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Lanthanide-Induced Nuclear Magnetic Resonance Shifts and Molecular Structure of L-Azetidine-2-Carboxylic Acid. II. Determination of Molecular Conformation

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Lanthanide-induced shifts (LIS) in ^{1}H NMR spectra of L-azetidine-2-carboxylic acid were observed in D_2O solution using Pr(III), Nd(III), and Eu(III) nitrates as shift reagents. By taking the LIS of one of the protons as reference, the shift ratios for the other four protons were calculated. The shifts ratio obtained were nearly constant for the three lanthanide ions, except for that with respect to the α proton which is located closest to the lanthanide ion. This indicates that the contribution of the contact shift is appreciable only for the observed LIS of the α proton and is negligible for the LIS's of the β and γ protons. The constancy of shift ratios assures, in addition, that the magnetic tensor is axially symmetric. Therefore, the LIS's of the β and γ protons may be used for the structure determination. For the β and γ protons, the ratios of LIS's intrinsic to the lanthanide-substrate 1 : 1 complex were corrected for the contribution of diamagnetic shifts due to complex formation which were estimated by the use of La(III) nitrate. The line broadening induced by Gd(III) was also observed. The structure analysis based on these data shows that the four-membered ring of L-azetidine-2-carboxylic acid is puckered with the dihedral angle of 25° and the carboxylate is in the equatorial position.

In order to utilize the lanthanide-induced nuclear magnetic resonance shift (LIS) to the quantitative discussion of molecular structure in solution, it seems to be a good practice to follow the procedures outlined below.

(a) The observed LIS may be a weighted average of those intrinsic to various stoichiometric complexes. The presence of a lanthanide-substrate 1 : 2 complex (abbreviated as LS_2) in addition to a 1 : 1 complex (LS) has been established in some cases.[1–3] The LIS intrinsic to the LS complex should be evaluated for the structure determination. In a preceding paper we discussed this problem and proposed an extrapolation method useful for evaluating the ratios of LIS's intrinsic to the LS complex.[3]

(b) The position of a nucleus (i) in the substrate molecule coordinated to a lanthanide ion may be determined by the use of the following equation, provided that the observed shifts arise solely from the dipolar interaction and that the magnetic tensor is axially symmetric.

$$\Delta\nu_i/\nu_0 = K\langle(3\cos^2\theta_i - 1)/r_i^3\rangle \qquad (1)$$

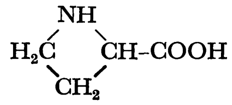
where ν_0 is the probe frequency, ν_i is the observed value of LIS, r_i is the length of the vector joining the lanthanide ion with the i th nucleus, and θ_i is the angle between this vector and the unique magnetic axis. Although two different expressions have been given to K by McConnel and Robertson[4] and by Bleaney *et al.*,[5] K can be treated as a constant characteristic of a lanthanide ion in applying this equation for the structure determination. Then, the ratios of observed LIS's of different nuclei should be nearly constant for various lanthanide ions, if the observed LIS's are predominantly due to the dipolar interaction[6] and the magnetic tensor is axially symmetric. This constancy of shift ratios for various lanthanides should be checked experimentally.[5]

(c) The correction for diamagnetic shifts induced by the complex formation should be made by observing the shifts with a diamagnetic lanthanoid La(III) or Lu(III).[5]

(d) The line broadening caused by Gd(III) should also be observed to supplement the LIS data.[5,7,8]

Recently Barry *et al.* applied these procedures to the structure determination of adenosine-5′-monophosphate[9] and cyclic 3′,5′-adenosine monophosphate.[10] We have applied them to L-azetidine-2-carboxylic acid (abbreviated as Az acid) in D_2O solution and have obtained satisfactory results which will be reported in this paper.

Az acid is a naturally occurring cyclic imino acid (see below) and is known to inhibit various reactions in many biological systems. The inhibitory action of Az acid is related to its incorporation into some proteins, where it replaces an equivalent amount of proline. The references dealing with biological activities of Az acid may be found in the paper by Berman *et al.*[11] in which the crystal structure of this compound has been reported. The information on the molecular structure of Az acid in aqueous solution will be of use in elucidating the molecular basis of its biological activities.



Experimental

^{1}H NMR spectra were observed in a similar way as described in the previous paper.[3] For the purpose of comparing the ratios of LIS's for various lanthanide ions, the successive addition of solid lanthanide nitrates to a substrate solution was employed, because it is experimentally simpler than either the successive dilution or the incremental dilution.

The structure analysis based on the LIS data was carried out by the programs PCS-I and II prepared in our laboratory.

Results and Discussion

Shift Ratios. As described above, it is important, before applying Eq. (1) to the structure determination, to check if the ratios of LIS's observed of different protons are constant for various lanthanide ions. For this purpose the LIS's of Az acid were observed in D_2O solution using the nitrates of praseodymium, neodymium, and europium as shift reagents.[12] Since Pr(III) and Nd(III) induced greater line broadening than Eu(III) did, the measurements with Pr(III) and Nd(III) were confined to the region of small ρ ($\equiv[L_0]/[S_0]$, where $[L_0]$ and $[S_0]$ are the total molar concentrations of the lanthanide ion and the substrate, respectively). This precluded the detailed analysis as applied previously to the results of the successive dilution and incremental dilution methods for the Az acid-Eu(III) system.[3] Therefore, in the present study, the results obtained by the successive addition method shown in Figs. 1—3 were compared. The LIS of proton A is taken as reference in these figures. It is noted that the shift ratio of proton E changes markedly for the three lanthanide ions, whereas the shift ratios for protons B, C, and D remain essentially constant.

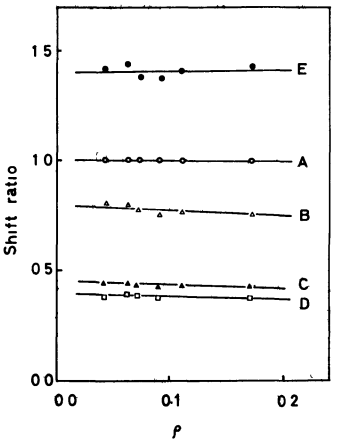


Fig. 1. The ratios of Pr(III)-induced shifts of L-azetidine-2-carboxylic acid observed by the successive addition method.
○, A; △, B; ▲, C; □, D; ●, E.

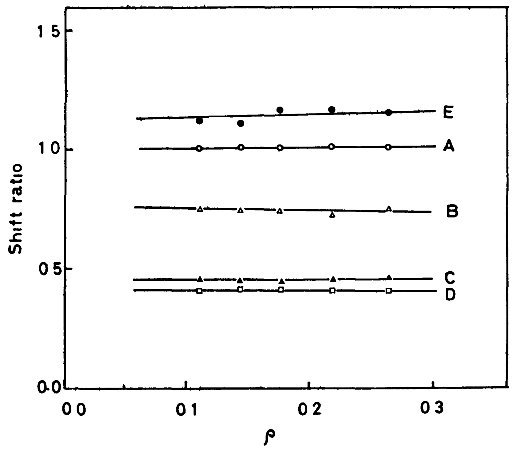


Fig. 2. The ratios of Nd(III)-induced shifts of L-azetidine-2-carboxylic acid observed by the successive addition method.
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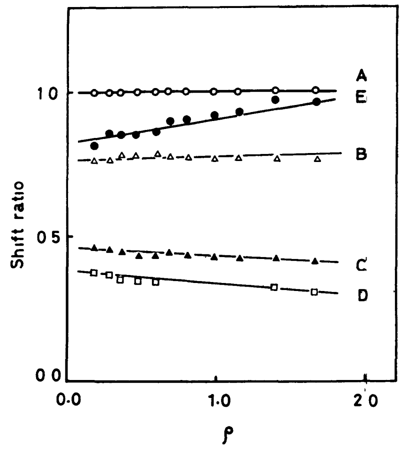

Fig. 3. The ratios of Eu(III)-induced shifts of L-azetidine-2-carboxylic acid observed by the successive addition method.
○, A; △, B; ▲, C; □, D; ●, E.

From these results it may be concluded in the first place that the contact shift makes a sizable contribution only to the observed LIS of proton E. The shift due to a contact mechanism is not expressed by Eq. (1). Accordingly, if the observed LIS's consist of contact and dipolar interaction terms, the shift ratios are not expected to be constant for various lanthanide ions. The contribution of a contact term to the shift of proton E may be explained by the fact that proton E is assigned to the α proton which is located nearest to the coordination site of the lanthanide ion;[3] the number of bonds between the α proton and the coordination site is smaller than that separating the β or γ proton from the coordination site.

In the second place the present results contain information on the axial symmetry of magnetic tensor. According to La Mar *et al.*[13] the shifts arising from the dipolar interaction may be expressed for the case with nonaxially symmetric magnetic tensor as

$$\Delta\nu/\nu_0 = k_1\langle(3\cos^2\theta-1)/r^3\rangle + k_2\langle(\sin^2\theta\cos 2\psi)/r^3\rangle \qquad (2)$$

where r, θ, and ψ are the polar coordinates of the nucleus under consideration, the Cartesian coordinates being taken along the principal magnetic axes with the lanthanide ion at the origin. If the observed LIS's are in accord with this equation, the shift ratios are expected to change their values for Pr(III), Nd(III), or Eu(III) which have different k_1 and k_2 values.[14] However, this is contrary to the results obtained for the shift ratios of protons A, B, C, and D. Therefore, it seems reasonable to conclude that the LIS's of these four protons are satisfactorily accounted for by Eq. (1) which is applicable to the axially symmetric case.

Correction for Diamagnetic Shifts Due to Complex Formation Observed LIS's usually consist of the diamagnetic as well as paramagnetic terms. In the present case the contribution of the diamagnetic term due to complex formation was found small compared with that of the paramagnetic term. However, the diamagnetic shift should be subtracted from the observed LIS's before an accurate structure determination is attempted. The contribution of the diamagnetic shift may be estimated by measuring the shift with a diamagnetic ion such as

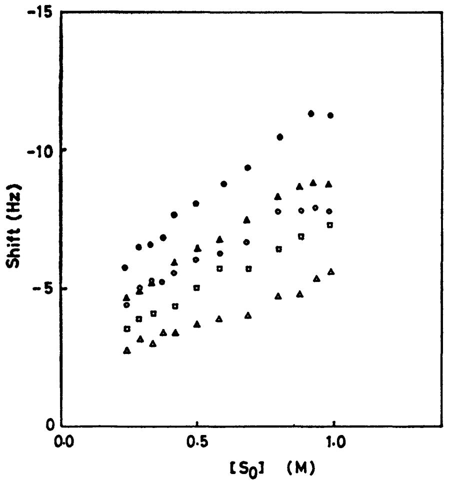

Fig. 4. La(III)-induced shifts of L-azetidine-2-carboxylic acid observed by the successive dilution method *vs.* total substrate concentration ($[S_0]$). D_2O solution, 37 °C, $\rho = 1.01$.
○, A; △, B; ▲, C; □, D; ●, E.

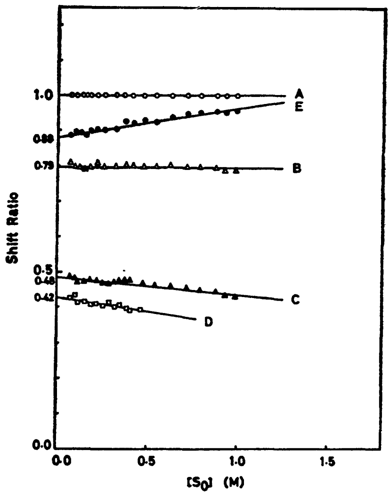

Fig. 5. The ratios of Eu(III)-induced shifts of L-azetidine-2-carboxylic acid observed by the successive dilution method and corrected for the diamagnetic shifts.
○, A; △, B; ▲, C; □, D; ●, E.

La(III). The assumption underlying this method is that the complex formed with La(III) has a structure almost identical to those formed by other lanthanide ions. The diamagnetic shifts observed by the successive dilution method for the Az acid-La(III) system are shown in Fig. 4. The low-field shifts were observed for all the protons. Using the results given in Fig. 4, the Eu(III)-induced shifts were corrected for the diamagnetic contributions. The shift ratios calculated from these corrected values for the Az acid-Eu(III) system are shown in Fig. 5. For the structure determination described below, the shift ratios of protons A, B, C, and D extrapolated to $[S_0]=0$ in this figure (1.00, 0.79, 0.48, and 0.42, respectively) will be used. These values correspond to the ratios of dipolar interaction terms in LIS's intrinsic to the LS complex.

Structure Analysis and Assignment of ^1H *NMR Spectrum*

As the number of observed LIS data is rather limited,

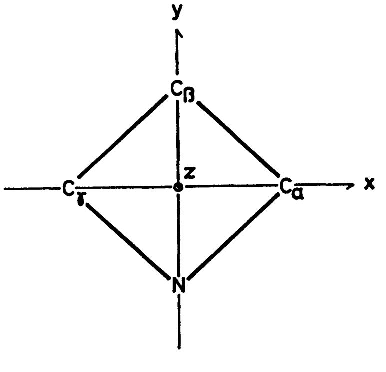

Fig. 6. The four-membered ring of L-azetidine-2-carboxylic acid and the coordinate system.

only the L-C_α distance and the ring-puckering coordinate are chosen as structural variables to be determined. The puckering mode of a four-membered ring can be best described by the coordinate (z) introduced by Chan *et al.*[15] The coordinates of atoms forming the azetidine ring (Fig. 6) are given as

$$\begin{aligned}
x_\alpha &= -x_\gamma = [(x_\alpha{}^\circ)^2-2z^2]^{1/2}\\
y_\alpha &= y_\gamma = 0\\
z_\alpha &= z_\gamma = z\\
x_\beta &= x_N = 0\\
y_\beta &= [(y_\beta{}^\circ)^2-2z^2]^{1/2}\\
y_N &= -[(y_N{}^\circ)^2-2z^2]^{1/2}\\
z_\beta &= z_N = -z \qquad (3)
\end{aligned}$$

where α, β, γ, and N represent the α, β, and γ carbons, and the nitrogen, respectively. When the molecule is planar, the α and γ carbons are located on the x axis and the β carbon and the nitrogen on the y axis. $x_\alpha{}^\circ$, $y_\beta{}^\circ$, and $y_N{}^\circ$ refer to the corresponding coordinates in the planar conformation. It is assumed that the four-membered ring always has a plane of symmetry (yz). The puckering mode described by Eq. (3) occurs without changing either the CC or CN bond length. The lengths of CC and CN bonds in the ring and the geometry around the α carbon and the carboxylate group are transferred from the structural parameters determined for this molecule in crystal.[11] As for the geometry around the β or γ carbons, it is assumed that the CH bond length and the HCH bond angle are 1.08 Å and 109°28′, respectively, and four CCH angles are all equal.

As for the coordination site of the lanthanide ion in the LS complex and the direction of symmetry axis of the magnetic tensor, it is most reasonable to assume that the lanthanide ion is on the bisector of the OCO angle and that the symmetry axis is collinear with the bisector.[16] This is in analogy with the lanthanide-mononucleotide complex where both the lanthanide ion and the symmetry axis are on the bisector of the OPO angle.[7,9,10] The shift ratios can now be calculated using Eq. (1).

On the other hand, four combinations (I-IV) are possible regarding the assignments of protons A, B, C, and D to *β-cis*, *β-trans*, *γ-cis*, and *γ-trans*, as given in Table 1 (*cis* and *trans* refer to the position of a proton

TABLE 1. ASSIGNMENTS OF PROTONS A, B, C, AND D[a)]

Proton	Assignment			
	I	II	III	IV
A	*β-cis*	*β-cis*	*β-trans*	*β-trans*
B	*β-trans*	*β-trans*	*β-cis*	*β-cis*
C	*γ-cis*	*γ-trans*	*γ-cis*	*γ-trans*
D	*γ-trans*	*γ-cis*	*γ-trans*	*γ-cis*

a) *Cis* and *trans* refer to the positions of protons with respect to the carboxylate group.

with respect to the carboxylate group). For each one of these four assignments the agreement factor (*R*) defined as[17–19)]

$$R=\left[\left\{\sum_i\left[\left(\frac{\Delta_1^i}{\Delta_1^s}\right)_{\text{obs}}-\left(\frac{\Delta_1^i}{\Delta_1^s}\right)_{\text{calc}}\right]^2\right\}\Big/\left\{\sum_i\left(\frac{\Delta_1^i}{\Delta_1^s}\right)^2_{\text{obs}}\right\}\right]^{1/2} \tag{4}$$

has been calculated for various values of the L-C_α distance and the *z* coordinate, so that a contour map as shown in Fig. 7 is obtained. This map shows that, for Assignment III, *R* is minimized only if the L–C_α distance is taken so short (2.3 Å) that the lanthanide ion partially penetrates into the carboxylate group. This indicates clearly that Assignment III should be discarded. The contour map obtained for Assignment IV is essentially the same as that shown in Fig. 7. Thus, Assignment IV is also discarded.

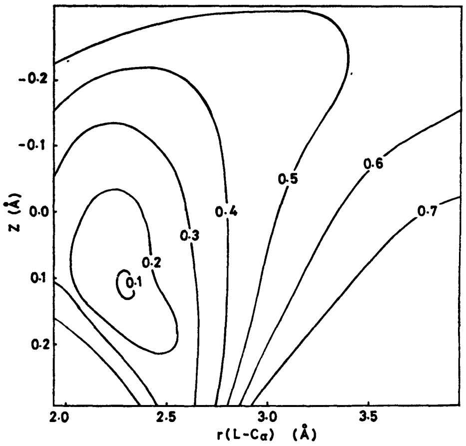

Fig. 7. The contour map of the agreement factor (*R*) for Assignment III.

Since only Assignments I and II remain acceptable, the assignments of *β* protons are now established; *i.e.*, A and B are assigned, respectively, to the *β-cis* and *β-trans* protons. The contour maps obtained for Assignments I and II are shown in Figs. 8 and 9, respectively. The minimum in the contour map in Fig. 8 corresponds to a conformation in which the ring is almost planar ($z\sim0$, Conformation I). On the other hand, the molecular structure corresponding to the minimum in the contour map in Fig. 9 has a puckered ring ($z=-0.125$ Å and the dihedral angle=25 °) with the carboxylate group in the equatorial position (Conformation II). (From the present analysis no information can be obtained about the internal-rotation angle around the C_α-CO_2^- bond.) It seems rather difficult

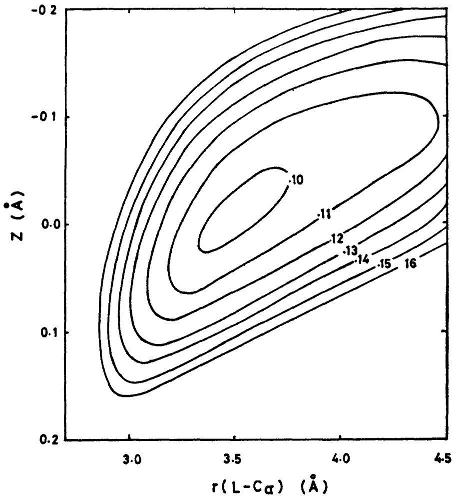

Fig. 8. The contour map of the agreement factor (*R*) for Assignment I.

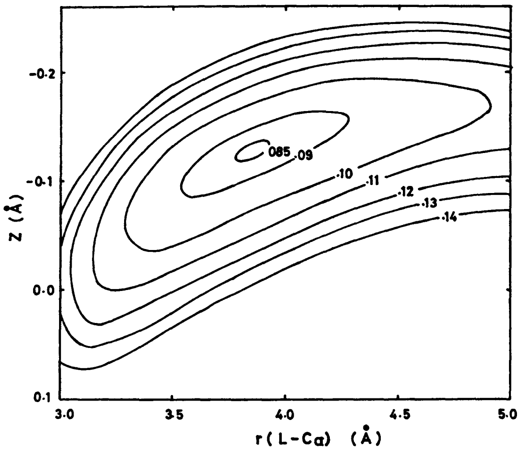

Fig. 9. The contour map of the agreement factor (*R*) for Assignment II.

to decide, from these results alone, which of the two conformations (therefore, which of Assignments I and II) is more probable, though the L–C_α distance in Conformation I (3.5 Å) is slightly shorter than the value (~4 Å) normally accepted.

The line broadening induced by Gd(III) has been found useful for solving the above-mentioned problem. Since the Gd(III)-induced line broadening is proportional to $1/r^6$ and independent of θ, the line-width data obtained for protons C and D are expected to be useful for determining which of these protons are closer to Gd(III). In Conformation I the distances from Gd(III) to C and D are 5.3 and 6.2 Å, respectively, whereas in Conformation II they are 6.5 and 6.3 Å, respectively. The ratios of the line broadening expected for protons, A, B, C and D in both conformations are given in Table 2. The spectra observed after adding extremely small amount of Gd(III) are shown in Fig. 10 for the lines assignable to almost pure transitions related to protons A, B, C, and D. Although the concentration of Gd(III) was not precisely determined, it was about 10^{-6} M for Fig. 10(b) and twice and three times of that for Figs. 10(c) and (d), respectively. The ratios

TABLE 2. THE RATIOS OF Gd(III)-INDUCED LINE BROADENING[a)]

Proton	Calcd Conformation I	Calcd Conformation II	Obsd
A	11.1	7.0	>3
B	2.8	2.5	>2
C	2.5	0.8	0.9
D	1.0	1.0	1.0

a) The broadenining of a line due to the transition of proton D is taken as reference.

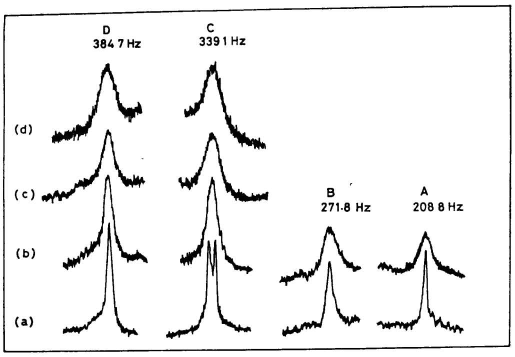

Fig. 10. Gd(III)-induced broadening of the lines assigned to the pure transitions of protons A, B, C, and D of L-azetidine-2-carboxylic acid. The concentration of Gd(III) is zero in (a), *ca.* 10^{-6} M in (b), twice and three times of *ca.* 10^{-6} M in (c) and (d), respectively.

of the line broadening thus observed are also given in Table 2. It is clear in this table that the observed ratios are in agreement with those expected for Conformation II. Thus, it may be concluded that Conformation II (therefore, Assignment II) is the real one.

It was found that the *R* value for Conformation II could be made as small as 0.038 by changing the bond angles N-C_α-COO^- and C_β-C_α-COO^- to 115.5 ° and 118.4 °, respectively, from the crystal data 117 ° and 113 ° with all the other parameters fixed as before. This changes the overall molecular conformation only slightly.

Conclusion

In utilizing the lanthanide-induced shifts and line broadening to the determination of molecular structure in solution, it has been shown necessary and rewarding to follow all the procedures outlined in the introductory part. In weakly acidic aqueous solution the four-membered ring of L-azetidine-2-carboxylic acid has a puckered conformation with a dihedral angle of 25 °, which compares with the angle of 11 ° as determined for this molecule in crystal.[11)] The carboxylate group is in the equatorial position. This molecular structure is depicted in Fig. 2 of the preceding paper.[3)]

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19) As for the definitions of Δ_1^t and Δ_1^s, see Eqs. (5) and (6) of reference 3.