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Molecular Order of a Liquid Crystal Investigated by Double-Quantum ^{13}C NMR

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Nuclear magnetic resonance (NMR) can give detailed information on molecular geometry because the dynamics of nuclear spins are sensitive to the through-space dipole–dipole (DD) interaction, which has an inverse-third-power distance dependence. In the isotropic phase, short-range dipole couplings may be estimated *indirectly* through the cross-relaxation rates, which are proportional to the square of the DD couplings. This method has developed into an essential tool for molecular structure determination.[1] In principle, longer-range and more detailed information is available from NMR measurements in the anisotropic liquid phase, where spectral splittings give *direct* measurements of the DD couplings.[2]

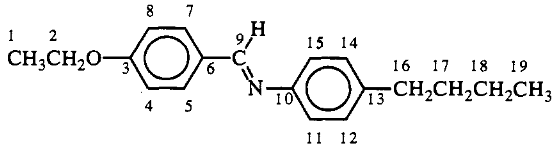
Dipole couplings in anisotropic systems have not been fully exploited mainly because ^{1}H NMR spectra are intractably complicated except for very small molecules. One approach to this problem has been to record high-order multiple-quantum ^{1}H spectra.[3] Unfortunately, although the number of spectral transitions is greatly reduced, the different dipolar couplings still remain seriously entangled. A more drastic method is to employ extensive ^{2}H labeling to isolate systems of small numbers of coupled ^{1}H spins.[4] However, this is chemically intensive and leaves problems of ^{2}H decoupling and assignment of the ^{1}H lines. Exploitation of heteronuclear DD couplings is feasible but requires complex multipulse sequences.[5]

In this communication we report a new approach which does not require isotopic labeling but still provides a clear separation of scores of useful dipolar couplings in molecules of reasonable size. Following similar experiments in isotropic liquids,[6] we use the special phase properties of double-quantum coherence (2QC) to filter out ^{13}C NMR signals originating from very rare naturally-occurring molecules with two ^{13}C spins. If ^{13}C–^{1}H couplings are effectively removed by suitably-modulated rf irradiation at the ^{1}H Larmor frequency, the four spectral lines from a given $^{13}C_2$ species are determined only by the two chemical shift values and the ^{13}C–^{13}C coupling and are very easy to assign and interpret. In principle, a molecule containing n carbon sites gives a spectrum with four lines from each of the $n(n-1)/2$ different $^{13}C_2$ species. Barring accidental degeneracies, contributions from different isotopomers are readily separated on the basis of their 2Q frequencies, which are the sums of the chemical shifts. The two-dimensional ^{13}C 2Q spectrum of a liquid crystal molecule contains a wealth of easily-interpreted structural and dynamic information.

So far we have only applied this method to neat nematic liquid crystals which orient spontaneously along the magnetic field. A two-dimensional 2Q ^{13}C spectrum of *N*-(*p*-ethoxybenzylidene)-*p*-*n*-butylaniline (EBBA) is shown in Figure 1. This spectrum was obtained by the standard two-dimensional pulse sequence[7]

$$(\pi/2) - \tau/2 - (\pi) - \tau/2 - (\pi/2) - t_1 - (\pi/2) - t_2$$

where the phases of all pulses are cycled according to a standard procedure to select NMR signals passing through 2Q coherence,[6] and τ was 1.0 ms. The displayed spectral region shows assigned peaks from ^{13}C spin pairs within the aromatic rings of the EBBA molecule. By using longer τ values, we have also detected ^{13}C pairs within the aliphatic side groups and between the chains and the core. Some of the observed couplings correspond to average ^{13}C–^{13}C distances of around 5.5 Å . Such long distances are difficult to assess cleanly by ^{1}H NOE measurements and are on the limits of feasibility for solid-state NMR techniques such as rotational resonance.[8]



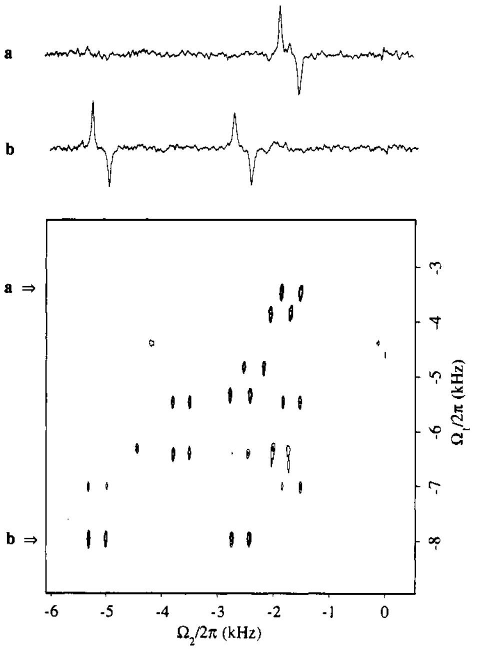


Figure 1. Part of a two-dimensional ^{13}C spectrum of EBBA obtained by employing the pulse sequence described in the text. The data set, 256 × 1024 points, was zero-filled to 1024 × 8192 points prior to Fourier transformation, and for each t_1 value 384 transients were recorded. The spectral width was 30 kHz in the Ω_1 dimension and 15 kHz in the Ω_2 dimension. The spectral axes are labeled in terms of rotating-frame frequencies, including the algebraic sign; the sense of the Ω_2 axis is conventional with "downfield" on the left and "upfield" on the right. The experiment was performed with a standard 10-mm high-resolution probe at a magnetic field of 4.7 T on a Bruker MSL200 spectrometer. The experimental temperature, 67 ± 1 °C, was obtained by measuring the proton chemical shift difference between the two peaks of ethylene glycol confined in a capillary centered in a tube of EBBA. The 2Q preparation time τ was 1.0 ms. In part a we show a slice along the Ω_2 axis which contains an A_2 subspectrum from carbons 4 and 8. The AX subspectrum in part b corresponds to spins at sites 3 and 5.

Some motionally-averaged dipolar couplings $\langle D_{ij}\rangle$, estimated from a series of 2Q spectra with τ values 1.0, 1.5, 2.0, and 4.0 ms are given in Table 1. To obtain these values the isotropic J couplings J_{ij}^{iso} were taken into account. These were estimated

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Table 1. Experimental ^{13}C–^{13}C Dipole Couplings in *N*-(*p*-Ethoxybenzylidene)-*p*-*n*-butylaniline

carbons	$\langle D_{ij}\rangle/2\pi$ (Hz)[a]	J_{ij}^{iso} (Hz)
4–8	116	3
5–7	117	3
11–15	118	3
12–14	120	3
2–4,8	−92	8
3–4,8	133	66
3–5,7	−158	4
3–6	−158	10
6–5,7	114	58
6–4,8	−154	3
5,7–11,15	−24	0
6–11,15	−33	0
13–11,15	−150	3
17–11,15	−35	0
17–12,14	−60	4

[a] The motionally-averaged dipole couplings are defined as $\langle D_{ij}\rangle = \langle -(\mu_0/4\pi)\gamma_C{}^2\hbar(1/2)(3\cos^2\theta_{ij}-1)r_{ij}{}^{-3} + \pi J_{ij}{}^{\text{aniso}}\rangle$, where r_{ij} is the spin–spin distance and θ_{ij} the angle between the applied magnetic field and the spin–spin axis. The accuracy of the $\langle D_{ij}\rangle/2\pi$ values is around 5 Hz.

from the literature[9,10] and are also given in the table. Appropriate correction for $J_{ij}{}^{\text{iso}}$ requires prior knowledge of the signs of $\langle D_{ij}\rangle$, which were guessed by reasonable geometric assumptions. The displayed values of $\langle D_{ij}\rangle$ are superpositions of the residual through-space dipolar coupling and the anisotropic part of the J coupling.[11] The couplings $\langle D_{4\,5}\rangle$, $\langle D_{4\,7}\rangle$, $\langle D_{11\,12}\rangle$, $\langle D_{11\,14}\rangle$, $\langle D_{7\,8}\rangle$, ... were also observed but are not reported in the table. Their assignment was uncertain due to an accidental coincidence between the 2Q frequencies of the relevant $^{13}C_2$ species.

A complete analysis of these results in terms of a dynamic conformational distribution will be given in a full article. However, some immediate remarks will be made. First, the fact that couplings 3–8 and 5–6 are markedly different suggests a distortion of the benzene ring, as suggested before,[3] or (possibly) significant differential J anisotropy. Second, the fact that the couplings between sites 3 and 4 and between sites 3 and 8 are identical strongly supports a motional model in which the aromatic moiety is rotating around its para axis, in addition to the overall rotation about the long molecular axis (these axes are in general not parallel). This dynamical model is also consistent with the observation that all four couplings 5,7–11,15 are identical.

A preliminary analysis of the ring dynamics may be performed in terms of local ordering matrices. Consider for example the aromatic ring of the benzylidene fragment. A local axis system may be attached to the ring with the z axis in the direction of the two para bonds and the y axis normal to the ring. This axis system corresponds to the principal axes of the local Saupe ordering matrix **S** provided that internal motion generates an effective C_{2v} symmetry.[2,12] A reasonable model which fulfills this would be 180° flips of the cores. For principal axes of **S** there are two quantities to determine, namely, S_{zz} and $S_{xx} - S_{yy}$. By fitting the couplings 3–5, 3–6, 4–6, 4–8, and 5–7 to the relevant expression[2] we obtained $S_{zz} = 0.45$ and $S_{xx} - S_{yy} = 0.024$ with a relative error in the fitted dipole couplings of less than 2%. Anisotropic J couplings were ignored, and hexagonal ring symmetry was assumed. A full spectral analysis, presented later, will concentrate more on long-range dipolar couplings for which J anisotropy is presumably negligible. This will allow the investigation of interesting issues such as the aliphatic chain conformations and the correlation in the motion of different molecular fragments. This will complement conventional approaches such as ^{2}H NMR spectroscopy.

The main disadvantage of the technique is its very low sensitivity. The results shown were made possible by two additional innovations. (i) We established a strong nuclear Overhauser enhancement of the longitudinal ^{13}C nuclear polarization by low-intensity ^{1}H irradiation for several seconds prior to each ^{13}C pulse sequence (a factor of 2–3 enhancement was typical). (ii) We decoupled ^{1}H from ^{13}C by modulated ^{1}H irradiation. The decoupler frequency was set to the center of the ^{1}H spectrum in the isotropic liquid phase. The decoupler field strength corresponded to a nutation frequency of $\omega_{1I}/2\pi = 10.4$ kHz. In practice we obtained adequate decoupling whenever the decoupler phase was changed by large angles in a periodic scheme on a millisecond time scale. Our specific sequence corresponded to flip angles {5990, 4979, 389, 5990, 4979, 389} and phases {90, 270, 90, 0, 180, 0}. The ^{1}H decoupling cycles were always initiated at the same point of the ^{13}C pulse sequence so as to allow the small cycling sidebands[13] of the $^{13}C_1$ species to be removed by the phase cycle. Despite these enhancements, acquisition of the spectrum shown in Figure 1 still took about 80 h of instrument time at our field of 4.7 T.

In summary, 2Q ^{13}C NMR in anisotropic liquids has great potential for extracting molecular conformational information. For neat liquid crystals, the sensitivity is satisfactory and the level of conformational detail is incomparable. For other systems, such as solute molecules or transmembrane peptides, the method may be combined with limited ^{13}C labeling.

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