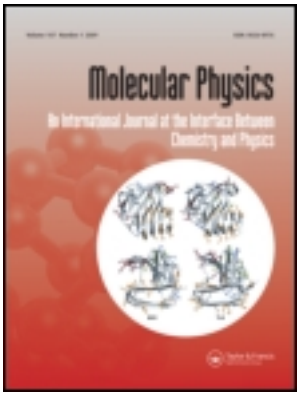

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Two-dimensional multiple quantum N.M.R. of isotopic mixtures in liquid crystals

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Two-dimensional multiple quantum N.M.R. of isotopic mixtures in liquid crystals

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A technique is presented for the experimental determination and assignment of dipolar coupling constants from the N.M.R. spectra of molecules dissolved in liquid crystals. The technique is applicable to larger molecules than have been studied to date. Random deuteration of the molecule results in a statistical mixture of partially protonated species which can be analysed by two-dimensional and multiple quantum N.M.R. techniques to determine and assign all the dipole coupling constants. Benzene is used as an illustrative example.

1. INTRODUCTION

Proton N.M.R. of molecules dissolved in liquid crystal solvents has been a useful means of determining averaged magnetic dipole coupling constants from which structural parameters can often be inferred [1]. Beyond a certain size and lack of symmetry, typically more than eight to ten hydrogens on an acyclic molecule, normal Fourier transform N.M.R. is not practical since the spectrum becomes intractably complex. For example, the proton N.M.R. spectrum of oriented benzene displays seventy six lines [2], whereas that of oriented hexane, illustrative of a class of flexible molecules whose conformational structure and motions are of interest, contains tens of thousands of transitions.

Two approaches to solving this problem can be envisaged. The first involves specific labelling with a few hydrogens (most simply pairs) in an otherwise perdeuterated molecule [3]. The advantages of this method are that the spectra and magnetic dipole coupling constants of these individual isotopomers are simple to analyse and assign. The disadvantage is that this approach can be synthetically quite demanding. The second approach, using the normal protonated molecule, involves exciting and detecting high n-quantum N.M.R. transitions. These are known to become simple and directly analysable as n approaches the number of spins [4]. An obvious advantage of this method is that no isotopic labelling is necessary, but, on the other hand, it is extremely difficult to obtain high n-quantum transitions (e.g. $n = 12$ in hexane) with practically useful sensitivity.

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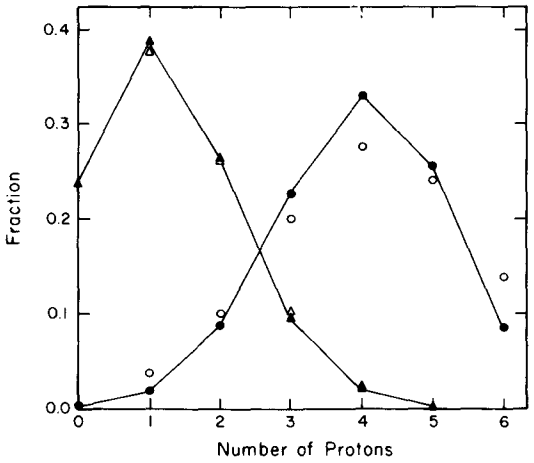

Figure 1. Mass spectral data from 33·9 per cent (0) and 78·7 per cent (Δ) randomly deuterated benzene. Open symbols are the fractions obtained from the measured relative intensities at masses 84 (0 protons) through 78 (6 protons). Solid symbols show the calculated statistical distributions.

Thus the first approach can be characterized as requiring difficult synthesis but straightforward N.M.R. whereas the second is easy on sample preparation but requires complex N.M.R. techniques.

In this paper, we describe a model study which incorporates the advantageous features of the above approaches. The idea is to deuterate randomly the compound to a certain level, yielding a statistical mixture of partially protonated molecules. The N.M.R. spectrum of this mixture will be extremely complicated, but if the spectra of the individual isotopomer constituents in the mixture can be separated out, then they may be simply analysed. This can be achieved by two-dimensional and multiple-quantum N.M.R. techniques. Two-dimensional N.M.R. allows correlation of lines arising from the same isotopomer and multiple quantum N.M.R. allows a classification of these isotopomers according to their number of spins and topology. The method is synthetically less demanding than specific labelling and the N.M.R. is easy if the level of protonation is such that n is not too high. As an example, randomly deuterated benzene is used, since its N.M.R. spectra in liquid crystals are well understood and it serves as an excellent prototype for this study.

2. Experimental

Randomly deuterated benzene was prepared by room temperature exchange of benzene with 85 per cent sulphuric acid in D_2O. A 33 per cent deuterated sample was selected from the reaction of C_6H_6 with D_2SO_4, and an 80 per cent deuterated sample was made from C_6D_6 with H_2SO_4. The reaction time was approximately 40 hours. Figure 1 shows the mass spectral analysis of each sample, together with the calculated curves for random deuteration based on the statistical equation

$$P(m) = d^{n-m}(1-d)^{m\,n}C_m,$$

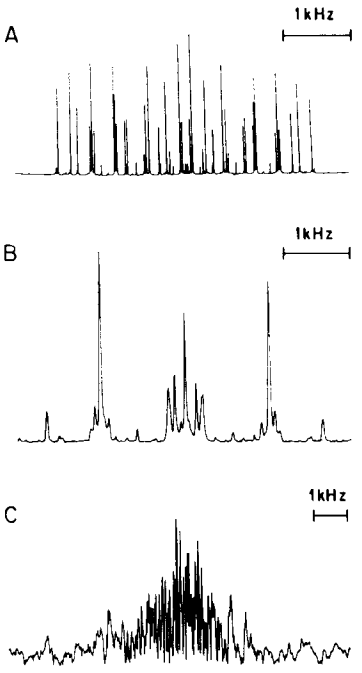

Figure 2. Proton magnetic resonance spectra of (*a*) benzene, (*b*) 78·7 per cent randomly deuterated benzene and (*c*) n-hexane in the nematic liquid crystal EK 11650.

where $P(m)$ is the probability of finding m protons, d is the fraction of deuteration and n is the total number of protons and deuterons in the molecule.

All samples were prepared by dissolving approximately 15 mole per cent of the appropriate deuterated benzene in the nematic liquid crystal EK 11650 (p-pentylphenyl-2-chloro-4-(pentylbenzyloxy)-benzoate) and were sealed in 5 mm N.M.R. tubes. The experiments were performed on a home-built spectrometer operating at 362 MHz for protons and interfaced to a VAX 11/730 minicomputer by a parallel MDB-DCHIB/DR11W data channel interface. Two dimensional data sets, up to 1 K × 4 K in size were recorded directly on the VAX for processing.

3. Dipole coupling constants

Figure 2 shows the spectrum of (*a*) benzene and (*b*) 80 per cent randomly deuterated benzene. (*b*) is a superposition of spectra from isotopomers with, in this case, 1–3 protons. (*a*) is a single isotopomer, the most highly protonated one. It is the high symmetry of benzene that reduces this spectrum to a simple one. In general, however, a molecule with 6 or more protons will have a complicated single quantum spectrum, illustrated by hexane (*c*). Spectrum (*b*) is simple and easy to interpret provided the subspectra of different isotopomers can be disentangled.

The spectra of diprotonated species provide the dipole coupling constants directly. Peaks arising from the same molecule can be correlated on a 2-dimensional COSY map [5]. These will be A_2 or, in the case of non-zero chemical shift, AB spectra. Two-dimensional dipolar A_2 type spectra consist of a pair

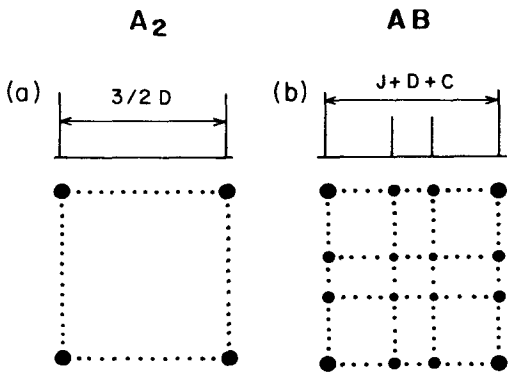

Figure 3. Schematic drawing of 2-dimensional patterns expected for (*a*) A_2 spin system and (*b*) AB spin system where $D_{AB} \geqslant \delta_{AB}$. The sides of the largest squares indicate the coupling constants, as shown. Here $D = D_{AB} + J_{AB}^{aniso}$, where D_{AB} is the dipole coupling between nuclei A and B, and J_{AB}^{aniso} is the anisotropic or pseudo-dipolar coupling. J_{AB} is the scalar coupling, δ_{AB} the difference in chemical shift and $C = \sqrt{[\delta_{AB}^2 + (J_{AB} - D_{AB}/2)^2]}$.

of lines in each frequency dimension, while AB type spectra give four lines in each dimension. The square patterns of diagonal and cross peaks for A_2 and a representative AB type, where $D_{ij} \geqslant (\delta_i - \delta_j)$ the chemical shift difference, are illustrated schematically in figure 3. The dipole coupling constants are simply extracted from the splittings.

Signals from monoprotonated molecules can easily be eliminated using a two-quantum filter. The 2-quantum-filtered COSY [6, 7] map for 80 per cent deuterated benzene is shown in figure 4. It was taken using the pulse sequence:

$$\left(\frac{\pi}{2}\right)_\phi - \frac{t_1}{2} - \pi_\phi - \frac{t_1}{2} - \left(\frac{\pi}{2}\right)_\phi - \tau_1 - \left(\frac{\pi}{2}\right)_x - \frac{\tau_2}{2} - \pi_x - \frac{\tau_2}{2} - \text{sample},$$

where ϕ is incremented by 90° while the receiver oscillates between 0 and 180°. The sample has 25 per cent diprotonated, 10 per cent triprotonated and ~2 per cent four-proton spin systems. No systems more complex than four spins are in effect present due to their low statistical probability. This important effect of random deuteration enables the clean use of n-quantum filters which usually work only as high pass filters, allowing n and higher spin systems to pass (although $n + 1$, $n + 3$, $n + 5$ ... spin systems can be inhibited by taking advantage of the oscillatory nature of their coherences), and clearly establishes a bandwidth of number of spins within which molecules must lie to be detected.

For benzene, there are three unique A_2 spectra, corresponding to the three dipole coupling constants. These are singled out in the COSY spectrum by their intensity and also because they each consist of only a pair of lines in one dimension. The three $|D_{ij}|$ values 1529, 307 and 193 Hz were obtained.

4. Assignment

The list of dipole coupling constants obtained from the diprotonated species can be assigned by examination and simulation of the spectra from the triprotonated species. The argument used is that the three coupling constants in a

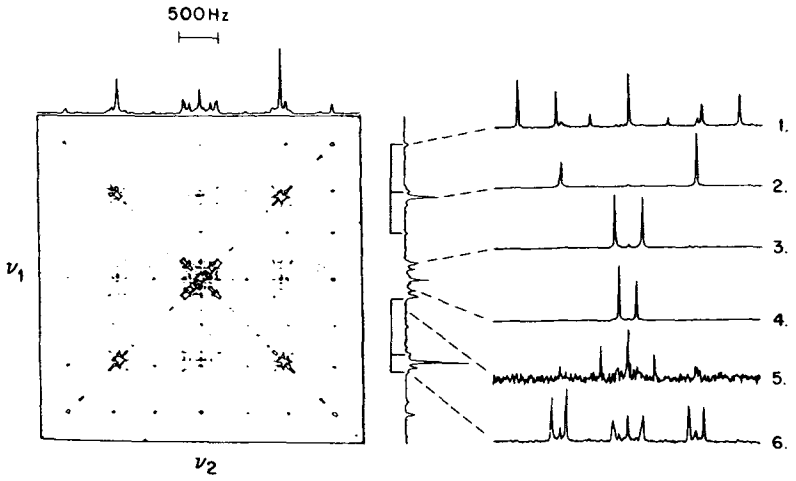

Figure 4. Two quantum filtered correlated spectrum of 78·7 per cent randomly deuterated benzene dissolved at 20 mole per cent in EK 11650. $1024t_1 \times 1024t_2$ points were recorded with deuterium decoupling and spectral widths of 10 kHz in t_1 and t_2, using the pulse sequence given in the text. 12 FIDs were collected for each t_1 point with a recycle delay of 5 s. The quadrant shown has been symmetrized along the diagonals. The slices indicate the six species present: 3 diprotonated (2), (3), (4) and 3 triprotonated (1), (5), (6).

triprotonated molecule must 'close' to form a triangle [8] and, since several cross-checks are possible, only the correct assignments will accurately simulate all possible triprotonated spectra. This process is illustrated schematically in figure 5 for a hypothetical 4 spin system. There are 6 unique dipole coupling constants, figure 5 (*a*), and only 4 possible triprotonated species, figure 5 (*b*). Thus it is impossible to have a triple with, for example, coupling constants 2 and 4, or 3 and

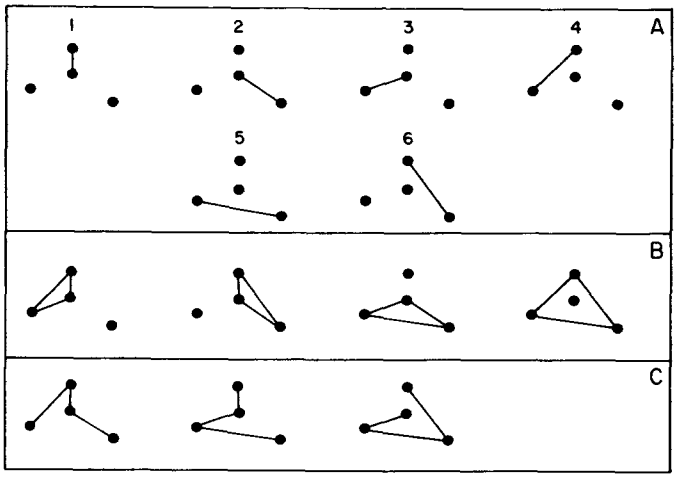

Figure 5. Illustration of procedure for the assignment of dipole couplings for a hypothetical 4 spin system. (*a*) Six unique coupled pairs, numbered 1 to 6; (*b*) the unique combinations of three couplings which connect three spins; (*c*) any other combinations of three couplings involve four spins and do not close.

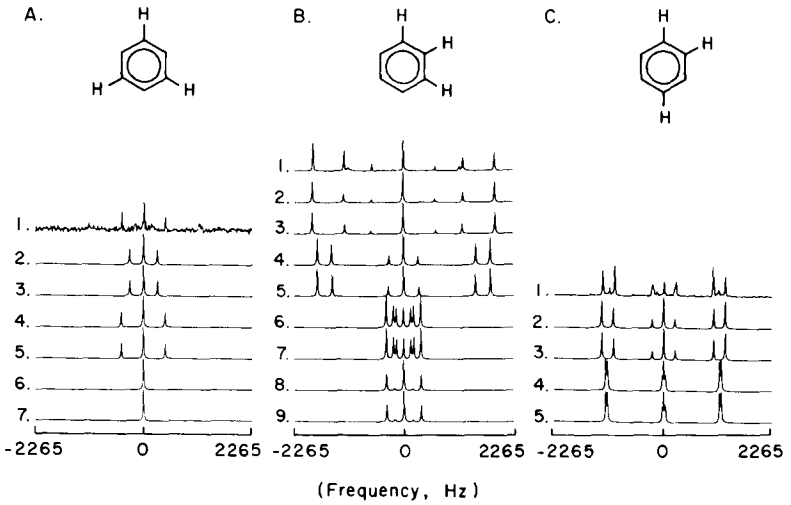

Figure 6. Simulations of spectra of triprotonated species. The experimental spectra can be simulated only by the correct assignments of D_{ij}. The dipole coupling constants used for the various simulations are listed in the table.

6, figure 5 (*c*). The final assignments are unambiguous, and the relative signs of the dipole coupling constants are obtained by this method. The absolute signs of the dipole coupling constants can be determined if the signs of J_{ij}, the indirect coupling constants, are known. These can be determined from studies in isotropic solution. Figure 6 illustrates the result for 80 per cent deuterated benzene, where the assignments are $|D_{\text{ortho}}| = 1529$, $|D_{\text{meta}}| = 307$, $|D_{\text{para}}| = 193$ Hz. It is known, from isotropic solution [9], that all J_{ij} are positive and for benzene opposite in sign to D_{ij}, therefore the dipole coupling constants are negative.

5. Multiple quantum N.M.R.

Multiple quantum N.M.R. [10] can be used to ascertain the correct assignments of the dipole coupling constants. Higher multiple quantum orders have fewer transitions, with line splittings given by combinations of the dipole coup-

Dipole coupling constants used for the simulated spectra of triprotonated benzenes shown in figure 6. Ortho, meta and para coupling constants are indicated by subscripts *o*, *m* and *p* respectively. All values are in Hz.

(*A*)	(*B*)	(*C*)
1,3,5 triprotonated benzene	1,2,3 triprotonated benzene	1,2,4 triprotonated benzene
(1) experimental	(1) experimental	(1) experimental
(2) $D_m = -193$	(2) $D_o = -1529$, $D_m = -307$	(2) $D_o = -1529$, $D_m = -307$, $D_p = -193$
(3) $D_m = +193$	(3) $D_o = +1529$, $D_m = +307$	(3) $D_o = +1529$, $D_m = +307$, $D_p = +193$
(4) $D_m = -307$	(4) $D_o = -1529$, $D_m = +307$	(4) $D_o = -1529$, $D_m = -307$, $D_p = +193$
(5) $D_m = +307$	(5) $D_o = +1529$, $D_m = -307$	(5) $D_o = +1529$, $D_m = +307$, $D_p = -193$
(6) $D_m = -1529$	(6) $D_o = +193$, $D_m = -307$	
(7) $D_m = +1529$	(7) $D_o = -193$, $D_m = +307$	
	(8) $D_o = -193$, $D_m = -307$	
	(9) $D_o = +193$, $D_m = +307$	

ling constants. Two-dimensional N.M.R. can be used to correlate coherences arising from the same spin system, and simulation of the experimental spectrum will confirm the assignments. Figure 7 (*a*) shows an experimental 5 quantum (ω_1) vs. 1 quantum (ω_2) correlation spectrum of a sample of 33 per cent randomly deuterated benzene in nematic phase. Only benzene and d_1 benzene have suffi-

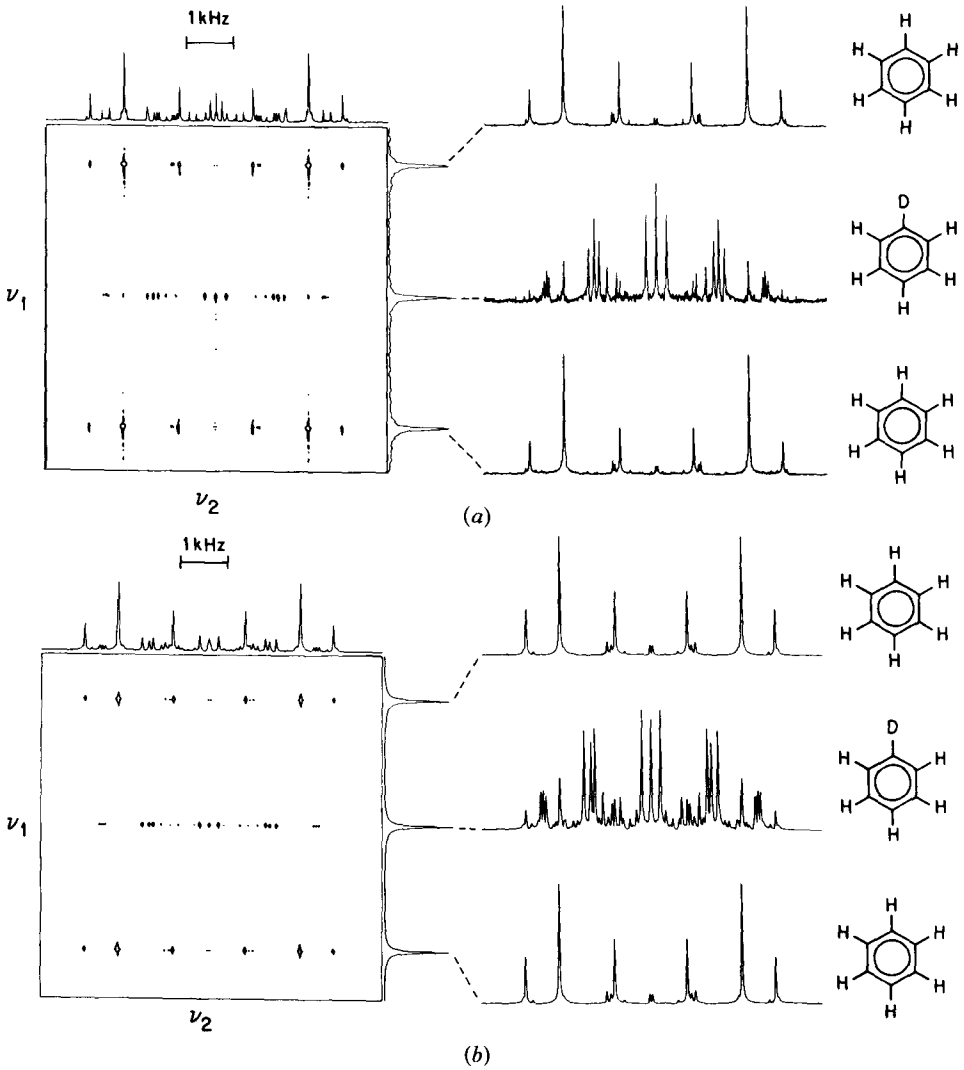

Figure 7. (*a*) 5 quantum (ν_1) vs. 1 quantum (ν_2) 2 dimensional correlated spectrum of 33·9 per cent randomly deuterated benzene dissolved at 15 mole per cent in EK 11650. The pulse sequence used selected for odd multiple quantum orders which were separated using time proportional phase incrementation [12, 13]. The phase ϕ was incremented in 26·7° steps. $3000t_1 \times 1024t_2$ points were recorded with deuterium decoupling and spectral widths of 125 kHz in ν_1 and 8 kHz in ν_2. 4 FIDs were collected per t_1 point with a recycle delay of 4 seconds. The data were zero filled to $4096t_1$ points prior to Fourier transformation. The plot shown is the 5 quantum section of ν_1, and has been symmetrized about $\nu_2 = 0$. The three projections along ν_2 clearly show benzene and d^1 benzene. Simulation of spectrum shown in figure 3 (*a*) using D_{ij} values determined from the 2 quantum filtered correlated spectrum.

cient protons to generate five quantum coherence, a singlet at $\omega_1 = 0$ for d_1 benzene and a doublet centred around $\omega_1 = 0$ for benzene. The simulated spectrum, resulting from the correct assignment of the dipole coupling constants, is shown in figure 7 (*b*). The dipole coupling constants can themselves be extracted from the $n - 1$ and $n - 2$ quantum spectra of an n-spin system [11]. The dipole coupling constants were determined with this method to be within experimental error of the values obtained from the COSY experiment.

6. Conclusion

We have shown that random deuteration can be usefully combined with multiple quantum N.M.R. to extract dipole coupling constants and spectroscopically separate mixtures. A high deuteration level was used to select for 2 and 3 proton containing isomers. Dipole couplings were extracted from the 2 proton isomers and the assignments and connectivities were determined using the 3 proton isomers. The assignments were verified using multiple quantum spectroscopy on a more highly protonated mixture. Work is in progress to apply these ideas to more complex systems.

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