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Extraction of ^1H–^1H and ^1H–^{13}C Dipolar Couplings from Spectra Acquired in Inhomogeneous Magnetic Fields

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Experimental procedures are presented for the extraction of ^1H–^1H and ^1H–^{13}C dipolar couplings from molecules oriented in high magnetic fields without a requirement for high field homogeneity. These couplings impose restrictions on structure in a manner similar to NOE distances and can serve as useful additional constraints for defining structure. The experimental procedures involve pulse sequences which refocus the effects of magnetic field inhomogeneities but which retain dipolar couplings, scalar couplings and, in cases of chemically distinct groups, chemical shift differences. The methods are important for the extension of experiments to higher fields and more complex media where field homogeneity is hard to maintain. The experiments are illustrated using samples containing sodium acetate-99% $^{13}C_2$ or alanine-99% $^{13}C_3$ dissolved in a nematic liquid crystal and oriented in an inhomogeneous magnetic field. Coupling constants obtained from the studies are in good agreement with couplings extracted from one-dimensional spectra obtained in homogeneous fields.

KEY WORDS Two-dimensional NMR Liquid crystals Dipolar couplings Field inhomogeneity

INTRODUCTION

Recent advances in macromolecular structure determination in solution have relied largely on measurements of dipole–dipole relaxation rates in high-resolution proton NMR spectra (i.e. nuclear Overhauser effects).[1] Very high and very homogeneous magnetic fields are required in the standard experiments employed to achieve the resolution of a sufficient number of resonances to provide the necessary geometric constraints for a structure determination. Even with commercial magnets approaching 14 T, studies have been relegated to readily soluble molecules of molecular weight less than 10 000 daltons.[2] It seems likely that fields could be pushed higher and the breadth of systems amenable to study expanded if requirements for homogeneous fields could be relaxed without losing information needed for specific assignments or distance determinations. Precedents for these types of experiments exist in two-dimensional J-resolved spectra[3] and in the earliest versions of scalar coupling correlated two-dimensional spectra, SECSY.[4,5] Although they have never been fully exploited for this purpose, these experiments are actually acquired in a mode whereby field inhomogeneity effects in the indirectly detected F_1 dimension cancel. More recently, Weitekamp *et al.*[6] have used a more general experiment in which N quantum coherence is allowed to evolve as part of a t_1 period, and then l quantum magnetization created from this coherence is allowed to evolve in an opposite direction for N times that part of the t_1 period to cancel inhomogeneity effects. Hall and Norwood have also proposed both the use of zero quantum experiments[7] and constant-time SECSY experiments[8] in *in vivo* metabolic work to allow for the refocusing of field inhomogeneity effects while retaining both chemical shift and scalar coupling information.

Although it is possible to modify the NOE experiments usually used to extract structural information to take advantage of the resolution offered by experiments such as those discussed above, this would be very costly in terms of acquisition time. The acquisition of spectra with adequate resolution in both F_1 and F_2 would, in fact, require extension of the above 2D experiments to a third dimension. Structural information can, however, be obtained from sources other than NOE experiments.[9] For example, at sufficiently high magnetic field strengths, molecules with sufficiently anisotropic susceptibilities orient and residual dipolar splittings can be observed.[10] Alternatively, molecules lacking adequate susceptibilities can be made to orient in applied magnetic fields using liquid crystal solvents.[11] Residual ^1H–^1H and ^1H–^{13}C dipolar splittings from ordered molecules can impose restrictions on allowed angles that can define structure in much the same way that restrictions on inter-proton distance from NOESY data sets are now used to define structures. Spectra from these molecules can be exceedingly complex owing to the extensive coupling and strong background ^1H signals. Use of heteronuclear filters can, however, eliminate much of this complexity.[12]

In this paper we present some straightforward extensions of J-resolved and SECSY pulse sequences which allow the measurement of ^1H–^1H and ^1H–^{13}C dipolar splittings for liquid crystal samples in inhomogeneous magnetic fields. We first consider a theoretical description of the effects of one of these sequences on AX_3 spin systems (A = ^{13}C, X = ^1H). We then present an application to the measurement of ^1H–^{13}C and ^1H–^1H dipolar splittings of sodium acetate-99% $^{13}C_2$ and

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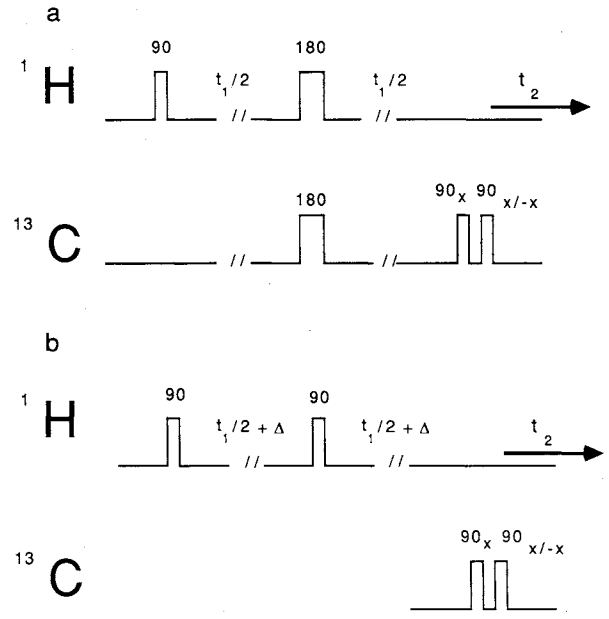

Figure 1. Pulse sequences used to extract ^1H–^1H and ^1H–^{13}C couplings from oriented molecules in inhomogeneous magnetic fields. Sequence (a) refocuses both inhomogeneity and chemical shift effects in ω_1, while sequence (b) retains the effects of chemical shift in ω_1.

alanine-99% $^{13}C_3$ dissolved in a lyotropic liquid crystal and oriented in an inhomogeneous magnetic field.

THEORY

Let us first consider an extension of the *J*-resolved experiment[3] to a heteronuclear case showing both scalar and dipolar coupling. No chemical shift resolution is obtained, but an ability to extract needed dipolar information is demonstrated. Figure 1a shows a pulse sequence used in this case. The sequence consists of a spin–echo which refocuses the effects of field inhomogeneity during t_1 followed by a ^{13}C filter which selects for ^1H magnetization coupled to ^{13}C. The effects of the sequence on an isolated AX_3 spin system can be placed on a quantitative basis by considering the product operator description of the evolution of the density matrix.[13]

The Hamiltonian describing the X spins in an oriented AX_3 spin system in an inhomogeneous magnetic field can be written as

$$\mathbf{H} = \mathbf{H}_z + \mathbf{H}_c \tag{1}$$

where $\mathbf{H}_z$ represents the contribution of $\mathbf{H}$ due to the Zeeman interaction and $\mathbf{H}_c$ represents scalar and dipolar coupling terms. We can write

$$\mathbf{H}_z = -[\omega_0 + \Delta\omega(x, y, z)]\mathbf{X}_z \tag{2}$$

where ω_0 is the Larmor frequency of spin X, $\Delta\omega(x, y, z)$ represents the field inhomogeneity contribution to $\mathbf{H}_z$ at the point (x, y, z) in the sample and $\mathbf{X}_z$ represents the z component of spin angular momentum associated with spin X. The coupling part of the Hamiltonian can be written as follows:

$$\mathbf{H}_c = 2\pi D \sum_{i>j} (3\mathbf{X}_{iz}\mathbf{X}_{jz} - \mathbf{X}_i \cdot \mathbf{X}_j) + 2\pi \sum_{i>j} J_{ij}\mathbf{X}_i \cdot \mathbf{X}_j + 2\pi D'\mathbf{X}_z \cdot \mathbf{A}_z \tag{3}$$

where J_{ij} is the scalar coupling between ^1H spins i and j, $\mathbf{X}_{iz}$ is the z component of angular momentum associated with proton i, $\mathbf{X}_z = \sum \mathbf{X}_{iz}$, $\mathbf{A}_z$ is the z component of spin angular momentum associated with the A spin and D and D' are the ^1H–^1H dipolar and ^1H–^{13}C couplings, respectively, given by

$$D = (\gamma_x{}^2\hbar)/(4\pi r_{ij}^3) \cdot \langle 1 - 3\cos^2\theta \rangle \cdot f_{HH}(\theta') \tag{4}$$

and

$$D' = (\gamma_X\,\gamma_A\,\hbar)/(2\pi r_{AX}^3) \cdot \langle 1 - 3\cos^2\theta \rangle \cdot f_{HC}(\theta') + J_{H-C} \tag{5}$$

In Eqns (4) and (5) the angular brackets denote a time average, θ is the angle between the methyl three-fold axis and the applied magnetic field, γ_X is the gyromagnetic ratio of spin X, $\hbar$ is Planck's constant divided by 2π, r_{ij} denotes the distance between spins i and j, J_{H-C} is the ^1H–^{13}C scalar coupling constant and $f(\theta')$ is a factor which scales the dipolar interaction due to motional averaging. For the axially symmetric scaling considered here, $f(\theta')$ is a function of the angle θ' that the dipolar vector makes with respect to the averaging axis:

$$f(\theta') = (3\cos^2\theta' - 1)/2 \tag{6}$$

It is clear that D and D' contain structural information. For the case of a methyl group θ' is 90° and 109.5° for the ^1H–^1H and ^1H–^{13}C vectors, respectively. In this case, $f_{HH}(\theta') = -0.50$ and $f_{HC}(\theta') = -0.33$.

The relationships

$$[\mathbf{X}_z, \mathbf{X}_i \cdot \mathbf{X}_j] = [\mathbf{X}_y, \mathbf{X}_i \cdot \mathbf{X}_j] = [\mathbf{X}_x, \mathbf{X}_i \cdot \mathbf{X}_j] = 0 \tag{7}$$

imply that the terms proportional to $\mathbf{X}_i \cdot \mathbf{X}_j$ do not contribute to the evolution of the spin system and hence can be omitted, yielding a simplified form of Eqn (3):

$$\mathbf{H}_c' = 2\pi \sum_{i>j} 3D\mathbf{X}_{iz} \cdot \mathbf{X}_{jz} + 2\pi D'\mathbf{X}_z \cdot \mathbf{A}_z \tag{8}$$

After the first 90° pulse of sequence 1a, the density matrix for the system can be written in terms of a single product operator, $\mathbf{X}_y$. The effects of t_1 evolution, including application of simultaneous ^1H and ^{13}C 180° pulses, can be described by

$$\rho(t_1) = \mathbf{R} \cdot (-\mathbf{X}_y) \cdot \mathbf{R}^{-1} \tag{9}$$

where $\mathbf{R}$ is an operator given by

$$\mathbf{R} = \exp(i\mathbf{H}t_1/2)\exp(-i\pi\mathbf{S}_x)\exp(-i\pi\mathbf{I}_x) \times \exp(i\mathbf{H}t_1/2) \tag{10}$$

and $\rho(t_1)$ is the density matrix after a total time delay t_1. Using commutator properties of the operators involved, Eqn (9) can be simplified to give

$$\rho(t_1) = \exp(i\mathbf{H}_c't_1) \cdot (\mathbf{X}_y) \cdot \exp(-i\mathbf{H}_c't_1) \tag{11}$$

which, on substitution of Eqn (8) for $\mathbf{H}_c'$, gives

$$\begin{aligned}\rho(t_1) = &- 2\mathbf{X}_x\mathbf{A}_z(\cos^2 3\pi Dt_1)(\sin \pi D't_1)\\ &- \sum_{i\neq k} 4\mathbf{X}_{iy}\mathbf{X}_{kz}\mathbf{A}_z(\cos 3\pi Dt_1)\\ &\times(\sin 3\pi Dt_1)(\sin \pi D't_1)\\ &+ \tfrac{1}{2}\sum_{i\neq j\neq k} 8\mathbf{X}_{ix}\mathbf{X}_{jz}\mathbf{X}_{kz}\mathbf{A}_z(\sin^2 3\pi Dt_1)\\ &\times(\sin \pi D't_1 + \text{terms not proportional to } \mathbf{A}_z\end{aligned} \tag{12}$$

Note that the effect of the simultaneous $\pi\{^1H, ^{13}C\}$ pulses is to refocus the effects of chemical shift and field inhomogeneities leaving no dependence on ω_0 or $\Delta\omega$. Couplings, however, continue to evolve.

Application of the next two ^{13}C pulses with the phase cycle depicted in Table 1 eliminates all terms not proportional to $\mathbf{A}_z$. Therefore, in considering the t_2 evolution that follows, we shall neglect these terms and consider only observable magnetization. We can write the density matrix evaluated at the point (x, y, z) in the sample as

$$\begin{aligned}\rho(x, y, z) \propto \{&2[\exp(i\pi D't_1)\exp(i\pi D't_2)\\ &- \exp(i\pi D't_1)\exp(-i\pi D't_2)]\\ &+ \exp[i\pi(D' + 6D)t_1]\exp[i\pi(D' + 6D)t_2]\\ &- \exp[i\pi(D' + 6D)t_1]\exp[i\pi(-D' + 6D)t_2]\\ &- \exp[i\pi(D' - 6D)t_1]\exp[-i\pi(D' + 6D)t_2]\\ &+ \exp[i\pi(D' - 6D)t_1]\exp[i\pi(D' - 6D)t_2]\\ &+ \text{complex conjugate terms}\}\\ &\times\{\mathbf{I}_y\cos[\omega_0 + \Delta\omega(x, y, z)t_2]\\ &- \mathbf{I}_x\sin[\omega_0 + \Delta\omega(x, y, z)t_2]\}\end{aligned} \tag{13}$$

Inspection of Eqn (13) shows that we should expect twelve resonances in a two-dimensional presentation. They occur at (F_1, F_2) frequencies of $(D'/2, D'/2)$, $(D'/2, -D'/2)$, $(D'/2 + 3D, D'/2 + 3D)$, $(D'/2 + 3D, -D'/2 + 3D)$, $(D'/2 - 3D, -D'/2 - 3D)$, $(D'/2 - 3D, D'/2 - 3D)$ and frequencies obtained by replacing D' and D with $-D'$ and $-D$ in the above expressions. In the F_1 dimension, where only coupling terms exist, these peaks are centered about 0 Hz, and in the F_2 dimension they are centered at the chemical shift offset $\omega_0/2\pi$, dispersed by homogeneity effects, $\Delta\omega/2\pi$.

Table 1. Phase cycling for the sequence in Fig. 1a

	^1H	
90°	180°	Receiver
X	X	X
X	X	-X
X	X	X
X	X	-X
X	X	X
X	X	-X
X	X	X
X	X	-X
	^{13}C	
180°	(90°)	(90°)
X	X	-X
X	X	X
Y	X	-X
Y	X	X
-X	X	-X
-X	X	X
-Y	X	-X
-Y	X	X

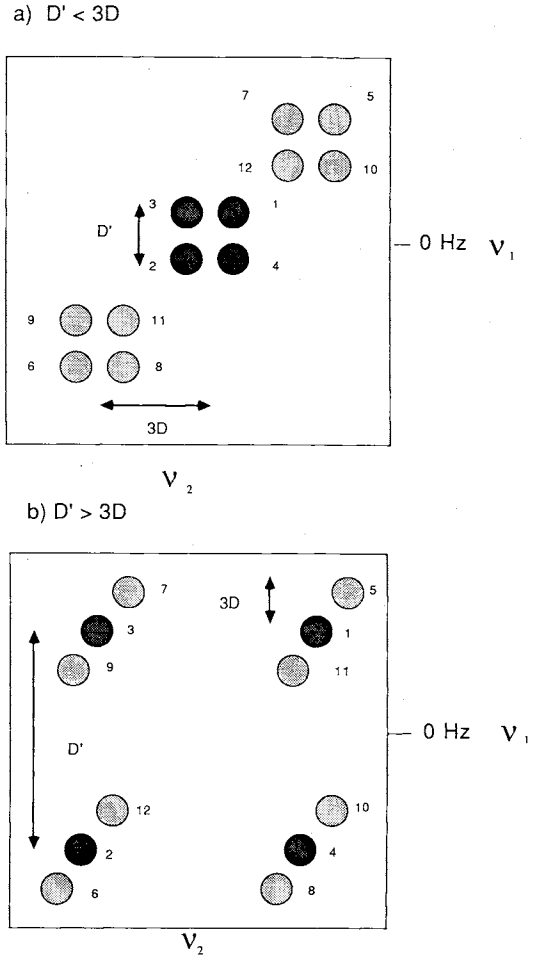

Figure 2. Theoretical contour plots obtained with the sequences in Fig. 1a for an AX_3 spin system with (a) $D' < 3D$ and (b) $D' > 3D$. The numbers adjacent to each multiplet component indicate how the components move on going from (a) to (b), while increasing color density of the multiplet components indicates increasing peak intensity.

The patterns in a two-dimensional plot can look surprisingly different depending on the relative sizes of the ^1H–^{13}C coupling, D', and the ^1H–^1H coupling, D. These patterns are illustrated for the cases $D' < 3D$ and $D' > 3D$ in Fig. 2a and b, respectively. In favorable cases these characteristic patterns allow an easy determination of the relative magnitudes of the ^1H–^{13}C and ^1H–^1H couplings.

A drawback of the sequence of Fig. 1a is that in addition to field inhomogeneity effects, chemical shift information is also eliminated. Chemical shift resolution can be restored by a number of pulse sequences. Figure 1b, together with the phase cycling in Table 2, illustrates one such example.

The effects of this sequence on a spin system can also be understood by a product operator treatment,[13] but a qualitative description suffices here. In a homonuclear

Table 2. Phase cycling for the sequence in Fig. 1b

	^{1}H	
90°	90°	Receiver
X	X	X
X	X	−X
X	Y	−X
X	Y	X
X	−X	X
X	−X	−X
X	−Y	−X
X	−Y	X
	^{13}C	
(90°)	(90°)	
X	X	
X	−X	
X	X	
X	−X	
X	X	
X	−X	
X	X	
X	−X	

AX spin system, for example, magnetization originating on spin A and processing with chemical shift ω_A and with a field inhomogeneity contribution ω_h during the first half of the evolution period is transferred to spin X by the action of the mixing pulse. Precession now occurs at a rate $-\omega_X$ and, neglecting the effects of diffusion, with an inhomogeneity contribution of $-\omega_h$. Thus, at $t = t_1$, the effects of the field inhomogeneity cancel and magnetization appears to have evolved at the difference in chemical shifts between the coupled spins. Both scalar coupling and dipolar effects associated with spins A and X are retained.

EXPERIMENTAL

The lyotropic liquid crystal phase used was originally reported by Lawson and Flautt.[14] It was prepared from a mixture of decyl sulfate, decyl alcohol, sodium sulfate and D_2O in the proportions 40:5:5:50 by weight. In all cases either sodium acetate-99% $^{13}C_2$ or alanine-99% $^{13}C_3$ was added to the solvent in amounts between 1 and 2% by weight.

All NMR spectra were acquired on a laboratory-built 490 MHz spectrometer operating in the Fourier transform mode. All spectra were recorded at 330 K. For spectra acquired with the sequence in Fig. 1a 100 experiments were obtained, each consisting of 1K complex points. Sixteen scans of the phase cycle were acquired with a relaxation delay of 4 s to give a total measuring time of 2.2 h. The data were processed with a straight Fourier transformation in t_2, a cosine-bell of the first 100 points in t_1, followed by zero-filling to yield a 256 × 1K real data matrix. Sweep widths of 5000 Hz in ω_2 and 800 Hz in ω_1 were obtained. The spectra were recorded in the magnitude mode.

A data set for the alanine-99% $^{13}C_3$ sample was acquired with the sequence of Fig. 1b. In this case 200 experiments, each consisting of 1K complex points, were obtained. Fifty-six scans were obtained per experiment, with a relaxation delay of 2.3 s to yield a measuring time of approximately 8 h. Δ was set to 15 ms to facilitate transfer of magnetization between the weakly coupled α- and β-protons of alanine. The data were processed with a cosine-bell weighting function applied to the first 200 points in t_2 followed by Fourier transformation. In t_1, a sine-bell weighting function shifted by 55° was applied and the data subsequently zero filled to 1K. Sweep widths of 5000 Hz in ω_2 and 2000 Hz in ω_1 were obtained and the data displayed in the magnitude mode.

All spectra were processed on a VAX 11/750 computer equipped with a Minimap array processor using software written by Dr D. Hare.

RESULTS AND DISCUSSION

Figure 3A shows a 1D ^{1}H NMR spectrum of sodium acetate-99% $^{13}C_2$ dissolved in the liquid crystal discussed above and oriented in a homogeneous magnetic field. The spectrum was acquired with a spin–echo sequence, 90°–τ–180°–τ–acquire, τ = 0.75 ms, to eliminate broad residual signals from the liquid crystal. Note that in this case $|D'| = 63$ Hz $< |3D| = 140$ Hz (i.e. the ^{1}H–^{13}C splitting, $|D'|$, is less than the ^{1}H–^{1}H dipolar splitting, $|3D|$). Figure 3B shows the spectrum of the same sample acquired under identical conditions, with the exception that the magnetic field was deshimmed. In this spectrum no information concerning chemical shifts or coupling constants can be obtained.

Information concerning couplings can be retrieved, however, by using the sequence in Fig. 1a. The sequence retains all dipolar and scalar couplings and refocuses chemical shift and magnetic field inhomogeneity effects. Figure 4 shows a contour plot presentation of the spectrum obtained from the sample in Fig. 3B. The pattern

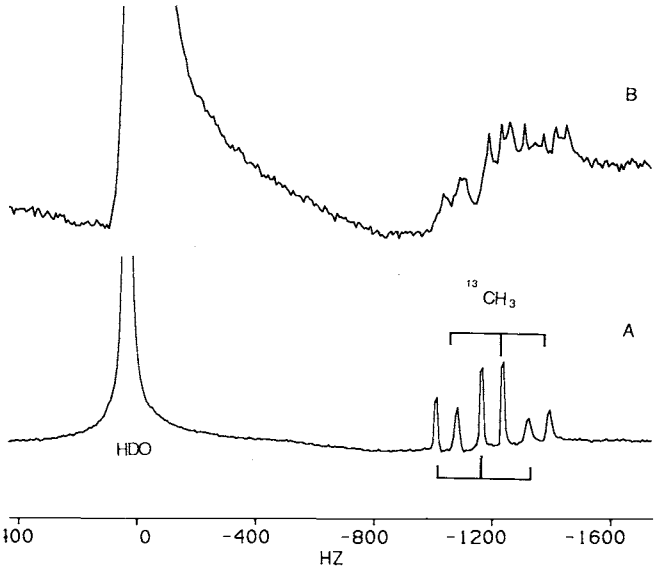

Figure 3. (A) High-resolution spectrum of sodium acetate-$^{13}C_2$ dissolved in a liquid crystal solvent at 330 K; (B) spectrum of the same sample acquired under identical conditions, with the exception that the magnetic field was initially deshimmed. Chemical shifts are relative to HDO.

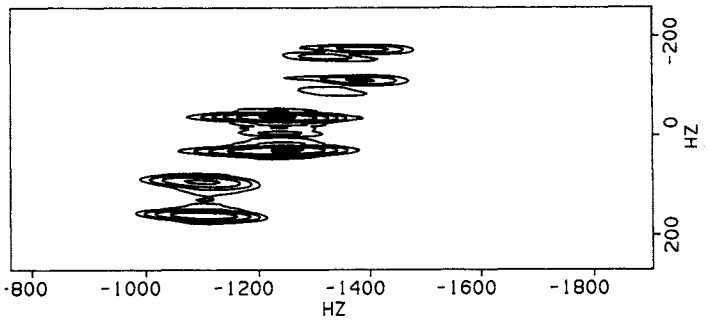

Figure 4. Contour plot presentation of the spectrum obtained from the sample in Fig. 3B using the sequence in Fig. 1a. Chemical shifts in F_2 are relative to HDO, and in F_1 the center of the spectrum is at 0 Hz. Spectral acquisition and processing parameters are indicated in the text.

is of the type shown in Fig. 2a. The resolution, however, is not adequate in the F_2 dimension to show resolvable splittings. The values of $|D'| = 68$ Hz and $|3D| = 131$ Hz, extracted from the spectrum in the F_1 dimension, are in good agreement with the values obtained from the 1D spectrum in Fig. 3A.

Figure 5A and B show 1D ^{1}H spectra for oriented alanine-99% $^{13}C_3$ in homogeneous and inhomogeneous magnetic fields, respectively. The spectra were acquired with a spin–echo sequence with $\tau = 0.75$ ms. Figure 5A shows that the coupling parameters for the methyl protons ($\nu = -1335$ Hz relative to HDO) are $|D'| = 114$ Hz and $|3D| = 42$ Hz. In addition, the methine proton at $\nu = -212$ Hz shows a splitting of 15 Hz due to the ^{1}H–^{13}C heteronuclear interaction, with the ^{1}H–^{1}H interaction too small to resolve.

Figure 6 shows a contour plot of the spectrum obtained from the sequence in Fig. 1a for the alanine sample oriented in an inhomogeneous field. Resolution in the F_2 dimension is again such that useful coupling information cannot be extracted. However, it is clear that this pattern resembles Fig. 2b, consistent with $3D < D'$. Once again, the effects of field inhomogeneity are refocused along F_1, allowing extraction of ^{1}H–^{1}H and ^{1}H–^{13}C coupling parameters. Values of $|D'| = 111$ Hz and $|3D| = 40$ Hz are obtained.

In many cases useful structural information can be obtained more readily from molecules labelled with ^{13}C in several sites. Knowledge of several ^{1}H–^{13}C dipolar splittings allows, in the case of rigid molecules, a determination of the relative orientation of the ^{1}H–^{13}C bonds. Extraction of data from multiply labelled molecules requires a pulse sequence which retains chemical shift resolution while refocusing magnetic field inhomogeneity effects. Figure 1b illustrates one such sequence.

The effects of this sequence are such that resonances in the ω_1 dimension are offset by the difference in chemical shift of the two coupled resonances, in this case the methyl and methine protons. Figure 7 shows the projection of the cross-peak centered at $(\omega_A - \omega_X/2, \omega_X)$ on to the F_1 axis for the AX_3 spin system of

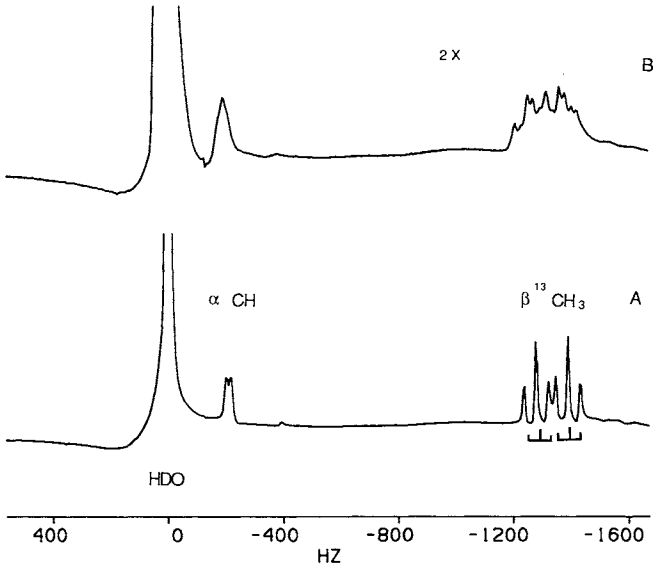

Figure 5. (A) High-resolution spectrum of alanine-$^{13}C_3$ dissolved in a liquid crystal solvent at 330 K; (B) spectrum of the same sample acquired in an inhomogeneous magnetic field. Chemical shifts are relative to HDO.

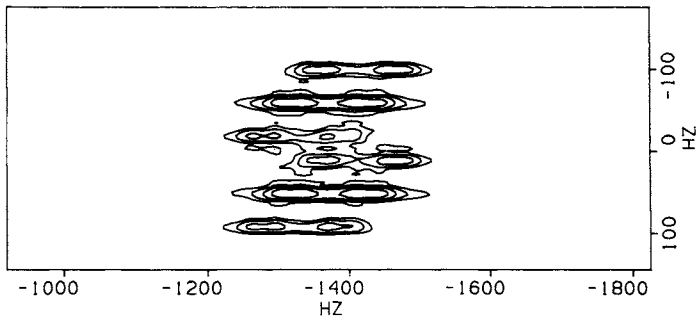

Figure 6. Contour plot presentation of the spectrum obtained from oriented alanine-$^{13}C_3$ using the sequence in Fig. 1a. Spectral acquisition and processing parameters are indicated in the text. Chemical shifts in F_2 are relative to HDO, and in F_1 the center of the spectrum is at 0 Hz.

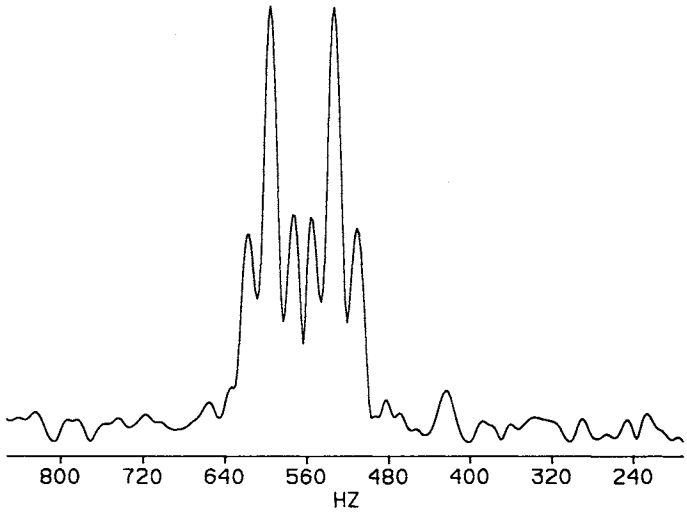

Figure 7. Projection of the cross-peak centered at $(\omega_A - \omega_X/2, \omega_X)$ on to the F_1 axis for the AX_3 spin system of alanine-$^{13}C_3$. The sequence in Fig. 1b was employed. See text for details. Chemical shifts in F_1 are relative to the center of the spectrum.

alanine-$^{13}C_3$ obtained with the sequence of Fig. 1b. Note that chemical shift resolution is retained, with resonances appearing at one half the difference in chemical shifts of the coupled spins (1123/2 Hz). Coupling information can be extracted from this spectrum by recalling that magnetization evolves on spins X and A, each for half of the evolution time. Since the predominant couplings are associated with the β-methyl group (see Fig. 5A), this implies a scaling down of the couplings by a factor of 2. A value of $|3D|/2 = 22$ Hz is obtained from the ^1H–^1H splittings in Fig. 7, which is in good agreement with the value obtained from the 1D experiment. The value of $|D'| = 125$ Hz, obtained from the spectrum in Fig. 7, is slightly larger than the actual value of $|D'| = 114$ Hz, since in addition to a geminal ^1H–^{13}C coupling there is a small vicinal ^1H–^{13}C coupling of 15 Hz. The ^1H–^{13}C splitting observed in the spectrum in Fig. 7 (63 Hz) is in good agreement with the average of the geminal and vicinal ^1H–^{13}C splittings (65 Hz) obtained from Fig. 5A.

In the case of complex spectra considerable simplification may be obtained by removing ^1H–^1H couplings. This can be accomplished by replacing the two evolution periods in the sequence in Fig. 1b with two constant time evolution periods.[15] Hall and Norwood[8] have developed such sequences for the measurement of high-resolution NMR experiments in the presence of inhomogeneous fields. In the present case, however, a knowledge of both the ^1H–^1H and ^1H–^{13}C interactions provides a way of obtaining both the sign and magnitude of the ^1H–^{13}C dipolar coupling. This can be seen as follows. The ratio of the ^1H–^1H dipolar splitting to the ^1H–^{13}C dipolar splitting for the AX_3 spin systems considered here is given by

$$r' = |D_{H-H}/D_{H-C}| = (1.5\gamma_H/\gamma_C)(r_{H-C}/r_{H-H})^3 \times |(3\cos^2 90 - 1)/(3\cos^2 109.5 - 1)| \quad (14)$$

which follows directly from Eqns (4) and (5). In Eqn (14), $r' \approx 2.2$, assuming standard bond geometries. For the case of oriented sodium acetate, values of $|D'| = |J_{H-C} + D_{H-C}| = 63$ Hz and $|D_{H-H}| = 140$ Hz are obtained. Noting that a typical value for J_{H-C} in a methyl group is *ca* 130 Hz, we conclude that $D_{H-C} \approx -67$ Hz or -193 Hz. Only the value of -67 Hz is consistent with $r' = 2.2$. In contrast, for the case of oriented alanine, values of $|D'| = |J_{H-C} + D_{H-C}| = 114$ Hz, $|D_{H-H}| = 42$ Hz and $J_{H-C} \approx 130$ Hz imply that $D_{H-C} \approx -16$ Hz or -244 Hz. Only the value of -16 Hz is consistent with $r' = 2.2$.

In liquid crystal samples it is frequently possible to decide whether D_{H-C} is greater or less than J_{H-C} via the fact that D_{H-C} scales with temperature- or concentration-dependent changes in order. In such cases the magnitudes of D_{H-C} and D_{H-H} can be used to determine geometries.

In summary, we have presented two pulse sequences which are useful for the extraction of homonuclear and heteronuclear dipolar couplings from molecules oriented in inhomogeneous magnetic fields. Useful structural information can be obtained which can, in some cases, supplement distance constraints obtained from cross-relaxation experiments. As NMR progresses to higher magnetic fields an increasing number of molecules will orient, and results from experiments such as these suggest that the application of ultra-high-field NMR to the study of the structure of molecules will be useful even in the presence of magnetic field inhomogeneity effects.

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REFERENCES

1. K. Wuthrich, *NMR of Proteins and Nucleic Acids*. Wiley, New York (1986).
2. T. A. Holak, Y. M. Kim, S. K. Kearsley and J. H. Prestegard, *Biochemistry* **27**, 6135 (1988).
3. W. P. Aue, J. Karhan and R. R. Ernst, *J. Chem. Phys.* **64**, 4226 (1976).
4. K. Nagayama, K. Wuthrich and R. R. Ernst, *Biochem. Biophys. Res. Comm.* **90**, 305 (1979).
5. K. Nagayama, Anil-Kumar, K. Wuthrich and R. R. Ernst, *J. Magn. Reson.* **40**, 321 (1980).
6. D. P. Weitekamp, J. R. Garbow, J. B. Murdoch and A. Pines, *J. Am. Chem. Soc.* **103**, 3579 (1981).
7. L. D. Hall and T. J. Norwood, *J. Magn. Reson.* **69**, 397 (1986).
8. L. D. Hall and T. J. Norwood, *J. Magn. Reson.* **109**, 7579 (1987).
9. Z. Luz, R. Poupko and E. T. Samulski, *J. Chem. Phys.* **74**, 5825 (1981).
10. E. W. Bastiaan, C. Maclean, P. C. M. Van Zijl and A. A. Bothner-By, *Annu. Rep. NMR Spectrosc.*, edited by G. A. Webb, Academic Press, New York, **19**, 35 (1987).
11. R. C. Long and J. H. Goldstein, *Mol. Cryst. Liq. Cryst.* **23**, 137 (1973).
12. G. Otting, H. Sen, G. Wagner and K. Wuthrich, *J. Magn. Reson.* **70**, 500 (1986).
13. O. W. Sorensen, G. W. Eich, M. H. Levitt, G. Bodenhausen and R. R. Ernst, *Prog. Nucl. Magn. Reson. Spectrosc.*, edited by J. W. Emsley, J. Feeney and L. H. Sutcliffe, Pergamon Press, Oxford, **16**, 163 (1983).
14. K. D. Lawson and T. J. Flautt, *J. Am. Chem. Soc.* **89**, 4589 (1967).
15. A. Bax and R. Freeman, *J. Magn. Reson.* **44**, 542 (1981).

Note added in proof: a relevant paper dealing with heteronuclear chemical shift spectroscopy in homogeneous fields has been brought to our attention: M. Gochin, D. T. Weitekamp and A. Pines, *J. Magn. Reson.* **63**, 431 (1985).