send for literature now. For details concerning Varian's new ADVANCE XL-400 Spectrometer, call the Varian sales office nearest you. Or write: ADVANCE XL-400, Varian Associates, D-070, 611 Hansen Way, Palo Alto, CA 94303.

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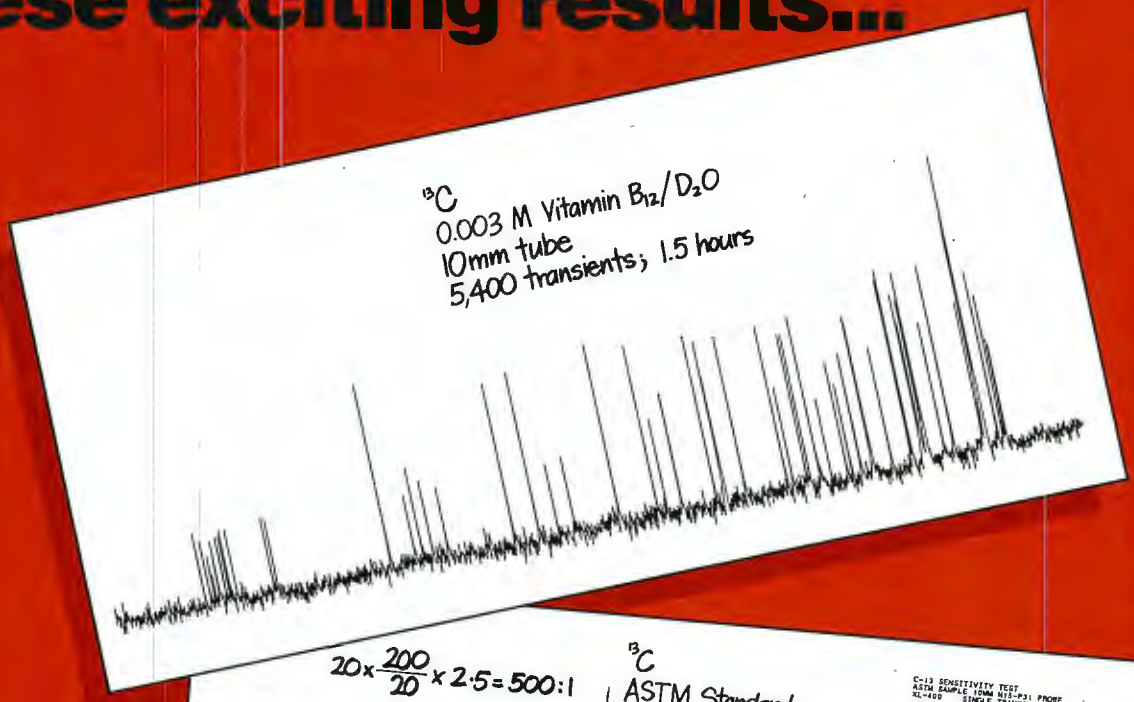

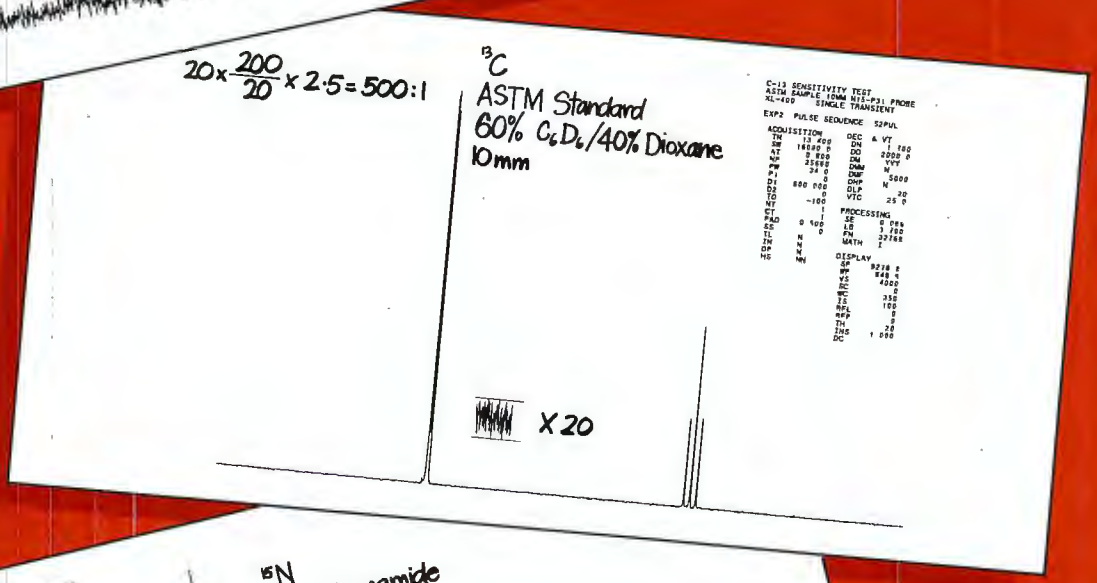

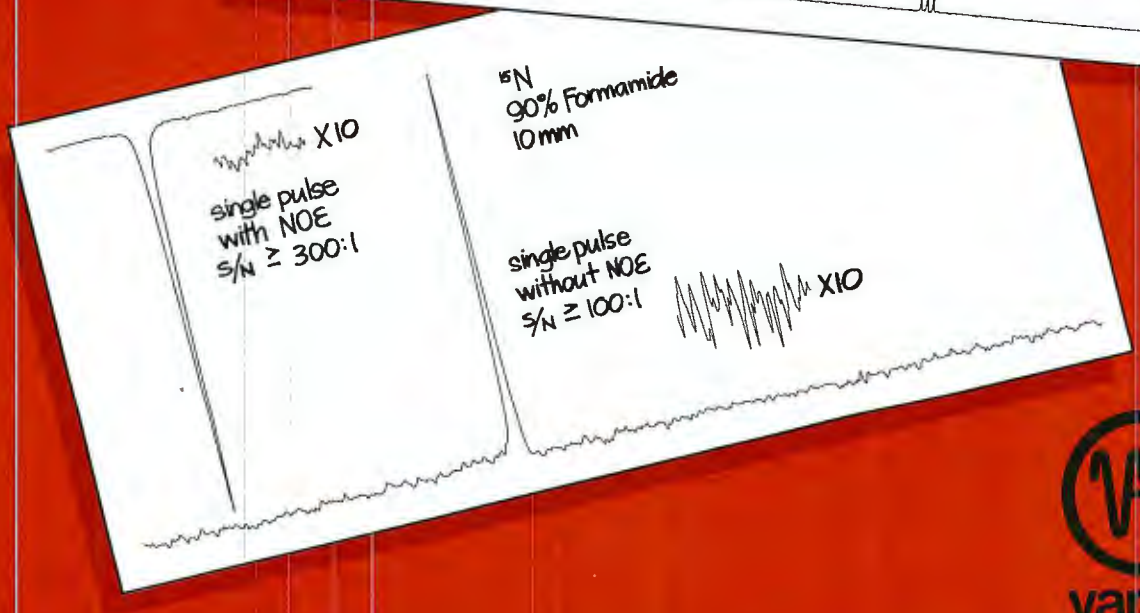

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ZÜRICH

Institut für Molekularbiologie und Biophysik

Gerhard Wagner, Erik R.P. Zuiderweg

HPM-Gebäude
Durchwahl-Nr.: 01/377 34 55
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Postadresse:
Institut für Molekularbiologie
und Biophysik
ETH -Hönggerberg
CH-8093 Zürich

1085

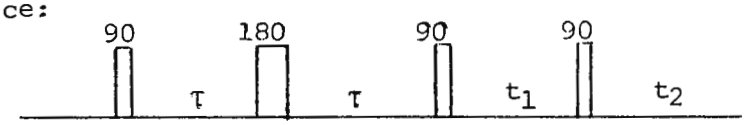
Prof. B. L. Shapiro
Editor and Publisher
TAMU NMR Newsletter
Texas A&M University
Dept. of Chemistry
College Station, Texas 77843
USA

Zurich, February 22, 1983

2D Double Quantum Spectra of Proteins

Dear Dr. Shapiro,

We report on the use of double quantum 2D NMR for the analysis of proton spectra of proteins. 2D NMR techniques such as COSY and SECSY (1) have been used successfully to identify spin systems of amino acid residues in proteins. These methods were difficult to apply, however, if coupled resonances were almost degenerate since then cross peaks are masked by the presence of a strong diagonal. We propose the use of double quantum 2D NMR to overcome this problem. We used the pulse sequence:



The first three pulses create multiple quantum coherence which is frequency labelled during t_1 and detected during t_2 after the final 90° pulse. In our experiment the delay τ was chosen for optimal 2Q transfer for coupled resonances with J = 16Hz. We have employed a 32 step phase cycling as proposed by Bax (2) to select for double quantum coherences present during t_1.

As an illustration the figure shows a double quantum 2D NMR spectrum of the basic pancreatic trypsin inhibitor (BPTI) in D_2O. All but 9 amide protons have been exchanged with deuterium. As an example of spin systems with almost degenerate resonances Thr 54 is traced in the spectrum. The coupling between the α- and β-proton resonances at 4.10 and 3.95ppm, respectively, is difficult to detect in a normal COSY spectrum, but is readily detectable in the double quantum experiment, due to the absence of a diagonal.

To our experience the analysis of this type of 2D spectra is as simple as for a COSY or SECSY spectrum with the following advantages:

1) No diagonal

2) The choice of τ for the preparation period allows the selection of subspectra with particular values of J-couplings.

3) No cancellation of antiphase cross peaks along ω_1 does occur, an effect that seriously reduces the intensity of cross peaks in COSY spectra of proteins.

Disadvantages are:

1) The need of a large spectral width in ω_1,

2) Loss of sensitivity due to T_2-relacation during the preparation pulse sequence. This effect is, however, partially compensated for by the absence of antiphase cancellation in ω_1 (see above).

Sincerely yours,

G. Wagner E.R.P.Zuiderweg

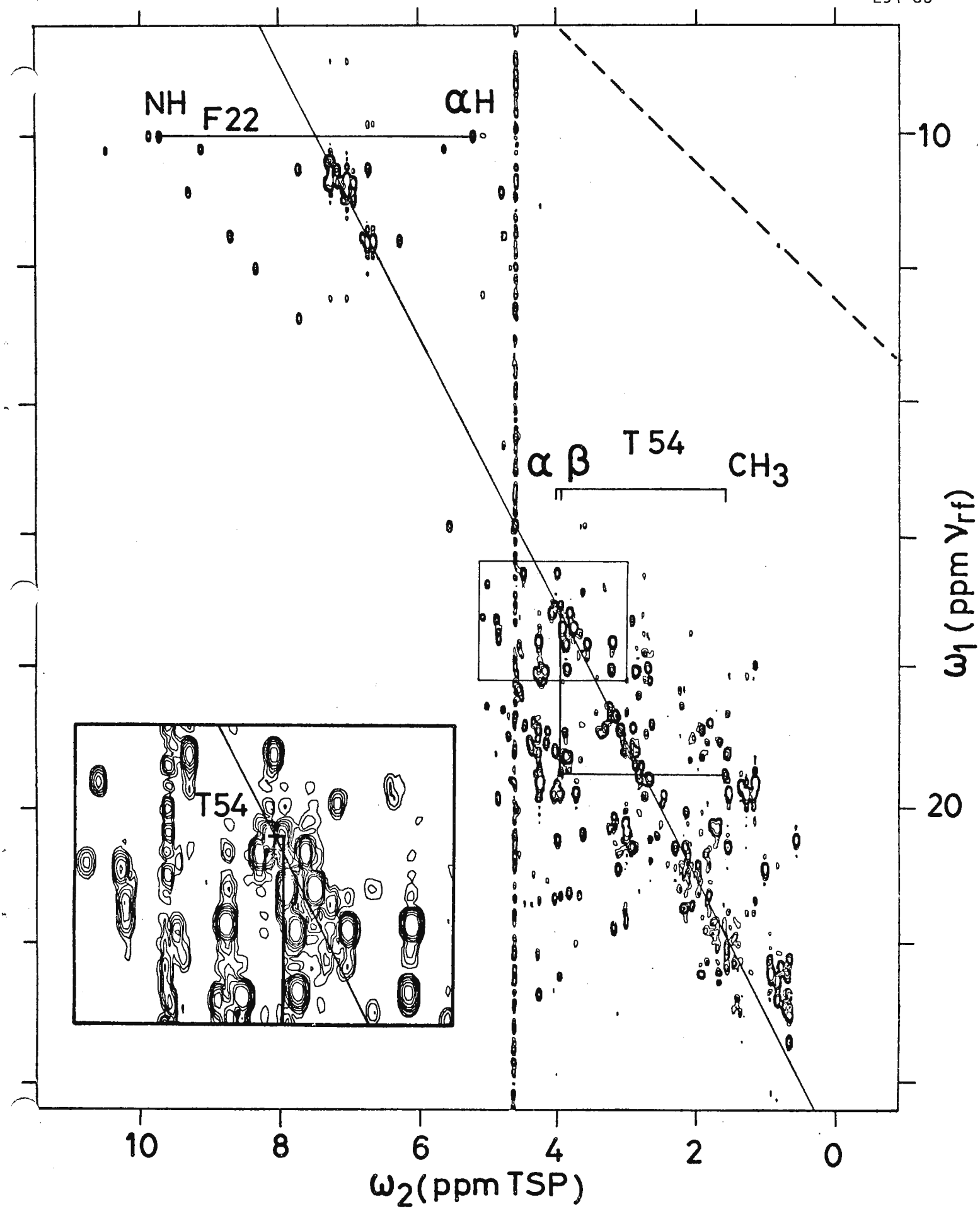
1. K. Nagayama, Anil Kumar, K. Wüthrich and R.R. Ernst, J. Magn. Res. 40, 321-334 (1980).

2. A. Bax: in Two-dimensional Nucl. Magn. Res. in Liquids, Delft University Press, D. Reidel Publishing Comp., Dordrecht, Boston, London (1982).

Figure Caption

500 MHz 2D double quantum spectrum of BPTI in D_2O at pD 6.3, 36°. The carrier was placed on the left hand side of the spectrum at 12.59 ppm from TSP. The ω_2-axis is calibrated relative to internal TSP, the ω_1-axis relative to the position of the carrier. The double quantum diagonal ($\omega_1=2\omega_2$) is drawn as a solid line,the single quantum diagonal ($\omega_1=\omega_2$) as a broken line. Connectivities are given for Phe 22 which has a slowly exchanging NH and for Thr 54 whose amide proton is exchanged against deuterium. The coupling between the α- and β- proton of Thr 54 is readily resolved. This spectral region is shown in the insert with an expanded scale to demonstrate how couplings between almost degenerate peaks can be observed which would be masked in a COSY experiment by the diagonal.

"Please credit this contribution to the subscription of Kurt Wüthrich".


NH F22
αH
α β T54 CH3
T54
10
20
ω1 (ppm γrf)
10
8
6
4
2
0
ω2(ppm TSP)

CHEMICAL INSTITUTE

UNIVERSITY OF AARHUS

DEPARTMENT OF ORGANIC CHEMISTRY
HANS JØRGEN JAKOBSEN

8000 Århus C, Denmark
Telephone (06) 12 46 33

Feb. 10, 1983
HJJ/BHN

Professor B.L.Shapiro
Department of Chemistry
Texas A & M University
COLLEGE STATION - Texas 77843
USA

Re: Polarization Transfer Pulse Sequence for Calibration of the ^1H Decoupler 90° Pulse Width

Dear Barry,

Recently we have made extensive use of the SINEPT polarization transfer pulse sequence (1) for natural abundance ^{15}N NMR studies on our 13 years old Varian XL-100-15 spectrometer using a supersensitive 18 mm probe (2). This sequence is especially useful for spectrometers without a proton phase shifter and also in many other respects.

However, calibration of the ^1H decoupler 90° pulse width for these studies may be very tedious using standard methods (e.g. (3)) without employing ^{15}N enriched samples. Thus, for such calibrations we have found the polarization transfer sequence shown in Fig.1 very useful. This sequence is most conveniently analyzed in terms of operator techniques which show that at the point of acquisition the density operator for a two-spin IS system (I = ^1H, S = ^{13}C,^{15}N) takes the form

$$\sigma_{Acq} = -m_H \sin\theta \sin 2\theta \sin(\omega_H \tau)\, 2I_z S_y \qquad [1]$$

In the equation [1] m_H is the equilibrium ^1H magnetization, θ the ^1H flip angle, and ω_H is the ^1H chemical shift relative to the ^1H transmitter. Optimum conditions for the calibration requires $\sin(\omega_H \tau) = 1$, i.e. the ^1H transmitter frequency must be adjusted to $\nu_{xmtr}(^1H) = (\frac{1}{2} + n)J_{IS}$, n = 0, ±1, ±2, etc., because $\tau = (2J)^{-1}$. A plot of the function $\sin\theta \sin 2\theta$ is given in Fig.2. This shows that for θ passing through 90° the doublet lines for the antiphased coupled spectrum become inverted and the intensity is zero for θ = 90°.

Fig.3 illustrates an application of the sequence for calibration of the 90° pulse width of the ^1H Gyrocode decoupler

for the V-4415 ^{13}C probe using an ENI model 320L (20W) power amplifier. The ^{1}H transmitter frequency was placed relative to the ^{1}H chemical shift of the $CHC\ell_3$ sample so as to obtain an optimum for the function $\sin(\omega_H \tau)$. A 90° pulse width of 51 μsec is obtained.

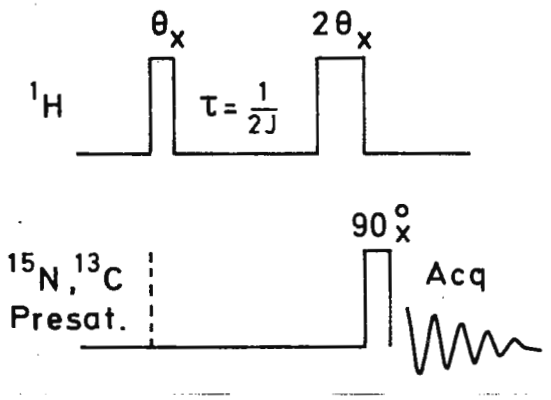


Fig.1. Polarization transfer pulse sequence for calibration of the ^{1}H decoupler 90° pulse width.

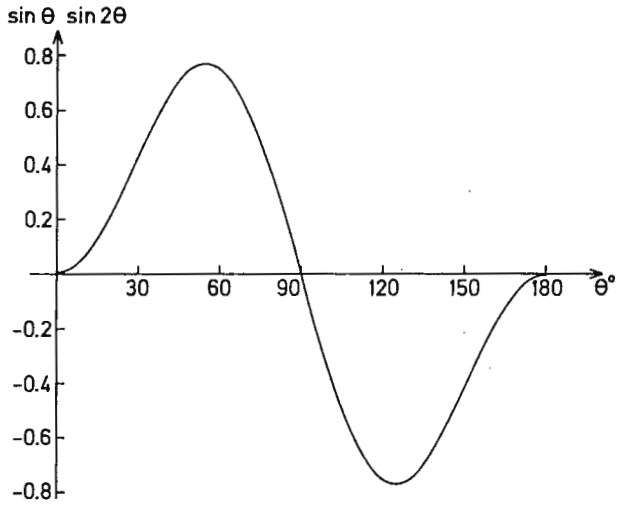


Fig.2. Plot of $\sin\theta \sin 2\theta$ versus the ^{1}H flip angle θ.

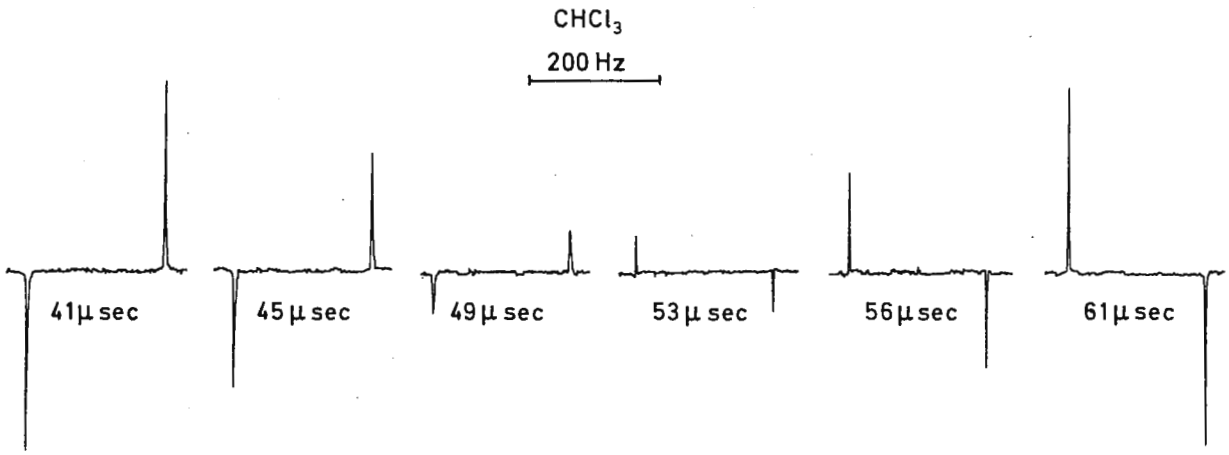


Fig.3. Polarization transfer enhanced coupled ^{13}C NMR spectra of $CHC\ell_3$ for calibration of the 90° ^{1}H pulse width. The pulse widths used in the sequence of Fig.1 are indicated below each spectrum.

Sincerely,

Henrik Bildsøe — Hans J.Jakobsen

(1) H.J.Jakobsen, O.W.Sørensen, and H.Bildsøe, J.Magn.Reson. 51, 157 (1983).
(2) P.Daugaard, P.D.Ellis, and H.J.Jakobsen, J.Magn.Reson. 43, 434 (1981).
(3) K.G.R.Pachler, J.Magn.Reson. 7, 442 (1972).

294-33

University of Edinburgh

Department of Chemistry

West Mains Road, Edinburgh, EH9 3JJ Scotland.

Yr. ref.:
Our ref.:

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Telephone 031 - 667 1081
Ext. 3245

16 February 1983

Professor B.L. Shapiro,
Department of Chemistry,
Texas A&M University,
College Station,
Texas 77843,
USA.

Dear Professor Shapiro,

Contour Shimming

I would like to describe a method of magnet shimming which we have used here for some years but which appears to be surprisingly little known elsewhere. It is intended to take a lot of the guesswork and mystery out of finding the right settings for the spinning shims, Z, Z^2, Z^3 and Z^4. For iron magnets these are Y, Y^2 (or CURV), Y^3 and Y^4, this description applies equally to both.

While even a novice can usually get the hang of shimming Z and Z^2 fairly quickly, the other two are much more tricky. Unfortunately the behaviour of Z and Z^2 and the attainable resolution are critically dependent on having Z^3 and Z^4 in the right place. These two high order shims are often woolly and vague in their behaviour, and it is quite difficult and time-consuming to locate the best position. The Z and Z^2 shims are, however, easily 'felt' into place, so the problem reduces to finding the best positions for the other two.

We draw a grid 10 cm x 10 cm and label one axis Z^3 and the other Z^4. Each combination of Z^3 and Z^4 setting is thus represented by a point on the grid. Z^3 and Z^4 are set to any values and then Z and Z^2 are optimised. The best lock level (or any other homogeneity criterion) is then written on the grid at the point represented by the Z^3 and Z^4 knob dial readings.

After writing in values for a few Z^3 and Z^4 combinations contour lines can be drawn connecting points of equal lock level. The best position then lies at the biggest peak of the surface now represented on the grid. If necessary, the process can be repeated with a larger scale grid over a narrower range of Z^3 and Z^4 settings. This method is thus exhaustive, as false maxima, represented by outliers of the main peak, asymmetric contours or long salients can quickly be found, and avoided. Poor regions are also clearly evident. The speed of the method is usually only limited by the relatively long settling time of the system after changing the Z^3 or Z^4 control.

Professor B.L. Shapiro 16 February 1983

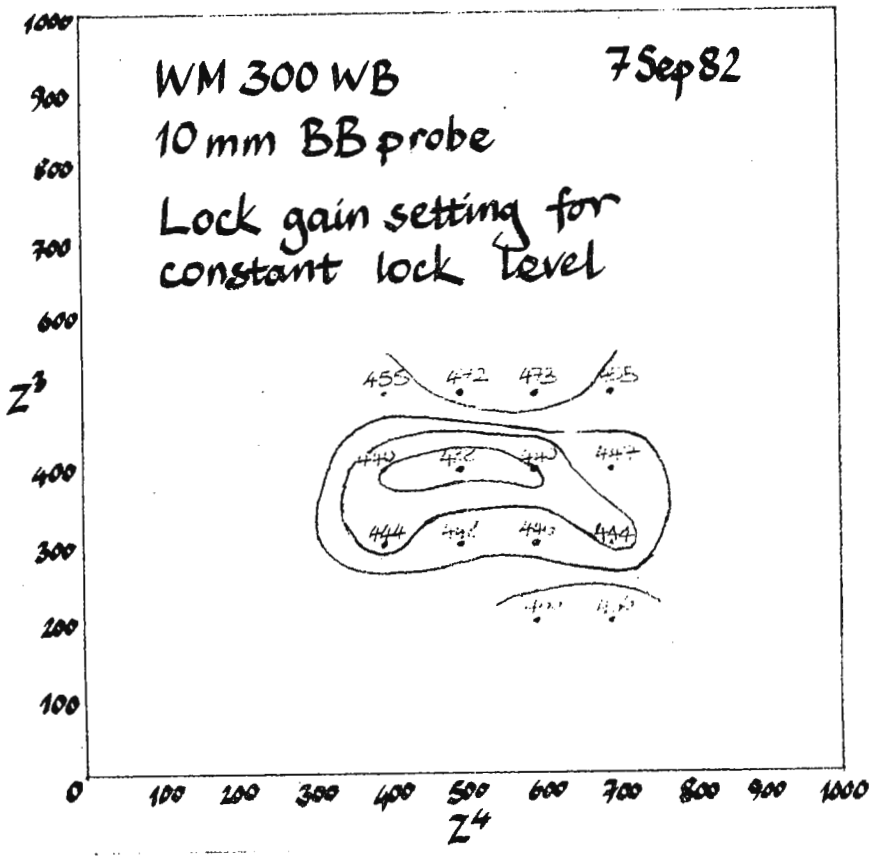
A particular advantage is evident on some Bruker spectrometers where the Z^4 shim has a large Z^0, (ie. main field) component. This leads to immediate loss of lock on twiddling the control. (I believe the Varian shim system automatically compensates for this effect). Contour shimming eliminates much of the twiddling and thus avoids lost lock, lost patience and lost temper.

Yours sincerely,

Alan S. Boyd

Dr. A.S.F. Boyd

Dr. I.H. Sadler



294-35

The University of Alabama in Birmingham
Comprehensive Cancer Center
205/934-5696

Dr. Bernard L. Shapiro
Department of Chemistry
Texas A&M University
College Station, TX 77843

February 7, 1983

Re: Postdoctoral Position Available

Dear Barry:

We are looking for a postdoctoral fellow to study metal-bleomycin interactions. The primary focus will be on iron complexes, which will be studies by NMR in conjunction with other spectroscopic methods. This fellow will collaborate closely with another fellow studying bleomycin complexes with nucleic acids. Other collaborators include investigators with expertise in synthesis of bleomycin fragments, in NMR of paramagnetic complexes and the Mossbauer spectroscopy.

We prefer someone who is just finishing or has just finished graduate school. Expertise in inorganic chemistry is highly desirable as is expertise in NMR, although we can provide training in NMR. A Bruker WH-400 spectrometer equipped with ^{1}H, ^{13}C and ^{31}P (broadbanded) probes is available with adequate spectrometer time. An NTC-300 equipped with multinuclear probes is available in the Chemistry Department. Access to other spectrometers -- uv, CD, fluorescence, EPR, etc. -- is available and a multiple technique approach is encouraged (when appropriate).

The project is supported by a five year NIH grant now into its first year. The salary is negotiable. The position, which is available immediately or at a date in the near future, has been created through the departure of other members of our laboratory. March 1 would be a realistic starting date.

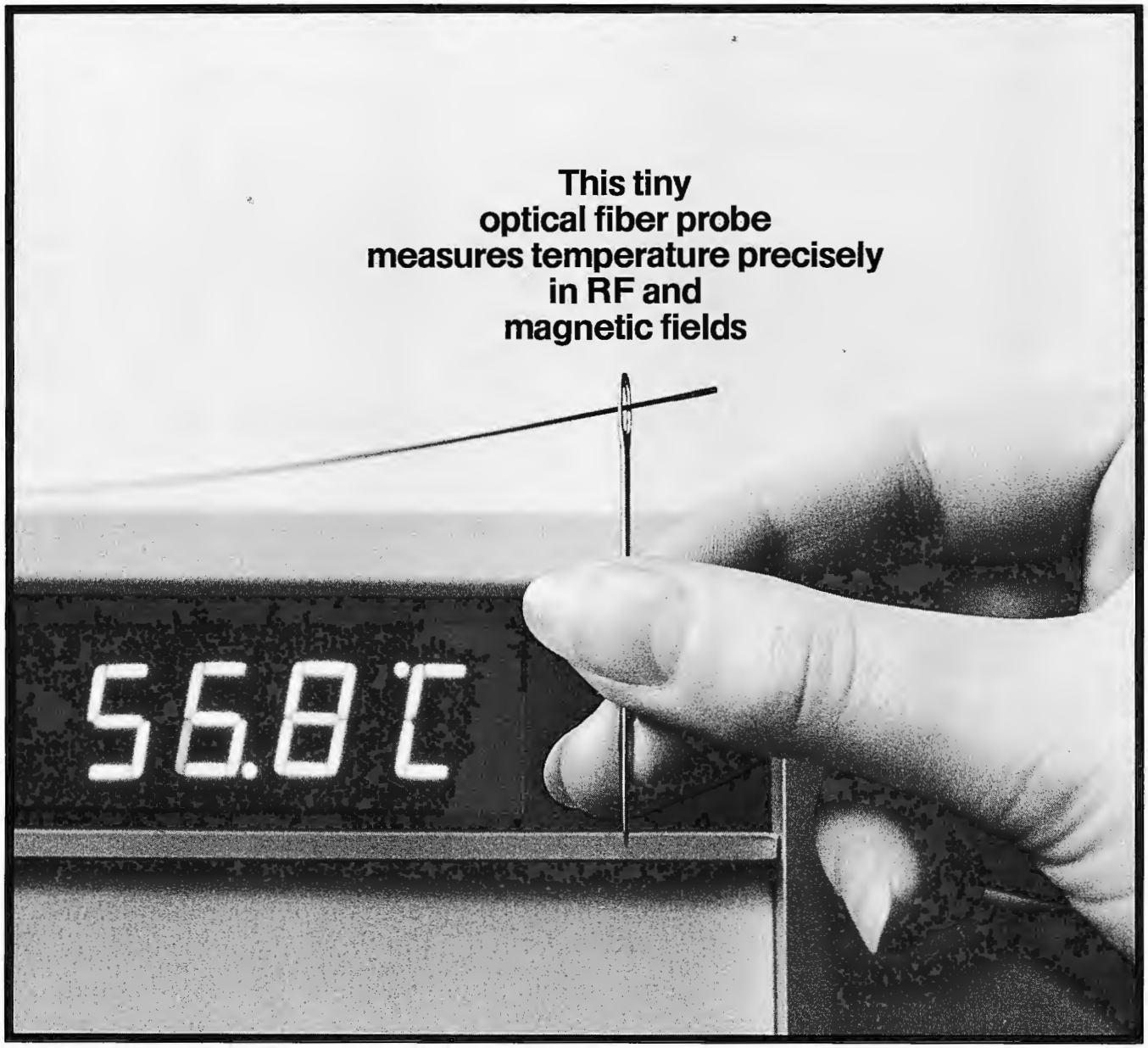
Please post this letter and advise suitable candidates to write to me or call (205) 934-5695.

Sincerely yours,

Jerry D. Glickson, Ph.D.
Professor of Biochemistry
Director, Cancer Center NMR Core Facility

P. S.: We anticipate that one or more positions in NMR studies of intact tissues will soon become available in our laboratory.

BREAKTHROUGH



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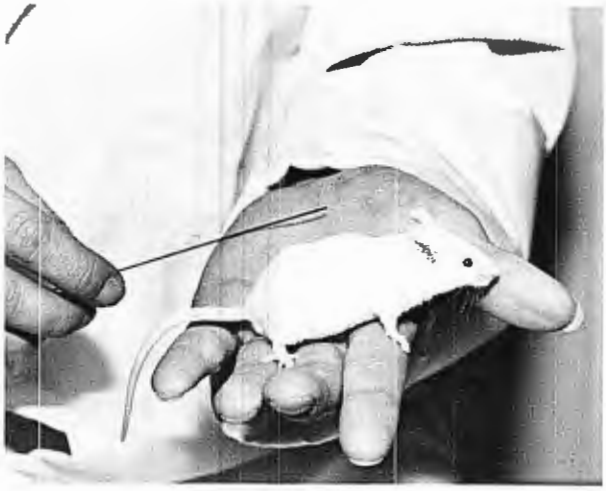

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3. Remainder of Range: ±0.6°C

Using Optional Precision External Temperature References:
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3. Remainder of Range: ±0.5°C

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Rear Panel Digital Output (Optional):
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Temperature References:
Two floating temperature reference wells, accurate to 0.25°C, are provided within the instrument for routine calibration. A high precision temperature reference, with two fixed point wells, is also available as an option.

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FREIE UNIVERSITÄT BERLIN
Fachbereich Chemie
Institut für Organische Chemie (WE 02)

FU BERLIN

Dr.Klaus Roth

Freie Universität Berlin,
FB Chemie, WE 02, Takustraße 3, 1000 Berlin 33

10.2.1983

Prof.B.Shapiro
Texas A&M University
College of Science
College Station, TX 77843

Dear Prof.Shapiro,

firstly I have to congratulate you to a new world record. The recent number of TAMU Letters arrived in central europe after an incredible short carriage time of seven weeks and two days. That means that all european readers have missed the several deadlines only by six weeks instead of normally seven or eight. Did you use a new designed bottle or did you simply use the atlantic instead of the pacific ocean for the bottle mail? What is the secret?

This remarkable speeding up prompts us to contribute a simple method for determination of time differences other than looking at the postage stamp.

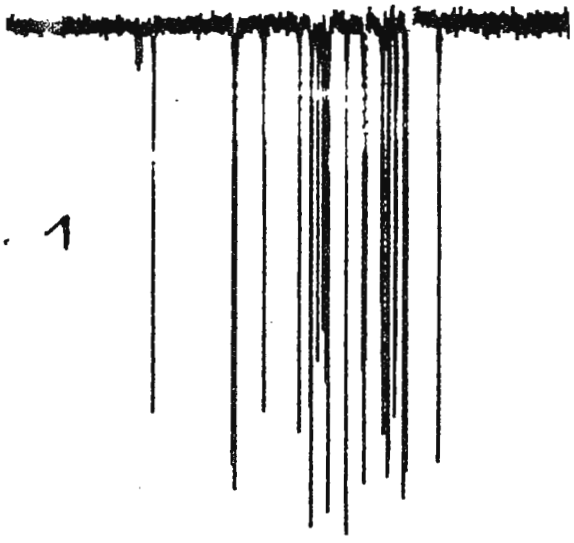
MEASUREMENT OF THE EXECUTION TIME OF BRUKER MICROINSTRA CTIONS

With our old WH-270 (old pulser board and CDC disc) we had problems with the timing in pulse sequences. Normally, the delay due to the execution of instructions within a microprogram must be compensated by a trial-and-error procedure. We wish to demonstrate an easy way of measuring these times exactly.

1. measurement of a normal carbon spin echo spectrum (e.g. cholesterylacetate = Dr.Hull's pet) , FT, proper phasing and storing the values of the phase corrections.

```
1 ZE
2 BB
3 D1
4 P1      90°
5 D2
6 P2     180°
7 D2
8 GO = 2
9 Exit
```

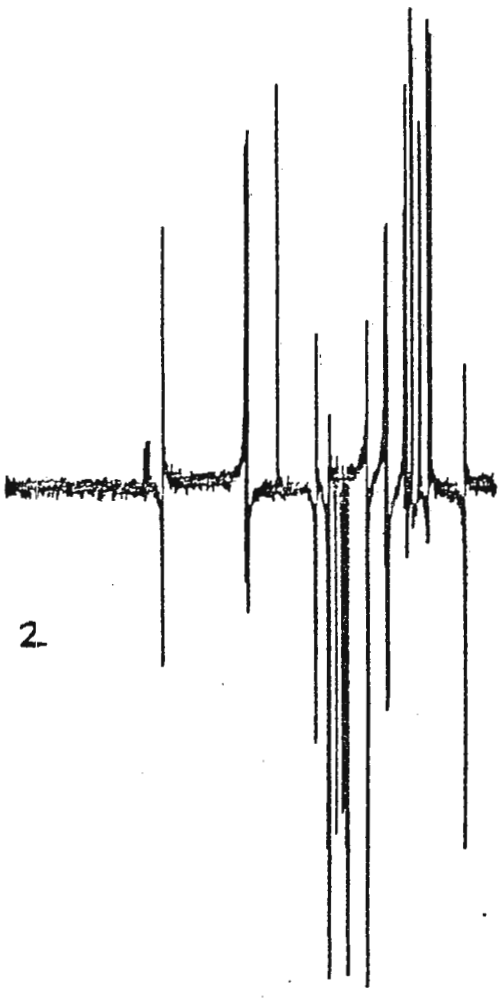
exp. 1



2. same experiment plus a (useless) BB instruction between step 7 and 8, after FT and phase correction with the parameters of the first experiment one observe a linear phase error of 360 degree within a width of 1840 Hz. This frequency dependent phase error is a result of an additional delay due to the execution of the BB instruction. Between this phase error and the time delay exists a simple relationship (see J.Magn.Resonance 38,65(1980)).

$$(\Delta\nu)_{360^\circ} = \frac{1}{\tau}$$

For the BB instruction one results τ = 0.54 ms.
Other typical values are:
DO = 0,45 ms
O2 = 3,85 ms



exp. 2

Sincerely Yours,

The University of Sydney

N.S.W. 2006

IN REPLY
PLEASE QUOTE:

TELEPHONE: 692 1122.

Professor B.L. Shapiro
Department of Chemistry
Texas A & M University
College Station, TX 77843
TEXAS, USA.

21st February, 1983

Dear Barry,

Assay of oxygen isotopes

In a recent ^{17}O NMR study we required a routine NMR method for assaying isotopically enriched water samples for ^{16}O, ^{17}O and ^{18}O. This was achieved indirectly by relying on the isotope induced shifts of the ^{31}P resonance in "oxygen labelled" trimethylphosphate.

The oxygen atom from water molecules can be incorporated easily into the P=O group of trimethylphosphate by reaction of the water sample with one equivalent of PCl_5 then with excess methanol.

$$H_2O^* + PCl_5 \rightarrow Cl_3P{=}O^* + 2HCl$$

$$Cl_3P{=}O^* + 3MeOH \rightarrow (MeO)_3P{=}O^* + 3HCl$$

The ^{31}P NMR spectrum of the "enriched" trimethylphosphate clearly shows the incorporation of each of the three oxygen isotopes (Figure 1). Molecules containing ^{16}O or ^{18}O give rise to singlet ^{31}P resonances with ^{31}P bonded to ^{18}O resonating ca. 5 Hz to higher field than ^{31}P bonded to ^{31}P bonded to ^{16}O ($CDCl_3$ solvent, 20° C at 121.5 MHz). Those molecules containing ^{17}O ($I=^{-5}/_2$) give rise to a sextet of broad lines ($W_{1/2}$ 51 Hz, spacing 160 Hz). Integration of the ^{31}P spectrum provides a measurement of the relative concentrations of the three species.

Please credit this contribution to the account of Professor S. Sternhell.

Yours sincerely

Leo Field

Dr L.D. Field
Dept. of Organic Chemistry

1. G. Lowe, B.V.L. Potter, B.S. Sproat and W.E. Hull, J. Chem. Soc. Chem. Commun., 733 (1979).

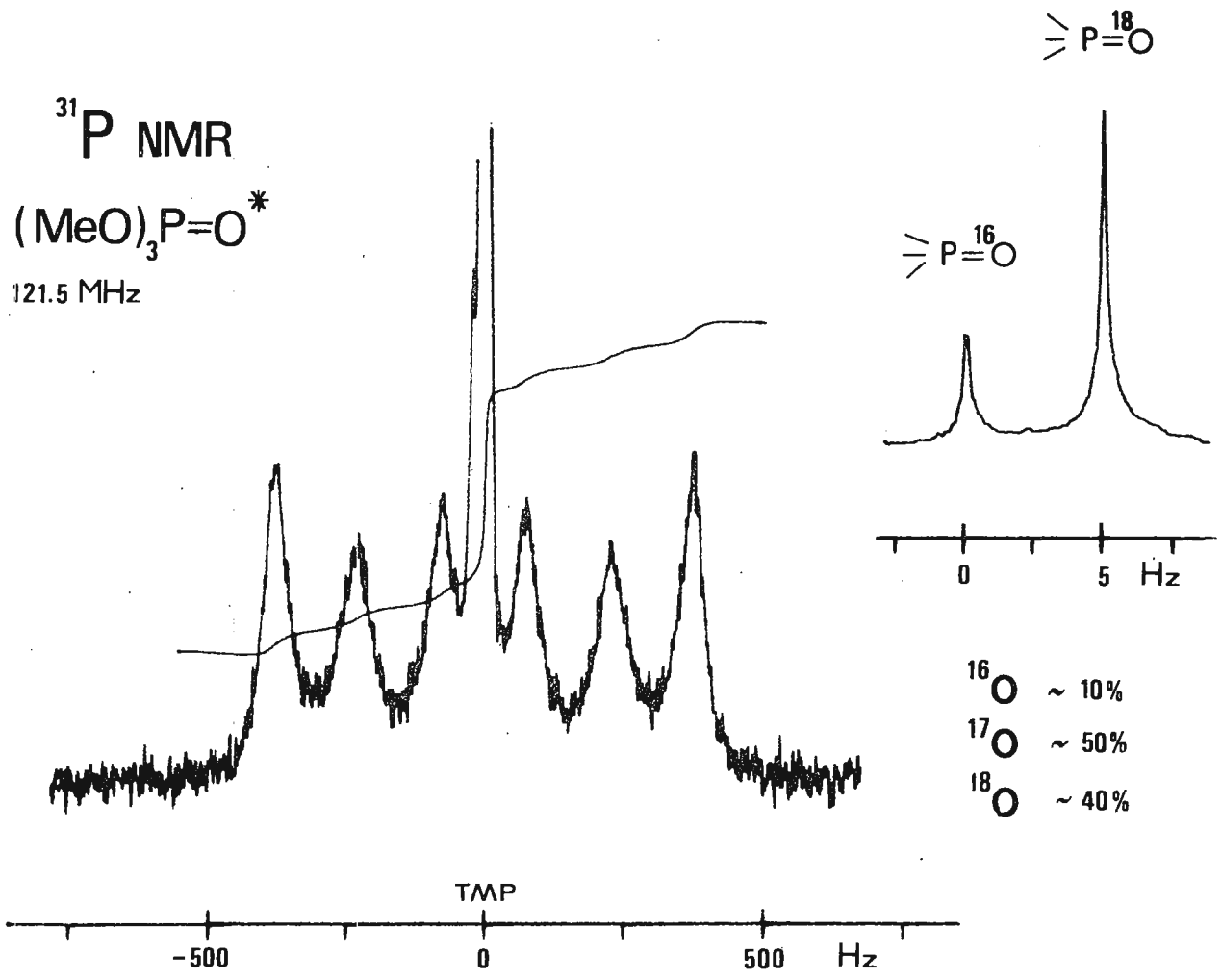


Figure 1 ^{31}P NMR spectrum of $(MeO)_3P=O^*$ in $CDCl_3$ at 20°C. Inset shows an expansion of the center of the spectrum.

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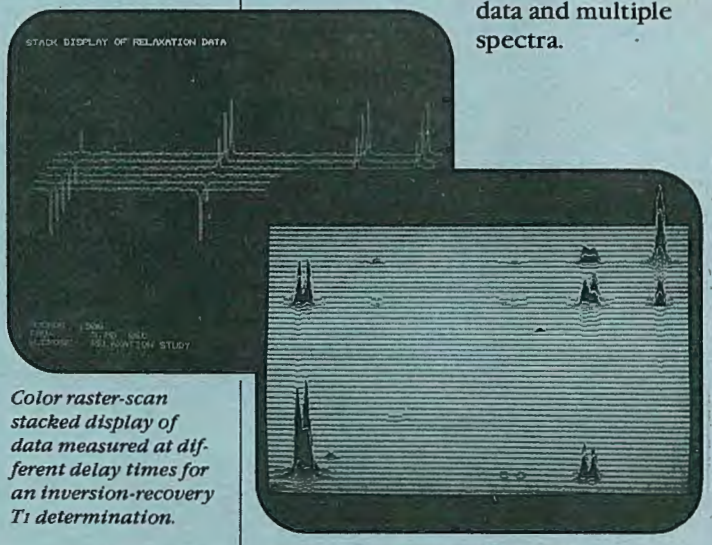

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The Nicolet NMR

THE SOLID LEADER in NMR

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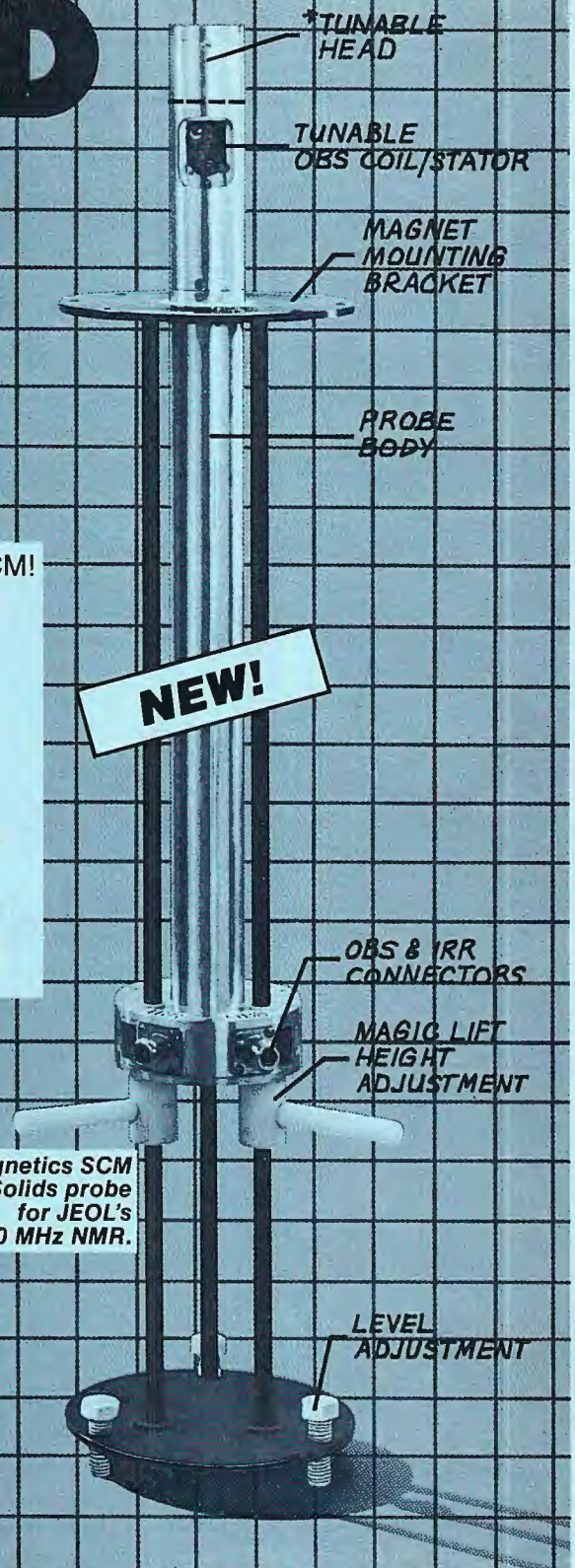

Chemagnetics SCM Solids probe for JEOL's 200 MHz NMR.

SOLIDS UPDATE – NOW AVAILABLE

- MULTI NUCLEAR SOLIDS PROBE FOR THE FX 270
- WIDE BORE MAGNET WITH VARIABLE TEMPERATURE SOLIDS FOR THE FX 200

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