THE NMR NEWSLETTER

No. 510

March 2001

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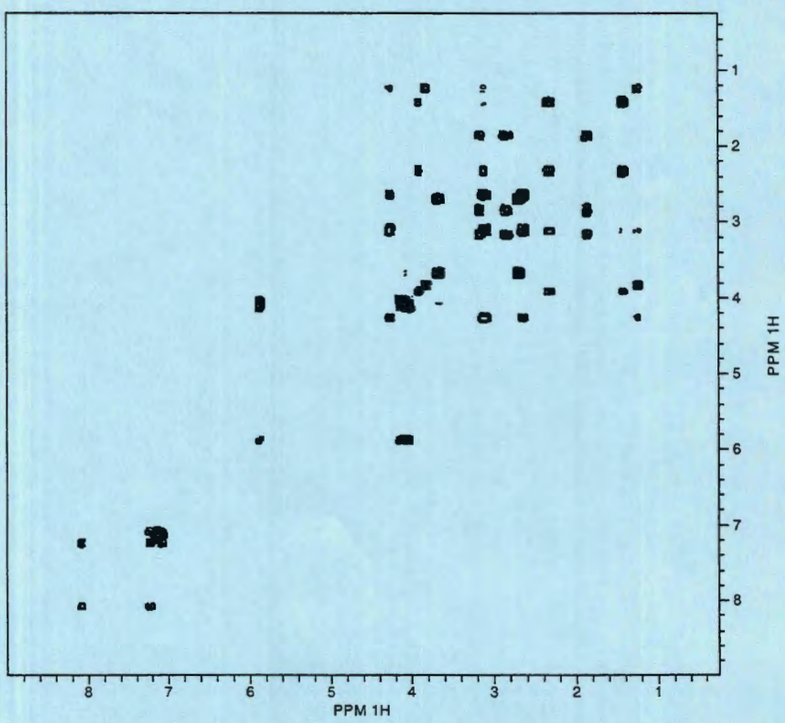

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FORTHCOMING NMR MEETINGS

PITTCON 2001, New Orleans, LA, **March 4-9, 2001**. Contact: The Pittsburgh Conference, Dept. CFP, 300 Penn Center Blvd., Suite 332, Pittsburgh, PA 15235-5503. Tel: 412-825-3220; Fax: 412-825-3224; E-mail: pittconinfo@pittcon.org.

42nd ENC (Experimental NMR Conference), Rosen Plaza Hotel, Orlando, Florida, **March 11-16, 2001**; Arthur G. Palmer, Chair: Agp6@columbia.edu; Contact: ENC, 1201 Don Diego Avenue, Santa Fe, NM 87505; (505) 989-4573; Fax: (505) 989-1073; E-mail: enc@enc-conference.org; Web: www.enc-conference.org.

ACS National Meeting, "Symposium on High Resolution NMR Spectroscopy of Polymers, " San Diego, CA, **April 1-5, 2001**; Contact: H. N. Cheng (hcheng@herc.com) or A. D. English (alan.d.English@usa.dupont.com); See Newsletter 505, 29.

Magnetic Resonance in Chemistry and Biology, XIth International Conference, Zvenigorod, Russia, **April 20-27,2001**. Contact: http://www.nmr.de/html/conf/zelino.shtml.

ISMRM 9th Scientific Meeting and Exhibition, and ESMRMB 18th Annual Meeting and Exhibition, Joint Annual Meeting, **April 21-27, 2001**, 10th Annual Meeting of the Section for Magnetic Resonance Technologists, and 17th Annual Meeting of the British Association of MR Radiographers, **April 20-22, 2001** Glasgow, Scotland, UK; Contact: ISMRM, P.O. Box 45690, San Francisco, CA 94145-0690; http://www.ismrm.org

ISMRM 9th Scientific Meeting and Exhibition; ESMRMB 18th Annual Meeting and Exhibition, Joint Annual Meeting. Glasgow, Scotland, **April 21-27, 2001**.Contact: ISMRM Central Office, 2118 Melvia Street, Suite 201, Berkeley, CA 94704. Tel: 510-841-1899; Fax: 510-841-2340; E-mail: info@ismrm.org.

Computational Aspects of Biomolecular NMR, Gordon Conference, "Il Ciocco", Barga (Pisa) Italy, **May 6-11, 2001**. Contact: Michael Nilges nilges@embl-heidelberg.de, or Dave Case case@scripps.edu.

continued on page 28

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Referenties:

Telefoonkontaktpersoon:

Bijlagen:

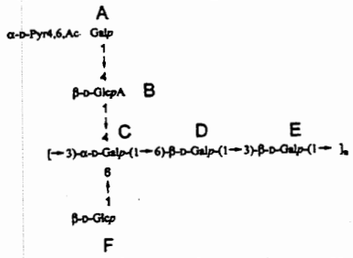
Gent, January 16th 2001 (received 1/29/2001)

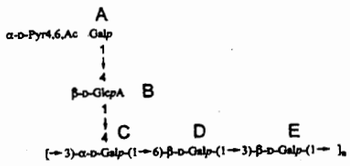
Prof B. L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303, USA

HOW WE IDENTIFY α,β1-6 LINKS IN COMPLEX CARBOHYDRATES

Dear Barry,

In order to assign the nature of the link between two residues in complex carbohydrates, the so-called integrated approach was proposed. With this technique the signals of the residues are assigned from COSY,1D-TOCSY and C,H-heterocorrelated experiments. For galactoses there is a bottleneck for the magnetization transfer between H-4 and H-5, so that identification of galactosyl H-6 resonances needs two TOCSY experiments, first with selective excitation of H-1 and then by exciting H-4. The ^{13}C-peaks are identified by GHSQC experiments, while the nature of the linkages can be derived unequivocally from GHMBC spectra optimised for ^{3}J-couplings such as existing between H-1 of one residue and C-n of the coupled residue.



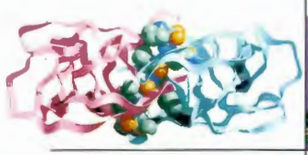


In the *Erwinia amylovora* bacteria studied, amylovoran occurs in two modifications, as a poly-hexasaccharide(in strains present on Malaceae host plants, see figure, left) and as a poly-pentasaccharide missing residue F (in strains on Rubus host plants, see figure, right). In the pentasaccharide obtained after treatment of the amylovorans with depolymerase, the presence of two β 1-6 linkages can be easily proved by an edited GHSQC. Indeed, connectivities with two separate protons are found at two different ^{13}C shifts between δ 66 and 72 ppm, the latter downfield shifted values being typical for O-substituted hydroxymethylene carbons involved in a glycosidic bond or in a pyruvate bridge. The hexasaccharide on the other hand showed similar responses but now at three different low field hydroxymethylene ^{13}C shifts. From the TOCSY patterns it was already derived that the sixth residue (F) is a β-D-glucopyranosyl moeity. In order to know to which residue it is linked, the shifts of the supplementary pair of H-6 resonances extracted from the GHSQC spectrum were carefully compared with the shifts found in the TOCSYsubspectra of units C, D and E. (Residue A is pyruvated and acetylated, and B is a glucuronic acid). Although H-1 of D and Eβ collapse so that it is difficult to distinguish their individual resonances in the TOCSY's, identical values for H-6 in the two types of spectra were found only for C, allowing to state that residue F is linked to C-6 of galactose C. Confirmation of this analysis was obtained also from a GHMBC-spectrum.

Sincerely Yours

André De Bruyn Roger Busson Martine Maes and Eric Messens

Krijgslaan 281 (S.4) 9000 GENT Telefax 09/264 49 98





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February 15, 2001 (received 2/21/2001)

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

Dear Dr. Shapiro, Re: Molecular Self-Association by NMR

In this note I would like to present 2.5 comments concerning molecular-self association probed by NMR. This is a common phenomenon that can have an important role in rationalizing pharmaceutical formulations/dosages for solutions. The NMR experiment is simple enough—just make a series of solutions with known total concentration of analyte and measure the 1-D chemical shift(s) of that compound as a function of its total concentration. In my experience the following practices, in addition to careful attention to chemical benchwork, are both useful and prudent.

0.5) Plot the NMR data using a semilog scaled abcissa. The sigmoidal transition from the limiting monomeric chemical shift at "infinite" dilution to the changing average chemical shift of the complex is more readily apparent with this representation, and one can easily see the (usually limited) segment of the total transition curve that practical solubility dictates.

1) Forget the reformulation of the fundamental equation to get straight lines on clever, re-parameterized plots. Just fit the data to the equation :

$$\delta_{obs} = F_{monomer}\delta_{monomer} + F_{dimer}\delta_{dimer} + ... + F_{n\text{-}mer}\delta_{n\text{-}mer}$$

F is the fraction of molecules in the indicated associated state, and is expressed at any given concentration in terms of stepwise self-association constants[1] and powers of the monomer concentration. For instance, for the simpliest case of dimerization

$$\delta_{obs} = \frac{b}{B}\delta_{monomer} + \frac{2K_2 b^2}{B}\delta_{dimer}$$

B is the total concentration of analyte, and b is the monomer concentration. The equation for trimers includes another association constant, chemical shift, and b to the third power. b is unknown in these equations, but it can be eliminated by solving for it in the conservation of mass relationship

$$B = b + 2K_2 b^2 + 3K_3 K_2 b^3 + ... + nK_n K_{n-1} \cdots K_2 b^n$$

For dimerization, b can be solved analytically and backsubstituted to give a closed, but messy, equation for δ_{obs}. The algebra for trimers involves cubic equations, and so on for higher complexes. A great convenience that works for all cases is to forget the algebra and let a PC do the work. I use MATLAB[2] to do the non-linear least squares fitting of the data with the routine "lsqcurvefit" in the Statistics Toolbox. This package is very nice in that it also produces confidence intervals on both the fit datapoints and the optimized parameters. But even more importantly, I can fit all resonance lines from the molecule to a common association constant simultaneously[3]. Thus for a dimer model, I fit all N datapoints per M resonance lines (N times M points) to (2M+1) parameters, rather than 3M parameters from fitting each line individually. For good datasets this process reduces the 95% confidence intervals on the obtained parameters. The same global fitting procedure also works for higher order models of association. Figure 1 shows the results of a global dimer fit to self-association data for solutions of quinine[3]. The results are in complete agreement with the values obtained by those authors using their FORTRAN calculation.

2) Having extolled the virtues of a general computational method to calculate higher order complexation data fitting, my next recommendation is to never use it – just stick with the dimer model

unless absolutely forced to invoke trimer, tetramers, etc. The reason is that no NMR dataset that I have seen covers a broad enough range of concentrations to define the sigmoidal curve well enough to justify the more complex models. Unless a large fraction of the complete curve is covered, just about any self-association model will satisfy the fit. To restate, if a dimer model fits the data well, any higher order model will also fit it well because the dimer case is a subset, and if you use more parameters to fit the same dataset, the residuals of the fit will be smaller, but that does not necessarily justify the the more complex model. This is especially the case when the added parameters have only a minor effect over the observed range of data, as is the usual case for higher order complexation.

Sincerely yours,

Paul.E.Fagerness@pharmacia.com

1. F.J.C.Rossotti and H. Rossotti, **J. Phys. Chem.**, Vol. 65, 926-930(1961).
2. MATLAB Version 12 with Statistics Toolbox Version 3, The MathWorks, Natick, MA
3. G. Uccello-Barretta, L. Di Bari, and P. Salvadori, **Magn. Reson. Chem.**, Vol 30, 1054-1063(1992).

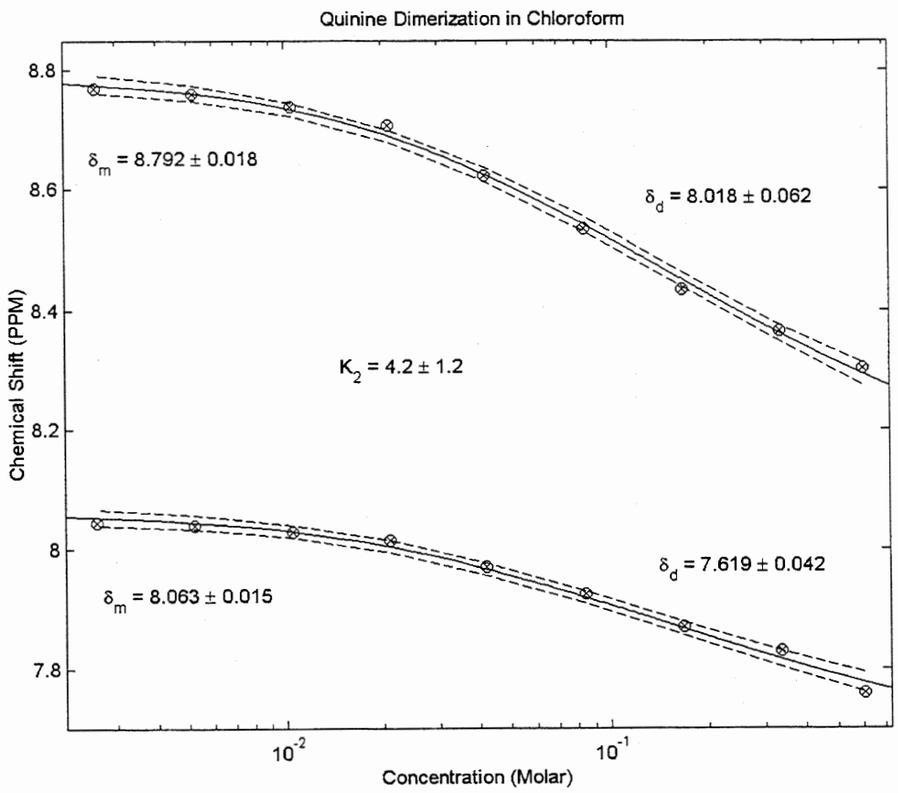

Figure 1. Data of reference 3 Table 2 fit with MATLAB. Only H-2 and H-3 chemical shifts are given. The dashed lines are 95% confidence intervals about the data values, and the error limits shown for parameters are 95% confidence intervals.

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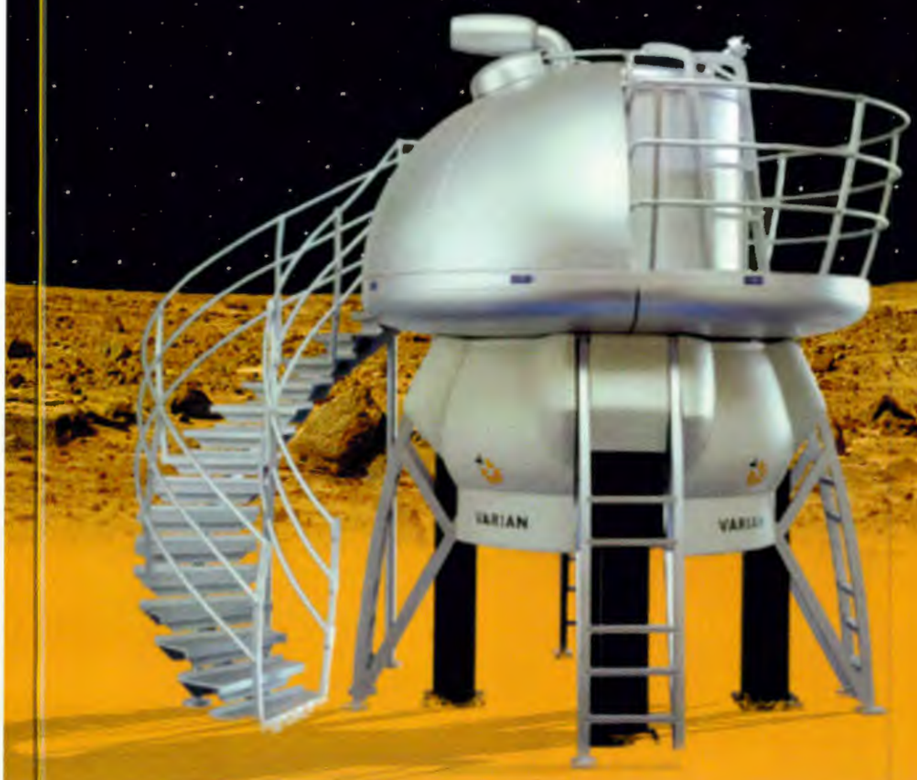

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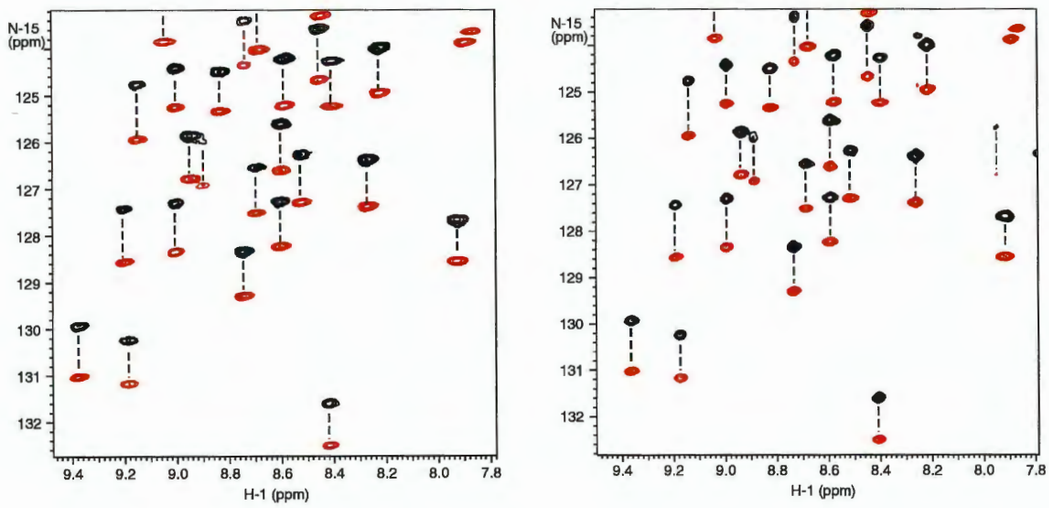
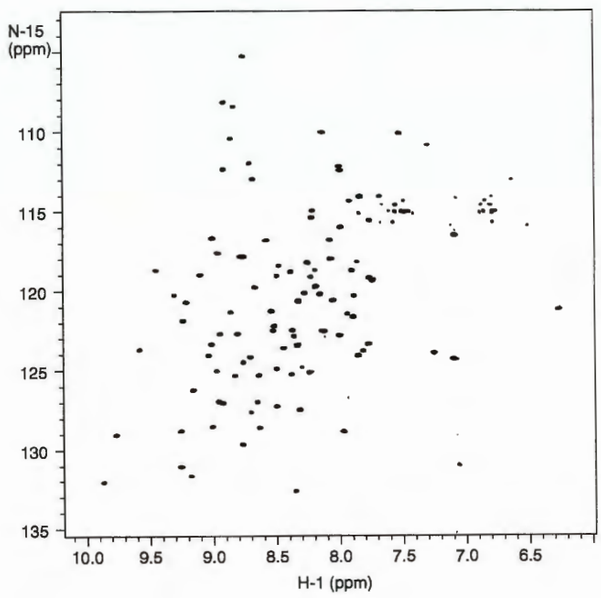


Measurement of residual dipolar N-H couplings at 900 MHz in partially oriented proteins with (right) and without the use of band-selective homonuclear decoupling.

TROSY at 900 MHz



The ^{15}N-^{1}H TROSY correlation spectrum of 6F1 1F2 module pair from the gelatin-binding domain of fibronectin. Sample courtesy of Prof. .I.D. Campbell of Oxford University.

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Alexej Jerschow and John W. Logan
Department of Chemistry, D62 Hildebrand Hall
University of California, Berkeley
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February 15, 2001
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(received 2/17/2001)

Dr. B.L. Shapiro
The NMR Newsletter
966 Elsinore Court
Palo Alto, CA 94303-3410
USA

Mixed Multiple Quantum Magic Angle Spinning Experiments

Dear Barry,

We would like to describe and demonstrate a variant of the multiple quantum magic angle spinning (MQMAS) experiment for quadrupolar nuclei. This new type of experiment maximizes the chemical shift (CS) and quadrupolar isotropic shift (QIS) scaling factors. The increase in resolution is demonstrated on a sample of aluminophosphate-41 (AlPO-41).

A resolution increase in the isotropic projection of a MQMAS experiment can be obtained by using two different [symmetric] multiple quantum coherences to refocus the 4^{th} rank quadrupolar broadening (instead of using one multiple quantum and a single quantum coherence). This type of methodology can in principle be applied to all spins $I > 3/2$, whether integer, or half-integer. We denote a general MQMAS experiment by MQNQ, which refers to M-quantum and N-quantum coherences (Figure 1 shows a schematic). Previously, 3Q1Q and 5Q1Q experiments have been used for $I = 5/2$.

The resulting spectrum is more spread out in frequency relative to the usual MQ1Q MAS experiment (Figure 2). The optimal choice of MQ and NQ coherences for a spin I is $2I$ and $2(I - 1)$, that is M = 5, and N = 3 for spin $I = 5/2$.

The spectra shown in Figure 3 were obtained using a Chemagnetics/Varian CMX Infinity 500 spectrometer. ^{27}Al NMR spectra were acquired at a Larmor frequency of 130.304 MHz. The AlPO-41 sample was in a 4 mm MAS rotor spinning at 15 kHz. A 20 kHz continuous decoupling field was applied to the protons. Extensive phase cycles were used to select the desired coherence pathway for each of the experiments. The spectra are referenced to aqueous $AlCl_3$, and are displayed on a ppm scale.

The peak labeled I in Figure 3A (5Q1Q) is further resolved in the 5Q3Q experiment in Figure 3B. Although the resolution of the peaks labeled I'', and I''' in Figure 3B do not appear well resolved, better separation can be attained by acquiring the spectrum in a different magnetic field (e.g. 9.4 T), since the QIS is field dependent. We believe that this new type of experiment might add to the usefulness of MQMAS experiments for quadrupolar nuclei.

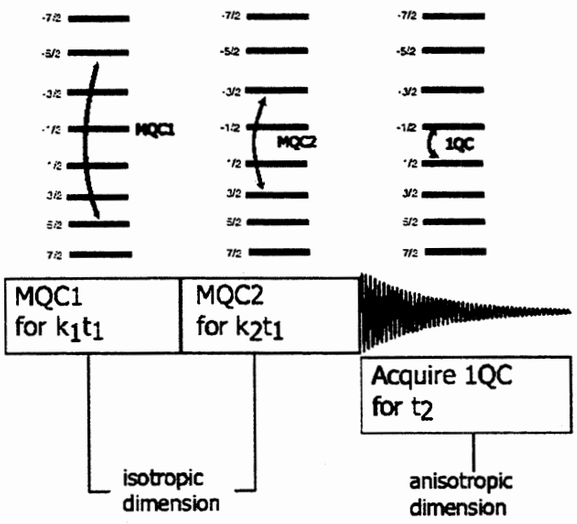

Figure 1: Schematic of a MQNQ experiment.

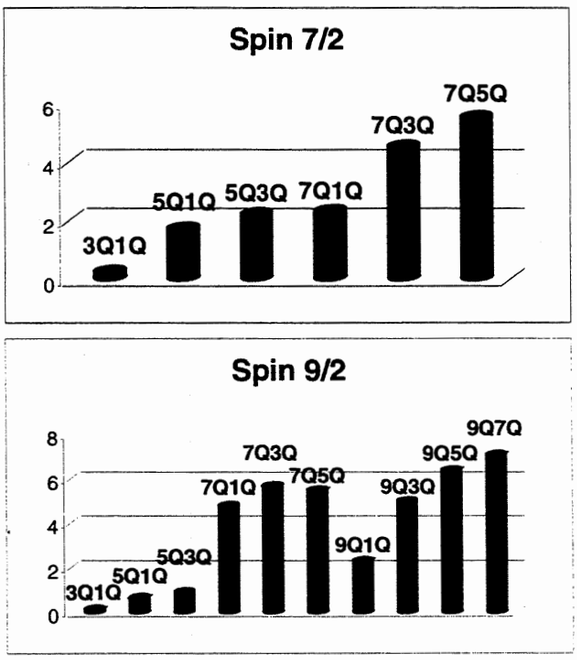

Figure 2: Chemical Shift scaling factors for different MQNQ experiments.

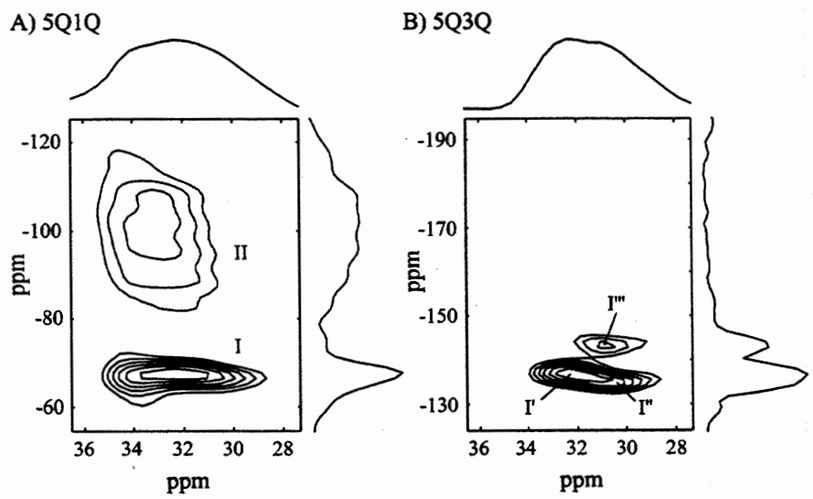

Figure 3: A) 5Q1Q and B) 5Q3Q ^{27}Al NMR spectra of AlPO-41.

Sincerely,

Alexej Jerschow

John W. Logan

P.S. Please credit this contribution to the Pinenuts account.

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at Urbana-Champaign

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mainzv@aries.scs.uiuc.edu

January 31, 2001 (received 1/31/2001)

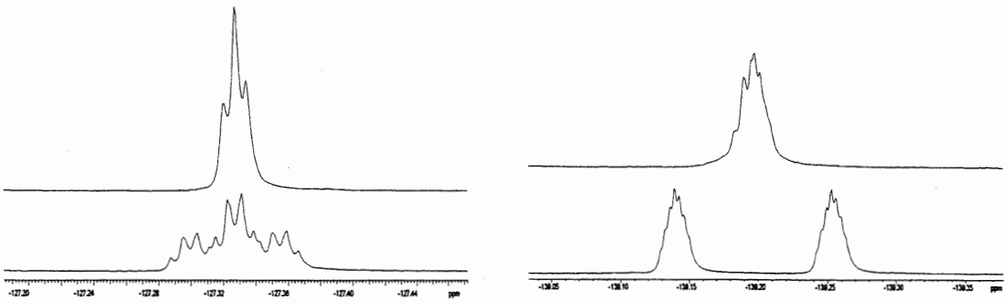
Title: ^{1}H-^{19}F Correlation Experiments Using QUAD Probes

Dear Barry,

Occasionally one of our users requires information on hydrogen/fluorine correlations. With basic selective decoupling ($^{1}H\{^{19}F\}$ or $^{19}F\{^{1}H\}$) and NOE experiments we are able to answer most of these questions. The capability of modern hardware to rapidly switch frequencies makes these experiments fairly simple if one has access to a suitable probe. In our case, we have a Nalorac QUAD probe with $^{1}H/^{19}F/^{13}C/^{31}P$ capabilities. The probe configuration of one probe input servicing both proton and fluorine frequencies allows for the manipulation of either nucleus with the use of a single transmitter. The only hurdles left are setting the hardware to deliver the correct frequencies and to observe one "high band" nucleus while decoupling another.

When we first considered a proton decoupled fluorine experiment (or vise versa) we were advised to use two transmitters, bandpass filters for both frequencies and couplers for routing the rf. Now while this approach will work there is an easier (and cheaper) method. Simply set up as if doing a "high band" homonuclear experiment. With a combined fluorine and proton probe channel the setup is straightforward and only slightly more involved than a proton/proton homonuclear decoupling experiment. It is simply a matter of setting the parameters to deliver the correct frequencies at the appropriate times. On our hardware, a Varian INOVA 500 with a Nalorac QUAD probe, the $^{19}F\{^{1}H\}$ experiment gives better results. The decoupling bandwith doesn't cover the entire proton chemical shift range so correct setting of the decoupler frequency is important.

For an $^{19}F\{^{1}H\}$ experiment on a Varian INOVA system one starts with the fluorine spectral parameters and sets dn to H1 and dof to the appropriate value determined in a separate experiment. The parameter homo is set to 'y' and dpwr is set to some reasonable value depending on the desired decoupling bandwidth. The $^{1}H\{^{19}F\}$ experiment is also possible provided the desired decoupling bandwidth is not too large. Below are fluorine spectra of 2,2,3,3-tetrafluoropropanol with and without proton decoupling.



The NOE experiment to determine the ^{19}F NOE to a specific proton is easier to visualize, as the two frequencies occur sequentially not simultaneously. With a sufficiently large offset (-29MHz on a 500MHz system) a fluorine resonance can be excited with the observe nucleus declared as hydrogen. Initial proton and fluorine spectra are required. The frequencies of these spectra can then be used to set the frequency of the saturation pulse of an NOE experiment to the desired fluorine resonance while the read pulse and acquisition frequency is set to the normal proton value. To determine the absolute enhancement, two FIDs are acquired. From the raw data, the intensities can be measured and compared or a difference spectrum created.

As the manipulation of numbers for the frequency offsets can be tedious and error prone we have created macros to calculate and set the frequencies from standard hydrogen and fluorine spectra. We have also modified Varian's cyclenoe macro to bring up suitable default values. If you are running an older version of VNMRX you need to be aware that the software may have set the limit of tof to ±100000Hz. This limit is a holdover from older hardware and acquisition software. If you are running VNMR4.3 or newer, tof is only limited by the frequency range of the spectrometer.

I hope this will be of interest to your readers. Please credit to Vera Mainz's subscription.

Sincerely,

Paul Molitor
Varian Oxford Instruments Center for Excellence in NMR Laboratory

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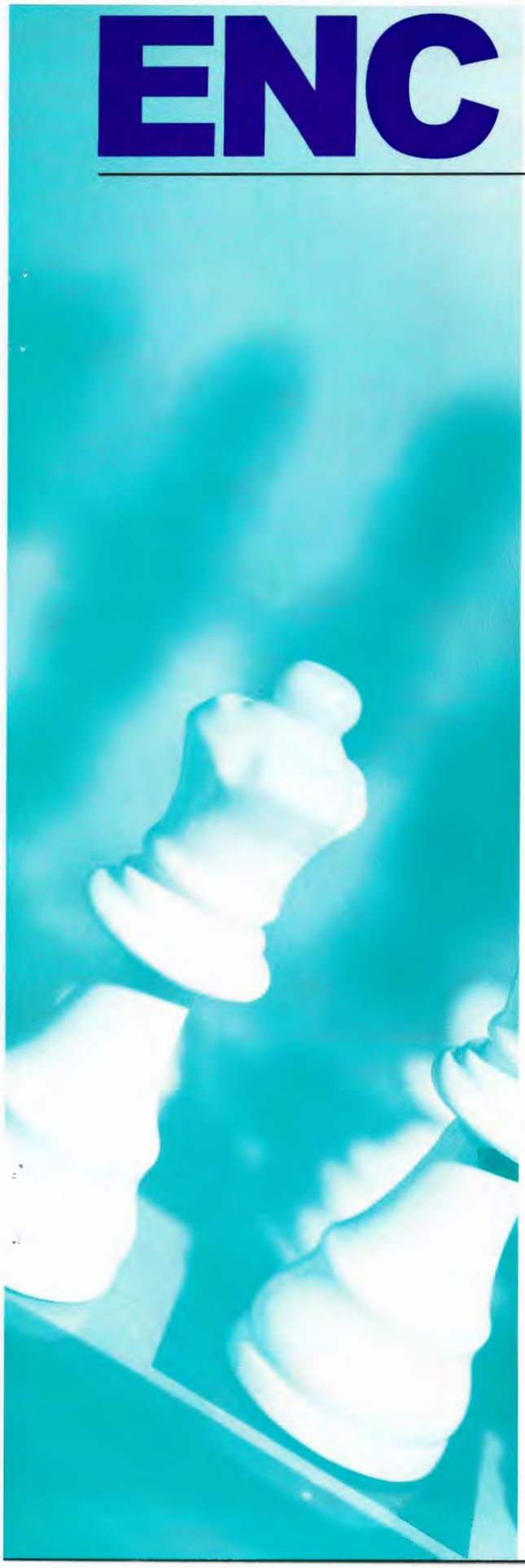

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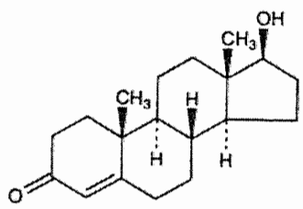
January 10, 2001 (received 2/3/2001)

Dear Barry,

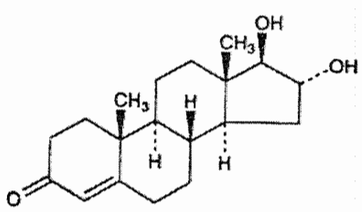
Negative NOE Signals in a Steroid

Testosterone, I, is often used as a substrate in metabolism research.



I.

The most common structural transformation is hydroxylation which can occur at almost any position. Traditionally, the site of hydroxylation has been determined by comparing the HPLC retention time with that of a standard. Because of instances of coelution, and perhaps for other reasons, several researchers have indicated that an NMR library of hyroxylated testosterones would be a very welcome additional source of structural information. Accordingly, this project has been undertaken and is progressing well. No unusual observations were encountered until we acquired NOE data on the 16α-hydroxy analog II.



II.

The initial appraisal of the NOE difference spectrum shown in A (irradiation of 18-methyl) was that we were experiencing an instrument problem. There was no reason to expect negative NOEs at 400 MHz from a molecular weight slightly above 300. Further, negative NOEs were not seen in any of the other hydroxylated analogs. Repeating this experiment doubling the delay to eight seconds resulted in a similar spectrum except for the chloroform signal which was now virtually nulled. Since the change in the solvent line was consistent with expectations, this implied that the experiment was probably working properly. The malfunction hypothesis had to be abandoned after the results shown in B and C were in hand. The appearance of positive and negative NOE signals in both experiments strongly suggested that all was well with the electronics and that the NOE findings were indeed valid.

An explanation that comes to mind is that we are seeing the effects of aggregation. Specifically, the results could be accounted for by stacking of the molecule and at least to some extent in a staggered arrangement (how else to account for an NOE signal from an A ring proton when a proton on the D ring is irradiated, and vice versa). This interpretation then led to a proposal by one of us (George Doss) that the aggregation might be disrupted by addition of a polar solvent. The prediction was confirmed experimentally. Reacquiring the 1D and 2D spectra after addition of 3-5% CD_3OD resulted in normal, i.e. positive NOE signals (D and E).

The structural requirements for the negative NOEs obviously are quite specific since these are not seen even in the 16β-hydroxy analog. It would be of interest to see what NOE experiments on the 16β-17α isomer would show.

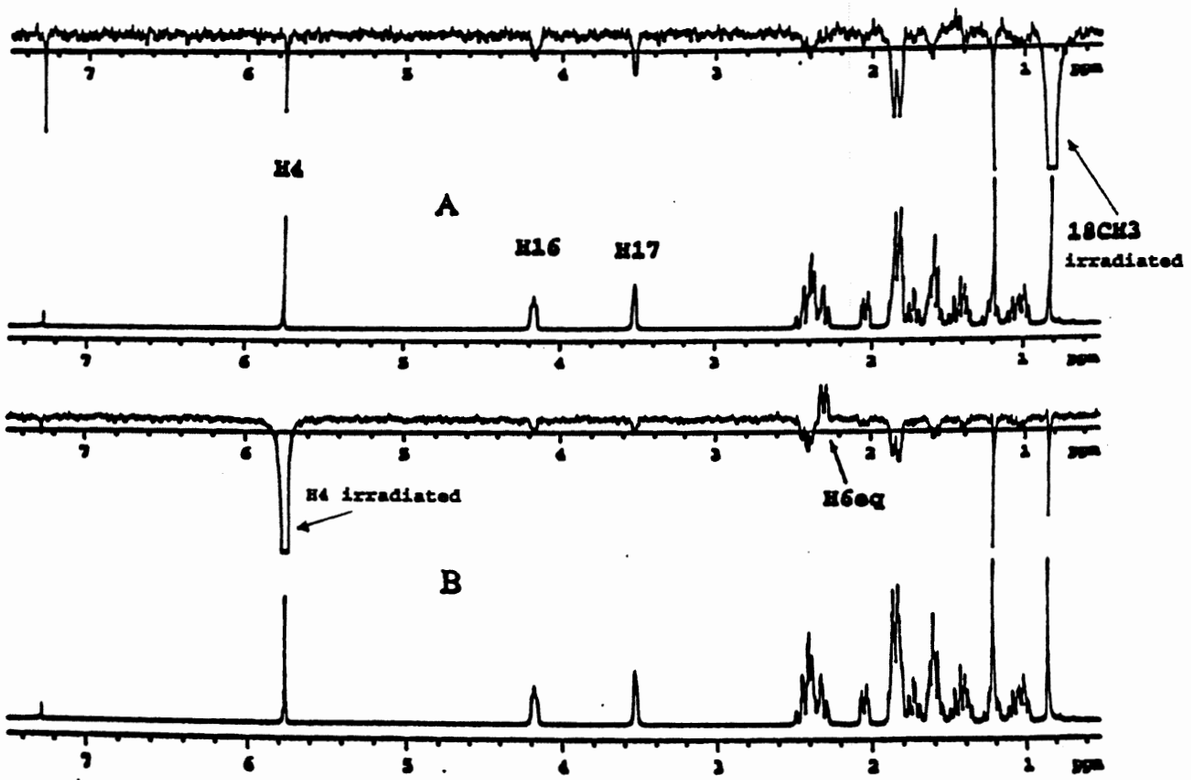
We recently came across a similar example of an anomalous NOE involving a molecular weight under 250. So perhaps this phenomenon is not as unusual as we had once thought.

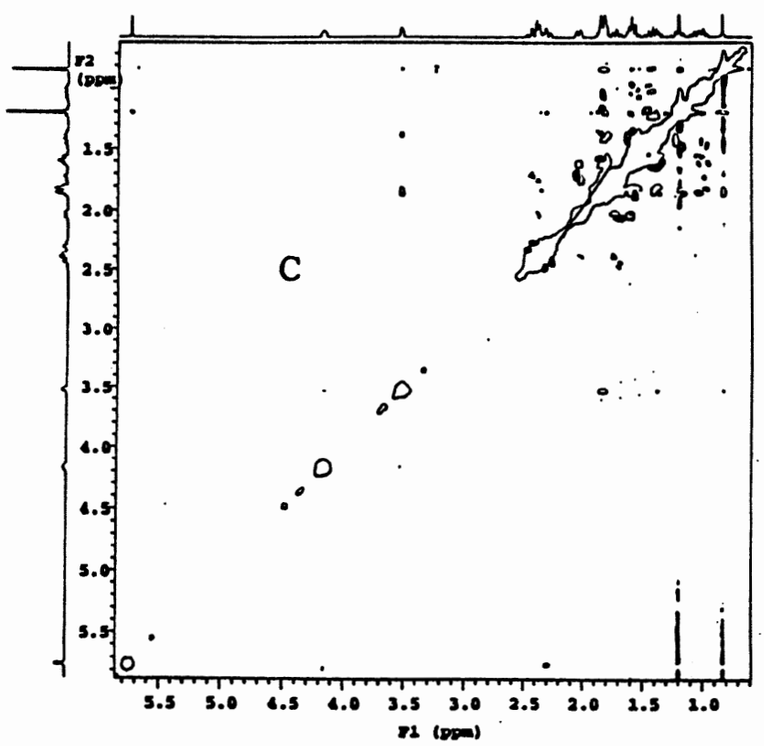
Regards,

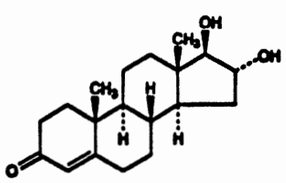
Byron

George Doss

Byron Arison George Doss


H4
A
H16
H17
18CH3
irradiated
H4 irradiated
H6eq
B
ppm


F2 (ppm)
C
F1 (ppm)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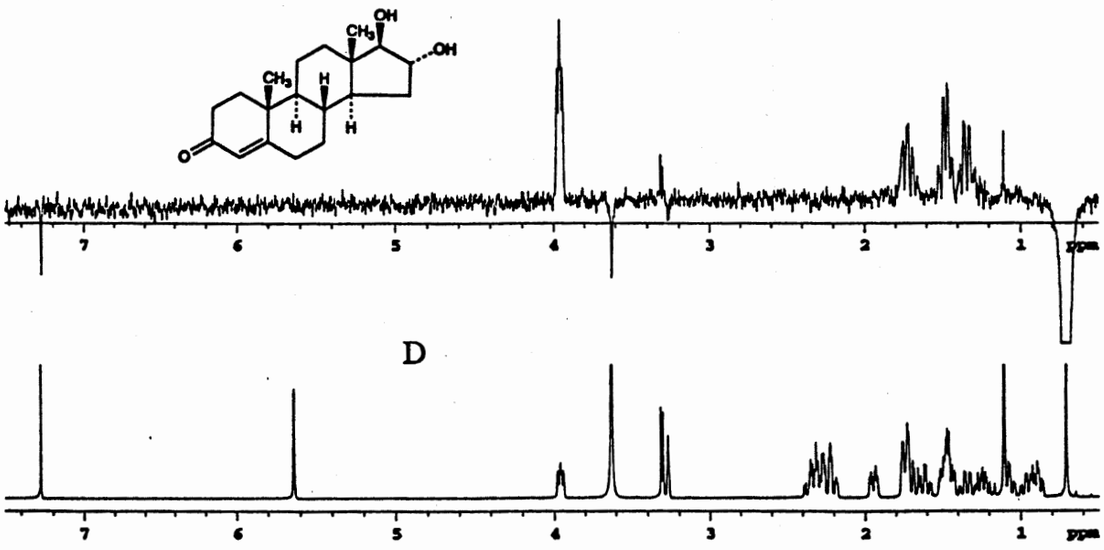
CH3
OH
CH3
H
H
H
O


OH
CH3
OH
CH3
H
H
H
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2
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ppm

D

7
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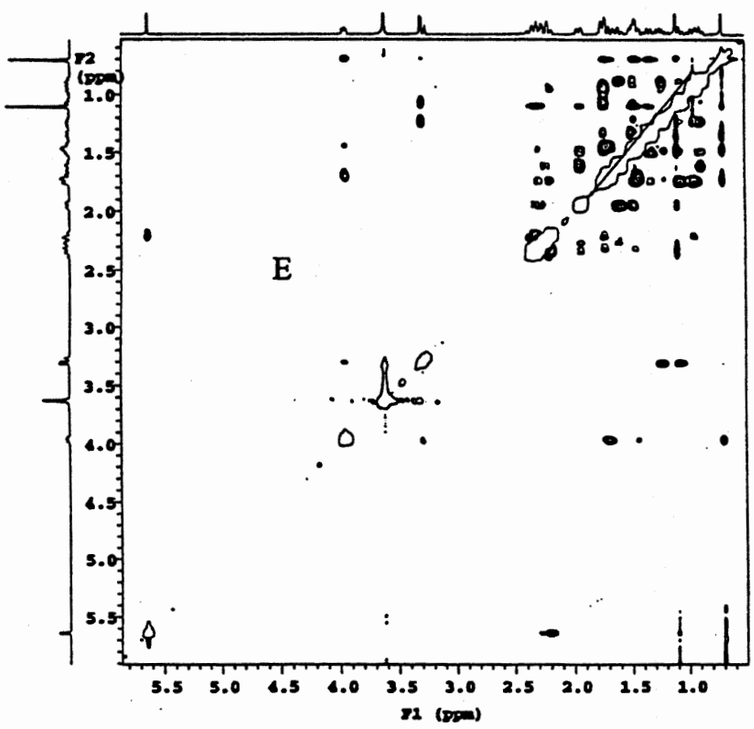
ppm


F2
(ppm)
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4.0
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5.5
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4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
F1 (ppm)

E

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January 18, 2001 (received 1/30/2001)

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

Re: Playing with heme and porphyrin

Dear Barry:

We were intrigued to learn that liver fatty acid-binding protein (LFABP) was once isolated as heme-binding protein and later reported to have higher binding affinity to heme than to long-chain fatty acids.[1] The possibility of heme binding is not obvious for a 10-strand β-clam protein structure containing 2 helices that cap the lipid entry to the β-clam cavity, though nitrophorin-1 exhibits a somewhat similar heme-binding motif. Armed with complete assignments of apo- and oleate-bound holo-LFABP, we decided to carry out some academic-style "SAR by NMR" experiments. We anticipated that the huge ring current would increase the dispersion of the proton shifts and the paramagnetic iron center would wipe out particular ^{15}N HSQC crosspeaks.

Not knowing much porphyrin and heme chemistry, and reluctant to introduce organic solvents or detergents into our lipophilic protein samples, we tried a number of way to coax hemin and protoporphyrin-IX into aqueous solution. We were delighted that both have good solubility in ammonium carbonate solution, but there were some problems with this procedure. For example, it was difficult make ferriheme from porphyrin in the presence of ammonium carbonate. The possibility that simple ammonium ion may be playing some tricks led us to finally settle on a protocol using stock solutions of hemin solution and protoporphyrin suspension, respectively, in 0.01 N NaOH. And although we might have liked to approach the 1-μM concentration of free heme in liver cells, not even the finest cryoprobe could yield HSQC data on 1 μM ^{15}N-LFABP samples!

Figure 1 shows the results of heme binding studies on a 52-μM ^{15}N-LFABP sample at pH 7 and 30°C. The proton shift range becomes narrower and the HSQC crosspeaks are rendered weaker and less distinct in general. The spectral features have changed to such an extent that most peaks can no longer be assigned by analogy with our apo- and oleate-LFABP assignments. Surprisingly in light of the affinity data cited above, the protein is restored to the oleate-bound holo form upon the addition of the fatty acid.

So far, it seems to us that LFABP's prowess in binding hemes may be overrated. There is certainly an interaction, but the binding affinity is nowhere near that of long-chain fatty acids, at least under our sample conditions. One factor that might interefere with the binding (competition) assay is the use of a Triton X-100 detergent, which may influence the partition of lipids and hemes to differing extents.

Sincerely,

Hsin Wang
NMR
Facility Manager

Ruth E. Stark
Professor of Chemistry
e-mail: stark@postbox.csi.cuny.edu

[1] J.M. Stewart, G.W. Slysz, M.A. Pritting, and U. Muller-Eberhard, *Biochem. Cell. Biol.* **74**: 249-255 (1996).

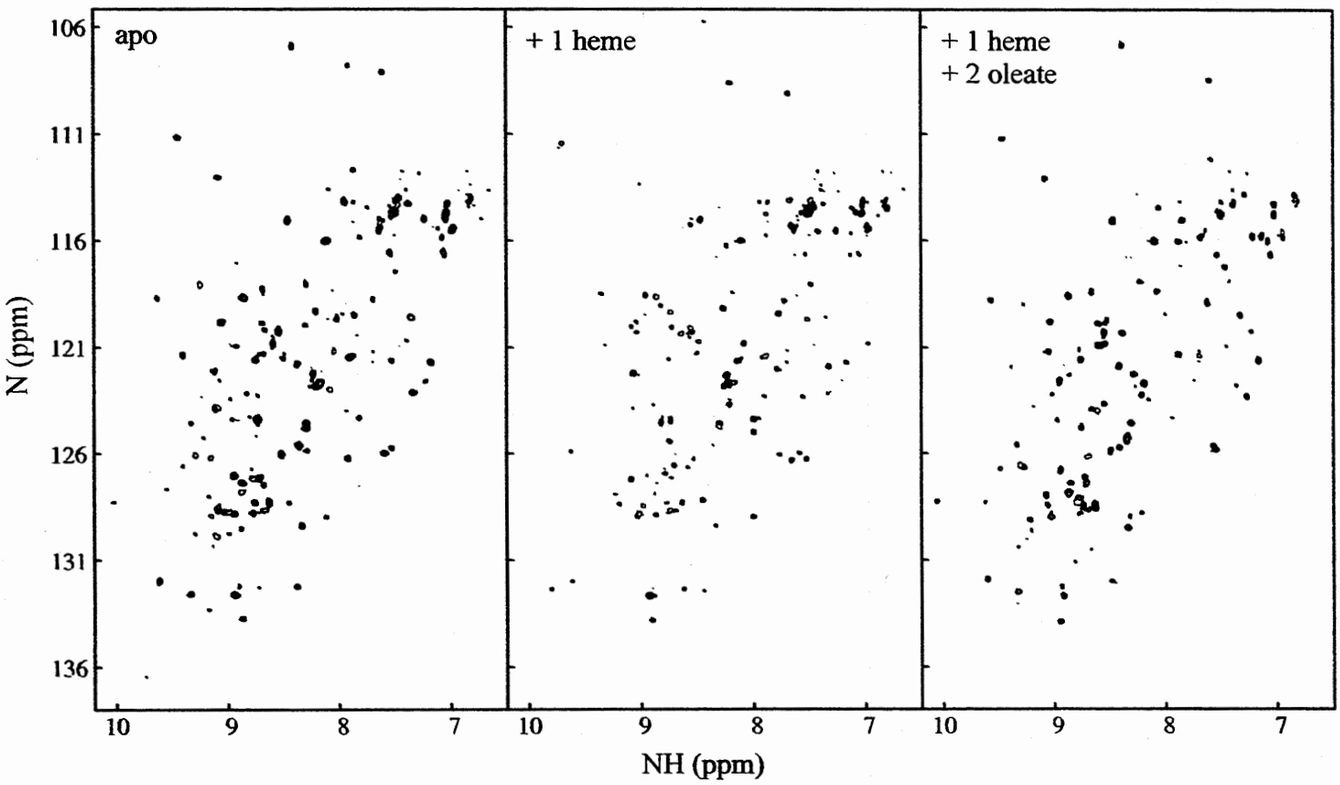

Figure 1. FHSQC spectra of a 52 μM LFABP at pH7, 30°C: (left) apo-LFABP, (center) with 1 eq. of heme, and (right) with 2 eq oleate. The spectrum on the right is nearly identical to 2eq oleate-bound holo-LFABP. Each spectrum took 22 hours to measure on our UnityInova 600 spectrometer.

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To NMR Newsletter 21.2.2001 (received 2/21/2001)

Inversion process and tautomerism in perylenequinones with helical structure : hypocrellins

Dear Barry,

Perylenequinones are a group of natural substances mostly of fungal origin, which exhibit intriguing stereochemical features and interesting biological activity, especially due to their photodynamic activity[1-10]. Among them, the hypocrellins, compounds extracted from the Chinese fungi *Hypocrella bambusae* and *Shiraia bambusicola* have been intensively investigated for their light-induced antitumor and antiviral activity. In particular, the energetic and dynamic properties of their electronic excited states have been the subject of numerous studies[7-9].

Hypocrellins share with many other natural perylenequinones an axial chirality due to the steric hindrance of the substituent groups, that leads to atropisomerism. The existence of stereogenic centers in the seven-membered ring, combined with the axial chirality, generates diastereoisomerism. In fact, other perylenequinones, such as cercosporin or phleichrome, undergo a thermal interconversion of the atropo/diastereoisomers at elevated temperatures, due to the high barrier of interconversion[1,6]. Furthermore, the 3,10-dihydroxy-4,9-perylenequinone system can exist in two main tautomeric forms, that we called tautomer I and tautomer II in fast equilibrium[5,6].

As a recent paper[7] reported the existence of a slow tautomeric equilibrium for hypocrellin A, we reinvestigated by NMR the behaviour of hypocrellins in solution, and report here the results of our investigation, that correct some of the results of Petrich et al.[7] and require a revision of the structure of shiraiachrome A.

The complete paper, as a part of our ongoing studies on stereochemistry and tautomerism of natural perylenequinones, will be published in *J. Chem. Soc. Perkin* 2, 2001.

The materials that we have investigated are a sample of hypocrellin (**1**) kindly provided years ago by Professor Breitmaier, and a sample of hypocrellin A (**2**), purchased from Molecular Probes Inc., that is the same source of the material examined by Petrich et al.[7]

Hypocrellin A (**2**) is the enantiomer of the original hypocrellin (**1**) isolated from *Hypocrella bambusae*, which presents axial chirality *M* (*R*) and configuration 14*R* , 16*S* [10] . We performed NMR experiments on both compounds **1** and **2** and the NMR data resulted identical, but the spectrum of the Molecular Probes sample showed the presence of four compounds (see Fig. 1). The signals labelled with **3** belong to the anhydroderivative formed by loss of water from C-14 and C-16, the signals labelled with **4** belong to another stereoisomer (shiraiachrome A), and finally the signals labelled with **5** and **6** belong to the atropisomers of **2** and **1** respectively, and are in dynamic exchange with each partner. The helix inversion process for these compounds is slow enough to be studied by NOESY-exchange NMR. The interconversion rate constants (k) of **1** into the atropisomer **6** were directly determined by quantification of the signal intensities of diagonal and cross peaks of the ROESY spectra , performed at different mixing times and temperatures. The k values, resulting by fitting of the experimental data in the appropriate equations, gave the activation parameters $\Delta G^{\#}$ = 65.5-66.6 kJ mol^{-1}, $\Delta H^{\#}$ = 76 kJ mol^{-1} and $\Delta S^{\#}$ = 37 J mol^{-1} K^{-1}. The barrier of the helix inversion process is lower with respect to other perylenequinones such as cercosporin and phleichrome,[2,3] because the presence of the seven-membered ring instead of the two chains reduces the steric hindrance and the conformational mobility. Although $\Delta G^{\#}$ and $\Delta H^{\#}$ are high enough, the entropy change, $\Delta S^{\#}$, is positive, thus favouring the inversion process.

The helix inversion also induces the inversion of the seven-membered ring, which adopts a twist-boat conformation in both atropisomers. The values of the dihedral angle C(1)-C(12b)-C(12a)-C(12), which shows the distortion of the perylenequinone system, have been obtained from energy minimisation of the structures derived from NOE data. They are in the range 25-29° for all stereoisomers.

It has been reported[7] that three significantly populated species of hypocrellin in the ground state are involved in an interconversion process. The three species have been identified as hypocrellin in the tautomeric states I and II, and its atropisomer in the tautomeric state I. This analysis implies that the tautomeric process is slow with respect to the NMR time scale. The involvement of the third species in the dynamic equilibrium was based on the wrong assignment of some resonances in the regions 2.5-6 ppm and 16-16.5 ppm, in part due to the impurities of the sample used. Actually some signals of the stereoisomer **4** (shiraiachrome A) have been attributed in Petrich's paper to a tautomeric species of hypocrellin in slow exchange.

The structure of **4** was then determined by accurate NMR experiments directly performed on the Molecular Probes sample, which contains as an impurity 14% of **4** . The most evident feature of the spectrum of **4** is the four-bond coupling of 2 Hz between one proton at C-13 and OH-14, which allowed together with the NOE results to derive the conformation of the seven-membered ring and the relative configuration. The similar Cotton effects of shiraiachrome A and hypocrellin **1** (known) showed that they must have the same axial chirality, thus the structure of shiraiachrome A (**4**) has been revised to *M(R), 14S, 16S.*

The lowfield portion of the NMR spectrum is reported in Fig.2 . All the phenolic OH signals were assigned by NOE experiments; the signals of 3,4-OH vs 9,10-OH in hypocrellin **1** was also attributed, as they show NOEs with 2-OMe and 11-OMe respectively. The signal at 16.06 ppm has been assigned in Petrich's paper to the third species in slow tautomeric exchange. Actually, the signal at 16.06 ppm, together with that at 16.15 ppm (partially hidden) belong to the two monodeuterated species (d_1) at the phenolic OH of hypocrellin. The deuteration process occurs slowly in acetone-d_6 solution, via the enol-tautomer of the solvent[6] (Fig 2a,b), but it can be improved by addition of D_2O (Fig. 2c).

These results confirm that there is no third species in dynamic equilibrium with hypocrellin **1** and its atropisomer **5** and that the tautomeric process is too fast to give rise to different signals of the two tautomers in the NMR spectrum.

The population of each tautomer can be obtained from the coupling constants between the proton of the phenolic OH groups and the adjacent carbon atoms, i.e. J(C3,OH) and J(C4,OH)[5], which in the specific case of **1** are 2.7 and 3.1 Hz resp. This corresponds to a 50% relative population for each tautomer. For cercosporin, the first known perylenequinone which exists in the tautomeric form I, J(C4,OH) is 5.2 Hz and J(C3,OH) is zero.[5] Finally, the very similar chemical shift values (6.4-6.5 ppm) found for compounds **1** - **6** show that the populations of the two tautomers do not change as a consequence of the helix inversion process.

1. U. Weiss, L. Merlini, and G. Nasini, *Prog. Chem. Org. Nat. Prod.* 1987, **52,** 1.
2. L. Merlini,G. Nasini, G.D. Andreetti, G. Bocelli and P. Sgarabotto, *Tetrahedron*, 1982, **38**, 2787.
3. A. Arnone, L. Camarda, L. Merlini and G. Nasini, *J. Chem. Soc., Perkin Trans. 1*, 1985, 1387.
4. A. Arnone, L. Merlini, R. Mondelli, G. Nasini, E. Ragg and L. Scaglioni, *Gazz. Chim. Ital.*, 1993, **123**,131.
5. A. Arnone, L.Merlini, R. Mondelli, G. Nasini, E. Ragg, L. Scaglioni and U. Weiss, *J. Chem. Soc. Trans. 2,* 1993, 1447.
6. S.Mazzini, L.Merlini, R.Mondelli, G.Nasini, E.Ragg and L.Scaglioni. *J. Chem. Soc. Trans. 2,* 1997, 2013.
7. A.Sirnov, D.H.Fulton, A.Andreotti and J.W.Petrich, *J.Am.Chem.Soc.,* 1999, **121,** 7979.
8. K.Das, K.D.Ashby, J.Wen and J.W. Petrich, *J. Phys.Chem.,* 1999, **103,** 1581 and 7949.
9. A. Datta, A.V. Smirnov, J. Wen, G. Churmanov and J.W. Petrich, *Photochem. Photobiol.* 2000, **71**,166.
10. W.S. Chen, I.-T.Chen, X.-Y. Wang, E. Friedrichs, H. Puff and E. Breitmaier, *Liebigs Ann Chem.,* 1981, 1880.

Sincerely yours

Rosanna Leonardo Scaglioni Stefania Mazzini

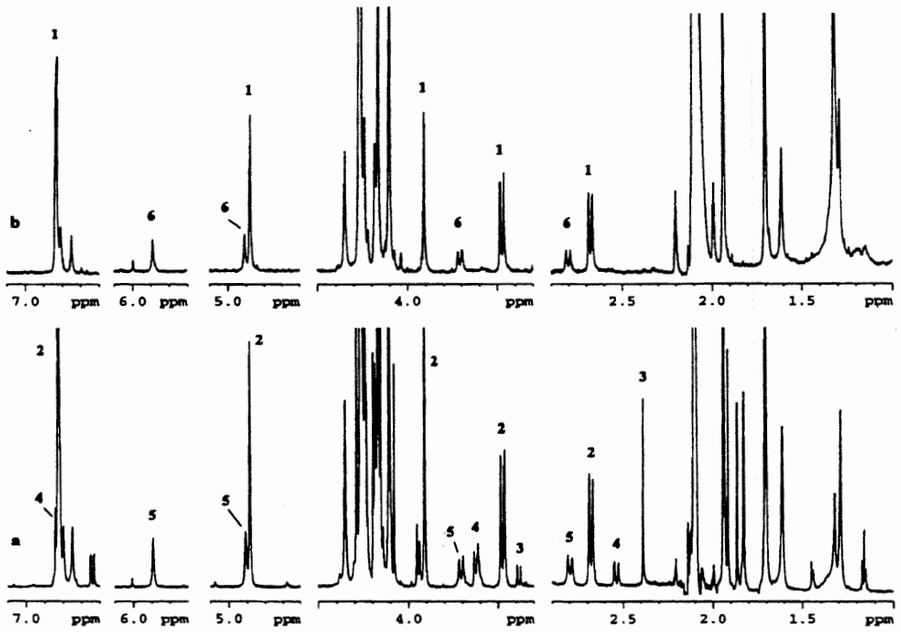


Fig.1 ^{1}H NMR spectra in [^{2}H]$_6$ acetone, at +5 °C of (a) the Molecular Probes sample and (b) hypocrellin **1** and the atropisomer **6**.

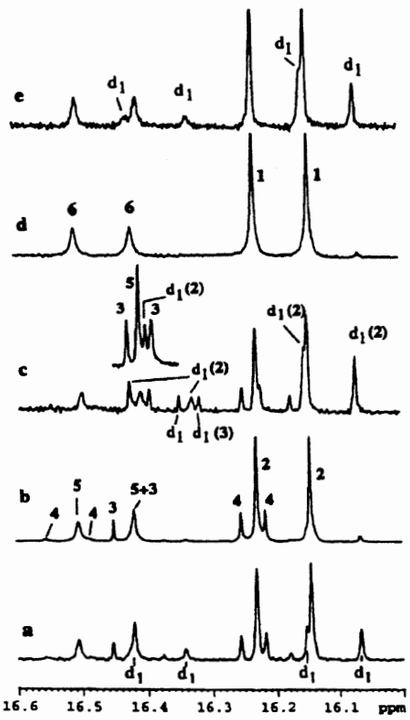
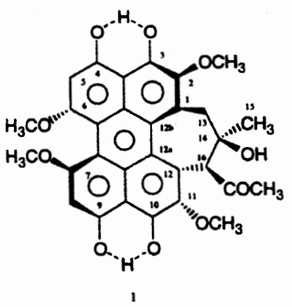
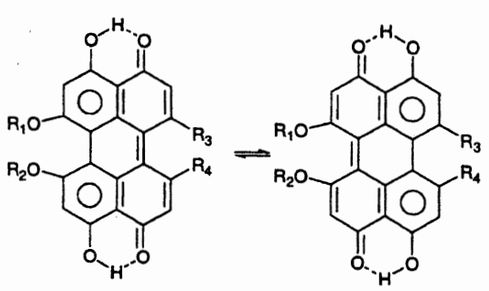


Fig.2 NMR spectra (phenolic OH protons region) in [^{2}H]$_6$ acetone at +5°C of: (a ,b, c) Molecular Probes sample; (a) 1D spectrum measured on a two-week old solution; (b) spectrum of a fresh solution; (c) the same after addition of D_2O; in the insert, the spectrum measured at -15°C; (d, e) hypocrellin **1**; (d) fresh solution; (e) after addition of D_2O.



1

1 *M*(R) ,14*R*,16*S*
2 *P*(S),14*S*,16*R*
4 *M*(R),14*S*,16*S*
5 *M*(R),14*S*,16*R*
6 *P*(S) ,14*R*,16*S*



I II

NEW MEXICO RESONANCE
A nonprofit research corporation

January 18, 2001 (received 2/10/2001)

Singing the praise of ancient test equipment for low frequency probe design and construction.

Dear Barry,

I point out a handy and economical device that dates back half a century: Boonton Q-meter, Model 160-A. It will measure coil inductance between 0.1μH and 100mH and the Q of the coil at frequencies up to 50 MHz. We use it in our lab for NMR probe construction but it quit working recently; no meter deflection no matter what. I removed the three tubes and took them to the local electronic surplus store but their tube checker was not working. Before leaving the store, I noticed another 106-A, identical to ours, on the shelf so I bought it for $50, thinking that was cheaper than the cost of three new tubes. It needed a new power cord and the cobwebs had to be removed but otherwise was in working order. Our old unit now works, also, after we cleaned the switch contacts. The main ones to clean are those that change the frequency bands and are on a large drum. We used an ordinary contact cleaner spray from electronic supply stores as well as quite a bit of wrist muscle; we also lubricated the bushings so the drum would turn more easily. We repaired a disconnected shield to the tuning capacitor assembly; this has solved a leakage current problem that caused a tingling sensation when we touched the case, not to mention unsteady readings.

A manual for this device can be purchased from Tucker Electronics or manual specialists like ManualMan (www.manualman.com) or A. G. Tannenbaum (www.agtannenbaum.com). There are other similar devices, Boonton Models 100-A, 170-A, and 260-A, but they are less common than the 160-A. Around 1970, Yokogawa Electric made a solid state equivalent of this Q-meter and HP still sells it (Model 4342A) but is quite expensive.

We use the Q-meter in conjunction with an equally old grid-dip meter (Megacycle Meter, Model 59) and a HP vector impedance meter, Model 4815A. [I bought a 4815A in working condition for $129.95 at the same surplus store some time back. Unfortunately, I have not been able to find a probe for it but it functions well as a spare.] For those of us doing fairly low frequency magnetic resonance, this is a great combination. We wind the coil and check its inductance and Q with the Q-meter. [Even if the coil was for a frequency higher than the 50 MHz limit, the instrument is still useful for comparison of coils. We use it with our 80.3 MHz coils all the time. By the way, the 4815A goes up to 110 MHz and the digital version (HP 4193A) does not go any higher.] Then we make a resonant circuit and make a quick determination of its resonance frequency with the grid-dip meter. The impedance can be adjusted with the vector-impedance meter to get it close to the desired value (e.g., 50 ohms) before the usual magic-T or quad-hybrid can be used.

Of course there are other ways to make these measurements. A standing wave bridge is a versatile instrument that is usually geared specifically to 50 ohm circuits. Solid state dip meters (e.g., from MFJ or antique Heathkit) work well, too, but the tube version (Model 59) has more output; it also comes in handy as a remote source of unmodulated "NMR" signal in the event that we need to go to an altogether new frequency, i.e., new nuclei, with a new coil. The ultimate but expensive solution is to buy a network analyzer but that is not really necessary at the lower frequencies.

Finally, the Q is not the end-all for NMR coils but is a good indication of how well a coil will work for NMR if other parameters stay the same. Chen and Hoult's *Biomedical Magnetic Resonance Technology* (Adam Hilger, 1989) has more details.

Sincerely,

Eiichi Fukushima

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Jan 19, 2001

(received 1/30/2001)

Dr. B.L. Shapiro
The NMR Newsletter
966 Elsinore Court
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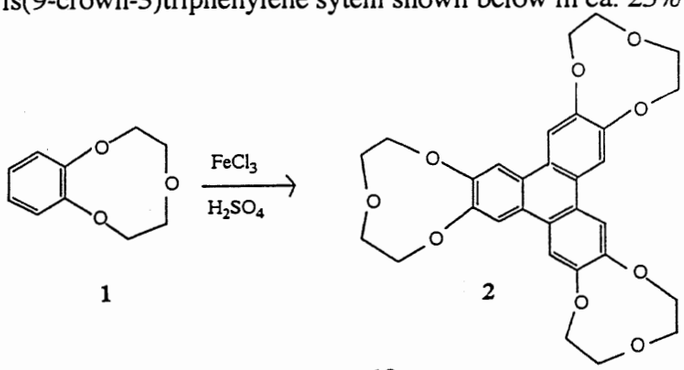
94303 USA

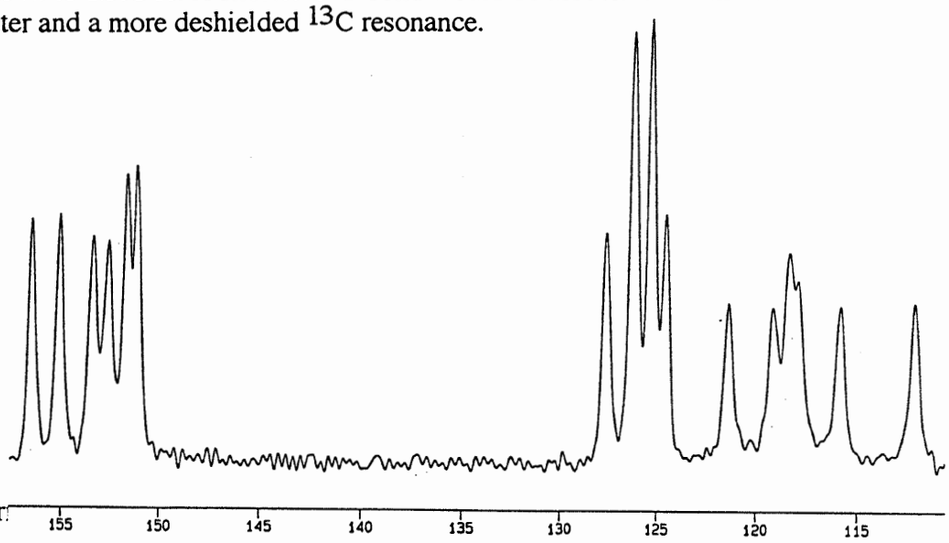
Title: Solid State ^{13}C NMR of tris(9-crown-3)triphenylene

Recently we have begun a program involving oxidation trimerization of a series of benz-annelated crown ethers to give tris(crown)triphenylene systems. These may be of interest as hexaalkoxy triphenylenes self assemble to make discotic liquid crystals. Our first foray into this area produced the tris(9-crown-3)triphenylene sytem shown below in ca. 25% yield.



1 2

Due to the 3-fold symmetry of this molecule, the ^{13}C NMR in solution shows only 5 resonances. However, in the solid state there is no symmetry (as found by X-ray crystallography) and the ^{13}C CPMAS spectrum of the aromatic region is shown below. All six aromatic C-O resonances are resolved in this beautiful spectrum. The range of chemical shifts in this region is 5.4 ppm. It is tempting to attempt a correlation with the X-ray derived aryl C-O bond lengths, which vary from 1.375 to 1.395 Å. A shorter C-O bond would be associated with increased double bond character and a more deshielded ^{13}C resonance.



G.W. Buchanan
Professor and Chair

Forthcoming NMR Meetings, continued from page 1:

13C Symposium and Training IX: Hepatic Gluconeogenesis, University of Texas Southwestern Medical Center, Mary Nell and Ralph B. Rogers Magnetic Resonance Center, Dallas, Texas, Thursday, **May 10, 2001**. Contact Janet Thach at janet.thach@utsouthwestern.edu. Please check our web page at http://www2.swmed.edu/rogersmr at a later date for details.

Gordon Research Conference on Magnetic Resonance, **June 17-22, 2001**, Roger Williams University, Bristol, Rhode Island (note the new, improved location !!!). Contacts: Rob Tycko, Chair, 301-402-8272, tycko@helix.nih.gov, and Kurt Zilm, Vice-Chair, kurt.zilm@yale.edu. Site description and application information available at http://www.grc.uri.edu.

IXth International Symposium on Magnetic Resonance in Colloid and Interface Science, St. Petersburg, Russia, **June 26-30, 2001**. Contact: Mrs. L. Ya. Startseva, Secretariat of ISMRCIS, Boreskov Institute of Catalysis, 5, Prosp. Akad. Lavrentieva, Novosibirsk, 630090, Russia. Tel: +7 (3832) 34-12-97; Fax: +7 (3832) 34-30-56; E-mail: star@catalysis.nsk.su.

Royal Society of Chemistry: 15th International Meeting on NMR Spectroscopy, Durham, England, **July 8-12, 2001**; Contact: Mrs. Paula Whelan, The Royal Society of Chemistry, Burlington House, London W1J 0BA, England; tel: +44 (0) 207-437-8656; fax: +44 (0) 207-734-1227; Email: conferences@rsc.org\; Use the subject header '01NMR15'

ESR and Solid State NMR in High Magnetic Fields, Stuttgart, Germany, **July 22-26, 2001**. Contact: Prof. Hans Paus, 2 Physikalisches Institut, Universität Stuttgart, Pfaffenwaldring 57, D-70550 Stuttgart, Germany. Tel: ++49-711-685-5223 or -5217; Fax: ++40-711-685-5285; E-mail: ampere2001@physik.uni-stuttgart.de.

43rd Rocky Mountain Conference on Analytical Chemistry, Denver, CO, **July 29 – August 2, 2001**; www.milestoneshows.com/rmcac

ISMAR 2001, Jerusalem, Israel, **August 19-24, 2001**; See http://www.tau.ac.il/chemistry/ISMAR.html.

14th European Symposium on Polymer Spectroscopy, Dresden, Germany, **September 2-5, 2001**. Contact: Institut für Polymerforschung Dresden e. V., ESOPS 14, Postfach 12 04 11, 01005 Dresden, Germany. Tel: +49 351 4658-282; Fax: +49 351-4658-214; E-mail: espos@ipfdd.de.

Sixth International Conference on Magnetic Resonance Microscopy, Nottingham, UK, **September 2-5, 2001**. http://www.magres.nottingham.ac.uk/conferences/2001/icmrm/

Fourth International Conference on Molecular Structural Biology, Vienna, Austria, **September 5-9, 2001**. Contact: Andreas Kungl, Austrian Chemical Society (GÖCH), Biochemistry Subgroup, c/o Institute of Pharmaceutical Chemistry, Univeristy of Graz, Universitätsplatz 1, A-8010 Graz, Austria. Tel: +43 316 380 5373; Fax: +43 316 382541; E-mail: andreas.kungl@kfunigraz.ac.at.

2nd Alpine Conference on Solid-State NMR, Chamonix-Mont Blanc, France, **September 9-13, 2001**; Contact: Alpine Conference Secretariat, Laboratoire STIM, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon Cedex 7, France; alpine.SSNMR@ens-lyon.fr; Tel. +33-(0)4 72-72-84-86/ 83 84; Fax. +33 (0)4 72 72 84 83; http://ens-lyon.fr/STIM/alpineweb,html

XXth International Conference on Magnetic Resonance in Biological Systems, Toronto, Ont., **August 25-30, 2002**. For further information check www.uwo.ca/chem/icmrbs/, or contact: mgordon@julian.uwo.ca

Additional listings of meetings, etc., are invited.

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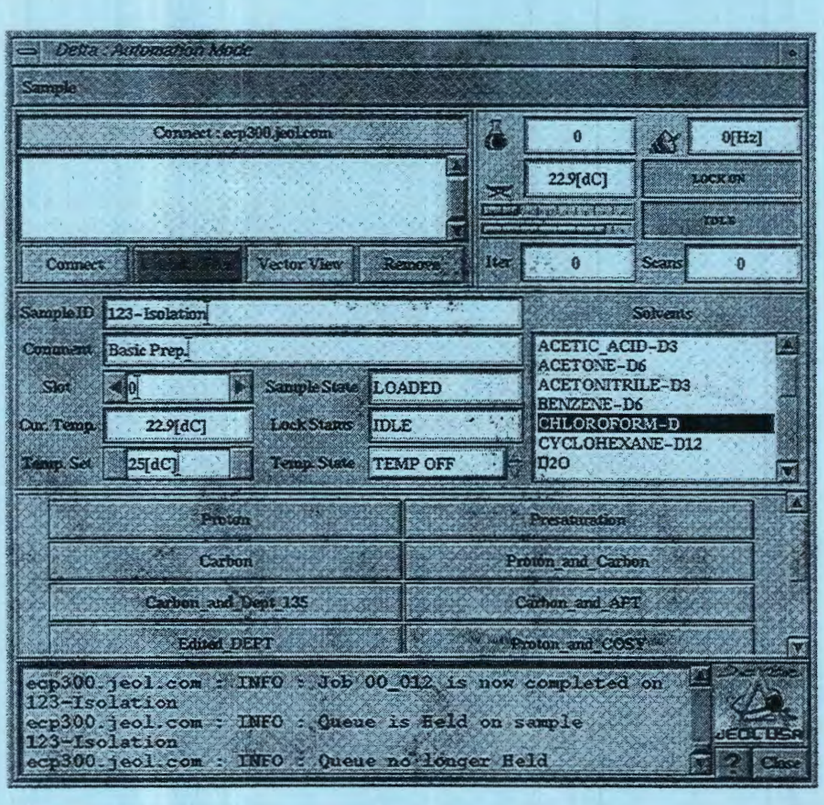

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