

## Dipolar Interactions in Molecules Aligned by Strong AC Electric Fields

Alexey Peshkovsky and Ann E. McDermott

Department of Chemistry, Columbia University, New York, New York 10027

Received March 27, 2000; revised July 7, 2000

We observed magnetization transfer and spectroscopic splittings due to dipolar couplings in the solution NMR spectra of neat nitrobenzene aligned using AC electric fields. Weak dipolar splittings have been previously observed for nitrobenzene in a DC electric field (T. M. Plantenga, *et al.*, *Chem. Phys.* 66, 1–9, 1982); the use of homogeneous pulsed AC fields has allowed us to establish stable experimental conditions, which were more tolerable to sample impurities and required no sample purification, and to carry out multidimensional experiments. A pulse sequence is discussed in which the electric field is present only for the indirect dimension: this sequence records the dipolar splittings for each proton in the indirect dimension; the direct dimension presents the isotropic chemical shift. Another pulse sequence is discussed that uses the applied electric field only in the mixing period to produce cross peaks between dipolar coupled pairs and correlate their isotropic chemical shifts. The order parameter describing molecular alignment was in good agreement with that previously determined from deuterium quadrupolar measurements of deuterated nitrobenzene in a similar range of electric fields:  $S_{\text{mol}} \sim 0.025\%$  for a field strength of 7.0 MV/m (rms). The dipolar splittings for ortho-meta, meta-para, and ortho-para protons were in qualitative agreement with the known geometry. © 2000 Academic Press

**Key Words:** nitrobenzene; dipolar coupling; NMR; order parameter; AC electric field.

## INTRODUCTION

Dipolar interactions provide a powerful tool for extracting angular and distance information in the context of molecular structure determination. For a pair of magnetically coupled nuclei in a rigid molecule, the Hamiltonian can be expressed as (2)

$$H = -\hbar\gamma(1 - \sigma_i)H_0I_z^i - \hbar\gamma(1 - \sigma_j)H_0I_z^j - \frac{\mu_0 \hbar^2 \gamma^2}{4\pi r_{ij}^3} \left( \frac{3}{2} \cos^2\theta - \frac{1}{2} \right) (3I_z^i I_z^j - \mathbf{I}^i \cdot \mathbf{I}^j), \quad [1]$$

where  $I_z^i$  is the  $z$  component of the angular momentum operator,  $\mathbf{I}^i$ , for the nucleus  $i$ , the magnetic field  $H_0$  is parallel to the  $z$  direction in the laboratory coordinate frame,  $\gamma$  is the gyromagnetic ratio of the nucleus,  $\sigma_i$  is the chemical shielding value for the nucleus  $i$ ,  $\mu_0$  is the magnetic permittivity of

vacuum, and  $\theta$  is the polar angle between the internuclear vector  $\mathbf{r}_{ij}$  and  $z$ . The dipolar splitting  $D$  described by the second term can be quantified as

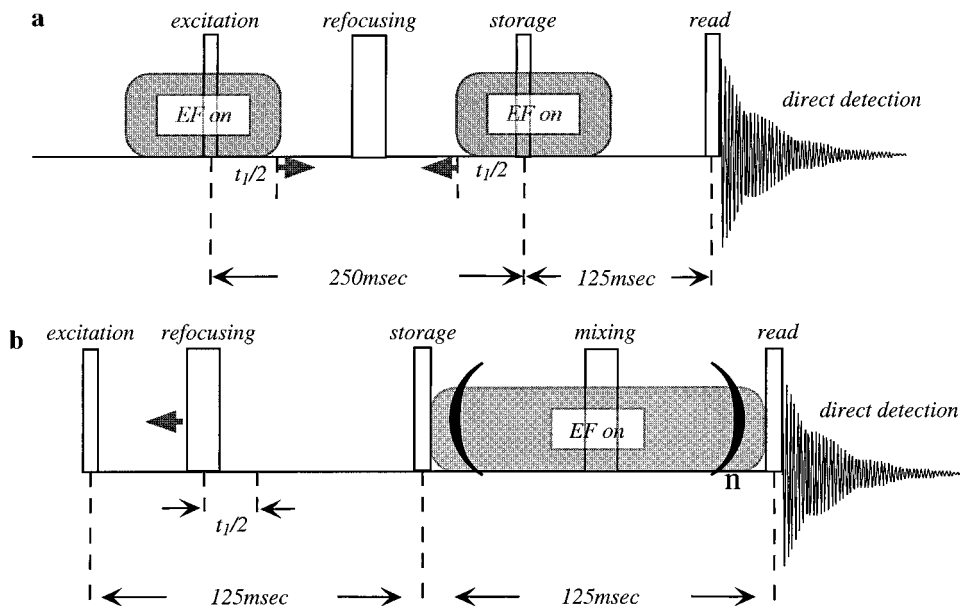
$$D = \frac{3}{8} \frac{\mu_0 \hbar \gamma^2}{\pi^2 r_{ij}^3} \left( \frac{3}{2} \cos^2\theta - \frac{1}{2} \right). \quad [2]$$

This term vanishes for a randomly tumbling liquid because, as the molecules tumble, all values of the angle  $\theta$  are rapidly sampled with equal probability, and the time average of the expression  $(\frac{3}{2} \cos^2\theta - \frac{1}{2})$  becomes zero. The interaction can, however, be recovered if tumbling molecules are given preferential orientation. If molecules which possess large magnetic susceptibility anisotropies are oriented with strong magnetic fields (3–5), or if molecules are mechanically ordered by a liquid crystalline medium (6–9), then the time average of the expression  $(\frac{3}{2} \cos^2\theta - \frac{1}{2})$  becomes nonzero. Mathematical details describing NMR of partially aligned systems, such as liquid crystals, can be found in the Ref. (10).

Electric fields can also be used to partially recover quadrupolar, dipolar, and chemical shift anisotropy interactions because they can introduce residual ordering in polar liquids. Thus, electric field NMR can provide an alternative tool for studying molecular structure. The first report of successful observation of the  $^1\text{H}$  dipolar couplings in para-nitrotoluene ordered with DC electric fields (11) was not confirmed by the subsequent experiments with the same molecule (12), as well as with nitromethane (13). Studies of  $^{14}\text{N}$  quadrupolar (14),  $^2\text{H}$  quadrupolar, and  $^1\text{H}$  dipolar splittings (1) were later performed, in which molecules with high dipole moments (nitrobenzene and ethylenecarbonate in nitrobenzene) were aligned with DC electric fields. Similar methods have been used to determine the shielding anisotropy for  $^{13}\text{C}$  in acetonitrile (15). For uniaxial alignment one can define an order parameter  $S_{\text{mol}}$  as

$$S_{\text{mol}} = \left\langle \left( \frac{3}{2} \cos^2\theta_{\text{mol}} - \frac{1}{2} \right) \right\rangle_E, \quad [3]$$

where the angled brackets stand for a time average, and  $\theta_{\text{mol}}$  is a polar angle between the direction of the electric dipole



**FIG. 1.** (a) The pulse sequence used to correlate the isotropic chemical shifts (in the direct dimension) with the dipolar splittings (in the indirect dimension) is shown. Dipolar coupling was reintroduced into the system by the molecular ordering of nitrobenzene with pulsed AC electric fields. The total length of the electric field pulses was kept constant, but their effect during the evolution period was modulated by incrementally moving them into the time window between the excitation and the storage pulses. A refocusing pulse in the middle of the evolution period served to exclude chemical shift evolution and to remove broadening due to the  $H_0$  inhomogeneity. (b) The pulse sequence used to correlate the chemical shift of dipolar-coupled pairs of protons is shown. The dipolar coupling introduced by the applied electric field was present during the mixing time, but not for the direct or indirect dimension. Time windows between the excitation and the storage pulses, as well as between the storage and the read (mixing time) pulses, were kept constant at 125 ms. Isotropic chemical shifts in the indirect dimension were detected by incrementally shifting the refocusing pulse toward the excitation pulse. A series of  $180^\circ$  pulses spaced by 0.5 ms was applied during the mixing time to provide a TOCSY-type longitudinal magnetization exchange, “untruncating” the dipolar coupling by regularly refocusing the chemical shift (17).

moment and the direction of  $H_0$ . The expected residual dipolar splitting frequency can then be expressed as

$$D = \tilde{D} \times S_{\text{mol}} \times \left( \frac{3}{2} \cos^2 \theta_{ij} - \frac{1}{2} \right), \quad [4]$$

where  $\tilde{D} = 3/8 \mu_0/\pi^2 \hbar \gamma^2/r_{ij}^3$  combines the fundamental constants and the constants specific to the nuclei under the observation, and  $\theta_{ij}$  is a polar angle between the internuclear vector  $\mathbf{r}_{ij}$  and the direction of the molecular electric dipole moment.

Earlier we reported experimental and theoretical investigations of molecular alignment of neat nitrobenzene- $d_5$  at various AC electric field strengths and temperatures, as well as of solutions with various concentrations of nitrobenzene- $d_5$  in nonpolar solvents using the deuterium quadrupolar interaction as a probe (16). In this paper, we describe measurements of dipolar splittings of meta- and ortho-proton signals in neat nitrobenzene.

## EXPERIMENTAL

A full description of the apparatus and the sample cells used in these experiments is given in Ref. (12). The spectra were

recorded on a Chemagnetics CMX II 400-MHz spectrometer. A homebuilt probe was used with a high-voltage line capable of delivering up to 12 kV (peak-to-peak) voltages to the sample. The high-voltage sample cells contained approximately 2  $\mu\text{l}$  of pure nitrobenzene and consisted of two parallel indium tin oxide glass electrodes, purchased from Delta Technologies (Stillwater, MN), spaced by 400  $\mu\text{m}$ . Silicone sealant, purchased from World Precision Instruments (Sarasota, FL), filled the gap between the electrodes, leaving a “disk”-shape bubble in the middle, which contained the liquid samples. Pulsed AC electric fields, rather than the DC fields, were utilized to reduce electrode polarization, electrode corrosion, sensitivity to impurities, electrical convection, electrolysis, and heating. Nitrobenzene (99%) was obtained from Aldrich (Milwaukee, WI), and no additional purification was necessary. The frequency of the AC electric field was 28.5 kHz.

## Pulse Sequences

In Fig. 1a we illustrate the two-dimensional pulse sequence, which we used to obtain spectra with the isotropic chemical shifts in the direct dimension and the dipolar splittings in the indirect dimension. The total length of the electric field pulses throughout the experiment was kept constant, as was the total

experimental time. Application of the electric field has the effect of heating the sample; this heating was estimated to be less than 1°C during each scan, on the basis of the sample resistance. Although this small heat load was compensated with cooling air, artifacts such as phase twist in the indirect dimension were observed unless a constant heat load during the experiment was applied. The field was applied during two time intervals, which were shifted through the constant time window between the excitation and the storage NMR pulses, with a symmetrical arrangement with respect to the refocusing pulse in the middle of the window. The window between the excitation and the storage pulses was chosen to be the  $J$  coupling “null” for all protons in nitrobenzene. The  $J$  coupling between neighboring protons is 8 Hz; because the time window was set to 250 ms, the spectra appeared to be largely unaffected by the I-bond  $J$  coupling.

Another pulse sequence (Fig. 1b) yielded two-dimensional spectra containing the isotropic chemical shifts in both dimensions, while the dipolar couplings during the mixing time caused the appearance of cross peaks. This pulse sequence is advantageous in the case of an inhomogeneous electric field, when the order parameter becomes nonuniform throughout the sample. The accuracy of the quantitative measurements is reduced for both types of experiments; however, this type of data acquisition still permits a qualitative analysis, while the first method can become inadequate, since the splittings may not be resolved due to the inhomogeneous line broadening. The electric field was applied during the mixing time between the storage and the read pulses. During this time period a series of 180° pulses, spaced by 0.5 ms, was also applied in order to refocus chemical shift and provide a longitudinal mixing scheme, analogous to early TOCSY experiments, in which the coupling was “untruncated” by effectively removing the chemical shift interaction (17). At the beginning of the mixing time the magnetization was placed along the  $z$ -axis, after which it evolved as

$$I_z^i \xrightarrow{\pi[J + D]\tau_m[3I_z^i I_z^j - \mathbf{I}_z^i \cdot \mathbf{I}^j]} \frac{I_z^i}{2} + \frac{I_z^j}{2} + \cos[2\pi(J + D)\tau_m] \left[ \frac{I_z^i}{2} - \frac{I_z^j}{2} \right] + \sin[2\pi(J + D)\tau_m][I_y^i I_x^j - I_x^i I_y^j], \quad [5]$$

where  $\tau_m$  is the mixing time,  $D$  is the dipolar coupling between nuclei  $i$  and  $j$ , and  $J$  is the scalar coupling within the same pair of nuclei. The  $I_x^i$  type terms remaining after the storage pulse were removed by phase cycling. The total mixing time (for the data presented in Fig. 4) was 125 ms, which was equal to a full  $J$  coupling period and to approximately half of the dipolar coupling period for ortho- and meta-protons at a 6.5 MV/m (rms) applied electric field. In absence of the electric field,  $D =$

0, and at the end of the mixing period  $\cos[2\pi(J + D)\tau_m] = +1$ , so no magnetization transfer occurred. When the electric field was set to 6.5 MV/m (rms),  $\cos[2\pi(J + D)\tau_m] = -1$ , and magnetization transfer was maximized. Thus, no  $J$ -induced cross peaks were expected to take place, while the dipolar-induced cross peaks were expected to be maximal. The time window between the excitation and the storage pulses was kept constant at 125 ms (one  $J$  coupling period). The refocusing pulse began at the center of the indirect dimension and was incrementally shifted toward the excitation pulse, which enabled the detection of the isotropic chemical shifts in the indirect dimension.

## RESULTS AND DISCUSSION

The distance between meta- and ortho-protons in nitrobenzene was determined to be 2.5 Å by ab initio HF (STO-3G) calculations of the geometry of nitrobenzene in Spartan (Wavefunction, Inc., Irvine, CA), resulting in a value for  $\tilde{D}$  of 23 kHz.

The 2D and the indirect dimension spectra for meta-protons, dipolar coupled to ortho-protons, as well as the geometry of the experiment, are illustrated in Fig. 2. The dipolar splittings for meta- and ortho-protons depend linearly on the square of the rms of the applied electric field as discussed in the review by Hilbers and MacLean (14). This dependence is plotted in Fig. 3. The relationship between the splittings (in hertz) and the field strength can be fit to the expression

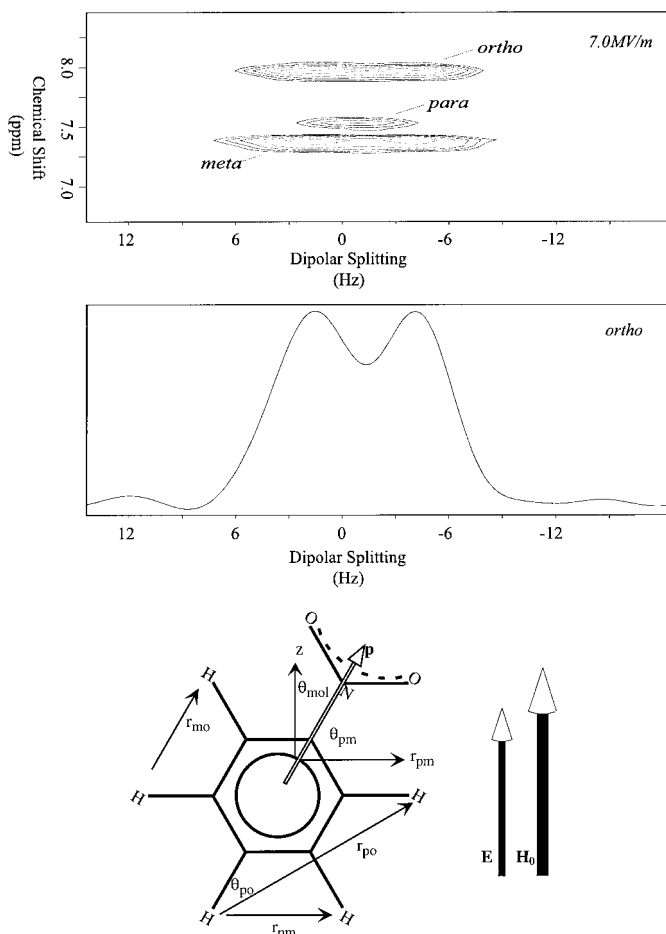
$$D = \tilde{D} \left( \frac{3}{2} \cos^2 \theta_{ij} - \frac{1}{2} \right) k E^2 = 1.16 \times 10^{-13} E^2, \quad [6]$$

where the slope  $k = (p/kT)^2 F/15 = 5.02 \times 10^{-18}$  is directly related to the molecular dipolar moment  $p$ , with a correction  $F$  (ranging from  $\sim 1$  for a low  $\epsilon$  to  $\sim 2$  for a high  $\epsilon$ ) related to the dielectric constant of the medium  $\epsilon$ , the shape of the molecule, and the resulting reaction fields, as discussed previously (16).

The splittings reported in our previous studies of the deuteron quadrupolar coupling for nitrobenzene (16) were governed by the analogous expression

$$Q = \tilde{Q} \left( \frac{3}{2} \cos^2 \theta - \frac{1}{2} \right) k E^2, \quad [7]$$

where  $\tilde{Q} = \frac{3}{2} e^2 q Q/h$  (Hz) collects the fundamental constants and the constants specific to the nucleus under observation, and  $\theta$  is a polar angle between the direction of the molecular electric dipole moment and the bond vector of the quadrupolar nucleus. The highest value for the order parameter  $S_{\text{mol}} = k \times E^2$  based on our previous experiments was 0.026% and occurred at the applied electric field strength of 7.0 MV/m (rms). The constant  $k = 5.24 \times 10^{-18} \text{ m}^2/\text{V}^2$  determined from this expression is in excellent agreement with that derived from

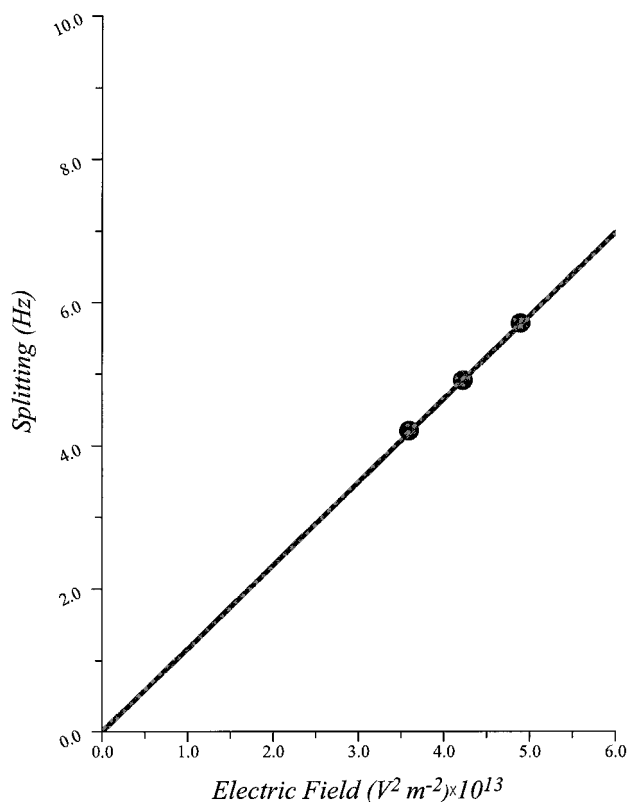


**FIG. 2.** The geometry of the experiment is shown along with a two-dimensional spectrum containing isotropic chemical shifts in the direct dimension and dipolar splittings in the indirect dimension. The dipolar shifts were reintroduced by the residual molecular ordering due to a 7.0 MV/m (rms) applied pulsed AC electric field. An indirect dimension slice showing the dipolar splitting for ortho-protons is also displayed. The vector  $\mathbf{r}_{mo}$  connecting coupled ortho- and meta-nuclei is parallel to the electric dipole moment of the nitrobenzene molecule,  $p$ , and forms an angle  $\theta_{mol}$  with the laboratory axis  $z'$ , which is parallel to the external magnetic field,  $H_0$ , and to the applied electric field,  $E$ . The two-dimensional plot shows that the dipolar splittings appear equally in the signals corresponding to ortho- and meta-protons, while the para-proton is apparently unaffected. The vector  $\mathbf{r}_{po}$  connecting the ortho- and para-nuclei is approximately 1.7 times longer than the ones connecting the neighboring protons. In addition, this vector and the vector  $\mathbf{r}_{pm}$  connecting the meta- and para-protons form angles  $\theta_{po}$  and  $\theta_{pm}$  equal to approximately 30 and 60° with  $p$ . Upon including the term  $(\frac{3}{2} \cos^2 \theta_{ij} - \frac{1}{2})$  in Eq. [4], one gets an approximately eight times reduction in the dipolar couplings for the po and pm pairs relative to the om pair.

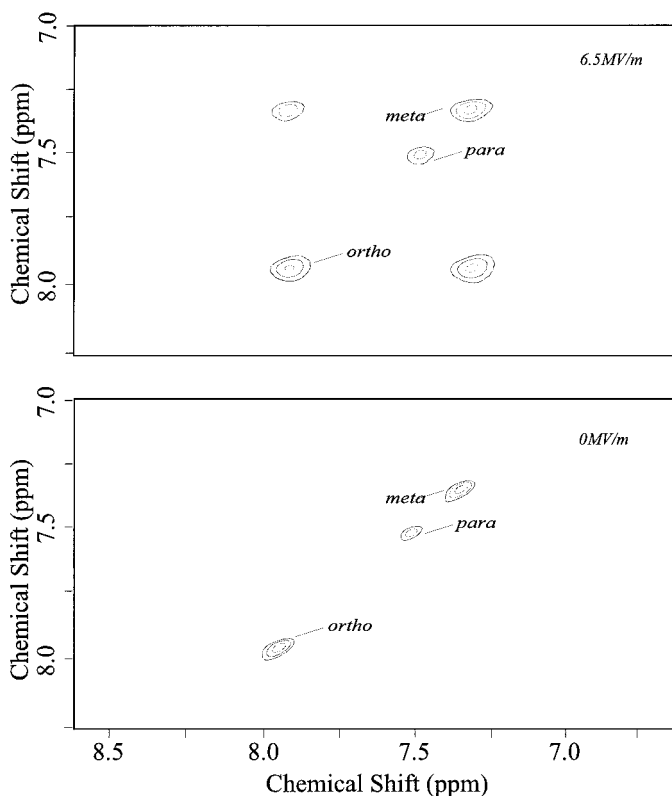
the experiments reported in this paper, within our estimated 5% experimental error. The dipole moment derived from the slope in Fig. 3 or Fig. 6 of Ref. (12) (7.3 D) is larger than the gas phase (4.2 D) value by a factor of approximately 1.7, presumably due to intermolecular interactions or cluster formation, as discussed previously (16).

Figure 4 illustrates two-dimensional spectra of nitrobenzene at 0 and at 6.5 MV/m (rms) electric fields, in which the electric field was applied for the mixing time only. In these spectra, both dimensions contained isotropic chemical shifts, so the corresponding peaks for para-, ortho-, and meta-protons appeared on the diagonal. The mixing time and the applied electric field strength were chosen in such a way that the interval was simultaneously a full  $J$  coupling period for ortho-protons (two periods for meta- and para-protons, which are  $J$  coupled to two neighbors) and approximately half of a period for the dipolar coupling between ortho- and meta-protons when the field was on. Thus, when no electric field was applied, no cross peaks were formed in the spectrum, and when a field of 6.5 MV/m (rms) was applied, strong cross peaks were observed between meta- and ortho-protons, as discussed quantitatively under Experimental.

A minor modification of this experimental setup could be used to determine the sign of the dipolar couplings. If the time window between the excitation and the storage pulses in the sequence shown in Fig. 1a or the mixing time in the sequence shown in Fig. 1b were not set to a  $J$  coupling “null,” but to



**FIG. 3.** The dependence of the dipolar splittings due to the residual molecular ordering on the square of the rms of the applied AC electric field is shown. The effective dipole moment of nitrobenzene, which was determined from the slope of the dependence, was in excellent agreement with that determined for nitrobenzene- $d_5$  in our previous studies (16). The  $R^2$  value for the line shown is larger than 0.99.



**FIG. 4.** Correlations of chemical shifts for dipolar-coupled pairs in nitrobenzene are shown; the pulse sequence in Fig. 1b, at 0 (rms) and 6.5 MV/m (rms), was used. The mixing time of 125 ms was chosen to simultaneously place the magnetization exchange due to  $J$  coupling at a minimum and the exchange due to the dipolar coupling at 6.5 MV/m (rms) applied electric field at a maximum. Strong dipolar cross peaks appear in the spectrum taken in the presence of the field and not in the zero-field control spectrum. Note that a  $J$  coupling pattern would lead to the formation of cross peaks between all neighboring proton pairs, not just between the ortho- and the meta-protons. Although not shown, weak cross peaks corresponding to the ortho-para and meta-para dipolar proton pairs were also observed.

another value, then the dipolar couplings between meta- and ortho-protons would either add to or subtract from the  $J$  couplings between them. In the case of the first pulse sequence, the sign of the dipolar couplings could be determined by noticing the changes in the indirect dimension peak splittings, and in the case of the second pulse sequence, the magnitude and the sign of the cross peaks would be affected.

Because AC electric fields can provide control over the time dependence of the order parameter in polar liquids, they can potentially be used to separate the effects of the tensorial interactions which depend on the molecular orientation, such as dipolar and quadrupolar couplings, from the ones which are independent from it, such as  $J$  coupling. For example, synchronization of refocusing pulses with the electric field would allow us to completely refocus the  $J$  interaction, but not dipolar, or vice versa. This could be useful for uncovering weak dipolar

couplings in systems containing large molecules and convoluted spectra.

## CONCLUSIONS

Pulsed AC electric fields were used to restore dipolar couplings between ortho- and meta-protons of nitrobenzene by inducing partial ordering of the molecules in the liquid. The dipolar couplings were demonstrated with two types of two-dimensional spectra. In one experiment, dipolar ordering due to the electric field was present during the indirect dimension only, while the direct dimension yielded the isotropic chemical shifts. In another experiment, both dimensions contained the isotropic chemical shifts, and the electric field was present only in the mixing period, so that dipolar couplings resulted in the formation of cross peaks between meta- and ortho-protons. The order parameter as a function of the applied electric field determined from these experiments was in an excellent agreement with that determined from our previous studies of the deuterium quadrupolar splittings for nitrobenzene- $d_5$  (16).

## ACKNOWLEDGMENTS

The authors thank Dr. Chad Rienstra for help with the longitudinal magnetization exchange pulse sequence. This project was supported by the Department of Energy (Grant DE-FG02-95ER14508), Training Program in Molecular Biophysics (2T32 GM08281), and R. C. Cottrell (CS 0046).

## REFERENCES

1. T. M. Plantenga, P. C. M. VanZijl, and C. MacLean, Studies of quadrupolar and dipolar electric field effects in the NMR spectra of binary mixtures of liquids, *Chem. Phys.* **66**, 1–9 (1982).
2. K. Schmidt-Rohr and H. W. Spiess, "Multidimensional Solid-State NMR and Polymers," Academic Press, London, 1994.
3. C. Gayathri, A. A. Bothner-By, P. C. M. VanZijl, and C. MacLean, Dipolar magnetic field effects in NMR spectra of liquids, *Chem. Phys. Lett.* **87**, 192–196 (1982).
4. J. R. Tolman, J. M. Flanagan, M. A. Kennedy, and J. H. Prestegard, Nuclear magnetic dipole interactions in field-oriented proteins—Information for structure determination in solution, *Proc. Natl. Acad. Sci. USA* **92**, 9279–9283 (1995).
5. P. C. M. VanZijl, C. MacLean, and A. A. Bothner-By, Angular correlation and diamagnetic susceptibilities studied by high field NMR, *J. Chem. Phys.* **83**, 4410–4417 (1985).
6. P. J. Bolon and J. H. Prestegard, COSY cross-peaks from H-1-H-1 dipolar couplings in NMR spectra of field oriented oligosaccharides, *J. Am. Chem. Soc.* **120**, 9366–9367 (1998).
7. N. Tjandra and A. Bax, Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium, *Science* **278**, 1111–1114 (1997).
8. M. R. Hansen, L. Mueller, and A. Pardi, Tunable alignment of macromolecules by filamentous phase yields dipolar coupling interactions, *Nat. Struct. Biol.* **5**, 1065–1074 (1998).
9. A. Bax and N. Tjandra, High-resolution heteronuclear NMR of human ubiquitin in an aqueous liquid crystalline medium, *J. Biomol. NMR* **10**, 289–292 (1997).
10. R. Y. Dong, "Nuclear Magnetic Resonance of Liquid Crystals," Springer-Verlag, New York, 1997.

11. A. D. Buckingham and K. A. McLauchlan, The absolute sign of the spin-spin coupling constant, *Proc. Chem. Soc.* 144, (1963).
12. R. E. Sears and E. L. Hahn, Upper limits to electric-field-induced nuclear magnetic dipole-dipole couplings in polar liquids, *J. Chem. Phys.* **45**, 2753 (1966).
13. J. D. Macomber, N. S. Ham, and J. S. Waugh, Upper limit to the electric-field effect on the NMR spectrum of nitromethane, *J. Chem. Phys.* **46**, 2855 (1967).
14. C. W. Hilbers and C. MacLean, NMR of molecules oriented in electric fields, *NMR Basic Prin. Progr.* **7**, 1-51 (1972).
15. L. Huis, F. J. J. Dekanter, and C. MacLean, Chemical shielding anisotropy of C-13 in CH<sub>3</sub>\*CN determined by NMR-spectroscopy of the dielectrically oriented molecule, *Mol. Phys.* **73**, 1077-1083 (1991).
16. A. Peshkovsky and A. E. McDermott, NMR spectroscopy in the presence of strong ac electric fields: Degree of alignment of polar molecules, *J. Phys. Chem.* **103**, 8604-8611 (1999).
17. L. Braunschweiler and R. R. Ernst, Coherence transfer by isotropic mixing: Application to proton correlation spectroscopy, *J. Magn. Reson.* **53**, 521-528 (1983).