THE
NMR
NEWSLETTER

No. 477

June 1998

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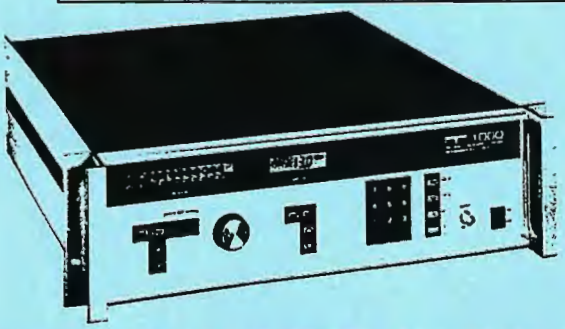

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Fifth International Conference on Heteroatom Chemistry, London, Ont., Canada, **July 5 - 10, 1998**. For details, see Newsletter 468, 40.

XIVth International Conference on Phosphorus Chemistry, Cincinnati. OH, **July 12 - 17, 1998**. For details, see Newsletter 468, 40.

NMR Symposium at the 40th Rocky Mountain Conference on Analytical Chemistry, Denver, CO, **July 27 - 30, 1998**. Contact: Dr. Robert A. Wind, Battelle/Pacific Northwest National Laboratory, P.O. Box 999, MS K8-98, Richland, WA 99352; (509) 376-1115; Fax: (509) 376-2303; Email: ra_wind@pnl.gov. See Newsletter 470, 8.

XVIIIth International Conference on Magnetic Resonance in Biological Systems, Tokyo Metropolitan University, **August 23 - 28, 1998**. Contact: Professor Masatsune Kainosho, Department of Chemistry, Tokyo Metropolitan University; +81-426-77-2544; Fax: +81-426-77-2525; e-mail: kainosho@raphael.chem.metro-u.ac.jp; http://icmrbs98.chem.metro-u.ac.jp

NMR Technologies: Development and Applications for Drug Design and Characterizations, Baltimore, MD, **October 29-30, 1998**; Contact: Jennifer Laakso, Cambridge Healthtech Institute, 1037 Chestnut St. Newton Upper Falls, MA 02164; 617-630-1300; Fax: 617-630-1325; chi@healthtech.com; http://www.healthtech.com/conferences/.

NMR Spectroscopy of Polymers, Breckenridge, Colorado, **January 24-27, 1999**; an International Symposium Sponsored by the Division of Polymer Chemistry, American Chemical Society; Organizers: P. T. Inglefield and A. D. English: Registration contact: Neta L. Byerly, Division of Polymer Chemistry, Inc., Virginia Tech, 201 Hancock Hall, M.C. 0257, Blacksburg, VA 24061; 540-231-3029; Fax: 540-231-9452; email: nbyerly@vt.edu.

40th ENC (Experimental NMR Conference), Clarion Plaza Hotel, Orlando, Florida, **February 28 - March 5, 1999**;immediately preceding Pittcon in Orlando; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87505; (505) 989-4573; Fax: (505) 989-1073; Email: enc@enc-conference.org.

continued on inside back cover

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Graduate Department of Biochemistry
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April 30 1998 (received 5/4/98)

What I Always Wondered About Coherence Selection, but was Afraid to Ask.

In treatments of spin coherence during multiple-pulse experiments it is often stated (see, for example, Ernst et al[1] p. 293) that quadrature detection selects the operator I_-, so that we should focus on phase changes of this operator. I have always been bugged by this statement because: 1. Quadrature detection is a purely engineering device. It is likely that many NMR instruments will not have quad detectors in the near future. "Digital quad detection" is really a fancy way to describe one step of a high frequency FT of a non-quad signal performed inside a DSP. 2. I_- is non-Hermitian, and its "expectation value" is trivially related to that of I_+ (they are complex conjugates of each other).

The answer to these questions is that the argument is both wrong and unnecessary, so it should not be invoked without proper qualification. I am quibbling to complain about this, but by avoiding a swindle and thinking correctly we might learn something interesting, or avoid errors.

This convention seldom has any bad consequence since the phase changes in $\langle I_- \rangle$ are in direct correspondence to the phase shift of the high-frequency observable $\langle I_x \rangle$ in the fixed frame. Further, the originators of this convention did a good deed by focusing on one bunch of matrix elements, thereby promoting a universal language. Finally, not every book repeats this argument (for example Freeman) though many do.

This statement comes from writing that the quad detector gives a signal $S = \langle I_x \rangle - i\langle I_y \rangle$ (equation A), and that $I_- = I_x - iI_y$ (equation B). Therefore, obviously $S = \langle I_- \rangle$ (see Ernst et al, equation 6.3.2). This is in error, in a slightly interesting way. The error is that the "i" = $\sqrt{-1}$ factors in these equations are not identical. Rewrite equation A as $S = \langle I_x \rangle - j\langle I_y \rangle$ where $j^2 = -1$, but ij is not equal to -1. The quantity j was introduced (in fact by me in J. Chem. Phys. 54, 1418 (1971), as "i") as a convenient way to organize data inside the computer, and has no deep physical significance. The relation between i and j may be a "hypercomplex" one similar to the relation between the hierarchies of hypercomplexity in 2D and higher NMR, as described in Ernst et al. p. 307-308, and implemented by States and Haberkorn. The argument would still be a swindle applied to quad coil detection[2] where both $\langle I_x \rangle$ and $\langle I_y \rangle$ are detected in the fixed frame. As everyone knows, hypercomplex numbers are related to quaternions (invented by Hamilton) and I tried to educate myself about these, to see if they could be useful other than as a description. I did not get any useful insight. If there are careful NMR papers on this topic I would appreciate learning of them.

Incidentally, electrical engineers now routinely use quad detection, but I have found only one book that explicitly treats the quad outputs as real and imaginary. I think they lose a lot by not doing so, but maybe they know that they will get confused. Sara Kunz and I discussed this point in the January JMR (hope you liked the cover!).

Al Redfield

Alfred G. Redfield

AGR/pm

---

[1] Ernst, Bodenhausen, and Wokaun, "Principles of NMR in 1 and 2 Dimensions", Oxford, 1987.
[2] Hoult, Chan, and Sank (1984) Magn. Reson. in Medicine 1, 339.

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Professor Barry Shapiro,
The NMR Newsletter,
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27 April 98
(received 5/4/98)

Radiation Undamping

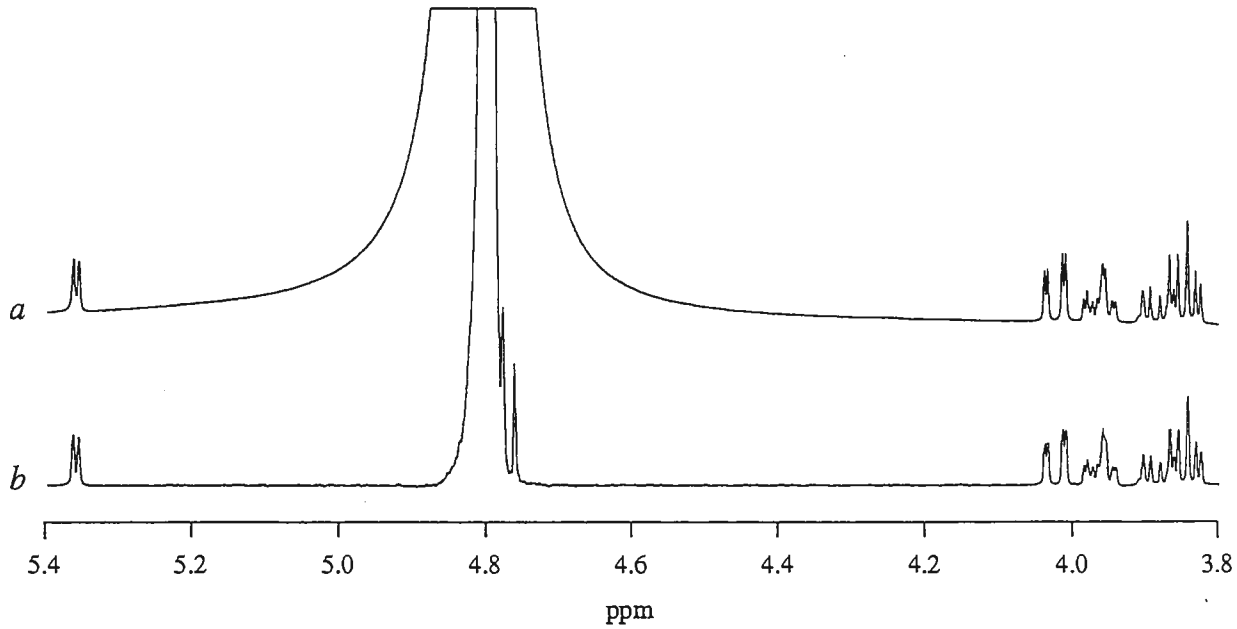
Dear Barry,

Long long ago as a postdoc at Saclay (France), I spent a very long time building a high resolution spectrometer from scratch, knowing that if I got the probe materials wrong, all the lines would be broad and the experiment useless. Not surprisingly, I found >200 Hz linewidths, even after redesigning the probe a couple of times. I was using a Pound spectrometer as the rf unit, and eventually it dawned on me that the problem was radiation damping (1), greatly enhanced by the positive feedback (regeneration) in the Pound box (2). Going to a conventional rf transmitter solved the problem. It turned out that this phenomenon had been anticipated by Lösche in East Germany, but I had overlooked this work until Jack Powles pointed it out. This cautionary tale served to sensitize me once and for all to the insidious dangers of radiation damping in high resolution NMR.

Now, some forty years later, this same demon is beginning to cause more widespread troubles in high resolution NMR when we use aqueous solutions in high polarizing fields. Consequently, several authors have proposed methods to suppress radiation damping by negative feedback (3), Q-switching (4) or the application of bipolar field gradients (5).

We have tried a different approach, compensating the radiation damping field (a torque about the -x axis) with a DANTE sequence of tiny rotations about the +x axis that just balance the effect of the radiation damping field. As the transverse NMR signal decays with time, the flip angle of the DANTE pulses is reduced at a matching rate. One complex data point is acquired in each interpulse interval. Once the DANTE frequency has been tuned to the water resonance, the inherent spectrometer stability ensures that it stays at resonance and maintains the correct phase. The water signal then decays quite slowly and the rest of the spectrum is unaffected.

Hervé Barjat (in Cambridge) performed the experiment on a 500 MHz Varian INOVA (in Palo Alto) using the remote "X-windows" facility. I was at Varian at the time and we had lots of help from Debbie Mattiello and



Howard Hill to sort out the tricky bits. A sample of D-glucose in 50% H_2O/D_2O at 35.5 °C serves as a test because the 8 Hz doublet of anomeric proton of the β isomer lies just underneath the water peak, which is broadened to 13.5 Hz by radiation damping. After compensation the water line narrows to 0.8 Hz, revealing the previously hidden doublet (see Figure).

This scheme has the advantage that it involves no modification to the radiofrequency circuitry and no significant loss in sensitivity, since the receiver duty cycle can be as high as 90%, and the Q-factor is unaffected. It is not, of course, a solvent suppression technique.

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4. C. Anklin, M. Rindlisbacher, G. Otting, and F. H. Laukien, *J. Magn. Reson. B* **106**, 199 (1995).
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5. V. Sklenar, *J. Magn. Reson. A.* **114**, 132 (1995).
   S. Zhang and D. G. Gorenstein, *J. Magn. Reson. A* **118**, 291 (1996).
   A. Bockmann and E. Guittet, *J. Biomolec. NMR,* **8**, 87 (1996).

Kindest regards,

Ray

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18 May 1998
(received 5/21/98)

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

Re: Measurement of Transverse and Longitudinal Cross-Correlation between ^{13}C-^{1}H Dipolar Interaction and
^{13}C Chemical Shift Anisotropy in a DNA Duplex

Dear Barry:

Recently, transverse relaxation interference (cross-correlation) between the ^{15}N CSA and ^{15}N-^{1}H dipolar interactions of peptide backbone amides has been quantitatively measured, and this cross-correlation was demonstrated to be directly proportional to the generalized order parameter S^2 (1). However, in principle, the strength of the relaxation interference depends on the angles between the unique axes of the CSA and dipolar tensors, so neither CSA values nor values of the spectral density $J(\omega)$ are determined from such transverse cross-correlation experiments. Measurement of the longitudinal cross-correlation rate can be readily carried out and, in combination with the transverse cross-correlation rate, can be useful for elucidating dynamics.

Figure 1 shows pulse schemes for quantitative measurement of transverse $R(^{13}C_X \rightarrow 2^{13}C_X{}^{1}H_Z)$ (top) and longitudinal $R(^{13}C_Z \rightarrow 2^{13}C_Z{}^{1}H_Z)$ (bottom) cross-correlation between ^{13}C-^{1}H dipolar coupling and ^{13}C CSA. The pulse sequence to record transverse CSA-DD cross-correlation rates of nitrogen, $R(^{15}N_X \rightarrow 2^{15}N_X{}^{1}H_Z)$, reported previously (1) is identical to the upper sequence of Figure 1 except for the gradient coherence selection with the sensitivity enhancement scheme (2), which is set for ^{13}C. The lower sequence is quite similar to the upper, although the ^{13}C magnetization relaxing during the cross-relaxation period 2Δ is longitudinal magnetization instead of transverse magnetization. Each pulse sequence is essentially a HSQC experiment with a transverse or longitudinal cross-relaxation period 2Δ inserted before the ^{13}C evolution period.To derive the longitudinal cross-correlation rate (lower pulse sequence), at least two independent measurements are required: the open ^{1}H 90° and composite $(90_y\text{-}220_x\text{-}90_y)$ 180° pulses are either applied (scheme A) or not applied (scheme B). The difference between scheme A and scheme B enables observation of cross-correlation effects during the period 2Δ. Likewise, at least two independent measurements are required to derive the transverse cross-correlation rate (upper pulse sequence). Thus, the ratio of the signal intensities obtained with schemes A and B becomes a simple function of the CSA-DD cross-correlation rates: $I_A/I_B(\text{trans}) = \tanh(2\Delta\, R(^{13}C_X \rightarrow 2^{13}C_X{}^{1}H_Z))$ and $I_A/I_B(\text{long}) = \tanh(2\Delta\, R(^{13}C_Z \rightarrow 2^{13}C_Z{}^{1}H_Z))$.

The sample used here was a DNA decamer duplex d(CATTTGCATC) : d(GATGCAAATG) in which every adenosine and guanidine is randomly fractionally enriched with 15% ^{13}C and 98% ^{15}N stable isotopes. The longitudinal and transverse ^{13}C CSA and ^{13}C-^{1}H dipolar cross-correlation rates were determined at three temperatures (average values are shown below). These longitudinal ^{13}C CSA and ^{13}C-^{1}H dipolar cross-correlation rates will aid us in analysis of molecular motion.

	30 °C	20 °C	10 °C
$R(^{13}C_X \rightarrow 2^{13}C_X{}^1H_Z)$	12.83 ± 2.50	16.70 ± 2.87	26.75 ± 5.52
$R(^{13}C_Z \rightarrow 2^{13}C_Z{}^1H_Z)$	1.92 ± 0.30	1.64 ± 0.26	1.64 ± 0.24

Sincerely,

Chojiro Kojima

Thomas L. James
Chairman, Department of Pharmaceutical Chemistry
Professor of Chemistry, Pharmaceutical Chemistry & Radiology

References

1. Tjandra, N., Szabo, A. and Bax, A. (1996) *J. Am. Chem. Soc. 118*, 6986-6991.
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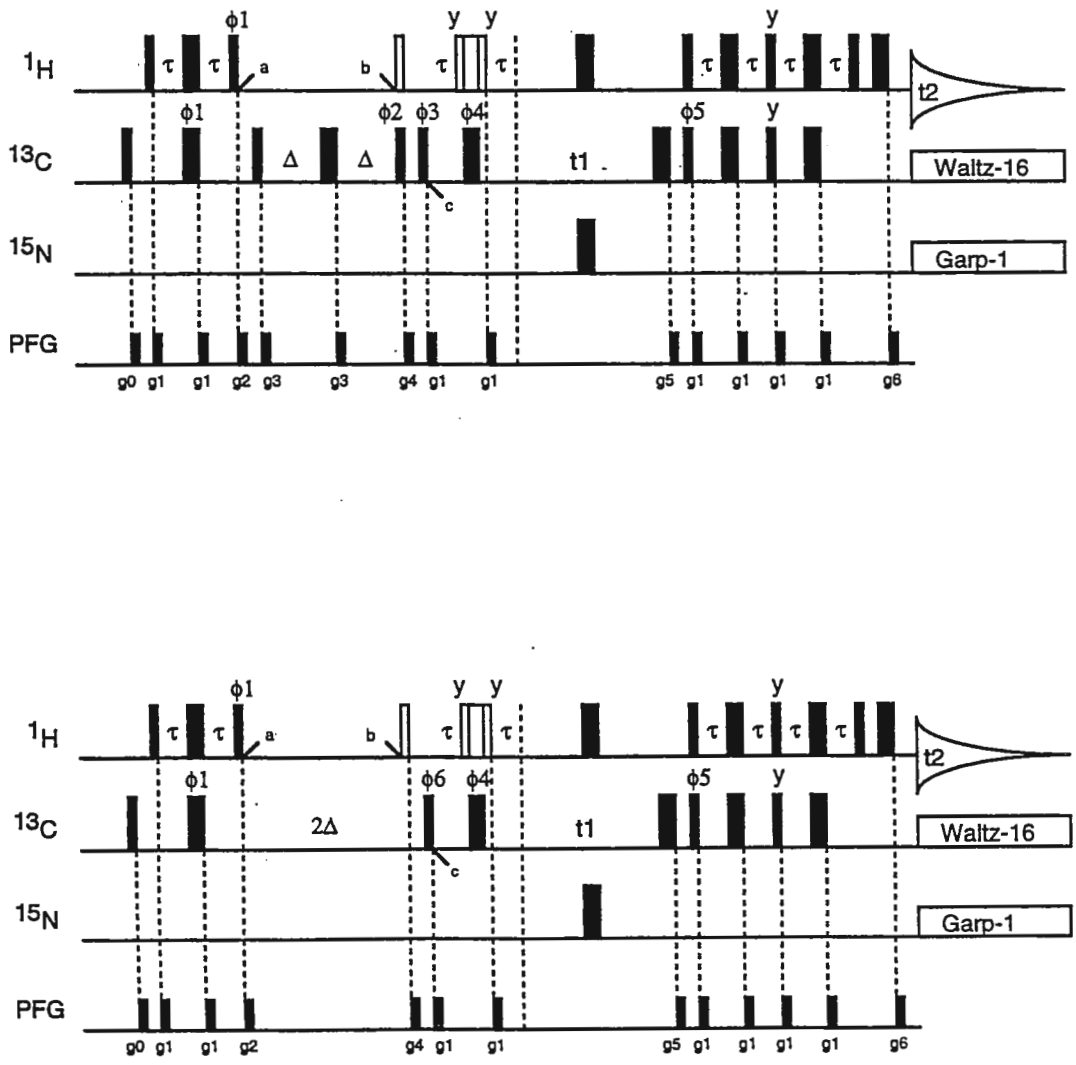

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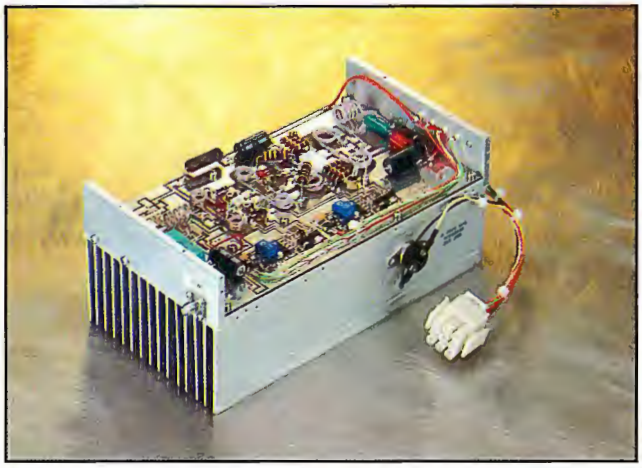

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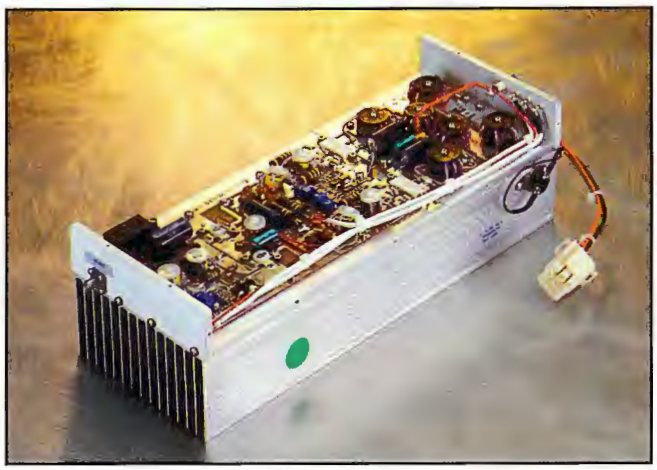

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SCIENTIFIC & MEDICAL PRODUCTS

COMPANY

AMT designs, develops and manufactures custom radio frequency (RF) and microwave power amplifiers for the wireless, scientific/medical and application specific industries. The company has been in business since 1984 and currently has over 60 employees, including 20 experienced engineers.

AMT has a worldwide reputation as a leading supplier of high power, solid state power amplifier products that operate at frequencies between 1 MHz and 3 GHz and provide RF power from several watts to several kilowatts. Its products are noted for their exceptional performance, highest quality and superior reliability.

The company's products are sold to numerous major corporations, universities and research centers throughout the world.

FACILITIES

AMT is located in Anaheim, California and occupies a 25,000 square foot facility allocated to engineering, manufacturing, quality assurance, marketing/sales, administration and finance.

Engineering areas include an R & D laboratory, a tool and die shop, mechanical design and drafting areas, an environmental testing laboratory and document control. The R & D laboratory is equipped with all of the latest design and testing equipment including intermodulation distortion simulators, network analyzers, spectrum analyzers, signal generators, noise figure meters and infrared (IR) scanners. The environmental testing laboratory includes equipment to simulate shock, vibration and thermal environments.

Manufacturing areas include a controlled access stock room, a 10,000 square foot assembly area and a production test area employing automatic testing. Also included is an environmental laboratory used for environmental stress screening of production products.

PRODUCTS

AMT's products vary in complexity from single modules, to rack-mounted amplifiers, to complete transmitter systems. The rack-mounted amplifiers and complete transmitter systems typically include detection/protection circuitry, built-in power supplies, front panel metering and digital and/or analog interface controls. Both forced air and/or water cooling are used, depending on the customer's requirements.

AMT's products feature highly reliable technical solutions designed for producibility and reliability. Producibility is enhanced through the use of surface mount components and circuit designs that eliminate the need for excessive alignment during the production cycle. High reliability is accomplished through the implementation of conservative thermal and RF circuit design and sophisticated self-protection schemes. Reliability is further enhanced during the design phase by employing detailed environmental testing.

These factors, along with computer driven automatic testing and environmental stress screening of the final product, ensure that the performance, quality and reliability meet AMT's exacting standards.

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Pharmacia & Upjohn

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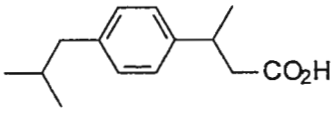
May 22, 1998 (received 4/23/98)

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

Sample Preparation and Handling in the SubMicro World

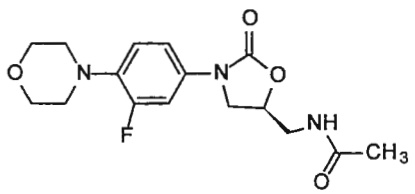
Dear Barry,

Our contributions the past several issues have addressed the performance and capabilities offered with a prototype 1.7 mm SubMicro Inverse-Detection Gradient or SMIDG™ NMR probe built for us by Nalorac. Performance notwithstanding, it is still necessary to get the sample into the tube and then into the spectrometer before what the probe contributes begins to matter. If sample preparation and the subsequent handling of the sample are deficient in technique, the difficulty in working with submicromole samples is proportionately increased. For these reasons, we thought it might be worthwhile to address sample preparation and handling in this contribution.



1

Much of the work our group at Pharmacia & Upjohn does is related to the isolation and characterization of impurities contained in candidate drugs and the study of the products of degradation after candidate drug molecules are subjected to various stress challenges, photo-oxidation, etc. To support this work, standard analytical and preparative HPLC methods are employed to isolate the sample(s) intended for study. At this point, pooled fractions from a preparative chromatographic system often contain contaminants that produce "chemical noise" in an NMR spectrum. To reduce the level of chemical noise-causing contaminants in our samples, pooled fractions containing the analyte of interest are subjected to cleanup *via* a chromatographic trapping procedure. The first step in the procedure is to reduce the percentage of organic mobile phase modifier (acetonitrile, methanol, etc.) in the pooled fraction by rotary evaporation or dilution with water. The resulting primarily aqueous solution is then pumped onto a 10 x 250 mm Kromasil C18 column which has been charged with water. This step traps the analyte on the head of the Kromasil column and irreversibly retains the majority of hydrophobic contaminants (mobile phase stationary phase, oil and grease from glassware, etc). Ionic mobile phase additives (trifluoroacetic acid, ammonium formate, etc) remaining from chromatographic isolation are then washed from the trapping column with 10 column volumes of water. The analyte is then eluted from the trapping column with 100% acetonitrile. Finally, the resulting analyte solution obtained from the trapping column is transferred to a specially cleaned (washed 3X with HPLC grade hexane) 10 ml conical vial and lyophilized. The dried samples are transferred, under vacuum, if necessary, to an argon atmosphere glove box where the NMR sample is prepared. The approximate sample recovery for this method of sample preparation is ~70%. For submicromole quantities of material, all sample preparation is done in the glovebox to avoid the introduction of water since a significant percentrage of the samples are prepared in d_6-DMSO. Preparation on the bench outside of the glovebox is undesirable. Using a 1 μmole sample of ibuprofen (**1**), for example, when the sample is prepared on the bench, the largest peak in the spectrum is the water that the sample picks up during preparation (see Figure 1). Note that the water peak is approximately the same size as the methyl doublet furthest upfield when all peaks are plotted on-scale.



linezolid (PNU-100766)

2

To illustrate the type of sample that can be obtained using the procedure just briefly outlined, a 50 μg sample of linezolid™ (**2**, PNU-100766) was subjected to preparative HPLC isolation and chemical noise cleanup. A 50 μg sample was injected onto an reversed phase, isocratic, preparative HPLC system employing an acetonitrile, water, and trifluoroacetic acid mobile phase. The linezolid peak was collected and the resulting fraction processed through the chemical noise cleanup procedure described above. Assming 70% recovery following chemical noise cleanup, the final, isolated sample was estimated to contain ~35 μg or about 0.1 μmole of sample.

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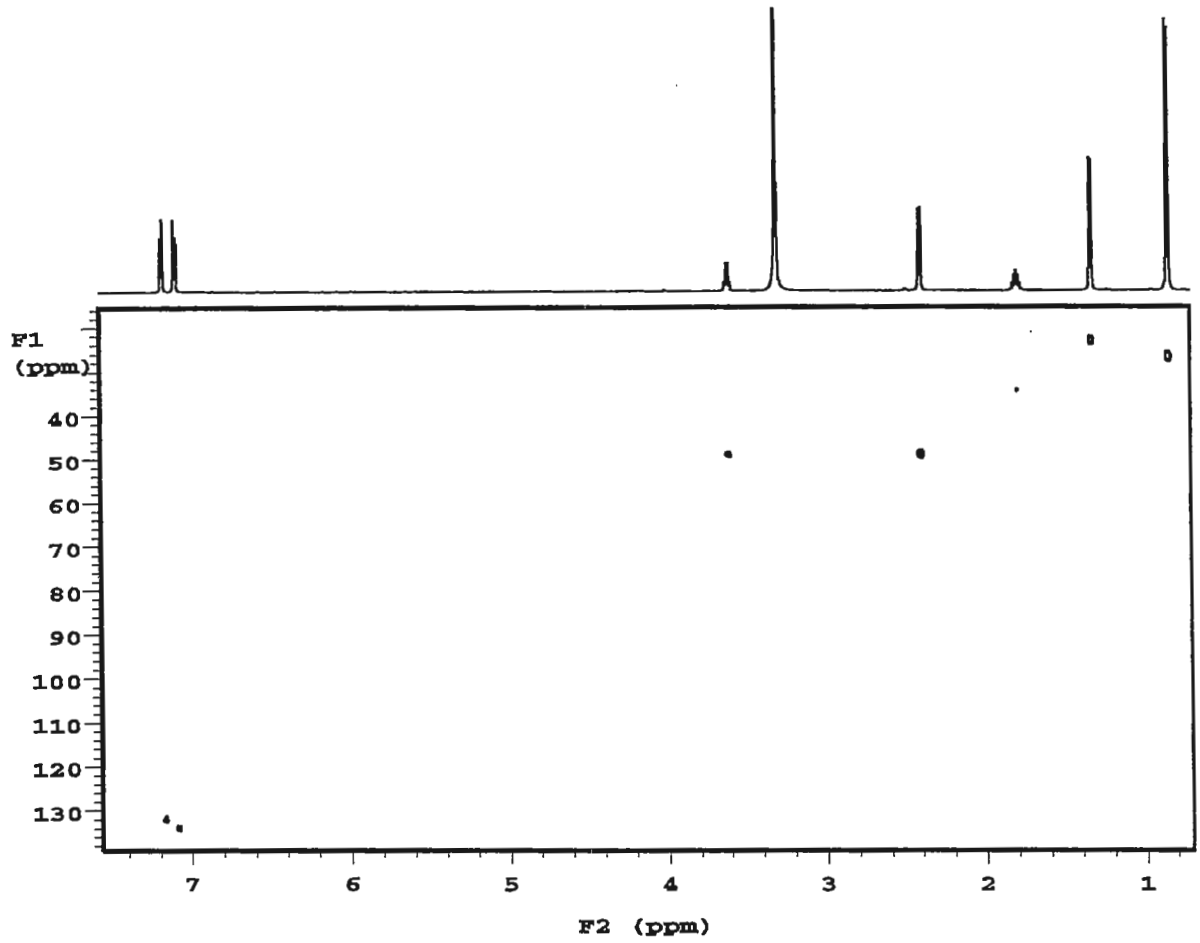

Figure 1. GHSQC spectrum of 1 μmole (206 μg) of ibuprofen dissolved in 25 μl 99.996% d_6-DMSO. The data were recorded as 2048 x 128 States-TPPI hypercomplex files with 1 transient accumulated/t_1 increment to give a total acquisition time of 6 min. All direct responses are visible, including the weak response for the methine of the sec-butyl group. The proton reference spectrum was acquired as a single transient following the application of a 90° pulse. The sample was prepared on the benchtop, giving rise to the intense water resonance at 3.3 ppm, which is the strongest resonance in the spectrum. For weaker samples, this level of water contamination would be totally unacceptable as noted in the text.

The isolated sample of linezolid was concentrated in the bottom of a conical bottom vial and then passed into the glove box for sample preparation. An aliquot of 28 μl of 99.992% d_6-DMSO (Cambridge Isotope Laboratories) was added to the sample vial using a flexible teflon needle (Hamilton #90624) small enough to be threaded to the bottom of the 1.7 mm NMR tubes attached to a Hamilton 50 μl gas-tight syringe. Mixing and complete dissolution were accomplished by slowly drawing the sample in and out of the teflon needle several times before drawing it into the needle for the final time. At that point, the teflon needle was inserted into the 1.7 mm NMR tube to the bottom of the tube. As the needle was then withdrawn the plunger of the syringe was slowly depressed to inject the sample into the tube. Considerable hangup of the solvent on the walls of the tube due to capillarity is possible so care should be taken during this step in the process. After the sample has been completely introduced to the tube, the tube should be shaken down to be certain that all solution hanging on the walls of the tube is down. We have found sample columns in the 1.7 mm tubes in the range of 20-22 mm (24-26.4 μl) to be well suited for ease of shimming. Sample volume in the tube can be calculated at 1.2 μl/mm of column height. This volume of solution can be easily transferred using the method just described if the initial sample is prepared with 28 μl of solvent.

The ^{1}H reference proton spectrum of the 50 μg sample of **2** subjected to the chromatographic workup and handling just described is shown plotted above a GHSQC spectrum of the antibiotic in Figure 2. The GHSQC spectrum was acquired as 2048 x 64 States-TPPI hypercomplex files in slightly <5 h with 128 transients accumulated/t_1 increment. The data were processed by linear prediction to 192 files in F_1 followed by zero-filing to 256 points prior to transformation. Gaussian multiplication was used used prior to Fourier transformation with the weighting function optimized to the data.

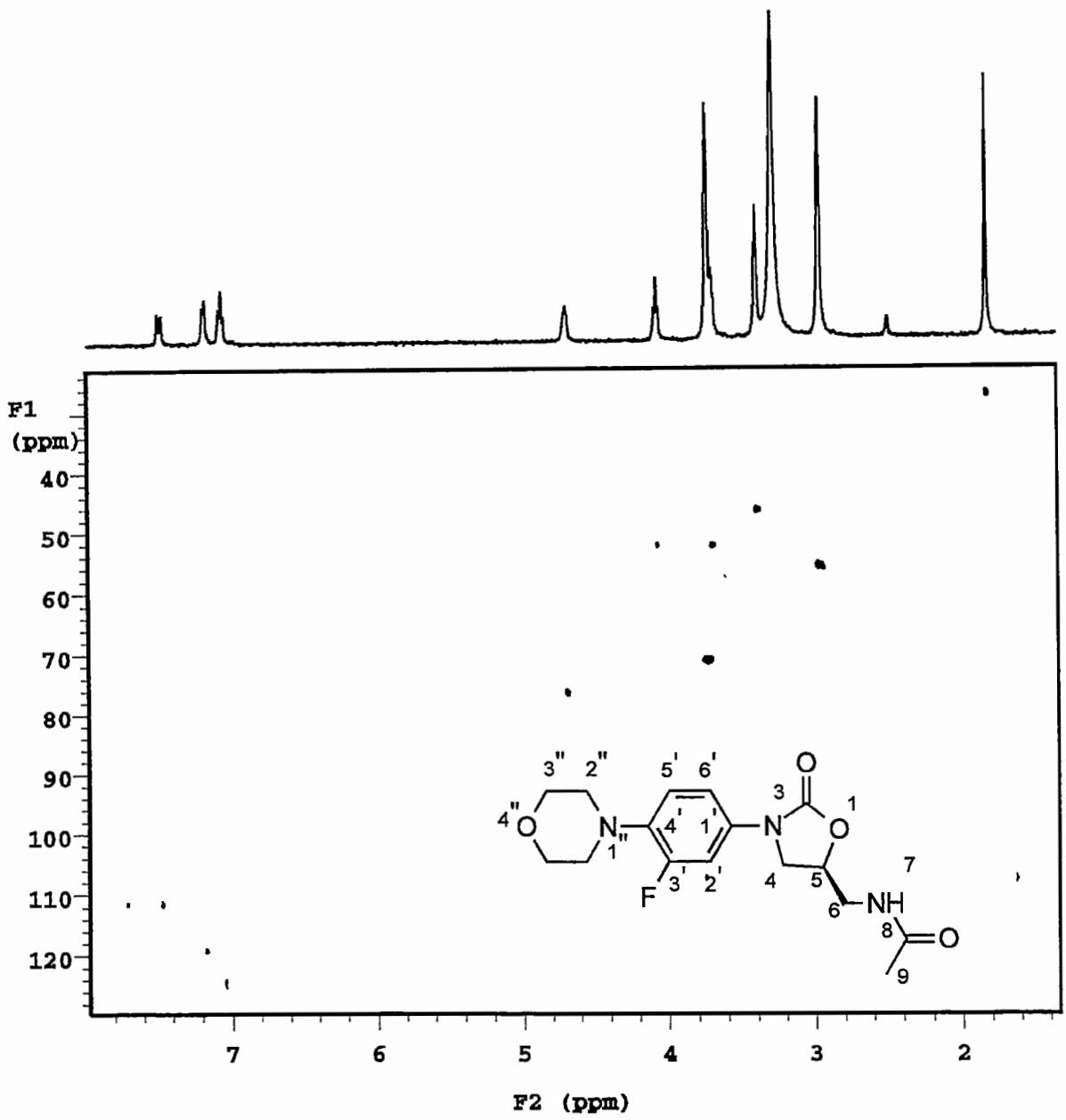

Figure 2. GHSQC spectrum of a 50 μg sample of the oxazolidinone antibiotic linzeolid (PNU-100766) subjected to the chromatographic workup described above. Sample recovery was estimated at 70%, affording a sample in the 1.7 mm SMIDG NMR tube, assuming complete transfer, of ~35 μg or ~0.1 μmole (mw 349). The data were acquired using a Varian INOVA 600 spectrometer equipped with a Nalorac Z•SPEC SMIDG™-600-1.7 probe. The ^{1}H reference spectrum plotted above the contour plot was plotted with all peaks on scale. The largest peak in the spectrum arises from residual water at ~3.3 ppm. The spectrum was acquired as 2048 x 64 States-TPPI hypercomplex files with 32 steady state transients and 64 transients accumulated/t_1 increment. Total acquisiton time was slightly <5 h. Data were processed using linear prediction to 192 files in F_1 and zero-filling to 256 points. Gaussian multiplication optimized for the data in both frequency domains was applied prior to Fourier transformation

As will be noted from the proton reference spectrum shown in Figure 2, the sample preparation just described affords a sample for NMR analysis that is free of any chromatogaphic artifacts and that is also reasonably dry. All of the protonated carbon responses are easily observed in the short <5 h data acquisition. In comparison, a sample of this size studied in a 3 mm probe would typically require at least an overnight acquisition to provide comparable data.[1-3] Long-range GHMBC data were accessible on the ~0.1 μmole sample of **2** in an overnight acquisition. In contrast, long-range data on a sample of this size in a 3 mm gradient inverse micro probe would generally be either out of reach or would require a full weekend of data acquisition. The lowest level 3 mm long-range data acquired in conjunction with a structure elucidation problem we are aware of was in the elucidation of the structure of crytolepicarboline, which was done on ~0.25 μmole (398 mw).[4] Work

recently reported from these laboratories has demonstrated that it is feasible to acquire long-range GHMBC data on samples as small as 0.04 μmole over a weekend. [4]

Regarding the water peak in the proton reference spectrum plotted above the contour plot in Figure 2, note that it is only slightly larger than the N-acetyl methyl singlet. In contrast, for the 1μmole ibuprofen sample preapred on the benchtop, the water was there the size of the methyl doublet, roughly five times the intensity of the corresponding water peak when prepared in the glove box as in Figure 2 (when the molar ratio of the two samples are considered). Furthermore, it is likely that the water in the spectrum in Figure 2 is residual to the chromatographic preparation of the sample. In contrast, the water in the spectrum in Figure 1 was absorbed by the solvent during preparation of the sample from a reference standard using serial dilution techniques. If the sample in Figure 1 had been chromatographically prepared, it is probable that the water resonance would have been correspondingly more intense.

In conclusion, SMIDG NMR probe technology now allows the spectroscopic characterization of samples that can be readily obtained from single semi-preparative HPLC isolation followed by treatment to reduce chemical noise in the sample arising from chromatographic artifacts, etc. This capability can be expected to greatly enhance the ability to characterize impurities and degradants of pharmaceuticals, their metabolites, combinatorial chemisitry products when the chemistry has led to unanticipated reaction products, and minor natural products. Many of these types of problems have in the past either been out of reach of all but proton NMR experiments, or have required extensive chromatographic effort with sample pooling to obtain sufficient quantities for even direct correlation inverse-detected heteronuclear experiments.

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3. G. E. Martin, J. E. Guido, R. H. Robins, M. H. M. Sharaf, P. L. Schiff, Jr., and A. N. Tackie, *J. Nat. Prod.*, **61**, in press (1998).
4. M. H. M. Sharaf, P. L. Schiff, Jr., A. N. Tackie, C. H. Phoebe, Jr., L. Hoard, C. Meyers, C. E. Hadden, S. K. Wrenn, A. O. Davis, C.W. Andrews, D. Minick, R. L. Johnson, J. P. Shockcor, R. C. Crouch and G. E. Martin, *Magn. Reson. Chem.*, **33**, 767-778 (1995).

Sincerely,



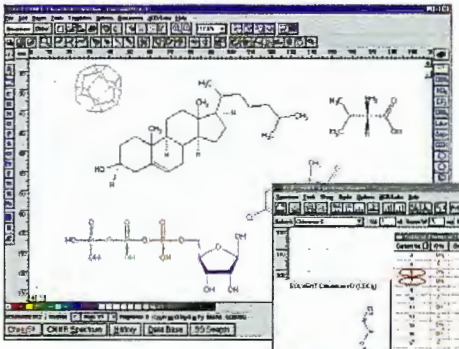
Brian D. Kaluzny Chad E. Hadden Russell H. Robins Gary E. Martin

The Expanding Universe of
Advanced Chemistry Development

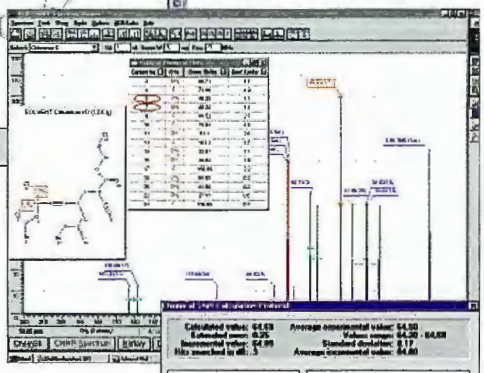
$^{1}H/^{13}C/^{19}F/^{31}P$ NMR Predictors

ACD/NMR Predictors provide the ability to calculate chemical shifts and, where appropriate, coupling constants for a variety of chemical structures. The ACD/NMR prediction suite has expanded now to include ^{1}H, ^{13}C, ^{19}F and ^{31}P prediction capability. The programs utilize our proprietary prediction algorithms developed over a period of many years, in conjunction with internal databases of experimental data collected from the open literature and verified for quality by our compilation team. The table indicates the size of the data files utilized for each nucleus.

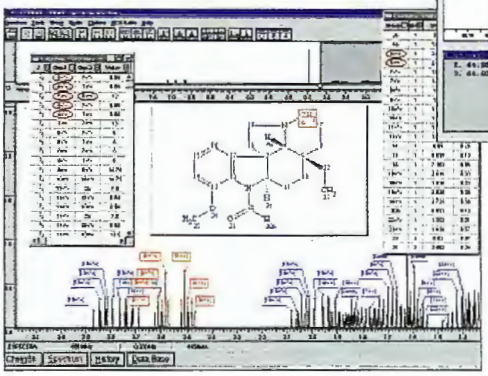
NUCLEUS	# STRUCTURES IN INTERNAL DB	# CHEMICAL SHIFTS	# COUPLING CONSTANTS
^{13}C	50,000	600,000	–
^{1}H	54,000	400,000	55,000
^{31}P	8,900	11,000	5,700
^{19}F	10,800	22,000	13,500



ChemSketch drawing interface



^{13}C prediction window showing solvent resonances, table of chemical shifts and confidence intervals



Calculation protocol in window for specific fragment

^{1}H prediction window with zoom-in, and tables of chemical shifts and coupling constants

Features of ACD/Predictor

- In general ACD/NMR Predictors calculate chemical shifts with an **accuracy of ± 3 ppm** or better (^{13}C NMR) and ± 0.3 ppm (^{1}H NMR)
- Calculated chemical shifts are always provided with 95% confidence intervals so that you can always know the reliability of the calculated values
- **Fully integrated with other ACD/NMR software**
- **Includes ACD/ChemSketch as an integrated editor** for drawing chemical structures and designing professional reports quickly and easily. This is a powerful molecular editor which allows automatic or customized numbering of molecules. Molecules, names, graphical objects, predicted spectra, and text which are displayed in ChemSketch can be cut and pasted into word processor programs.
- **Contains the History Window** which displays all the structures used for spectral calculations during the current program session.
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- Allows you to change the spectrum scale and chemical shift units, **zoom** in any spectral region.
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For contact information, see reverse side

The Expanding Universe of
Advanced Chemistry Development

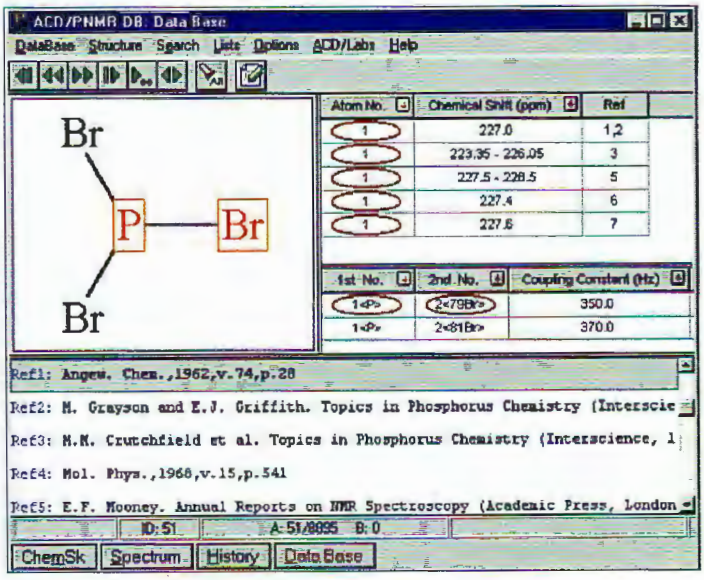
$^{1}H/^{13}C/^{19}F/^{31}P$ NMR Databases

ACD/NMR DBs allow you to view chemical shifts and coupling constants for known compounds!

The optional user-accessible internal data bases add-ons contain:

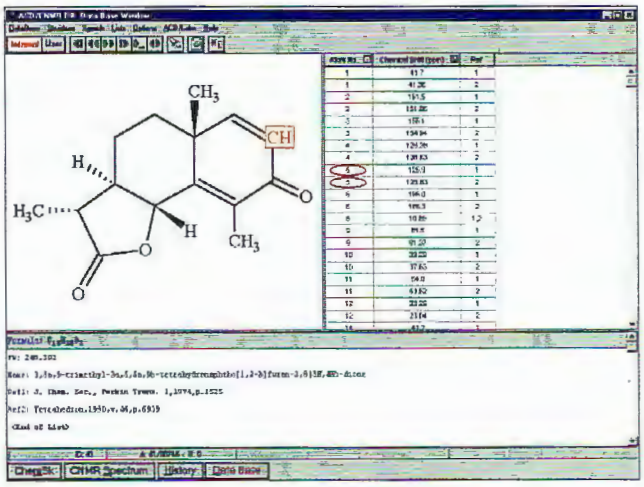
^{1}H NMR DB: over 54 000 structures
^{13}C NMR DB: over 50 000 structures
^{19}F NMR DB: over 10 800 structures
^{31}P NMR DB: over 8 900 structures
^{13}C NMR DB of Natural Products and analogs : over 5200 structures

Each database includes original literature references, molecular formula, molecular weight and IUPAC names which can be searched and viewed. Search capability also includes structure and substructure, and searching by chemical shifts and coupling constants.

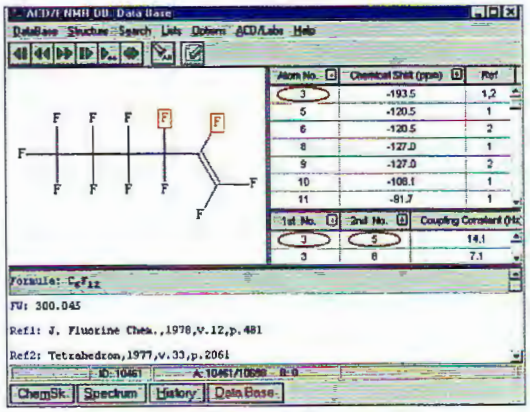


Phosphorus Database window showing chemical shifts, coupling constants, and references

CNMR DB window showing chemical shifts, references, formula and IUPAC name



Fluorine Database window showing chemical shifts, coupling constants, and references



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Professor J. C. Lindon

BIRKBECK COLLEGE
University of London

12 May 1998
(received 5/20/98)

Dr. B.L. Shapiro
The NMR Newsletter,
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CA 94303 USA

Dear Barry,

Directly-coupled CE-NMR and CEC-NMR in Drug Metabolism

Direct coupling of HPLC with NMR spectroscopy is now a routine commercially-available technique which has proved useful in many areas of analytical chemistry particularly for drug metabolite identification and the information content of such studies has been extended by the further hyphenation with mass spectrometry. There have been a number of extensions to the approach involving other types of separation and Jonathan Sweedler has reported developments in capillary electrophoresis coupling to NMR (CE-NMR). This methodology and that of capillary electrochromatography (CEC-NMR) has now been evaluated using an extract of human urine containing the metabolites of acetaminophen. This work has been a collaboration with Ian Wilson at Zeneca Pharmaceuticals, and Professors Ernst Bayer, Klaus Albert and their research teams at the University of Tübingen, Germany. The metabolism of acetaminophen (4-hydroxyacetanilide) has been studied extensively using NMR spectroscopy and it is known that the two major metabolites are the glucuronide and sulfate conjugates of the phenolic hydroxyl group. More recently the metabolism of acetaminophen has been used by us as a model system to investigate the usefulness of both HPLC-NMR and HPLC-NMR-MS.

1.5 ml of human urine was collected 3 hours after a normal therapeutic dose of acetaminophen and was extracted by passing it down a C18 solid phase extraction cartridge previously conditioned with methanol and HCl and the fraction containing the metabolites was washed off with 100% methanol and evaporated to dryness. The experimental arrangement for the CE-NMR and CEC-NMR experiments has been reported by the Tubingen group (Anal. Chem., in press). The continuous-flow CE-NMR experiment gave the result shown in Figure 1(a) which is viewed as a contour plot with CE separation time on the vertical axis and the NMR chemical shift on the horizontal axis. The peaks spread throughout the figure arise from formate from the buffer at δ8.4, residual water in the D_2O buffer at δ4.7 and a small amount of glycine which remained from earlier use of a glycine-containing buffer at δ3.5. In addition at separation times of 49 min and 73 min sets of peaks related to acetaminophen can be observed. The first eluting can be assigned to acetaminophen glucuronide from the known aromatic proton and N-acetyl methyl chemical shifts taken together with the diagnostic shifts of the glucuronic acid moiety. The second set of acetaminophen-related resonances show aromatic proton peaks consistent with the sulfate conjugate of acetaminophen. Finally, a third component is detected in the continuous-flow CE-NMR experiment and the NMR spectrum of this component is consistent with the endogenous compound hippurate. This shows the expected aromatic proton resonances together with a singlet from the glycyl methylene group.

Directly-coupled CEC-NMR spectroscopy was also achieved using the same sample. The result is shown in Figure 1(b). The CEC-NMR result is similar to that from the CE-NMR experiment with the glucuronide conjugate eluting first at 36 min, followed by the sulfate at 40 min and then hippurate at 50 min. Individual rows from the continuous-flow CEC-NMR experiment are shown in Figure 2 with the assignments as marked.

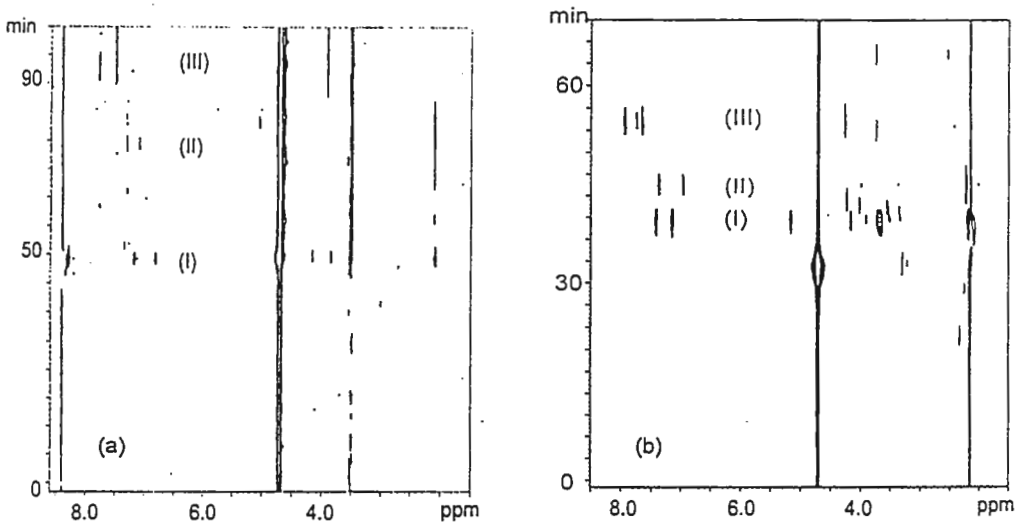
For CE-NMR, based on the observed signal-noise ratio seen in the NMR spectrum and given the known volume of the CE-NMR detection cell, it is estimated that approximately 10 ng of each metabolite was detected with a quality of result which would have allowed unambiguous identification of the molecules. Although CE-NMR remains a

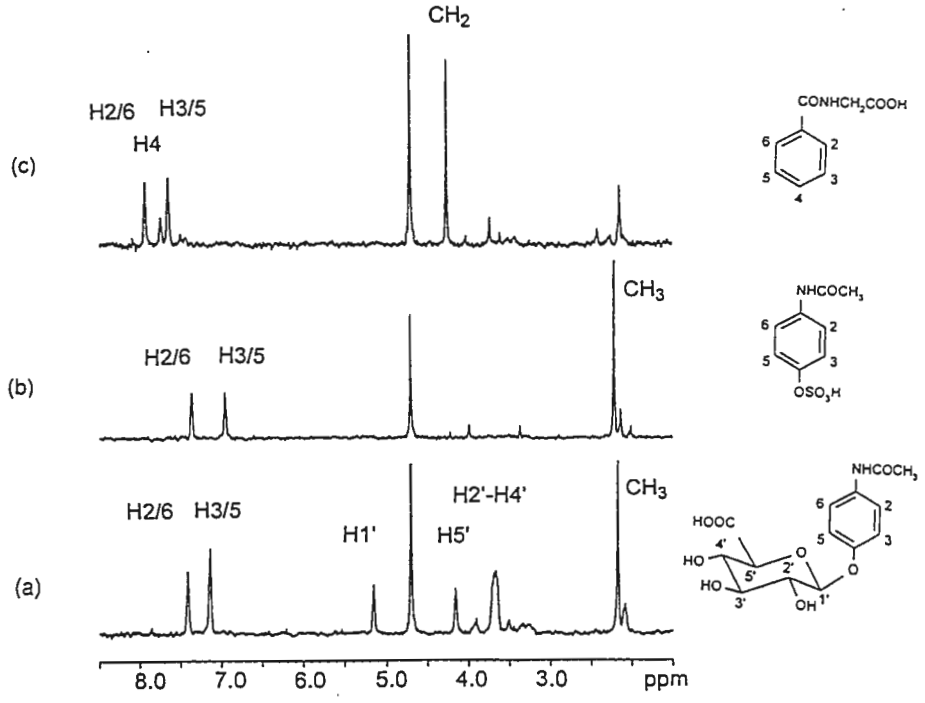
technically difficult procedure it has potential for identification of small quantities of analytes. The CEC-NMR spectra show better signal-noise ratio and this is a combination of both greater loading and active NMR detection volume.

Yours sincerely,

John Lindon

Jeremy Nicholson





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Microsoft: Just Say NO!

May 15, 1998
(received 5/19/98)

Dear Dr. Shapiro:

As many of your readers know, I have previously extolled the virtues of using TecMag-based NMR systems that operate with Apple Macintosh computers. What with Apple's declining market share and TecMag's own decision to offer PC-based systems, many of your readers might think the Apple platform dead for new users or old ones seeking to upgrade. Not so.

This past year I decided to replace three ailing Macintosh II*ci* computers, all of which ran TecMag instruments in my lab. With the help of Bruce Berkoff of UMAX Corporation (http://www.supermac.com), I obtained three UMAX Supermac (Apple-clone) computers. All three are 603*e*-based machines running at 180Mhz and retail for considerably less than $1,000. I also placed an order with TecMag for three upgrade kits. The installation was accomplished with no fuss by my first-year PhD student Greg Roberts; we now have three considerably more powerful NMR instruments, at least in term of data processing and multi-tasking.

TecMag's upgrade kits contained everything needed for the transition. Each kit included a National Instruments PCI 96-bit interface board, PCI to NuBus adapter boards, and new headers for boards inside our Aries and two Libras. The upgrades for two of the systems were $650 each, including TecMag's 20% academic institution discount. For our oldest Libra with the NBE board, the upgrade kit was $950. This kit included an extra adapter board that ran from the clone to the Libra's NBE board. Both of the adapter boards for this system connect to the same board in the clone, so no additional expansion slots are tied up. Each system required a board that ran from the clone to the spectrometer's MACINT1 board.

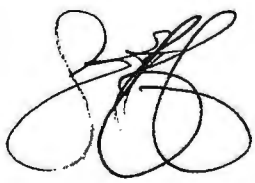
Three headers needed to be replaced on two boards for the Aries and the newest Libra. Four headers needed to be replaced on three boards for the old Libra; the extra header was for the NBE board. One of the Libra's had old wire-wrapped boards. Replacing the headers for that system only required careful use of tweezers to pull out the old ones. No soldering was involved. The other Libra and Aries had printed circuit boards, which have soldered connections to the headers. Each of the pins on the headers are fragile and closely spaced, so removing the old connections and soldering the new ones with your typical lab soldering iron would be very difficult and not worth the risk of damaging the expensive headers that probably paid for some TecMag vacations. Our electronics shop had some gear that they use for circuit boards, so they replaced the headers for two of the spectrometers.

PowerMac versions of the latest MacNMR software were available for free by downloading from the TecMag website. A few drivers also had to be replaced; these too were also available on their website.

It's certainly not as exciting as laser-polarized xenon, but any contribution I can make to insuring competitiveness in the computer market place is worth it.

Please credit this contribution to the Raychem account.

Sincerely,



UMEÅ UNIVERSITY
Department of Organic Chemistry
Prof Ulf Edlund

1998-04-20
(received 4/28/98)

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

Dear Barry,

PLS Modeling of FID Data Preprocessed by Orthogonal Scatter Correction.

The aim of this approach is to find a suitable way of handling an independent matrix of complex spectra (X) and its relation to a dependent matrix of properties (Y, i.e. reactivities, toxicities, or other activities). Small systematic information in X, related to Y, is commonly hidden in other dominant variation in X (instrumental and or sample instabilities, temperature effects, different measuring conditions etc.) . If these unwanted systematic variation is of similar magnitude as the the variation sought for, then normal PLS regression can handle this using FID´s or frequency spectra in X. However, if the looked-for variation is minor then orthogonal scatter correction (OSC) on the FID´s turned out to be quite useful i.e. OSC removes variation in X which is not related (orthogonal) to Y.

A typical set of CP/MAS NMR spectra of dissolving pulps could be illustrative. Using PLS modelling on 17 FID´s without OSC resulted in 5 components totally explaining 85.5 % of the quality variables in Y. However, three of the components described most of the variation in X (98.6 %) but almost no variation in Y (1.5 %). Performing OSC on the FID´s as a preprossesing method improved the predictive ability of the procedure significantly. Only 3 components were significant explaining 99.2 % in Y (cross-validated value, see below). The resulting loading spectrum after FT (showing only those carbon signals related to the Y changes), showed amazing improvement relative to the experiment using unpreprocessed data.

Best regards

Ulf Edlund

Project	OSC2MAT									
Model	**OSC FID model**									
Dataset	OSC2MAT									
Type	PLS									
NObs	17	NVarX	2048	NVarY	1					
Title										
A	**R2X**	**R2X(cum)**		**Eig**	**R2Y**	**R2Y(cum)**		**Q2**	**Limit**	**Q2(cum)**
1	**0.686**	**0.686**	**11.664**	**0.970**	**0.970**	**0.967**	**0.097**	**0.967**	**R1**	**1**
2	**0.168**	**0.854**	**2.860**	**0.013**	**0.983**	**0.380**	**0.097**	**0.980**	**R1**	**1**
3	**0.028**	**0.882**	**0.472**	**0.015**	**0.998**	**0.584**	**0.097**	**0.992**	**R1**	**1**

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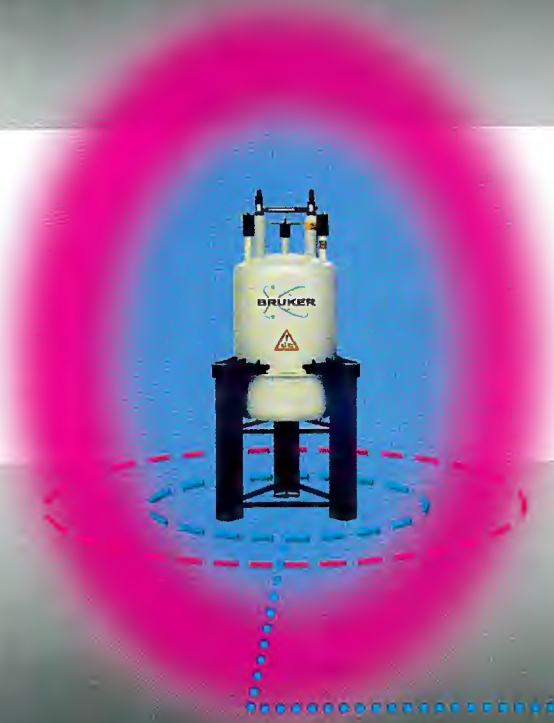

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Dr. B. L. Shapiro
The NMR Newsletter
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Palo Alto, CA 94303

e-mail: nmra@po.iams.sinica.edu.tw

May 15, 1998
(received 5/21/98)

The Influence of Adsorbed Benzene on Na-sites in NaY Zeolite Investigated by 2D Triple-quantum ^{23}Na MAS NMR Spectroscopy

Dear Prof. Shapiro:

The effect of benzene adsorption on ^{23}Na resonance and the quadrupolar interaction in NaY zeolites is studied by two-dimensional triple-quantum MAS ^{23}Na NMR spectroscopy. The pulse program is based on the two-pulse sequence proposed by Frydman et al. [1], and adds a z-filter pulse [2] to reduce the errors from the phase-cycling.

The 2D-spectrum of dry NaY zeolite shows two kinds of ^{23}Na signal, the gaussian-like down-field peak along SQ-axis may be correlated to SI-Na and the high-field peak with quadrupole-splitting to SII-Na [3,4]. In Fig. 1, with increasing benzene-loading, the SII-Na signal changes from a quadrupole-splitting lineshape to a gaussian-like lineshape and displaces to a new position in the 2D-spectra. For SI-Na, the calculated isotropic chemical shifts (δ^{CS}) are -7.3, -7.1, -6.5, -6.1 and -6.1 ppm referred to solid NaCl, for the NaY zeolites with loadings of 0, 0.3, 0.8, 2.0 and 5.0 C_6D_6 per supercage. This reflects a small effect of down-field shift of SI-Na in the vicinity of adsorbed benzene molecules on SII or the 12R window site [5]. Also, the calculated second-order-quadrupolar-effect (SOQE) parameters are 1.3, 1.2, 1.2, 1.3 and 1.2 MHz for the same loadings. In addition, for benzene-adsorbed SII-Na, the calculated δ^{CS} parameters are -21.9, -22.5 and -23.3 ppm for the loadings of 0.8, 2.0 and 5.0 C_6D_6 per supercage, and the calculated SOQE parameters are 2.3, 1.9 and 1.7 MHz for the same loadings. The ring current effect of benzene may give rise to the up-field trend of the δ^{CS} parameter for SII-Na adsorbed with benzene. Nevertheless, adsorption of benzene may reduce the electric field gradient at SII-Na as well and results in a decrease in magnitude of SOQE for SII-Na.

Sincerely yours.

Kan-Nian Hu

Lian-Pin Hwang

Kan-Nian Hu

Lian-Pin Hwang*

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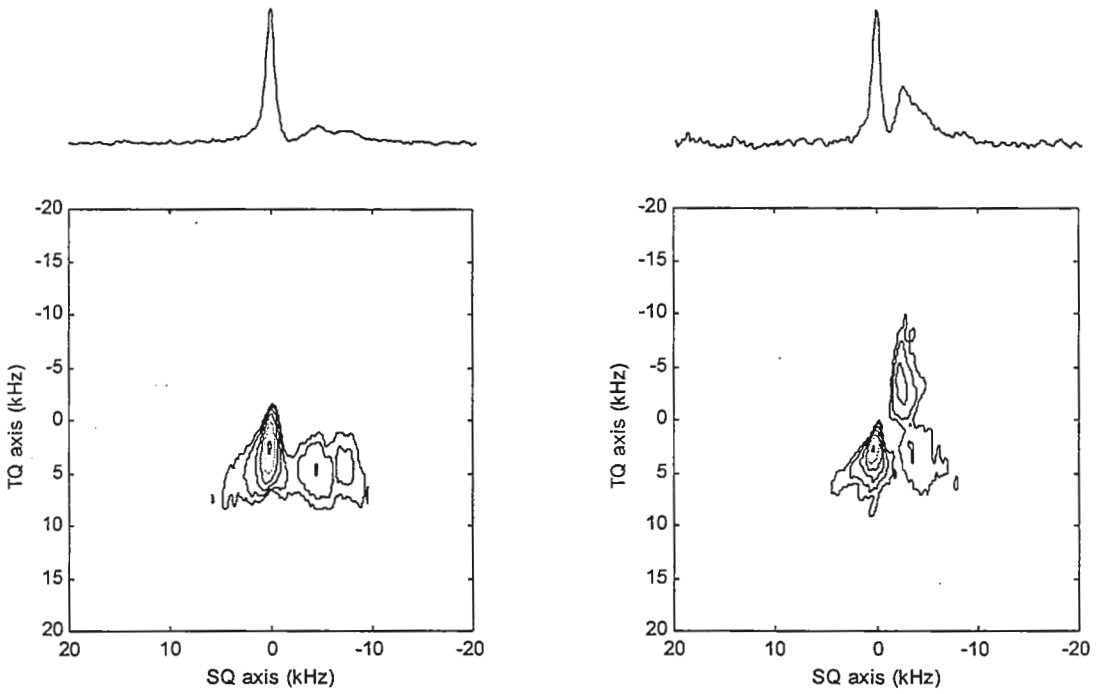

dry NaY

NaY loaded with 0.8 C_6D_6 per supercage

Reference:
[1] A. Medek, J. S. Harwood, L. Frydman, J. Am. Chem. Soc. 117 (1995) 5317.
[2] J. P. Amoureux, C. Fernandez, S. Steuernagel, J. Magn. Reson. A 123 (1996) 116.
[3] H. Koller, B Burger, A. M. Schneider, G. Engelhardt, J. Weitkamp, Microporous Material 5 (1995) 219.
[4] M. Hunger, P. Sarv, A. Samoson, Solid State Nuc. Magn. Reson. 9 (1997) 115.
[5] B. L. Su, J. Chem. Soc. Faraday Trans. 93 (1997) 1449.

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405-744-5920
FAX 405-744-6007

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

May 4, 1998
(received 5/8/98)

Dear Dr. Shapiro,

^{1}H NMR spectroscopy of paramagnetic heme proteins has provided a wealth of information on the electronic and molecular structure of the heme prosthetic group and the nearby amino acids.[1] In comparison, much less information is available about the ^{13}C resonances arising from carbons in the prosthetic group. This stems from the inherently low sensitivity associated with the observation of ^{13}C nuclei, and from the lack of a general process for isotopic labeling of the heme.

Although many of the problems associated with the poor sensitivity encountered with ^{13}C NMR experiments of paramagnetic heme proteins have been overcome with indirect-detected experiments such as HMQC,[2] the assignment of quaternary carbons in paramagnetic hemes continues to be challenging. Hetero-correlated experiments based on the relatively large value of $^1J_{CH}$ (HMQC) typically meet the condition $T_2^{-1} < {}^1J_{CH}$. Consequently, development of coherence and antiphase cancellation are less problematic in HMQC than in experiments based on $^3J_{HH}$, such as COSY. In fact, HMQC has been successfully applied to a number of heme proteins to obtain ^{1}H and ^{13}C assignments corresponding to heme CH_n units.[2,3] In contrast, the application of hetero-correlated experiments to the detection of long range ^{1}H-^{13}C correlations in fast relaxing systems has been less successful.[2,4] This is largely due to the fact that the condition $T_2^{-1} > {}^2J_{CH}$ is typically encountered in paramagnetic hemes. Consequently, the assignment of quaternary carbons, which are typically obtained with HMBC experiments in diamagnetic molecules, are not readily obtained in fast relaxing paramagnetic heme proteins.

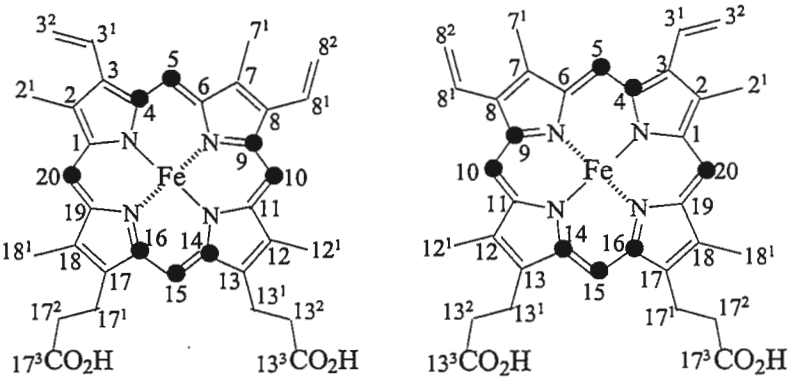
We have recently reported a biosynthetic approach for the isotopic labeling of heme which may be applicable to most heme proteins with removable hemes.[5] In this letter we wish to communicate that the assignment of quaternary carbons in a paramagnetic active site has been carried out with a ^{13}C-^{13}C double quantum coherence experiment, INADEQUATE,[6] and a protein containing doubly-labeled heme, taking advantage of the relatively large value of $^1J_{CC}$.

The heme in mitochondrial cytochrome b_5 was labeled as shown in Fig. 1, utilizing 5-^{13}C-δ-aminolevulinic acid as a source of ^{13}C-label. The INADEQUATE spectrum obtained from ^{13}C-labeled-heme cytochrome b_5 is shown in Fig. 2. We believe that this is the first demonstration of a ^{13}C-^{13}C double quantum coherence experiment applied to a paramagnetic heme incorporated into a protein. Furthermore, it is clear that all the expected type I and type III correlations[7] are present, thus demonstrating the potential applicability of the INADEQUATE experiment to paramagnetic heme active sites.

Yours sincerely,

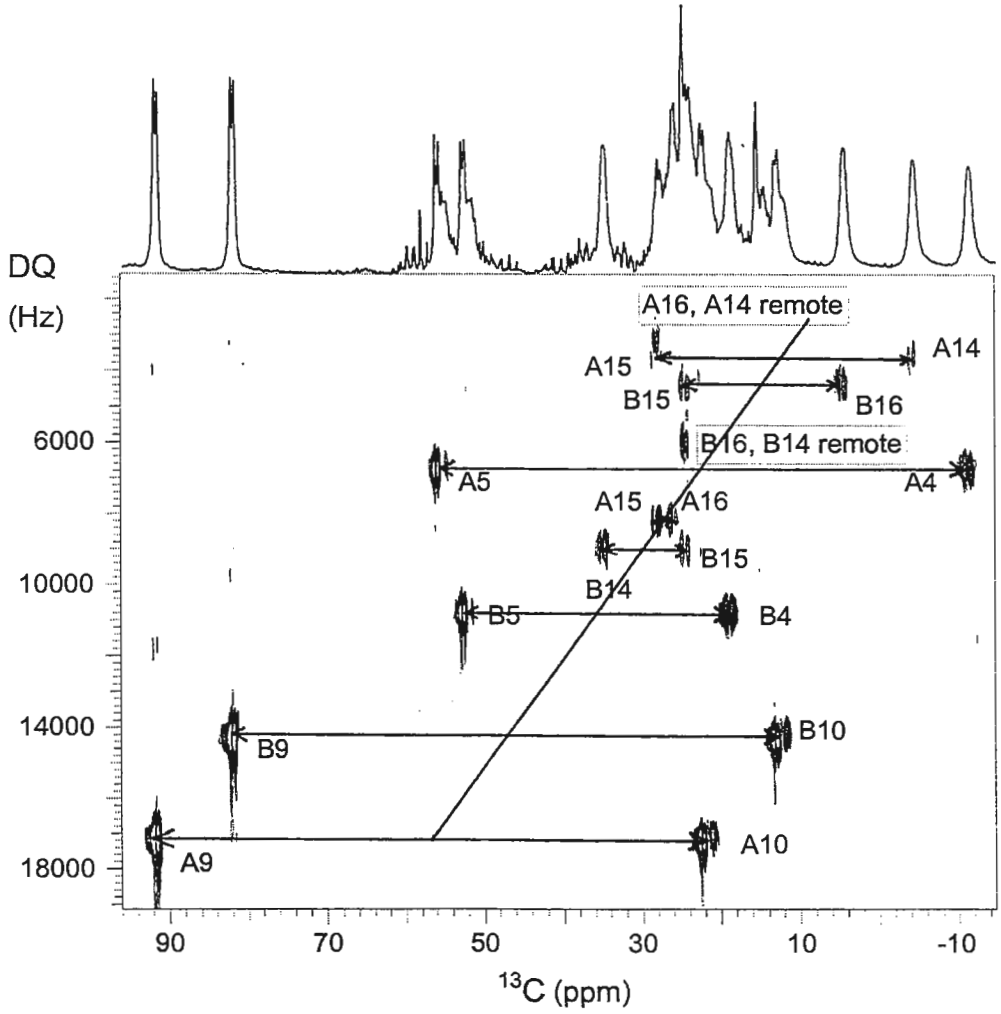
Mario Rivera Feng Qiu

Fig. 1:



Isomer A of OM Cytochrome b5 Isomer B of OM Cytochrome b5

Fig. 2:



References:

1) Biological Magnetic Resonance, Berliner, L., and Reuben, J. Eds. (1993) Vol 12, Plenum Press.
2) Timkovich, R. A. *Inorg. Chem.* (1991) 30, 37.
3) Turner, D. L., Costa, H. S., Coutinho, I. B., Legall, J., and Xavier, A. V. *Eur. J. Biochem.* (1997) 474.
4) Rodríguez-Marañón, M. J., Qiu, F., Stark, R. E., White, S. P., Zhang, S. P., Zhang, S. I., Foundling, S. I., Rodríguez, V., Shilling, C. L., Bunce, R. A., and Rivera, M. (1996) 35, 16378.
5) Rivera, M., and Walker, F. A. (1995) 230, 295.
6) Bax, A., Freeman, R., Frenkiel, T. A., and Levitt, M. H. (1980) 43, 478.
7) Braunshweiler, L, Bodenhausen, G. and Ernst, R. R. (1983) 48, 535.

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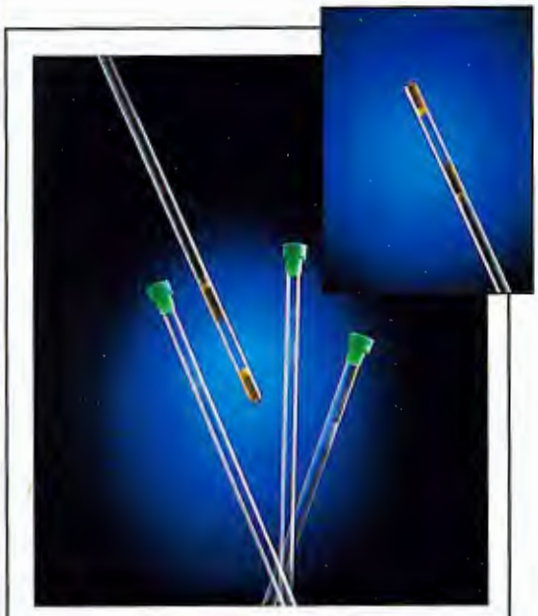

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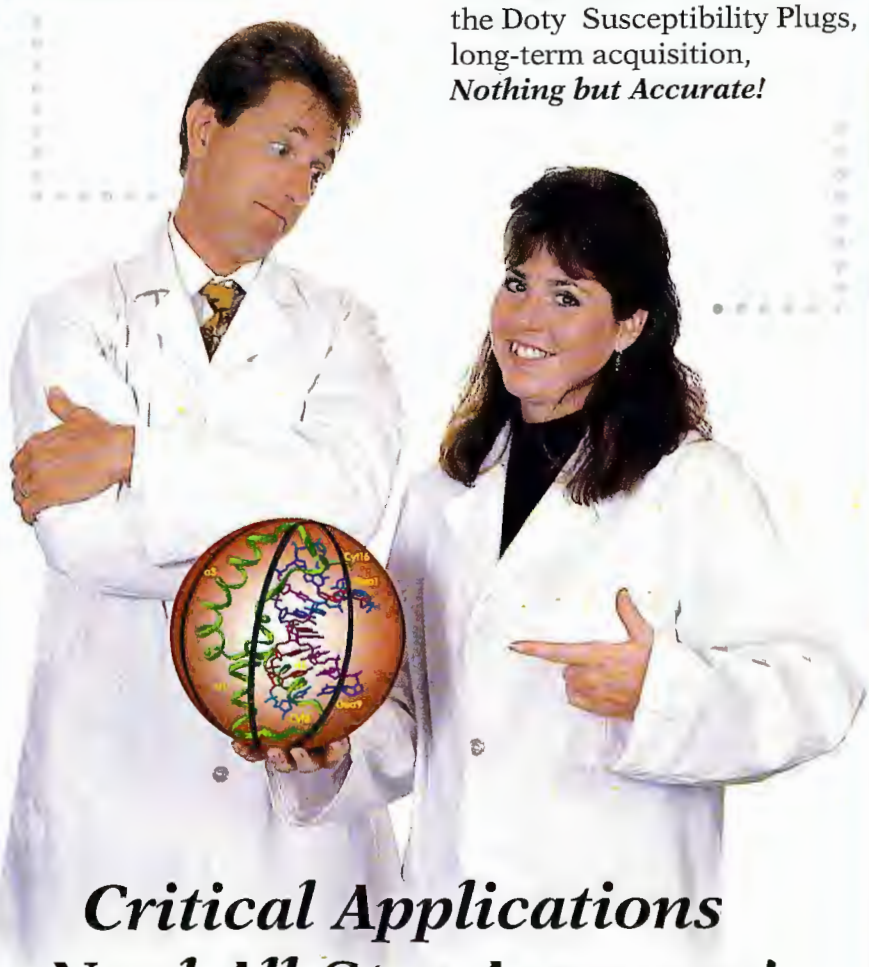

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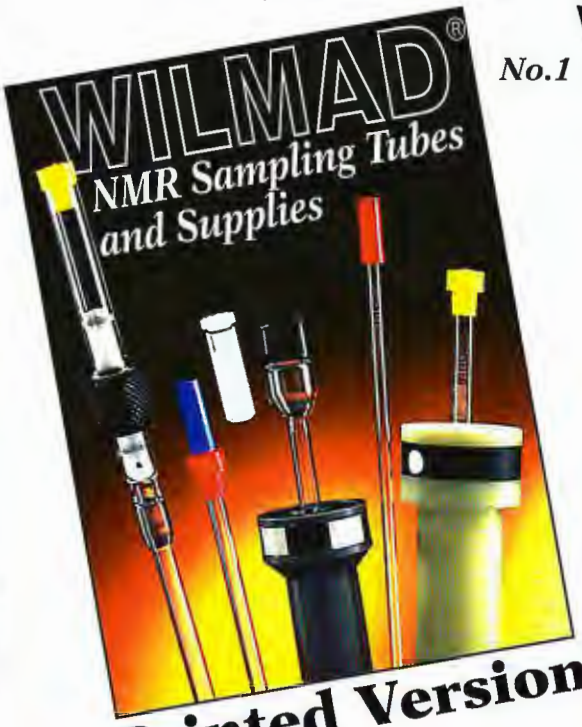

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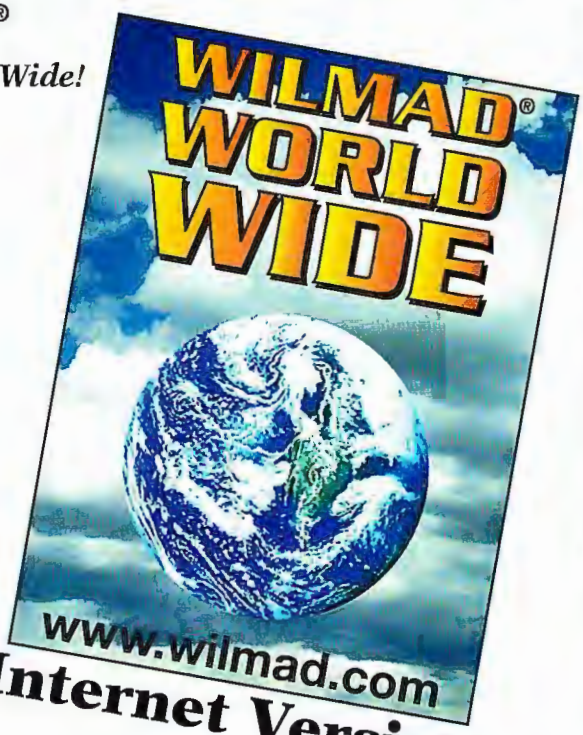

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(received 5/16/98)

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

Dear Barry

You don't need to be stuck with the signal from the glue

Some little while ago a co-worker visited our Lab to use the dedicated ^{19}F probe on our elderly JEOL GSX spectrometer. He planned to make preliminary measurements on some metabolite samples prior to running ^{19}F detected LC-NMR. This instrument has been the model of reliability since it was purchased in 1985. Looking back in our service records reveals no more than 2 days per year can be attributed to unplanned downtime (*aka* breakdown). I suspect you have guessed what comes next - the instrument wouldn't work. After some cursing and swearing we all felt better and decided to try our luck on our Varian Inova instrument, which was not specified to include a dedicated ^{19}F probe.

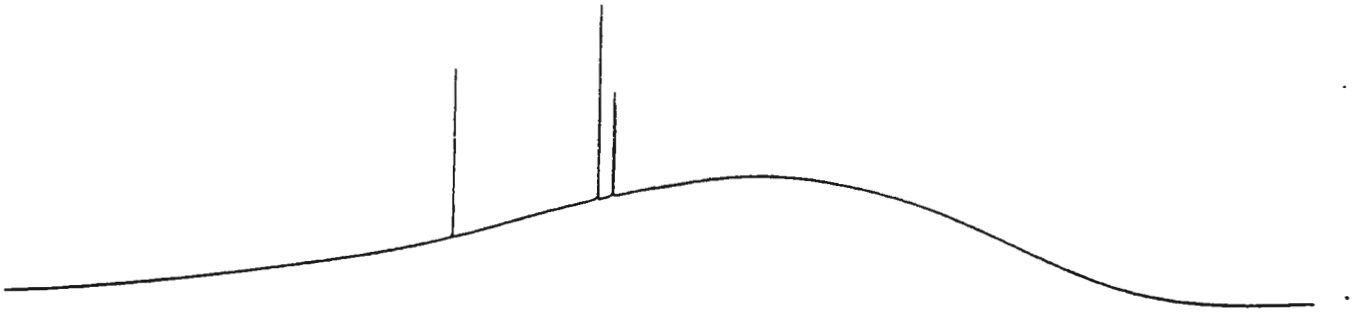
We already knew that the Varian probes we have (Nano•Probe™, ID5 and SW5) can be tuned to observe ^{19}F using the proton channel. We also knew that these probes have a broad background signal which we attribute to fluoridated glues used in the construction of the probes. This observation equally applies to non ^{19}F dedicated probes from other manufacturers - our JEOL autotune probe also has the same signal. The signal appears at around -120 ppm with respect to $CFCl_3$ and has a half height width of some 80 ppm.

We decided that we would prefer not to have this broad signal present, so we tried to eliminate it using a spin echo sequence. This worked fine as regards removing the broad signal but left us with datasets in which first order phase correction was, more or less, impossible.

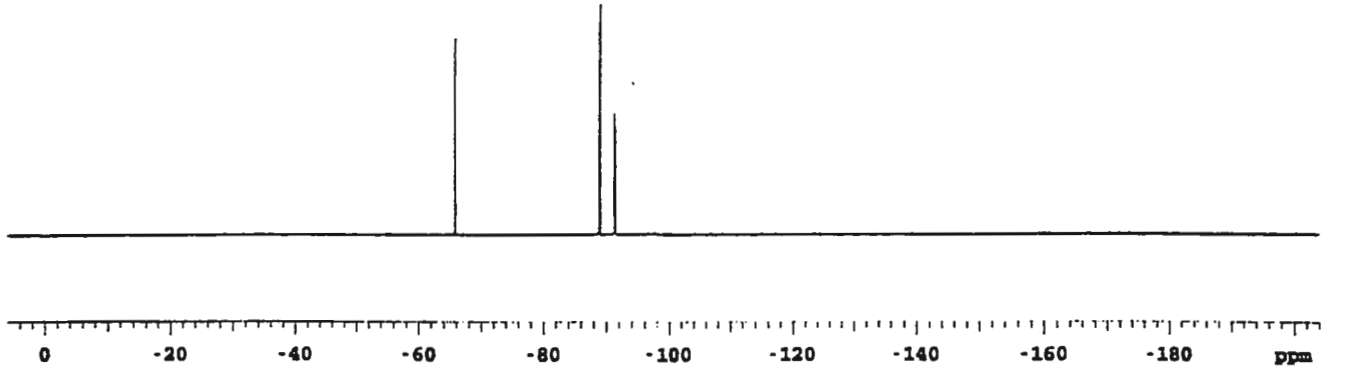
Martin then suggested we try linear prediction. He remembered the talk by W Reynolds at the 1997 International NMR Meeting in the UK, which gave compelling reasons why we should use LP on 2D data sets. So we tried it on our 1D ^{19}F spectrum.

By using backwards linear prediction over the first 0.25-1 ms of the spectrum FID we achieved a completely flat baseline over our routine 200 ppm sweep-width. We found that LP was easily set up as standard processing parameters in the Varian software and now use it on a routine basis. The following spectra show the improvement which can typically be achieved

a ^{19}F spectrum processed without LP



b ^{19}F Spectrum processed with LP



Please credit this contribution to Lydia Chang's account.

Yours sincerely

Paul *Martin*

Paul Stanley Martin Kipps

The NMR Newsletter - Book Reviews

Book Review Editor: István Pelczer, Dept. of Chemistry, Princeton University, Princeton, NJ 08544

"Spin Choreography
Basic Steps in High Resolution NMR"

by

Ray Freeman

Spektrum Academic Publishers (UK)/University Science Books (USA);
1997; 391 pages, $65.00 (hardcover); ISBN 1-901217-04-3

I don't know if Ray Freeman likes the films of Federico Fellini, but I could not help associating this one-of-a-kind book with those films, especially Amarcord. There is a whole cavalcade of basic knowledge, well-developed conclusions, little tricks and secrets, all presented with lots of artistry, love of the subject, and superior elegance. This association was further supported by the way how Professor Freeman chose the title for his new book, comparing the dance of nuclear spins choreographed by pulse sequences to that of ballet dancers. There is one point, however, where this association breaks down; "Spin Choreography" is much more than looking to the past. We find not only those basic ideas and results started at the dawn of high resolution NMR spectroscopy, but also lots of brand new applications, as revolutionary as very broad band decoupling using adiabatic pulses, for example. At the same time, this book is edited in a highly systematic fashion, carries a lot of educational value, and can be useful material for any university course.

The book consists of twelve chapters, and an Appendix – explaining an intentionally modest number of acronyms (with original literature references) – and a five-page Index. Each chapter starts with a content summary, and is completed with carefully selected references. These references embrace practically the whole history of high resolution NMR, making direct connections between the late forties/early fifties and the upcoming end of the century. The first three chapters provide the basic tools for proceeding with the rest efficiently (Energy Levels; Vector model, and Product Operator Formalism).

In the following nine chapters one will find some condensed matter – quite an encyclopedia of most of the fundamental NMR phenomena and examples of their use. Discussion of spin echoes is followed by that of soft radio frequency pulses, touching on some aspects of multidimensional spectroscopy. Those readers (most of us, I guess), who would miss the enlightening illustrations well-known from "A Handbook of NMR" and the many lectures of Prof. Freeman, will be compensated by titles (and attitude), such as "Separating the wheat from the chaff" (Chapter 6). This chapter is about selection of information of interest against artifacts or unwanted magnetization of any kind, using phase cycling, gradients, various filters and data processing approaches.

Continued

Broadband decoupling, making heteronuclear experiments feasible at very high fields, is the subject of the next chapter. The discussion starts from basic theory, surveys various approaches popular at times (many still being used), concluding with the use of adiabatic pulse techniques. Two-dimensional spectroscopy in the next chapter covers the basic homo- and heteronuclear correlation techniques, including multiple-quantum and J-correlations, with a brief look at higher dimensionality and applications for isotope-labeled substances. The nuclear Overhauser effect is discussed in an independent chapter (Chapter 9).

"In defense of noise" is the title of the next chapter, where various sources of noise, its suppression, treatment of noisy data in data processing, and, as the title hints, use of noise (in stochastic excitation and noise decoupling, for example) are discussed. It is rare to see such a thoughtful summary of this aspect of signal processing and data treatment. The last two chapters deal with water, including suppression techniques and radiation damping, and various ways of measuring J-coupling constants, in both time and frequency domain.

Each chapter focuses on a more-or-less stand-alone subject, which leads to some overlap and repetition, but each time in a different context. The text is well supported with lots of graphical illustrations and example spectra (real or simulated), and all processes are described with the aid of product operators. This book can be recommended to anyone who uses NMR and wants to learn more about the subject. It makes an excellent source for university courses, and serves as a reference book, too. And, above all, it is pleasant reading, even in the evening when the music is on – let the spins dance!

István Pelczer
Department of Chemistry
Princeton University
Princeton, NJ 08544

Postdoctoral Position

Opening for a postdoctoral position at Clark University, Worcester, MA, (about 40 miles west of Boston) in the areas of **biomolecular NMR** and **protein dynamics.** If you are experienced & motivated in some of the following areas:

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- heteronuclear multidimensional NMR: methods and applications
- molecular dynamics computer simulations; molecular modeling
- spin dynamics and quantum computing
- protein expression / peptide synthesis

please contact *Rafael Brüschweiler* by mail or e-mail. Starting date is end of August or later. Provide your *CV* and names & e-mail addresses of three individuals who can be asked for reference.

current address:
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Laboratorium für Physik. Chemie
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8092 Zürich
Switzerland
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E-mail: brueschweiler@nmr.phys.chem.ethz.ch

new address (from mid August):
Prof. Rafael Brüschweiler
Carlson Chair of Chemistry
Department of Chemistry
Clark University
950 Main Street
Worcester, MA 01610-1477, U.S.A.

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HARVARD UNIVERSITY

DEPARTMENT OF CHEMISTRY AND CHEMICAL BIOLOGY

Shaw G. Huang, Ph.D.
Director, Magnetic Resonance Lab

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May 14, 1998
(received 5/20/98)

Dr. Barry Shapiro
The NMR Newsletter

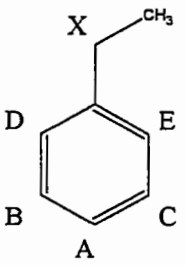
Dear Barry:

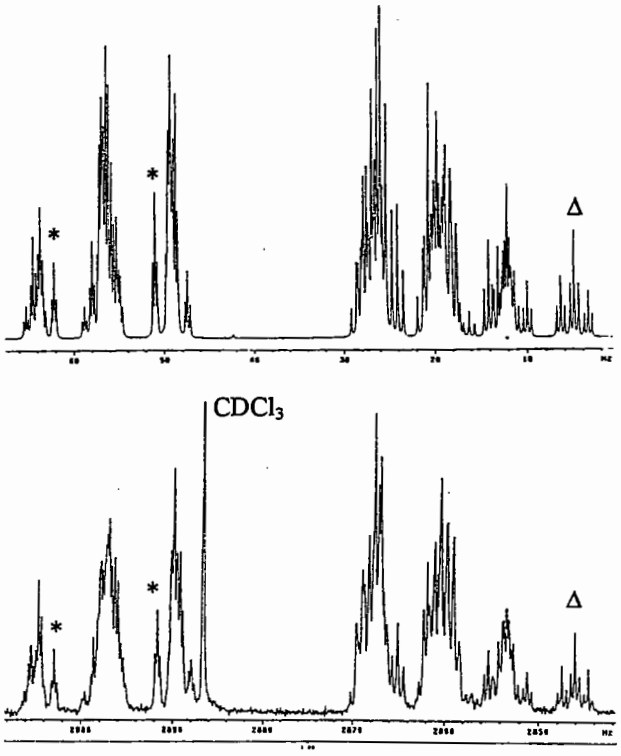
Traditionally, it is a common practice for NMR spectroscopists to use ortho-dichlorobenzene in D_6-acetone as a reference sample to check the resolution, and 0.1 % ethylbenzene in $CDCl_3$ as a reference sample to check the sensitivity of NMR spectrometers. However, many people have found that it is more difficult to shim the magnet field using D_6-acetone than using $CDCl_3$, which, in fact, is the most commonly used NMR solvent in many institutions. For these reasons, we have been using simply one reference sample, 0.1 % ethylbenzene in $CDCl_3$, to check both the sensitivity and the resolution of our spectrometers. To check the resolution using this sample, we just examine the aromatic proton region, which is composed of several multiplets of an $ABCDEX_2$ spin system. The para-proton has a ttt pattern located at the high-field side of this region. Specific attention is paid to the tt pattern of the most high-field multiplet (marked with Δ in the spectra below). If this tt multiplet can be nicely resolved, the shimming should be more than adequate. Further improvement of the shimming can resolve the smaller triplets belonging to the meta-protons (the peaks marked with * in the spectra below). These triplets are due to the 5-bond coupling between the meta-protons and the two methylene protons on the side-chain, which is about 0.25 Hz. The following spectra show a typical spectrum that we obtain on our 400 MHz NMR spectrometers (bottom trace), and a simulated spectrum (top trace, with 0.1 Hz linewidth added) with a set of properly chosen parameters (not optimized by iteration due to the interference of the large solvent peak). Note that the 6-bond coupling between the para-proton and the methylene protons is 0.44 Hz, which is almost twice as large as the 5-bond coupling. The 4-bond coupling between the ortho-protons and the methylene protons is about 0.63 Hz.

We believe that this new practice can be accepted by the NMR community as a more convenient and practical way of checking the resolution and the sensitivity using just one reference sample. Of course, in order to provide enough digital resolution, the memory size used for both the FID and the spectrum should be at least 128K.

Sincerely yours,

Shaw G. Huang





Chemical Shifts (Hz)

A=12.85, B=C=55.85
D=E=23.3, X=-1000

Coupling Constants (Hz)

JAB=JAC=JBD=JCE=7.38
JAD=JAE=1.36
JAX=0.44
JBC=1.80
JBE=JCD=0.35
JBX=JCX=0.25
JDE=1.60
JDX=JEX=0.63

mayo

May 7, 1998 (received 5/11/98)

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

Mayo Foundation
200 First Street SW
Rochester, Minnesota 55905
507-284-2511

Department of Biochemistry
and Molecular Biology

Separation of Chemical Exchange and Cross-Relaxation by Proton Dilution

Dear Barry:

We have studied the influence of proton dilution on cross-relaxation and have derived a Taylor expansion formula that describes the normalized cross-peak intensity of a 2D exchange spectrum, a_{ij}, as a function of the cross-relaxation rate, σ_{ij}, mixing time, τ_m, and the probability of finding a proton simultaneously at both sites i and j (1):

$$a_{ij} = \frac{A_{ij}(\tau_m)}{A_{ii}(0)} = \delta_{ij} - \frac{p_{ij}}{p_i}\sigma_{ij}\tau_m + O(\tau_m^2) \; . \qquad [1]$$

For a random isotope exchange $p_{ij} = p_i p_j$ and the apparent magnetization exchange rate is scaled by the probability of finding a proton at the partner spin site,

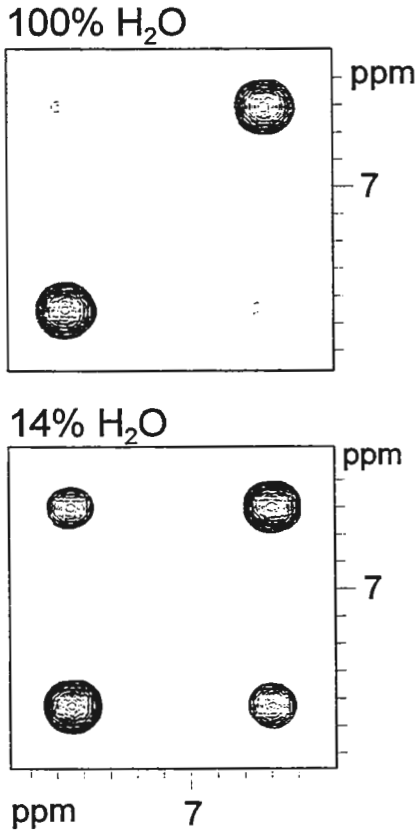
$$a_{ij} = \delta_{ij} - p_j \sigma_{ij}\tau_m + O(\tau_m^2) \; . \qquad [2]$$

In contrast to that, it is easy to show that the chemical exchange is independent of the protonation of either site,

$$a_{ij} = \delta_{ij} + k_{ij}\tau_m + O(\tau_m^2) \; , \qquad [3]$$

where k_{ij} is the chemical exchange rate constant. Of course, one needs a proton to observe the exchange at all; however, the normalized cross-peak intensity is independent of the degree of isotope exchange. When both cross-relaxation and chemical exchange are taking place between the same spin sites, we obtain

$$a_{ij} = \delta_{ij} + (k_{ij} - p_j \sigma_{ij})\tau_m + O(\tau_m^2) \; . \qquad [4]$$



The chemical exchange between the sites guarantees that $p_i = p_j = p$. In the extreme narrowing limit, where $\sigma > 0$, cross-relaxation and chemical exchange can cancel each other ($k - p\,\sigma = 0$) leading to the false conclusion that the system is rigid and the observed spins are far apart. For constant k and σ the cancellation occurs at a particular degree of protonation, $p = k/\sigma$. Thus, by recording the experiment at two different dilutions, the presence of simultaneous cross-relaxation and chemical exchange can be revealed. As an experimental example, we have used the side-chain NH_2 group of free glutamine in which the protons exhibit cross-relaxation due to the close proximity and chemical exchange due to the rotation about the partial double C-N bond. The experiment is performed at 300 MHz in water and by manipulating the temperature, we were able to achieve almost complete elimination of the cross-peaks (top panel). However, addition of D_2O (at the same temperature) dilutes the protons, suppresses the cross-relaxation and the strong chemical exchange cross-peaks are clearly seen (bottom panel). From the spectra recorded at a few dilutions, one obtains the chemical exchange rate constant as the intercept at $p = 0$, and the cross-relaxation rate constant from the slope of the apparent cross-relaxation rate $k - p\,\sigma$ measured as a function of proton concentration p.

References: 1. Zs. Zolnai, N. Juranić, and S. Macura, *J. Biomol. NMR*, in press.

Sincerely yours,

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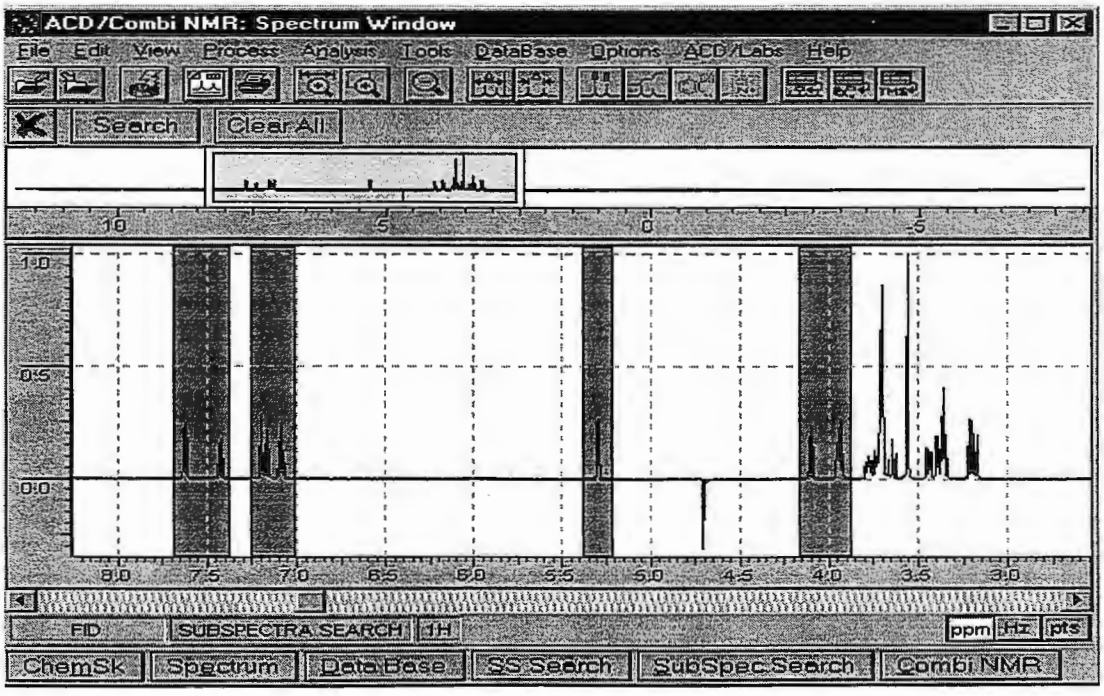
Combinatorial NMR - Applications of NMR Prediction Algorithms

May 6th, 1998
(received 5/11/98)

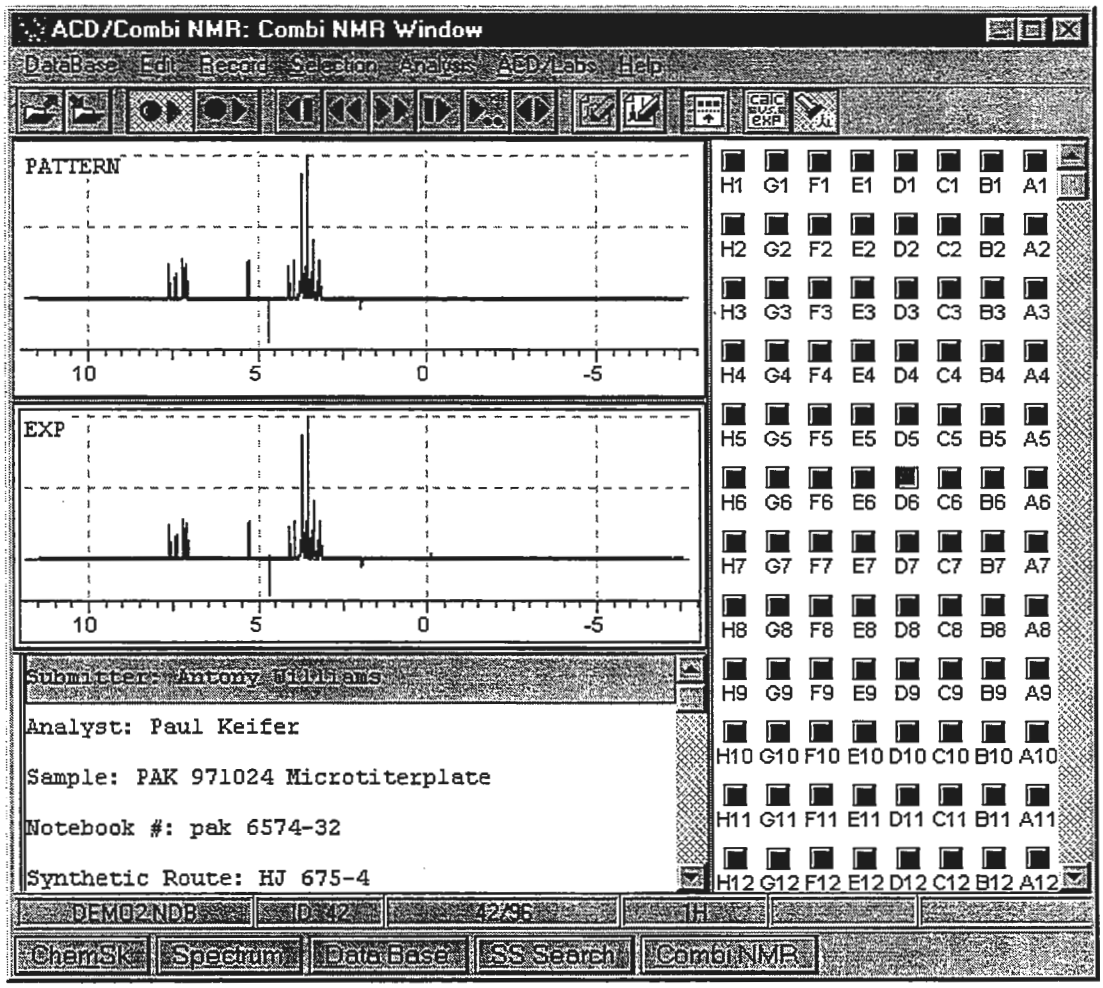
Dear Barry,

As a result of the shift towards high throughput screening and combinatorial analytical methods, we at Advanced Chemistry Development are pursuing any possibility to support this exciting field. In collaboration with Varian NMR Instruments, specifically Evan Williams and Paul Keifer, we have enhanced our NMR software offerings to support Combinatorial NMR applications. ACD/Combi-NMR presently allows the user to access NMR data directly from the spectrometer and process using a Group Macro processing feature. We have used this to process the data obtained from a 96-well plate but have tested the bulk processing on over 200 FIDs. An alternative of course is to obtain the appropriate set of phasefiles from the spectrometer. Following processing the spectra automatically populate a database identical to those generated in our ACD/NMR Manager product.

The Combi/NMR module can be used to allow spectral, subspectral or multi-subspectral searching with the "hits" displayed on a 96-well plate format using color coding to show responses to the search. An example is shown below where the multi-subspectral search was performed on the spectrum immediately below and matches, spectra containing the highlighted subspectra, were located within the 96-well plate as shown at right. Green highlighted buttons indicate the hits with the lower screen indicating one of these hits and the upper screen showing the pattern for matching.



ACD/Combi NMR therefore allows the user to remove the data from the spectrometer and perform necessary data handling at the desk. Following this data handling the Combinatorial plate data sets can be stored as individual databases or merged into a larger overall database containing up to 500,000 spectra. Each entry in the database can be associated with up to 16,000 textual user data fields that are fully searchable through the database interface. These of course could include Notebook #'s, Chemist's Name, Solvent details, Synthesis ID # or whatever.



Following acquisition of a Combi data set the user has the opportunity to add suggested structures and associate them with particular spectra in the dataset. Presently this can be done directly through our integrated ChemSketch structure drawing package (which can read ChemDraw, ISIS.skc, molfiles or many other formats). Using the ACD H1 NMR prediction algorithms we generate predicted spectra for each of the suggested structures and display them on screen for direct visual comparison with the experimental spectra. This comparison could obviously be performed just by manually screening for matches but we perform a statistical analysis based on the differences in shifts between the experimental and predicted spectra and produce a Combi-result factor which varies between 0 and 1, 1 being a perfect match. The obtained values are then displayed using color coding to display ranges for the match factors. Databasing these spectra including

structures allows future searches of groups of such databases by structure or substructure as well as the user definable textual data.

Our future improvement plans for this development project include quantitation capabilities, ignoring exchangeable protons as an option during the spectral matching procedure and utilizing user databases containing structures and assignments pertinent to the chemistry of the user or laboratory in question. We look forward to our continuing collaborations with Varian as well as suggestions from your readers regarding possibly useful features that could be incorporated into ACD/Combi NMR.

Best wishes Barry!

Tony Williams
tony@acdlabs.com

Are Computers Male or Female?

Five reasons to believe computers are male:

1. They have a lot of data, but are still clueless.
2. They are supposed to help you solve problems, but half the time they ARE the problem.
3. As soon as you commit to one you realize that, if you had waited a little longer, you could have obtained a better model.
4. In order to get their attention, you have to turn them on.
5. Big power surges knock them out for the rest of the night.

Five reasons to believe computers are female:

1. No one but the Creator understands their internal logic.
2. The native language they use to communicate with others is incomprehensible to everyone else.
3. The message "Bad command or file name" is about as informative as, "If you don't know why I'm mad at you, then I'm certainly not going to tell you."
4. Even your smallest mistakes are stored in long-term memory for later retrieval.
5. As soon as you make a commitment to one, you find yourself spending half your paycheck on accessories for it.

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

May 18, 1998
(received 5/21/98)

Dear Barry,

We currently have the following two openings available in our laboratory. Interested applicants should send a current CV or resume to me at : Steve Maple, Eli Lilly and Company, Lilly Corporate Center, DC 3811, Indianapolis, IN 46285.

Analytical Chemist - LC/NMR - BS, MS
We seek a highly motivated scientist with NMR experience to become the primary operator for a new 600 MHz LC-NMR system supporting the structure elucidation of metabolites of novel drug candidates. The successful candidate will have knowledge of the practical aspects of NMR spectroscopy, a fundamental understanding of organic chemistry, excellent problem solving and communication skills, and the ability to work in a team-based environment. HPLC, LC-NMR, MS, and UNIX skills are definite pluses.

Postdoctoral Fellow --- LC-NMR and LC-NMR-MS
A position is available involving the development, automation, and applications of these two techniques. The successful candidate will have a recent Ph.D. degree with an emphasis in either NMR or MS. An aptitude for instrumental design, computer programming, excellent problem solving skills, and the ability to work in a team-based environment are also required. Instrumentation available in the laboratory includes Varian 300, 500, and 600 MHz NMR systems (two equipped for LC-NMR) and three MS instruments (a triple quadrupole, TOF, and a 3T FTICR) equipped with various ionization sources. This position provides a unique opportunity with competitive salary and benefits.

Regards,

Steve

Steven R. Maple

Address all Newsletter correspondence to:

Dr. B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303.
650-493-5971* - *Please call only between 8:00 am and 10:00 pm, Pacific Coast time.*

Deadline Dates

No. 478 (July)	26 June 1998
No. 479 (Aug.)	24 July 1998
No. 480 (Sept.)	21 Aug. 1998
No. 481 (Oct.)	25 Sept. 1998
No. 482 (Nov.)	23 Oct. 1998

* Fax: 650-493-1348, at any hour. Do not use fax for technical contributions to the Newsletter, for the received fax quality is very inadequate.

* E-mail: shapiro@nmrnewsletter.com

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Forthcoming NMR Meetings, continued from page 1:

Spin Choreography - a symposium in appreciation of Ray Freeman, Cambridge, England, **April 8-11, 1999**; web site: http://mchsg4.ch.man.ac.uk/mcmr/RF.html; fax: c/o M.H.Levitt +46-8-15 2187; email: mhl@physc.su.se.

41st ENC (Experimental NMR Conference), Asilomar Conference Center, Pacific Grove, CA, **April 9-14, 2000**; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87505; (505) 989-4573; Fax: (505) 989-1073; Email: enc@enc-conference.org.

Additional listings of meetings, etc., are invited.

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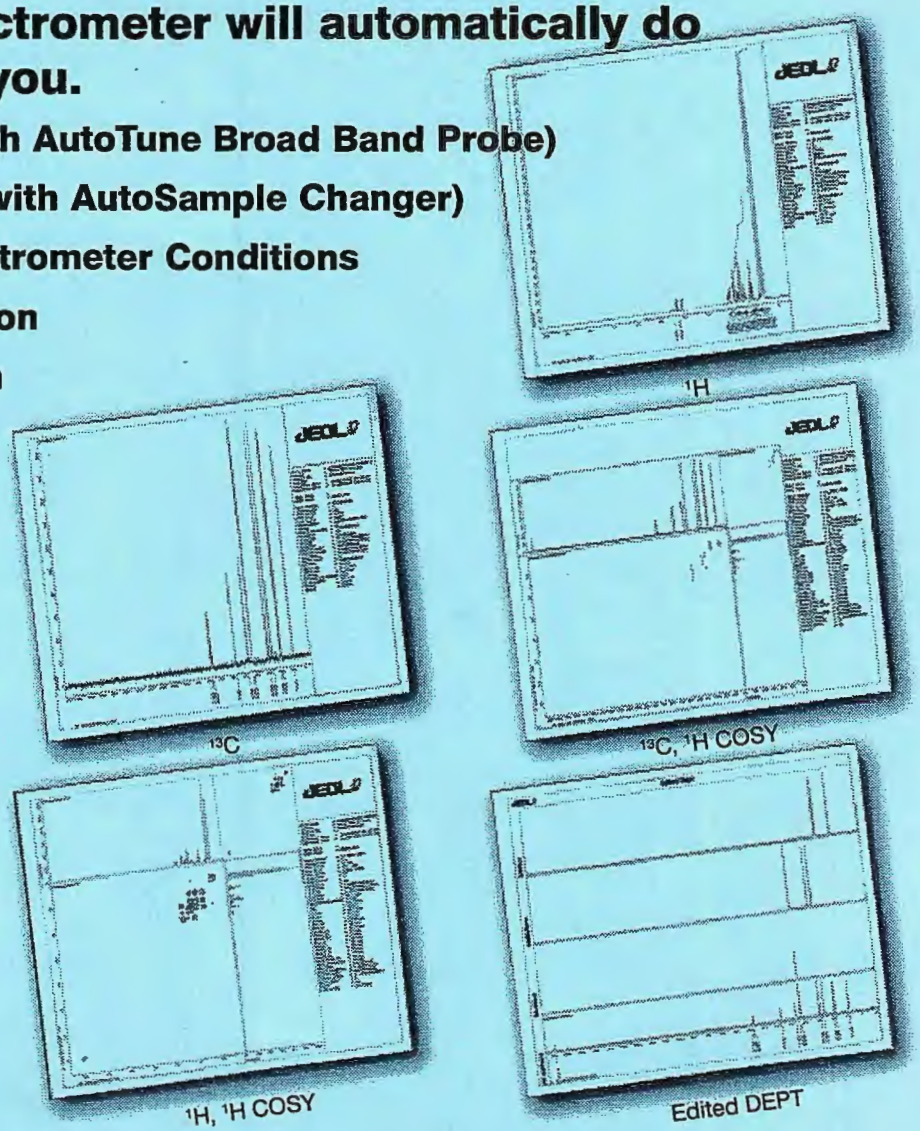

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