

for most of the splitting. Excellent agreement is also observed in the galactose series.

The accuracy of these additive calculations indicates the general validity of the additivity principle and thus justifies the use of a simple retro-additive approach to the interpretation of complex CD spectra. Application of selective bichromophoric derivatization to complex stereochemical problems can provide for interpretable information-rich CD spectra of nanomolar quantities. Such an approach is currently being applied to oligosaccharide microanalysis, wherein the free hydroxyls are derivatized with one type of chromophore and the linkage positions are tagged with a second chromophore. Resulting subunits are then fully characterized by UV (to determine chromophoric ratios) and CD measurements.

Acknowledgment. The work was supported by NIH grant AI 10187. One of us (J.T.V.) thanks the Comunidad Autónoma de Canarias—Caja General de Ahorros de Canarias for a fellowship.

Registry No. BBCC, 104375-86-2; BCCB, 104375-87-3.

Determination of Dipole-Dipole Couplings in Oriented *n*-Hexane by Two-Dimensional NMR

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Received May 9, 1986

NMR spectra of molecules partially oriented in liquid crystals contain information about the anisotropic dipole-dipole interactions of the spins. It is of interest to recover the intramolecular dipolar coupling constants D_{ij} between each pair of spins since D_{ij} is proportional to $\langle r_{ij}^{-3} \rangle$ and quantitatively describes the molecular structure and the motions affecting the structure.¹ However, in conventional NMR spectroscopy the number of detectable transitions becomes intractably large as the number of active spins increases, and the individual lines may no longer be resolvable. In *n*-hexane, which is the molecule of interest here, the number of distinct transitions, even taking into account the symmetry of the molecule, can be as large as 60 000, concealing the 16 unique dipolar couplings between protons.

Several techniques have been proposed to overcome this problem. One useful approach is selective isotopic substitution, e.g., selective positioning of protons in otherwise deuterated molecules and measurement of the NMR spectrum under conditions of deuterium decoupling.^{2,3} This allows an arbitrary reduction of the number of active spins but requires demanding synthetic effort. Another possible solution is the observation of high-order multiple-quantum spectra in order to reduce the redundant information in single-quantum NMR spectra.⁴

We give here a preliminary report of an alternative method to analyze spectra and determine structures of molecules dissolved in liquid crystals. The basic idea is the use of randomly deuterated samples (a synthetically facile undertaking) which contain a mixture of all possible isotopomers of a molecule. This approach has been used before in the analysis of some cyclic compounds^{3,5,6} in nematic liquid crystals. A highly deuterated sample will contain mostly isotopomers with just a few protons. Thus, the task of

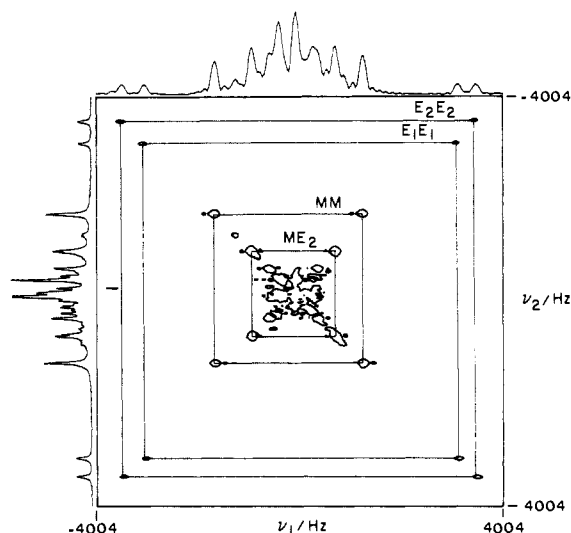


Figure 1. Two-quantum filtered COSY-type spectrum of 81% randomly deuterated *n*-hexane, 23 mol % in EK 11650, taken with the pulse sequence described in the text. Deuterium-decoupled FIDs, 128 × 1024 points, were collected on a 360-MHz spectrometer (nonquadrature in t_1) with a spectral width of 16 667 Hz in both dimensions and a recycle delay of 5 s. For each t_1 point, 200 FIDs were accumulated while the double-quantum mixing time τ_1 was incremented from 0 to 9800 μ s in 200- μ s steps after every fourth FID. For τ_2 , 4 ms was used. The data set was zero-filled to 512 × 2048 points prior to Fourier transformation. The square patterns which give the dipole coupling constants are illustrated for four of the 16 proton pairs.

Table I. List of Dipole Coupling Constants Obtained for the Eight AB and Eight A₂ Coupling Patterns

sites	$D_{ij}^{a,c}$	$D_{ij}^{b,c}$	sites	$D_{ij}^{a,c}$	$D_{ij}^{b,c}$
E ₁ E ₁	3974	3968	E ₁ E ₂	186	183
E ₁ E ₁	713	706	E ₁ E ₂	81	81
E ₁ E ₁	609	612	MM	1876	1862
E ₂ E ₂	4487	4482	MM	206	203
E ₂ E ₂	190	189	ME ₁	386	382
E ₂ E ₂	43	48	ME ₁	322	314
E ₁ E ₂	1616	1626	ME ₂	1034	1041
E ₁ E ₂	1086	1106	ME ₂	598	591

^aThe COSY-type experiment. ^bThe INADEQUATE-type experiment. ^cThe dipole coupling constants D_{ij} are defined by the equation $\mathcal{H}_D = \sum_{i < j} D_{ij} (3I_{zi}I_{zj} - I_i I_j) / 2$.

interpreting one complex spectrum is reduced to one of analyzing a large number of simple but overlapping spectra. The crucial point is the ability to recognize the individual signals which derive from the same spin system (arise from one isotopomer). This can be achieved by the combined application of two-dimensional NMR⁷ and multiple-quantum NMR^{8,9} techniques as is shown below for the case of *n*-hexane.

An 81% randomly deuterated sample of *n*-hexane was synthesized by exchange of *n*-hexane in the gaseous phase with D₂ over Pd on charcoal at 190 °C. It was determined by mass spectroscopy to have a statistical distribution of isotopomers. Most molecules have between 0 to 5 protons on the chain. Figure 1 shows a two-quantum filtered^{10,11} COSY-type spectrum of the mixture, taken with the pulse sequence

$$(\pi/2)_\phi - t_1 - (\pi/2)_\phi - \tau_1 / 2 - \pi_x - \tau_1 / 2 - (\pi/2)_x - \tau_2 / 2 - \pi_x - \tau_2 / 2 - \text{sample}$$

The phase ϕ is incremented in 90° steps, alternating the receiver

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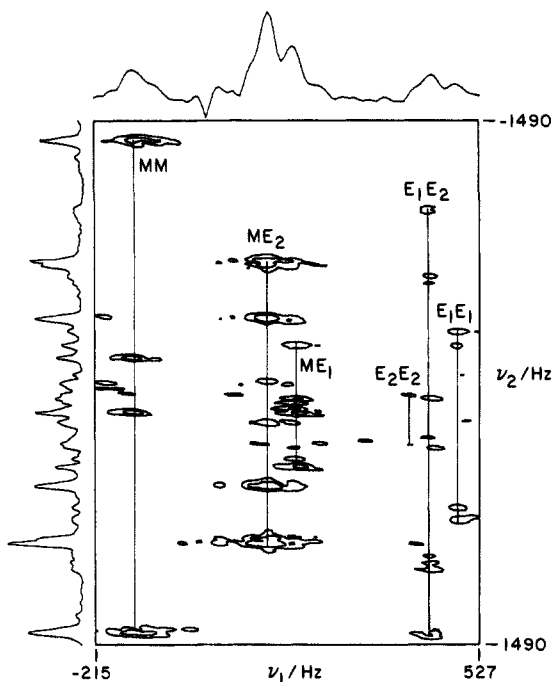


Figure 2. Part of a double-quantum vs. single-quantum spectrum of the same sample as in Figure 1, obtained by using the pulse sequence described in the text. Deuterium-decoupled FIDs, 128×1024 points, were recorded on a 360-MHz spectrometer with quadrature phase detection in both dimensions and a recycle delay of 5 s. The spectral width was 3.3 kHz in ν_1 and 7.35 kHz in ν_2 . τ_1 was 250 μ s and τ_2 4 ms. Vertical lines parallel to the single-quantum axis illustrate the six double-quantum frequencies of molecules with two protons along which the A_2 - and AB-type spectra can be identified.

phase between 0 and 180°. The spin-echo delay τ_2 eliminates any contribution to the signal from the liquid crystal. The double-quantum filter time τ_1 is varied to average out all double-quantum coherences not refocused at the end of τ_1 . The spectrum effectively contains only signals from two proton isotopomers, since signals from one and three proton isotopomers are eliminated by the two-quantum filter. Four or more proton molecules do not contribute due to a combination of low statistical probability of occurrence and a wide range of isomers. The COSY-type spectrum thus has 16 subspectra of the type A_2 or AB. Signals in the same spin system can easily be identified by off-diagonal correlations which form square patterns with diagonal peaks. All 16 dipole couplings could be read off this map and from a similar experiment in which a π pulse was applied in t_1 . The dipole coupling constants thus determined are listed in Table I, together with a site assignment based on chemical shift.

The same information was corroborated in a second 2D-IN-ADEQUATE-type¹² experiment. The pulse sequence used was $(\pi/2)_\phi - \tau_1 / 2 - \pi_\phi - \tau_1 / 2 - (\pi/2)_\phi - t_1 - (\pi/2)_x - \tau_2 / 2 - \pi_x - \tau_2 / 2$ -sample

where ϕ is incremented by 90° while the receiver oscillates between 0 and 180°. Here two- and one-quantum signals were correlated in a two-dimensional map as shown in Figure 2. Six vertical lines were produced parallel to the one-quantum axis, corresponding to the six possible double-quantum frequencies

$$2\nu_M, \nu_M + \nu_{E_1}, \nu_M + \nu_{E_2}, 2\nu_{E_1}, 2\nu_{E_2}, \nu_{E_1} + \nu_{E_2}$$

(The notation used is M = methyl protons, E_1 = C-2 methylene protons, E_2 = C-3 methylene protons.) The six slices along the one-quantum axis each contain A_2 or AB subspectra corresponding to their group type; thus there are two MM, two ME_1 , two ME_2 , three E_1E_1 (two shown), three E_2E_2 (one shown), and four E_1E_2

(three shown) subspectra. Subspectra are easily identified by their symmetric disposition around the central chemical shift position in ω_2 . Spin systems with more than two protons in general produce two-quantum spectra lying outside the chemical-shift range, since they are dominated by dipole couplings. They may have inner lines of low intensity, which will not correspond, except by accident, to any of the six double-quantum frequencies. Different two-quantum preparation times result in different relative intensities of the subspectra. Table I includes a list of dipole coupling constants obtained with two preparation times, 250 μ s and 2.5 ms. The values of D_{ij} from the COSY-type and INADEQUATE-type experiments agree rather well. It remains to assign the couplings constants to specific pairs of protons on the molecule. These couplings can be used to test various theoretical models of conformational motions for hydrocarbon chains in anisotropic environments.¹³⁻¹⁵ The fact that D_{ij} 's for a molecule with 14 protons can be determined bodes well for the application of two-dimensional and multiple-quantum NMR to structure and motions of oriented molecules.

Acknowledgment. This work was supported by the Director, Office of Basic Energy Sciences, Material Science Division of the U.S. Department of Energy, and by the Director's Program Development Funds of the Lawrence Berkeley Laboratory under Contract DE-AC03-76SF00098. K.V.S. acknowledges support by the Swiss National Science Foundation.

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Assignment of Secondary Amide ¹⁵N Resonances of Bleomycin A₂ by Two-Dimensional Multiple-Quantum ¹H-¹⁵N Shift-Correlation NMR Spectroscopy

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Received April 7, 1986

As part of our program to delineate the solution conformation of metal and nucleic acid complexes of the bleomycins (Bleo),¹ Figure 1a, we have assigned the ¹⁵N NMR resonances of Bleo A₂, the most abundant congener of these antineoplastic antibiotics.^{2,3} Because isotopic enrichment of this antibiotic cannot readily be achieved, our experiments were performed at natural abundance in aqueous solution by using the recently introduced two-dimensional multiple-quantum ¹H-¹⁵N shift-correlation NMR spectroscopic method.⁴⁻¹⁰ This method is several orders of

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(1) The abbreviations used: Bleo, bleomycin; NMR, nuclear magnetic resonance; DMS, (3-aminopropyl)dimethylsulfonium salt; BIT, 2'-(2-aminoethyl)-2,4'-bithiazole-4-carboxylic acid; Thr, L-threonine; Val, 4-amino-3-hydroxy-2-methyl-n-valeric acid; His, L-erythro- β -hydroxyhistidine.

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