

A High-Resolution 3D Separated-Local-Field Experiment by Means of Magic-Angle Turning

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A 3D separated-local-field (SLF) experiment based on the 2D PHORMAT technique is described. In the 3D experiment, the conventional 2D SLF powder pattern for each chemically inequivalent carbon is separated according to their different isotropic chemical shifts. The dipolar coupling constant of a C–H pair, hence the bond distance, and the relative orientation of the chemical-shift tensor to the C–H vector can all be determined for the protonated carbons with a single measurement. As the sample turns at only about 30 Hz in a MAT experiment, the SLF patterns obtained approach those of a stationary sample, and an accuracy in the measurement similar to that obtained on a stationary sample is expected. The technique is demonstrated on 2,6-dimethoxynaphthalene, where the ^{13}C – ^1H separated-local-field powder patterns for the six chemically inequivalent carbons are clearly identified and measured. The observed dipolar coupling for the methoxy carbon is effectively reduced by the fast rotation of the group about its C_3 symmetry axis. The average angle between the C–H bond direction and the C_3 rotation axis in the OCH_3 group is found to be about 66° . © 1997 Academic Press

INTRODUCTION

It has been known for more than a decade (1–4) that the relative orientation of a chemical-shift tensor with respect to the direction of a C–H vector, as well as the C–H bond distance, can be obtained from a 2D separated-local-field (SLF) experiment on a stationary powder sample. Unfortunately, when there are several chemically distinct nuclei in the sample, overlap of several broad SLF patterns often prevents the separation necessary for their individual identification and measurement. The SLF experiment was thus limited to rather simple systems with only one chemically inequivalent carbon (2, 4) or to single crystals where high-resolution spectra are obtained (1, 3). In the latter case, the dipolar coupling at a specific molecular orientation may be obtained. The 2D SLF experiment has been incorporated into a 2D exchange experiment to produce a 3D experiment in which dipolar coupling information is used to simplify spectral assignments; however, its application is again limited to single crystals (5). The SLF experiment has also been applied

to a spinning sample in a MAS experiment (6) where the spectral overlap caused by the chemical-shift anisotropy interaction (CSA) is eliminated at a sample spinning rate larger than the width of the CSA. However, the high resolution achieved by MAS comes at the expense of information on the shift tensor.

Ideally, one would like a 3D experiment in which the SLF pattern for each carbon is isolated by its isotropic shift. Such a 3D SLF experiment is possible by employing the MAT experiment (7–9). It has been demonstrated in an initial report (10) that the SLF powder pattern for complex molecular structures may be obtained with a 3D PHORMAT experiment to which a dipolar evolution dimension is introduced in the third dimension. Originally, homonuclear dipolar decoupling during the dipolar evolution period was not used, though better results can be obtained with homonuclear dipolar decoupling during the dipolar evolution period. Such a 3D SLF pulse sequence based on the PHORMAT experiment with Lee–Goldburg homonuclear decoupling is reported in this paper. A typical application is demonstrated with 2,6-dimethoxynaphthalene, where the C–H-resolved SLF powder pattern for each individual carbon is obtained. The method reported in this paper may be considered as an alternative to the 3D ^{15}N – ^1H SLF experiment recently reported by Opella's group (11), where a combination of PISEMA (12) and the original Gan version of MAT (7) was used.

EXPERIMENTAL

The experiments were performed on a CMX-400 NMR spectrometer with ^{13}C and ^1H Larmor frequencies of 100.623 and 400.124 MHz, respectively. A MAS probe with a 7.5 mm rotor and a spinning speed regulator system from Chemagnetics were used for the experiment. A rotor synchronization system described previously was also used to precisely trigger the pulses at $\frac{1}{3}$ of the rotor cycle as required by the PHORMAT experiment (9). The sample, 2,6-dimethoxynaphthalene, was purchased from Aldrich and was used as received.

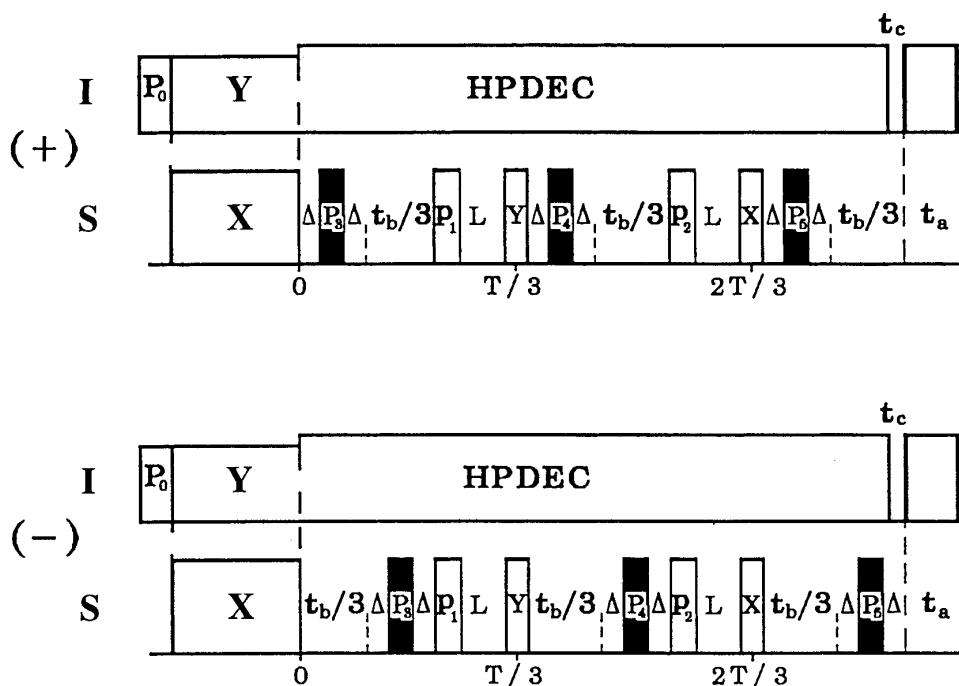


FIG. 1. The pulse sequence for the 3D MAT separated-local-field (SLF) experiment. ^1H homonuclear decoupling is applied during the t_c period.

The pulse sequence for the 3D SLF experiment is shown in Fig. 1. The main body of the “+” and “-” sequences are the basic PHORMAT pulse sequences when $t_c = 0$, and the phase cycling for each pulse as well as the way to process PHORMAT data are given in Ref. (9). The period t_c indicated in the pulse sequences is the corresponding dipolar evolution time variable. During the t_c period, homonuclear dipolar decoupling is applied. Flip back of the proton magnetization at the end of the data acquisition is used, which is found to give better S/N for systems with relatively long ^1H T_1 values. A short recycle delay time may be used if the $T_{1\rho}$ of the system is long. The reasoning behind the flip-back pulses is explained as follows: During the CP and decoupling period before t_c , the proton magnetization is locked along the Y axis of the rotating frame; during t_c , the magnetization is locked along the effective field direction, e.g., the magic-angle axis if a Lee–Goldburg decoupling is applied. At the end of the t_c , a second proton high power decoupling is applied along the Y axis. The magnetization is relocked along the Y axis; hence, a flip-back pulse will store the remaining magnetization back to the main field direction.

The experiment can be performed in one of two ways: First, the echo time Δ is chosen to be a constant with a value equal to Δ_0 plus the maximum value of t_c , i.e., $\Delta_{\text{const}} = \Delta_0 + t_{c1} - t_c$. When t_c increases, t_{c1} decreases correspondingly. In the second method, Δ increases with t_c through the relation, $\Delta = t_c + \Delta_0$. Δ_0 (about $30 \mu\text{s}$) is the time required to suppress the dead time associated with probe ring-down

and receiver recovery. The PHORMAT experiment works satisfactorily for any value of Δ since an ideal chemical-shift echo is formed for a rotating sample regardless of the value of Δ . However, greater S/N is always obtained in the second method because the T_2 effect is minimized.

The flip-flop Lee–Goldburg (FFLG) (13) sequence is used for homonuclear dipolar decoupling. Our experimental results show that t_c can be either incremented by a full FFLG cycle or alternatively incremented by half of the FFLG cycle. Both alternatives have essentially the same efficiency in narrowing the linewidth in the dipolar evolution dimension for a given decoupling power. If a half FFLG cycle is used, the spectral width for the dipolar dimension is twice that obtained by incrementing the full FFLG cycle. This half cycle approach is especially useful for carbon-13 systems with strong C–H coupling when the available decoupling field strength is limited, e.g., less than about 60 kHz. On our CMX-400 NMR system, a proton decoupling field of 67.6 kHz is readily achieved. The resulting cycle time for a full FFLG cycle is $24 \mu\text{s}$. In our experiment, t_c is incremented by half of the cycle time (i.e., $12 \mu\text{s}$) with the alternative increment of the +LG and –LG half cycles. The corresponding decoupler offsets are +47,778 Hz for the +LG and –47,778 Hz for the –LG pulses, respectively. The experimentally determined scaling factor for the FFLG sequence is found to be essentially the theoretical value of 0.577, yielding an effective spectral width of 144 kHz for the dipolar dimension of the 3D SLF experiment.

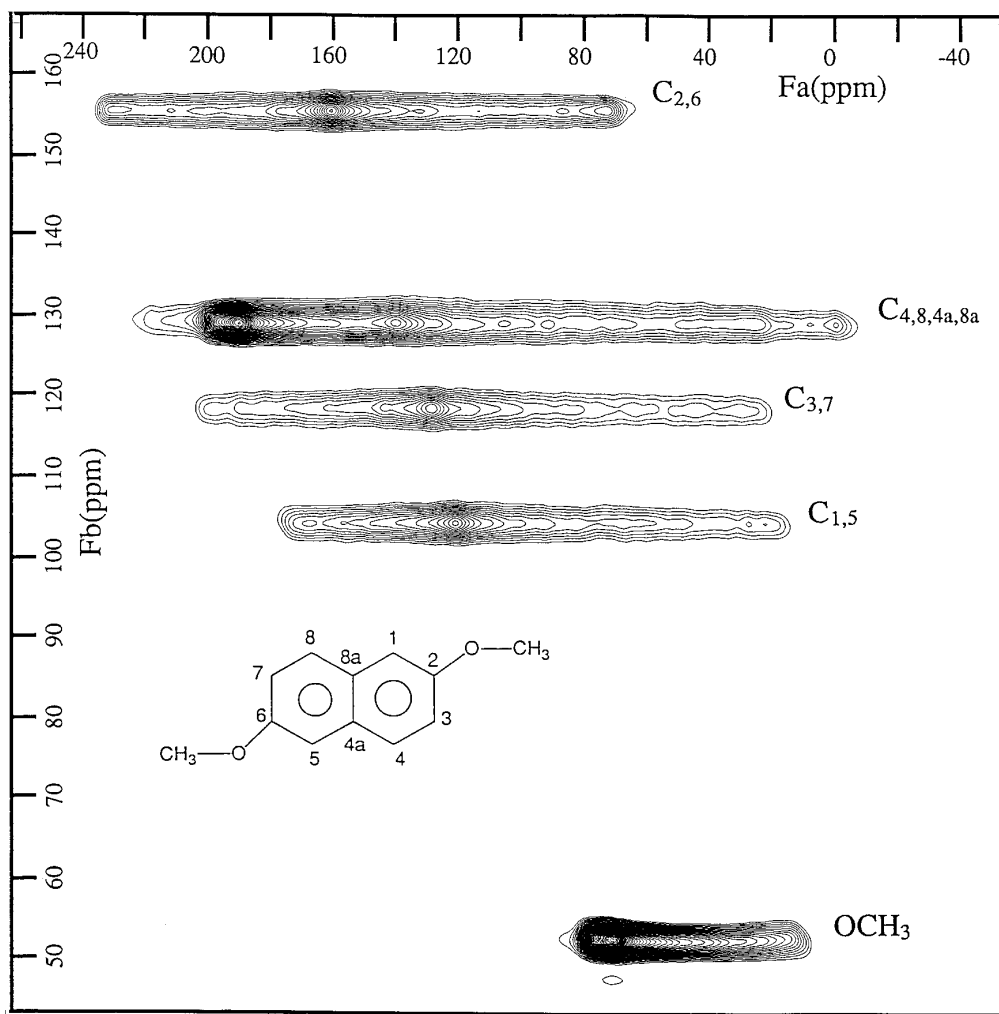


FIG. 2. The projection of the 3D MAT SLF of 2,6-dimethoxynaphthalene to the F_a - F_b spectral plane. Only a central portion of the 2D spectrum is shown. The contour interval is 5%, and the lowest plotted contour is 2% of the maximum peak height. The chemical shifts are referenced to TMS via HMB as a secondary reference with a chemical-shift value of 17.3 ppm for the methyl carbon. Gaussian line broadening of 3.2 and 1.8 ppm was applied in the acquisition (F_a), and the isotropic chemical-shift (F_b) dimensions, respectively.

The 3D MAT SLF data were acquired by obtaining sets of 2D PHORMAT data by incrementing t_c through the relationship $\Delta = \Delta_0 + t_c$. The increment of t_c is 12 μs and the +LG and -LG pulses were incremented alternatively. Thirty-two t_c values ranging from 0.1, to 372.1 μs were acquired. A total measurement time of about 8 days was required. The data were obtained at a rotor frequency of 30 Hz and varied by only 0.17 Hz during the eight-day data acquisition period.

The 2D PHORMAT data at each specific value of t_c were acquired using the following experimental parameters. The cross-polarization contact time was 4 ms, $\Delta_0 = 25 \mu\text{s}$, the cross polarization and the decoupling fields were 67.6 kHz, and the recycle delay time was 1 s. Acquisition dimension (t_a) FIDs with 256 complex points were transformed to

spectra with a 80 kHz spectral width. The 2D data were collected at 32 different values of the isotropic shift evolution variable t_b incremented by periods of 80 μs . This results in a maximum evolution time of 2.56 ms and an isotropic-dimension spectral width of 12.5 kHz. The eight types of free-induction decays (9) were acquired with the (+) and (-) PHORMAT pulse sequences using a total of 640 scans at each different t_b value. A measurement time of 5.7 h was required for acquiring a 2D PHORMAT data at each specific t_c value.

RESULTS AND DISCUSSION

The experimental results for 2,6-dimethoxynaphthalene (2,6-DMN) are summarized in Figs. 2-4. The projection of

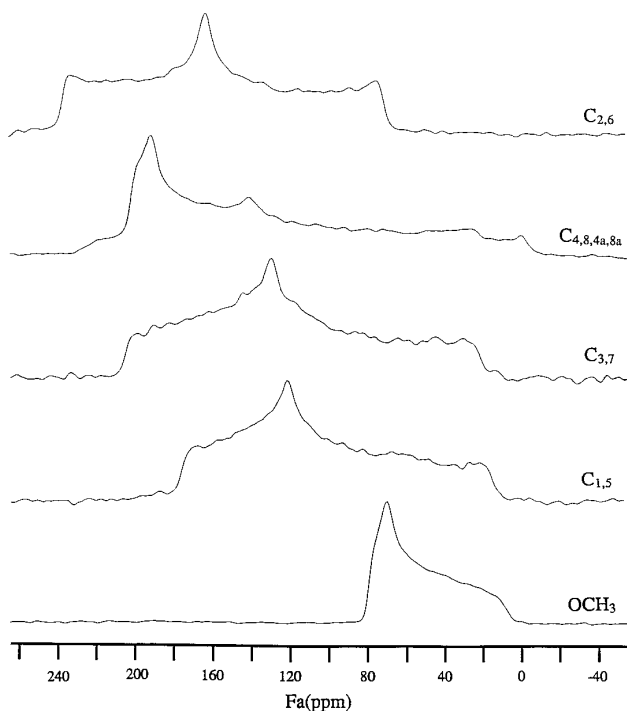


FIG. 3. The powder patterns for each chemically inequivalent carbon in 2,6-dimethoxynaphthalene obtained by taking a slice through the 2D spectrum shown in Fig. 2. Slices were taken at the center of the corresponding isotropic chemical-shift position.

the 3D SLF spectrum onto the F_a - F_b spectral plane is shown in Fig. 2. This is essentially the PHORMAT spectrum obtained for a dipolar evolution time variable of $t_c = 0$ μ s. The powder patterns were obtained for each carbon by simply taking a spectral slice at the corresponding isotropic chemical-shift position: those for $t_c = 0$ are shown in Fig. 3. The 2D SLF powder pattern for each carbon shown in Fig. 4 are then obtained by Fourier transforming the sliced spectra with respect to the time variable t_c .

It is clear that the 2D ^{13}C - ^1H SLF powder patterns for each carbon in 2,6-DMN are successfully isolated with this method. The dipolar coupling peaks with a ratio of roughly 1:3:3:1 in OCH_3 are clearly presented in the 2D SLF pattern of OCH_3 in Fig. 4. A dipolar coupling constant of about 5.7 kHz is found by visual inspection. It is known that the C-H bond distance of the methyl group is around 1.095 \AA , yielding a 22.85 kHz dipolar coupling constant. The reduction in dipolar coupling is apparently caused by the fast rotation of the methyl group around its C_3 symmetric axis. The angular information about the C-H vector with respect to the C_3 rotation axis of the OCH_3 group can be readily derived in the following.

The secular part of the dipole-dipole interaction Hamiltonian for the CH_3 system is given by (14).

$$\mathbf{H} = \sum_j A_j S_z I_{zj}, \quad j = 1, 2, 3$$

$$A_j = D_j [1 - 3 \cos^2(\theta_j)]$$

$$D_j = \gamma_1 \gamma_S / r_j^3, \quad [1]$$

where $D_j = \gamma_1 \gamma_S / r_j^3$ is the dipolar coupling constant in frequency units for a rigid system, and θ_j is the angle between the main field direction and the C-H_j vector direction for the j th bonded proton. Assuming that the motion is a continuous rotation about the C_3 symmetric axis, the angle between the C_3 axis and the main field direction is θ'_j , the angle between the C-H_j and the C_3 axis is β , and the angular rotation speed is ω with $\omega t = \phi$. The following relationship is obtained:

$$\cos(\theta_j) = \cos(\beta) \cos(\theta'_j) - \sin(\beta) \sin(\theta'_j) \cos(\phi). \quad [2]$$

Then, in the expression for A_j in Eq. [1]

$$\begin{aligned} 1 - 3 \cos^2(\theta_j) &= 1 - 3 \cos^2(\beta) \cos^2(\theta'_j) \\ &\quad - 3 \sin^2(\beta) \sin^2(\theta'_j) \cos^2(\phi) \\ &\quad + \frac{3}{2} \sin(2\beta) \sin(2\theta'_j) \cos(\phi). \quad [3] \end{aligned}$$

The average value of $\cos^2(\phi)$ and $\cos(\phi)$ over a complete rotation is $\frac{1}{2}$ and 0, respectively, so that

$$\begin{aligned} \langle 1 - 3 \cos^2(\theta_j) \rangle_{\text{rot}} \\ = \left(\frac{3}{2} \sin^2(\beta) - 1 \right) (3 \cos^2(\theta'_j) - 1). \quad [4] \end{aligned}$$

Thus, if the frequency of rotation $\omega/2\pi$ is larger than the frequency width of the rigid-lattice resonance, then the effective dipolar coupling constant is scaled by a factor of $[\frac{3}{2} \sin^2(\beta) - 1]$. Using the experimentally determined dipolar coupling constant of 5.7 kHz for the OCH_3 group, we obtain

$$\frac{3}{2} \sin^2(\beta) - 1 = (5.7 \text{ kHz}/D),$$

where $D = \gamma_1 \gamma_S / r^3$ is the dipolar coupling constant for a rigid system. If a C-H bond distance of 1.095 \AA is used, one obtains a value of 65.9° for the angle β , and the O-C-H angle is 114.1° , which is very close to the expected angle of 109 - 112° found in the literature.

Compare the 2D SLF powder pattern with those of solid benzene reported by Linder *et al.* (4). The 2D SLF powder pattern for the OCH_3 carbon in 2,6-DMN is clearly a superposition of two patterns of similar kinds with a ratio of roughly $\frac{1}{3}$, a consequence of the rapid motional averaging of the methyl group. The δ_{33} component should be oriented

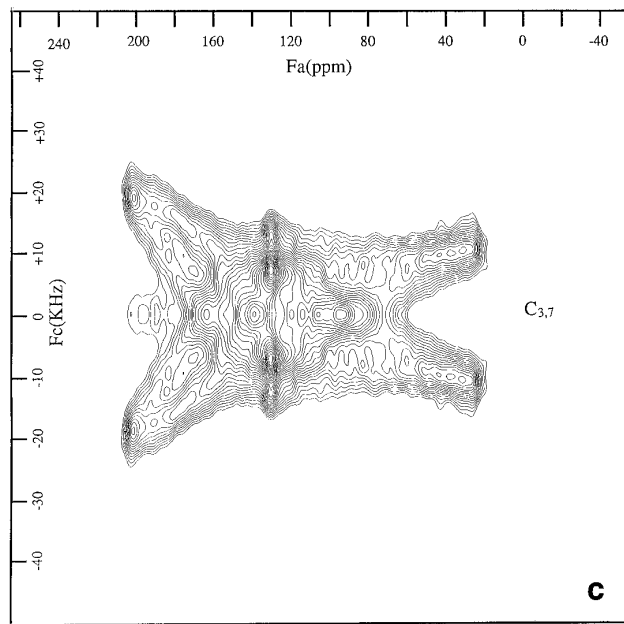
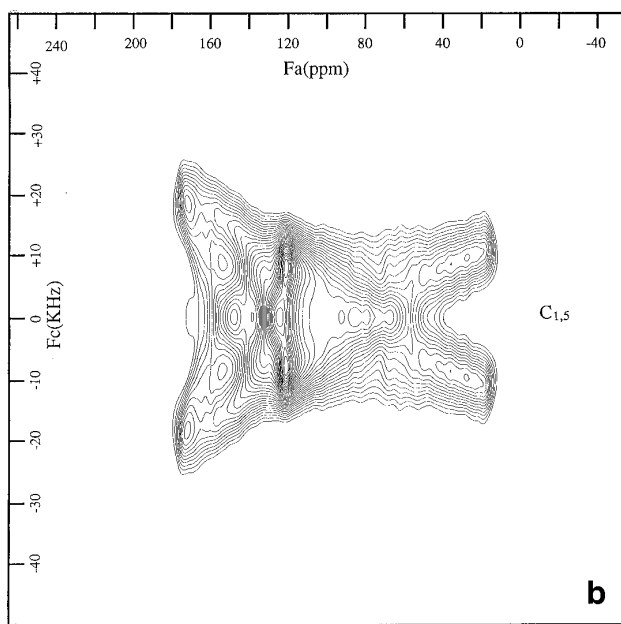
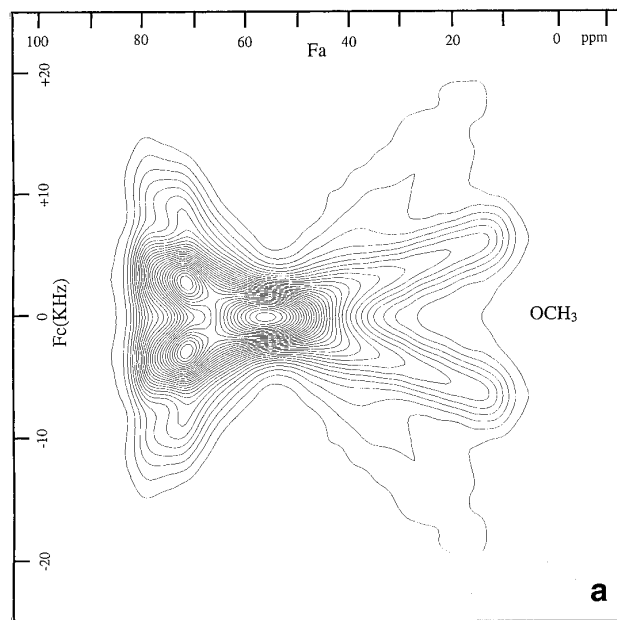


FIG. 4. The resolved 2D SLF powder patterns are given for each chemically inequivalent carbon in 2,6-dimethoxynaphthalene.

roughly along the direction of the O–C bond direction, and the δ_{11} and δ_{22} components are perpendicular to the O–C bond direction.

The 2D SLF pattern for $C_{1,5}$ displays the characteristic 2D SLF powder pattern of a CH carbon when the δ_{33} component is perpendicular to the aromatic ring, and the δ_{11} component is along the C–H bond direction and lies in the aromatic plane [see the theoretical patterns in Ref. (4)]. Similar patterns are also found for $C_{3,7}$ and $C_{4,8}$ even though the isotropic chemical shift of $C_{4,8}$ is superimposed on that of $C_{4a,8a}$. For

these protonated aromatic carbons, a dipolar coupling constant of roughly 22.7 kHz can be found by visual inspection, resulting in a C–H bond distance of 1.098 Å, a typical value expected in aromatic systems.

The 2D SLF patterns of the nonprotonated bridgehead carbon $C_{4a,8a}$ and the methoxy substituted aromatic carbon $C_{2,6}$ show no dominant dipolar couplings among the CSA powder patterns, indicating that the effect of the dipolar coupling from the remote protons to different orientations of the crystallite is roughly the same.

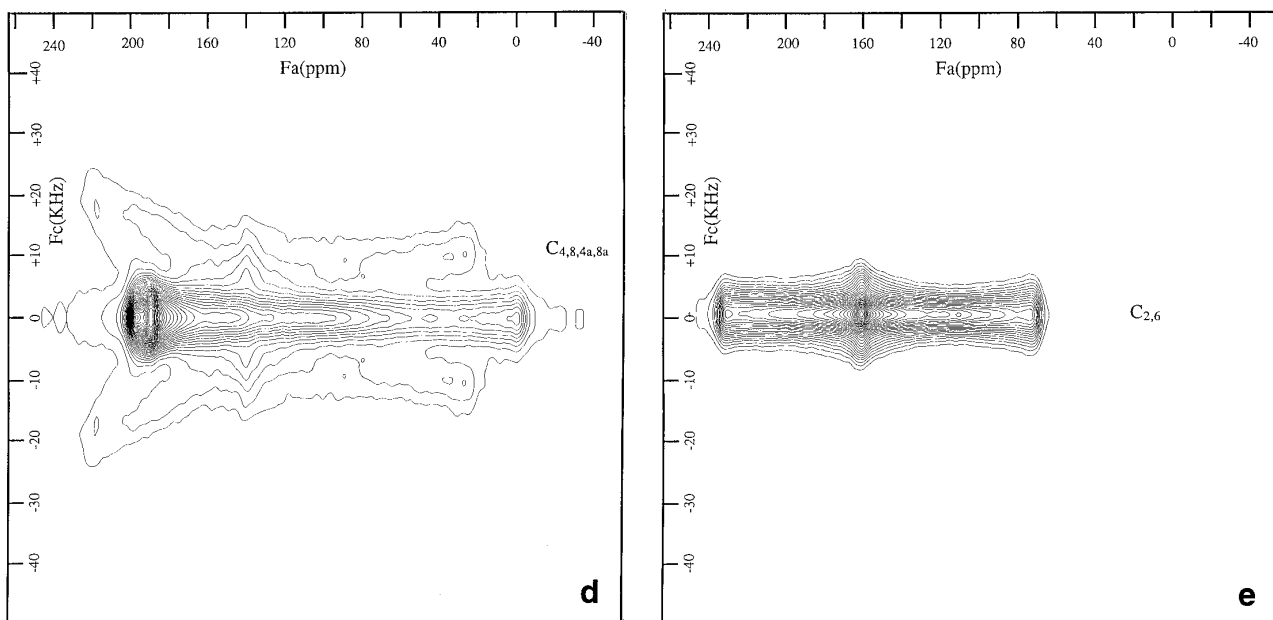


FIG. 4—Continued

In general, the 2D resolved SLF powder pattern for a C–H pair can be a complex shape, depending on the relative orientation of the CSA tensor with respect to the C–H bond vector (4). In order to accurately extract the bond distance and the orientation information, a theoretical simulation, which includes the effect of slow sample rotation (15), is necessary. We are in the process of constructing a simulation package for this type of experiment.

It should be pointed out that the method presented in this paper differs from the 3D separated-local-field experiment reported by Opella's group (11) in the following four aspects: (a) The PHORMAT sequence eliminates the dead-time effect associated by probe ring-down and receiver recovery and produces a perfectly phased spectrum with no shear operation as required by the original Gan sequence. (b) The dipolar evolution dimension is introduced in the echo segment with no need of matching for the ^{13}C channel. This makes the experiment easier to execute. (c) In this scheme, a scaling factor of 0.577 produces a larger observable spectral width for the dipolar dimension at a given proton decoupling power. This is especially useful when the available proton decoupling power is limited or when very large dipolar coupling is encountered. (d) The observable spectral width can be further enlarged alternatively by incrementing the +LG and –LG Lee–Goldburg half cycles.

CONCLUSIONS

The resolved 2D separated-local-field powder patterns may be obtained for chemically inequivalent carbons even

in complex molecular structures using the 3D MAT SLF experiment suggested in this paper. The dipolar coupling constant of the C–H pair, the bond distance, and the relative orientation of the chemical-shift tensor for the protonated carbons to the C–H vector can all be determined with a single measurement. The observed dipolar coupling in a methyl group is effectively reduced by the fast rotation of the group about its C_3 symmetric axis. The average angle between the C–H bond direction and the C_3 rotation axis in a OCH_3 system is found to be about 65.9° . Because the sample turns very slowly in a MAT experiment, i.e., 30 Hz, the SLF patterns obtained on a slowly rotating sample approaches that of a stationary sample, yielding an accuracy in the measurement similar to that obtained on a stationary sample.

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