Conformational Studies of Lanthanide Complexes with Carboxylate Ligands

By BARRY A. LEVINE, JANET M. THORNTON, and ROBERT J. P. WILLIAMS*
(*Inorganic Chemistry Laboratory and Biophysics Laboratory, South Parks Road, Oxford*)

Summary The conformations of lanthanide complexes of indol-3-ylacetate in solution are shown to change along the lanthanide series.

THE binding of a carboxylate ligand to a paramagnetic Ln^{III} cation results in a perturbation of the n.m.r. resonances of the ligand and this can be quantitatively related to the spherical spatial co-ordinates of the nuclei under study.[1,2] The resulting pseudocontact shift for each resonance is given by equation (1) for the case of rhombic symmetry,

$$\Delta\nu/\nu_1 = D_1(3\cos^2\theta_1/r_1^3) - D_2(\sin^2\theta_1\cos 2\phi_1/r_1^3) \quad (1)$$

where r_1 is the distance of the particular nucleus from the paramagnetic centre, θ_1 is the angle between the principal symmetry axis, z, and the vector r_1 joining the nucleus and the paramagnetic centre and ϕ_1 is the angle defining the direction of the vector r_1 with respect to the x and y axes. The induced relaxation by Gd^{III} is isotropic and varies inversely as the sixth power of r_1.

When ratios of shifts of different nuclear resonances are found to be independent of lanthanide cation, it is reasonable to assume that the shifts have their origin in dipolar coupling, that the geometry of the Ln^{III} ligand complex is the same along the lanthanide series and that, to a good approximation, the observed anisotropy of the magnetic susceptibility of the complex has axial symmetry.[3] Equation (1) then reduces to its first term only and ratios of shifts, together with relaxation time or line broadening data, can be used to map out the Ln^{III} complex conformation. The exploration of the conformation of flexible molecules is greatly assisted if the molecule contains a rigid unit which gives rise to several observable n.m.r. resonances for then the protons of the frame can be used to define uniquely the position of the metal ion and its magnetic axis before a search for the more flexible part of the molecule is made. The indole ring of indol-3-ylacetate (IA) provides just such a rigid framework (it occurs in peptides as tryptophan) and we have therefore taken it as the first $R\text{-}CO_2^-$ molecule for conformational study. The six ring proton positions (the NH proton is observable in water) of the indole ring give rise to twelve measurable quantities (shifts and broadenings) which are used to define the five parameters of the metal position and its magnetic axis. Subsequently the link from the indole ring to the metal through the acetate residue can be completed by using the n.m.r. shift and relaxation data of the $>CH_2$ group.

Since fast exchange occurs between the Ln^{III} and (IA) only an average of the resonances of the free and complexed ligand is observed by n.m.r. spectroscopy. At pH 6, *i.e.* above the pK_a of the ligand, at 25 °C, and for a ligand concentration of 0·004M, only the 1 : 1 complex was observed, as was shown by the constancy of the ratios of shifts on different protons from metal to ligand concentration ratios of 1 : 30. By taking such ratios we do not need to know the values of the stability constants or the values of the fully bound shifts, for a conformation search.[1] Although both these last quantities are readily obtained from the experimental data their accuracy is not beyond question.

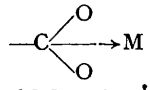
The experimentally obtained ratios of shifts of the protons, extrapolated to zero ionic strength, were corrected by the diamagnetic La^{III} shifts which were very small. The diamagnetic contribution using Lu^{III} as blank was found to be the same and so can be assumed not to vary along the lanthanide series. Shifts on the indole ring protons relative to the $>CH_2$ are in the Table. The possibility of a contact contribution to the induced shift of the $>CH_2$ was investigated, but eliminated after considering the ratio of the observed $>CH_2$ shift relative to the proton shifts in the ring for the early members of the series of lanthanide cations, in whose complexes the contact contribution is expected to be relatively large.[3]

TABLE

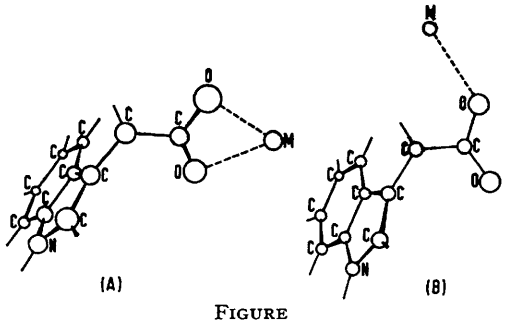
Experimental shifts (p.p.m.) and shift ratios induced by Ln^{III} binding to indol-3-ylacetate.

Ln^{III}	ΔCH_2[a]	CH_2 shift at a 25 : 1 ratio of metal–ligand	H-2	H-4	H-5	H-6	H-7
Pr	−7·61±0·28	−6·69±0·08	35±3[b]	30±3	1·5±2	2·5±2	6±2
			(34±3)	(30±3)	(1·5±2)	(3±2)	(6±2)
Pr[c] (calc.)			33	30	2	3	6
Nd	−2·78±0·12	−2·45±0·03	33±4	25±4	−0·5±2	2±2	6±2
			(34±4)	(27±4)	(1·5±2)	(3±2)	(6±2)
Sm	−0·42±0·04	−0·37±0·01	39±5	27±4	2±3	5±3	8±3
Eu	+3·05±0·13	+2·68±0·02	35±3	29±3	−1±2	1±2	6±2
Tb	−30·6±1·1	−26·9±0·4	26±4	13±3	−6±2	−1±2	3±2
Dy	−46·7±2·1	−41·1±0·6	30±3	24±3	−2±2	1±2	5±2
Ho	−24·9±0·9	−21·9±0·4	27±3	13±3	−6±2	−1±2	4±2
Er	+3·2±0·4	+2·82±0·21	12±4	−50±5	−35±4	−14±3	−5±3
Tm	+7·65±0·42	+6·73±0·37	4±3	−90±8	−49±5	−17±3	−6±2
			(20±3)	(−15±4)	(−18±4)	(−6±2)	(1±2)
Tm[c] (calc.)			9	−86	−53	−18	−8
Yb	+7·14±0·21	+6·28±0·11	27±3	−4±3	−15±3	−3±2	3±2
			(30±3)	(12±3)	(−7±3)	(0±2)	(4±2)

[a] A negative shift represents a shift to low field. [b] % shift relative to $-CH_2$; values in parentheses refer to those found at 82 °C. [c] Conformation calculated by computer.

The constancy of shift ratios for the members of the lanthanide series before Gd^{III}, invariant with temperature in the range 20—85 °C, demonstrates effective axial symmetry for these complexes. The computed conformation, Figure (A), gave very good agreement (Table) with both these shift ratios and with Gd^{III} and Pr^{III} relaxation measurements as well as with the published crystal structure of indol-3-ylacetic acid.[4] Binding of the lanthanide cation to the carboxylate is bidentate, the effective z-axis being along the  direction and with a metal-oxygen distance of 2·2 ± 0·1 Å. We thus conclude that the conformation of peptides can be studied using lanthanide probes bound to the carboxylate terminus and that special advantages accrue if the peptides contain tryptophan.

For the later members of the lanthanide series the shift ratios change somewhat (and systematically) for each metal, a strong deviation being found in the case of the Tm^{III} complex. The relative line broadening by Tm^{III} was also very different from that observed with Gd^{III} and Pr^{III}. In order to interpret these data we decided to search for conformations exactly as in the cases of the earlier lanthanide cation complexes, *i.e.* on the basis of an axially symmetric complex and assuming isotropic broadening. The computed position of the metal, Tm^{III}, relative to the indole ring was then found to be such that the symmetry axis, the z-axis, lies along the oxygen–metal bond. This computed conformation, Figure (B), differs from that of the Pr^{III} complex not only in the orientation of the indole ring relative to the metal, but also in that binding to the carboxylate occurs through one oxygen only. Very good agreement with both experimental shift (Table) and Tm^{III} relaxation data was found with a metal–oxygen distance of 2·5 ± 0·15 Å. Although this search procedure using data from one lanthanide cation is open to question, the number of parameters which define the conformation is twelve and the conformation is therefore well defined.



FIGURE

The change in the conformation of the complex, Figure (A) to (B), really a movement of the metal ion with respect to the co-ordinating ligand, can be thought of as a change from a bidentate binding of a carboxylate to a monodentate binding at an ionic radius of *ca.* 0·85 Å. In the series Ge^{II}, Sn^{II}, Pb^{II} acetate complexes the same change from bidentate to monodentate carboxylate co-ordination occurs at Sn^{II}, radius 1·15 ± 0·05 Å.[5] Whatever the cause of this change in structure with change in radius it could be profoundly important in the case of biological ligands not so much in terms of the thermodynamics of binding but in its kinetic consequences.

This work is supported by the M.R.C. R.J.P.W. is a member of the Oxford Enzyme Group.

(*Received, 20th June* 1974; *Com.* 725.)

[1] C. D. Barry, J. A. Glasel, A. C. T. North, R. J. P. Williams, and A. V. Xavier, *Nature*, 1971, **232**, 236.
[2] F. J. C. Rossotti, 'Contributions to Coordination Chemistry in Solution,' ed., E. Hogfeldt, Swedish Nat. Sci. Res. Council, Stockholm, 1972, p. 263.
[3] C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *J.C.S. Dalton*, 1973, p. 2662.
[4] I. L. Karle, K. Britts, and P. Gun, *Acta Cryst.*, 1964, **17**, 496.
[5] N. W. Alcock, H. M. Powell Symposium, Oxford, April 1974.