Conformation of Hydroxy-L-proline in Aqueous Solution. Comparison of Results from Lanthanide Ion Probe Measurements and Proton Coupling Constants

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The conformation of hydroxy-L-proline in D_2O solution has been studied by analysing its ^1H chemical shifts and spin-lattice relaxation times perturbed by the binding of lanthanide(III) ion. Spin couplings, including a large long-range coupling constant (2.22 Hz) between the protons bonded to the β- and δ-carbons, remain unaltered by the binding of La^{III}. Thus, conformational changes upon complex formation are absent. The assignment of protons in this study which utilizes the lanthanide probe is in agreement with previous result from analysis of the coupling constants. The conformations estimated independently by these two approaches are similar, although from a more quantitative point of view the puckering angles ω_1 (between the planes formed by C_α, C_β, and N and by C_β, C_δ, and N) and ω_2 (between the planes formed by C_β, C_δ, and N and by C_β, C_γ, and C_δ) differ by *ca.* 20 and 15°, respectively, between the two conformations.

THE elucidation of conformations of biological molecules in solution is an essential step in understanding their physiological functions. N.m.r. spectroscopy has been widely used for this purpose mainly through the analysis of spin-coupling constants, chemical shifts, and the nuclear Overhauser effect. Structure determination using chemical shifts and relaxation perturbations induced by paramagnetic lanthanide ions [1] has also been initiated and interesting results have been obtained for biologically important molecules.[2] However, some of the conclusions obtained by this method (hereafter called the 'lanthanide ion probe method') are not in exact agreement with the results derived from the analysis of spin-coupling constants. For instance, a computer search for the conformation which best fits the chemical shift and relaxation perturbation data observed for adenosine 5'-monophosphate [2b] and cytidine 5'-monophosphate [2e] has resulted in a single conformation (or a family with conformationally very similar members) for each nucleotide, whereas recent studies of spin-coupling constants have shown that nucleotide structures in solution are a dynamic average of a number of rapidly interconverting conformers.[3] Whether the single conformation obtained by the lanthanide ion probe method is an average [2b] or the most populated conformation appears to be an open question.[4] In the case of nicotinamide mononucleotide, a mixture of conformers obtained by the lanthanide ion probe method alone has been found to disagree with the results from the coupling constants.[5]

Undoubtedly, the lanthanide ion probe method should be applied with caution to the conformational analysis of flexible molecules, and it is good practice to examine the conformation estimated by the lanthanide ion probe method in the light of coupling constants (or any other data) whenever they are available. However, the information obtainable from the coupling constants may not be fully reliable, because it depends on the empirical parameters in the Karplus equation.[6] Therefore, it is important to check, for some key molecules which are less flexible, how consistent are the conformations

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[1] B. C. Mayo, *Chem. Soc. Rev.*, 1973, **2**, 49; A. F. Cockerill, G. L. O. Davies, R. C. Harden, and D. M. Rackham, *Chem. Rev.*, 1973, **73**, 553; J. Reuben, in ' Progress in N.M.R. Spectroscopy,' eds. J. W. Emsley, J. Feeney, and L. H. Sutcliffe, Pergamon, Oxford, 1973; ' N.M.R. in Paramagnetic Molecules: Principles and Applications,' eds. G. N. La Mar, W. D. Horrocks, jun., and R. H. Holm, Academic Press, New York, 1973; ' Nuclear Magnetic Resonance Shift Reagents,' ed. R. E. Sievers, Academic Press, New York, 1973; R. A. Dwek, ' Nuclear Magnetic Resonance in Biochemistry,' Clarendon, Oxford, 1973.

[2] (*a*) C. D. Barry, A. C. T. North, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *Nature*, 1971, **232**, 236; (*b*) C. D. Barry, J. A. Glasel, R. J. P. Williams, and A. V. Xavier, *J. Mol. Biol.*, 1974, **84**, 471; (*c*) C. D. Barry, D. R. Martin, R. J. P. Williams, and A. V. Xavier, *ibid.*, p. 491; (*d*) C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *J.C.S. Dalton*, 1974, 1762; (*e*) C. D. Barry, C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *ibid.*, p. 1765; (*f*) B. A. Levine, J. M. Thornton, and R. J. P. Williams, *J.C.S. Chem. Comm.*, 1974, 669; (*g*) A. D. Trifunac and J. J. Katz, *J. Amer. Chem. Soc.*, 1974, **96**, 5233; (*h*) D. K. Lavallee and A. H. Zeltmann, *ibid.*, p. 5552; (*i*) G. N. La Mar and D. L. Budd, *ibid.*, p. 7317; (*j*) F. Inagaki, S. Takahashi, M. Tasumi, and T. Miyazawa, *Bull. Chem. Soc. Japan*, 1975, **48**, 853, 1590.

[3] (*a*) C. Altona, and M. Sundaralingam, *J. Amer. Chem. Soc.*, 1973, **95**, 2333; (*b*) F. E. Evans and R. H. Sarma, *J. Biol. Chem.*, 1974, **249**, 4754; (*c*) D. B. Davies and S. S. Danyluk, *Biochemistry*, 1974, **13**, 4417.

[4] J. Feeney, *Nature*, 1974, **252**, 633.

[5] B. Birdsall, N. J. M. Birdsall, J. Feeney, and J. Thornton, *J. Amer. Chem. Soc.*, 1975, **97**, 2845.

[6] (*a*) M. Karplus, *J. Chem. Phys.*, 1959, **30**, 11; (*b*) L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Pergamon, Oxford, 1969, 2nd edn., chs. 4—2 and 4—4.

estimated independently by the lanthanide ion probe method and by the coupling constant approach.

Hydroxy-L-proline is a cyclic imino-acid occurring principally in collagen. The conformation of this molecule in the crystal and that in aqueous solution have been studied by the analyses of X-ray diffraction [7] and of the spin coupling constants,[8] respectively. The latter work together with a recent study on a closely related molecule (fluoro-L-proline)[9] has shown that coupling constants provide quantitative information on the conformation of these five-membered rings. Thus, the study of hydroxy-L-proline is expected to give a good test of the consistency of the lanthanide ion probe method and the coupling constant approach and to provide results useful in future studies of flexible amino-acids and peptides.

EXPERIMENTAL

Materials.—Hydroxy-L-proline was purchased from Nakarai Chemical Co. and used without further purification. The nitrates of Eu$^{\mathrm{III}}$, Pr$^{\mathrm{III}}$, Gd$^{\mathrm{III}}$, and La$^{\mathrm{III}}$ with purity $>99.9\%$ were obtained from the same company and used for measuring the induced shifts or relaxation time perturbations.

Preparation of Samples.—The lanthanide-induced shifts were observed in D_2O solution by the successive dilution method.[2j,10] The concentration of hydroxy-L-proline in the initial solution was 0.780, 0.362, and 0.812M for the measurements with Eu$^{\mathrm{III}}$, Pr$^{\mathrm{III}}$, and La$^{\mathrm{III}}$, respectively. The molar ratio (ρ) of a lanthanide ion to the substrate was kept constant throughout a series of measurements (ρ 0.850, 0.828, and 0.867 for Eu$^{\mathrm{III}}$, Pr$^{\mathrm{III}}$, and La$^{\mathrm{III}}$, respectively). The 'pH' (uncorrected pH meter reading) was in the range 4.6—5.0, so that the zwitterion is the predominant species. A Radiometer PHM 26 pH meter was used with a long, thin Ingold combination electrode (Catalogue No. 6030–04). For the measurements of the Gd$^{\mathrm{III}}$-perturbed proton relaxation times, 0.369M solutions containing additionally a small amount of Gd$^{\mathrm{III}}$ (20—60μM) were prepared. A 1.0M solution was used for observing the ^{13}C spin-lattice relaxation times.

N.m.r. Measurements.—The n.m.r. spectra were obtained with a Hitachi R-22/FT spectrometer operating at 90 MHz for ^{1}H and 22.6 MHz for ^{13}C. The probe temperature was kept at 37°. The chemical shifts for protons were determined by a frequency counter using a frequency-sweep mode. Sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) was used as an internal standard. Spin–lattice relaxation times (T_1) were obtained by means of a standard $180°$–τ–$90°$ pulse sequence.

Data Analysis.—The ^{1}H n.m.r. spectrum of hydroxy-L-proline was analysed as a system of six nuclei using the programs NMRTRY, NMRFIT, and NMRPLOT developed in our laboratory [2j] and the Hitac 8800/8700 system at the Computer Centre of the University of Tokyo. The search for a best-fit conformation based on the induced-shifts and relaxation data was performed by the programs PCS-I and -II also developed in our laboratory.[11]

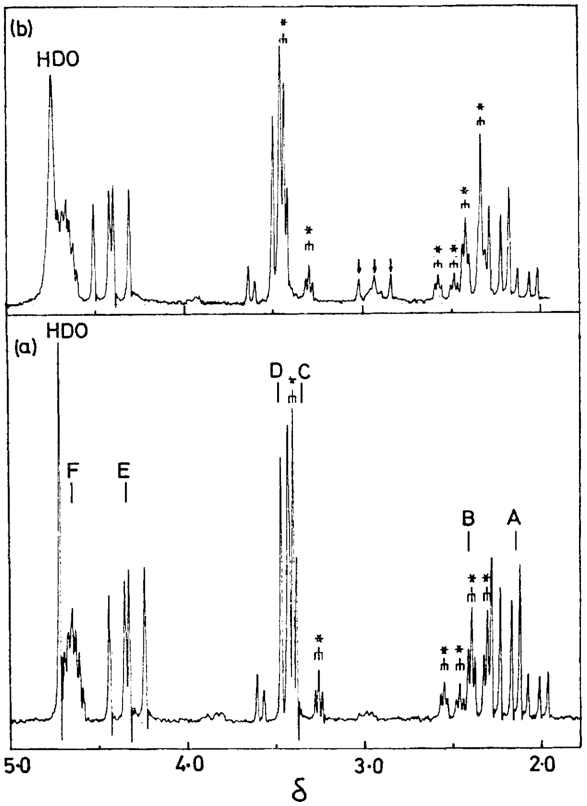

FIGURE 1 ^{1}H N.m.r. spectra of hydroxy-L-proline (0.54M in D_2O; 'pH' 4.5; 37°): (a), without La$^{\mathrm{III}}$, and (b) with 0.47M-La$^{\mathrm{III}}$. A—F indicate the chemical shifts of H_A—H_F downfield from DSS. The triplets with asterisks are the splittings due to the long-range coupling between H_B and H_C. The peaks with arrows are due to DSS

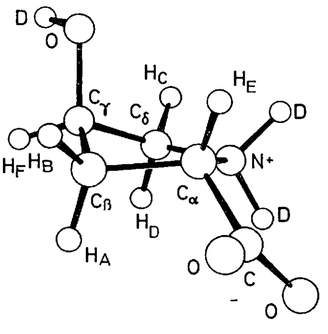

FIGURE 2 Molecular conformation of hydroxy-L-proline as estimated by the lanthanide ion probe method

RESULTS AND DISCUSSION

Analysis of Coupling Constants.—The 90 MHz ^{1}H n.m.r. spectrum of 0.54M-D_2O solution of hydroxy-L-proline is shown in Figure 1(a). The groups of peaks A—F may be assigned to six protons,[8,12] *viz.*, A and B to the β-, C and D to the δ-, E to the α-, and F to the γ-protons (see Figure 2 also). The analysis of the 60

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[7] J. Donohue and K. N. Trueblood, *Acta Cryst.*, 1952, **5**, 419.

[8] R. J. Abraham and K. A. McLauchlan, *Mol. Phys.*, 1962, **5**, 195, 513.

[9] J. T. Gerig and R. S. McLeod, *J. Amer. Chem. Soc.*, 1973, **95**, 5725.

[10] T. A. Wittstruck, *J. Amer. Chem. Soc.*, 1972, **94**, 5130.

[11] F. Inagaki, M. Tasumi, and T. Miyazawa, submitted to *Bull. Chem. Soc. Japan*.

[12] L. Pogliani and M. Ellenberger, *Spectroscopy Letters*, 1973, **6**, 261.

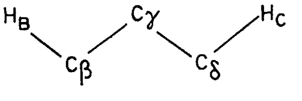
MHz spectrum was carried out previously [8] by dividing the system into two parts consisting of three (A'B'X) and of four (ABXR) nuclei. In the present study a complete system of six nuclei has been treated and the results are listed in Table 1. The wide range of values of the vicinal coupling constants indicates clearly that this molecule exists predominantly in a single conformation. Moreover, a large long-range coupling between H_B and H_C (2.22 Hz) suggests that this conformation contains the W-like arrangement of atoms (A).[6b,13] The vicinal coupling constants may be utilized in a more detailed discussion of the conformation.[8]

TABLE 1

^1H Chemical shifts (δ) and coupling constants (J) of hydroxy-L-proline

δ [a]		J/Hz [b]			
δ_A	2.1534	J_{AB}	−14.21	J_{CD}	−12.63
δ_B	2.4164	J_{AE}	10.11	J_{CF}	1.47
δ_C	3.3552	J_{AF}	4.33	J_{DF}	3.77
δ_D	3.4781	J_{BC}	2.22	J_{EF}	0.60
δ_E	4.3423	J_{BE}	7.95		
δ_F	4.6591	J_{BF}	1.70		

[a] ^1H Chemical shifts are downfield relative to the methyl signal of DSS. The r.m.s. error is $< \pm 0.0004$ p.p.m. [b] J_{AC}, $J_{AD'}$, J_{BD}, J_{CE}, and J_{DE} were assumed to be zero. The r.m.s. error is $< \pm 0.05$ Hz.



(A)

Spectrum observed in the Presence of La^{III}.—One of the questions often raised against the lanthanide ion probe method is whether or not the conformation of the substrate molecule obtained by this method is free from any effect caused by the binding of the lanthanide ion. An answer may be obtained by examining the spectrum observed in the presence of La^{III} or Lu^{III}. These diamagnetic ions would form complexes isomorphous with those formed by the paramagnetic lanthanides. If no appreciable change in the coupling constants is caused by the addition of La^{III} or Lu^{III}, it may then be concluded that no conformation change of the substrate molecule occurs upon complex formation with the lanthanide ions.

In Figure 1(b) the spectrum of hydroxy-L-proline in the presence of La^{III} is shown. The concentrations of the substrate and La^{III} are 0.54 and 0.47M, respectively. If the association constants for this system are assumed to be equal to those of the L-azetidine-2-carboxylic acid–lanthanide system,[2j] *ca.* 60% of the substrate molecules are bound to La^{III} under the above conditions. Between Figures 1(a) and (b) no difference in the pattern of splittings is found, though small shifts (the ' complex formation ' shifts) are induced and line widths are slightly broader in Figure 1(b). The splittings with asterisks are due to the long-range coupling mentioned above. These remain unchanged in Figure 1(b), indicating strongly that the complexation does not alter the W conformation of the free molecule.

Lanthanide-induced Shifts.—The ^1H chemical shifts induced with Eu^{III} and Pr^{III} were observed by the successive dilution method. The observed shifts were corrected for the ' complex formation ' shifts which were estimated using diamagnetic La^{III}.[14] Next the ratios of shifts of protons H_B—H_F relative to that of H_A were calculated and extrapolated to zero substrate concentration. Such a procedure was previously shown to be appropriate for obtaining experimentally the shift ratios intrinsic to the 1 : 1 complex between the substrate molecule and the lanthanide ion.[2j,15] The shift ratios thus obtained with Eu^{III} and Pr^{III} are listed in Table 2.

TABLE 2

Lanthanide-induced shifts and relaxation rate perturbations relative to the H_A proton of hydroxy-L-proline

	Induced shift			Relaxation rate ($1/T_{1M}$)	
Proton	Observed [a]		Calculated	Observed [b]	Calculated
	Eu^{III}	Pr^{III}		Gd^{III}	
H_A	1.00	1.00	1.000	1.00	1.000
H_B	0.80	0.75	0.795	0.52	0.488
H_C	0.35	0.33	0.395	0.12	0.094
H_D	0.41	0.37	0.450	0.22	0.189
H_E [c]	0.78	1.22		1.28	1.290
H_F [d]	0.36	0.34	0.385		

[a] The estimated experimental error is ±0.03 for Eu^{III} and ±0.05 for Pr^{III}. [b] The estimated error is ±0.05. [c] The shift ratio of H_E was not used in the conformational analysis (see text). [d] The relaxation rate of H_F was not observed due to the overlapping of the HDO peak.

For H_B, H_C, H_D, and H_F, similar values of the shift ratios were obtained with the two lanthanides. This means [14a] that the induced shifts for these protons are mainly pseudo-contact in origin and that the susceptibility tensor has effectively axial symmetry.[16] Thus, such shift ratios may be used for the conformational analysis in terms of the McConnel–Robertson equation.[17] However, for H_E, different values of the shift ratios were obtained with the two lanthanide ions and were not useful in the conformational analysis. The contact shift seems to make a significant contribution to the observed shift of H_E, which is located closest to the binding site (the carboxylate group) for the lanthanide ion. A similar result was obtained also for the α-proton of L-azetidine-2-carboxylic acid.[2j]

Gd^{III}*-induced Relaxation Rate Enhancement*.—Gd^{III} is

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[13] M. Barfield and B. Chakrabarti, *Chem. Rev.*, 1969, **69**, 757.

[14] (*a*) B. Bleaney, C. M. Dobson, B. A. Levine, R. B. Martin, R. J. P. Williams, and A. V. Xavier, *J.C.S. Chem. Comm.*, 1972, 791; (*b*) K. Tori and Y. Yoshimura, *Tetrahedron Letters*, 1973, 3127; (*c*) D. J. Chadwick and D. H. Williams, *J.C.S. Perkin II*, 1974, 1202.

[15] F. Inagaki, M. Tasumi, and T. Miyazawa, *Bull. Chem. Soc. Japan*, 1975, **48**, 1427.

[16] J. M. Briggs, G. P. Moss, E. W. Randall, and K. D. Sales, *J.C.S. Chem. Comm.*, 1972, 1180; B. F. G. Johnson, J. Lewis, P. McArdle, and J. R. Norton, *J.C.S. Dalton*, 1974, 1253; D. J. Chadwick and D. H. Williams, *J.C.S. Perkin II*, 1974, 1903; W. D. Horrocks, jun., *J. Amer. Chem. Soc.*, 1974, **96**, 3022; G. N. La Mar and E. A. Metz, *ibid.*, p. 5611.

[17] H. M. McConnel and R. E. Robertson, *J. Chem. Phys.*, 1958, **29**, 1361.

a good relaxation probe for conformational studies,[2a,b,18] because it has a long electron-spin relaxation time and its 4*f*-electron shell is well screened. The relaxation rates for the nuclei of a Gd^{III}-bound molecule are to a good approximation proportional to $\langle r_i^{-6} \rangle$, where r_i refers to the distance between Gd^{III} and the *i*th nucleus. In the present study the change of the spin–lattice relaxation time (T_1) with the addition of a small amount of Gd^{III} was followed. In Figure 3 the observed spin–lattice relaxation rates ($1/T_1^{obs}$) are plotted against the concentration of Gd^{III}.

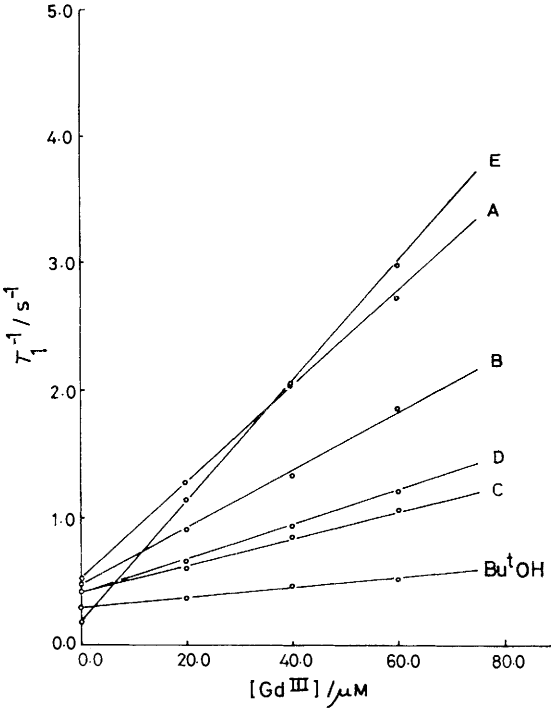

FIGURE 3 Dependence of the spin–lattice relaxation rate ($1/T_1^{obs}$) of H_A–H_E on the concentration of Gd^{III}. The concentration of hydroxy-L-proline was 0.369M. Bu^{t}OH (*ca.* 4mM) was used as internal standard

The spin–lattice relaxation rates ($1/T_{1M}$) for the nuclei in the Gd^{III}-bound molecule were evaluated as follows. If the exchange of the substrate molecule between the free and Gd^{III}-bound states is fast, the observed relaxation rate may be given by equation (1) [2b] where *f* is the

$$1/T_1^{obs} = f/T_{1M} + (1 - f)/T_{1A} + 1/T_{1b} \qquad (1)$$

fraction of bound molecule and T_{1A} is the spin–lattice relaxation time of a free molecule. The last term ($1/T_{1b}$) in equation (1) is an average of the change in the relaxation rate due to the presence of paramagnetic species in solution and is proportional to the total concentration of Gd^{III} at least in the present experimental conditions. Thus, equation (2) applies where β

$$1/T_{1b} = \beta\,[\mathrm{Gd^{III}}] \qquad (2)$$

is the proportionality constant. Since the total concentration of Gd^{III} is much lower than that of the substrate ([S$_0$]), *f* may be substituted by $K[\mathrm{Gd^{III}}]/(1 + K[\mathrm{S_0}])$ where *K* is the association constant. Equation (1) may now be rewritten as (3). The coefficient

$$1/T_1^{obs} = (\alpha + \beta)[\mathrm{Gd^{III}}] + 1/T_{1A} \qquad (3)$$

$$\alpha = K(1/T_{1M} - 1/T_{1A})/(1 + K[\mathrm{S_0}]) \qquad (4)$$

($\alpha + \beta$) in equation (3) corresponds to the slope of the plot in Figure 3, and β may be estimated from the slope of a similar plot obtained for the methyl resonance peak of t-butyl alcohol (*ca.* 4 mM) which was used as an internal standard (also shown in Figure 3). The α value thus obtained may be regarded equal to $(1/T_{1M}) \times K/(1 + K[\mathrm{S_0}])$, since T_{1M} is much smaller than T_{1A}. For a conformational analysis, the ratio of the relaxation rate of a nucleus relative to that of a standard nucleus is required. Such ratios for H_B—H_E relative to the relaxation rate of H_A are listed in Table 2.

Conformational Analysis and Assignment of ^{1}H *N.m.r. Spectrum*.—Only two ring-puckering co-ordinates were chosen as the structural parameters to be determined because of the limited number of observed data on the chemical shift and the relaxation time perturbations. The puckering of a five-membered ring may be described by two dihedral angles ω_1 and ω_2, the former being the angle between the planes formed by C_α, C_β, and N and by C_β, C_δ, and N and the latter between those formed by C_β, C_δ, and N and by C_β, C_γ, and C_δ. These angles are defined in the range of $180° \geq \omega_1, \omega_2 > -180°$ with the sign being decided as follows. Consider first the planar ring ($\omega_1 = \omega_2 = 180°$) where the carboxylate and hydroxy-groups are placed below and above the ring, respectively. Then, the sign of ω_1 is taken positive for the upward displacement of C_α and that of ω_2 negative for the upward displacement of C_γ.

The lengths of skeletal bonds and the external bond angles were fixed to the values determined for this molecule in a crystal.[19] The bond angles within the ring were varied from the values in the crystal according to the changes in ω_1 and ω_2. The tetrahedral angle was assumed for the angles H_E-C_α-CO_2^-, H_A-C_β-H_B, H_F-C_γ-O, and H_C-C_δ-H_D. All the protons were placed at symmetrical positions with respect to the local ring structure.

It has been shown that the early members of the series of lanthanide ions like Eu^{III} and Pr^{III} are on the bisector of the OCO angle and that the symmetry axis of the susceptibility tensor is collinear with this bisector.[2f,j] In the present study this was assumed and later supported by the good agreement between the observed and the calculated results described below.

The shift ratios can now be calculated using the McConnel–Robertson equation. The ratio of the spin–lattice relaxation rate of the *i*th nucleus relative to that of the *s*th nucleus is usually given by equation (5). This

$$(1/T_{1M})_i/(1/T_{1M})_s = \langle r_i^{-6} \rangle / \langle r_s^{-6} \rangle \qquad (5)$$

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[18] G. N. La Mar and J. W. Faller, *J. Amer. Chem. Soc.*, 1973, **95**, 3817.

[19] J. Donohue and K. N. Trueblood, *Acta Cryst.*, 1952, **5**, 419.

equation is valid, provided that the two relaxation rates are controlled by identical correlation time mechanisms.[2b] By reasoning which is described in the Appendix, this assumption seems to be correct for the nuclei under study.

For various sets of ω_1 and ω_2, the agreement factor (R) defined in equation (6) [20] is calculated. In equation (6)

$$R = \left\{ \frac{\sum_i (p_i^{\text{obs}} - p_i^{\text{calc}})^2 + \sum_i (q_i^{\text{obs}} - q_i^{\text{calc}})^2}{\sum_i (p_i^{\text{obs}})^2 + \sum_i (q_i^{\text{calc}})^2} \right\}^{1/2} \qquad (6)$$

p_i and q_i refer to the shift ratio and the relaxation ratio for the ith nucleus, respectively. From the contour map of R thus obtained (using p_i^{obs} for Eu^{III} *), the most probable conformation of hydroxy-L-proline can be determined.

For the assignment of H_A–H_D, the following two combinations are possible. In Assignment I H_A, H_B, H_C, and H_D are assigned to the β-*trans*-, β-*cis*-, δ-*cis*-, and δ-*trans*-positions, respectively, whereas in Assignment II they are assigned to the β-*cis*-, β-*trans*-, δ-*trans*-, and δ-*cis*-positions, respectively. Here, *cis* and *trans* refer to the positions with respect to the carboxylate group. Accordingly, the contour map of R was calculated for each assignment. For Assignment I a rather large value of R (0.13) is obtained at the minimum and the corresponding conformation is an unrealistic one ($\omega_1 = 108$ and $\omega_2 = -146°$). This indicates that Assignment I should be discarded. On the other hand, the contour map of R shown in Figure 4 was obtained for Assignment II. The minimum of the contour map in Figure 4 has a small R value (0.037) and corresponds to a reasonable conformation ($\omega_1 = 165$ and $\omega_2 = -149°$) depicted in Figure 2. Almost identical contour maps were obtained even when the distance between Ln^{III} and C_α was changed slightly (by *ca.* ± 0.1 Å) from 4.2 Å † for which the map in Figure 4 was calculated. Therefore, Assignment II is undoubtedly the correct one. The shift ratios and the relaxation ratios calculated for the above conformation are compared with the observed values in Table 2.

Comparison of the Structural Information.—The two ring-puckering parameters ω_1 and ω_2 for the hydroxy-L-proline molecule in a crystal were found to be *ca.* 180 and −163°, respectively.[19] Both of these values differ by *ca.* 15° from those obtained by the lanthanide ion probe method. The bond angles within the ring obtained by the lanthanide ion probe method differ from the crystal values by *ca.* 2° on an average (maximum *ca.* 7° for the angle C_α–C_β–C_γ). Such differences are not surprising in view of (1) the presence of several intermolecular hydrogen bonds in the crystal which may affect the molecular structure and (2) some assumptions used in the lanthanide ion probe method.

The vicinal coupling constants listed in Table 1 agree with the values obtained by Abraham and McLauchlan within 0.3 Hz [8] and also with those obtained by Gerig and McLeod for fluoro-L-proline within 0.8 Hz.[9] The ring conformations obtained by these two groups are quite similar with each other. The former authors obtained 180 and −127° for ω_1 and ω_2, respectively, whereas the latter gave 176 and −129°. The difference between these ω_2 values obtained from the coupling constant analyses and that in the crystal is quite large. The bond angle C_β–C_γ–C_δ obtained by Gerig and McLeod (94.6°) deviates considerably from the other four angles (102—105°) within the ring and also from the corresponding one in the crystal (103.9°).

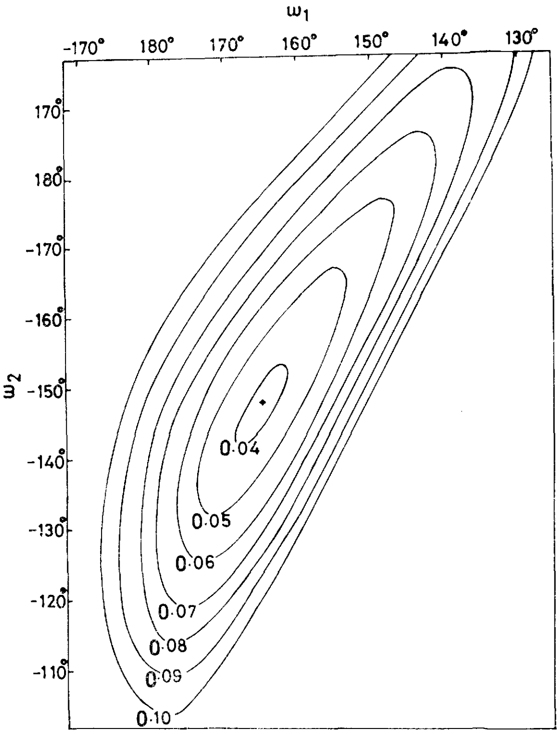

FIGURE 4 Contour map of the agreement factor (R). The angles ω_1 and ω_2 are defined between 180 and −180° (see text)

Thus, both conformations estimated independently by the lanthanide ion probe method and by the coupling constant analysis are shifted from that in a crystal. The degree of this shift seems slightly larger for the conformation obtained by the coupling constant analysis. Since both methods use various assumptions, a more detailed comparison of the results would probably be of little value. However, it should be emphasized that the results obtained by the two methods are qualitatively in agreement. The same assignment of protons was established independently by the two methods. The **W** conformation of the H_B–C_β–C_γ–C_δ–H_C part predicted from a large value of the long-range coupling constant

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* Since Eu^{III} induced smaller broadening of lines than Pr^{III}, the shift data obtained with Eu^{III} were more reliable.

† This is equivalent to the Ln–O distance of 2.4 Å, which is in the range generally accepted.[2f,21]

[20] M. R. Willcott, III, R. E. Lenkinski, and R. E. Davis, *J. Amer. Chem. Soc.*, 1972, **94**, 1742; R. E. Davis and M. R. Willcott, III, *ibid.*, p. 1944.

[21] G. E. Hawkes, D. Leibfritz, D. W. Roberts, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1973, **95**, 1659.

between H_B and H_C is seen in the molecular structure estimated by the lanthanide ion probe method (the internal rotation angles around the C_β–C_γ and C_γ–C_δ bonds are 150 and $-156°$, respectively).

In Figure 5 the six vicinal coupling constants in Table I are plotted against the corresponding dihedral angles determined by the lanthanide ion probe method. The curves indicate the values calculated by the Karplus equation using the coefficients of Gerig and McLeod (solid line) [9] and those of Altona and Sundaralingam (dashed line).[3a] The agreement between the observed points and the calculated curves is reasonable, and the observed points may be brought to either of the calculated curves by shifting dihedral angles by *ca.* 20° at most.

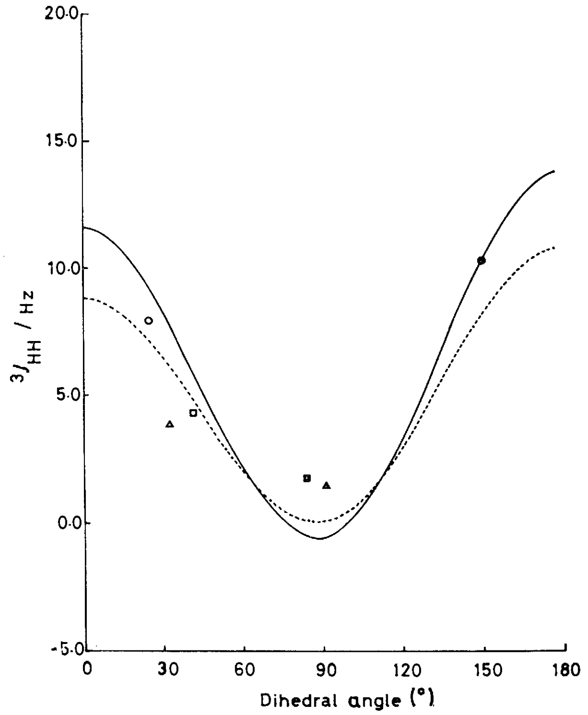

FIGURE 5 Plot of vicinal coupling constants *versus* the dihedral angle: ● J_{AE}; ○ J_{BE}; □ J_{AF}; ■ J_{BF}; ▲ J_{CF}; △ J_{DF}. The curves indicate the values calculated by the Karplus equation using the coefficients of Gerig and McLeod (solid line) [9] and those of Altona and Sundaralingam (dashed line) [3a]

Conclusions.—The conformations obtained by the lanthanide ion probe method and by the coupling constant analysis are qualitatively consistent as far as hydroxy-L-proline is concerned. It seems rather difficult to decide which of the two methods gives a better estimate of the molecular structure in solution. However, the present study indicates that any conclusion derived from the lanthanide ion probe method should agree with the information from a careful analysis of coupling constants at least to the extent implied in Figure 5.

APPENDIX

Equation (5) is based on the assumption that the spin–lattice relaxation rates of the *i*th and *s*th nuclei are controlled by identical correlation time mechanism. Whether internal motions like a ring puckering make different contributions to the total spin–lattice relaxation rates of various nuclei of the Gd^{III}-bound complex may be estimated from the following study on the effective correlation times of free hydroxy-L-proline.

The spin–lattice relaxation time of a ^{13}C nucleus is primarily determined by the dipolar interaction with the proton(s) directly bonded to the carbon atom.[22] Then, the spin–lattice relaxation time (T_1) of a ^{13}C nucleus is related to the effective correlation time (τ^{eff}) by equation (7)

$$1/T_1 = \hbar^2\gamma_H^2\gamma_C^2\tau^{eff}\sum_{j=1}^{N} r_j^{-6} \qquad (7)$$

where r_j is the length of the *j*th CH bond and *N* is the number of protons bonded to the carbon atom. The spin–lattice relaxation times observed for C_α, C_β, C_γ, and C_δ of hydroxy-L-proline are 2.7_0, 1.4_9, 2.8_0, and 1.4_7 s, respectively, and the corresponding τ^{eff} values calculated by equation (7) are 1.7_3, 1.5_6, 1.6_6, and $1.5_8 \times 10^{-11}$ s, respectively. The similarity of these τ^{eff} values indicates that internal motions make uniform contributions, if any, to the spin–lattice relaxation rates of the four ring carbons. This suggests also that the spin–lattice relaxation rates for all the protons in the Gd^{III}-bound complex are controlled by effectively identical mechanisms.

[5/1323 *Received*, 4*th July*, 1975]

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