TEXAS A&M UNIVERSITY

NMR NEWSLETTER

No. 430

July 1994

Continued on inside back cover

TABLE 1 DEUTERATED SOLVENTS

Cat. No.	Description	Formula	Min. Iso.	Density	MP	BP (°C)	$-\chi_v \times 10^6$ @ (°C)
D-11							0.551 (32)
D-120							
D-13							0.460 (20)
D-121	Aceonte-d_6 + 1% TMS		99.8%				
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D-14							
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FORTHCOMING NMR MEETINGS

8th International Symposium on Molecular Recognition and Inclusion, Ottawa, Ontario, Canada, **July 31 - August 5, 1994**; Contact: H. Morin-Dumais, Steacie Institute for Molecular Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, ON K1A 0R6, Canada; (613) 993-1212; Fax: (613) 954-5242 See TAMU NMR Newsletter 427, 38

Solid-State NMR Symposium, 36th Rocky Mountain Conference on Analytical Spectroscopy, Denver, CO, **July 31 - August 5, 1994**; Contact: R. E. Botto, Chemistry Divn., Argonne Natl. Lab., Argonne, IL 60439; (708) 522-3524; Fax: (708) 252-92882 See TAMU NMR Newsletter 424, 46.

2nd Meeting, Society of Magnetic Resonance, San Francisco, California, **August 6 - 12, 1994**; Contact: SMR Berkeley Office, 1918 University Ave., Suite 3C, Berkeley, CA 94704; Tel. (510) 841-1899; Fax: (510) 841-2340.

Gordon Conference on Order/Disorder in Solids, New London, New Hampshire, **August 7 - 12, 1994**; Contact: Prof. M. A. White, Dept. of Chemistry, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J3; Tel. (902) 484-3894; Fax: (902) 494-1310. See TAMU NMR Newsletter 421, 44.

XVIth International Conference on Magnetic Resonance in Biological Systems, Veldhoven, The Netherlands, **August 14 - 19, 1994**; Organizing Committee: M. J. A. de Bie, C. W. Hilbers, R. Kaptein; Contact: Secretariat XVIth ICMRBS, Bijvoet Center for Biolmolecular Research, Padualaan 8, NL-3584 CH Utrecht, The Netherlands; Tel. +31 30 53 2652/2184/3801; Fax: +31 30 53 7623/54 0980.

Ampere Summer School on Magnetic Resonance with Spatial Resolution, Eichstätt, Bavaria, Germany, **September 2 - 8, 1994**; Contact: L. D. Hall or B. Blümich - See TAMU NMR Newsletter 426, 56.

FACSS XXI (21st Annual Conference of the Federation of Analytical Chemistry and Spectroscopy Societies, St. Louis, Missouri, **October 2 - 7, 1994**; Held jointly with the MMRS-5 meeting *(v.i.)*. Contact: FACSS National Office, 198 Thomas Johnson Drive, Suite S-2, Frederick, MD 21702-4317; Phone: (301) 846-4797. See TAMU NMR Newsletter 428, 28.

5th Missouri Magnetic Resonance Symposium, St. Louis, Missouri, **October 5 - 6, 1994**; Held jointly with the FACSS meeting *(v.s.)*. Contact: FACSS Natl. Office, 198 Thomas Johnson Dr., Suite S-2, Frederick, MD 21702-4317; Phone: (301) 846-4797. See TAMU NMR Newsletter 428, 28.

Continued on page 52

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF CHEMISTRY
Lensfield Road
Cambridge CB2 1EP

Ray Freeman, FRS
John Humphrey Plummer
Professor of Magnetic Resonance

Telephone: **(0223) 336450**
FAX (UK): **(0223) 336362**
(International): **+44 223 336362**

Professor Bernard L Shapiro,
Editor/Publisher* TAMU NMR,
966 Elsinor Court,
Palo Alto,
California 94303
USA

31 May 94
(received 6/6/94)

Fellgett Rides Again

Dear Barry,

In the abstract to his famous Summer School lecture on 2D spectroscopy, Jeener(1) already anticipated that his new technique would have a sensitivity comparable to the equivalent 1D experiment carried out in the same total time. This "2D multiplex advantage" has been cited as the reason why there is so much multidimensional spectroscopy performed these days. (Could it be that simple inertia leads us to use the existing 2D packages rather than program an alternative 1D experiment ?).

The precious multiplex (Fellgett) advantage need not be lost when we set up a small number of 1D experiments. It is well known to those who know these things that to weigh N different objects it is better to put them all on the scales at the same time, carefully partitioning them between left and right-hand pans in N different permutations, than to weigh each object separately. The key is the appropriate Hadamard (2) matrix, for example for N=4 objects A, B, C, D,

	A	B	C	D
First weighing	+	+	+	+
Second weighing	+	+	−	−
Third weighing	+	−	+	−
Fourth weighing	+	−	−	+

where "+" indicates an object on the left-hand pan and "–" an object on the right-hand pan. Summing the results gives four times the weight of A, but any random and uncorrelated errors are only doubled with respect to a single weighing. The combination (+ – – +) gives four times the weight of D and so on. In general the "signal to noise" increases as the square root of N. The multiplex advantage has been restored. This was exploited at a very early date (1949) in infrared spectroscopy (3) and more recently in magnetic resonance imaging (4,5) and high resolution NMR (6-8).

Moral: Don't despair if you like planning to do the key experiments by 1D rather than accumulating everything in the 2D mode, you can buy back the sensitivity advantage by performing all N experiments together, alternating the phases according to the rows of the Hadamard matrix and separating the individual traces afterwards according to the columns of the same matrix.

Kindest regards,

Ray

Ray Freeman

(1) J. Jeener, Ampere International Summer School, Basko Polje, Yugoslavia, 1971.
(2) J. Hadamard, Bull. Sci. Math. **17**, 240 (1893) [yes, 1893!].
(3) M. J. E. Golay, J. Opt. Soc. Am. **39**, 437 (1949).
(4) R. J. Ordidge, A. Connelly, and J. B. Lohman, J. Magn. Reson. **66**, 285 (1986).
(5) L. Bolinger and J. S. Leigh, J. Magn. Reson. **80, 162** (1988).
(6) C. Müller, and P. Bigler, J. Magn. Reson. A. **102,** 42 (1993).
(7) V. Blechta and R. Freeman, Chem. Phys. Lett. **215**, 341 (1993)..
(8) V. Blechta, F. Del Rio-Portilla and R. Freeman, Magn. Reson. Chem. **32**, 134 (1994).

**Also scholar, bon vivant, raconteur, and a great disappointment to his mother. . .*

R - 3 out of 4 correct. Not bad.

B

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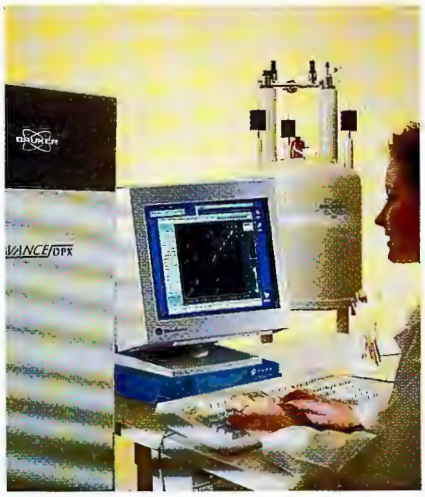

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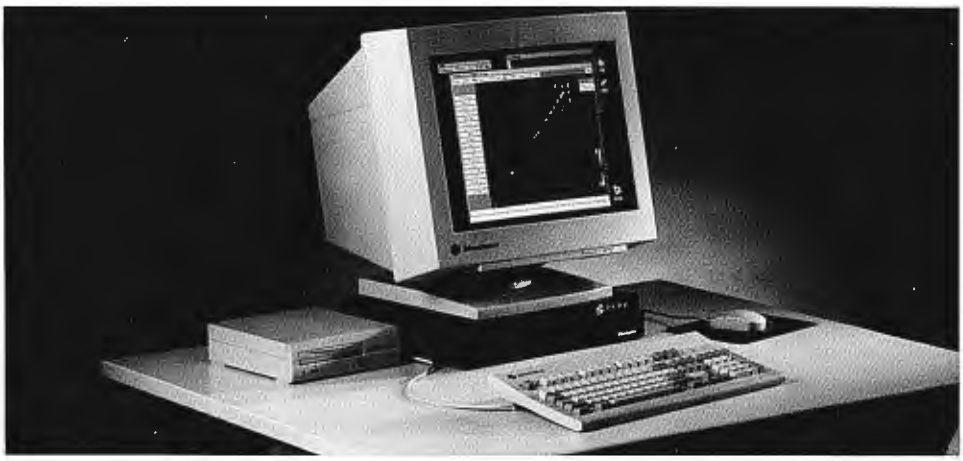

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UNIVERSITY OF DELAWARE

DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

University of Delaware
Newark, Delaware 19716

(received 5/21/94)

May 16, 1994

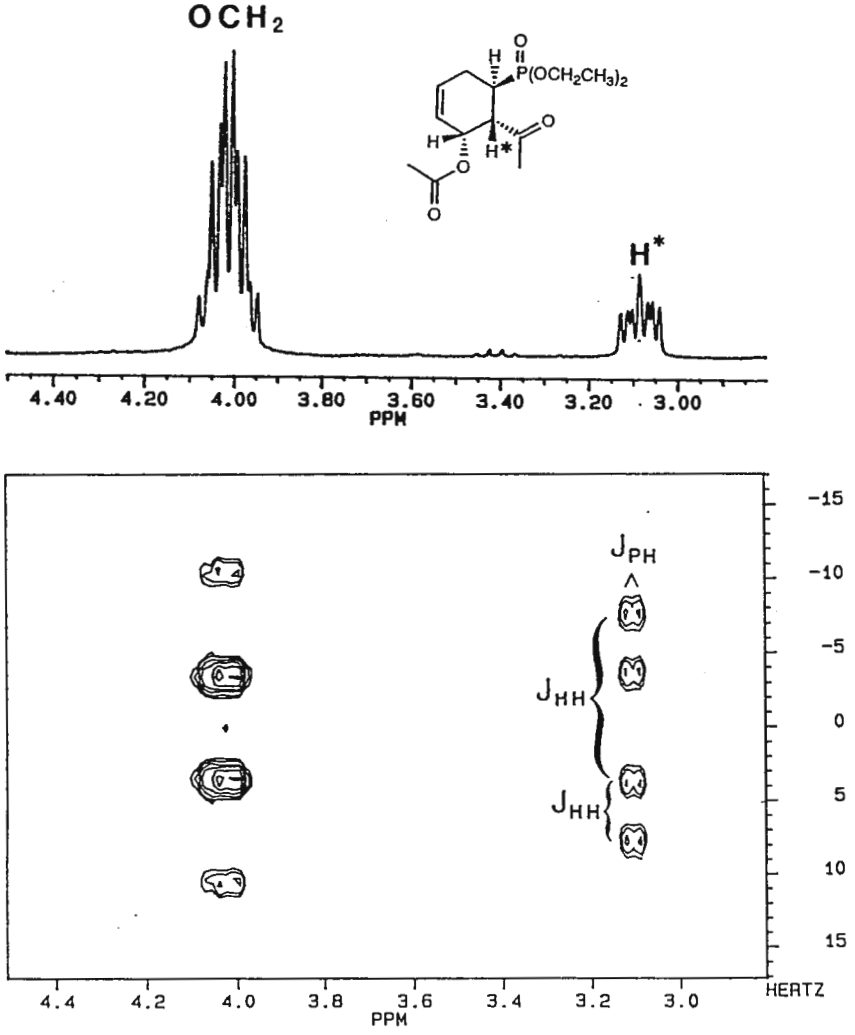
Distinguishing Homonuclear and Heteronuclear J-Coupling Constants in Phosphonates

Recently, we have been interested in the synthesis of myo-inositol phosphonates. We ultimately want to compare the activity of synthetic phosphonates with naturally occuring myo-inositol phosphates, important intermediaries in a variety of biochemical pathways. We have been exploring a Diels-Alder cycloaddition as the probable first step in this synthesis, and we need to be able to determine the relative amount of endo v.s. exo product formed. Consequently, we need to know the stereochemical configuration of the phosphonate product(s) formed.

The structure of the initial phosphonate product, shown in the figure below, contains three chiral centers, yielding four possibilities for the relative stereochemistry of the three protons attached to these chiral carbons: trans-trans, trans-cis, cis-trans, or cis-cis. In a 6-membered ring, the vicinal coupling constants between protons on adjacent carbons are generally large (6-14 Hz) if the two protons are trans diaxial and small (<5 Hz) if the protons are diequatorial or axial/equatorial (cis). Therefore, the relative stereochemistry of both proton pairs associated with the three chiral centers can be determined by measurement of the two vicinal coupling constants for these proton pairs. Unfortunately, the splitting patterns observed for each of these protons is complicated by 2-, 3-, and 4-bond $^{1}H/^{31}P$ coupling associated with the phosphonate group. Hence, the homonuclear coupling constants are difficult to measure, especially on older spectrometers which lack the capability to decouple ^{31}P while observing ^{1}H. Identification of ^{1}H coupling constants from selective proton decoupling experiments is precluded in this case by severe spectral overlap in the upfield region of the 250 MHz proton spectrum.

One experiment which works well to solve this problem, even on an old instrument such as our Bruker WM 250 NMR, is two-dimensional J-resolved spectroscopy. In this experiment, splitting due to heteronuclear coupling behaves the same as splitting due to chemical shift differences, and the heteronuclear coupling constant can be measured from the splitting in the f2-dimension. By contrast, the splitting along the f1-dimension is affected solely by homonuclear J-coupling and can be used to measure homonuclear coupling constants. An expansion of the 250 MHz 1D and 2D J-resolved spectra, containing the OCH_2 protons of the phosphonate ester groups and the proton α to the acetyl group (labeled H^*), is shown in the figure below. For both proton signals, the splitting patterns observed in the 1D spectrum are complicated by heteronuclear coupling. Despite this complication, the two homonuclear and one heteronuclear coupling constants associated with H^* (α to acetyl group) can be measured easily from the J-resolved spectrum. The doublet structure due to heteronuclear coupling is visible as splitting in the f2-dimension, and $^{3}J_{HP}$ was measured to be 7.0 Hz. The doublet of doublets structure arising from homonuclear coupling is visible along the f1-axis, and the two coupling constants were measured as 4.0 and 11.5 Hz. The proton α to the acetate group is isolated from other proton signals and could be decoupled selectively. This selective decoupling experiment enabled the larger coupling constant (11.5 Hz) to be assigned to coupling between H^* and the

proton α to the phosphonate group. Therefore, the smaller coupling constant is between H* and the proton α to the acetate group. These results imply that the geometry of the former proton pair is trans diaxial, as expected since these protons were trans in the precursor molecule, while the latter proton pair are in a cis (axial-equatorial) configuration, as expected based on the endo rule for the Diels-Alder reaction.



The example described above shows how 2D J-resolved spectroscopy, a somewhat neglected experiment, can be used to measure both heteronuclear and homonuclear coupling constants. This experiment is simple and fast and can be performed easily, even on old spectrometers. Furthermore, the results are easy to interpret, even by inexperienced users.

Please credit this to the account of Cecil Dybowski.

Sincerely,

Martha Bruch

Keith Herzog

Cynthia McClure

Wesleyan UNIVERSITY

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DEPARTMENT OF CHEMISTRY

Conversion of VNMR & Felix Spectra into Adobe Illustrator Format

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

Wednesday, June 1, 1994
(received 6/6/94)

Dear Barry:

We like to be able to extensively annotate our spectra for publication and slides. The best program for doing this, in our opinion, is the Macintosh version of Adobe Illustrator. However, the plot output of Varian VNMR or Biosym FELIX can not be directly put into Adobe Illustrator format. In a previous communication we showed how multiple conversions could be used to accomplish this. Recently we have found a shareware program which does the conversion quite nicely in a single step as well as a commercial program which works even better.

HPGL method. Save HPGL format plots from either VNMR or FELIX. These are then sent via ftp to a Macintosh. The shareware program EPSfilter2.1a1 (copyright by Bryce Fowler and available on any of the Merit Macintosh sites on the net- use Mosaic or its equivalent to go to University of Michigan GOpherBLUE [gopher.archive.merit.net/11/.software-archives/] and follow the links to the graphics section) converts HPGL to Adobe Illustrator. Conversion of a typical 2D spectrum requires a minute or two. The program claims to convert postscript to Illustrator but this feature does not work in our hands.

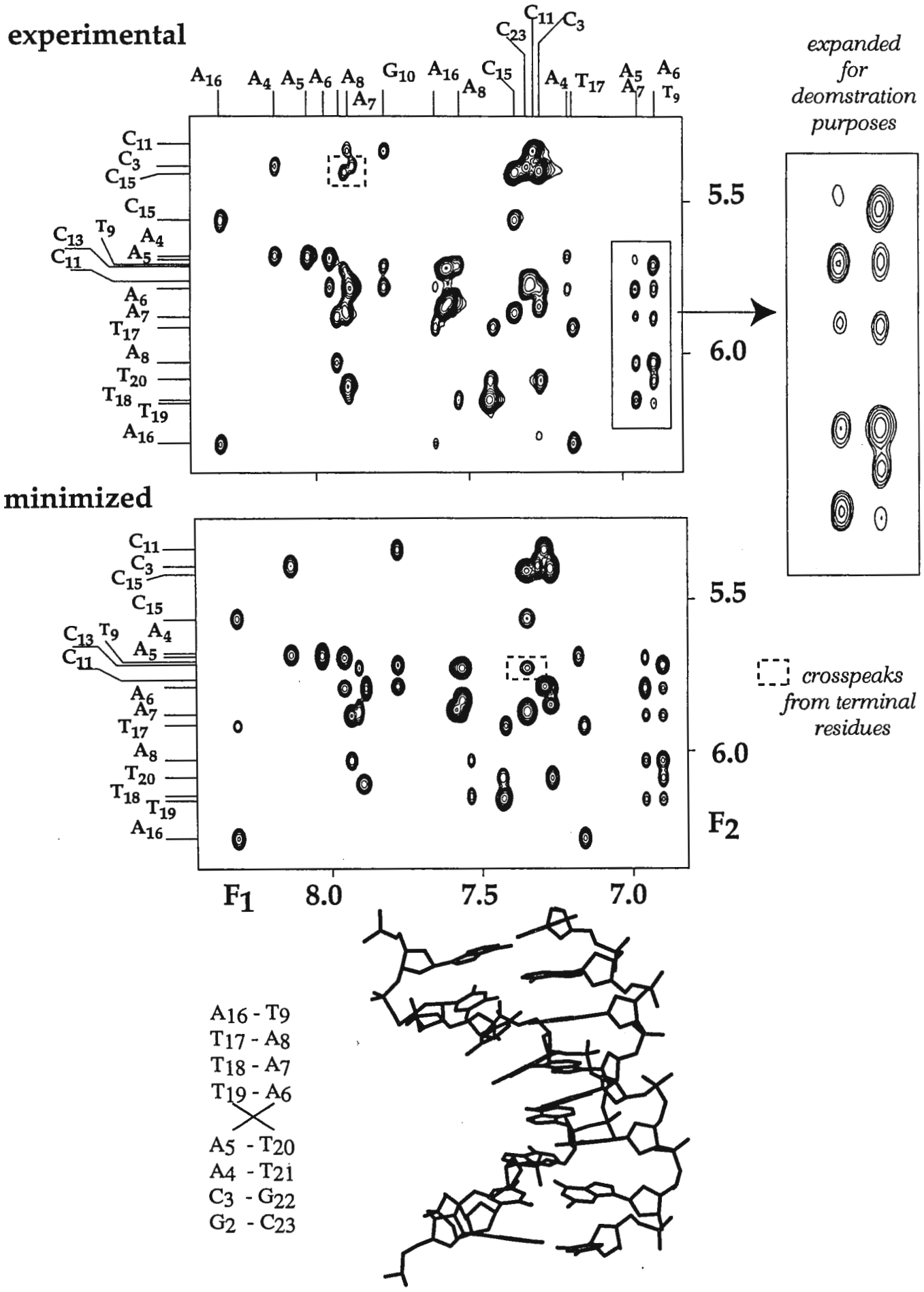
Postscript method. Save postscript format plots and then ftp these over to the Macintosh. Adobe Acrobat Distiller converts the postscript files into "portable document format" which they call pdf. The pdf files can be opened and edited by Illustrator. This route eliminates the editing of the axis and so on required in the HPGL route. Distiller is supplied with Illustrator 5.5.

We have also used IslandDraw on a SUN to do the annotations. In our opinion this program is slow and awkward compared to Illustrator. We also tried out Illustrator on the SUN but found this to be slow and awkward compared to the Macintosh verstion.

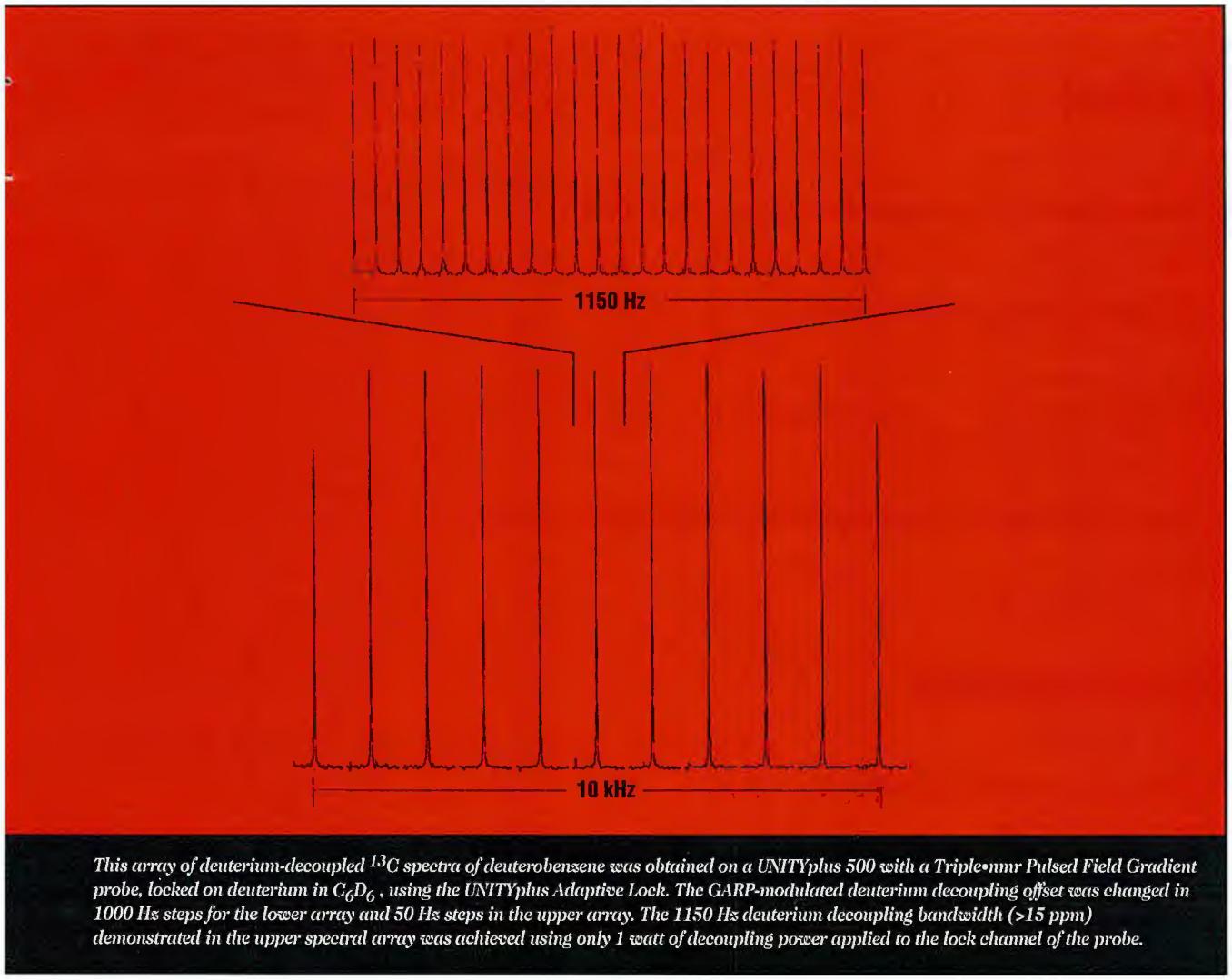
The attached spectra compare the experimental data on a duplex DNA containing an A-tract with the data predicted by a structure obtained by restrained molecular dynamics. This shows the combination of material from various sources into a single figure. The region of the DNA which bends is also shown along with the sequence of this portion of the DNA.

Sincerely,

Philip Bolton


experimental
minimized
expanded for deomstration purposes
crosspeaks from terminal residues
F1
F2
8.0
7.5
7.0
5.5
6.0
A16 - T9
T17 - A8
T18 - A7
T19 - A6
A5 - T20
A4 - T21
C3 - G22
G2 - C23

Maintain Deuterium Lock During Deuterium Decoupling



This array of deuterium-decoupled ^{13}C spectra of deuterobenzene was obtained on a UNITYplus 500 with a Triple•nmr Pulsed Field Gradient probe, locked on deuterium in C_6D_6, using the UNITYplus Adaptive Lock. The GARP-modulated deuterium decoupling offset was changed in 1000 Hz steps for the lower array and 50 Hz steps in the upper array. The 1150 Hz deuterium decoupling bandwidth (>15 ppm) demonstrated in the upper spectral array was achieved using only 1 watt of decoupling power applied to the lock channel of the probe.

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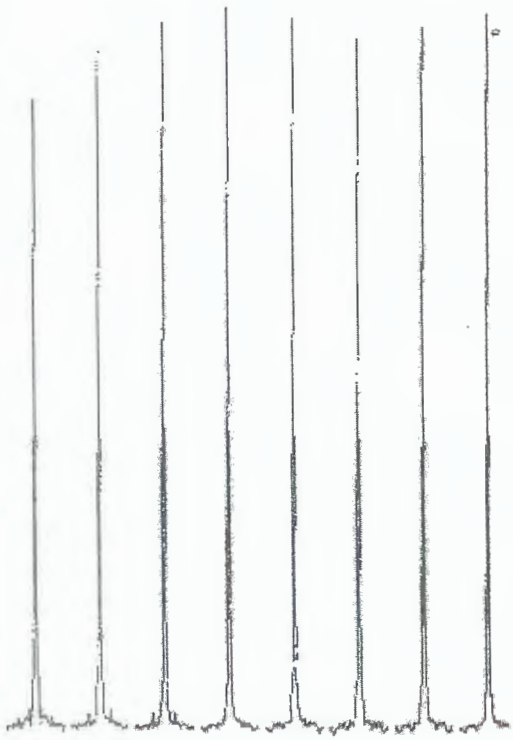

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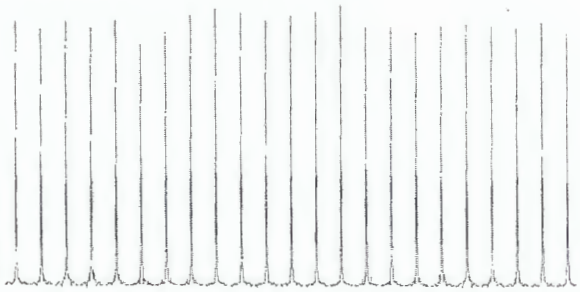

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May 19, 1994
(received 5/31/94)

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

More nmr measurements of flavone

Dear Professor Shapiro:

Flavone is the parent molecule or a widely distributed class of natural products which have been extensively studied. The total syntheses of 5,6,7,8--tetradeuteroflavone and 2',3',4',5',6'-pentadeuteroflavone that we described two decades ago, allowed us[1] to assign all hydrogen and carbon chemical shifts, as well as all hydrogen-hydrogen coupling constants.
As an extension of these isotopically labelled compounds, we now describe the measurements performed on 4-^{13}C-flavone, which has been prepared in our laboratory with an isotopical enrichment of 92.7%.

Heteronuclear carbon-hydrogen coupling constants:

The proton signals owing to the aromatic *ortho* disubstituted ring (A-ring) of flavone appear as a first order four nuclei spin-spin system for which all coupling constants are accurately known[1]. This four proton spin-spin system changes to a heteronuclear first order five nuclei spin-spin system, in which only ^{5}J(C-4,H-7) is missing, when the carbonyl carbon is labelled with ^{13}C. The corresponding spin-spin systems of the A-ring of the molecules are depicted in the Figure, which clearly shows the presence of the additional couplings in the case of the labelled molecule. These additional coupling constants are summarized in the Table.

Homonuclear carbon-carbon coupling constants:

The observed one-, two- and three-bond carbon-carbon coupling constants of the ^{13}C labelled molecule are summarized in the Table. The ^{1}J(C-4,C) values appear normal, as also is the magnitude of ^{2}J(C-4,C-8a): however ^{2}J(C-4,C-2) and ^{2}J(C-4,C-5) are absent, Regarding ^{3}J(C,C), the values also seem normal.

Induced isotope shifts:

Measurements on a mixture of flavones show $^{1}\Delta^{13}C(^{13/12}C)$ values of -15.5 and -16.2 ppb for C-3 and C-4a, respectively. In addition a $^{3}\Delta^{13}C(^{13/12}C)$ value of +3.7 ppb is observed C-1', which corresponds to a rather large three-bond induced isotope shift.

1) P. Joseph-Nathan, J. Mares, M.C. Hernández and J.N. Shoolery, *J. Magn. Reson.*, **16**, 447 (1974).

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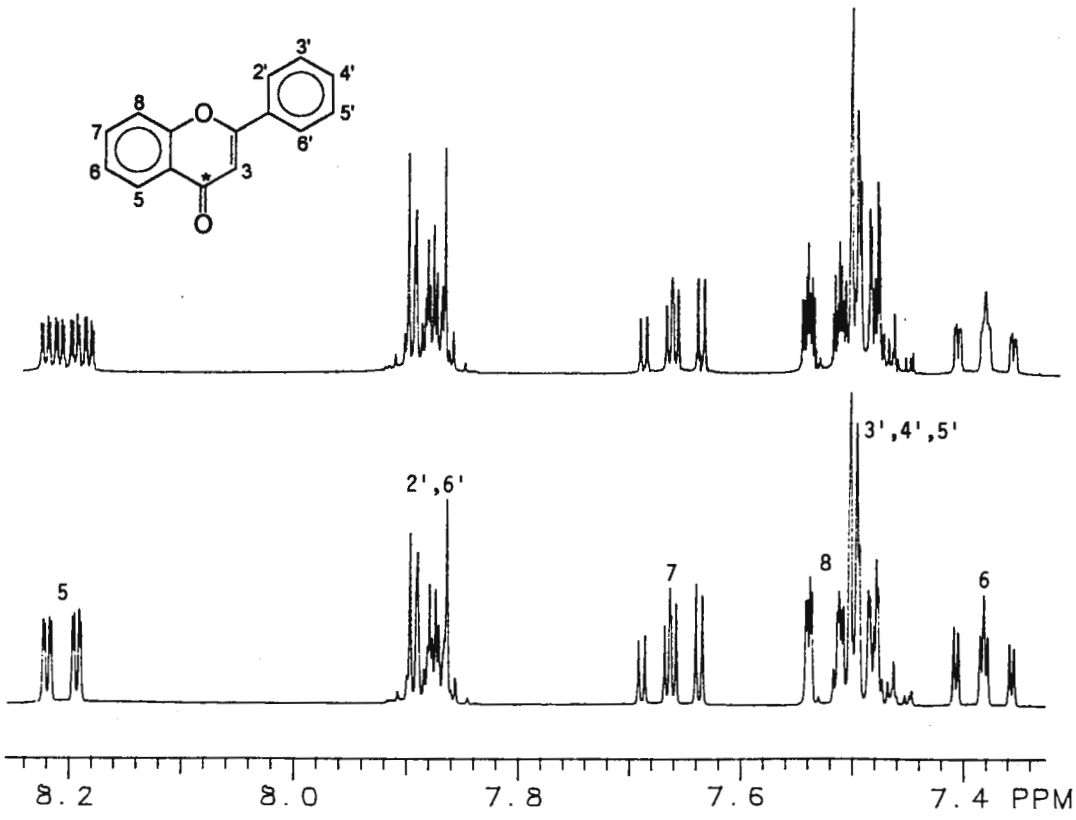

Homonuclear and heteronuclear coupling constants of C-4 in flavone-4-^{13}C.

n	C	J(Hz)	n	H	J(Hz)
1	3	57.7	2	3	2.0
1	4a	54.1	3	5	3.8
2	8a	1.4	4	8	1.6
3	6	3.5	4	6	0.3
3	8	2.0			
3	1'	3.0			

Sincerely yours,

Pedro Joseph-Nathan

Martha Sonia Morales-Ríos

Pharmaceutical Research
2800 Plymouth Road Phone: 313-996-7000
Ann Arbor, MI
48105

PARKE-DAVIS
People Who Care

(received 5/31/94)

Prof. Bernard L. Sharpiro, Editor
TAMU Newsletter
966 Elsinore Court
Palo Alto, CA 94303

Computer Program for Calculating Root-Mean-Square-Deviation to facilitate protein structure alignment

Dear Prof. Shapiro:

We have written a UNIX based C program to calculate the root-mean-square-deviation (rmsd) of a group of structures from PDB files. The user chooses a group of files by inputting the user specification as it is in UNIX: for example, test*.pdb would select all files test1.pdb, test2.pdb, test12.pdb, etc. Next, the user specifies which atoms are to be selected from the files: for example, C*, N* would give all atoms C, CA, CB, N, etc. and just * would give all atoms. Finally the user specifies the name of the output file to be generated.

The output file consists of the number of each residue and its rmsd. They are one space apart, so that the file may be exported to Microsoft Excel® or any other spreadsheet or charting program, where a chart can be produced showing the results.

The rmsd is calculated from either the average structure or any specified input structure. The following formula is used to calculate the average rmsd:

$$\text{rmsd} = \frac{1}{Ns} \sum_{0}^{Ns} \sqrt{\sum_{i=0}^{i<Na} \frac{(Xi-Xj)^2 + (Yi-Yj)^2 + (Zi-Zj)^2}{Na}}$$

where Xi, Yi, and Zi are the x,y, and z coordinates for each atom; Xj, Yj, and Zj are the average or reference atom's x,y, and z coordinates; Na is the number of atoms in this residue; and Ns is the number of structures.

The following is an example of the program using test pdb files.

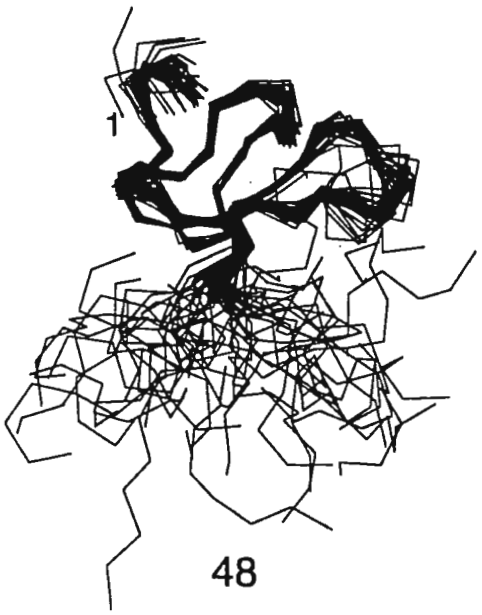

Figure 1. Example Structures for Calculation.

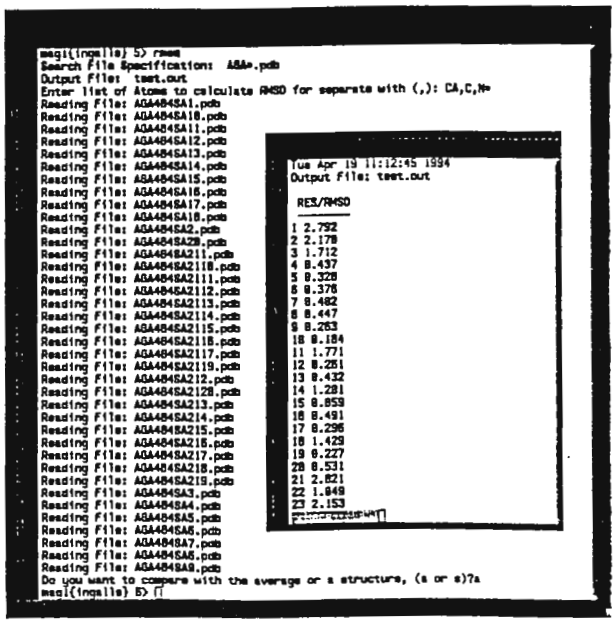

Figure 2. Example Input and Output.

Figure 1 shows the ensemble of structures used for calculating the rmsd. Figure 2 shows the input to the program for this example in the larger window and the smaller window shows the first 23 residues and their rmsd's of the output file. This output file was then read into Microsoft Excel®, a line chart of the average rmsd's for each residue was produced and is shown in Figure 3.

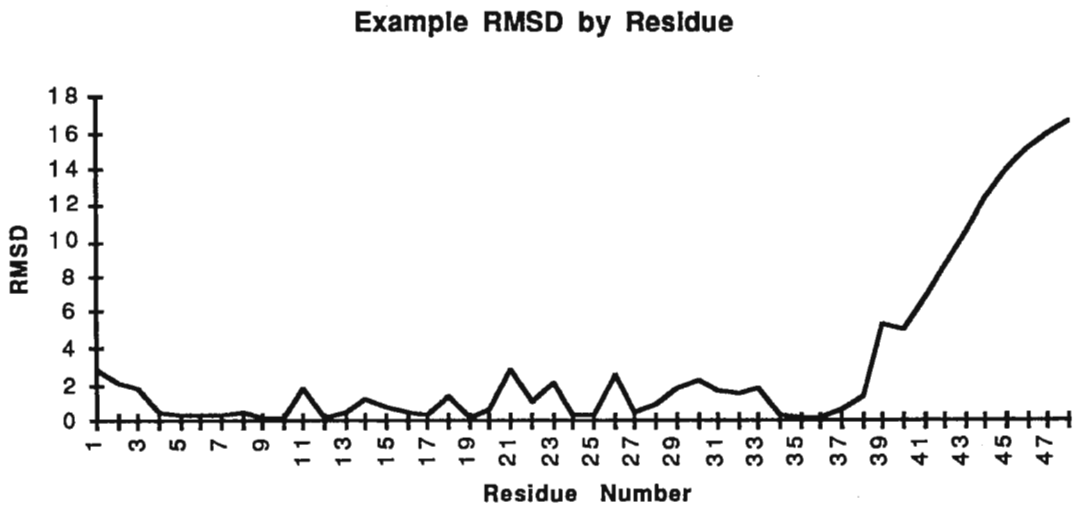

Figure 3. Example RMSD by Residue output from Microsoft Excel®.

The source code for this program is available by sending E-mail via internet to (ingalls@aa.wl.com) or by sending a written request to Warner-Lambert / Parke-Davis attn. Chris Ingalls, 2800 Plymouth Rd. Ann Arbor, MI 48105.

Please credit this contribution to D. Omecinsky.

Sincerely yours,

Chris Ingalls Michael D. Reily

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Prof. Dr. Bernhard Blümich

Tel. ++49 241 80 6421
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31. Mai 1994 (received 6/6/94)

RWTH Aachen / SB Chemie
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RWTH, Makromolekulare Chemie
Worringer Weg 1, D-52056 Aachen

Dr. B. L. Shapiro
TAMU NMR Newsletter
968 Elsinore Court
Palo ALto, CA 94303, USA

Relaxation Analysis of Aging in SBR

Dear Barry,

having moved to Aachen University of Technology about a year ago, we are continuing our work on the characterization of thermal oxydative aging of rubbers. The experiments are still being done at the Max-Planck Institute for Polymer Research in Mainz, until the new equipment arrives in Aachen. The basic idea is to exploit the manifold of NMR parameters to generate image contrast. In the past, we analyzed magnetization decay curves for each pixel in terms of a single relaxation time assuming exponential relaxation. This, of course, is a rather crude simplification. In fact, G. Simon and H. Schneider have published various papers on relaxation of transverse magnetization in cross-linked rubbers, where they observe a non-exponetial decay, which can be parametrized in terms of a Gaussian contribution and two Lorentzian ones (see e.g. *Makromol. Chem. Macromol. Symp.* **52** (1991) 233). The Gaussian part derives from the motion of the inter cross-link chains, a slow Lorentzian from motion of dangling chains, and a very slow one from the sol content of the rubber. The latter often is too weak to be observable. From the time constant of the Gaussian decay quantitative values of the cross-link density can be derived with very good results for a variety of rubbers. For this reason, we wanted to apply the Simon-Schneider analysis in our investigations of aging in rubbers.

Taking SBR as a technologically interesting rubber, because it is car-tire tread material, we found rather rapid decays. Therefore, the beginning of the decay could not be determined, as it falls within the experimental deadtime from pulses and switching gradients. Consequently the Gaussian component could not be analyzed in a straight forward manner. We ended up defining a cross-link parameter δ, which measures the ratio of the Gaussian amplitude I' to the total magnetization amplitude I at time Δt shifted from zero time (Fig. 1a). This parameter varies nicely over the cross section of and aged SBR sheet as one starts at $\Delta r = 0$ in the center of the sample and moves towards

the surface at Δr = 700 µm (Fig. 1b). By measuring the NMR cross-link parameter δ for a series of SBR samples which differed only in the cross-link density and correlating it with standard torque data, which are used in the rubber industry to characterize the cross-link density, we found a linear relationship (Fig. 1c). This demonstrates, that not only the time constant of the Gaussian relaxation component, but also its amplitude are directly related to the cross-link density. It seems to us, that the relative amplitudes are in fact easier to evaluate than the time constants. Further work on the multi-parameter analysis of relaxation in rubbers for the characterization of aging processes is in progress.

With kind regards,

Bernhard Blümich

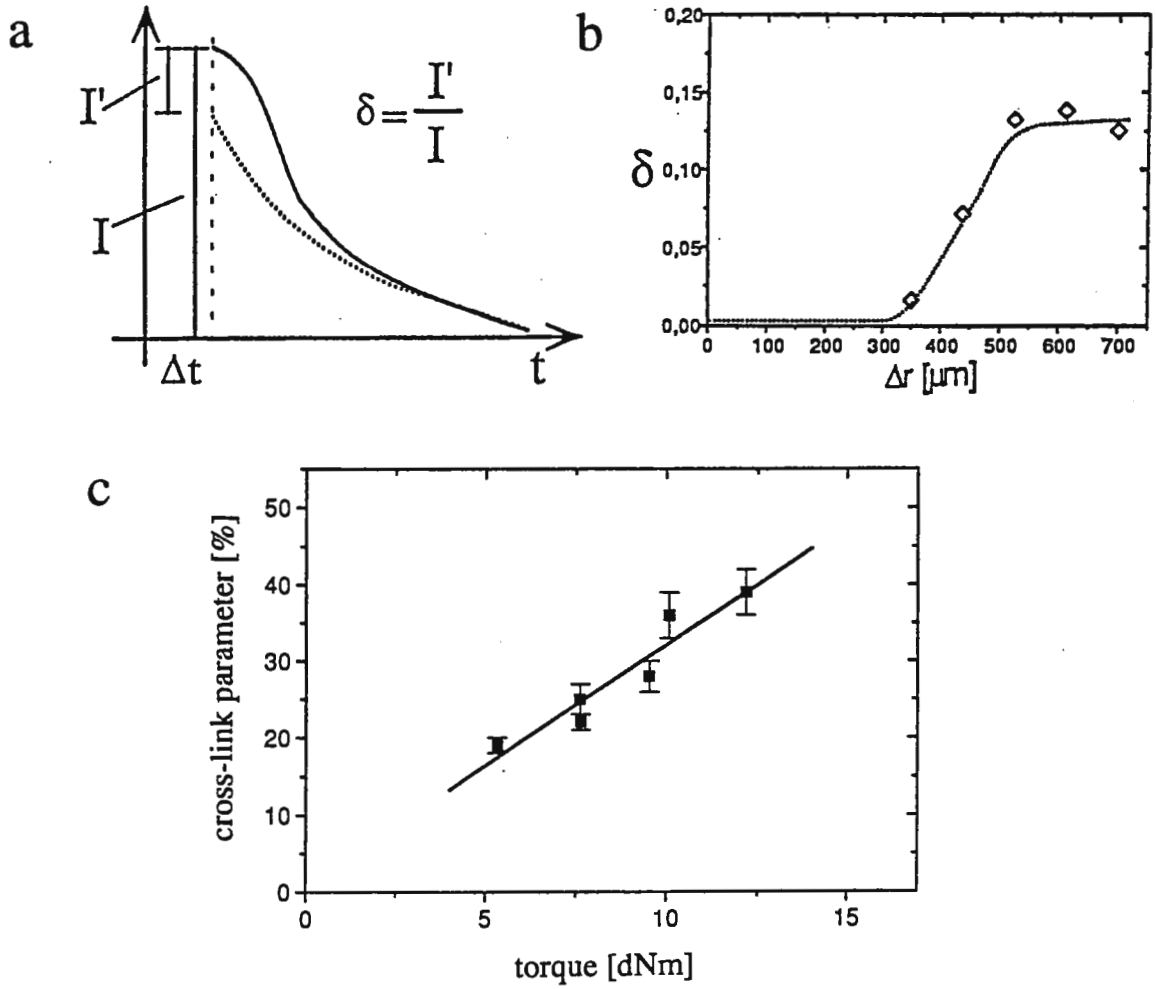

Fig. 1: Analysis of thermal aging in SBR. a) Definition of the cross-link parameter δ in terms of the amplitude of the Gaussian relaxation component. b) Variation of δ over half the diameter of a sheet of SBR which has been aged in air at elevated temperature. c) Correlation of the cross-link parameter with torque values for SBR samples with different cross-link densities.

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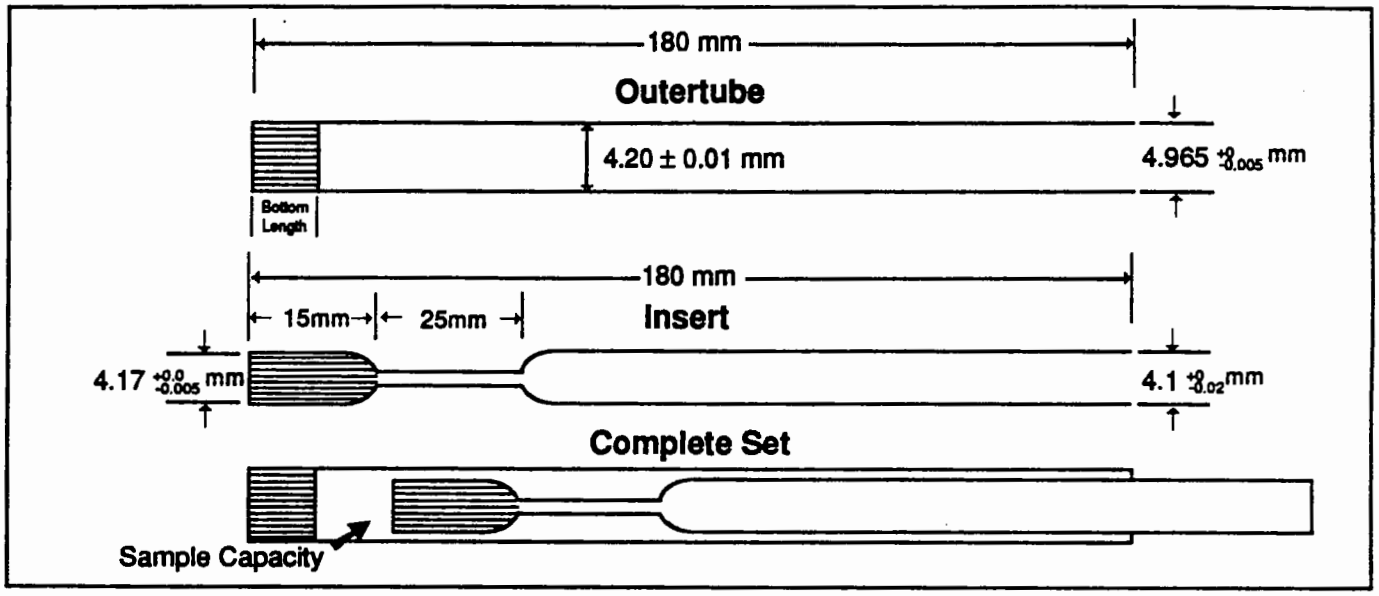

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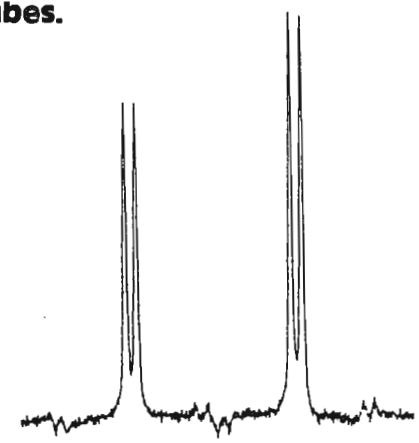
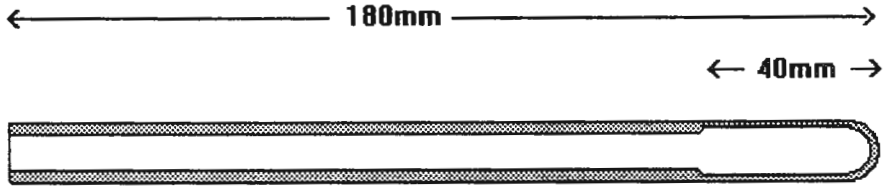


Fig.1a Fig.1b

The spectra of 20mm sucrose in D_2O were obtained with a single scan without apodization prior to Fourier transformation on a Bruker AMX-600 spectrometer at 298 K. By using Shigemi high quality 5mm standard tube (Fig.1a) and the Shigemi highly sensitive thin wall 5mm tube (Fig.1b), the spectra confirms a sensitivity enhancement of about 10%.



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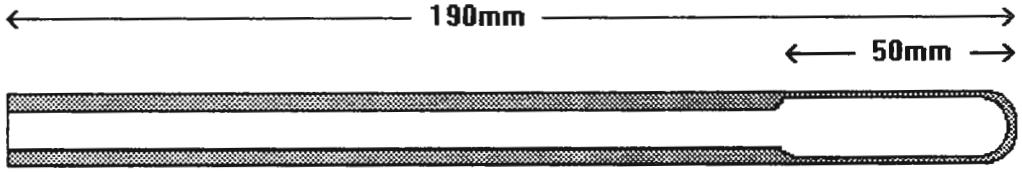

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Dr. Bernard Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303

May 20, 1994
(received 5/23/94)

Title: HIGHER C-13 SENSITIVITY (A RECORD ?)

Dear Dr. Shapiro,

I have evaluated the SHIGEMI 'symmetrical NMR microtube' for C-13 applications with limited sample quantity. These sample tubes are claimed to have a magnetic susceptibility which matches that of D2O. I used the BMS-005V sample tube for the results shown here. The spectra are from a sample of 400 µg sucrose in 200 µl D2O.

SEARLE An acquisition time of 993 ms, with no relaxation delay, was used to acquire 3600 scans in one hour.

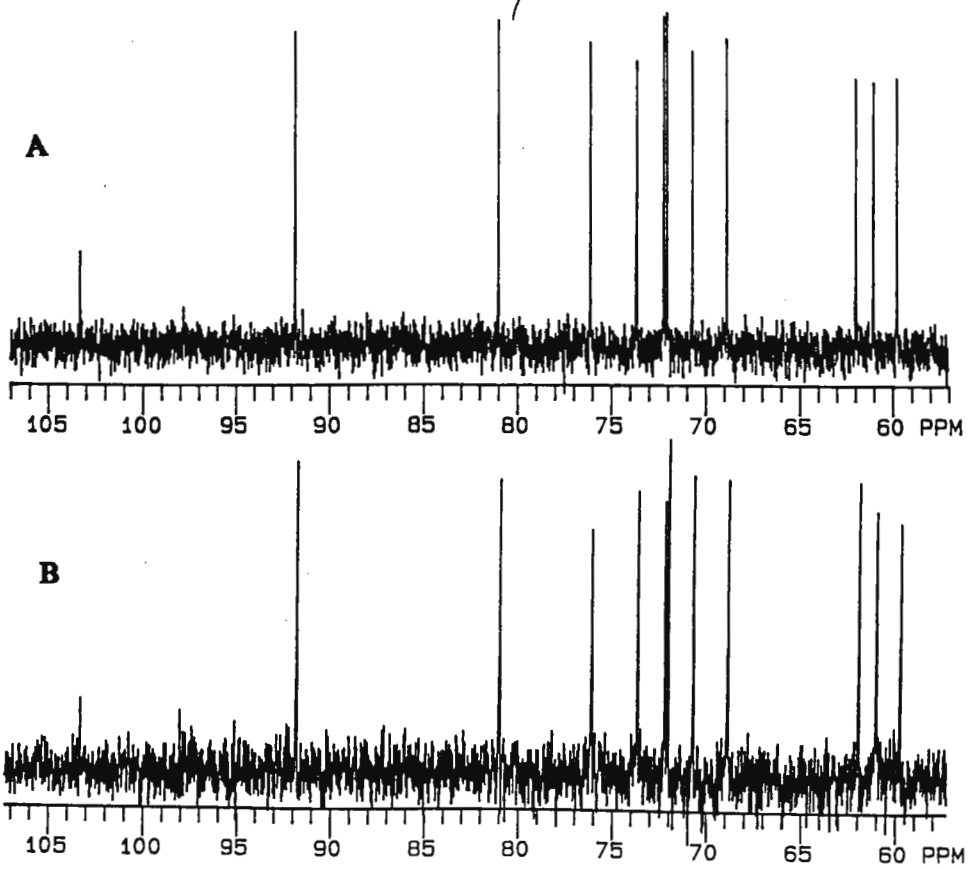
I used less than a 90 degree flip-angle in order to see the anomeric signal, in this very dilute solution.

A) is from our VXR-500 using a 'switchable' probe.

B) is from our VXR-400 using a 'switchable' probe.

Regards,

Robert W. Dykstra



Carleton UNIVERSITY

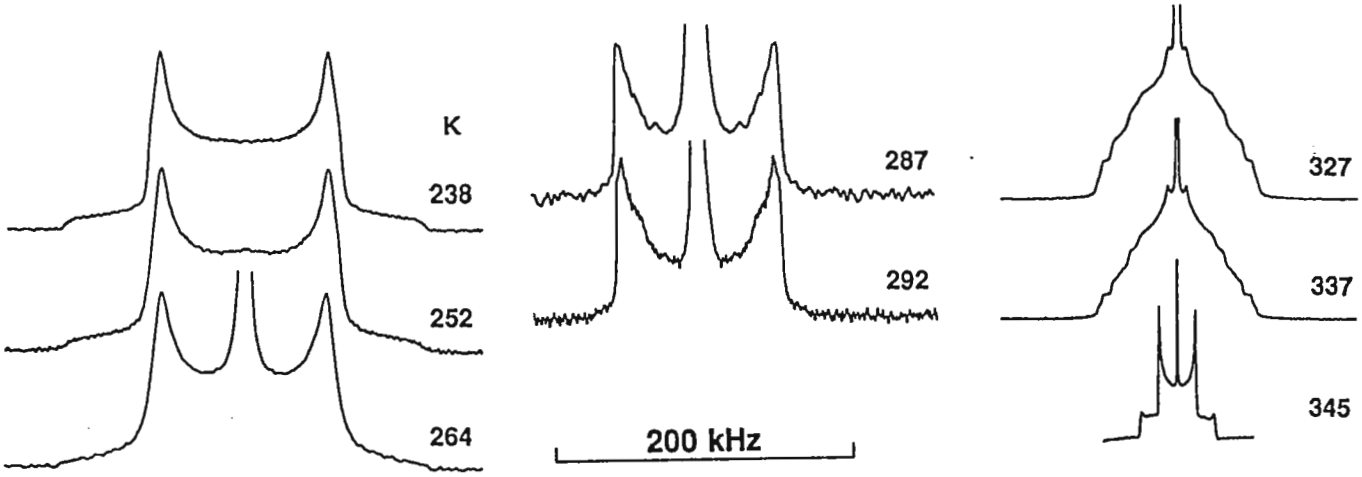
Department of Chemistry
203 Steacie Building
1125 Colonel By Drive
Ottawa, Canada K1S 5B6
Tel: (613) 788-3841
Fax: (613) 788-3749

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

May 31, 1994
(received 6/6/94)

Title: Solid State Molecular Motion In 15-crown-5.NaI As Studied By ^2H NMR

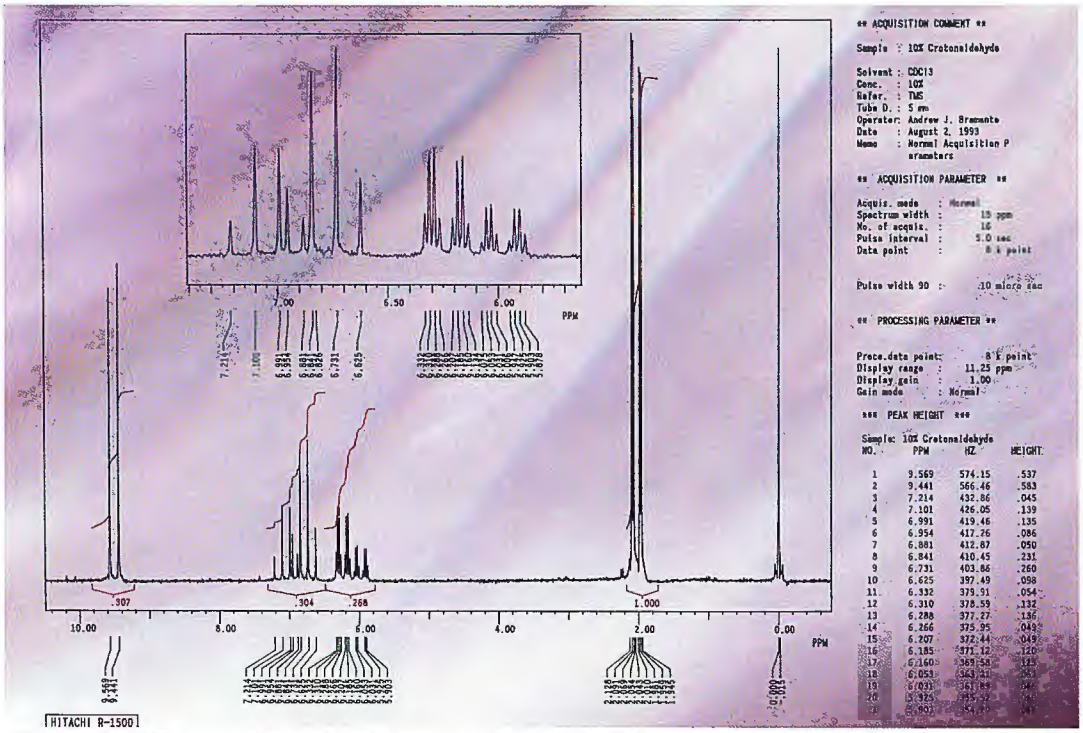
PhD student Marielle Gerzain has synthesized 15-crown-5.d8 and we are in the process of examining the large ampltitude motion of the macrocyclic ring in a series of 15-crown-5 complexes. Below are shown some representative solid state ^2H spectra of the NaI complex over a range of temperatures. The static lineshape is not reached until about 250K. Also we have detected a phase transition near 314K which has been verified via Differential Scanning Calorimetry.



Although the detailed analysis of the lineshapes is not complete, it appears that there is a "merry-go-round" type motional situation here, somewhat akin to what we found in some 18-crown-6 complexes a few years ago (1).

1. C.I Ratcliffe, J.A. Ripmeester, G.W.Buchanan, J.K. Denike. JACS 114, 3294 (1992)

G.W.Buchanan
Professor of Chemistry
Director, Ottawa-Carleton Chemistry Institute



This R-1500 FT-NMR spectrum of crotonaldehyde represents a 16 pulse acquisition; each pulse was 10 µsec with a pulse interval of 5 seconds.

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May 27, 1994 (received 6/4/94)

NMR Spectroscopy of Supercooled Sugars

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

Dear Dr. Shapiro,

At ambient temperatures, the hydroxyl protons of carbohydrates in aqueous solutions are in fast chemical exchange with bulk water protons, thus escaping NMR observation and subsequent broader attention. Several researchers, including ourselves, have attempted to overcome this problem by dissolving carbohydrates in mixed solvents (water/acetone, water/methanol) with the aim of slowing down the chemical exchange by lowering the temperature to approximately –10°C while retaining aqueous conditions. The question remains, however, the degree to which these conditions differ from a pure water environment. We report here the observation of sugar hydroxyl proton resonances under supercooled conditions, employing pure water as a solvent; their patterns are distinct from those observed in mixed solvents. To supercool carbohydrates in water we are currently using capillary tubes (1.5 mm i.d.; Kimble Products) which allow to reach temperatures close to –18°C. At this temperature and pH~6.7, the exchange rates of the sugar hydroxyl protons with water slow down to a few exchanges per second. This allows us to obtain 2D scalar, chemical exchange or dipolar correlated spectra. An example of a 2D COSY spectrum obtained on supercooled sucrose is shown in Fig. 1. NOESY experiments performed on some supercooled di- and trisaccharides enabled us to confirm that many of the intramolecular hydrogen bonds inferred from their crystal structures persist in solution [Poppe & Van Halbeek (1994) *Nature Structural Biology* **1**(4), 215-216]. We are hopeful that these "cold" experiments will permit the examination of the solution structure and solvation of carbohydrates in more detail than formerly possible. This kind of research will undoubtedly benefit from current developments in microprobe design. Unhindered by difficulties in securing the necessary amount of sugar material, our experiments, thus far, have been performed with 5-mm probes; those conditions result in a slow radiation damping rate at the cost of low signal-to-noise ratio in the spectra.

Sincerely,

Herman van Halbeek

Leszek Poppe Herman van Halbeek

CCRC Building: 220 Riverbend Road, Athens, Georgia 30602-4712 USA
CCRC Telephone: 706-542-4401 Facsimile: 706-542-4412
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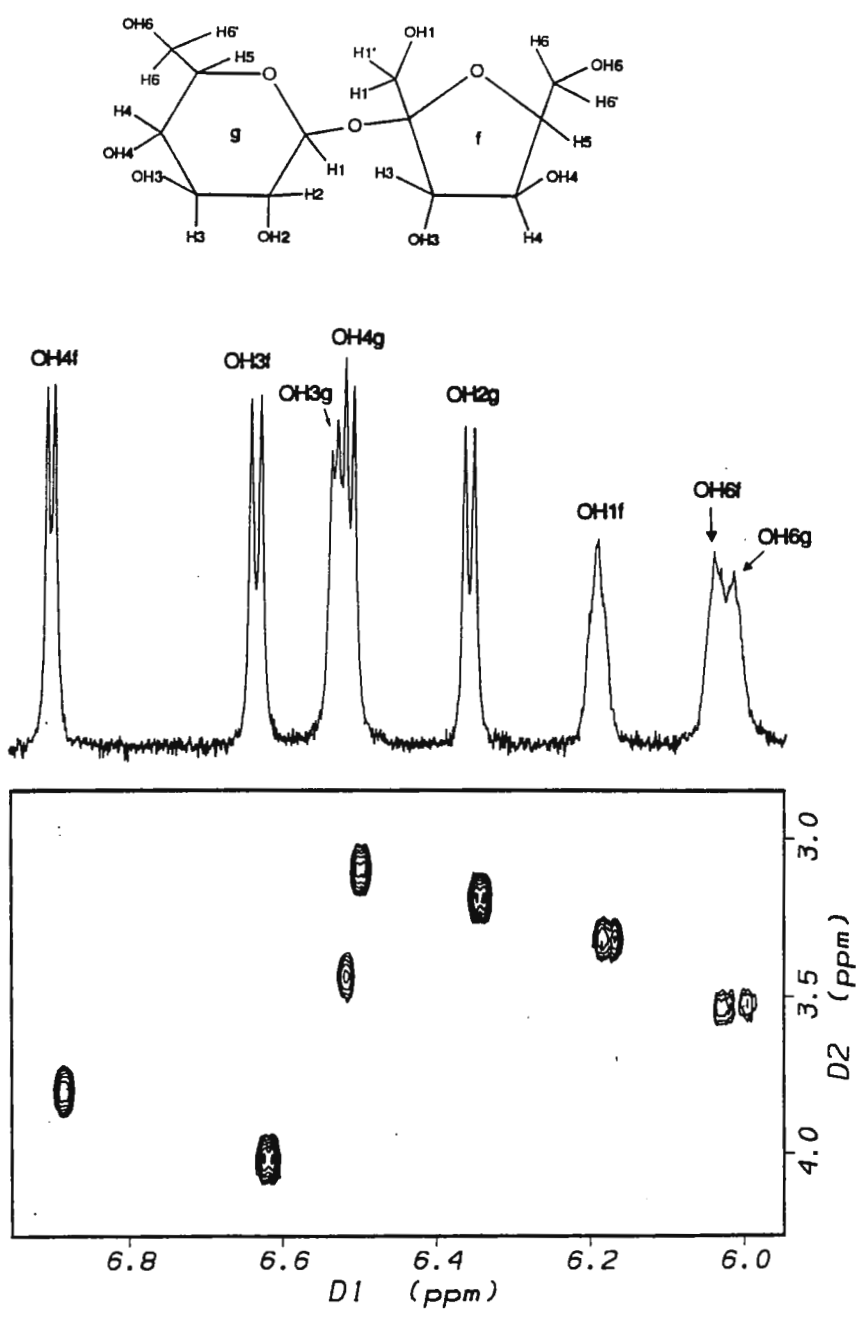

Figure 1 ^1H spectra of sucrose in water (90% H_2O/10% D_2O) at -17 °C using Jump-Return water suppression technique. The bottom spectrum is the expansion of 2D COSY spectrum.

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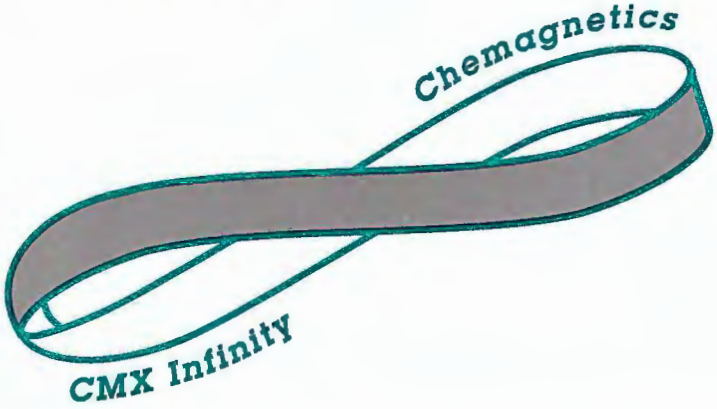

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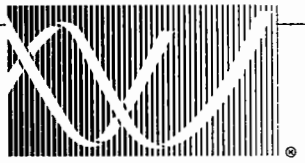


C A B M

CENTER FOR ADVANCED BIOTECHNOLOGY AND MEDICINE
679 Hoes Lane • Piscataway, NJ 08854-5638

Protein NMR Laboratory

June 14, 1994
(received 6/18/94)

Dear Dr. Shapiro,

GRIM REAPER: A UTILITY FOR UNIX DISK SPACE MANAGEMENT

Disk space, or lack thereof, is a constant problem in a multiuser protein NMR laboratory environment. Although the majority of disk space available to lab members is considered temporary data storage space, these data partitions seemed to always be full. We considered fully automated mechanisms for deletion of old files, but decided against it since it seemed arbitrary and too draconian. Neither of us felt comfortable with the idea of the computer deciding which files to delete. Instead we decided in favor of a compromise between fully automated deletion and manual deletion of files by utilizing a process that automatically selects files as candidates for deletion, with deletions occurring only when a normal non-privileged user runs a separate interactive program. This allows human judgement to remain in the file deletion process but minimizes the time involved in finding the biggest and oldest files.

Here are the specifications for our system design:

- All file ages refer to the last access or modification time, whichever is later.
- Only files in specific disk partitions used for temporary data storage are candidates for deletion.
- Any files younger than 10 days should not be considered.
- Any files older than 30 days are deletable.
- Between 10 and 30 days, the largest files are deletable.
- The identification of candidate files for deletion is automatic but actual file deletion only occurs with a "y" or "n" choice typed by a (non-super) user.

The resulting "reaper" utility has the following three parts: reaper-candidate, reaper-index and grim-reaper (with the name "reaper" itself unfortunately already taken for a Unix system command). Two of these, reaper-index and grim-reaper, are written in the C language, with the executables installed in a standard location, such as /usr/local/bin. The reaper-candidate utility is a small shell script which is run nightly by cron and generates the list of candidate files. It uses reaper-index to generate in integer index for each file which is then sorted in descending order. The reaper-index utility generates the index using the weighted sum of the file's access time and size. The

grim-reaper utility is the process that has file access and deletion privileges as "super user" but is run by a normal user and allows only the files selected by reaper-candidate to be deleted. All file deletions with grim-reaper are logged using the UNIX syslog facility with a message indicating the date, file name and user who performed the deletion. The reaper-index program takes file names as an argument and returns a two column list with an integer index and filename. For efficiency, it ignores all files smaller than a particular size. For very old files, the change in the index with time simulates the growth of a colony of bacteria.

Below is an example of the interactive use of grim-reaper to perform the file deletions.

```
> grim-reaper

Considering file "/tmp4d/data/celda/exp2/datdir/data"
-rw-rw-r--   1 celda    nmr      4194784 Feb  1 10:36
/tmp4d/data/celda/exp2/datdir/data
DELETE IT? (y, n, q): n

Considering file "/tmp4d/data/lwang/cspb_2/b_021/b_21.out.Z"
-rw-rw-r--   1 hli      nmr       760643 Feb  1 13:04
/tmp4d/data/lwang/cspb_2/b_021/b_21.out.Z
DELETE IT? (y, n, q): y
executing /bin/rm -f /tmp4d/data/lwang/cspb_2/b_021/b_21.out.Z

Considering file
"/tmp3d/data/chien/cc.I.086.msx1.3dnoesyhsqcpfg.fid/fid"
-rw-r--r--   1 rios     nmr      75729044 Apr 20 20:56
/tmp3d/data/chien/cc.I.086.msx1.3dnoesyhsqcpfg.fid/fid
DELETE IT? (y, n, q): q
```

The result is deletion of an old 760K byte file.

One basic policy that allows this to work is that *all* primary data sets (i.e. time domain data sets) are backed up by users onto optical media within one or two days of data collection. These can then be rapidly read onto the system for processing and analysis. Of course, people in the lab have now learned to "touch" all files read onto the system from optical disks or magnetic tape to ensure that they are marked with a date corresponding to their latest use on the system. They also are careful (we hope!) to back up important processed data sets before they leave the lab for more than 10 days.

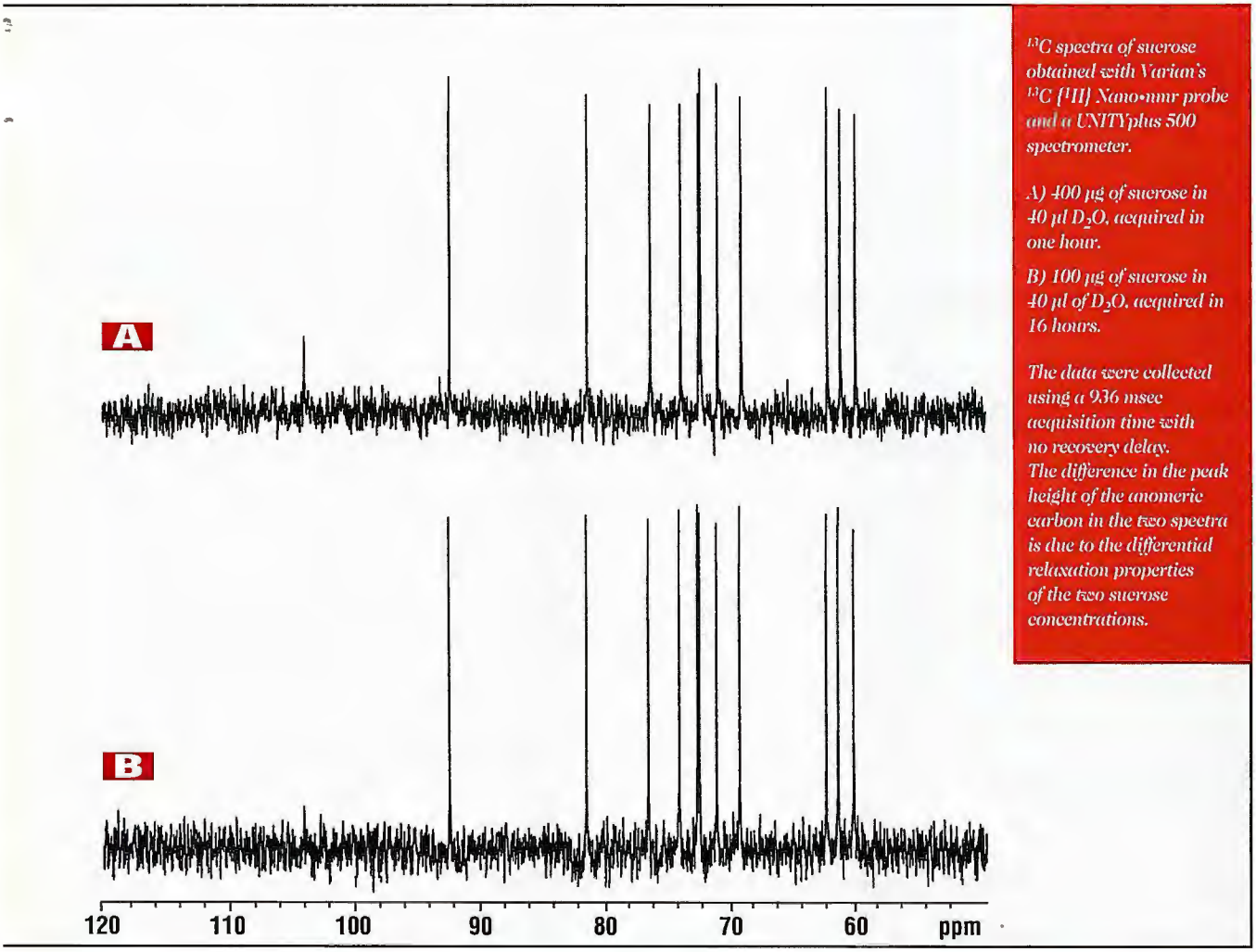
Any laboratory using large temporary data files, including both NMR and crystallography groups, should find reaper to be a useful utility. The programs and shell script are available by electronic mail request.

Sincerely,

George Elkins
elkins@nmrlab.cabm.rutgers.edu

Gaetano T. Montelione
guy@nmrlab.cabm.rutgers.edu

Highest ^{13}C Sensitivity for the Smallest Samples



^{13}C spectra of sucrose obtained with Varian's ^{13}C {^1H} Nano•nmr probe and a UNITYplus 500 spectrometer.

A) 400 µg of sucrose in 40 µl D_2O, acquired in one hour.

B) 100 µg of sucrose in 40 µl of D_2O, acquired in 16 hours.

The data were collected using a 936 msec acquisition time with no recovery delay. The difference in the peak height of the anomeric carbon in the two spectra is due to the differential relaxation properties of the two sucrose concentrations.

Only from Varian's new ^{13}C {^1H} Nano•nmr Probe

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Varian's innovative ^{13}C[^1H} Nano•nmr probe now allows you to obtain the highest sensitivity ^{13}C spectra on microgram samples, with a sensitivity surpassing any other standard technology probe on the market.

The ultra-small sample volume of the Nano•nmr probe reduces solvent artifacts and permits exceptionally short pulse widths for uniform bandwidth excitation. The unique, leading-edge Nano•nmr probe spinning technology enables you to acquire high resolution, multidimensional ^{13}C spectra with ease.

Transcend the current limits of ^{13}C detection with Varian's ^{13}C {^1H} Nano•nmr probe and the UNITY*plus* spectrometer and expand your capabilities to include heteronuclear multidimensional structure determination of ultra-small, microgram samples.

For more information, contact the Varian office near you.

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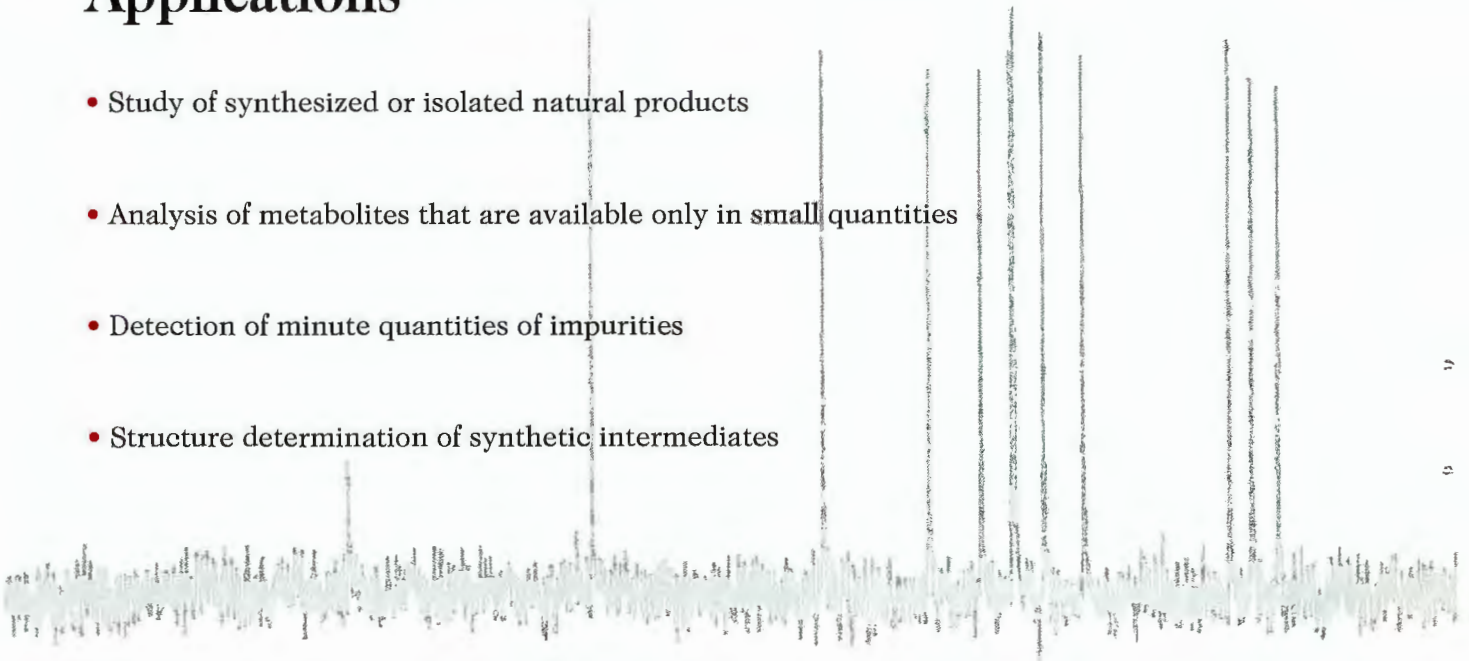
Varian's New ^{13}C {^{1}H} Nano•nmr Probe Expands Your Opportunities

Advantages

- Reach the highest sensitivity ^{13}C spectra on microgram quantities of sample
- Achieve uniform excitation over the entire spectral width with short pulse widths
- Acquire uncompromised high-resolution spectra with high effective dynamic range
- Obtain fewer solvent artifacts using the ultra-small sample volume
- Perform multi-dimensional structure elucidation experiments on ultra-small samples

Applications

- Study of synthesized or isolated natural products
- Analysis of metabolites that are available only in small quantities
- Detection of minute quantities of impurities
- Structure determination of synthetic intermediates



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May 3, 1994 (received 5/24/94)

Bernard L. Shapiro, Ph.D.
Editor, Texas A&M NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94503

Heteronuclear Nano-Probes™ but Not Quite Nanograms Yet

Dear Barry,

Well, don't have a coronary -- as promised, this is the work referred to in our last Newsletter contribution. As we noted in the introduction to our comments on the Varian homonuclear ($^1H/^{19}F$) Nano-probe™ we had also recently received a heteronuclear version of the probe which uses the same 40 μl cell for the acquisition of low level ^{13}C spectra. Never ones to be bashful about trying new hardware, the following represent the first results we were able to obtain with the probe and our impressions.

We elected, once again, to use the small alkaloid cryptolepine (MW 232 Da) as a model compound for the study. As a starting point we prepared a sample containing 400 μg (1.72 μmoles) dissolved in 40 μl of 99.96% d_6-DMSO (Cambridge). The ^{13}C reference spectrum acquired on this sample is shown in Figure 1 after 32 and 512 transients. You'll note on comparing the spectra that the protonated carbon resonances are all up and discernible in the 32 transient spectrum but that it takes more signal-to-noise (s/n) for any of the quaternary carbons to be discerned clearly. To be versatile, we would like a probe on which proton reference spectra can be acquired on the decoupler coil; the proton reference spectrum of cryptolepine is also shown as one of the traces presented in Figure 1 and was certainly acceptable for a 1 transient acquisition.

Versatility is clearly one of the best attributes of the Nalorac Z•SPEC® micro probes that we have been heavily utilizing in our laboratories. Hence, we were interested in pushing the Varian heteronuclear Nano-probe™ to see how it would perform with heteronuclear shift correlation experiments such as the "old standby" HETCOR experiment. Using the 400 μg sample of cryptolepine, a HETCOR experiment was set up to store data after 16, 32, 48, and 64 transients/t_1 increment. The data were acquired as 256 x 64 points with spectral widths of 2104 and 1175 Hz in F_2 and F_1, respectively. The 90° pulse widths were 6.6 μsec at tpwr = 55 for the X pulses and 27 μsec at a power of 61 for proton pulses from the decoupler. The data after 16 transients (20 min acq time) are shown in Figure 2A flanked by the proton and carbon reference spectra from Figure 1. Although there is some spurious noise present, the only noise response which might be misinterpreted as real is the response defined by several contours at F_1/F_2 frequencies of 8.94/130.61 (response denoted by *). Although this response is near the chemical shift of a proton and an unrelated carbon resonance, the peak is clearly spurious. Allowing the acquisition to complete the phase cycle (32 transients/file) affords the data shown in Figure 2B. It will be clearly noted that the offending spurious response is no longer observable. Clearly, the heteronuclear Nano-probe™ offers excellent submilligram sensitivity for ^{13}C-detected heteronuclear shift correlation experiments. Assuming the probe's response characteristics will be linear for samples down to the range of perhaps ~0.5-1.0 μmole, the probe offers excellent carbon sensitivity coupled with at least the capability of performing ^{13}C-detected heteronuclear shift correlation experiments for those labs that must have access to very low level carbon reference spectra. We still need to evaluate the probe's performance on 2D experiments with more challenging samples and for long-range heteronuclear shift correlation experiments such as CSCMLR[1] and FLOCK.[2]

Pushing the probe much further, we had occasion to acquire a carbon reference spectrum of an enzymatically-prepared sample of the epoxide metabolite of 1-ethylphenoxathiin 10,10-dioxide (BW1370U87).[3] The spectrum of 1-(2'-oxiranyl)phenoxathiin 10,10-dioxide, acquired overnight in 28,400, transients is shown in Figure 3. Overall s/n is approx. 5:1. All of the protonated carbons are readily identifiable. The quaternary carbons, given experience in the phenoxathiin series, are discernable but quite weak. Following the acquisition of an HMQC spectrum using a Nalorac

Z•SPEC® MID-500-3 μ inverse probe, which is shown in Figure 4, the sample was quantitated against a synthetically prepared reference standard and found to contain 30 μg of the epoxide (0.11 μ mole).

In summary, the new Varian heteronuclear Nano-probe offers the means of acquiring ^{13}C spectra on very small samples in quite reasonable periods of time. As such, the probe offers a superb complement to the capabilities of the Z•SPEC® micro inverse probes offered by Nalorac Cryogenics Corp. As we develop more experience with the use of the Nano-probes™ we'll continue to share this information with the readers of the Newsletter.

Best reagrds,

Gary E. Martin
Div. of Organic Chemistry

John P. Shockcor
Div. of Pharmacokinetics
& Drug Metabolism

Ronald C. Crouch
Div. of Organic Chemistry

References:

1. A.S. Zektzer, M.J. Quast, G.S. Linz, G.E. Martin, J.D. McKenney, M.D. Johnston, Jr., and R.N. Castle, *Magn. Reson. Chem.*, 24, 1083 (1986).
2. W.F. Reynolds, S. McLean, M. Perpick-Dumont, and R.G. Enriquez, *Magn. Reson. Chem.*, 27, 162 (1989).
3. J.P. Shockcor, R.M. Wurm, I.S. Silver, R.C. Crouch, and G.E. Martin, *Tetrahedron Lett.*, in press (1994).

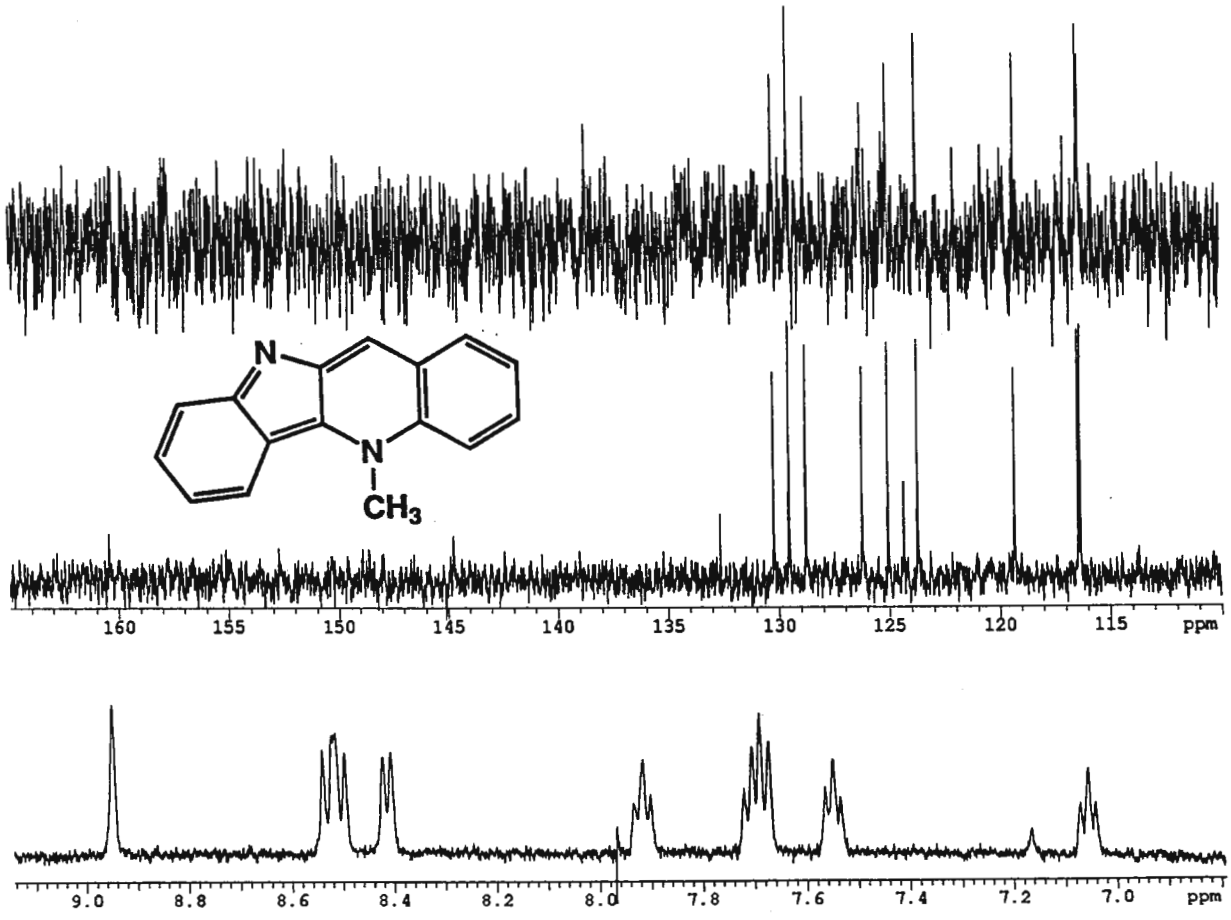


Figure 1. ^{13}C spectra of cryptolepine (MW 232 Da) acquired on a 400 μg sample (1.72 μmole) in 40 μl of d_6-DMSO. (TOP) Aromatic region of the spectrum after 32 transients; (MIDDLE) aromatic region of the spectrum after 512 transients; (BOTTOM) ^{1}H reference spectrum off the decoupler coil — 1 transient.

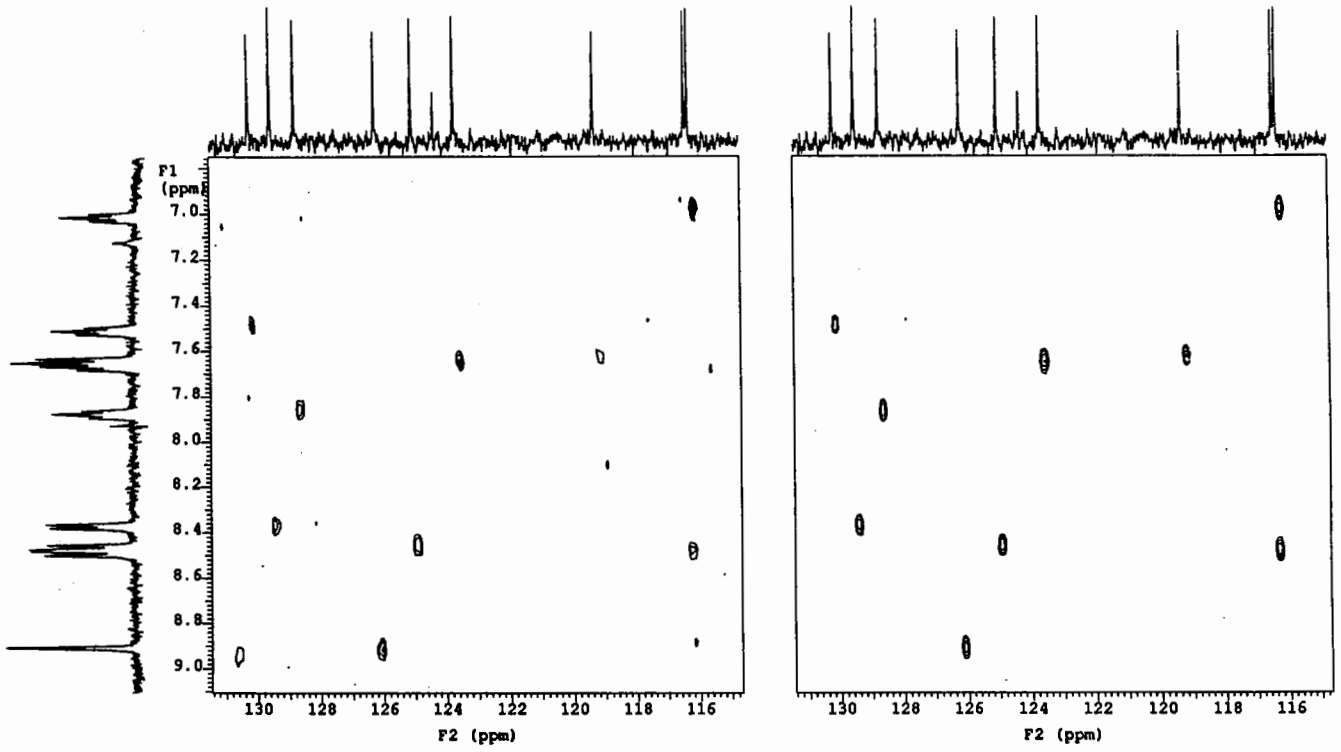


Figure 2. ^{13}C-Detected HETCOR spectra of 400 μg of cryptolepine in 40 μl d_6-DMSO. Left panel spectrum after 16 transients/file (20 min acq.); right panel spectrum after 32 transients/file (40 min acq.). The spectra are flanked by the proton and carbon reference spectra from Figure 1.

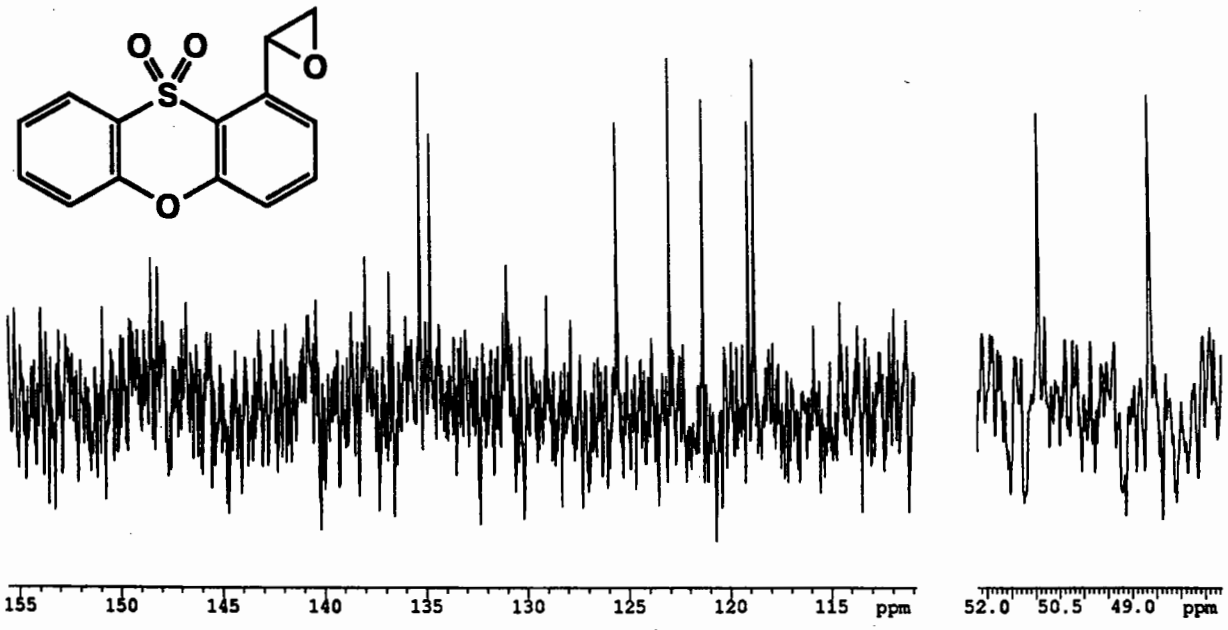


Figure 3. ^{13}C reference spectrum of 30 μg (0.11 μmole) of 1-(2'-oxiranyl)phenoxathiin 10,10-dioxide (BW1370U87) in 40 μl d_6-DMSO acquired overnight (28,400 transients) using the heteronuclear Nanoprobe™.

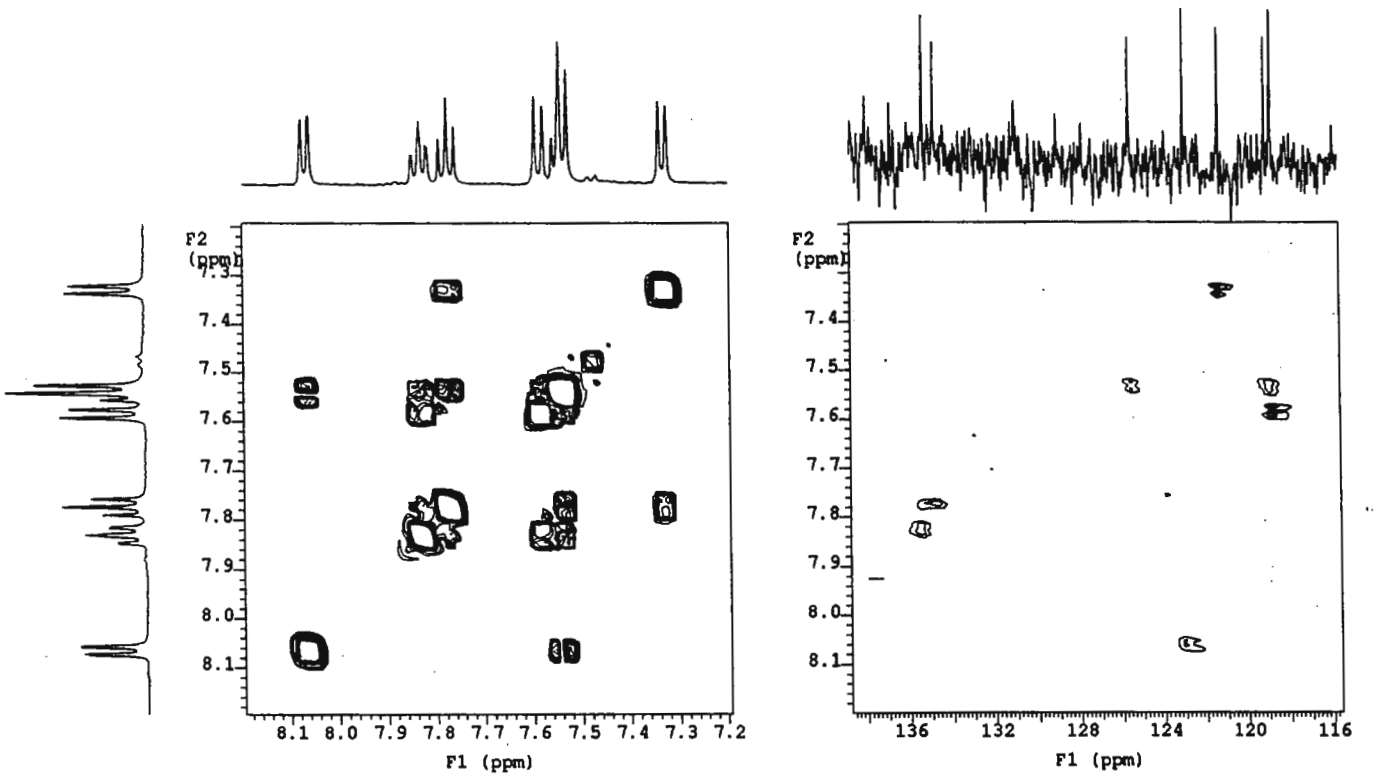


Figure 4. COSY and HMQC spectra of a 30 μg sample (0.11 μmole) of 1-(2'-oxiranyl)phenoxathiin 10,10-dioxide. The former (left) was acquired in 40 μl d_6-DMSO using a Varian homonuclear Nano-probe™ following the acquisition of the 13C reference spectrum shown in Figure 3. The latter (right) was acquired in 130 μl d_6-DMSO overnight using a Nalorac Z•SPEC® MID-500-3 micro inverse probe. The HMQC spectrum is flanked by the ^{13}C spectrum acquired using the Varian heteronuclear Nano-probe™ and a proton reference spectrum acquired with the micro inverse probe.

UNIVERSITY OF THE PACIFIC
College of the Pacific
Department of Chemistry

(received 6/23/94)

Dear Barry

Etoposide Hydration and Solution Conformation

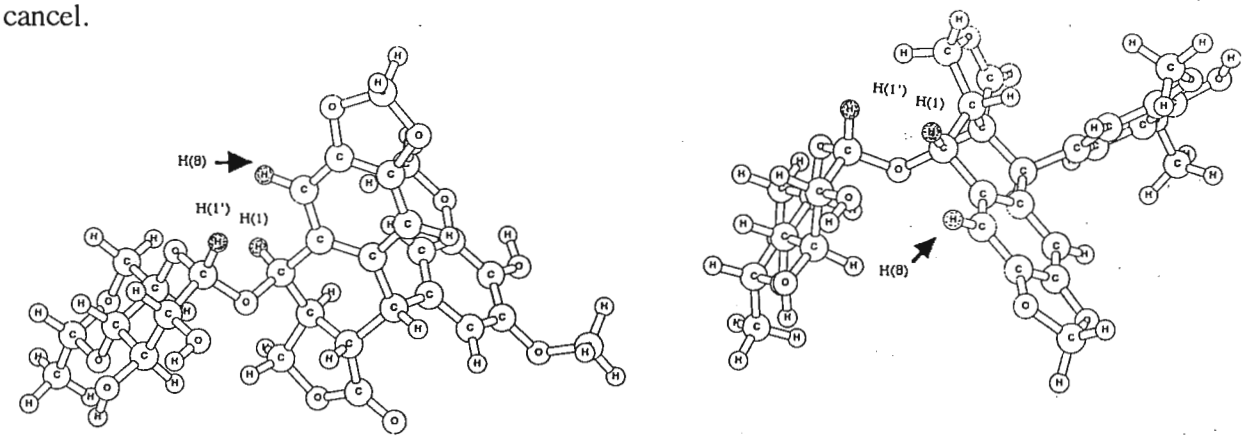
M. J. Minch, R. C. Vasavada, S. Patil, C. Brackett and B. Jasti
Departments of Chemistry, Medicinal Chemistry and Pharmaceutics
University of the Pacific
Stockton, CA 95211

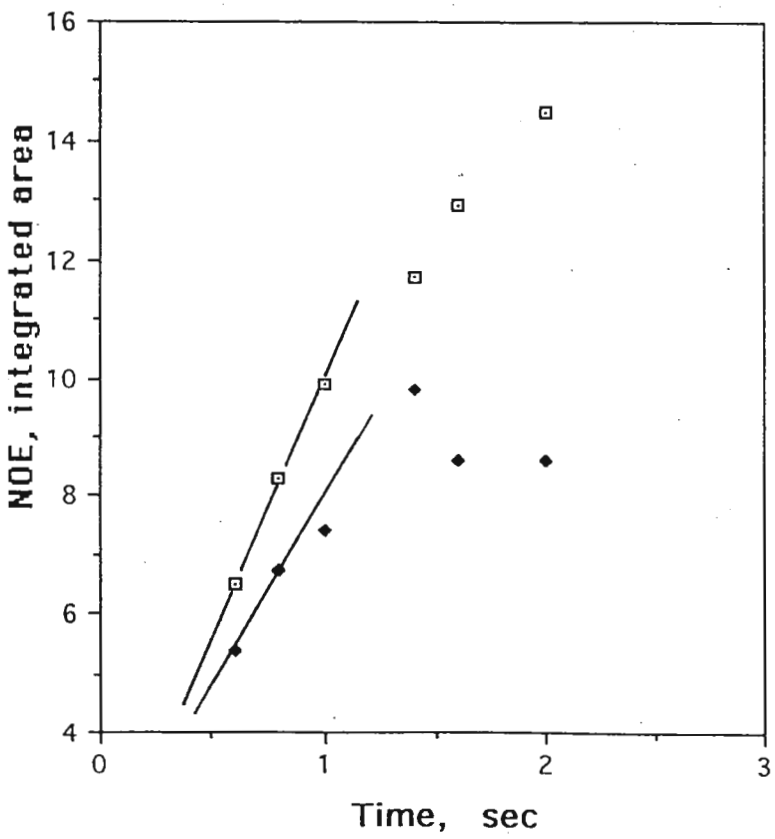
The antitumor drug etoposide has a structure in which two rigid ring systems are linked by a flexible glycosidic bond. A 4, 6-O-ethylidene β-glucopyranosyl group is linked to a substituted tetrahydronaphthalene system. MM2 molecular modeling of this compound indicates that two possible orientations (+178°, -99°) of the sugar ring system about the glycosidic dihedral angle [C(2')-C(1')-O-C(1)] have about the same energy. Since this angle serves as a central hinge between two large ring systems, variations in it markedly influence the overall molecular shape and any stereochemical fit between drug and receptor. Moreover we have found that the relative concentration of water to etoposide in $CDCl_3$ determines the chemical shifts of the anomeric proton of the glycosidic subunit and of the glycosidic OH protons. Dilute solutions of etoposide, regardless of water content, show sharp OH resonances as doublets. The OH resonances are both broadened and downfield shifted at higher concentrations (> 0.01 M) of etoposide in $CDCl_3$, especially if several equivalents of water are present. Since no other changes in chemical shift and coupling constants occur as a function of etoposide or water concentrations, these changes probably reflect changes in the relative orientation of the two ring systems and not changes in the conformation of either ring. We have been interested in using NOEs to learn about the average orientations of these ring systems with respect to each other. Since each ring system moves as a rigid unit, changes in the relative orientation of the ring systems will not alter intra-system NOEs but will change NOEs between neighboring protons on different ring systems. Consequently we have examined the ratio of the slopes of the NOE buildup curves for various pairs of protons. If proton A is irradiated then the ratio of the rates of buildup of NOEs for protons X and R are related to the distances between A and X, which are in different ring systems, and between A and a reference proton R within the same ring system. Since this latter distance is invariant to ring reorientation and can be calculated from model structures, it serves as a reliable ruler.

$$\frac{S_{A\rightarrow X}}{S_{A\rightarrow R}} = \frac{r_{AR}^6}{r_{AX}^6} * \left(\frac{J(\omega)_X}{J(\omega)_R} \right)$$

If the relaxation of the protons in either ring system are similarly governed by the overall tumbling of the molecule, then the spectral density terms cancel and relative distances can be calculated from the sixth root of the NOE ratio.

For example with wet concentrated etoposide we find that irradiation of the aromatic proton H(8) (see arrow) gives nearly equivalent buildup of NOE for H(1') and H(1), the protons on either side of the glycosidic bond. Similarly we see NOE buildup in the other two protons if either H(1') or H(1) are irradiated. We do not see NOE build up in carbohydrate ring protons if other protons in the tetrahydronaphthalene ring are irradiated, e.g. H(2) and H(3). This is consistent with the lowest energy structure (below left) and rules out the next most likely conformation (below right). We are currently applying this approach to more dilute and very dry solutions to ascertain what conformational changes accompany the striking changes in OH resonances. Temperature effects are also being studied to ascertain the level of motion about the glycosidic hinge and the validity of our assumption that the J(ω)s cancel.





EASTMAN

Research Laboratories
Kingsport, Tennessee 37662

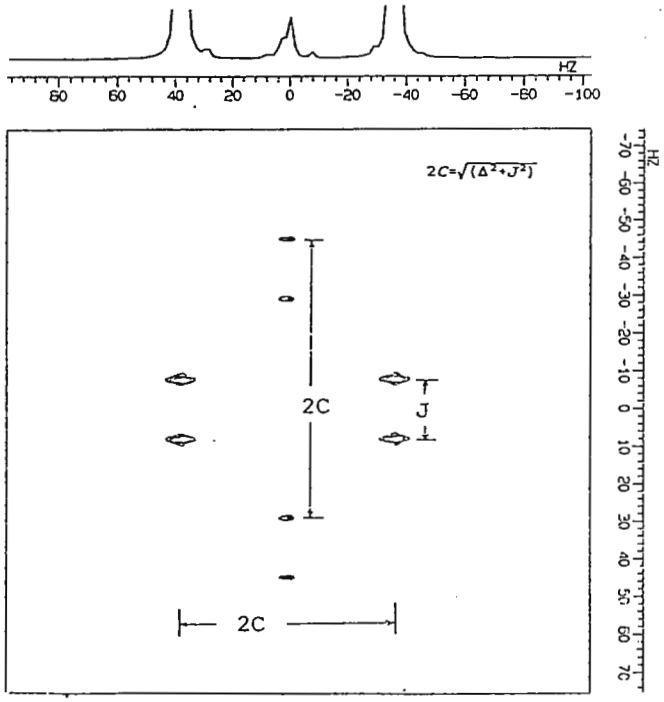
Eastman Chemical Company

June 6, 1994 (received 6/10/94)

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

Dear Barry,

Lately we have examined the 2D J-resolved spectra of molecules containing several strongly coupled proton systems. As is well known, there are additional peaks that appear in these spectra. Also, projections of the tilted spectra do not yield the exact chemical shifts of the coupling pairs. To better understand these spectra, a simple spreadsheet was set up to simulate the peak positions using the equations given in the literature (Bodenhausen and coworkers, Journal of Magnetic Resonance, 31, 75-95, 1978). The 2D J-resolved spectrum of a textbook AB system, citric acid, is shown in the figure. The spreadsheet allowed us to notice a few useful facts about J-spectra of strongly coupled systems. For example, note that it is possible to relate the separation of the outer peaks to the "length" of the center strip. This fact can be quite helpful in untangling a complex spectrum with several sets of strongly coupled spins.



In J resolved spectra of ABX systems, the center "strip" consists of 8 responses that often form a zigzag pattern vertically. The zigzag pattern results when there is a difference between the AX and BX coupling constants. In this case also it is possible to relate the separation between the outer responses to the height of the center strip. A more complete account of this work will be submitted to Concepts in Magnetic Resonance.

Mike

Michael D. Meadows
Senior Research Chemist
Eastman Chemical Company
phone: 615-229-8826
internet: mmeadows@emn.com

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

May 11, 1994
(received 6/2/94)

Dr. B.L. Shapiro
TAMU NMR Newsletter
966 Elisnore Court
PALO ALTO, California
U.S.A. 99303

Title: Cheaper fibre optic link

Dear Barry,

We recently updated our Bruker WM-360 to an AM-360. Since we already had a standalone ASPECT-1000 Data Station for off-line processing and archiving on optical disc, we elected to connect the new system to the Data Station's existing fibre optic network. In order to cut costs, we had our Campus Network Services (CNS) people supply and install the fibre optic cable.

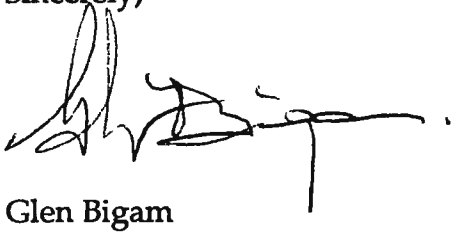
Since CNS would have had to order a minimum of 500 M ($2,800 Cdn) of the 4-strand 100 μ dia. fibre cable that Bruker uses, they chose instead to install the standard 62.5 μM cable used in the campus network. With an eye to future expansion, they installed 120 M of 12-strand 62.5 μM cable for about 1/3 the cost (~ $850 Cdn). We now have the option of adding two more instruments at a cost of about $400 each, which is the cost of the jumpers to convert from the standard ST to the SMA connectors used by Bruker.

Unfortunately, it didn't work. A little consultation with the Hewlett Packard Optoelectronics Design Catalog convinced us we needed more light. So we replaced the existing transmitter diodes (HFBR-1202) with higher power ones (HFBR-1454 — about $30 each).

Unfortunately, this didn't work either. This time, a little calculation showed that we had too much light. Reducing the forward current in the transmitter diodes from 100 ma to 60 ma cleared up all the problems and the system works very well. As a bonus, CNS tells us that we should be able to operate with a cable length up to 1 km if we ever need it.

Please credit this to Tom Nakashima's subscription.

Sincerely,



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Glen Bigam

MICROSAMPLE PROBES

In the past various attempts have been made in the NMR industry to design probes for milli-, micro- and even high nanogram amounts of sample. However, these probes typically suffered from a number of drawbacks which rendered them not very useful in practice: some designs use solenoidal coils which make it impossible to load/eject the sample at the top of the magnet; instead the probe has to be removed from the magnet to change a sample. Moreover, these probe designs are primarily useful for 1H-only coils, but cannot be readily built as broadband inverse or inverse triple-resonance probes. Typically, lineshapes for older microsample probes were not satisfactory for biological NMR experiments which require water suppression.

BRUKER INTRODUCES a new and unique series of 2.5 mm probes for microsample applications.

Our user friendly design offers:

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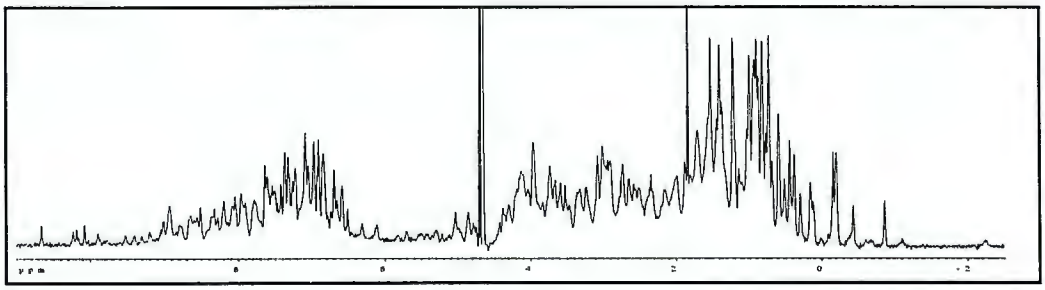
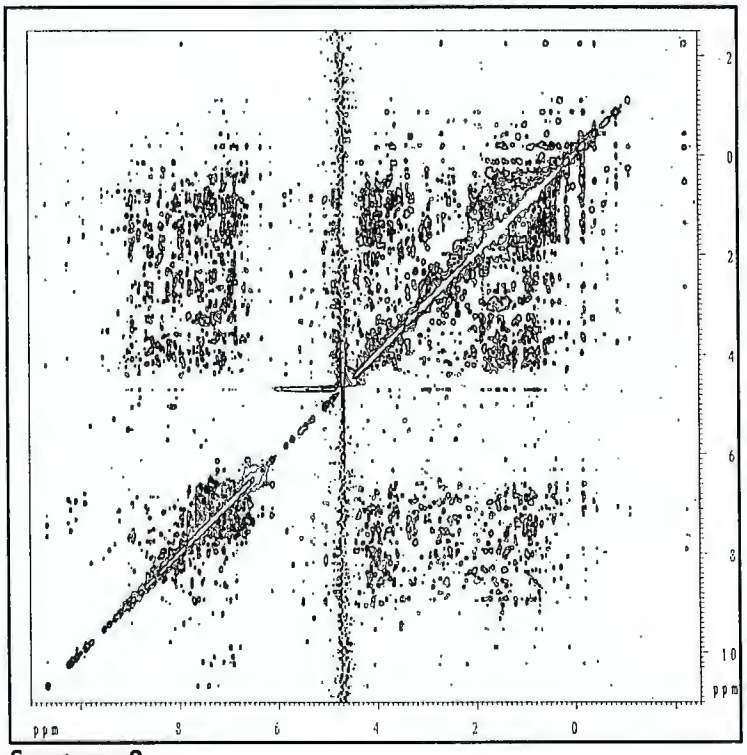
- **gradients available for GRAdient SPectroscopy**



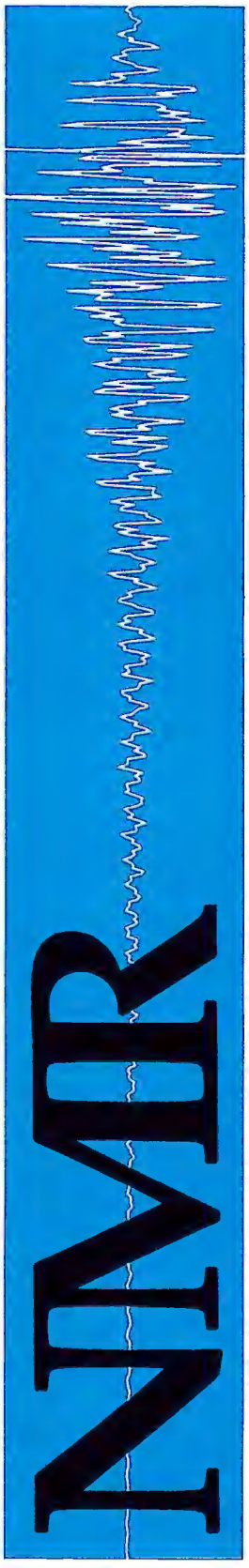
Spectrum 1



Spectrum 2

Spectrum 1: 64 scans of 1 milligram of lysozyme in 0.1 milliliter of 90% H_2O / 10% D_2O. The first increment of a NOESY experiment demonstrates the excellent lineshape and water-suppression capability.

Spectrum 2: 2D NOESY with 150 msec mixing time at 12 1/4 hours acquisition time.



BRUKER

NMR

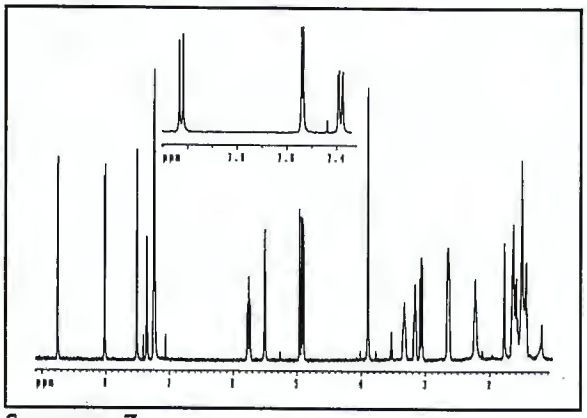
Spectrum 3: A 10 minute acquisition on 10 micrograms of quinine in $CDCL_3$. Note the excellent resolution for the aromatic peaks in the inset.

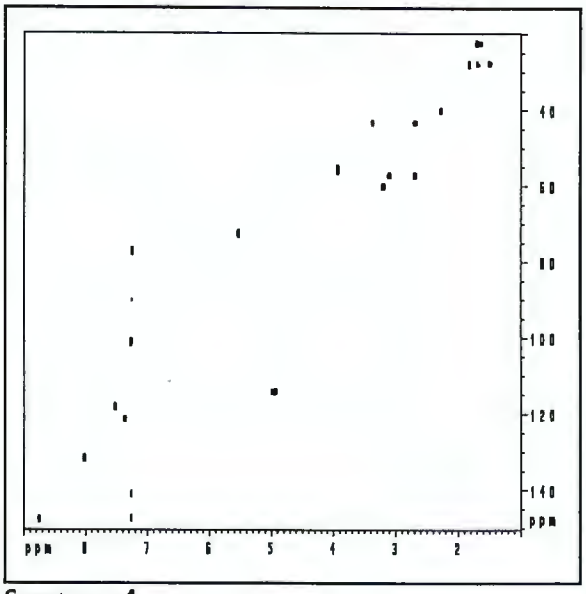
Spectrum 4: 2D HMQC at 12 hours. Same sample as spectrum 3.

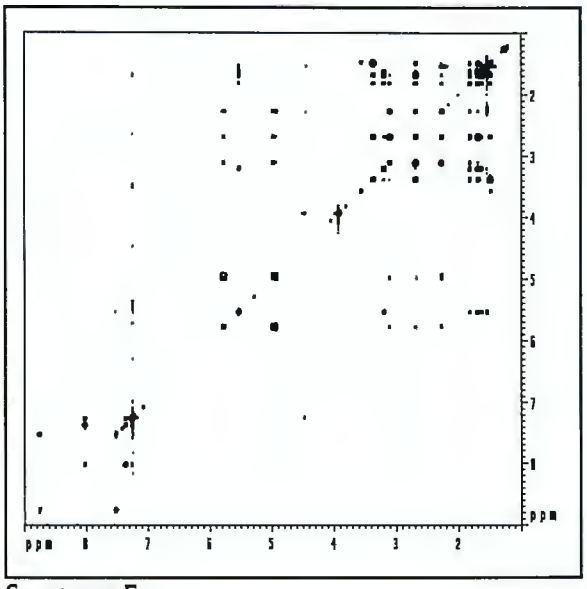
Spectrum 5: A 2D TOCSY with 3 hour acquisition time, same sample.

Experimental details:
All spectra shown here were acquired on an **AMX 600** equipped with the high-dynamic range **SE 451™** receiver, which is essential for measuring small sample amounts with good sensitivity. A **BOSS2™** (Bruker Orthogonal Shim System) was used for optimal lineshape, and the probe was a 2.5 mm inverse triple-resonance probe [$^1H\{^{13}C,^{15}N\}$]. All spectra were acquired non-spinning, and 90° pulse widths were <5 microseconds for ^1H, <12 microseconds for ^{13}C, and <40 microseconds for ^{15}N.

This novel generation of Bruker 2.5 mm probes ideally complement the standard 5 mm and 8 mm probes (for solubility-limited cases) in biological NMR applications.


Spectrum 3


Spectrum 4


Spectrum 5

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF CHEMISTRY
Lensfield Road
Cambridge CB2 1EP

Ray Freeman, FRS
John Humphrey Plummer
Professor of Magnetic Resonance

Telephone: **(0223) 336450**
FAX (UK): **(0223) 336362**
(International): **+44 223 336362**

Professor Barry Shapiro,
Editor TAMU NMR,
966 Elsinor Court
Palo Alto, California 94303
USA.

6 June 94
(received 6/11/94)

"NMR and More"

Dear Barry,

Just a few days ago I sent in a contribution entitled *"Fellgett rides again"* in which I cited Jeener's famous Basko Polje lecture that introduced 2D and taught us all that NMR would never be quite the same again. For years I have kept precious copies of the abstract and subsequent text from this Ampere Summer School talk, but of course it could not be properly cited since it had never been published. One had to take it on trust.

I am very pleased to say that this is no longer the case. To commemorate the 80th anniversary of Anatole Abragam, a *festschrift* was held in Gif-sur-Yvette on the 4 June 1994 in which some distinguished resonators presented papers and several more contributed to a new book "NMR and More" edited by Maurice Goldman and M. Porneuf and published by *Les Editions de Physique, Avenue du Hoggar, Zone Industrielle de Courtaboeuf, B.P.112, F-91944 Les Ulis cedex A, France.* On the day, a specially bound copy of this book was presented to Abragam (to his great surprise).

The point I would like to make here is that Jean Jeener has now reproduced his lecture notes "Pulse Pair Techniques in High Resolution NMR" as a chapter in this book, pages 265 to 278. Now anyone can check how clearly the new two-dimensional method was described in the abstract prepared in 1971. Unfortunately Jean still declines to show the actual two-dimensional spectra which were dogged by spectrometer phase instabilities (*plus ça change . . .*) but at least we now have a proper reference to this amazing work.

Kindest regards,

Ray

Ray Freeman

Southern Methodist University
Department of Chemistry
Dallas, Texas 75275

Professor Bernard L. Shapiro
TAMU NMR Newsletter
966 Elsinore Court
Palo Alto, California 94303

June 19, 1994

(received 6/24/94)

Dear colleagues,

After certain political changes the scientific community in Ukraine, as well as in other former Soviet Union countries, is experiencing serious economic difficulties. It is difficult to get money not only to purchase new pieces of equipment, but even for servicing existing ones.

I am grateful to the editors of this journal for the possibility to appeal to its readers. The Institute of Bioorganic Chemistry in Kiev (Ukraine) needs the Multifunctional Probe System "OMNI PROBE" (which includes a frequancy synthesizer, wide-band probe module, wide-band local oscillator, and a wide-band preamplifier) in order to turn the JEOL-FX90Q NMR spectrometer into a multinuclear observation unit. If you have one, which is not in use (as well as other parts for this spectrometer, even if they are out of order), you can help us very much with your donation.

I would like to extend this idea. If you have old spectrometers or other equipment, which you are planning to get rid of, you would help us survive this difficult time of change by donating these items to our Institute. Thank you.

Dr. Igor Shevchenko
Tel: (214)768-2445
(214)265-8914 (home)
Fax: (214)768-4089

We are grateful to Southern Methodist University and the University of Texas at Dallas for their generous donations.

FOX CHASE
CANCER CENTER

Department of Nuclear Magnetic Resonance
and Medical Spectroscopy

7701 Burholme Avenue
Philadelphia, Pennsylvania 19111
215 728 3049
FAX 215 728 2822

June 17, 1994
(received 7/22/94)

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

Differentiation Between Phosphorylethanolamine and Phosphorylserine in Extracts of Human Sarcoma Tumors

Dear Dr. Shapiro,

^{31}P NMR spectra of human cancers are generally characterized by increased phosphomonoester (PME) and phosphodiester (PDE) peaks, low phosphocreatine and relatively alkaline pH (1). The PME peak is of particular interest since decreases in its concentrations may be an early predictors of human tumor response to therapy (2-4). In order to understand the biochemical mechanisms leading to the elevation of PME's in tumors it is important to definitively identify the component(s) of this peak. Using localized CSI, ^{1}H decoupled ^{31}P spectroscopy, we have assigned this PME peak to phosphorylethanolamine (PEth) on the basis of its in-vivo chemical shift. While our assignment is in agreement with several previous reports (5,6), this identification remains in some doubt because the chemical shift of phosphorylserine (PSer) is very close to that of PEth over a broad pH range.

In order to determine the conditions under which PEth and PSer can be differentiated in an extract, a mixture of PEth and PSer was titrated over a pH range from 5.25 to 8.0. Under these conditions the two metabolites can be clearly resolved at pH 6.5 and below. In a tumor extract however, due to solution conditions, PEth and PSer continue to coresonate down to pH 6.0 (Fig. 1A & 1B. The two resonances became clearly separable by lowering the pH of the extracts to 5.5 (Fig. 1C). Using this information, we are presently analyzing extracts from sarcoma tumors. Thus far, in several extracts, we have confirmed that essentially the entire peak previously assigned to phosphorylethanolamine in these tissues is indeed PEth.

Definitive identification of the phospholipid-related phosphomonoesters in tumors is an important first step in understanding the biochemical mechanism underlying these changes. Analysis of tumor extract spectra at pH 5.5 combined with spiking of the samples with authentic PEth and PSer offers a method for reliable and reproducible differentiation between these two metabolites.

Sincerely

Benjamin Szwergold Nanci Aiken Truman R. Brown

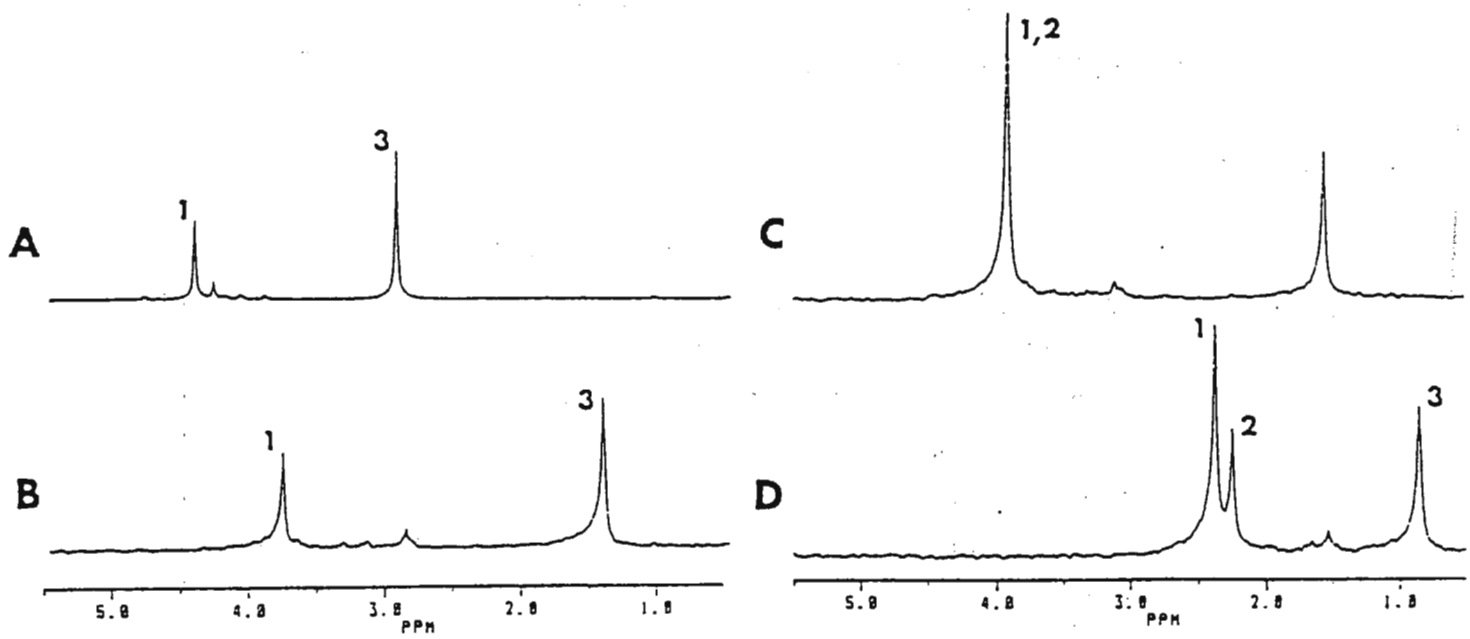


Fig.1 **A)** A ^{31}P NMR spectrum of an extract of a liposarcoma at pH 7.5. **B)** Same extract at pH 6.25. **C)** Same extract as in **B** following the addition of PSer. and PEth. **D)** Same extract as in **C** at pH 5.5. Peak identification: 1 - phosphorylethanolamine, 2 - phosphorylserine, 3 - inorganic phosphate.

1. Negendank, W. *NMR in Biomed.* 5:303-324 (1992)
2. Koutcher, J.A. et al. Magn. Reson. Med. 16:19-34 (1990)
3. Redmont, O.M. et al. Magn. Reson. Med. 25:30-44 (1992)
4. Sanuki, E. et al. 10th Ann. Mtg. SMRM (abstract) pp. 587 (1991)
5. Maris, J.M. et al. *N. Eng. J. Med.* **312**:1500-1505 (1985)
6. Navon et al. P.N.A.S. 75:891-895 (1978)

UNIVERSITY OF ST. ANDREWS

SCHOOL OF CHEMISTRY,
THE PURDIE BUILDING,
THE UNIVERSITY,
ST. ANDREWS,
FIFE KY16 9ST
SCOTLAND

Tel: (0334) 463815 (Direct Line)
(0334) 476161 (Switchboard)
FAX (0334) 463808
E-mail fgr@st-andrews.ac.uk

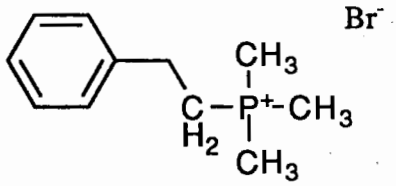
From: Dr F. G. Riddell

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

08 - 06 - 94
(received 6/20/94)

Dear Barry,

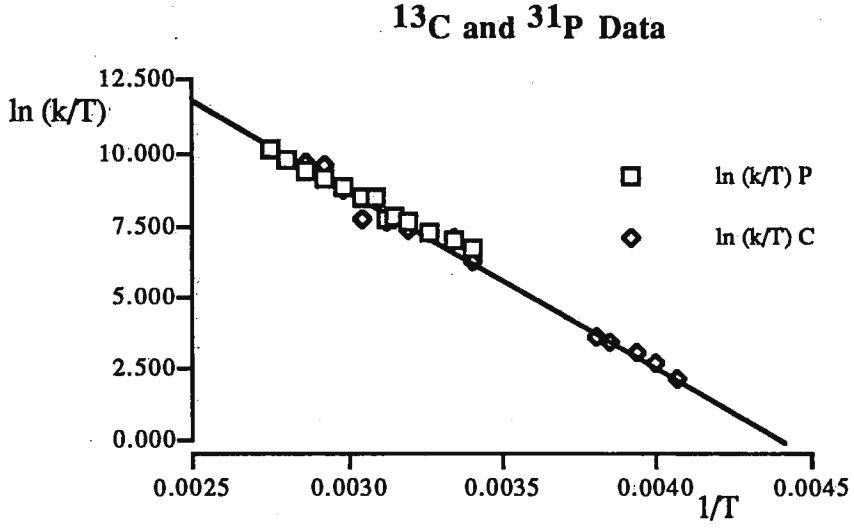
We have been having a lot of fun looking at alkyl group rotations in solids. Most recently we have turned our attention to hetero atom containing groups such as trimethylammonium and trimethylphosphonium. The latter is very attractive since it contains two NMR active nuclei suitable for solid state investigations: ^{13}C in the methyls and ^{31}P in the centre of the group. The first phosphonium salt we looked at was (1) synthesised in collaboration with Prof F. Fülöp in Szeged in Hungary.



(1)

This compound shows both dynamic line shape changes in its ^{13}C CP/MAS spectrum and changes in $T_{1\rho}$ in the ^{13}C and ^{31}P spectra consistent with a rotation process about the C-PMe_3 bond. What is very satisfying is that the rotation rates obtained from the ^{13}C line shapes and $T_{1\rho}$ measurements fall on the same activation plot. What is even more delightfully satifying is that the rates from the ^{31}P $T_{1\rho}$ measurements do so too (Figure One). The icing on on the cake is that the ^{13}C-^{1}H and the ^{31}P-^{1}H dipolar interactions calculated from our two sets of $T_{1\rho}$ data are in the correct ratio for the γ values. For a discussion of the theory see reference 1.

The good straight line obtained from three complementary but different NMR methods involving two nuclei and the range of temperatures explored gives us confidence in the remarkable activation parameters: $\Delta H^{\neq}$ = 54.4 kJ.mol^{-1}; $\Delta S^{\neq}$ = +86.4 J.K^{-1}.mol^{-1}. Look at that enormous entropy of activation! What does it mean for a solid? We have some ideas, but any comments would be welcome.



One further added bonus is that the J coupling between ^{31}P and ^{31}C of ca 50 Hz can be seen with a little help from resolution enhancement.

Thanks are due to Professor John Strange of The University of Kent for helpful advice and discussions.

Best wishes,
Yours sincerely,

Frank

Frank Riddell

Martin Rogerson

Reference 1; F. G. Riddell, S. Arumugam, K. D. M. Harris, M. Rogerson and J. H. Strange, J. Amer. Chem. Soc., 115, 1881 (1993).

Lilly

Lilly Research Laboratories
A Division of Eli Lilly and Company

Lilly Corporate Center
Indianapolis, Indiana 46285
(317) 276-2000

April 27, 1994
(received 5/27/94)

All Zirconia Rotors Are Not Created Equal

Dear Dr. Shapiro,

We at Eli Lilly & Co. have recently established a Materials' Science solid-state spectroscopy lab, which is responsible for the characterization of crystal and amorphous forms of our small molecule drug compounds both as bulk materials and in formulations. As a means to this end, we have purchased a Varian Unity 400 MHz solid-state NMR system, equipped with, among others, two CP/MAS probes. As new contributors to TAMU we would like to share our unusual finding regarding the curious and frustrating problem of arcing on our new system.

Shortly after our 7 mm VT CP/MAS probe was installed it began to arc. Shown in Figure 1 is a typical "arced" fid for hexamethylbenzene. This result was obtained with the 7 mm probe irrespective of the samples and rotors used (both zirconia and silicon nitride are used in our lab). Interestingly, arcing was never seen using our 5 mm VT CP/MAS probe. Subsequent to eliminating the possibility of spectrometer malfunction, our probe was returned to Varian at which time arcing was visually observed in the coil. After the severely damaged coil was replaced, we were advised to either avoid using our zirconia rotors, which were probably causing the arcing, or to sacrifice resolution and signal-to-noise by using them with much lower decoupler power levels.

We were surprised to learn that zirconia, a popular rotor material which is thought to be very inert, could be responsible for the observed arcing. Then it occurred to us that arcing was never observed with our 5 mm probe for which we have only silicon nitride rotors. Additionally, zirconia is known to be a solid electrolyte, i.e. conductive, when defects are present, especially at high temperatures[1]. The conductivity of zirconia coupled with the fact that our rotors are severely discolored (ZrO_2 rotors are typically white to pale yellow; ours are pale tan-green) led us to believe that the rotors were in fact defective. We are currently using our silicon nitride rotors exclusively and have not experienced any problems with arcing on the 7 mm probe. We contend that the arcing previously observed with this probe while using the silicon nitride rotors occurred only after the coil integrity was initially compromised using zirconia. Our theory of defective rotors will be tested as soon as a new batch of zirconia rotors arrives.

Please credit this contribution to the account of Dr. Doug Dorman.

Sincerely,

Paula A. Longo Susan M. Reutzel

[1] West, A. R. *Solid-State Chemistry and its Applications*; John Wiley & Sons, 1987.

^{13}C CP/MAS
7 mm Probe
HMB

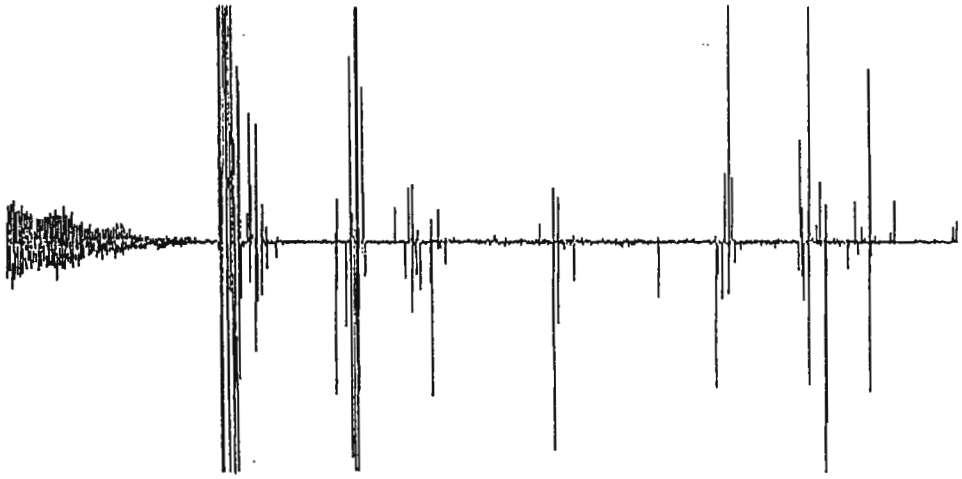


Figure 1.

FORTHCOMING NMR MEETINGS, *Continued from page 1.*

Symposium on "NMR as a Structural Tool for Macromolecules: Current Status and Future Directions, Indianapolis, IN, **October 30 - November 1, 1994**; Contact: Ms. Padmini Nallana, Coordinator, NMR Symposium, Dept. of Physics, Indiana University Purdue University Indianapolis, 402 N. Blackford St., Indianapolis, IN 46202-3273; Tel. (317) 278-1263; E-mail: PADMINI@INDYVAX.IUPUI.EDU; Fax: (3172) 274-2393. See TAMU NMR Newsletter 425, 31.

36th ENC (Experimental NMR Conference), Boston, MA, **March 26 - 30, 1995**; Contact: ENC, 815 Don Gaspar, Santa Fe, NM 87501; (505) 989-4573; Fax: (505)989-1073

12th International Meeting on NMR Spectroscopy, Sponsored by the Royal Society of Chemistry, Manchester, England, **July 2 - 7, 1995** [sic]; Contact: Dr. J. F. Gibson or Ms. G. B. Howlett - See TAMU NMR Newsletter 415, 5; Phone: (44-71) 437-8656; Fax: (44-71) 437-8883.

ISMAR 1995, Sydney, NSW, Australia, **July 16-21, 1995**; Contact: Dr. Les. Field, Dept. of Organic Chemistry, Univ. of Sydney, Sydney, NSW 2006, Australia. Phone: +61-2-692-2060; Fax: +61-2-692-3329; Email: ismar-95@biochem.su.oz.au Also, see TAMU NMR Newsletter 419, 26.

Additional listings of meetings, etc., are invited.

♣ ♦ ♥ ♠ ♣ ♦ ♥ ♠

Have you received your 1994-95 invoice? If not, please call!
If you have received your invoice, please be sure it has been processed for payment.
Many thanks for your continued cooperation.

BLS

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♣ ♦ ♥ ♠ ♣ ♦ ♥ ♠

All Newsletter correspondence should be addressed to

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

(415) 493-5971 - *Please call only between 8:00 am and 10:00 pm, Pacific Coast time.*

Deadline Dates

No. 432 (September)	26 Aug. 1994
No. 433 (October)	23 Sept.1994
No. 434 (November)	21 Oct. 1994
No. 435 (December)	18 Nov. 1994

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