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#### FORTHCOMING NMR MEETINGS

- Second International Conference on Magnetic Resonance Spectroscopy, Heidelberg, Germany, September 6-10, 1993; Contact: Dr. Winifried Kuhn, Fraunhofer Inst., Ensheimer Str. 48, DW-6670 St. Ingbert, Germany; Phone: (49) 6894-89738; Fax: (49) 6894-89750, or Dr. Bernhard Bluemich, Max-Planck-Inst. for Polymer Research, Postfach 3148, D-6500 Mainz, Germany; Phone: (49) 6131-379125; Fax: (49) 6131-379100.
- 1993 FACSS Meeting, Detroit, Michigan, October 17-22, 1993; Contact: H. N. Cheng, Hercules, Inc., Research Center, 500 Hercules Road, Wilmington, DE 19808; Phone: (302) 995-3505; Fax:. (302) 995-4117. See TAMU NMR Newsletter 411, 10.
- Pacific Conference, Pasadena, California, October 19-23, 1993; Contact: Ms. B. Belmont, Pacific Conference, 14934 S. Figueroa St., Gardena, CA 90248; Phone: (310) 538-9709.
- <u>1993 CABM Fall Symposium on "Macromolecular Recognition"</u>, Piscataway, NJ, October 21-22, 1993; Program Organizer: G. T. Montelione; Contact: Linda Van Derveer, Symposium Coordinator, Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, NJ 08854; Phone: (908) 235-5309; Fax: (908) 235-4850. See Newsletter <u>419</u>, 30.
- 35th ENC (Experimental NMR Conference), Asilomar Conference Center, Pacific Grove, California, April 10 15, 1994; 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 [sic]; Contact: Dr. Wm. A. Bubb, Secretary, Univ. of Sydney, Dept. of Biochemistry, Sydney, NSW 2006, Australia. See TAMU NMR Newsletter 419, 26.

Additional listings of meetings, etc., are invited.

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DEPARTMENT OF CHEMISTRY

Magnetic Shielding of a 19" Color Monitor

Dr. Barry Shapiro TAMU Newsletter 966 Elsinore Court Palo Alto, CA 94303 Wednesday, July 7, 1993 (received 7/12/93)

Dear Barry:

We have recently installed a SUN computer and monitor near our spectrometer. Even though the 19" color monitor was placed outside of the 5 gauss line there was significant distortion of the images on the monitor. Attempts to remove the distortion by degaussing the monitor, moving the screen around and the like did not meet with success. Thus, we decided to construct a wood box with mu metal lining to shield the screen. Mu metal is a very effective magnetic shield and is quite flexible. The box is 25" tall, 26" deep and 14" wide. A 5" by 14" cutout in the back of the box was made to allow running in the power and connection cables. The box was lined with AD-MU-80 mu metal, thickness of 0.1 inch, obtained from Ad-vance Magnetics of Rochester, IN. A total of 18 feet, at \$27.65/foot, of 15" wide strips of mu metal was required to cover the interior of the box at one layer of thickness. For ventilation purposes two louvered vents were added to each side of the box and for aesthetic purposes the box was covered with formica and some other trim. The box works quite well and there is no visible distortion of the images on the monitor. The ventilation provided by the simple vents appears to be adequate for the monitor. The monitor was degaussed after being placed in the box.

The scientific support services at Wesleyan could construct similar boxes for other users if there is interest. Please contact Thomas Castelli at 203-344-8544 extension 2752.

Sincerely,

J-G CHU.

Thomas Castelli

Philip\_Bolton

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6/30/93

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

Dear Barry

#### Conformational Analysis of the Amino Acid Side Chain of Sinefugin

Quite some time ago we reported an NMR study of the conformational forms of S-adenosyl-L-methionine (SAM) and S-adenosyl-L-homocysteine (SAH), two important cofactors of methyl transferases (JACS, 103, 6015-6019 (1981)). The conformation of Sinefugin, an antibiotic analog of SAM and SAH, is therefore of interest to us since it is a potent inhibitor of SAM utilizing methyl transferases. Sinefugin is a conformationally complex molecule with a flexible  $\alpha$ ,  $\delta$ -diamino acid side chain, a nonplanar furanose ring and either *syn* or *anti* adenine ring orientations. Of special challenge to us is the side chain which, even at 500 MHz, affords a complex pattern of overlapping diastereotopic methylene protons (H<sub>5R</sub>, H<sub>5S</sub>, H<sub>βR</sub>, H<sub>βS</sub>, H<sub>γR</sub>, H<sub>γS</sub>) with up to 13 coupling constants all falling within less than 1 ppm. Resolving this complex pattern and determining the average conformation of the side chain from the NMR coupling constants has proven far from trivial.



The overlapping cross peaks from closely spaced proton resonances make DQF COSY spectra undecipherable. However pH titration studies permitted us to distinguish peaks from *gamma* and *beta* protons since the *gamma* amino group is deprotonated preferentially upon going to higher pH. Conventional 1D spectra, of various pH solutions, with and without coherent decoupling at the  $\alpha$  - or  $\delta$ -protons was enough to get a start on the assignments, which were refined iteratively by spectrum simulation.

The most intersting feature of this pH dependence is the unusual behavior of the 5', 5" proton signals. Serianni (*Magn. Reson. Chem.*, 28, 324-330 (1990)) and others have established that the prochiral 5' and 5" -protons of nucleosides are diastereotopic with the pro-R proton appearing upfield. We were suprised to observe that both of these protons in Sinefugin have the same chemical shift at pH 7 and appear as a "triplet" centered at 2.21 ppm (J<sub>ave</sub> = 4.5 Hz), Fig 1. At higher pH (eg. 9.60) the peaks separate and move upfield to 2.108 and 2.026 ppm with a complex coupling pattern (J<sub>4'5S</sub> = 8.8, J<sub>4'5R</sub> = 3.5, J<sub>65S</sub> = 3.5, J<sub>65R</sub> = 9.9 and J<sub>5S5R</sub> = -15.3 Hz), Fig 2. The first explanation that comes to mind is that at low pH, when both amino 419~6

groups are protonated, there is essentially free rotation about the  $C_4$ - $C_5$  and  $C_5$ - $C_{\delta}$  bonds with roughly equal populations of the staggared conformations and a curious averaging of chemical shift effects on the prochiral protons. At higher pH when the  $\delta$ -amino is largely deprotonated, there are unequal conformation populations so that unequal coupling constants are observed. Coupling constants for each staggared form can be estimated by the Altona method and the observed couplings can then be used to calculate the percentage of each contributing conformation by solving two sets of three simultaneous equations. The results are consistent with the extended (tg, tg) conformation of Fig 3.



Fig. 2 pH 9.60

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(received 7/16/93) July 15, 1993

### Solvent Suppression in HCC(CO)NH-TOCSY Experiments

Using Pulsed-Field Gradients

Dear Dr. Shapiro,

Pulsed-field gradients (PFGs) provide an important approach for selective detection or cancellation of heteronuclear coherence (Hurd and John, 1991). In these experiments, magnetic field gradients with carefully tuned amplitudes are used to defocus and then selectively refocus magnetization transfer pathways of interest, while simultaneously defocusing (or homospoiling) undesired magnetization states. Over the last year we have implemented and optimized an extensive family of phase sensitive heteronuclear PFG experiments. An important pair of experiments which we use in determining sequential resonance assignments in proteins are HCC(CO)NH-TOCSY (Logan et al., 1992, Montelione, et. al., 1992) and HCCNH-TOCSY (Logan et al., 1992, Lyons and Montelione, 1993) which have been modified to use PFG's for H20 solvent suppression.

Examples of 2D PFG HCC(CO)NH-TOCSY spectra recorded with and without H2O solvent preirradiation are shown in Figure 1. In Figure 2 is a comparison of traces from these same two spectra.

As can be seen in Figure 1, deleterious effects of solvent preirradiation are avoided in PFG HCC(CO)NH-TOCSY. Comparison of traces in Figure 2 shows a significant attenuation of  $H^{\alpha}$ -H<sup>N</sup> cross-peaks when preirradiation is used. This is because solvent preirradiation also saturates  $H^{\alpha}$  resonances. Although attenuation due to preirradiation is less severe for HCC(CO)NH-TOCSY cross peaks involving sidechain aliphatic protons, these are also attenuated by spin-diffusion and saturation-transfer effects. Overall, significantly better triple resonance data are obtained using PFGs in place of preirradiation for water solvent suppression.

Sincerely Yours, Singre Wang Lingre L. Wang

Juy /m. T. aetano T. Montelione





Figure 1. Comparison of 2D PFG\_HCC(CO)NH-TOCSY spectra. (A) 2D spectrum collected with DIPSI-3 <sup>13</sup>C-<sup>13</sup>C spin lock (23.5 ms) without water preirradiation. (B) 2D spectrum recorded with the same isotropic mixing scheme using weak (<50 Hz decoupler field strength) water preirradiation. These data were recorded using 100% uniformly <sup>13</sup>C- and <sup>15</sup>N-enriched sample of the Z-Domain of Protein A (Lyons, et. al., 1993), dissolved at a concentration of 2 mM at pH 6.5 and temperature of 30° C. The spectra were collected for about 20 hours each on a Varian Unity 500 NMR spectrometer.



Figure 2. Comparison of traces along the detection dimension of 2D PFG\_HCC(CO)NH-TOCSY with and without water preirradiation. (A)  $H^{\alpha}$ - $H^{N}$  region. (B)  $H^{\beta}H^{N}/H^{\gamma}-H^{N}/H^{\delta}-H^{N}$  region.

#### **REFERENCES:**

Hurd, R. E. and John, B. K., J. Magn. Reson. **91**, 648 (1991). Logen, T. M., et al., FEBS Letts.**314**, 413 (1992). Montelione, G. T., J. Am. Chem. Soc. **114**, 10974 (1992). Lyons, B. A. and Montelione, G. T., J. Magn. Reson. **B 101**, 206 (1993). Lyons, B. A., et al., Biochemistry, in press, (1993).

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(received 7/19/93)

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

#### RE: TmDOTP5- AS AN IN VIVO SODIUM SHIFT REAGENT

Dear Barry:

After joining the Rogers Magnetic Resonance Center, I (DEW) am pleased to have the opportunity to engage in research in my long-standing interest in NMR of biological tissue, including nuclei such as <sup>23</sup>Na. There is much interest in conventional magnetic resonance spectroscopy (MRS) here, and this contribution describes some of the latest research on in vivo sodium MRS.

The <sup>23</sup>Na signals in the various compartments of a biological tissue all have the same chemical shift and one of the methods for separating these signals is the use of paramagnetic anionic shift reagents (SRs). These SRs are largely membrane impermeable, diffuse into all extracellular space, and competetively bind with Na+ and other cations in this space and induce chemical shifts in their resonances.

We have recently tested a new SR, thulium(III) 1,4,7,10-tetraazacyclododecane 1,4,7,10- tetrakis-(methylene phosphonate) (TmDOTP<sup>5-</sup>) to the *in vivo* rat kidney, as an extension to the earlier *in vivo* applications to rat liver, brain, and heart. One jugular vein of 400-500 g. rats was used to infuse anesthesia and the other to infuse the 80 mM SR. The right kidney was tied off and the exposed left kidney was examined with a surface coil tuned to <sup>23</sup>Na at 53 MHz. The infusion of SR was started at 2 ml/hr and increased to 8 ml/hr over a period of 9 minutes. When a chemical shift of about 8 ppm was achieved for the extracellular peaks, the infusion rate was reduced to 2 to 3 ml/hr to maintain this shift. NMR spectra were collected at various times during a period of a half hour. Blood and urine samples were separately analysed by <sup>23</sup>Na MRS.

Figure 1 compares in vivo <sup>23</sup>Na spectra obtained after infusion of TmDOTP<sup>5-</sup> (left) and DyTTHA<sup>3-</sup> (right). The former shows three distinct peaks : an unshifted resonance at 0 ppm, a resonance at about 10 ppm, and a third resonance at about 35 ppm. Subsequent experiments run using blood and urine samples collected from these animals indicate that the unshifted resonance is the intracellular sodium signal, the 10 ppm resonance is from interstitial and vascular sodium and the most shifted resonance represents filtrate sodium (figure 2).

These results show that TmDOTP<sup>5-</sup> is very effective as an *in vivo* <sup>23</sup>Na SR in the kidney. Compared to DyTTHA3-, it produces much better resolution at much lower concentrations. Consequently, it may thus be a viable alternative to current SRs, with great potential for in vivo application.

Sincerely.

Seshan

N. Bansal M. J. Germann A. D. Sherry C. R. Mallov

D. E. Woess



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Fig. 1 : Comparison of *in vivo* <sup>23</sup>Na spectra from rat liver obtained during infusion of TmDOTP<sup>5-</sup> (left) and DyTTHA<sup>3-</sup> (right). The times indicated in the middle of the stacked plots is at the beginning of data acquisition. The infusion coses at the corresponding time intervals are shown at the left for TmDOTP and at the right for DyTTHA<sup>3-</sup>.



Fig. 2 :  ${}^{23}$ Na MR spectra from blood, urine and *in vivo* rat kidney. The correspondence of the peaks show that the most shifted peak is from  ${}^{23}$ Na present in filtrate, the less shifted peak is from vascular and interstitial  ${}^{23}$ Na and the unshifted resonance is the intracellular  ${}^{23}$ Na signal. The slight increase in the chemical shift of the urine peak as compared to that of the most shifted peak *in vivo* can be attributed to the increased concentration of shift reagent in the urine.



## OVERSAMPLING AND DIGITAL FILTERING

The AVANCE<sup>TM</sup> DMX spectrometer series, introduced at the ENC 1993, is pioneering the use of modern digital signal processing technology in analytical NMR spectroscopy. Oversampling and subsequent digital filtering have many advantages, including increased dynamic range of the ADC system, perfect phase linearity, constant filter magnitude response, flexibility in programming filter functions, etc.

The figure shows a practical NMR example of the benefits of oversampling and digital filters. A single scan of a sample of 0.1% ethylbenzene was measured on an AVANCE DMX 600 with simultaneous acquisition.



**Figure A:** sweep width of 10,000 Hz shows aromatic peaks at 3,000 Hz **Figure B:** sweep width of 5,000 Hz using analog filter, aromatic peaks folded **Figure C:** sweep width of 5,000 Hz using digital filter, aromatic peaks are not visible







Digital filters provide very sharp cut-offs when compared to traditional analog filters. A typical 4-pole analog antialiasing filter provides an attenuation of approximately 80 dB per decade in the cut-off region. Analog filters with somewhat steeper cut-offs are available but lead to strong phase nonlinearities, and consequently to baseline distortions in NMR.

For comparison, digital filters have much sharper transitions, and can provide a slope of 800 dB per decade in cut-off regions. At the point where 4-pole analog filters have their famous "3dB point", digital filters can provide already close to 100 dB attenuation. In addition, phase nonlinearities essentially do not exist in digital filters.

The single scan of 0.1% ethylbenzene measured on an AVANCE DMX 600 with simultaneous acquisition, shows an example of the benefits of oversampling and digital filters. **Figure A** displays a sweep width of 10,000 Hz, chosen to show the aromatic region above + 3,000 Hz. In **Figure B** a traditional 5,000 Hz sweep width analog filter was employed, and the aromatic peaks are attenuated but visibly folded about the negative axis (simultaneous mode). Finally, **Figure C** presents the same 5,000 Hz sweep width generated using oversampling followed by digital filtering. There is not even the slightest trace of a folded peak, while the baseline remains flat, and the magnitude response is constant over the sweep width pass-band region.

Oversampling and digital filtering provide extremely steep filter cut-offs, extremely flat magnitude response in the pass-band region, and near-ideal phase linearity. Together they effectively prevent any folding (aliasing) of undesirable peaks or noise from outside the passband, a benefit particularly for multidimensional NMR spectroscopy.

### THE ALBERT EINSTEIN COLLEGE OF MEDICINE of Yeshiva University **Jack and Pearl Resnick Campus** 1300 Morris Park Avenue, Bronx, NY 10461

Department of Physiology and Biophysics Nuclear Magnetic Resonance Laboratory

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

July 16, 1993 (received 7/19/93)

### USE OF SODIUM TRIPLE QUANTUM FILTERED DIFFERENCE SPECTROSCOPY TO MEASURE CHANGES IN CARDIAC [Na<sup>+</sup>], WITH PACING

Dear Barry,

Most mammalian hearts show an increase in contractile force during increased heart rate. According to the sodium pump lag hypothesis, an increase in sodium influx during increased heart rate is not matched immediately by an increased sodium efflux and results in increased intracellular sodium (1). Increased intracellular sodium leads to reduced calcium efflux via the sodium/calcium exchanger and the accumulation of intracellular calcium results in increased contractility. Recently Elgavish reported the experimental verification of the lag theory in rat hearts perfused with solution containing the shift reagent  $Dy(PPP_i)_2$  (2). On the contrary, Butwell et al. reported the observation of no significant alteration in intracellular sodium with increasing heart rate over a wide range of heart rates in rat hearts perfused with a solution containing the shift reagent Tm(DOTP)<sup>-5</sup> (3). We felt that the discrepancy might arise from the differing perfusate free calcium concentration used in the two studies (<0.7 mM in the study with  $Dy(PPP_i)_2$  and 3.9 mM in the study with Tm(DOTP)<sup>-5</sup>). In 1974 Forester and Mainwood reported the effect of external calcium on the frequency-tension curves of rat papillary muscle (4). There were no increases in tension with frequency when calcium was 2.5 mM or more. Small positive responses in tension with frequency were found to develop in the range 1.0 - 1.5 mM calcium and larger positive responses developed at lower concentrations of calcium.

Because shift reagents can alter the ionic environment of the heart we chose to test our hypothesis by examining the effect of stepped up cardiac pacing on the triple quantum filtered (TQF) sodium signal of the rat heart perfused at physiological calcium concentration (1.25 mM). Figure 1A shows the increase in the TQF signal of a perfused heart when heart rate was raised from 156 to 300 bpm. For comparison of signal amplitude the signal obtained at 300 bpm is shown in Figure 1B. TQF spectra resulted from signal averaging 960 transients and were acquired as previously described (5). The TQF difference spectrum (1A) clearly demonstrates an increase in the TOF detected sodium at increased cardiac pacing rate. Since we detect no significant difference in the TQF spectra obtained during pacing when external calcium is increased to 2.5 mM it appears that there is negligible effect of pacing on the extracellular sodium TQF signal and that the TQF difference spectrum represents increased intracellular sodium. The TQF difference spectra thus allow detection of an alteration in intracellular sodium during cardiac pacing without disturbing the physiological ionic environment of the heart and may be useful in other physiological situations.

Sincerely,

Linda A. Jelicks

Rajelins

Raj K. Gupta

### 419-18

References

**FIGURE 1** 

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- 4.G. V. Forester and G. W. Mainwood, Pflugers Arch. 352, 189-196 (1974).
- 5. L. A. Jelicks and R. K. Gupta, Magn. Reson. Med. 29, 130-133 (1993).



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Dr. Bernard Shapiro TAMU NMR Newsletter 966 Elsinore Court Palo Alto, CA 94303 July 21, 1993 (received 7/23/93)

Dear Dr. Shapiro,

Title: A VXR-400 Preamplifier modification

Using the automatic sample handling capabilities of our NMR spectrometers significantly increases our productivity. A switchable probe set for 1H and 13C, and a sample changer allow data collection over-night or throughout the entire week-end from a number of samples. However, automated operation of our VXR-400 can be aborted by intense 1H signals from a sample containing 40 mg or more solute. When the computer controlled process can not reduce the spectrometer gain sufficiently to avoid an 'overload' condition, an interruption occurs and production ceases. A method to preclude this problem is for the spectroscopist to make two NMR samples from the material available, one dilute for 1H, and another for 13C analysis. This approach increases sample handling time and spectrometer time, thereby reducing productivity.

To eliminate this shortcoming, we have modified the VXR-400 preamplifier to incorporate a computer controlled 20 dB attenuator at the preamplifier output. We used one of the 4 spare computer-control lines found on the 'XL Interface Board' which are controllable in pulse sequences. Ms. Lillian Garcia, the VXR-400 operator, modified the standard 1H pulse sequence to include the control of one of these signals, renamed the modified sequence, and selects the modified sequence when there are concentrated samples to run. (This includes the neat temperature calibration standards.)

Our test results indicate that the receiver's signal-tonoise ratio (S:N) is decreased by 45% only when the preamp output is attenuated by 20 dB, but is otherwise unaffected. This slight decrease in S:N is insignificant when highly concentrated samples are used. We can now routinely use samples which contain up to 350 mg in automation simply by selecting the appropriate pulse sequence.

A simplified block diagram of the VXR-400 preamplifier is shown in Fig. 1. The location of the modification is shown in a dashed block. Fig. 2 shows schematically the computer-controlled 0/20 dB attenuator circuit. A control signal of +3 to +5 Vdc will give minimum attenuation, and a 0 to +0.6 Vdc signal provides 20 dB attenuation of the radiofrequency signal. The on/off switching time is less than one microsecond.

Regards Dykstra Rober



## Fig. 1 VXR-400 Pre-amplifier



- C1-C4: 10nF
- L1-L3: 8.2 µH
- D1-D2: Hewlett-Packard 5082-3039 PIN DIODE

## Fig. 2 0/20 dB Attenuator

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HMQC

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Figure 2: Phase-sensitive HETCOR spectrum of 600µg Sucrose acquired in 2 hours with a Z•SPEC MD 400 probe.



Figure 3:  ${}^{13}C$  spectrum of 1.0mg Prostaglandin D2 obtained in 2 hours with a Z•SPEC MD 400 probe.



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Figure 4: HETCOR of 1.0mg Prostaglandin D2 obtained in 14 hours with a Z•SPEC MD 400 probe.



Telephone: +61 2 692 2597 Facsimile: +61 2 692 4726 Department of Biochemistry The University of Sydney

NSW 2006 Australia

14 July 1993 (received 7/22/93) L. Dr Bernard & Shapiro TAMU NMR Newsletter 966 Elsinore Court PALO ALTO CA 94303 USA

Dear Professor Shapiro,

We recently carried out work on the diffusion of molecular hydrogen in aqueous solutions containing protein (1). Hydrogen is the smallest molecule that can be used to probe the physical environment inside cells. During this work we measured the T<sub>1</sub> of the hydrogen in aqueous solution, at 1 atmosphere of pressure at 298°K, and obtained a value of 2.1 s, which is much longer than the value of 0.07 s predicted for pure dipole-dipole relaxation using the Debye equation to estimate the rotational correlation time of the molecule.

We have now measured the T<sub>1</sub> of hydrogen in D<sub>2</sub>O as a function of temperature. The results are shown in Fig.1. It can be seen that the temperature dependence of T<sub>1</sub> is very small. The activation energy is much lower than that expected for a dipolar relaxation mechanism. It appears that the relaxation of hydrogen in aqueous solution has both the dipolar and spin-rotation contributions which also occur in the gas phase. We are currently attempting to determine the relative contribution of each mechanism to the relaxation time.

Yours sincerely,

Dorchop

**BOB CHAPMAN** 

HAGGAI GILBOA

PHILIP

(1) Kuchel, P.W., Chapman, B.E. and Lennon, A.J. (1993) Diffusion of Hydrogen in Aqueous Solutions Containing Protein Pulsed Field Gradient NMR Measurements. J. Magn. Reson. (in press).





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Dr Bill Bubb Secretary:

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

Dear Professor Shapiro,

### ISMAR 1995 in SYDNEY

The International Society of Magnetic Resonance will hold its 1995 conference in Sydney, Australia at the University of Sydney from 16-21 July, 1995. The conference will incorporate the biennial Australian Magnetic Resonance meeting and commemorate the 50th anniversary of the discovery of NMR.

The conference will provide an extensive scientific program in Australia's number one tourist destination. Sydney also offers accommodation to cater for all budgets and good international and domestic transport connections. Further details may be obtained from Dr. Les Field, Chair ISMAR-95 (E-mail: ismar-95@biochem.su.oz.au).

Yours sincerely,

Bill Bubb

For the ISMAR-95 Committee:

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Prof. Dr. B. L. Shapiro TAMU-NMR Newsletter 966 Elsinore Court Palo Alto, CAL. 94303 USA MARBURG. DEN 13.7.93 (received 7/24/93) TELEFON (064 21) 28-1 DURCHWAHL: (064 21) 28 TELEX 482 372 TELEFAX (064 21) 28 55 47

### 13C,199Hg-Correlation

Dear Barry,

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we are still looking through the periodic system and search for possible applications of 2D  $^{13}C$ , nnX correlations. Enclosed is a spectrum of (CH<sub>3</sub>)<sub>2</sub>Hg, where such a correlation was obtained by the HMQC method under proton decoupling in about half an hour, the sample was half concentrated. Note the strong isotope effect of the mercury atoms<sup>1</sup> bound to  $^{13}C$ , which, remarkably, is downfield. We hope that these 2D  $^{13}C^{199}$ Hg correlations might be useful in more complicated organomercury compounds.

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<sup>1</sup>J. Jokisaari, K. Räisänen, Mol. Phys. 36, 113-23 (1978).



SYMPOSIUM October 21-22, 1993 Piscataway, New Jersey

## Macromolecular Recognition

Helen Berman Steven Ealick Michael Hecht Anthony Kossiakoff Shawn Ramer Catherine Strader William DeGrado Juli Feigon Mitsuhiko Ikura Mark Matteucci Alanna Schepartz Alexander Wlodawer

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Figure 1: 1D spectrum of 1.5 mM protein dissolved in  $250\mu$ l H<sub>2</sub>O solution.

A protein, 11kDa, was dissolved in 250µl H<sub>2</sub>O solution (pH 3.8) containing 5% D<sub>2</sub>O for frequency lock and packed into the Shigemi Microtube. This spectrum was measured at 37°C on a Bruker AMX500 spectrometer with scan times 64. The solvent signal was suppressed by using low power RF irradiation.

Figure 2: 2D NOESY spectrum of the same sample in Shigemi Microtube.

This 2D NOESY spectrum ( $\tau m=150$  msec) of the protein in 250µl H<sub>2</sub>O solution was measured with scan times 32 on a Bruker AMX500 spectrometer. The observed data matrix size was 1024 ( $t_2$ ) x 200 ( $t_1$ ) complex points. This matrix was processed with zero filling along  $t_2$  dimension and resulted in a final data matrix of 1024 (F2) x 512 (F1) real points. It should be noted that baseline correction and digital processing were **not** applied to remove the water signal.

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Dr. Bernard Shapiro 966 Elsinore Court Palo Alto, CA 94303 June 29, 1993 (received 7/2/93)

Pb-207 Anisotropic Chemical Shifts

Dear Barry,

The investigation of spin-1/2 nuclei has held a special attraction, perhaps because of the simplicity of the spectroscopy.  $^{207}$ Pb is such a nucleus, that should be relatively easy to detect. Indeed, solid-state lead NMR studies date to the late 1950's,<sup>1</sup> although anisotropic chemical shifts were not always reported. The shift should be a rather strong function of the covalency, the disposition of neighboring ions, and the state of the lead ion. Below are preliminary results of the anisotropic chemical shifts of a number of lead compounds determined at Delaware. These are probably accurate to ±20 ppm in most cases, although we have yet to do a careful error analysis.

| Anisotropic<br>Compounds (Re  | Chemical<br>elative to  | Shifts<br>Tetramet  | of Lead<br>hyllead)   |  |  |
|---|---|---|---|--|--|
| Compound<br>$PbO_2$<br>$PbI_2$<br>$PbBr_2$<br>$PbTiO_3$<br>$Pb(SCN)_2$<br>$PbCl_2$<br>$PbCC_4$<br>$PbCC_3$<br>$PbF_2$<br>$Pb(NO_3)_2^1$<br>$PbSO_4$ | $\sigma_1$<br>6423<br>129<br>-697<br>-2001<br>-837<br>-1493<br>-3657<br>-1797<br>-2313<br>-2528<br>-3476<br>-3273 | $\sigma_2$<br>5244<br>-14<br>-850<br>-991<br>-1835<br>-1623<br>-2038<br>-2263<br>-2483<br>-2528<br>-3476<br>-3448 | $\sigma_3$<br>3950<br>-206<br>-1257<br>-991<br>-2071<br>-2026<br>-2038<br>-2656<br>-3075<br>-2954<br>-3531<br>-3840 |  |  |
| <sup>1</sup> We use as a secondary standard $Pb(NO_3)_2$ ,<br>which can be referenced to tetramethyl-<br>lead through the literature.]              |   |   |   |  |  |

Because of the large shift range [+11000 to -5000 ppm, reported in the literature] and the large anisotropies of some of the resonances, one has several problems: (1) initially finding the resonance; (2) accounting for distortions over the range of the resonance in fitting the spectrum to extract NMR parameters. Once the parameters are obtained, one sees <sup>207</sup>Pb anisotropic chemical shifts are extremely sensitive to the local environment.

One of the nice things about this work is that it gives us the opportunity to work with others. Dale Perry, of the Lawrence Berkeley Laboratory, gave us very pure materials to examine, an important point when working with lead compounds since they exhibit various polymorphs. Guenther

Neue, of the University of Dortmund, has contributed immensely to the analysis and contributed his own special insights into the spectroscopy. We are very fortunate to have them as colleagues.

ours truly, Professor

<sup>1</sup>Rocard, J. M.; Bloom, M.; Robinson, L. B. Can. J. Phys. **1959**, 37, 22. The 8<sup>th</sup> International Symposium on Molecular Recognition and Inclusion will be held in Ottawa, Ontario, Canada from July 31 to August 5, 1994. For further information please contact Mrs. Huguette Morin-Dumais, Steacie Institute for Molecular Sciences, National Research Council of Canada, 100 Sussex Drive, Ottawa, Ontario, Canada, K1A 0R6. Telephone: (613) 993-1212, FAX: (613) 954-5242, E-Mail: ISMRI@NED1.SIMS.NRC.CA.

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### Information

The 8<sup>th</sup> International Symposium on Molecular Recognition and Inclusion will focus on the rapidly advancing field of supramolecular chemistry including all aspects of molecular recognition and inclusion. As part of the main symposium, a special "Lock and Key" symposium will be held to commemorate the 100<sup>th</sup> anniversary of Emil Fischer's seminal paper in which the lock and key analogy was first proposed. The symposium will focus on molecular recognition and will include all chemical and biological aspects (structure, catalysis, complex formation biomimetic reactions, self assembly, sensors, drug design, new and modified hosts). Additional areas to be covered include: physical methods and computation (advances and applications to molecular recognition and guest-host chemistry) and the solid state (new inclusion hosts, microporous solids, clathrates, inclusion compounds, intercalates, chemistry in confined spaces, clusters). The program will consist of 35-40 plenary and invited lectures as well as contributed presentations in poster format. Abstracts of oral and poster presentations will be available to participants in booklet form.

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> 23 - 06 - 93 (received 6/28/93)

Professor B. L. Shapiro, 966, Elsinore Court, Palo Alto, California 94303, USA

Dr F. G. Riddell

### Solid State Conformational Analysis

Dear Barry,

From:

One of the things that Martin Rogerson and I have been doing recently with our MSL500 is searching for intramolecular motions in solids, particularly bond rotations. We have now found plenty of new examples where we have been able to measure the rates of rotations of methyl, ethyl, t-butyl, t-amyl and phenyl groups in crystalline organic solids. The i-propyl group eludes us still, but it was in searching for an example of i-propyl group rotation that we stumbled across an example in which we could observe the rates of both methyl and phenyl rotation.

The compound in question is (1). In the CP/MAS spectrum one of the two N-methyl groups reduces in intensity until it disappears as the temperature is lowered (Figure one). This is accompanied by a quite dramatic reduction in  $T_{1\rho}$  for the methyl carbon. We attribute this behaviour to the rate of rotation of the methyl group being comparable to the precessional frequency of the spin locking field. This enhances the  $T_{1\rho}$  relaxation causing poor cross polarisation and the dramatic reduction in signal intensity. This explanation is confirmed when single 90° pulses are applied followed by high power <sup>1</sup>H decoupling. The methyl group remains visible but broadened.



To confirm that there is something odd about one of the N-methyl groups we gave a sample of the compound to our crystallographer, Phil Lightfoot. He tackled the structure using the newly emerging powder diffraction technique. Indeed, one of the N-methyls is very oddly positioned. It has a dihedral angle of only 17<sup>o</sup> to the nearest methyl in the i-propyl group and also shows the closest approach of any carbon to the bromide ion. These are undoubtedly the reasons for its restricted rate of rotation. At higher temperatures the phenyl group rotation rate seems to affect the spectrum with what appears to be a coalescence phenomenon of four of the six lines (2 overlapped) visible at low temperatures. The C(1) and C(4) resonances in the phenyl on the rotation axis are not involved. At temperatures above coalescence the  $T_{1p}$  effect is again apparent and the intensity of the coalesced peaks is considerably reduced so that only the other two carbons are visible.

My prejudices, when we started this work, suggested that only the most symmetrical groups should rotate at appreciable rates in the restricted environment of a solid. My prejudices were wrong. We now see that alkyl group rotations, even of t-amyl groups, are an integral part of the normal molecular conformational motions that occur in solids. We still have to find an example of i-propyl groups doing their thing but I am confident that it will not be long before we come up with an example.





Figure Two

Best wishes, Yours sincerely,

Frank

Frank Riddell

Martin Rogerson

293 263 263 248 234



Figure One

P. Up yor

Phil Lightfoot

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14 July 1993 (received 7/17/93)

Dr B L Shapiro TAMU NMR Newsletter 966 Elsinore Court Palo Alto, CA94303 USA

Dear Dr Shapiro

### Transferred NOE Revisited and Applied to Large Protein Systems.

We, like others, have reinvestigated and expanded the use of transferred NOE experiments. The experiment has frequently been used in NMR studies of protein-ligand interaction under exchanging conditions. It is a combined phenomenon consisting of cross-relaxation and chemical exchange between different states. Its quantitative interpretation, however, requires the use of a rather large system of coupled differential equations. In order to overcome some of the reported difficulties, we have adopted a *complete relaxation matrix approach* which looks at the whole protein-ligand system; other previously reported applications of this approach have only examined the intra-ligand TNOEs, with restrictions on the influence of the protein. Much of our approach and the mathematics required are reported in reference 1.

The system under study is the interaction between the protein Chloramphenicol Acetyl Transferase (CAT) (Mr = 75kD) and Coenzyme A. We are particularly interested in the use of TNOE to determine chemical exchange rates and the results of these attempts are shown in Figs. 1 and 2. Fig.1 shows the theoretical NOE intensity dependence on the mixing time under different chemical exchange rates ( $K_{-1}$ ). Fig.2 shows a comparison between the experimental (points) and calculated (solid lines) mixing time difference of TNOE cross-pear intensities. From Fig.2 the best agreement between experimental and calculated intensities was achieved when a protein-ligand complex lifetime of 2ms and a protein correlation time of 80ns were used in the calculations.



Fig. 1 Mixing time dependence of TNOE cross-peak intensities for CoA proton pairs in the CAT-CoA system: (a)  $C^{PB}H^2/C^{PB}H^3$ , 1.78Å; (b) C1'H/C4'H, 3.0Å; (c) C8H/C3'H, 4.5Å; (d)  $C3'H/C^{P8}H_3$ , 7.7Å, at a ligand:protein ratio of 10 and different exchange rates (x-, (k<sub>-1</sub>): 10 ( • ), 100 ( O ), 500 ( • ), 1000 ( • ). The equilibrium constant in all cases was  $5x10^{-5}M$ .



Fig. 2 Comparison between experimental (points) and calculated (solid lines) mixing time dependence of TNOE cross-peak intensities for CoA proton pairs in the CAT-CoA system: (a) C<sup>PB</sup>H<sup>2</sup>/C<sup>PB</sup>H<sup>3</sup>, 1.78Å; (b) Cl'H/C4'h, 3.0Å. A ligand:protein ratio 10 was used.

419-44

We have therefore shown that by careful use of the TNOE experiment, it is possible to obtain kinetic data.

Please credit this contribution to the account of Gordon Roberts.

Yours sincerely,

Lith

. 15 m

Lu Yun

Igor

Ref. 1. L.Y.Lian, I.L.Barsukov, M.J.Sutcliffe, K.H.Sze, and G.C.K.Roberts, "Protein-Ligand Interactions: Exchange Processes and Determination of Ligand Conformation and Protein-Ligand Contacts", in Methods in Enzymology (1993), in press.

All Newsletter Correspondence

Should Be Addressed To:

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

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