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# Selective Detection of the Proton NMR Spectra of Molecules Containing Rare Spins at Natural Abundance in Liquid Crystalline Samples

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**It is shown that the proton NMR spectra of molecules containing rare spins at natural abundance dissolved in a liquid crystalline solvent can be obtained free from the strong lines from the spectrum of the abundant isotopomer by the 2D HSQC NMR experiment. The technique can also give the individual chemical shifts of the rare spins, and, for a molecule containing another abundant nucleus, such as fluorine, the rare spin–$^{19}$F total anisotropic couplings are also obtained. The usefulness of the technique is demonstrated for molecules containing $^{13}$C as the rare spins.** 

## INTRODUCTION

The NMR spectra of samples dissolved in liquid crystalline solvents can be analyzed to yield a set of partially averaged dipolar couplings, $D_{ij}$, between the NMR-active nuclei. The set of $D_{ij}$ may then be used to investigate the structure and orientational order of the solute molecules, and in addition, if they are flexible, their conformational distribution. The spectra become increasingly complex as the number of interacting nuclei increases, but in principle it should be possible to analyze a resolved spectrum. The spectra are dominated by lines from the molecules containing only the isotopically most abundant nuclei, so that for a hydrocarbon this is the isotopomer containing just $^1$H and $^{12}$C.

It is a great advantage to be able to determine the dipolar couplings between the protons and carbons, and in principle this can be achieved either by recording and analyzing the $^{13}$C spectrum or by detecting the weak lines in the proton spectrum from isotopomers containing just one $^{13}$C. The direct detection of the $^{13}$C spectra of these 1% abundant molecules is often impracticable because of low signal-to noise ($S/N$), and in addition overlap of the spectra from different isotopomers severely complicates their analyses. Even when direct detection is possible it is more advantageous to obtain the couplings from the proton spectra of these isotopomers since this ensures that both $^1$H–$^1$H and $^1$H–$^{13}$C couplings are obtained under exactly the same conditions of temperature and concentration, both factors having a very large effect on their values. Detecting the $^{13}$C satellites in a proton spectrum of an oriented sample has proved possible for several molecules, but in general this approach has inherent difficulties. First, some of the lines will be obscured by the strong lines from the all-$^{12}$C isotopomer. Second, when there is more than one group of chemically equivalent carbon nuclei the different $^{13}$C satellite spectra will often overlap and be difficult to assign. And last, but not least, the presence of impurities can produce lines of comparable intensities to those from the molecule of interest, and these may be difficult to identify.

We will demonstrate here that these problems can be overcome by using the two-dimensional heteronuclear single-quantum coherence experiment referred to usually as HSQC (*1, 2*). The principle of this experiment is based on the INEPT method for the transfer of spin polarization between coupled nuclei (*3*). This transfer depends on the presence of a coupling between the $^1$H and $^{13}$C nuclei, for example, and hence it can be used to discriminate between the all-$^{12}$C isotopomers and those containing a $^{13}$C nucleus when detection is made of the carbon spectrum. However, such a single $^{13}$C detected INEPT experiment has the major disadvantage of low sensitivity. The 2D HSQC experiment removes this disadvantage by having two INEPT transfer steps, first from protons to carbons and second in the reverse direction, followed by detection of the proton resonances of only the 1% naturally abundant $^{13}$C-labeled molecules.

For isotropic samples a transfer of spin polarization occurs only if the nuclei interact through a scalar coupling $^nJ_{CH}$. The transfer efficiency depends on the magnitude of $^nJ_{CH}$, which is usually easy to predict, and this allows easy optimization of the experiment. The magnitude of $^nJ_{CH}$ usually decreases rapidly with increasing $n$, such that the INEPT transfers become of too low efficiency when the number of bonds between the $^{13}$C and $^1$H nuclei is $>2$. For liquid crystalline samples spin polarization can be transferred by dipolar as well as scalar coupling. Dipolar couplings for samples dissolved in thermotropic liquid crystalline solvents are usually much larger than scalar couplings, and are of appreciable value between all the $^{13}$C and $^1$H nuclei in a molecule, which essentially removes any constraint on the value of $n$. However, the dipolar couplings are not

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**FIG. 1.** Structure and atomic labeling of (left) 1-chloro-2-bromoethane and (right) 1,2-dibromo-2,2-difluoroethane.

easily predictable, and this might make optimization of the INEPT transfer difficult to achieve.

We show here that the 2D HSQC experiment is extremely useful for liquid crystalline samples, and has the potential for widespread application. To demonstrate this we have chosen two examples whose structures and atomic labeling are shown in Fig. 1. The first is a sample of 1-chloro-2-bromoethane dissolved in a nematic solvent ZLI 1132. The all-$^{12}$C molecules give an AA′ BB′ spectrum from which it is possible to obtain four dipolar couplings, $D_{AA'}$, $D_{BB'}$, $D_{AB}$, and $D_{AB'}$. Each of the two single $^{13}$C isotopomers gives an AA′BB′X type spectrum, and these add two $^{13}$C–$^{1}$H dipolar couplings, $D_{AX}$ and $D_{BX}$, for each isotopomer, thus increasing to eight the number of dipolar couplings that can possibly be obtained. The second sample is 1,2-dibromo-2,2-difluoroethane also dissolved in ZLI 1132 and now the spin systems of the single $^{13}$C isotopomers are of type AA′KK′X. The proton spectrum of the all-$^{12}$C isotopomer yields the couplings $D_{AA'}$, and $D_{AK} + D_{AK'}$ while the 2D HSQC proton-detected experiment on the single $^{13}$C isotopomers adds two $D_{AX}$ and two $D_{KX}$ couplings. For both of these molecules it was possible to detect a sufficient number of lines from single $^{13}$C isotopomers directly in either the $^{1}$H or the $^{19}$F spectra and hence determine the values of the $D_{CH}$, $D_{CF}$, and $D_{FH}$ which can then be compared with the results obtained by the 2D HSQC experiments.

## EXPERIMENTAL

The experiments were performed on a Varian 600-MHz spectrometer with a dual $^{13}$C/$^{1}$H inverse detection probe which can be used to produce a magnetic field gradient in the direction, $z$, of the static field $B_0$. The samples were contained in 5-mm-o.d tubes, and a deuterium lock signal was provided by having a $D_2O$ sample located in a central, coaxial capillary. The 90° proton pulses were of length 5.6 $\mu$s, while those for $^{13}$C were 16.4 $\mu$s.

The sequence of RF and field gradient pulses used is shown schematically in Fig. 2, and is based on developments of the basic 2D HSQC method proposed by Li and Montelione (*4*) and by Willker *et al.* (*5*). The field gradient pulses serve to eliminate signals from the molecules not containing a $^{13}$C nucleus. Note that the ratio of the areas of the two gradient pulses is set to be in the ratio $\gamma_H/\gamma_C$ in order to select coherence transfer between single-quantum $^{13}$C and single-quantum $^{1}$H. The delay $\tau_1$ is fixed so as to produce a good transfer of spin polarization. For an isotropic sample of a molecule like 1-chloro-2-bromoethane the value of $\tau_1$ should be set to $1/(4^1 J_{CH})$, which has only a weak molecular or site dependence and so is easily chosen in advance of the experiment. In the case of an isotropic sample of 1,2-dibromo-2,2-difluoroethane efficient proton–carbon INEPT transfers require $\tau_1 = 1/(4^1 J_{CH})$ for the isotopomer with $^{13}$C on site 1, and $\tau_1 = 1/(4^2 J_{CH})$ when the $^{13}$C is at site 2, which again can easily be guessed in advance. For liquid crystalline

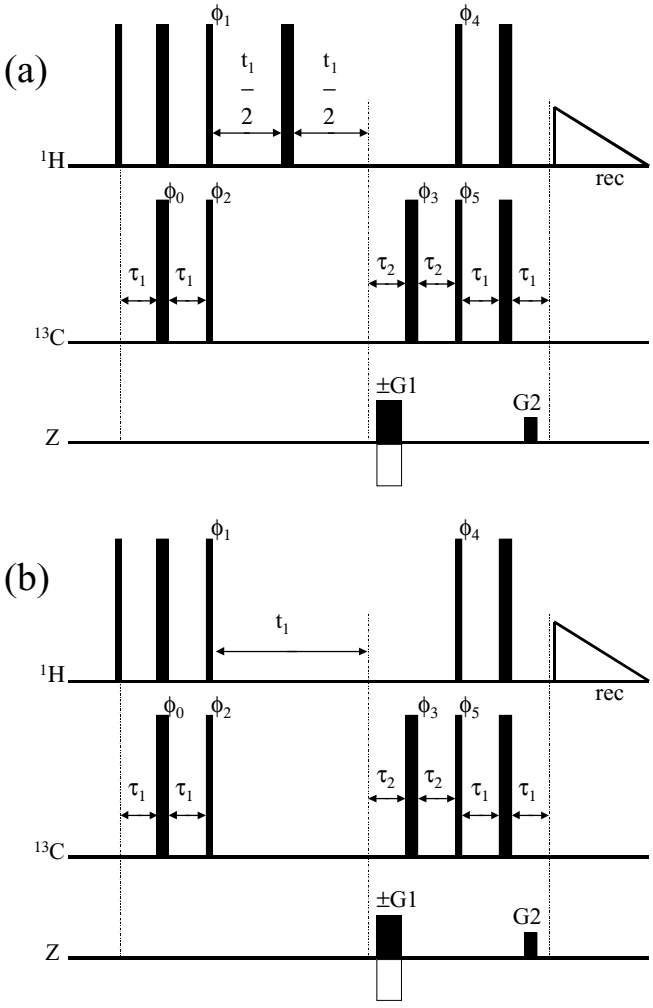



**FIG. 2.** Pulse sequences used to obtain HSQC spectra (a) without and (b) with coupling between $^{1}$H and $^{13}$C in the $F_1$ dimension. The pulses are either 90° (narrower width) or 180°. Note that the difference between the two sequences is the presence in (a) of a 180° pulse on the protons in the middle of the $t_1$ period. The RF field strengths used for the 90° pulses were 51 kHz for $^{1}$H and 15.2 kHz for $^{13}$C. The delay $\tau_2$ was long enough to include the $z$-gradient pulse G1 (32 G/cm, 2.5 ms) plus 100 $\mu$s recovery delay. The second $z$-gradient pulse G2 was of amplitude 16 G/cm and duration 1.25 ms. All RF pulses are along the $x$ axis unless otherwise indicated. The phases were $\phi_0 = x$; $\phi_1 = 2y, 2(-y)$; $\phi_2 = x, -x$; $\phi_3 = x, -x$; $\phi_4 = 4x, 4(-x)$; $\phi_5 = 8x, 8(-x)$; receiver $= x, -x, -x, x, 2(-x, x, x, -x), x, -x, -x, x$. Quadrature detection in $F_1$ was achieved by alternating the sign of G1.

samples the transfer depends on the total couplings ${}^nT_{CH} = {}^nJ_{CH} + 2{}^nD_{CH}$, which can be strongly sample and site dependent. Note too that ${}^nJ_{CH}$ is usually much smaller than ${}^nD_{CH}$, and may also be of the same or opposite sign. For the present samples it was discovered that a good $S/N$ could be obtained when $\tau_1$ was set to be 1.8 ms, which is approximately $1/(4{}^1J_{CH})$. To investigate whether this was a fortuitous result a series of simulations of the HSQC experiment were performed using the program NMRSIM obtained from Bruker Spectrospin.

The nematic liquid crystalline solvent, ZLI 1132, was obtained from Merck Ltd.

## RESULTS

Figure 3 shows the 2D spectrum obtained for 1-chloro-2-bromoethane with the sequence shown in Fig. 2a. The value of $\tau_1$ was initially chosen to be 1.8 ms, which is approximately $1/(4{}^1J_{CH})$, and this gave good intensities for spectra corresponding to both the single $^{13}$C molecules. In fact, $1/(4{}^1T_{CH}) = 0.1$ ms and $1/(4{}^2T_{CH}) = 1.6$ ms for C1, with similar values for C2. Our main interest is in the $F_2$ cross sections at the positions of the carbon chemical shifts, which correspond to the carbon-13-coupled proton spectra of the two isotopomers, and for $\tau_1 =$ 1.8 ms the cross section for C1 showed distortions in many of the lines. These are probably caused by instability of the temperature, and hence of the orientational order, over the long recording time for the spectrum. A 2D spectrum recorded with $\tau_1 = 2.8$ ms had better proton cross sections, and these were used in the subsequent spectrum analyses.

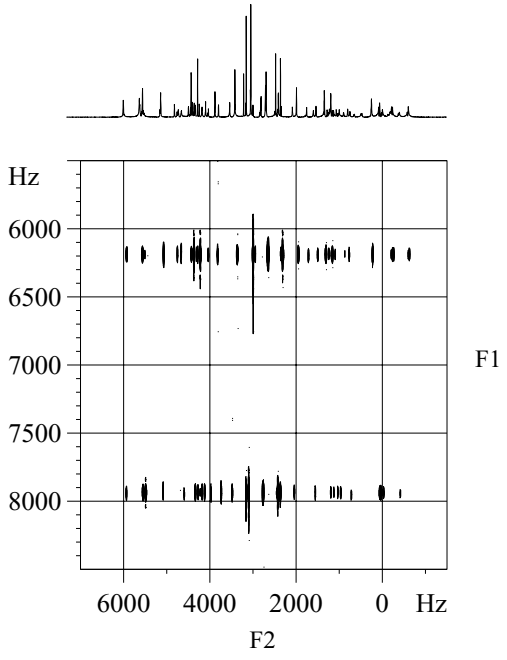



**FIG. 3.** The 600-MHz 2D HSQC spectrum of 1-chloro-2-bromoethane dissolved in the nematic solvent ZLI 1132 obtained with version (a) of the pulse sequence in Fig. 2. The delay $\tau_1$ was fixed at 1.8 ms. The delay $t_1$ was incremented in 500 steps of 40 $\mu$s to give a spectral width after Fourier transformation of 25 kHz and a digital resolution of 50 Hz in the $F_1$ dimension. The 90° proton pulse width was 5.6 $\mu$s, and the 90° carbon pulse was 16.4 $\mu$s. The free induction decays were recorded for 0.4 s into 16 K words computer memory giving a digital resolution in $F_2$ of 2.5 Hz. A 90° shifted sinebell function was applied in both dimensions prior to Fourier transformation. The spectrum is displayed in magnitude mode.

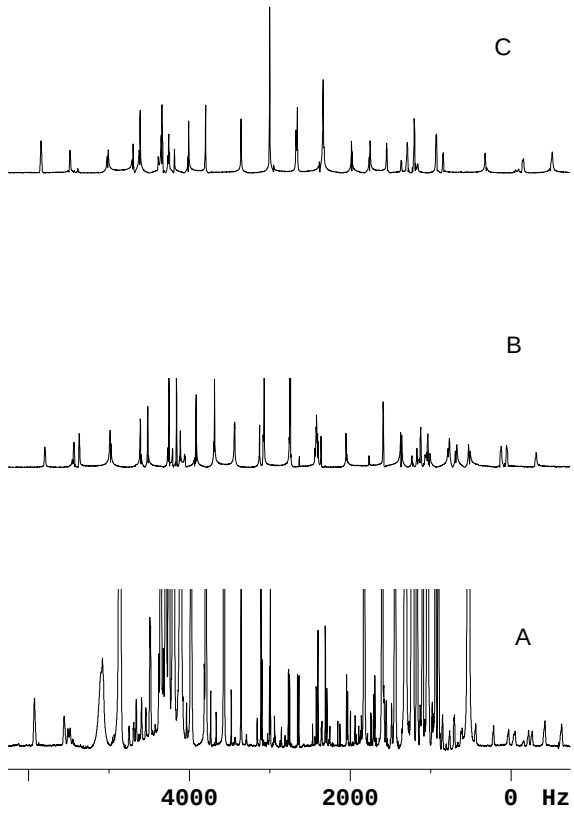


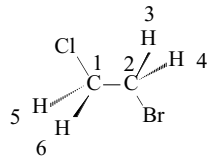

**FIG. 4.** (A) The normal 1D 600-MHz spectrum of 1-chloro-2-bromoethane in ZLI 1132 expanded vertically to show the $^{13}$C satellites. (B) and (C) are sections parallel to the $F_2$ axis at the points corresponding to $\delta_{C1}$ and $\delta_{C2}$ in Fig. 3.

The projection onto the $F_1$ axis gives the proton-decoupled $^{13}$C spectrum. This contains two strong lines corresponding to the resonances from C1 and C2, and also a number of weaker lines, which we attribute to $^{13}$C nuclei in the liquid crystalline solvent. The relative intensities from solute and solvent molecules depends on the value of $\tau_1$, but those from the solute were stronger for both values of $\tau_1$ used in our experiments. The positions of the two lines from the solute molecules gives the relative chemical shift of the two carbons. The shielding constant, $\sigma_i$, for a nucleus in a uniaxial liquid crystalline sample is given by

$$\sigma_i = \sigma_{0i} + \sigma_{ai}, \qquad [1]$$

where $\sigma_{0i}$, the scalar part, is independent of the solute orientational order, while $\sigma_{ai}$, the total anisotropic part, is not. Note

**TABLE 1**
**Dipolar Couplings, $D_{ij}$, in Hertz, and Chemical Shifts, $\delta_{ij}$, in ppm, Obtained for a Sample of 1-Chloro-2-bromoethane Dissolved in the Nematic Solvent ZLI 1132**




| | From the normal 600-MHz proton spectrum | | | From the proton-detected 2D HSQC spectrum | |
|---|---|---|---|---|---|
| | No $^{13}C$ | $^{13}C$ on C1 | $^{13}C$ on C2 | $^{13}C$ on C1 | $^{13}C$ on C2 |
| | | | Nematic solution | | |
| $D_{56}$ | $1064.3 \pm 1.1$ | $1063.5 \pm 0.7$ | $1064.6 \pm 0.8$ | $1059.0 \pm 1.0$ | $1061.0 \pm 0.5$ |
| $D_{35} = D_{46}$ | $211.3 \pm 0.6$ | $208.6 \pm 0.8$ | $210.0 \pm 0.5$ | $208.4 \pm 1.2$ | $210.1 \pm 0.6$ |
| $D_{36} = D_{45}$ | $183.1 \pm 0.7$ | $182.2 \pm 0.9$ | $182.4 \pm 0.4$ | $184.2 \pm 1.0$ | $182.4 \pm 0.6$ |
| $D_{34}$ | $1032.2 \pm 1.0$ | $1032.5 \pm 1.1$ | $1032.2 \pm 0.2$ | $1028.9 \pm 0.9$ | $1028.0 \pm 0.6$ |
| $D_{23} = D_{24}$ | | | $1118.1 \pm 0.3$ | | $1114.1 \pm 0.8$ |
| $D_{25} = D_{26}$ | | | $82.0 \pm 1.3$ | | $83.6 \pm 0.6$ |
| $D_{13} = D_{14}$ | | $75.0 \pm 1.1$ | | $78.3 \pm 1.4$ | |
| $D_{15} = D_{16}$ | | $1179.6 \pm 0.8$ | | $1172.0 \pm 1.3$ | |
| $\delta_{53}$ | $0.186 \pm 0.002$ | $0.183 \pm 0.003$ | $0.187 \pm 0.002$ | $0.192 \pm 0.005$ | $0.192 \pm 0.002$ |
| $\delta_{12}$ | | | | $11.58 \pm 0.01$ | |
| | | | Chloroform solution | | |
| $J_{15}$ | $153.8 \pm 0.1$ | | | | |
| $J_{13}$ | $-3.1 \pm 0.1$ | | | | |
| $J_{23}$ | $155.1 \pm 0.1$ | | | | |
| $J_{25}$ | $-3.7 \pm 0.1$ | | | | |
| $J_{34}$ | $-8.5 \pm 0.1$ | | | | |
| $J_{35}$ | $5.9 \pm 0.1$ | | | | |
| $J_{36}$ | $9.7 \pm 0.1$ | | | | |
| $J_{56}$ | $-9.5 \pm 0.1$ | | | | |

*Note.* The chemical shifts and scalar couplings, $J_{ij}$, in hertz, obtained from samples dissolved in chloroform are also given.

that both parts will have a contribution from the bulk magnetic susceptibility of the sample in addition to terms of a molecular origin. We are concerned here only with noting that the difference in shielding, that is, the chemical shift $\delta_{12}$, between the two carbons is not expected to be the same as that for an isotropic sample. In fact, the value of $\delta_{12}$ measured for a sample dissolved in $CDCl_3$ is 12.67 ppm, while for the liquid crystalline sample it is 11.58 ppm. It is not expected that the sign of $\delta_{12}$ will change between isotropic and liquid crystalline phases for the $^{13}C$ nuclei in these substituted ethanes, whose orientational order is relatively small, and so we can confidently assign the peak at lower field to being carbon 1.

The $F_2$ cross-sections through the 2D spectrum at the $F_1$ values corresponding to the carbon resonances are shown in Fig. 4, and are the proton spectra of the two single $^{13}C$ isotopomers. The $F_2$ sections were analyzed separately by the conventional technique of using only the line frequencies, and this yielded the parameters in Table 1. It was then possible to identify lines in the normal proton 1D spectrum from each of the two isotopomers, and again to analyze these separately. The NMR parameters determined from the 1D and 2D experiments are given in Table 1. First, note that the values of the $D_{ij}$ obtained from the 1D experiment for the three isotopomers are in close agreement, indicating that the substitution of $^{12}C$ by $^{13}C$ has a negligible effect on either the orientational order or the vibrational averaging in this molecule. Second, the two sets of data obtained from the 2D experiment are also in very good agreement with each other, but some of the values of the $D_{ij}$ obtained from the 2D and 1D experiments differ by more than the sum of the standard deviations. This difference probably arises because of a small change in temperature between the two experiments.

The absolute overall intensity and the relative intensities of the lines in the $F_2$ sections shown in Fig. 4 are expected to depend strongly on the value chosen for $\tau_1$. This dependence has been explored by simulating the response of the spin system of both of the isotopomers, $Cl^{13}CH_2CH_2Br$ and $ClCH_2{}^{13}CH_2Br$, to the same pulse sequence as in the 2D HSQC, but keeping all the delays fixed except $\tau_1$ which was varied in steps of 50 $\mu$s between 3 $\mu$s and 5 ms. The total integrated intensity $I(\tau_1)$ of each spectrum is shown in Fig. 5 as a function of $\tau_1$. As expected for a strongly coupled group of spins the value of $I(\tau_1)$ is always finite, and is a maximum at about $\tau_1 = 1.6$ ms, but

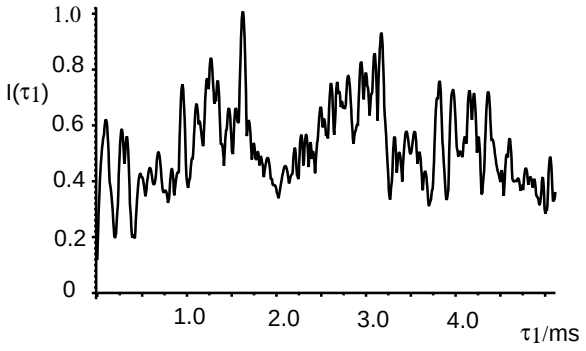



**FIG. 5.** The total integrated intensity $I(\tau_1)$ of spectra simulated for the isotopomers $Cl^{13}CH_2CH_2Br$ and $ClCH_2{}^{13}CH_2Br$ when in an ordered phase and with the parameters given in Table 1 and obtained with the sequence in Fig. 2a. All the delays in the simulated sequence were fixed, except for $\tau_1$, which was varied in steps of 50 $\mu$s from 3 $\mu$s to 5 ms.

when $\tau_1$ is $>0.5$ ms there is always $\geq 0.4$ of the maximum intensity. The relative intensities of the lines in each of the simulated spectra vary quite strongly with $\tau_1$, and this is shown in Fig. 6, in which simulated spectra are presented corresponding to a selected range of values of $\tau_1$. Figure 6 also contains a normal 1D spectrum simulated with the same values of the chemical shifts and coupling constants. These results show that there are significant differences in intensity between some of the lines and those simulated for the 1D normal spectrum. For these isotopomers $\tau_{1\,\max}$ is very close in value to $1/4T_{13} = 1.7$ ms. The spectrum simulated for $\tau_1 = 1/4T_{15} = 0.103$ ms is shown in Fig. 6c, and it is seen that many lines are of very low intensity, and so this would not be a good choice for the fixed delay in a real experiment. In fact, such a short value of $\tau_1$ is not practicable

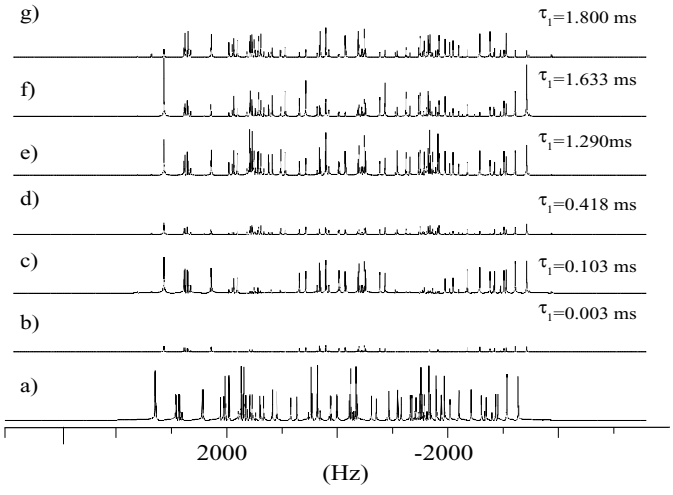



**FIG. 6.** Proton spectra for $Cl^{13}CH_2CH_2Br$ and $ClCH_2{}^{13}CH_2Br$ obtained by (a) simulation of the normal 1D spectrum with the parameters in Table 1 and (b–g) simulations of the 1D HSQC experiment with values of the delay $\tau_1$ as shown against each spectrum.

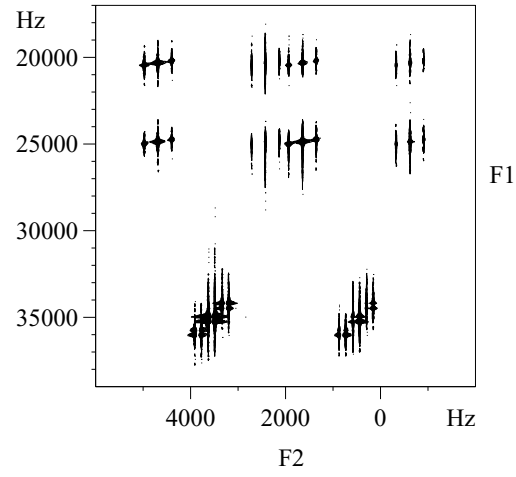



**FIG. 7.** The 600-MHz 2D HSQC spectrum of 1,2-dibromo-2,2-difluoroethane dissolved in the nematic solvent ZLI 1132, and obtained with version (b) of the sequence in Fig. 2. The delay $\tau_1$ was fixed at 1.8 ms. The delay $t_1$ was incremented in 256 steps of 25 $\mu$s to give a spectral width after Fourier transformation of 40 kHz and a digital resolution of 160 Hz in the $F_1$ dimension. The 90° proton pulse width was 5.6 $\mu$s, and the 90° carbon pulse was 16.4 $\mu$s. The free induction decays were recorded for 0.4 s into 16 K words computer memory giving a digital resolution in the $F_2$ dimension of 1.25 Hz. A 90° shifted sinebell function was applied in both dimensions prior to Fourier transformation. The spectrum is displayed in magnitude mode.

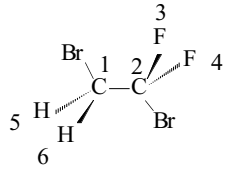
in real experiments of the type used here since it does not allow sufficient time for application of the gradient pulses. Note that the simulated spectrum for $\tau_1 = 1.8$ ms $(=1/4J_{15})$, which was the initial, trial value used in the real experiments, does give a reasonable, relative intensity distribution and an acceptable overall intensity, but it is not the optimum choice. The choice of $\tau_1 = 1.8$ ms was guided by the idea that some of the lines in the proton spectra would depend strongly on the smaller values of ${}^nT_{CH}$, and a guess that these might be close in value to ${}^1J_{CH}$. This gave a reasonable spectrum for this particular sample, as did $\tau_1 = 1.6$ ms, because $I(\tau_1)$ lies in the range 0.4 to 1.0 for the range $\tau_1 = 0.5$ to 5.0 ms.

Figure 7 shows the 2D HSQC spectrum of 1,2-dibromo-2,2-difluoroethane dissolved in ZLI 1132, which was obtained with version (b) of the pulse sequence, that is, without refocusing of the carbon–proton couplings in the $t_1$ domain. The interval $\tau_1$ was again set to the value, 1.8 ms, which would maximize the transfer efficiency for an isotropic sample: for this sample ${}^1J_{CH} = 156.7$ Hz, ${}^1D_{CH} = 1058$ Hz, ${}^2J_{CH} = -3.5$ Hz, and ${}^2D_{CH} = 72$ Hz.

The projection onto $F_1$ shows a clear triplet of doublets structure for C2, and a doublet of triplets for C1. The triplet structure is attributed to coupling between the carbons and the fluorines, and it was confirmed by a simulation of the 2D spectrum using NMRSIM that the splitting is $(J_{CF} + 2D_{CF})$. The simulation also confirmed that the doublet structure arises from coupling to the two protons and is equal to $2(J_{CH} + 2D_{CH})$. The doublet structure arises because the center line of the triplet expected

**TABLE 2**

**Dipolar, $D_{ij}$, and Scalar, $J_{ij}$, Couplings in Hertz, and Chemical Shifts, $\delta_{ij}$, in ppm, Obtained for a Sample of 1,2-Dibromo-2,2-difluoroethane Dissolved in the Nematic Solvent ZLI 1132**




| | From the normal 600-MHz proton spectrum | | | From the proton-detected 2D HSQC spectrum | |
|---|---|---|---|---|---|
| | No $^{13}$C | $^{13}$C on C1 | $^{13}$C on C2 | $^{13}$C on C1 | $^{13}$C on C2 |
| | | | Nematic solution | | |
| $D_{56}$ | $1019.4 \pm 0.2$ | $1019.5 \pm 0.9$ | $1019.8 \pm 0.3$ | $1013.0 \pm 0.4$ | $1012.3 \pm 0.5$ |
| $D_{35} + D_{45}$ | $278.9 \pm 0.5$ | $276.3 \pm 0.5$ | $279.4 \pm 0.5$ | $278.5 \pm 0.5$ | $277.0 \pm 0.5$ |
| $D_{15} = D_{16}$ | | $1057.9 \pm 0.9$ | | $1053.4 \pm 0.5$ | |
| $D_{25} = D_{26}$ | | | $71.8 \pm 0.3$ | | $70.1 \pm 0.6$ |
| $D_{13} = D_{14}$ | | | | $59 \pm 0.5$ | |
| $D_{23} = D_{24}$ | | | | | $541 \pm 1$ |
| $D_{34}$[a] | 521.0 | 521.0 | 521.0 | 521.0 | 521.0 |
| $\delta_{12}$ | | | | $82.1 \pm 0.01$ | |
| | | | Chloroform solution | | |
| $J_{15}$ | $156.7 \pm 0.1$ | | | | |
| $J_{25}$ | $-3.6 \pm 0.1$ | | | | |
| $J_{23}$ | $-304.5 \pm 0.1$ | | | | |
| $J_{13}$ | $\pm 27.3 \pm 0.1$ | | | | |
| $J_{35}$ | $13.3 \pm 0.1$ | | | | |
| $J_{45}$ | $12.6 \pm 0.1$ | | | | |

*Note.* The $^{13}$C chemical shifts and scalar couplings to proton, $J_{ij}$, in hertz, obtained from samples dissolved in chloroform are also given.

[a] Estimated from a $^{19}$F spectrum of a similar sample taken at 338 MHz.

for coupling with two protons in a 1D experiment does not evolve in INEPT transfers. The observed triplet splitting on C2 of $\pm 783 \pm 3$ Hz gives $D_{23} = 541 \pm 1$ or $-241 \pm 1$ Hz. This compares with a value of $D_{23} = 539.8 \pm 0.4$ Hz measured from a $^{13}$C spectrum recorded on the same sample, and whose sign was determined by recording spectra in liquid crystalline solvent mixtures having an anisotropy in their magnetic susceptibility, $\Delta\chi$, close to zero (*6*). In such solvents it is possible to record spectra corresponding to the director being both parallel and perpendicular to the magnetic field and hence to obtain the relative signs of the scalar and anisotropic spin–spin couplings.

The doublet splitting on C2 of $\pm 293 \pm 2$ Hz gives $D_{25} = 75.1 \pm 0.5$, or $-71.5 \pm 0.5$ Hz, compared with a value of $71.0 \pm 0.6$ Hz obtained from the proton spectrum, where again the sign has been determined by experiments on $\Delta\chi = 0$ mixtures.

The splittings along $F_1$ for C1 are consistent with $D_{15} = 1054 \pm 1$ Hz, which compares with a value of $1053.4 \pm 0.5$ Hz obtained from the proton spectrum (see Table 2), and $D_{13} = 55 \pm 5$ Hz, compared with $59.0 \pm 0.5$ Hz.

The separation along $F_1$ of the centers of the lines from C1 and C2 gives the chemical shift difference as $\delta_{12} = 82.1$ ppm, compared with the value obtained for a sample dissolved in chloroform of 80.66 ppm.

Figure 8 is the 2D HSQC spectrum of 1,2-dibromo-2,2-difluoroethane dissolved in ZLI 1132, which was obtained with version (a) of the pulse sequence, that is, with refocusing of the carbon–proton couplings in the $t_1$ domain. The splitting in the carbon dimension from coupling to the fluorines is now more clearly evident; however, the values measured for the $D_{CF}$ couplings from this spectrum are less precise than those obtained from Fig. 7. This is evident from the variations in the values obtained from different line separations, and we attribute this to a lack of precision in the refocusing of the CH couplings (*7*, *8*).

Figure 9 shows the $F_2$ cross sections of the peaks from carbons 1 and 2 in Fig. 7, and for comparison the normal 1D proton spectrum is expanded vertically to show the satellite lines. Note that the 1D spectrum contains a considerable number of lines from impurities which are comparable in intensity to the satellite lines. Table 2 compares the data obtained from analysis of the cross sections in the 2D HSQC experiment with that obtained by analysis of the $^{13}$C satellite lines in the normal proton and fluorine spectra. The agreement between the $D_{ij}$ values obtained by the two methods is similar to that for the

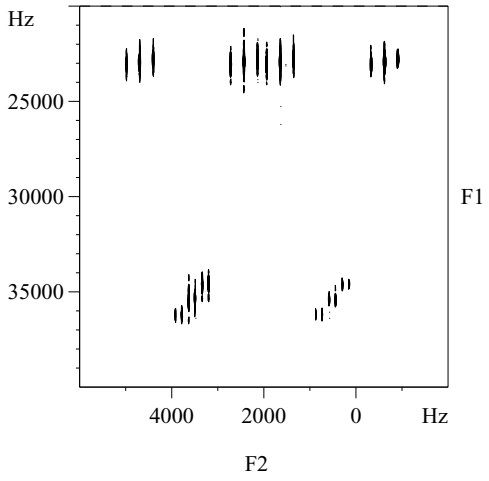



**FIG. 8.** The 600-MHz 2D HSQC spectrum of 1,2-dibromo-2,2-difluoroethane dissolved in the nematic solvent ZLI 1132, and obtained with version (a) of the sequence in Fig. 2. The delay $\tau_1$ was fixed at 1.8 ms. The delay $t_1$ was incremented in 256 steps of 25 $\mu$s to give a spectral width after Fourier transformation of 40 kHz and a digital resolution of 160 Hz in the $F_1$ dimension. The 90° proton pulse width was 5.6 $\mu$s, and the 90° carbon pulse was 16.4 $\mu$s. The free induction decays were recorded for 0.4 s into 16 K words computer memory giving a digital resolution in the $F_2$ dimension of 1.25 Hz. A 90° shifted sinebell function was applied in both dimensions prior to Fourier transformation. The spectrum is displayed in magnitude mode.

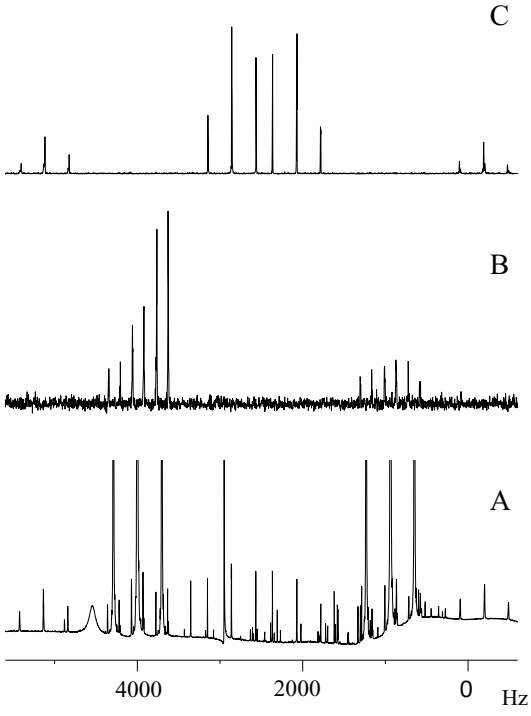



**FIG. 9.** (A)The normal 1D 600-MHz spectrum of 1,2-dibromo-2,2-difluoroethane in ZLI 1132 expanded vertically to show the $^{13}$C satellites. (B) and (C) are $F_2$ cross sections corresponding to the peaks in Fig. 7 which stem from carbons 1 and 2.

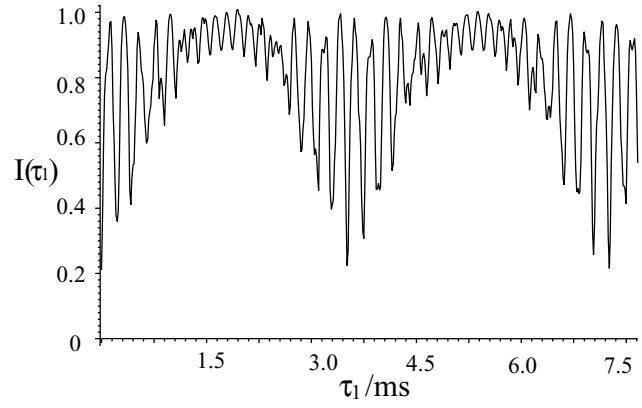



**FIG. 10.** The total integrated intensity $I(\tau_1)$ of spectra simulated for the isotopomers Br $^{13}CH_2CF_2Br$ and $BrCH_2{}^{13}CF_2Br$ when in an ordered phase and with the parameters given in Table 2 and obtained with the sequence in Fig. 2b. All the delays in the simulated sequence were fixed, except for $\tau_1$, which was varied in steps of 50 $\mu$s from 3 $\mu$s to 5 ms.

1-chloro-2-bromoethane, and can be attributed to small differences in temperature in the two experiments.

We have explored the dependence on $\tau_1$ of the intensities of the lines in the HSQC experiment on this molecule by simulating the response to the pulse sequence by NMRSIM. In this molecule, unlike 1-chloro-2-bromoethane, the two singly $^{13}$C containing isotopomers are expected to show a very different dependence of the intensity with $\tau_1$. This is because of the different magnitudes of the couplings which can transfer magnetization between $^{13}$C and $^{1}$H. Thus, in the isotopomer $Br^{13}CH_2CF_2Br$ there is a large $^1T_{CH}$ coupling, whereas in $BrCH_2{}^{13}CF_2Br$ there is just one $^2T_{CH}$. In Fig. 10 the dependence on $\tau_1$ of the total intensity in the $F_2$ simulated spectrum is shown. The data are for both isotopomers and were obtained by summing the spectra from simulations on the separate species. We can see clearly two oscillations, one rapid and one slow, associated with $^1T_{CH}$ and $^2T_{CH}$, respectively.

## CONCLUSIONS

The 2D HSQC experiments on these two substituted ethanes dissolved in a nematic solvent yield a wealth of information. For both molecules it was possible to obtain values of $^nD_{CH}$ and the relative chemical shifts of the $^{13}$C nuclei. In the present examples the values of $\delta_{12}$ were used only to assign the peaks, but they can also be used in the analysis of the $^{13}$C spectra of these and similar samples. In this case being able to obtain their values in this direct way can be a considerable advantage.

The proton-detected 2D HSQC experiment on the sample of 1,2-dibromo-2,2-difluoroethane yielded values for $|^nJ_{CF} + 2^nD_{CF}|$. The generality of this indirect detection of coupling between $^{13}$C and a nucleus whose resonance has not been excited in the experiment needs to be explored further, but it promises to make these types of experiment even more attractive for liquid crystalline samples.

The present experiments were deliberately done on samples where it was possible to obtain the values of the dipolar couplings to $^{13}C$ by single-pulse acquisition, so that it was possible to validate the data obtained by 2D HSQC. Experiments have also been performed on more complex molecules for which the dipolar couplings were not known in advance, and it has proved an easy task to choose an interval $\tau_1$ which gives a good intensity for all the carbon satellite spectra.

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## REFERENCES

1. G. Bodenhausen and D. Ruben, *Chem. Phys. Lett.* **69,** 185 (1980).
2. C. J. Turner, *in* "Encyclopedia of Nuclear Magnetic Resonance" (D. M. Grant and R. K. Harris, Eds.), p. 2335, Wiley, Chichester (1996).
3. G. A. Morris and R. Freeman, *J. Am. Chem. Soc.* **101,** 760 (1979).
4. Yu-Chi Li and G. T. Montelione, *J. Magn. Reson.* **101,** 315 (1993).
5. W. Willker, D. Leibfritz, R. Kerssebaum, and W. Bermel, *Magn. Reson. Chem.* **31,** 287 (1993).
6. J. W. Emsley and D. L. Turner, *J. Chem. Soc. Faraday Trans.* **77,** 1493 (1981).
7. A. G. Avent, J. W. Emsley, and D. L. Turner, *J. Magn. Reson*. **52,** 57 (1983).
8. N. Suryaprakash, D. Merlet, and J. W. Emsley, to be published.