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Orientations of the ¹⁷O electric-field-gradient tensor and chemical shift tensor in benzamide: NMR of dipolar coupled spins

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Abstract

We report a solid-state ¹⁷O NMR study of [α -¹³C, ¹⁷O] benzamide. The orientations of the ¹⁷O electric-field-gradient and chemical shift tensors were determined from analysis of magic-angle spinning and stationary ¹⁷O NMR spectra. The largest electric-field-gradient component lies in the amide plane and perpendicular to the ¹³C-¹⁷O dipolar vector, whereas the intermediate electric-field-gradient component is along the C=O bond. It is also found that the principal component of the ¹⁷O chemical shift tensor with the least shielding, δ_{11} , is approximately 18° off the C=O bond and that the component with the most shielding, δ_{33} , is perpendicular to the amide plane. The present study confirms our earlier results of quantum chemical calculations. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

Recently we attempted to determine the orientations of the ¹⁷O electric-field-gradient (EFG) tensor and chemical shift (CS) tensor for the carbonyl oxygen in amides [1–3]. Our approach was to obtain the *relative* orientation between the ¹⁷O EFG and chemical shift tensors by analyzing NMR spectra of a stationary powder sample at more than one magnetic field strength, and then deduce the *absolute* tensor orientations in the molecular frame from quantum chemical calculations. Recent advances in computational chemistry have made it possible to accurately evaluate NMR tensors at least for light elements [4–6]. As a result,

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quantum chemical calculations have become an increasingly important tool even to experimentalists. However, it is always desirable to obtain experimental confirmation for the results of quantum chemical calculations. The ideal experimental NMR technique for determining the absolute orientation of an NMR tensor in the molecular frame is single-crystal NMR. Unfortunately, single-crystal NMR experiments require large single-crystal samples that very often are difficult to obtain. Growing large single-crystals containing ¹⁷O labels is even more challenging since the quantity of the ¹⁷O-labeled sample is usually small.

In this contribution, we chose to use an alternative approach to determine the absolute orientation of ¹⁷O NMR tensors in the molecular frame. In particular, we synthesized singly and doubly isotopic labeled benzamide samples, [¹⁷O]

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benzamide and $[\alpha^{-13}C, {}^{17}O]$ benzamide. Using the ${}^{13}C{-}^{17}O$ dipolar vector as an internal reference in the molecular frame, we were able to determine the absolute orientations of the ${}^{17}O$ chemical shift and EFG tensors in benzamide. Although NMR of dipolar coupled spins has been extensively studied for spin-1/2 systems [7–14], we are unaware of any study in which the half-integer quadrupolar nucleus under direct observation is dipolar coupled to a spin-1/2 nucleus.

2. Experimental

2.1. Sample synthesis

Water (¹⁷O 52.8% atom) was obtained from ISOTEC (Miamisburg, Ohio). Benzoic acid-¹⁷O₂ was prepared by reacting α , α , α -trichlorotoluene and H₂¹⁷O in a sealed tube at 120°C for 15 h. Benzoic acid-¹⁷O₂ was then converted to [¹⁷O] benzoyl chloride (¹⁷O 52.8% atom) by refluxing thionyl chloride and benzoic acid-¹⁷O₂ for 1 h followed by distilling off the excessive thionyl chloride. [¹⁷O] benzoyl chloride was synthesized by reacting [¹⁷O] benzoyl chloride with ammonia in methanol at room temperature for 3 h.

2.1.1. $[\alpha^{-13}C, {}^{17}O]$ Benzoyl chloride

A total of 0.344 g (2.8 mmol) of $[\alpha^{-13}C]$ benzoic acid (¹³C 99.2 atom% obtained from CDN Isotopes, Pointe-Claire, Quebec, Canada) was refluxed with excessive thionyl chloride for 1 h. $[\alpha^{-13}C]$ Benzoyl chloride was obtained after removing the remaining thionyl chloride by distillation. 0.05 g (2.8 mmol) of H₂¹⁷O was added to $[\alpha^{-13}C]$ benzoyl chloride. The reaction tube was then sealed and left at 60°C for 5 h, which yielded solid $[\alpha^{-13}C, {}^{17}O]$ benzoic acid when cooled to room temperature. $[\alpha^{-13}C, {}^{17}O]$ Benzoic acid was readily converted to $[\alpha^{-13}C, {}^{17}O]$ benzoyl chloride by the same procedure as mentioned above.

2.1.2. $[\alpha^{-13}C, {}^{17}O]$ Benzamide

A cold ammonia-methanol solution was added dropwise to $[\alpha$ -¹³C, ¹⁷O] benzoyl chloride until the temperature was stabilized. The solution was stirred at room temperature for 3 h. The solution was then poured into cold water and the white product was extracted with ethyl ether. The product was characterized by IR, NMR and melting point. The theoretical ¹⁷O enrichment level in $[\alpha$ -¹³C, ¹⁷O] benzamide is 26.4%.

2.2. Solid-state NMR

Solid-state ¹⁷O NMR spectra were recorded on a Bruker Avance-500 spectrometer operating at 500.13 and 67.78 MHz for ¹H and ¹⁷O nuclei, respectively. Polycrystalline samples were packed into zirconium oxide rotors (4 mm o.d.). A Bruker 4 mm MAS probe was used for both MAS and static experiments. The sample spinning frequency was 14 kHz in the MAS experiments. The RF field strength for ¹⁷O was approximately 80 kHz. In the ¹⁷O static experiments, the whole-echo acquisition approach [15] was used to avoid acoustic ringing from the probe and to enhance sensitivity. In particular, the pulse width for the first and second pulses was 1 and 2 µs, respectively. The delay between the first and second pulses was 120 µs, which is sufficiently long to allow the detection of the whole echo signal. To the whole-echo NMR signal was applied a large first-order phase correction to obtain the distortion-free line shape. A recycle delay of 10 s was used in all experiments. Chemical shifts were referenced to a liquid H₂O sample. Spectral simulations were performed with WSOLIDS (Drs. Klaus Eichele and Rod Wasylishen, Dalhousie University).

3. Results and discussion

Fig. 1 shows the experimental and calculated ¹⁷O MAS NMR spectra of $[\alpha^{-13}C, {}^{17}O]$ benzamide obtained with a spinning rate of 14 kHz. The spectrum exhibits a typical central-transition NMR line shape arising only from the second-order quadrupole interaction. This is because the ${}^{13}C{}^{-17}O$ dipolar interaction is completely averaged by MAS and ${}^{1}J({}^{13}C, {}^{17}O)$ is negligibly small. The only indirect spin–spin coupling constants between ${}^{13}C$ and ${}^{17}O$ nuclei reported in the literature are those for acetone (${}^{1}J({}^{13}C, {}^{17}O) = 22$ Hz) [16], CO (${}^{1}J({}^{13}C, {}^{17}O) = 16.4$ Hz) [17] and CO₂(${}^{1}J({}^{13}C, {}^{17}O)$



Fig. 1. Experimental (upper trace) and calculated (lower trace) ¹⁷O MAS NMR spectra of $[\alpha$ -¹³C, ¹⁷O] benzamide at 11.75 T. Spinning rate, 14 kHz. Recycle delay, 10 s. Number of transients, 1306.

= 16.1 Hz) [17]. These provide an upper limit for ¹J(¹³C, ¹⁷O) in amides. Analysis of the ¹⁷O MAS spectrum yields the following NMR parameters: $\delta_{iso} = 300 \pm 1$ ppm, $e^2 qQ/h = 8.40 \pm 0.01$ MHz and $\eta = 0.37 \pm 0.02$. These parameters are identical to those reported in our earlier study of [¹⁷O] benzamide [1].

Before we present detailed experimental ¹⁷O NMR results for stationary samples, it is worth discussing briefly the basic principles of dipolar NMR spectroscopy. For magnetically dilute spin-1/2 nuclei, an NMR spectrum obtained for a stationary powder sample often exhibits a line shape known as the chemical shift powder pattern. From the three singularities of the chemical shift powder pattern, it is straightforward to obtain the principal components of the chemical shift tensor. In the absence of any local molecular symmetry, it is generally difficult to know the directions of these principal components in the molecular frame of reference. When the spin-1/2 nucleus under observation is adjacent to another spin-1/2 nucleus of different kind, spectral features known as the dipolar splittings [13,14] are often observed at the singularities of the chemical shift powder pattern. The magnitude of the dipolar splittings can be written as

$$\Delta v_{ii} = R_{\rm DD} (1 - 3\cos^2 \vartheta_{ii}) \tag{1}$$

where $R_{\rm DD}$ is the dipolar coupling constant, $(\mu_0/4\pi)(1/r^3)\gamma_1\gamma_2(h/4\pi^2)$, and ϑ_{ii} is the angle be-

tween the dipolar vector and the *i*th principal component of the chemical shift tensor (i = 1, 2, 3). The general appearance of a dipolar NMR spectrum is simply a sum of two overlapping chemical shift powder patterns [14]. From the experimental dipolar splittings, it is possible to obtain the orientations of the principal elements of the chemical shift tensor with respect to the dipolar vector. The essence of the dipolar NMR approach is that the dipolar vector serves as an internal reference.

For half-integer quadrupolar nuclei, the situation is more complex. Here we will focus only on NMR spectra from the central-transition $(-1/2 \leftrightarrow +1/2)$. Under such circumstances, two types of second-rank tensors are operative, e.g., chemical shift tensor and EFG tensor. The resultant NMR spectrum usually exhibits more singularities than does a normal chemical shift powder pattern. Detailed theoretical analysis of such NMR powder spectra can be found in the literature [18-22]. In general, it is possible to obtain information about the relative orientation between the chemical shift and EFG tensors from analyzing stationary NMR spectra obtained at multiple magnetic fields. Of course, the absolute orientations of these tensors in the molecular frame of reference are still unavailable from such an analysis.

Similar to the dipolar NMR for spin-1/2 nuclei, if the quadrupolar nucleus under observation is dipolar coupled to a neighboring spin-1/2 nucleus, dipolar splittings will also be observed at spectral singularities. However, in this case, the singularities may not correspond to the positions of any principal component of either chemical shielding or EFG tensors. Rather these singularities are known as the critical frequencies or critical points [19]. In general there are up to six critical points in a powder NMR spectrum arising from a combination chemical shift and second-order quadrupolar interactions. As illustrated in Fig. 2A, a typical ¹⁷O NMR powder spectrum without any dipolar coupling exhibits both steps (labeled as S) and peaks (labeled as P). In order to illustrate the basic features of dipolar NMR spectra for halfinteger quadrupolar nuclei, three different situations where a neighboring spin-1/2 nucleus is present are considered in Fig. 2B-D. Here the



Fig. 2. Typical ¹⁷O NMR powder spectrum (A) and dipolar spectra (B, C, D). The positions of the six critical frequencies are labeled as S1, S2 and S3 for steps and P1, P2, and P3 for peaks. In (B), (C) and (D), the orientation of the dipolar vector is given.

direction of the dipolar vector in the principalaxis-system (PAS) of the EFG tensor was defined by (ϕ, θ) where θ is the polar angle and ϕ the azimuthal angle.

Fig. 2B shows the theoretical NMR line shape when the dipolar vector lies along the largest component of the EFG tensor, V_{ZZ} . Clearly dipolar splittings are observed at all critical points. As expected, the magnitude of each dipolar splitting depends on the relative orientation of the dipolar vector with respect to the direction that gives rise to the critical frequency. For example, as seen from Fig. 2B, the dipolar splitting at the critical point S2 is approximately two times larger than those at S1 and P1. The dipolar splittings at P2 and P3 are very small. As seen from Fig. 2C, when the dipolar vector is at (0°, 90°), which means that the dipolar vector is along the smallest EFG component, V_{XX} , the dipolar splittings at S1 and S2 are much smaller than that at P1. Similarly, when the dipolar vector is along the intermediate EFG component, V_{YY} , the largest dipolar splitting in the ¹⁷O NMR spectrum appears at S1, as illustrated in Fig. 2D.

The experimental and calculated stationary ¹⁷O NMR spectra of [¹⁷O] benzamide and [α -¹³C, ¹⁷O] benzamide are shown in Fig. 3. The analysis of the ¹⁷O NMR spectrum for [¹⁷O] benzamide has been described in detail, in our previous studies [1–3]. From the analysis, we obtained the following parameters: $\delta_{11} = 500 \pm 20$, $\delta_{22} = 400 \pm 20$, $\delta_{33} = 0 \pm 20$ ppm, $\alpha = 8 \pm 5^{\circ}$, $\beta = 90 \pm 2^{\circ}$, $\gamma = 72 \pm 5^{\circ}$ where the three Euler angles define the relative orientation between the EFG and chemical shielding tensors. These results suggest that V_{XX} is parallel to δ_{33} and that δ_{11} makes an angle of $18 \pm 5^{\circ}$ with respect to V_{YY} [1–3].

Comparison of the NMR spectra shown in Fig. 3 clearly reveals the presence of dipolar splittings at the critical points of the spectrum for $[\alpha^{-13}C, {}^{17}O]$ benzamide. The largest dipolar splitting was observed at approximately 580 ppm (denoted as S1), 3868 ± 50 Hz. Meanwhile the dipolar splitting at 200 ppm (P1) is much smaller, 1616 ± 50 Hz. The smallest dipolar splitting is at 20 ppm (P3), 1176 ± 50 Hz. As mentioned earlier, analysis of the ¹⁷O NMR spectrum of [¹⁷O] benzamide yielded the relative orientation between the ¹⁷O EFG and chemical shielding tensors. The observation of dipolar splittings in the ¹⁷O NMR spectrum of $[\alpha^{-13}C, {}^{17}O]$ benzamide allowed us to obtain the orientation of the dipolar vector in the PAS of the EFG tensor. In this case, we found that the use of a dipolar vector direction of (90°, 90°) in spectral simulation yielded the best agreement between the observed and calculated line shapes. This result means that the ¹³C–¹⁷O dipolar vector is along the intermediate component, V_{YY} . The dipolar coupling constant, $R_{DD}(^{13}C, ^{17}O)$, used in the spectral simulation was -2100 ± 50 Hz, which corresponds to a C=O bond length of



Fig. 3. Experimental (A) and calculated (B) stationary ¹⁷O NMR spectra of [¹⁷O] benzamide at 11.75 T. Recycle time, 10 s. Number of transients, 2924. Experimental (C) and calculated (D) stationary ¹⁷O NMR spectra of [α -¹³C, ¹⁷O] benzamide at 11.75 T. Recycle delay, 10 s. Number of transients, 4991.

 1.249 ± 0.009 Å. This is in excellent agreement with that determined from a low-temperature neutron diffraction study, 1.245 Å [23].

Interestingly, the observed dipolar splitting at S1, 3868 \pm 50 Hz, is nearly twice of $R_{DD}(^{13}C, ^{17}O)$, which means the direction of S1 is nearly parallel to the C=O bond. Using Eq. (1), we calculated the angle between the direction of S1 and the C=O bond to be $13 \pm 2^{\circ}$. Since S1 represents the highest frequency end of the spectrum, to a very good approximation, it corresponds to the direction of the tensor component with the least shielding, δ_{11} . Therefore, the value of $13 \pm 2^{\circ}$ is also consistent with our previous result that δ_{11} makes an angle of $18 \pm 5^{\circ}$ relative to the C=O bond [1–3]. Of course, the precise direction of S1 must be calculated using the equations given by Chu and Gerstein [19].

The orientations of the ¹⁷O NMR tensors in the molecular frame of benzamide are depicted in Fig. 4. The largest component of the EFG tensor, V_{ZZ} , is in the amide plane and perpendicular to the C=O bond. The smallest EFG tensor component, V_{XX} , is perpendicular to the amide plane. For the ¹⁷O chemical shielding tensor, the principal component with the largest shielding, δ_{33} , is perpendicular to the amide plane. Most importantly, the tensor component with the least shielding, δ_{11} , is $18 \pm 5^{\circ}$ off the C=O bond. These orientations



Fig. 4. Illustration of the orientations of the 17 O NMR tensors in benzamide.

confirm our earlier results of quantum chemical calculations [1–3].

Finally, it should be mentioned that the fact that the dipole–dipole coupling tensor is axially symmetric imposes an intrinsic limitation on the dipolar NMR approach. That is, dipolar NMR spectra are invariant to any simultaneous rotation of the NMR tensors about the dipolar vector. In the present case, since the tensor component associated with the most shielding, δ_{33} , is known to be always perpendicular to the amide plane, the absolute orientations of the ¹⁷O NMR tensors

depicted in Fig. 4 can be unambiguously determined. Based on results of the present and previous studies [1–3], we can conclude that the absolute orientations of the ¹⁷O NMR tensors for an amide functional group show very little variations among different amides. However, the magnitude of the ¹⁷O NMR tensors is very sensitive to both inter- and intra-molecular interactions. It is this latter aspect of the ¹⁷O NMR tensors that may be useful in future solid-state ¹⁷O NMR studies of chemical and biological systems.

4. Conclusions

We have utilized the ${}^{13}C{-}^{17}O$ dipolar interaction in [α - ${}^{13}C$, ${}^{17}O$] benzamide to obtain the orientations of ${}^{17}O$ NMR tensors in the molecular frame. The results for [α - ${}^{13}C$, ${}^{17}O$] benzamide confirmed our earlier theoretical results, which strongly suggests that the current quantum chemical calculations are capable of yielding reliable results for ${}^{17}O$ NMR tensor orientations. Since both experimental and theoretical studies suggest that the ${}^{17}O$ NMR tensor orientations in primary amides are similar to those in secondary amides, the present results for benzamide are also relevant to the situation in a peptide bond. The information obtained from the present study provides a foundation for future solid-state ${}^{17}O$ NMR studies of peptides and proteins.

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