J. B. Stothers
\( ^{13} \text{C} \) NMR of Some B-NOR and A-HOMO-B-NOR Steroids \( \ldots 1 \)

C. E. Holloway
T1 Studies of Sterically Crowded Organotin Derivatives \( \ldots 3 \)

E. A. Yue
A-60A or A-56/60A Wanted \( \ldots 4 \)

C. M. Griswold
A Variable Frequency Pulsed Spectrometer \( \ldots 5 \)

S. L. Gordon and K. Wuthrich
Positive Results from Negative NOE’s \( \ldots 7 \)

T. L. James
Postdoctoral Position \( \ldots 14 \)

G. Bigam
Decitex - Nova Interface \( \ldots 16 \)

J. R. Alger, I. M. Armitage and J. D. Otvos
\( ^{31} \text{P} \) Saturation Transfer on a Bruker HFX-90;
Postdoctoral Position Available \( \ldots 17 \)

J. A. G. Drake, D. W. Jones and H. Pakdel
Coupling-Constant Errors with LAOCOON III;
Calculations in Heterocyclic Compounds \( \ldots 19 \)

K. D. Berlin
Novel Solvent Induced Shifts in \( \alpha \)-Methylene-
\( \gamma \)-Butyrolactones Attached to Rigid Systems \( \ldots 21 \)

G. N. LaMar
NMR Spectroscopist Position Open \( \ldots 24 \)

M. D. Johnston, Jr.
Two-Step Equilibria the Fastest Way \( \ldots 25 \)

J. J. Duifjes, C. Erkens and J. Lugtenberg
\( ^{13} \text{C} \) NMR Spectrum of a Pyrryl-s-triazine \( \ldots 27 \)

G. Gatti
The \( ^{13} \text{C} \) Methyl Shift in Models of Regio-
Irregular Polypropylene \( \ldots 29 \)

F. Fodd, C. Ramoni and G. Vicari
Proton Magnetic Relaxation Studies of Dextran Derivatives \( \ldots 31 \)

P. C. Lauterbur
T2; Scendental Manganization, or How to Relax
Your Dogs and Rats \( \ldots 33 \)

P. S. Pregosin
Multinuclear NMR Studies on the Pt-SN System;
Postdoctoral Position Available \( \ldots 35 \)

G. J. Martin, M. L. Martin and M. L. Filleux
Is \( ^{15} \text{N} \) Spectroscopy a Good Way for Evaluating
Nitrogen Lone-Pair Delocalisation? \( \ldots 40 \)

W. Ritchey and A. Olson
Sale on NMR and EPR Instrumentation \( \ldots 42 \)

D. L. Harris and K. A. Koehler
Metal Ion NMR for the Study of the pH Dependence
of Metal Ion Binding to Macromolecules \( \ldots 43 \)

M. Mattingly and R. Rowan III
Interfacing a Clock to an XL-100 \( \ldots 45 \)

D. E. Westmoreland
In the Trenches with a Rodenticide Metabolite \( \ldots 47 \)

---

A monthly collection of informal private letters from Laboratories of NMR. Information contained herein is solely for the use of the reader. Quotation is not permitted, except by direct arrangement with the author of the letter, and the material quoted must be referred to as a “Private Communication”. Reference to the TAMU NMR Newsletter by name in the open literature is strictly forbidden.

These restrictions apply equally to both the actual Newsletter participant-recipients and to all others who are allowed access to the Newsletter issues. Strict adherence to this policy is considered essential to the successful continuation of the Newsletter as an informal medium of exchange of NMR information.
Consummate care in the storage and preparation of spectroscopic samples is just as integral a part of good spectroscopic practice as running the investigation or analyzing the spectra. And consummate care, of course, begins with equipment.

Our new, expanded Wilmad line of vials, storage and septum bottles, and a broad variety of stoppers, caps, and septa help materially to simplify the handling, storage, and preparation of samples . . . eliminate expensive sample loss . . . and save unnecessary waste of time and money.

Wilmad vials and bottles are manufactured of top-quality borosilicate glass to prevent any pH modification of the contents. The variety of caps available match any sampling or storage need. Snap caps of polyethylene, open-top types with elastomer septa, aluminum seals with Teflon-faced septa . . . whatever you need we now carry in stock.

Write or call for our new Catalog 781.
For those who expect more in FT NMR Spectrometers ... it's JEOL

The FX60Q, FX90Q & FX100 Feature:
- (DQ) DIGITAL Quadrature Detection System
- Multi-Frequency TUNEABLE Probe observation
- Dual Frequency probes
- 4-channel DIGITAL phase shift
- (DPS)
- Comprehensive auto-stacking system
- Foreground/Background system
- Computer based pulse programmer with Multiple Pulse Sequence Generator
- CPU Expandable to 65K words (MOS)
- 2-channel 12 bit AD/DA
- T amused spin locking system
- Disc storage systems
- Multi-Mode HOMO/HETERO decoupling capabilities
- Programmable Variable Temperature Unit
- Simplex Y/Curvature gradient controller
May 12, 1978.

Dear Barry:

Although literally hundreds of steroids have been examined by $^{13}$C nmr, there seems to be a dearth of $^{13}$C results for A-homo-B-nor and B-nor steroid skeletons. Recently, I have had the opportunity to examine a few of the latter in connection with one of Ed Warnhoff's projects and some of these results may be of interest to some Newsletter readers. At the same time, I can perhaps settle the issue raised by your pink letter.

Acid-catalyzed opening of the oxide ring in 3x,5α-oxido-A-homo-B-norcholestan-3-one (1) with BF$_3$-Et$_2$O at room temperature gave a single alcohol containing a fully substituted double bond. Clearly, a backbone rearrangement had occurred, the extent of which was of some interest. The $^{13}$C spectrum revealed the 18-methyl carbon at 11.0 ppm and signals corresponding to those for C-13 and C-15 to C-27 in many cholestan-3-ones (1) were evident. Further, in the presence of Eu(fod)$_3$, the olefinic signals were more strongly affected than any of the methine signals except for that from the carbonyl carbon. It follows that a Westphalen-type of rearrangement had occurred to produce 2. The aforementioned shieldings agreed well with those for a variety of 5-methyl-19-nor-5β-cholesten-9(10)-enes (2). At lower temperatures, the oxide opening gave the Δ$^+$- and Δ$^-$-unsaturated alcohols 3 and 4 as well as 2. Not unexpectedly, half of their signals correspond closely with those found for many cholestan-3-ones while the remainder could be assigned to specific carbons in the usual way; these results are listed in the Table for 2-4. Interestingly, the carbonyl carbons in 2 and 4 are equivalent as are the carbonyl protons. However, the half-widths of the proton absorptions were 20 and 11 Hz, respectively, from which the favored conformation of the A ring can be deduced as sketched in A and B below. Three examples in the B-nor series were also available (5, 6 and 7) for which the corresponding results are included in the Table. A comparison of these data with those for the corresponding cholestan-3-one derivatives showed that pronounced differences (up to 14 ppm) occur for the B-ring carbons. In general, the shieldings for C-11, 12, and C-15 to C-27 are similar in both series. This is also true for C-1, 2 and 3 in the unsaturated cases but significant differences for these centres are found for the saturated ketone 2. This is not surprising because of the appreciable geometrical differences between 5α-cholestan-3-one and its B-nor analog 7. The preparation and characterization of these compounds will appear in ref. 3. The complete results together with those for several additional examples in the A-homo-B-nor series will be submitted for publication shortly.

I trust this will satisfy the demand of the pink letter.

J. B. Stothers

$^{13}$C shieldings (ppm from TMS, CDCl$_3$ solutions) for 2-7

<table>
<thead>
<tr>
<th></th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-4a</th>
<th>C-5</th>
<th>C-6</th>
<th>C-8</th>
<th>C-9</th>
<th>C-10</th>
<th>C-18</th>
<th>C-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>19.6</td>
<td>39.8</td>
<td>75.9</td>
<td>33.5</td>
<td>35.3</td>
<td>49.2</td>
<td>43.8</td>
<td>43.0</td>
<td>136.7</td>
<td>140.0</td>
<td>11.0</td>
<td>26.7</td>
</tr>
<tr>
<td>4</td>
<td>32.4</td>
<td>33.2</td>
<td>75.9</td>
<td>40.6</td>
<td>23.5</td>
<td>153.9</td>
<td>127.0</td>
<td>45.0</td>
<td>56.5</td>
<td>48.9</td>
<td>12.4</td>
<td>18.7</td>
</tr>
<tr>
<td>3</td>
<td>34.2</td>
<td>30.8</td>
<td>67.0</td>
<td>32.4</td>
<td>113.7</td>
<td>156.9</td>
<td>38.5</td>
<td>38.9</td>
<td>59.8</td>
<td>46.4</td>
<td>12.4</td>
<td>16.2</td>
</tr>
<tr>
<td>5</td>
<td>37.3</td>
<td>32.0</td>
<td>71.6</td>
<td>36.6</td>
<td>149.0</td>
<td>125.4</td>
<td>46.2</td>
<td>62.5</td>
<td>44.8</td>
<td>12.3</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>35.4</td>
<td>33.7</td>
<td>199.3</td>
<td>122.5</td>
<td>179.0</td>
<td>34.7</td>
<td>38.4</td>
<td>58.1</td>
<td>44.1</td>
<td>12.4</td>
<td>17.4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>32.4</td>
<td>35.3</td>
<td>214.7</td>
<td>44.4</td>
<td>44.3</td>
<td>38.4</td>
<td>40.1</td>
<td>55.1</td>
<td>39.8</td>
<td>12.3</td>
<td>24.9</td>
<td></td>
</tr>
</tbody>
</table>

![Chemical Structures](image1.png)

1. ![Chemical Structure](image2.png)
2. ![Chemical Structure](image3.png)
3. ![Chemical Structure](image4.png)
4. ![Chemical Structure](image5.png)
5. ![Chemical Structure](image6.png)
6. ![Chemical Structure](image7.png)
7. ![Chemical Structure](image8.png)
Dear Professor Shapiro:

Studies of Sterically Crowded Organotin Derivatives

We have been examining the applicability of $T_1$ studies for identifying and characterising barriers to internal rotation (eg. Axelson & Holloway, Can. J. Chem. 54 2820 (1976)) in a number of sterically crowded molecules. Where overall isotropic rotational reorientation can be assumed, the analytical procedure is a relatively simple one bearing in mind of course, the structural effects pointed out by Blunt and Stothers (J. Magn. Res. 27, 515 (1977)).

We decided to examine some organotin compounds lying around the lab, which were originally designed to be as sterically hindered as possible with a view to isolating optical enantiomers. In some of these a ligand bears an anisochronous pair of methyl groups, which give rise to two methyl resonances. The question could be asked whether they do so because of the intrinsic asymmetry of the molecule or because they are locked in specific sites. In the latter case, each site could also differ with respect to the rotational barrier of each individual methyl group. A fairly typical example is shown in the figure below for tertiarybutylbenzylneophylphenyltin run as a pure liquid with an external $D_2O$ lock, at $35^\circ$C on a CFT 20.

![Diagram of organotin compound]
Assuming isotropic reorientation of this relatively heavy molecule, the shortest NT₁ value representing the most rigid parts of the system is about 0.3. The phenyl group on the tin has a slightly longer NT₁ which may be due to some residual motion whereas the benzyl and neophyl phenyl rings appear to have the shortest NT₁, perhaps due to intermolecular dipolar relaxation (all nOe values are maximum). The τ-butyl methyl groups are probably spinning, but not fast enough to be called free rotors (which would require their T₁ values to be about 3(NT₁)). The neophyl anisochronous methyls seem to be in more restricted environments than the τ-butyl methyls, and also each have slightly different rotational barriers.

We are currently looking for systematic trends in a series of these compounds, the chemistry and nmr of which have been previously reported (Kandil & Holloway, J.C.S. Dalton 1421 (1973) and Axelson, Kandil & Holloway, Can.J.Chem. 52, 2968 (1974)).

Sincerely yours,

C.E. Holloway
Associate Professor
Chemistry Department
Professor Bernard L. Shapiro  
Department of Chemistry  
Texas A&M University  
College Station, TX 77843

Dear Dr. Shapiro:

We thought Newsletter readers would be interested in a variable frequency pulsed spectrometer which we have built here at Virginia. This instrument will be used mainly for water proton relaxation measurements on enzyme and membrane systems, but we have already observed lithium-7 resonances and feel that this machine has the sensitivity to be useful with a variety of nuclei.

As you know, magnets suitable for such a system are hard to come by. New ones are of course expensive, and good used magnets are hard to find. After writing to all the major chemistry departments in the U.S. and Canada, we finally bought a very well preserved magnet and power supply from a Varian DA-60 spectrometer in the lab of Ted Schaefer at the University of Manitoba. To circumvent the problems of frequent replacement (at high cost!) of the 304 TL tubes in the 2100B magnet power supply, we had Rolf Tschudin install his solid state pass bank, which has been operating since August, 1977 with no problems. This cleverly designed unit of Rolf's consists of 34 high voltage power transistors, each on its own plug-in heat sink, which, along with several other circuit boards, fit on one large rack in the space vacated by the 304 TL's.

The probes, r.f. circuitry and pulse programmer for this unit were designed and built for us by Don Vickers and Tom Hill at SEIMCO, New Kensington, Pennsylvania. Their elegantly-designed consoles are now in use in various labs around the country, but we have their first set of broad band, cross-coil probes installed now in our unit. The two probes cover the ranges of 4-15 MHz and 15-60 MHz and are equipped for temperature control via a standard Varian V-4343 Temperature Controller. With a 50 Vpp signal at the transmitter coils, the pulse widths required for a 90° pulse are

<table>
<thead>
<tr>
<th>Frequency (MHz)</th>
<th>Pulse Width (µsec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Frequency Probe</td>
<td></td>
</tr>
<tr>
<td>4 MHz</td>
<td>9.5 µsec</td>
</tr>
<tr>
<td>6 MHz</td>
<td>10.4 µsec</td>
</tr>
<tr>
<td>8 MHz</td>
<td>13.0 µsec</td>
</tr>
<tr>
<td>11 MHz</td>
<td>17.0 µsec</td>
</tr>
<tr>
<td>15 MHz</td>
<td>16.0 µsec</td>
</tr>
<tr>
<td>15 MHz</td>
<td>23.0 µsec</td>
</tr>
<tr>
<td>22 MHz</td>
<td>25.0 µsec</td>
</tr>
<tr>
<td>30 MHz</td>
<td>25.0 µsec</td>
</tr>
<tr>
<td>42 MHz</td>
<td>24.0 µsec</td>
</tr>
<tr>
<td>60 MHz</td>
<td>28.0 µsec</td>
</tr>
</tbody>
</table>

May 2, 1978
Since we run this instrument unlocked, using only the V-3508 solid state superstabilizer, titrations of a sample are rapid and convenient. Frequency changes are also quite easy and take only a few minutes, since the magnet and power supply, with Tschudin's modification, stabilize quickly.

We are presently using this spectrometer to examine the binding of CrATP and other nucleotide analogs to the (Na⁺ + K⁺)-ATPase and other similar enzymes. Most of our measurements are of T₁ and T₂, but the versatile pulse programmer from SEIMCO allows choice of other pulse sequences. For example, T₁'s can be easily measured on this instrument. We will be happy to provide more information on this instrument and modifications to interested parties.

Please credit this contribution to Dr. Bruce Martin's "account".

Sincerely,

[Signature]

Charles M. Grisham
Assistant Professor of Chemistry

P.S. The ENI Model 310 L Power Amplifier is ideal for this system, since it has a frequency range of 250 kHz - 110 MHz. The frequency counter we use is a Data Precision Model 585 and it is impressive for its range (250 MHz), precision (8 digits), small size (5 1/2" x 1 3/4" x 3 1/2") and best of all, its small price (~ $300.00)!
Positive Results from Negative NOE's

Dear Barry:

The negative proton-proton NOE's that occur in protein NMR spectra at high fields offer an attractive means for obtaining protein structural information. This is because the enhancements are largely determined by intramolecular dipole-dipole interactions. The interpretation of these NOE's, however, is often complicated by large cross-relaxation terms involving spins other than the irradiated and observed nuclei. In order to obtain NOE difference spectra which are more easily interpreted, we have been using the transient NOE technique, introduced by Solomon in his classic study of HF. We apply a selective inversion pulse on an individual line of the $^1$H NMR spectrum, and monitor the resulting NOE difference spectrum as a function of time. The intensity of each line in the transient NOE spectrum builds up by spin diffusion at a characteristic rate. After reaching a maximum, the lines decay to zero via spin lattice relaxation. The initial rate of intensity increase depends only on the cross-relaxation coefficients between the irradiated and observed nuclei, and is simply related to proton-proton distances in the three-dimensional structure of the protein.

In Figure 1, we show some experimental results for horse ferrocytochrome c, a heme protein of molecular weight 12,500. The positions of the Met 80 resonances $-\text{C}^4\text{H}-\text{C}^5\text{H}_2-\text{C}^1\text{H}_2-\text{S}-\text{C}^6\text{H}_3$ are indicated at the top of the figure. Notice that each Met 80 methylene proton has a different resonance frequency. The bottom trace is the steady state NOE difference spectrum, which results in...
from irradiation of the $\gamma$ resonance at -1.8 ppm, with negative Overhauser enhancements appearing as positive peaks. The upper traces are transient NOE difference spectra for different delay times $\tau$ after inversion of the $\gamma$ resonance. At $\tau=0$, the large signal at -1.8 ppm corresponds to the inverted line while the smaller signal at -3.7 ppm corresponds to the geminal methylene proton. The latter signal is the result of spin diffusion during the 15 msec inversion pulse. For increasing values of $\tau$, the pulsed line decreases in intensity, while the other lines grow with initial rates which are in agreement with what we expect from the proton-proton distances of the Met 80 residue. A more detailed account of these experiments will be presented in a forthcoming publication.

With best wishes,

Yours sincerely,

Sidney L. Gordon
Visiting Professor

Kurt Wüthrich

References:
360 MHz Fourier transform $^1$H steady state and transient NOE difference spectra of a 0.008 M solution of horse heart ferrocytochrome c in 0.05 M deuterated phosphate buffer, pD = 6.8, $T = 49^\circ$. The steady state NOE difference spectrum is the result of 2000 accumulations and the transient NOE difference spectra are each the result of 1000 accumulations. The steady state NOE's were obtained by applying a 2s low power saturating pulse followed by a $90^\circ$ observation pulse. The transient NOE's were obtained by applying an 15 ms inversion pulse followed, after a delay time $\tau$, by a $90^\circ$ observation pulse. The difference spectra were obtained by subtracting the spectra with NOE's from reference spectra obtained by offsetting the irradiation pulse to -5 ppm.
HIGHER RESOLUTION SPECTROMETER SYSTEMS

The most extensive range of Fourier transform and CW spectrometer systems with outstanding performance . . . from 14 to 83 kG.

WP-60
60 MHz for Protons
2 - 10 mm Tubes
Pre-tuned Probes

Inexpensive routine FT Spectrometers operating at 14 and 18.8 kG respectively. Both have full multinuclear and experimental capability. They are distinguished in their price range by their unequalled resolution, sensitivity and ease of operation.

WP-80 DS
80 MHz for Protons
2 - 10 mm Tubes
Pre-tuned Probes

The industry standard for large iron magnet systems. It features a broadband multinuclear capability and advanced data system with multi-task capabilities. The 15-inch magnet draws low power and insures the highest resolution and sensitivity.

WH-90 DS
90 MHz for Protons
2 - 15 mm Tubes
Pre-tuned Probes
Broadband Observation and Probes

WP-200
200 MHz for Protons
2 - 15 mm Tubes
Pre-tuned Probes

Bruker's new routine superconducting spectrometer — revolutionary in performance and price. With approximately 25 l of helium usage per month, all barriers to the use of supercon technology have now been broken. The WP-200 exhibits outstanding resolution and sensitivity and is fully multinuclear. The data system is the most advanced of its type using a 24 bit word length and foreground/background capability.

THE WP-200 — A NEW GENERATION IN NMR SPECTROSCOPY
**WH-180**
180 MHz for Protons
5–30 mm Tubes
Broadband Multinuclear Observation

**WH-270**
270 MHz for Protons
2–15 mm Tubes
Pre-tuned Probes
Broadband Multinuclear Observation

**WH-360**
360 MHz for Protons
2–15 mm Tubes
Pre-tuned Probes
Broadband Multinuclear Observation

These three spectrometers offer the highest possible performance available today. With optimum sensitivity through the use of large sample tubes (WH-180), or maximum field strength (WH-360), each one of these systems meets the most demanding research needs. The superconducting magnets used combine the optimum in bore size, resolution and low helium consumption.

In addition, combination systems are available. For example: In the WH-270/180 spectrometer, a 42 and 63 kG magnet is operated from a single console.

**CW OPTION** Using microprocessor technology, any of the Bruker high resolution spectrometers can be obtained in a CW version, or this unit can be obtained as a standard accessory. Pre-programmed parameters are available for routine scans, etc., and through a calculator type keyboard, specific sweep widths and sweep times can be entered. The unit also incorporates a homonuclear decoupler and Indor system.
**CXP SERIES**

**HIGH POWER PULSE SPECTROMETERS**

- Frequency Range: 4 to 100 MHz
  Also available with Supercon magnet systems at 180 and 270 MHz
- NEW! Completely computer-controlled Digital Pulse Program Generator with a high time resolution (10 nsec).
  It is capable of generating all practical pulse sequences known to NMR spectroscopists today and provides the user with the opportunity to invent and experiment with his own programs.
- Accessories: A comprehensive range for high power pulse experiments
  1. T1p capability
  2. Variable temperature operation
  3. Double resonance in solids
  4. Complete Fourier transform systems
  5. Multi-pulse solid state experiments
  6. Magic angle spinning

**BRUKER EPR SPECTROMETERS**

**ER-200 TT**

- New breakthroughs in EPR instrumentation — a routine instrument with research performance
- Automatic bridge balancing
- Push-button tuning
- 3 detection channels
- 1st or 2nd derivative detection
- ENDOR and TRIPLE attachment

**ER-420**

- EPR at O-BAND 34.0—35.0 GHz
  X-BAND 9.2—10.0 GHz
  S-BAND 3.0—4.0 GHz
  UHF-BAND 0.2—0.8 GHz
- ENDOR ACCESSORY UNIT
  High power with interchangeable probe heads
  Low power with low temperature systems
- VARIABLE TEMPERATURE EPR/ENDOR
  3.6 to 573° K
  Liquid N2/He Insert Dewars
  Liquid He Cryostat Systems (metal, glass, quartz)
- N.M.R. field measurement/marking with self-tracking auto-lock Gaussmeter
- Computer interfaced for maximum versatility in data handling

**ER-10**

- EPR-MINISPEC with auto-tuned X-Band bridge, air cooled 4" electromagnet,
  auto-recycle power supply and variable temperature compatibility

For more information, call or write:

BRUKER INSTRUMENTS, INC.
Manning Park
Billerica, Mass. 01821
Tel: 617 272-9260

BRUKER INSTRUMENTS, INC.
530 Beall Avenue
Rockville, Maryland 20850
Tel: 301 789-4441

BRUKER INSTRUMENTS, INC.
1801 Page Mill Road, Suite 212
Palo Alto, California 94304
Tel: 415 325-6677

BRUKER SPECTROSPIN LTD.
2410 Dunwin Drive, Unit 4
Mississauga, Ontario, Canada
May 3, 1978

Re: Post-doctoral Position

Dear Barry:

A post-doctoral position is available for an individual interested in studying mobility in proteins and membranes. In particular, the $T_1$ method for studying translational motion (1) and the off-resonance $T_1$ technique we have been developing for examining rotational motions (2,3) will be utilized. A background in the area of molecular motions or in specific labeling of proteins and phospholipids would be useful but not essential.

Interested applicants should forward their curriculum vita and arrange for three letters of recommendation to be sent. Salary for the position is twelve to thirteen kilobucks per annum. The University of California is an Equal Opportunity/Affirmative Action employer.

Yours truly,

Thomas L. James
Assistant Professor of Chemistry and Pharmaceutical Chemistry


May 16, 1978

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

Re: Decitek - Nova Interface

Dear Barry,

As any Digilab owner will tell you, the time required for loading the Digilab DPNMR program via the teletype reader makes a bed a necessary item of laboratory furniture. Failing to convince our purchasing department of this evident fact, we did the next best thing and acquired a WP-60 with a Decitek High Speed Reader. Interfacing the Decitek to the Nova computer cut our loading time from about 5 hours to 15 minutes. Perhaps some of your readers can make use of this interface.

The first thing to do is to determine whether or not your Nova Teletype Board has a 4011 Paper Tape Reader Option (IC's U50-55,67,68,70,71,U83-U88). If not, a schematic and kit containing the necessary IC's and small parts may be obtained from Datagen and soldered onto the board. (We'll be happy to supply more information.) That done, note that on the lower edge of the Nova back plane are pins for five 20-pin connectors. The one labelled P-7 is factory wired to the HSR portion of the I/O board. A 20-pin socket (A-MP16/48-I) and harness must be made up to connect P-7 to a 25 pin Cinch socket (DB-25F) mounted on the computer rear panel with the other I/O connectors.

A length of 20 conductor ribbon cable and two 25-pin Cinch plugs (DB-25P) connect the computer and HSR. Alternate conductors should be grounded at the computer end of the cable to minimize cross-talk and noise pick-up.

Finally, a Mode Selector plug (see diagram) must be wired and plugged into the 16-pin DIP socket in the Decitek.
<table>
<thead>
<tr>
<th>Back Plane</th>
<th>Nova Signal Name</th>
<th>Nova Cannon Connector</th>
<th>Decitek Cannon Connector</th>
<th>Decitek Signal Name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gnd</td>
<td>1</td>
<td>11</td>
<td>DATA6</td>
</tr>
<tr>
<td>A59</td>
<td>CH6</td>
<td>2</td>
<td>11</td>
<td>DATA6</td>
</tr>
<tr>
<td>A61</td>
<td>CH5</td>
<td>3</td>
<td>10</td>
<td>DATA5</td>
</tr>
<tr>
<td>A63</td>
<td>CH4</td>
<td>4</td>
<td>9</td>
<td>DATA4</td>
</tr>
<tr>
<td>A65</td>
<td>CH3</td>
<td>5</td>
<td>7</td>
<td>DATA3</td>
</tr>
<tr>
<td>A67</td>
<td>CH2</td>
<td>6</td>
<td>6</td>
<td>DATA2</td>
</tr>
<tr>
<td>A69</td>
<td>CH1</td>
<td>7</td>
<td>5</td>
<td>DATA1</td>
</tr>
<tr>
<td></td>
<td>+5V</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A71</td>
<td>STOP</td>
<td>9</td>
<td>3</td>
<td>PULSE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gnd</td>
<td>10</td>
<td>25</td>
<td>Gnd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>A57</td>
<td>CH7</td>
<td>12</td>
<td>12</td>
<td>DATA7</td>
</tr>
<tr>
<td>A49</td>
<td>CH8</td>
<td>13</td>
<td>13</td>
<td>DATA8</td>
</tr>
<tr>
<td>A47</td>
<td>GO</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A77</td>
<td>SPKT</td>
<td>16</td>
<td>2</td>
<td>FLAG</td>
</tr>
<tr>
<td>A75</td>
<td>RDRDY</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+5V</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A73</td>
<td>FWD+STOP CONTR</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gnd</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please credit this to Dr. Nakashima's account.

Sincerely,

Glen Bigam

GB/SS
Dear Professor Shapiro:

For the past couple years we have been using $^{31}$P NMR to monitor the enzyme-phosphate intermediates which lie along the reaction pathway of *E. coli* alkaline phosphatase. We have shown that the covalent (E-P) and noncovalent (E·P) complexes give rise to resonances which are well resolved from that of the product of the reaction, inorganic phosphate (P) (Fig. 1a). Spurred by the recent interest in using magnetization transfer to extract kinetic information from enzymatically catalyzed reactions, we decided to test the feasibility of performing saturation transfer experiments on the alkaline phosphatase system using our modified Bruker HFX-90.

The frequency for the homonuclear decoupling pulse was selected digitally from a General Radio GR 1061 frequency synthesizer with the usual array of in-line mixers to attenuate the off resonance (several KHz) frequency and reduce saturation effects during acquisition. Unique perhaps to our old system (Bruker HFX-90) was the availability of an extra set of fixed orthogonal coils in the probe arm which we found to be ideally suited for the introduction of the saturating pulse. This procedure provided much better isolation than could be obtained from a directional coupler. $^1$H decoupling was also employed in these experiments using the Helmholtz coils on the insert.

For the experiment shown in Fig. 1, saturating pulses of 1.0 sec. (indicated by the arrows) were positioned off resonance (la) or on E-P, E·P and P, respectively. Each spectrum (22,500 transients) was of 2.4 mM enzyme plus 9.6 mM phosphate, pH 5.5 contained in 0.8 ml in a 10 mm tube. The data clearly show that the intensities of the nonsaturated species are reduced upon saturation of the species with which they are in slow exchange. The magnitude of these intensity losses allows one to calculate the rate constants $k_1$, $k^{-1}$, $k_2$, and $k^{-2}$ using a simple 3-site exchange formalism. In fact, if all three species are sequentially saturated as in Fig. 1 the system is overdetermined, allowing checks to be made on the reliability of the experimental data as well as the proposed enzyme mechanism.
One of us (IMA) has a postdoctoral position available for 1 year. Applicants with experience in applying multinuclear NMR techniques to biological systems would be preferred.

Sincerely,

J.R. Alger
Ian M. Armitage
J.D. Otvos


\[
E - P \xrightleftharpoons[100 Hz]{K_{-1}} E \cdot P \xrightleftharpoons[{K_{-2}}]{K_{2}} E + P_i
\]

Figure 1
COUPLING-CONSTANT ERRORS WITH LAOCOON III CALCULATIONS IN HETEROCYCLIC COMPOUNDS

If some of the transition frequencies overlap, errors in the observed line frequencies of a high-resolution n.m.r. spectrum can lead to doubts about the uniqueness, and uncertainties in the magnitudes, of the computed transition parameters (1,2).

When these are refined iteratively by a program such as LAOCOON III, so that r.m.s. discrepancies between observed and calculated line frequencies are minimized, examination of the residual errors between observed and calculated frequencies can lead to a misleadingly optimistic impression of the parameter accuracy achieved. Thus Ewing (3) concluded that 2.5 is a realistic figure for the underestimation of such errors.

Recently J.A.G.D. has had occasion to make around 20 independent determinations (separate iterative analyses from independently measured 220 MHz $^1$H spectra) each for several closely-coupled four-spin systems. Provided inter-ring coupling can be neglected, these ABCD systems are characterized by four aromatic chemical shifts and six coupling constants. The data on 5H-dibenzocyclohepten-5-one (I) and related compounds (II,III) and on fluoren-9-one (IV) [TAMUNMR Newsletter 221-16 (Feb. 1977)], which have close chemical shifts (spread over narrow ranges of 0.4 p.p.m. or less for II, III, and IV), provide an interesting comparison between LAOCOON-calculated probable errors in coupling constants, $J$, (assumed independent of the conditions) and the deviations (assumed normally distributed) derived from separate analyses of spectra recorded under different conditions of solvent (CDCl$_3$ or CS$_2$), concentration and temperature.

For each of the six coupling constants in the four compounds, the Table shows the mean $J$(from 22 analyses of I, 12 of II, 16 of III, and 21 of IV), the mean LAOCOON probable error (PE), the r.m.s. deviation $q$ from the set of replicate analyses, and the ratio $q$ /PE between these. The mean LAOCOON r.m.s. errors for I-IV are 0.04, 0.04, 0.03, and 0.04 Hz respectively. If 2$q$ is taken as an acceptable indicator of the error in $J$, then the present data ($q$ /PE average is 3.6) suggest that the individual LAOCOON probable errors in $J$ be multiplied by a factor of 7.

With apologies for being so late with this contribution.

Yours sincerely,

J.A.G. Drake

D.W. Jones

Hooshang Pakdel
TABLE: Mean H-H coupling constants and their LAOCOON probable errors (PE/Hz) and experimental e.s.d.s (σ_e/Hz) for ABCD systems in aromatic regions of I, II, III, and IV.

<table>
<thead>
<tr>
<th>Coupling</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>J/Hz</td>
<td>PE</td>
<td>σ_e</td>
</tr>
<tr>
<td>J_{1,2}</td>
<td>7.79</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td>J_{1,3}</td>
<td>1.24</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>J_{1,4}</td>
<td>0.47</td>
<td>0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>J_{2,3}</td>
<td>7.30</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>J_{2,4}</td>
<td>1.42</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>J_{3,4}</td>
<td>7.79</td>
<td>0.02</td>
<td>0.10</td>
</tr>
</tbody>
</table>

References

Dear Barry:

I am sorry for the delay in getting in our contribution. We have been examining some alpha methylene-γ-butyrolactones of late. The two isomers show the dramatic effect of polar versus nonpolar solvents on the shifts in rigid systems. At the moment we have no explanation for the upfield shift of the vinyllic proton, which is anti to the carbonyl group, when the solvent changes from hexadeuterioacetone to DCCl₃. In contrast, the syn proton is shifted downfield. Any suggestions would be welcomed.

Mp 84-85°  
60.88 (0.86)
6.05 (6.02)

Mp 83-84°  
60.89 (0.87)
6.20 (5.56)
65.64 (5.59)

I shall try not to be so late next time with this letter but the lab has loaded with sample requests of late.

Sincerely yours,

K. D. Berlin
Regents Professor
A hot performer at a cool 4.2°K

Varian introduces:
The XL-200 superconducting FT NMR spectrometer

In a cost- and resource-conscious world, the new XL-200 with 47-K superconducting magnet makes a lot of sense. To begin with, its high-field performance and advanced design come in a truly affordable package. And economy characterizes the XL-200 spectrometer in other ways, too—such as the low-loss dewar unit, which lets the system operate over three months on only 25 liters of liquid helium!

The basic instrument is designed for $^1$H (200 MHz) and $^1$C (50.3 MHz) observation, but it will accommodate a host of other nuclei with the optional 20-80 MHz broadband accessory.

The XL-200's data management system tops all conventional concepts of versatility and convenience. There are two processing units working in tandem—one 32 bits wide and very fast for data acquisition, the other programmed in a high-level language and extremely flexible for data manipulation. Both operate continuously and, together with the XL-200's full complement of built-in I/O devices, offer you unique multi-tasking capability and high sample throughput.

And that's only the beginning of a long list of features which could read like your own NMR wishlist:
- 47-K Nb-Ti superconducting magnet with 50-mm bore
- 25 liters liquid He dewar capacity; 3-month refill interval
- 35 liters liquid N$_2$ dewar capacity; 14-day refill interval (45 days with optional refrigerator)
- 5- and 10-mm samples standard; other sample sizes optional
- Broadband probes covering 20-80 MHz and 180-212 MHz ranges
- Flexible mix/match RF system with fixed-frequency sources such as $^1$H, $^13$C, $^1$F, and $^31$P
- Compatible with RF synthesizer for broadband multinuclear operation
- 50-kHz spectral widths with quadrature phase detection
- Automatic $^1$H internal field/frequency stabilization with exclusive AutoLock™ circuit
- $^1$H homo/heteronuclear decoupler for a wide variety of gated modes
- Programmable 32K CPU for data processing and multitasking
- Independent 32-bit parallel processor with dedicated random-access memory for spectrometer control and data acquisition
- Built-in I/O devices include solid-state keyboard; 5M-word moving-head disk with dual platter (one removable); high resolution raster scan storage/display oscilloscope; 32-column line printer; 500 x 240 mm X-Y recorder.

If you would like the balance of the features to compare with your wishlist, write Varian Associates, Inc., Box D-070, 611 Hansen Way, Palo Alto, CA 94303.
Varian Sales Offices

CALIFORNIA
9901 Paramount Boulevard
Downey, CA 90240
Phone: (213) 927-3415
375 Distel Circle
Los Altos, CA 94022
Phone: (415) 968-8141

COLORADO
4665 Kipling, Suite 1
Wheatridge, CO 80033
Phone: (303) 425-0413

GEORGIA
6650 Powers Ferry Road
Suite 100
Atlanta, GA 30339
Phone: (404) 955-1392

ILLINOIS
205 W. Touhy Avenue
Park Ridge, IL 60065
Phone: (312) 825-7772

KENTUCKY
Executive Park, Suite 110
Louisville, KY 40207
Phone: (502) 897-0171

MARYLAND
4701 Lydell Drive
Cheverly, MD 20781
Phone: (301) 772-3683

MASSACHUSETTS
400 Totten Pond Road
Waltham, MA 02154
Phone: (617) 890-8430

NEW JERSEY
25 Hanover Road
Florham Park, NJ 07932
Phone: (201) 822-3700

NEW YORK
6489 Ridings Road
Syracuse, NY 13206
Phone: (315) 437-6464

OHIO
25000 Euclid Avenue
Euclid, OH 44117
Phone: (216) 261-2115

TEXAS
Plaza Southwest
5750 Bintliff Drive, Suite 202
Houston, TX 77036
Phone: (713) 763-1800

WASHINGTON
300 120th Avenue
Bldg. 2, Suite 230
Bellevue, WA 98005
Phone: (206) 454-2910

Instrument Division sales offices and representatives are located in: Algeria, Argentina, Australia, Austria, Benelux, Brazil, Canada, Chile, China, Colombia, Costa Rica, Denmark, Eire, Finland, France, Germany, Great Britain, Greece, Holland, Hong Kong, Iceland, India, Iran, Israel, Italy, Japan, Korea, Lebanon, Malaysia, Mexico, Morocco, New Zealand, Norway, Pakistan, Peru, Philippines, Puerto Rico, Portugal, Scandinavia, South Africa, Spain, Sweden, Switzerland, Thailand, Turkey, United States, Venezuela.
May 26, 1978

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

Dear Dr. Shapiro:

The University of California, Davis, will have an opening for an NMR spectroscopist for our new Biological Magnetic Resonance Facility, as described below. We would appreciate it if this notice can be made available to all potential candidates in your Department.

NMR Spectroscopist Position Open

Assistant Research NMR Spectroscopist to supervise new Biological Magnetic Resonance Laboratory consisting of 200 MHz and 360 MHz Multinuclear FTNMR Spectrometers. Candidate must show strong evidence for productive research, as position involves advising and collaborating with biological science faculty as well as pursuing independent research. Responsibilities also include spectrometer maintenance and development, supervising one or more technicians as well as training and scheduling users. Ph.D. in Chemistry or equivalent degree, thorough background in FTNMR and hardware/software experience essential; some experience in biological FTNMR applications highly desirable. Salary $17,500 - $20,500, depending upon qualifications and experience. Send curriculum vitae, bibliography and three letters of reference to Professor G.N. La Mar, Department of Chemistry, University of California, Davis, CA 95616. The final date of application for the position will be July 17, 1978.

In compliance with federal and state laws and University policy, the University of California does not discriminate on the basis of race, color, national origin, religion, sex, handicap, age, or against disabled veterans or Veterans of the Vietnam era. The University of California is an affirmative action/equal opportunity employer.

Sincerely yours,

Gerd N. La Mar
Professor of Chemistry
Co-director, UCD Biological Magnetic Resonance Facility
May 22, 1978

Dear Barry:

Often in handling multiple-step equilibria by fast-exchange NMR, problems are encountered in doing statistics on the resultant shift vs. concentration curves. A rigorous treatment requires highly accurate first and second derivatives of concentrations and concentration-dependent functions. These, in turn, are best facilitated by exact, closed-form, solutions for concentrations. We give below a solution for two-step equilibria which is totally unambiguous and fool-proof. Previously published solutions, although correct, were too general to be of use when statistical applications of a rigorous nature were carried out (i.e., solutions including standard errors of equilibrium constants and/or bound shifts). In the results below, things which can be deduced by simple algebra are omitted; only the more difficult things are shown in detail.

The following system is solved for \([AB]\):

\[
A + B = AB \quad \text{and} \quad AB + B = AB_2
\]

This has respective equilibrium constants of \(K_1\) and \(K_2\). \([AB_2]\) may be obtained from simple algebraic manipulation after \([AB]\) has been found. Now, let \(\rho = A/B\) (the relative formalities of \(A\) and \(B\)) and let \(k = K_1/K_2\). Then, if we let \(x = [AB]\) for notational convenience, solution of the following cubic equation will give us the desired concentration:

\[
x^3 + a_2x^2 + a_1x + a_0 = 0
\]

Here,

\[
a_0 = \rho k^2 B_0^2/[K_1(k - 4)],
\]

\[
a_1 = (B_0^2 k(1 - 2\rho) + k^2[B_0(1 + \rho) + 1/K_1](1/K_1))/(4 - k),
\]

and,

\[
a_2 = k[1/(1/K_1) + 2\rho B_0/(4 - k)].
\]

If \(k = 4\), these equations are not used and are replaced by a simple quadratic form not shown. For the more common occurrence \((k \neq 4)\), we write the complete solution. First, we let

\[
q = (3a_1 - a_2^2)/9,
\]

\[
r = [(a_1a_2 - 3a_0)/6] - (a_2/3)^3,
\]

THE UNIVERSITY OF SOUTH FLORIDA IS AN AFFIRMATIVE ACTION EQUAL OPPORTUNITY INSTITUTION
and \[ D = q^3 + r^2. \]

Now, if \( D > 0 \), we have the relatively simple solution
\[ [AB] = x = s_+ + s_- = a_2/3 \]
where
\[ s_\pm = (r \pm \sqrt{D})^{1/3} \]
and we use the real cube roots (positive or negative). This first case is totally unambiguous since there is only one real root with \( D > 0 \). However, if \( D \leq 0 \), there are three real roots; the physically relevant root is determined by whether \( k < 4 \) or \( k > 4 \). Below are given the "recipes" for these two possibilities.

**\( k < 4 \) case:**

Let \( t = (r^2 + |p|)^{1/2} \) and \( \Omega = [\text{arc} \cos(r/t)]/3 \).

Then, \[ [AB] = x = 2t^{1/3} \cos(\Omega) - a_2/3. \]

**\( k > 4 \) case:**

Use the \( x \) as obtained immediately above (for \( k < 4 \)) and go a little further by letting
\[ Q = a_2 + x \quad \text{and} \quad R = -a_2/x. \]

Then, \[ [AB] = [-Q + \sqrt{Q^2 - 4R}]/2. \]

The above solutions are totally unambiguous and represent the fastest way to calculate \([AB]\) and, hence, \([AB^2]\). Needed derivatives can be obtained from these equations (with an appropriate amount of suffering).

Hopefully, some papers applying these—and derivations for more complicated systems—will appear as papers in the near future.

Sincerely yours,

Milton D. Johnston, Jr.
Assistant Professor of Chemistry

SUGGESTED TITLE: Two-step equilibria the fastest way
Dear Professor Shapiro;

$^{13}$C NMR spectrum of a pyrryl-s-triazine

Recently we prepared

Its $^1$H noise decoupled $^{13}$C NMR spectrum shows the following features (solvent: hexadeuteroacetone, 30°C, chemical shifts relative to T.M.S.)

<table>
<thead>
<tr>
<th>Chemical Shift</th>
<th>Assignment</th>
<th>Coupling Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.0</td>
<td>CH$_3$</td>
<td></td>
</tr>
<tr>
<td>14.4</td>
<td>CH$_3$</td>
<td></td>
</tr>
<tr>
<td>115.2</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>123.0 t</td>
<td>C</td>
<td>2.3 Hz</td>
</tr>
<tr>
<td>135.7</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>140.1</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>170.2 t</td>
<td>C</td>
<td>14.5 Hz</td>
</tr>
<tr>
<td>171.5 dd</td>
<td>C = C</td>
<td>225.5 Hz</td>
</tr>
</tbody>
</table>

The $^{13}$C NMR spectrum at -10°C shows additional splitting in the signals corresponding to 3C and 5C (all other features are the same within experimental error).

These facts can be understood by assuming that rotation around the 1-7 bond at -10°C is slow on the NMR time scale giving an observable
chemical shift difference between C3 and C5 of 0.2 ppm. At 30°C the rotation is that rapid that only the averaged chemical shift of C3 and C5 can be observed.

The compound lacking the 8 and 10 methyl groups does not show a difference between the C3 and C5 signals at -10°C or 30°C. Clearly showing that the 8 CH₃ has a profound influence on the rotation barrier between the two ring systems. Its ¹³C NMR spectra at -10°C and 30°C are identical and very similar to the 30°C ¹³C NMR spectrum of the dimethyl derivative.

Sincerely Yours,
Jeroen Jacob Duijfjes Cees Erkelens Johan Lugtenburg

Signals of C₁, C₃ and C₅ in the ¹³C NMR spectrum of the dimethyl-pyrryl-s-triazine. (¹H noise decoupled, solvent hexadeutero-acetone, temperature: -10°C).
Dear Dr. Shapiro,

in a work on the structural characterization of models of regioirregular polypropylene we have measured the $^{13}$C chemical shift of the methyl groups (underlined position) in the following hydrocarbons:

The observed methyls can be present in the possible arrangements of irregular polypropylene. The obtained data suggest that it may be possible to estimate the chemical shift of a methyl in a paraffinic chain through an additivity scheme of the kind introduced by Grant and Paul and making allowance for configurational effects.

Thus the methyl shift is given by the relation

$$\nu = 17.99 + \Sigma N_i P_i + \Sigma R_{ij}$$
where \( N \) is the number of methyl groups having a definite distance from and a steric relation with the observed methyl, \( P \) is the additivity parameter characterizing both the distance \((\gamma, \delta, \epsilon, \zeta)\) and the steric relationship (erithro or treo) and \( R_{ij} \) is a parameter which describes the effect of the proximity of two methyl substituents and depends on their steric relation.

The values of the parameters we have found are the following:

<table>
<thead>
<tr>
<th>( P_1 )</th>
<th>( P_\gamma )</th>
<th>( P_\delta )</th>
<th>( P_\epsilon )</th>
<th>( P_\zeta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>erithro</td>
<td>-3.05</td>
<td>0.77</td>
<td>0.16</td>
<td>0.05</td>
</tr>
<tr>
<td>treo</td>
<td>-4.83</td>
<td>0.22</td>
<td>0.06</td>
<td>-0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>( R_{ij} )</th>
<th>( R_{\delta \zeta} )</th>
<th>( R_{\delta \epsilon} )</th>
<th>( R_{\gamma \epsilon} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>e e</td>
<td>0.02</td>
<td>0.17</td>
<td>-0.12</td>
</tr>
<tr>
<td>e t</td>
<td>-0.10</td>
<td>-0.06</td>
<td>-0.40</td>
</tr>
<tr>
<td>t e</td>
<td>-0.15</td>
<td>-0.33</td>
<td>-0.14</td>
</tr>
<tr>
<td>t t</td>
<td>-0.20</td>
<td>-0.39</td>
<td>-0.52</td>
</tr>
</tbody>
</table>

These parameters can be used in the study of the spectra of irregular polypropylene and of copolymers of ethylene with propylene.

A detailed report is in press on Macromolecules.

Best regards

G. Gatti
Proton Magnetic Relaxation Studies of Dextran Derivatives

Dear Prof. Shapiro,

NMR spectroscopy has been indicated as a powerful tool for the structural determination of polysaccharides. Chemical shifts and coupling constants from either $^1$H or $^{13}$C spectra have been used for identifying the anomeric configuration of glycosidic linkages, as well as the location of substituents on the carbohydrate moieties. In particular, a comparison of NMR and other chemical methods (e.g. methylation followed by the use of combined gas liquid chromatography-mass spectrometry (glc-ms)), has been carried out on native dextrans isolated from various bacterial strains. Although methylation data provide more precise values for the degree of branching, NMR spectroscopy has been shown to provide more specific information on the nature of anomeric linkages (1). Moreover, NMR has the additional advantages of being a non-destructive technique and allowing the identification of non-carbohydrate organic material possibly present in the preparation.

Another advantage of NMR lies in the possibility of providing insight into the nature of molecular motions of these polysaccharides, through the study of spin-lattice and spin-spin relaxation times, determined at the level of the individual chemical groups. $^{13}$C T1 relaxation measurements, carried out by Benesi and Gerig (2) on linear,α(1→6)-linked dextrans of different molecular weight (T40, MW 4 x 10$^4$; T250, MW 2.3 x 10$^5$; T2000, MW 2.10$^6$; Pharmacia Fine Chemicals), as well as on a cross-linked dextran, Sephadex G-75, have shown that a) the relaxation of all carbon atoms in these polymers is overwhelmingly dominated by dipolar interactions; b) the small segments of the polymer are able to execute rapid local motions that are relatively independent of the overall conformation of the polymer in solution; c) all relaxation data (T1, T2, NOE) can be consistently analyzed in terms of anisotropic reorientation of the monomeric units (considered as ellipsoids of revolution) in which "the molecular motion of the glucose residues about the axis roughly defined by the polymer chain directions is about 16 times easier than reorientation normal to the chain direction" (2).

In an attempt to make use of the potentiality of NMR relaxation methods for assessing the structure and mobility of these polysaccharides in relation to their immunochmical properties, we have carried out proton magnetic relaxation measurements (100 MHz, 37°C, C = 2 mg/ml, in D$_2$O saline solution) on O-stearoyl dextrans (OSD) prepared by reacting increasing amounts of stearoyl groups (esterified at the C-3 position) with dextran T70 (MW 7 x 10$^4$) (3). Immunochmical studies on dextran T70 and its O-stearoyl derivatives (stearoyl contents ca. 0.4, 1.4, 2.7, 3.9 % w/w respectively) had shown that, although all these compounds precipitate with a rabbit anti-dextran serum, the two derivatives with higher stearoyl contents (2.7 and 3.9 %)
exhibit some loss of specificity (3). Our NMR studies are aimed at assessing whether the observed changes in immunochemical properties can be related to changes of chain flexibility possibly induced by increasing stearoyl contents.

H peak assignments on dextrans were based on a) a comparison of the spectral features of oligosaccharides of the isomaltose series; b) peak assignments worked out at 300 MHz by De Bruyn et al. (4) for the α-D-glucopyranosyl-α-D-glucopyranose and "shift-increments" determined vs. α-D-glucopyranose; c) double resonance experiments. Although at 100 MHz the signals arising from the saccharide rings are strongly coupled and partially overlapping to each other, a careful analysis of the partially relaxed spectra and double resonance experiments allowed us to assign the bands arising from H3-H2-H4 and to H5-H6A-H6B respectively.

As a comparison, 1H T 1 studies have also been carried out on linear dextrans of different molecular weight (MW = 2·106, 7·106, 2·107) and on the above mentioned stearoyl derivatives. Our results have shown that: 1) in agreement with the 13C T 1 results by Benesi and Gerig (2), the T 1 values of all proton groups are maintained practically constant (within ±5%) in the dextrans of the various sizes tested; 2) by increasing the stearoyl content of dextran T 70 the a (1→6)-linked anomeric proton shows slight but reproducible decreases in 1H T 1 from 270 ms (T 70, OSD 0.4%) to 250 ms (OSD 1.4%) to 235 ms (OSD 2.7% and 3.9%). The 1H T 1 values of H3, H2 and H4 are practically kept constant at a value of 320 ± 10 ms up to a stearoyl content of 2.7% whereas they drop to 270 ± 10 ms in OSD 3.9%. T 1 values of H5 and H6 are kept constant within experimental errors, for all samples tested.

The selective changes observed for the various groups, at the level of H3 H2 and H4, as well as -although to a minor extent- at the level of the anomeric proton, would suggest that, rather than to simple micelle-like aggregation, these changes should be attributed to a modified mobility of the monomeric units induced by an increase in the stearoyl content. 13C NMR measurements are now in progress for further testing these tentative conclusions, in terms of rotational diffusion and segmental flexibility of the chains. If our preliminary conclusions are confirmed these studies would point to a possible conformational role of the antigenic determinants in the immunochemical specificity of polysaccharides and to the potentiality of NMR relaxation techniques in further elucidating the molecular mechanisms governing the interaction of these antigens with specific antibodies.

(Franca Podo) (Carlo Ramoni) (Giuseppe Vicari)
Laboratorio di Biologia Cellulare e Immunologia, Istituto Superiore di Sanità, Roma

May 25, 1978

Dear Barry:

As a prelude to zeugmatographic imaging experiments with Relaxation Contrast Reagents, we have been carrying out a number of experiments on the effect of Mn++ on water proton relaxation times in tissues. The first measurements were on rat plasma and on samples of myocardium (heart muscle) from pigs and dogs (don't you long for the good old days when the standard test sample was potable?). Now, however, we are shooting manganous solutions into the veins of dogs and rats, and then looking at the water proton T1 values and the Mn contents of various normal organs and of normal and ischemic myocardium. To make a long (and still very incomplete) story short, the table below shows the 4 MHz T1 changes produced after 1 hour in some rat organs by non-lethal Mn++ doses of 0.1 mmol/kg body weight, and the sketch shows the relaxation rates in pieces of a dog's heart in which an ischemic region was produced by tying off coronary arteries at the points indicated, injecting the same dose of Mn++ in saline solution an hour later, and then sacrificing the animal after another half hour. Not only are different organs differently affected, probably largely because of differences in Mn++ uptake, but the poorly perfused region of the heart shows a much smaller effect than the regions still supplied with a normal flow of blood. It may be that such enhanced T1 contrast will be useful in NMR zeugmatographic imaging in living animals and humans, and we hope to test that idea soon.

This work has been done in collaboration with a visitor, M. Helena Mendongoa Dias, of the Instituto Superior Tecnico, Lisbon, and with A.M. Rudin and M.J. Glucksman, with funding from the NIH and from a V.A. Grant to M.J. Jacobson at the Northport VA Hospital and the SUNY/SB Department of Surgery.

Best regards.

Yours truly,

Paul C. Lauterbur
Professor of Chemistry

PCL:eg
<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>0.1 mmol/kg Mn&lt;sup&gt;++&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>656</td>
<td>465</td>
</tr>
<tr>
<td>heart</td>
<td>363</td>
<td>47</td>
</tr>
<tr>
<td>lung</td>
<td>404</td>
<td>221</td>
</tr>
<tr>
<td>liver</td>
<td>123</td>
<td>29</td>
</tr>
<tr>
<td>spleen</td>
<td>244</td>
<td>186</td>
</tr>
<tr>
<td>muscle</td>
<td>278</td>
<td>262</td>
</tr>
</tbody>
</table>

(T<sub>1</sub>)<sup>-1</sup> values (in sec<sup>-1</sup>) for dog myocardium after injection of Mn<sup>++</sup> at 0.1 mmol/kg. The (T<sub>1</sub>)<sup>-1</sup> value for myocardium without Mn<sup>++</sup> injection is about 3. Note the region of decreased relaxation enhancement distal to the ligations.
Dear Prof. Shapiro,

Catalytic systems containing magnetically active metals and ligands provide fruitful ground for multinuclear studies, and the system cis-^{195}\text{PtCl}_2(^{31}\text{PR}_3)_2 + ^{117,119}\text{SnCl}_2 represents one such example. The product resulting from the interaction of these two molecules (see equation) can be readily identified using a combination of \(^{195}\text{Pt}, ^{119}\text{Sn}, ^{31}\text{P}\) and \(^1\text{H}\) methods.

\[
\text{cis-PtCl}_2(\text{PR}_3)_3 + \text{SnCl}_2 \rightarrow \text{trans-PtCl(SnCl}_3(\text{PR}_3)_2}
\]

The appearance of a two-bond tin-phosphorus coupling \(^{31}\text{P}\) spectrum as well as the chemical shift of the coordinated tin prove that the two metals lie within one coordination sphere. The one-bond platinum-phosphorus coupling \(^{195}\text{Pt}\) and \(^{31}\text{P}\) spectra) and the nature of the second order \(^1\text{H}\) spectrum support a trans configuration of the phosphorus atoms, although the starting material may be seen to have cis geometry. Finally, the number of phosphorus atoms attached to the metal (good catalysts are, by definition, labile) is confirmed by the \(^{195}\text{Pt}\) spectrum. NMR and transition metal chemistry continue to enjoy a profitable partnership.

There will be a position for a postdoctoral student in our lab beginning September of this year. The work will involve the nmr of catalytic systems and interested parties may write to me directly.

Please credit this contribution to the account of Prof. L. M. Venanzi.

Sincerely yours,

Dr. P. S. Pregosin
The R-600 control panel is simple to operate, easy to understand.
NOW...
EFFORTLESS FT NMR

Perkin-Elmer's Model R-600 is a high-performance, low-cost instrument for routine proton observation at 60 MHz. It's the first commercially available NMR spectrometer with a dedicated digital microcomputer that doesn't require a computer expert. The R-600 utilizes the proven R-24 Series magnet system. And because it's a permanent magnet NMR, there are no special requirements for water or power. Just plug it in.

So it's easy to operate, easy to install. And with an extremely low price tag and operating cost, it's also easy on your budget.

**MULTIPLE USES**
All you need are microgram samples to get routine spectra from the Model R-600. Consequently, you can apply NMR in many new areas. You can get unequivocal identification of many LC and GC fractions. You can use NMR to analyze trace amounts of impurity or isolated natural product when these are all you have available.

**MULTIPLE SAVINGS**
Even if you have a complex FT NMR spectrometer now, you still need the Model R-600. Your large unit is usually tied up with time-consuming $^{13}$C experiments. Besides, adapting it to proton capability would be tedious or costly. Adding a Model R-600 will give you the extra NMR you need, save money, and get your work done on time.

With superb sensitivity, the R-600 lets you run routine experiments on a small scale. Your sample requirements drop from milligram sizes to 500 micrograms or less. But you'll still get the same quality spectra.

**SIMPLE OPERATION**
Not only is the microcomputer easy to operate, it also does most of the work. And the R-600 is the first FT NMR with controls arranged for operation like a conventional continuous wave instrument. Programming was designed by an NMR spectroscopist, so operational parameters and commands are user oriented. With just ten keys, the control panel simplifies setting the operating conditions and readout of the measurements.

**EASY T,**
The Model R-600 has other advantages. For instance, its two Auto-T modes enable you to run a complete T experiment while specifying the fewest parameters. There's also a solvent suppression mode to minimize interfering peaks such as water.

**GET ALL THE FACTS**
Learn how the Model R-600 can simplify your NMR life. Request our literature describing its long list of benefits. Ask for a demonstration. Write Perkin-Elmer Corp., Main Ave., MS-12, Norwalk, CT 06856. Or call Tom Proulx or Jim Hannon at 203-762-1776.
Cher Barry,

Je vous prie de nous excuser pour le retard mis à vous envoyer notre contribution qui concerne l'utilisation de la spectroscopie $^{15}$N à la prévision de la délocalisation électronique dans différentes séries de composés N-X.

Nous avons établi plusieurs corrélations entre les énergies d'activation Ea ou les enthalpies libres d'activation $\Delta G^\ddagger$ de processus de rotation génée monomoléculaire, autour d'une liaison N-X et le déplacement chimique $\delta^{15}$N. Lorsque la barrière est essentiellement d'origine $\delta^{15}$N et que les phénomènes intermoléculaires ou stériques ont une importance relative négligeable dans une série de composés, la corrélation présente une très bonne linéarité (cf. figure).

Nous avons ainsi étudié :
- des thioamides $(\text{CH}_3)_2\text{N-CSR}$
- des amides $(\text{CH}_3)_2\text{N-COR}$, $(\text{CH}_3\text{CH}_2)_2\text{N-COR}$, $\text{H}_2\text{N-COR}$, $\text{CH}_3\text{HN-COR}$
- des anilines et énaminines : $\text{-C=C-N}(\text{CH}_3)_2$, $\text{-C=C-N}(\text{CH}_3\text{CH}_2)_2$, $\text{C}_6\text{H}_5\text{-N}(\text{CH}_3)_2$, $\text{C}_6\text{H}_5\text{N}(\text{CH}_3\text{CH}_2)_2$, $\text{C}_6\text{H}_5\text{NH}_2$
- des composés diazotés : $(\text{CH}_3)_2\text{N-N} = \lambda$, $\text{A} \equiv \text{CRR'}$, O, O$_2$, N-Ar

et avons observé que les valeurs des pentes (b) des corrélations sont comprises entre 0,1 et 0,3 kcal/ppm.

Plusieurs remarques peuvent alors être faites :

1) La RMN de l'azote $^{15}$N est une méthode très précise et très sensible pour obtenir des valeurs de délocalisation en terme de hauteur de barrière. En effet, une valeur $\delta^{15}$N peut être mesurée d'une façon reproductible à ± 0,1 ppm et la délocalisation électronique peut être calculée avec une précision supérieure à 0,05 kcal/mole$^{-1}$.

2) La RMN de l'azote $^{15}$N est une méthode juste (accurate) lorsque des renseignements relatifs sont recherchés : comparaison des valeurs de délocalisation d'un composé à l'autre dans une série, variation en fonction du solvant ou de la concentration etc... La méthode est moins juste lorsque les hauteurs absolues des barrières sont calculées au moyen des corrélations car l'erreur sur l'ordonnée à l'origine peut être importante (elle n'est d'ailleurs pas plus grande que les erreurs systématiques liées aux mesures de RMN dynamique).

3) La RMN de l'azote $^{15}$N est aussi une méthode prospective car elle permet de prévoir des valeurs de barrières qui n'avaient pas pu être mesurées antérieurement. Le dernier exemple exploité au laboratoire est particulièrement
illus: la tétraméthyl urée (CH₃)₄N-CO-N(CH₃)₂ (TMU) a été abondamment étudiée par les techniques de RMN dynamique mais aucun phénomène de coalescence n'a pu être mis en évidence jusqu'à ce jour tant en résonance lH que ¹³C. La valeur du déplacement chimique δ¹⁵N = -315,3 ppm permet cependant de prévoir une barrière de 11,6 Kcal.mole⁻¹ qui devrait correspondre à un phénomène de coalescence aux alentours de 210/230°C. Incités par cette constatation, nous avons pu effectivement faire apparaître deux signaux ¹³CH₃ à basse température en utilisant une solution de TMU et de Eu(FOD)₃ dans CD₂Cl₂ ΔG° = 11,1 Kcal.mole⁻¹.
June 1, 1978

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

SALE ON NMR AND EPR INSTRUMENTATION

Dear Professor Shapiro:

We are having a sale here at Case Western Reserve on some of our NMR spectrometers, and one EPR, and thought some of the readers might know of someone starting on low-budget NMR/EPR, or could use these instruments for back-up parts, or could make use of their associated magnets (all Varian):

- HA 100 with Fourier Transform, $^1$H and $^{13}$C observe
- HA 60 with high impedance magnet
- HA 60 with low impedance magnet
- A 60A with spin decoupler and V.T. S:N = 22:1
- A 60 with V.T. S:N = 15:1
- V 4502-14 EPR with X-band bridge, and 12 inch wide gap (2.625 in.) low impedance magnet

We're not going out of the NMR/EPR business, but rather making room and raising funds toward some newer instrumentation. Those interested may write, or call us at (216) 368-3589 or 3658.

Sincerely,

William Ritchey

Alan Olson

AO:fs
cc: W. Ritchey

Department of Chemistry
Dear Professor Shapiro:

Metal Ion NMR For The Study of The pH Dependence of Metal Ion Binding to Macromolecules.

Traditional methods of investigation of the interactions between metal ions and macromolecules, such as equilibrium dialysis, are cumbersome. In a fast-exchanging system it is possible to probe the pH-dependence of metal ion binding rapidly and with small expenditure of protein (a few milligrams) by the monitoring of linewidth (and, thereby, transverse relaxation) changes in the NMR signal of the metal ion upon association/dissociation of the complex.

The protein involved in this study was a calcium ion-binding fragment, Fragment-1, of a blood coagulation protein, prothrombin. At the La$^{3+}$ concentration employed, it has been established in other studies that not only are the high affinity ion-binding sites occupied, but also an undetermined number of relatively non-specific, lower affinity sites. Effects seen are therefore an average of those characterizing the various individual sites.

The general dependence of the linewidth of the $^{139}$La$^{3+}$ resonance, with and without added protein, on pH is illustrated in the accompanying figure. Total Fragment-1 concentration was $4.48 \times 10^{-5}$ M in 0.15 M sodium chloride, 50 mM MES buffer pH initially 6.0 (adjusted with triethylamine). The symbol o refers to runs carried out in the presence of a total lanthanum ion concentration of 6 mM. pH was varied by addition of microliter amounts of 1 N hydrochloric acid slowly added to the vigorously stirred protein:lanthanum sample. Sample volume was 6.0 ml. Sweep width was 5,000 Hz. Approximately 200,000 transients were accumulated per spectrum. Lanthanum ion linewidth controls were run under exactly the same conditions, but in the absence of fragment-1. The symbol refers to an experiment organized as follows: the pH of a sample containing fragment-1 ($4.77 \times 10^{-5}$ M) in 6 mM Lanthanum chloride, 5 mM calcium chloride, 0.15 M sodium chloride 50 mM MES buffer, pH initially 6.0 (adjusted with triethylamine). The symbol corresponds to lanthanum controls run under the same conditions, including ion concentration, but in the absence of fragment-1. In all cases adjustment was from high to low pH values.

A small decrease in $^{139}$La$^{3+}$ linewidth occurs on decreasing the pH from 6.0 to 5.2. Below pH 5.0 a broad titration process, typical of protein carboxyl groups, is indicated until a pH value of 3.4 is reached. Below this value the linewidth plateaus prior to falling off below pH 2.5 to approach that of unbound $^{139}$La$^{3+}$. Studies designed to allow more detailed interpretation of these observations are ongoing.
Spectra were recorded at 14.13 MHz on a Varian XL100 FT spectrometer in 18mm spinning tubes with vortex plugs. The instrument was modified for multinuclear operation in the manner described by Marshall et al. (Marshall, A.G., Hall, L.D., Hutton, M., and Sallos, J., J. Mag. Res., 13, 392 (1974). The 18mm probe was made by Nicolet Technology, Inc.

Yours sincerely,

David L. Harris
Department of Chemistry

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

Dear Barry:

Interfacing a Clock to an XL-100

When making $T_1$ or other measurements requiring long delays between pulses, a spectrometer operator frequently loses track of the time since the last pulse. This could be useful information: if the spectrometer should lose lock between pulses, one might have time to reestablish lock before the next pulse, without having to delay or abort the experiment. More commonly one might wish to touch up the homogeneity prior to each pulse. Or, when using a new data acquisition program one might wish to time the pulse delays, to ensure they are what they should be.

To solve these problems we have been using a National Semiconductor Corporation MA 1002-C clock chip, which is a 24-hour clock with LED display. Using the schematic shown in Figure 1, we interface the clock to our XL-100 spectrometer. The clock resets on each pulse (or manually), and displays either minutes:seconds (up to 9:59) or hours:minutes (up to 23:59) since the last pulse. When not in use for timing pulse delays, the clock can be used to display the time of day.

Sincerely yours,

Mark Mattingly  
Robert Rowan, III

MM/RR/ssc
SCHEMATIC #87-109-813

COMPONENTS
IC1 NE555
IC2 SN7400
S1 Push button; NC, momentary
S2, S3 DPDT toggle switch
Q1, Q2 2N3906
Q3 2N5458

RESISTANCE IN Ω
CAPACITANCE IN µF

FIGURE 1. MA 1002-C/XL-100 INTERFACE
Professor B. L. Shapiro  
Department of Chemistry  
Texas A & M University  
College Station, Texas 77843

SUBJECT: In the Trenches with a Rodenticide Metabolite

June 2, 1978

Dear Professor Shapiro,

Recently, I was involved in identifying a series of metabolites of the rodenticide Vacor®, which has the structure:

\[
\begin{align*}
\text{CH}_2-\text{NH-C-NH}_2 \quad \text{O} \quad \text{NO}_2
\end{align*}
\]

One metabolite which was formed in dog liver had been isolated by column chromatography on XAD-2 and Bio Sil A, preparative TLC, and reverse-phase HPLC. The sample of 34 µg of metabolite obtained was run with a 1 mm microinsert on a Varion XL-100 and gave the spectrum shown in Figure 1. Although we have never had contamination problems before, the spectrum was strongly reminiscent of unwanted paramagnetic ions! The sample was treated by passage over a microcolumn of Chelex 100 and 20 µg of the metabolite reisolated and examined again by NMR, giving the spectrum shown in Figure 2. From these data the metabolite was identified as nicotinamide. Re-examination of Figure 1 shows that the H2 and H6 peaks were preferentially broadened and shifted compared to H4 and H5, which implies specific rather than nonspecific broadening has occurred due to complex formation involving the pyridine nitrogen. I attempted to duplicate the spectrum of Figure 1 by adding various paramagnetic ions to pure nicotinamide, but could not get a spectrum which matched well. Thus the exact nature of the problem remains a mystery, although the Chelex 100 treatment is recommended for anyone who encounters a similar situation.

Yours truly,

David G. Westmoreland

DGW:pt
FEATURES INCLUDE:

- 3.5T superconducting magnet with 10 cm room-temperature bore.
- Straight-through access to sample area.
- Quick-disconnect probes for rapid changeover.
- 5, 12 and 20 mm sample tubes as standard, 30 mm optional.
- Quadrature phase detection as standard.
- Computer-controlled audio filter from 100 Hz to 51,100 Hz in 100 Hz steps.
- Nicolet 1180 data system with simultaneous acquisition, processing and plotting.
- Digital plotter with plot lengths selectable from 1 cm to 900 cm.

OPTIONS INCLUDE:

- NT-150 MF: broad-band multi-nuclei observe for 4 to 60 MHz.
- NT-150 CP: optimized system for Waugh-Pines cross-polarization studies.

For more information or to discuss your applications, please telephone or write.

NICOLET TECHNOLOGY CORPORATION
145 East Dana Street
Mountain View, California 94041
Phone: 415/969-2076
While you’re working in the foreground... your FX is working in the background*

examples:
- Fourier transformation
- Data massage
- Basic programming
- $T_1/T_2$ calculation
- Plot/print/CRT display
- Spin simulation

examples of acquisition:
- $T_1/T_2$
- Auto stacking
- Multi-mode
- Pulse programmed
- Kinetic
- Long term

*Foreground/Background system

The FX60Q, FX90Q & FX100 features:
- (DQD) Digital Quadrature Detection System
- Multi-Frequency TUNABLE Probe observation
- Dual Frequency probes
- 4-channel DIGITAL phase shifters (DPS)
- Comprehensive auto-stacking system
- Foreground/Background system
- Computer-based pulse programmer with Multiple Pulse Sequence Generator
- GPU Expandable to 65K words (MOS)
- 2-channel 12-bit AD/DA
- $T_1/T_2$ spin locking system
- Disc storage systems
- Multi-Mode HOMO/HETERO decoupling capabilities
- Programmable Variable Temperature Unit
- Simplex Y/Curvature Gradient controller

JEOL
235 Birchwood Ave., Cranford, NJ 07016
201-272-9820