

Nitrogen-15 Chemical Shift Tensors in L-Histidine Hydrochloride Monohydrate

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Abstract: ¹⁵N chemical shift tensors in L-histidine hydrochloride monohydrate were determined from orientation-dependent studies of single crystals. In contrast to a previously reported case of a nitrogen in a heterocyclic ring, the imidazole ring nitrogen tensors in the present compound are of moderate size (both about 230 ppm). It is suggested that this is due to the fact that both nitrogens are protonated. The principal components of the tensors are oriented approximately along the molecular symmetry directions of the ring. The least shielded element σ_{33} lies approximately along the NH bond direction, and the most shielded element σ_{11} is perpendicular to the plane of the imidazole ring. The NH₃⁺ tensor is rather small (<12 ppm). The data suggest that high-field solution ¹⁵N NMR experiments will not be plagued with shift anisotropy broadening, and for similar reasons high-field magic angle spinning spectra of solids will not exhibit inordinate numbers of rotational sidebands. A comparison of the relative sizes of a variety of ¹⁵N shift and the corresponding ¹⁴N quadrupole tensors is made, and an anomaly with regard to NH₃⁺ groups is pointed out.

Introduction

The development of high-resolution solid-state NMR techniques has made possible the study of chemical shift tensors, and in the past few years, tensors for a number of different nuclei (e.g., ¹H, ¹⁹F, ³¹P, and ¹³C) in a variety of functional groups have been studied.^{1,2} To date, however, there has been a conspicuous absence of data on ¹⁵N chemical shift tensors, presumably because of the difficulty of detecting nuclear signals from this low-abundance, low-gyromagnetic ratio spin. The exceptions to this statement are reports on NH₄NO₃ and glycine,³ nitrobenzene,⁴ and pyridine,⁵ which relied heavily on enriched samples. Nevertheless, it can be expected that ¹⁵N will receive more attention in the near future for a number of reasons. First, the increased availability of high-field spectrometers and the development of polarization transfer techniques for both solids and liquids provide a means of at least partially circumventing the acute sensitivity problem presented by ¹⁵N. Spectrometers operating in the vicinity of 12 T are now beginning to appear, and two methods for transferring proton polarization to ¹⁵N, which has been common practice in solid-state spectroscopy,⁶ have recently been demonstrated for liquids. Specifically, *J* cross polarization⁷ and INEPT⁸ allow rapid acquisition of ¹⁵N solution spectra, and both circumvent the variable NOE which sometimes results in a vanishing small ¹⁵N signal. Secondly, cross polarization can be combined with magic angle sample spinning (MASS) to yield at least pseudo-isotropic spectra of solids.⁹ Finally, of the three or four nuclei which occur abundantly in biological systems (for example, proteins and nuclei acids), nitrogen is very often functionally important because of its ability to form hydrogen bonds, etc. Thus, if the sensitivity problem can be overcome, then ¹⁵N would be an ideal candidate for investigating biopolymers.

Data on ¹⁵N shift tensors are important for investigations of both solids and solutions, although for slightly different reasons. Sensitivity considerations dictate that ¹⁵N spectroscopy should be performed at high fields, but at some point shift anisotropy relaxation will begin to dominate solution line widths, particularly of biopolymers. Examples of this phenomenon have already been observed in the case of ¹⁹F-labeled proteins.¹⁰ Thus, if ¹⁵N shift tensors were anomalously large, then any gain in sensitivity from high-field observation would be partially offset by shift anisotropy line broadening. The report of an ~770-ppm tensor in pyridine⁵ draws particular attention to this point. Alternatively shift anisotropy line broadening can be a useful source of information on molecular motion. That is, if this line broadening is observed and a reasonable model for the molecular motion can be postu-

lated, then this broadening can be used to obtain molecular correlation times provided the requisite rigid lattice tensor data are available.¹⁰ In MASS spectra of solids, sidebands appear when the spinning rate, ν_r , is less than the size of the shift tensor, $\Delta\sigma$ (in hertz),¹¹ the number of sidebands being

$$N \simeq [(\Delta\sigma/\nu_r) - 1]$$

At high fields *N* is obviously going to be larger and thus data on the size of shift tensors will be useful in assigning centerbands and sidebands in such spectra and/or determining the optimal spinning rate for MASS experiments. Finally, one of the most chemically interesting applications of solution NMR is the study of intra- and intermolecular chemical exchange. In solids the same type of phenomena can be observed, but in such cases the opportunity to observe the anisotropy of the motion is present.^{12,13} Moreover, the types of spectra observed are strongly dependent on the details of the motional process, for example, whether it is discrete or continuous reorientation, and on the relative orientation of the axis of motion with respect to the shift tensor. Of course, the rate of the motion with respect to the size of the shift tensor influences the line shapes. These statements are applicable to both MASS and stationary powder spectra, and in both cases a knowledge of the shift tensor is requisite for understanding the spectra.

For these reasons we have undertaken a series of studies of various ¹⁵N shift tensors and here we report the results of a single crystal investigation of L-His·HCl·H₂O. This compound was

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Table I. Principal Values and Direction Cosines of the Imidazole ¹⁵N Shielding Tensors in L-His·HCl·H₂O^a

N	principal value, ppm	shift tensor direction cosines			xyz direction cosines			$\frac{1}{3} \sum \sigma_{ii}$
		a	b	c	a	b	c	
N2	38.8	0.8682	0.4919	-0.0652	z: 0.8669	0.4944	-0.0640	165.8
	198.1	-0.4751	0.7861	-0.3954	y: -0.4268	0.6696	-0.6078	
	260.5	0.1432	-0.3743	-0.9162	x: 0.2576	-0.5542	-0.7915	
N3	35.1	0.8674	0.4947	-0.0539	z: 0.8656	0.4960	-0.0692	152.2
	170.1	0.0578	0.0074	0.9983	y: 0.1861	-0.1903	0.9639	
	251.3	-0.4942	0.8691	0.0222	x: -0.4649	0.8472	0.2570	
NH ₃ ⁺	$\Delta\sigma < 12$							

^a The principal values are in ppm relative to external powdered (NH₄)₂SO₄ and the direction cosines are referred to the *abc* unit cell axes. Also included are direction cosines of two Cartesian *xyz* coordinate systems chosen to facilitate an understanding of the molecular orientation of the tensors. Specifically, *z* and *z'* are perpendicular to the plane of the imidazole ring, *x* and *x'* bisect the CNC angles, and *y* and *y'* are orthogonal to these directions. These are shown in Figure 3.

chosen for study because it is easily crystallizable and has a known crystal structure,¹⁴ and crystals can be doped with paramagnetic Cu²⁺ to shorten the proton *T*₁.¹⁵ In addition, for the compound studied here, the imidazolium ring is protonated and is a formally aromatic five-membered heterocyclic ring. Consequently, it affords an opportunity to determine if the large shift anisotropy exhibited by pyridine is a property of "aromatic" heterocycles or if it is due to the low symmetry at the nitrogen which results from the pyridine nitrogen being nonprotonated. Finally, histidine is a widely occurring residue in proteins, with considerable functional significance at enzyme active sites. It could therefore be a useful intrinsic probe in ¹⁵N NMR studies of solid and liquid crystalline biopolymers or in solution studies of such molecules, particularly in cases where selective ¹⁵N labeling is possible.

Experimental Section

Single crystals of L-His·HCl·H₂O were grown by slow evaporation from an aqueous solution containing Cu²⁺ ions.¹⁵ Three suitable crystals were cut and mounted on machined Kel-F buttons which in turn were mounted in the goniometer of the NMR probe. Spectra were taken in 10° angular increments using a multiple contact version of the direct observation double resonance experiment described by Pines et al.⁶ with a cross polarization time of 2 ms and a 2-s recycle delay. Typically about 20 min was necessary to obtain a spectrum with 2 suitable signal-to-noise ratio. Line positions were referenced to solid powdered (NH₄)₂SO₄ which exhibits an isotropic resonance line. The data were analyzed according to standard procedures.¹⁶

Results and Discussion

Crystallographic studies indicate that the space group of L-His·HCl·H₂O is *P*2₁2₁2₁, and the unit cell contains four molecules which are related by the transformations *xyz*, $\bar{x}yz$, *x* $\bar{y}z$, and *xyz*.¹⁴ Thus, in a general orientation we should observe four magnetically inequivalent molecules, and because there are three nitrogens per molecule, a total of 12 lines should appear in the spectrum. When the rotation axis coincides with one of the crystallographic twofold axes, this number is reduced theoretically by a factor of 2. However, in the case of L-His·HCl·H₂O a further reduction is evident as is illustrated in the spectrum of Figure 1. The lines on the right of this spectrum, which was obtained from the a rotation with $\theta = 0^\circ$, are due to the ring nitrogens, while the line on the left is due to the four NH₃⁺ moieties. In essentially all crystal orientations we observe the type of behavior exhibited in this spectrum; that is, the NH₃⁺ lines are degenerate, or almost so, while the ring nitrogen lines are split by 100–200 ppm. This is further illustrated by the rotation plot shown in Figure 2 which exhibits data for rotation approximately about the a crystallographic axis. Here we discern that the NH₃⁺ exhibits essentially no angular dependence, while the four ring nitrogen lines move over about 100 ppm as the crystal is rotated through 180°. From Figures 1 and 2 we can surmise that ¹⁵N shift tensors for NH₃⁺ groups are quite small and those for the protonated imidazolium

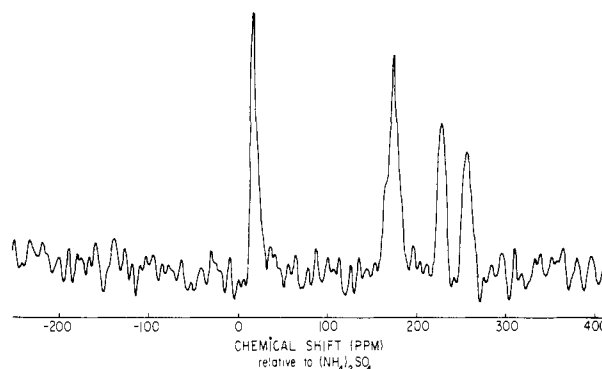


Figure 1. Natural abundance ¹⁵N crystal spectrum obtained from L-His·HCl·H₂O. The a unit cell axis was approximately along rotation axis of the NMR goniometer. Note, the single line on the left is due to the NH₃⁺ moieties which indicates that the shift tensors for these groups are very small. The lines on the right are due to the imidazole nitrogens.

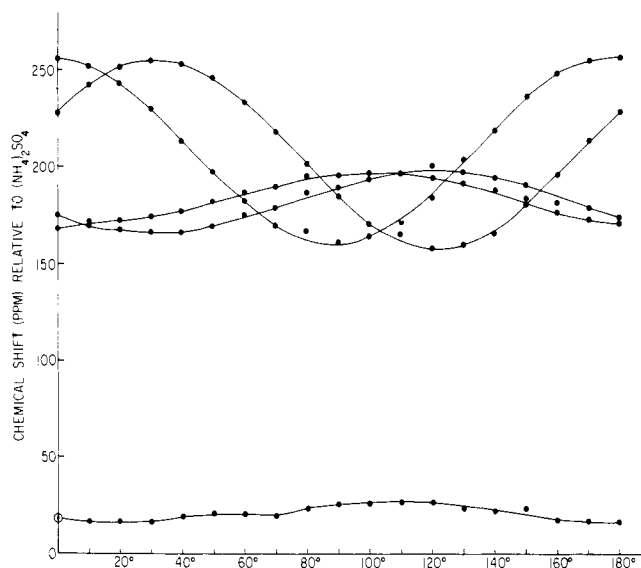


Figure 2. Rotation plot obtained from the a rotation illustrating the small angular dependence of the NH₃⁺ lines and the relatively large angular dependence of the imidazole lines.

ring are of moderate size, ~200 ppm. By combining data from Figure 2 with data from the two other approximately orthogonal crystal rotations we obtain the principal values and direction cosines for the ¹⁵N tensors given in Table I. Also listed in this table are direction cosines for two orthogonal coordinate systems whose orientations were chosen to illustrate the molecular orientations of the tensors. Specifically, *z* and *z'* were taken as perpendicular to the imidazole ring, the *x* and *x'* bisect the CNC angle, and the *y* and *y'* are orthogonal to these directions. The orientation of these coordinate systems are shown in Figure 3 along with the orientations of the imidazole nitrogen tensors.

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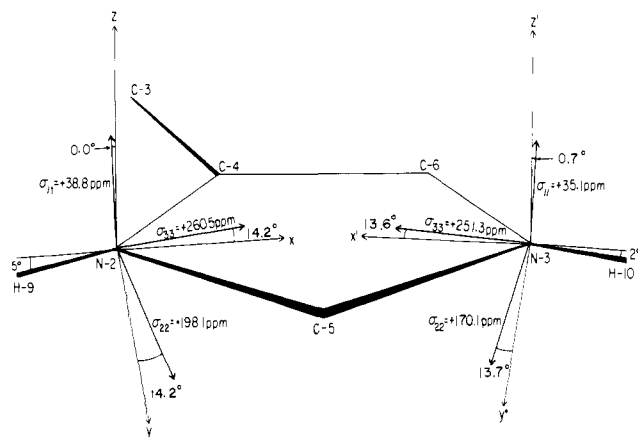


Figure 3. Illustration of the molecular orientation of the ^{15}N imidazole tensors. The xyz coordinate systems were chosen so that z is perpendicular to the ring, x bisects the CNC angle, and y is orthogonal to these orientations. The principal axes of the shift tensor lie near these molecular "symmetry" directions.

As can be discerned from Table I, the ^{15}N imidazole tensors are not unusually large. Both tensors have a breadth of about 230 ppm which is a factor of about 3.5 smaller than was found in pyridine, where a breadth of 782 ppm was observed,⁵ and a factor of two smaller than in nitrobenzene, where a breadth of 429 ppm was reported.⁴ In addition, both tensors are axially asymmetric, the asymmetry parameters being 0.49 and 0.69 for N2 and N3, respectively. The difference in the trace of the two tensors is 13.6 ppm which is considerably larger than the 2.4-ppm difference observed between the two nitrogens in solution at low pH.¹⁷ The difference in the size of the shift tensors observed in L-His-HCl-H₂O, and in other ^{15}N tensors as well, when compared with that of pyridine, is undoubtedly due in part to the fact that the nitrogen in pyridine is not protonated. As a consequence the symmetry at the nitrogen is lower and the shift tensor is larger. Evidence in support of this view is available from other experiments to be reported in a future publication.¹⁸ In particular, ^{15}N spectra obtained from samples of His lyophilized from a pH 8.6 solution show two overlapping powder patterns. One of these is nearly identical in breadth with the tensors for the protonated nitrogen discussed here and has been assigned to the N2 nitrogen. However, the other tensor which has been assigned to the N3 nitrogen via specific isotopic enrichment is almost 400 ppm wide. At pH 8.6 this nitrogen is expected to be nonprotonated¹⁷ and this establishes that the lower symmetry induces a substantial change in the ^{15}N shift tensor.

The molecular orientations of the tensors are illustrated in Figure 3, and we see that the principal components are near the molecular symmetry directions. For instance, σ_{11} is almost perpendicular to the plane of the imidazole ring and σ_{33} is very nearly along the N-H vector for both nitrogens. Correspondingly, the σ_{22} element is approximately orthogonal to the NH direction and in the plane of the imidazole ring. These results are quite similar to those found for ^{13}C tensors in aromatic rings. For example, in durene¹⁹ and in the polyalkybenzenes²⁰ the σ_{11} component of the aromatic ^{13}C tensor is perpendicular to the ring and the σ_{33} component is along the C-CH₃ direction.

The very small shift anisotropy of the NH_3^+ group ($\Delta\sigma < 12$ ppm) has also been observed in other cases. For example, it has

been reported in glycine³ and also observed in lysine,²¹ asparagine,²² and in Gly-Gly-HCl-H₂O.²³ Similarly, NH_4^+ ions, for instance, in $(\text{NH}_4)_2\text{SO}_4$, have a vanishingly small anisotropy as was mentioned above. An interesting comparison can be made between the size of these shift tensors and the corresponding ^{14}N quadrupole couplings. In NH_4^+ ions both the shift anisotropy and the quadrupole couplings are small. For instance, in $(\text{NH}_4)_2\text{SO}_4$ or $\text{NH}_4\text{H}_2\text{C}_2\text{O}_4 \cdot 1/2\text{H}_2\text{O}$ the quadrupole coupling is of the order of 100 kHz and the shift anisotropy is ≈ 10 ppm.²⁴ In other less symmetric cases the shift tensors increase to 150–200 ppm and correspondingly there is a substantial increase in the quadrupole couplings. For His-HCl-H₂O the imidazole shift tensors are ~ 230 ppm and the quadrupole couplings are 1.29 and 1.46 MHz with $\eta = 0.97$ and 0.30, for N2 and N3, respectively.²⁵ Likewise, in peptide nitrogens the shift tensors are about 150 ppm²³ and $(e^2qQ/h) \sim 3$ MHz. In contrast to this trend are the data for NH_3^+ groups. In particular, even though the shift tensors are small the quadrupole couplings are rather large. For glycine $e^2qQ/h = 1.18$ MHz and $\eta = 0.54$ at room temperature²⁶ but $\Delta\sigma \approx 10$ ppm; the same is true for L-His-HCl-H₂O. In view of this apparent anomaly, and the relatively large amount of data available on both shift and quadrupole tensors, these cases should prove interesting for quantum chemical calculations.

In the Introduction, it was mentioned that one reason for investigating ^{15}N shift tensors was to determine if shift anisotropy broadening will be an important relaxation mechanism in high-field solution studies. In addition, the relationship between the size of the shift tensors and the number of sidebands observed in MASS spectra provided another motivation for this work. In view of the moderate size shift tensors observed for L-His-HCl-H₂O, it appears that solution experiments can be performed at currently available fields without undue line broadening. In fact, the experiments could probably be performed at two to three times higher fields before these effects become important. In addition, MASS ^{15}N spectra taken at spinning speeds of 2–4 kHz will exhibit only weak sidebands except in cases where nonprotonated nitrogens occur, and in this situation one can expect ~ 2 pairs of relatively weak rotational sidebands.

Conclusions

In summary, it appears that ^{15}N chemical shift tensors exhibit some unexpected as well as some expected properties. In particular, the relatively small size of NH_3^+ -type sensors is somewhat surprising in view of the previously known size of the corresponding ^{14}N quadrupole couplings. In contrast, both the size and molecular orientation of the imidazole ring ^{15}N tensors is similar to those observed for ^{13}C aromatic tensors. In view of these results, it can be said that high-field solution ^{15}N investigations will not be plagued with shift anisotropy broadening effects except for cases like pyridine or His at high pH where the breadth of the tensor increases substantially because the nitrogen is nonprotonated. For similar reasons ^{15}N MASS spectra of protonated nitrogens will consist primarily of centerbands with weak sidebands. But for nonprotonated species ca. two pairs of sidebands will be observed.

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