hinderance caused by the pyrrolidine *N*-CH_3 would cause the LSR/I^- moiety to migrate to the back side of the pyridine ring.

Rotation of the Ln ion results in the motionally averaged orientation of the complex depicted in Figure 2 which is fixed within the statistical uncertainty of ±5.7° for angles θ_L and ϕ_S.

The association is visualized as an interaction between the LSR and the anion (iodide) which results in the paramagnetic effect experienced by nuclei of the cation or substrate. It would appear that the association is such that the overall net charge of the LSR/anion moiety remains negative and that the initial charge affinity of the anion is not greatly changed, as evident by the calculated lanthanide position. When the X-ray data reported for *N*-methylpyridinium iodide are used as a model, the closest approach of the iodide to the pyridine ring was reported as 3.76 Å from the nitrogen and 3.73 Å above the plane of the ring. The results from this study position the Ln ion approximately 3.87 Å above the C(6)–H(6) bond (or the plane of the pyridine ring). Although there is no direct evidence presented here supporting the actual position of the iodide, when viewing molecular models it is not unreasonable to depict the iodide as being sandwiched in between the LSR and the cation in a linear fashion as shown in Figure 2. The usual, directly coordinated, Ln distances derived from LSR interactions are reported as approximately 2.5–2.65 Å. Significantly larger distances are observed with ion pair complexes of LSR's.

Conclusion

The use of LSR's and LRR's as tools for quantitating the time-averaged solution geometry of an organic salt proves to be a workable technique. The results of this study show that LIS and relaxation data, taken separately or combined, yield structural information comparable to data obtained from studies of directly coordinated Ln complexes. Calculations of this type rarely produce a single point that will exactly define the time-averaged position of the lanthanide ion; however, the fits obtained from the present data strongly suggest that the probability of such a point is not an incorrect approximation. Ion pair complexes of this type result in induced shifts that are primarily dipolar in origin with no significant contact contribution. Consequently, the information obtained from distance and angle calculations serves to define the position of the anion in solution or to quantitate the solution geometry of the cation.

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Registry No. 1, 21446-46-8; Er(fod)$_3$, 17978-75-5; Eu(fod)$_3$, 17631-68-4; Ho(fod)$_3$, 18323-97-2; Pr(fod)$_3$, 17978-77-7; Yb(fod)$_3$, 18323-96-1; Gd(fod)$_3$, 18323-96-1.

Lanthanide Induced Shift and Relaxation Rate Studies of Aqueous L-Proline Solutions

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Abstract: The structure of aqueous L-proline at pH 3 has been studied with use of the lanthanide-induced-shift technique. Paramagnetic nuclear shifts and enhancement of nuclear-relaxation rates have been measured for all ^{13}C and ^1H nuclei of L-proline in the presence of ten different lanthanide cations. The relaxation-rate data indicate that isostructural complexes are formed between proline and all ten lanthanide cations. An attempted separation of the observed lanthanide-induced shifts into pseudocontact and contact components with use of the Reilley method (C. N. Reilley, B. W. Good, and R. D. Allendoerfer, *Anal. Chem.*, **48**, 1446 (1976)) gave inconsistent results for the α and carboxyl carbons indicating that either the shifts at these nuclei have a nonaxial dipolar contribution or the hyperfine coupling constant is lanthanide ion dependent. The pseudocontact shifts at C_β, C_γ, and C_δ and at all protons conform well to the axial symmetry model with the lanthanide ion principal symmetry axis intersecting a single oxygen atom 2.9 ± 0.1 Å from the lanthanide cation.

The trivalent lanthanide cations have found widespread use as NMR shift and relaxation probes of the dynamic solution conformations of molecules.[1] The shift method relies upon the measurement of paramagnetic shifts in several nuclei of a molecule, a purification of each measured LIS of any contact-shift contribution, and a comparison of the resulting pseudocontact shifts with those calculated from an appropriate dipolar model which contains both distance and angular parameters. The interpretation of relaxation data to obtain relative Ln^{3+}–nuclei distances is more direct but requires the assumption of an identical correlation time for all nuclei in the molecule. Recent LIS studies on some simple carboxylates[2,3] and amino acids[3,4] have suggested that these ligands do not form isostructural complexes with all lanthanide cations. Elgavish and Reuben have used relaxation data to show that sarcosine forms isostructural complexes with all lanthanide cations[5] and proposed[6] that the earlier Ln^{3+}-ligand structural changes resulted from a choice of improper models for the interpretation of LIS data. In this paper, we report lanthanide-induced shifts and relaxation rates for all ^{13}C and ^1H nuclei in the complexes of L-proline with ten different paramagnetic cations. The relaxation data show that isostructural complexes are formed with all ten lanthanide cations. The pseudocontact LIS data for all nuclei except the carboxyl and α carbons conform nicely to the effective axial symmetry dipolar model while the aforementioned carbon shifts contain an additional contribution which could lead to incorrect conclusions about structural alterations along the Ln^{3+} series.

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Experimental Methods

Lanthanide chlorides were prepared from the lanthanide oxides (a gift from the Molybdenum Corp.) and standardized by titration with EDTA with xylenol orange as the indicator. L-Proline (Sigma grade) was dissolved in 99.7% D_2O (Merck) containing 1% *tert*-butyl alcohol, which served as an internal chemical-shift standard. All pH measurements were made with an Orion Model 701A digital meter equipped with a combination glass electrode and were not corrected for the deuterium isotope effect.

The ^{13}C and ^1H NMR spectra were recorded on a JEOL FX-200 spectrometer. Spin–lattice relaxation rates were measured at a constant probe temperature of 25 ± 1 °C by using the inversion recovery method at 50.1 MHz and 199.5 MHz for ^{13}C and ^1H, respectively. The paramagnetic contributions to the relaxation rates, T_{1P}^{-1}, were determined by subtracting the relaxation rates for a proline solution containing La^{3+} from the rates for a proline solution containing an identical concentration of paramagnetic cation. Corrections for outer-sphere effects were also made by further substracting the difference in relaxation rate for *tert*-butyl alcohol in the paramagnetic sample vs. that in the La^{3+} sample. Errors in the resulting corrected T_{1P}^{-1} are expected to be better than ±10%. The computer program, PDIGMGD, assesses the best agreement between calculated and observed relaxation rates at each metal ion position from normalized standard deviations (*R* factors) then holds that position for the cation and varies the principal symmetry axis of the complex to obtain the best agreement between calculated and observed LIS values.

Theory

The shift induced at a nucleus of a molecule upon binding of a paramagnetic lanthanide cation to that molecule could arise from a through-bond contact or a through-space dipolar or pseudocontact interaction.

$$\Delta_{\mathrm{obsd}} = \Delta_{\mathrm{c}} + \Delta_{\mathrm{d}} \qquad (1)$$

The contact shift[7] is proportional to the spin expectation value, $\langle \mathrm{Sz} \rangle$, of the particular paramagnetic lanthanide and the scalar hyperfine coupling constant, *A*, while the dipolar shift[8] is proportional to a magnetic constant, *D*, characteristic of each lanthanide, two crystal field coefficients, $A_2{}^0$ and $A_2{}^2$, and two geometrical functions which relate the distance and spherical coordinates of the nucleus under observation in a coordinate system with the lanthanide cation at the origin. When a lanthanide–

$$\Delta_{\mathrm{obsd}} = A\langle \mathrm{Sz} \rangle + D(A_2{}^0G + A_2{}^2G') \qquad (2)$$

$$\text{where } G = \frac{3\cos^2\theta - 1}{r^3} \text{ and } G' = \frac{\sin^2\theta\cos 2\phi}{r^3}$$

ligand complex has axial symmetry or becomes axially symmetric as a result of intramolecular motions or rearrangements,[9,10] the term in G' disappears and the pseudocontact shift becomes proportional to $(3\cos^2\theta - 1)/r^3$. G' may also be accidentally zero for several nuclei in a molecule, i.e., when $\theta = 0°$ or $\phi = 45°$, 135°, 225°, or 315°. If one assumes that a ligand forms isostructural complexes with all lanthanide cations and the crystal-field coefficients $A_2{}^0$ and $A_2{}^2$ are constant along the series, the observed pseudocontact shifts for a given nucleus will be proportional to the *D* values for each lanthanide cation. Similarly, if the overlap between ligand and lanthanide ion orbitals is independent of the lanthanide cation (i.e., *A* is constant for a given nucleus), the measured contact shifts for a given nucleus will be porportional to the $\langle \mathrm{Sz} \rangle$ values for each lanthanide cation. This is the basis of the temperature-independent method for separating contact and pseudocontact contributions to measured shifts for a series of lanthanide complexes first introduced by Reilley et al.[11] One need not assume effective axial symmetry for successful use of this method, but $A_2{}^0$ and $A_2{}^2$ must be independent of the lanthanide cation. Equation 2 may be rearranged into two linear forms,

$$\Delta_{\mathrm{obsd}}/\langle \mathrm{Sz} \rangle = A + \frac{D}{\langle \mathrm{Sz} \rangle}(A_2{}^0G\ A_2{}^2G') \qquad (3)$$

$$\Delta_{\mathrm{obsd}}/\mathrm{D} = A\frac{\langle \mathrm{Sz} \rangle}{D} + (A_2{}^0G + A_2{}^2G') \qquad (4)$$

It has been noted[6,12] that eq 3 should be used when the experimental shifts are largely dipolar in origin and eq 4 used when contact contributions predominate.

The paramagnetic contribution to the relaxation rate of a given nucleus is given by,[1]

$$\frac{1}{T_{1\mathrm{P}}} = \frac{P_{\mathrm{M}}}{T_{1\mathrm{M}}} = \frac{Kf(\tau_{\mathrm{c}})}{r^6} \qquad (5)$$

where P_{M} is the fraction of complexed ligand, $T_{1\mathrm{M}}^{-1}$ is the relaxation rate of the fully bound ligand, *K* is a constant characteristic of a given nucleus (i.e., ^{13}C or ^1H) and lanthanide, and $f(\tau_{\mathrm{c}})$ is a function of the correlation time that dominates the relaxation process. If τ_{c} is constant for all nuclei in a small molecule such as L-proline, then the ratio of relaxation rates for two different ^{13}C or ^1H nuclei in a molecule is a direct measure of their relative distances from the paramagnetic cation.

$$\frac{(T_{1\mathrm{P}}{}^{-1})_1}{(T_{1\mathrm{P}}{}^{-1})_2} = \frac{(r^{-6})_1}{(r^{-6})_2} \qquad (6)$$

Results

Complex Formation. The stability constants between L-proline and nine lanthanide cations were measured at pH 3 as described for L-alanine.[4] No indication of complex formation greater than 1:1 stoichiometry was evident in ligand titrations (at constant $[Ln^{3+}]$) or metal ion titrations (at constant [proline]). An analysis of the data for a single ML complex yields stability constants of 1.08 ± 0.32 M^{-1} for the nine different lanthanide cations. This may be compared with values of 0.7 ± 0.1 M^{-1} for lanthanide–alanine complex formation under these same experimental conditions and with 2.5 ± 0.5 M^{-1} for 1:1 lanthanide–proline complexes at pH 3.5.[13] Elgavish and Reuben[14] have analyzed their NMR shift data for two lanthanide cations with L-alanine at pH 4.5 with a model which assumes that complexes of 1:1 and 2:1 stoichiometry are formed. They calculated values of 4.3 and 3.2 M^{-1} for ML formation with two different lanthanide cations and 1.1 and 0.8 M^{-1} for ML_2 formation but most importantly also found that the calculated fully complexed shifts for ML and ML_2 were identical within experimental error. This indicates that NMR shift data can be compared from one lanthanide cation to another even though the stoichiometry of complex formation in these weak complexes may be uncertain.

Lanthanide-Induced Shifts. The high-resolution ^1H and ^{13}C spectra of 1 M L-proline solutions were measured in the presence of 0.03–0.40 M lanthanide chlorides. The lanthanide-induced shift at each nucleus normalized to 0.2 M Ln^{3+} is given in Table I. The proton, C_γ, and C_δ shifts for each lanthanide have the same sign as the published *D* value[8] for each ion and are therefore dominated by pseudocontact interactions. The C_o, C_α and C_β shifts do not follow these same trends, indicating that they contain a significant contact component. This is verified by the Gd^{3+}-induced shifts which are entirely contact in origin. None of the carbon shifts in this complex are devoid of a contact component and the magnitude of the shifts is clearly in the order $C_\alpha > C_o \gg C_\beta \gg C_\gamma > C_\delta$.

The contact- and pseudocontact-shift component to each measured LIS may be separated and compared by using two of Reilley's methods.[12] Method C_1 relies upon a measurement of the Gd^{3+} shifts to determine the hyperfine coupling constant, *A*,

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Table I. Lanthanide-Induced Shifts in the ^{1}H and ^{13}C Resonances of L-Proline (1 M) at pH 3[a]

	^{1}H					^{13}C				
Ln^{3+}	δ_{H_α}	$\delta_{H_{\beta_1}}$	$\delta_{H_{\beta_2}}$	δ_{H_γ}	δ_{H_δ}[b]	δ_{C_o}	δ_{C_α}	δ_{C_β}	δ_{C_γ}	δ_{C_δ}
Pr	−0.61	−0.33	−0.44	−0.15	−0.13	+0.24	−4.62	−0.27	−0.30	−0.27
Nd	−0.17	−0.13	−0.11	−0.04	−0.04	+3.77	−5.44	+0.08	−0.21	−0.12
Eu	+0.19	+0.28	+0.40	+0.13	+0.10	−6.46	+11.15	−0.40	+0.18	+0.13
Gd	*c*	*c*	*c*	*c*	*c*	−22.4	+30.45	−1.90	+0.38	+0.15
Tb	−4.62	−1.55	−2.34	−0.73	−0.42	−23.33	+18.49	−4.15	−1.10	−1.12
Dy	−7.07	−2.85	−4.43	−1.53	−1.23	−30.39	+8.75	−5.87	−2.25	−2.20
Ho	−3.61	−1.27	−1.95	−0.68	−0.61	−16.12	+8.59	−2.93	−1.01	−1.06
Er	+1.16	+0.66	+0.93	+0.26	+0.16	+2.99	+9.66	+0.41	+0.45	+0.34
Tm	+2.62	+1.08	+1.66	+0.53	(i) +0.25 (ii) +0.53	+8.80	+6.83	+1.42	+0.80	+0.72
Yb	+1.03	+0.45	+0.76	+0.22	+0.20	+3.97	+2.03	+0.72	+0.38	+0.34

[a] Normalized to 0.2 M Ln^{3+}. A negative sign denotes a downfield lanthanide-induced shift. [b] These values represent an average LIS for the two nonequivalent δ protons. The two δ protons were resolved in several of the complexes at high cation concentrations but only the Tm^{3+} complex showed separate resonances as indicated at 0.2 M Tm^{3+}. [c] These resonances were too broad for an accurate chemical shift measurement at 0.2 M Gd^{3+}.

Table II. Analysis of Lanthanide-Induced Shifts by Eq 3 and 4 for the Entire Lanthanide Series vs. Division into Two Groups of Cations

	all Ln data (eq 3)			all Ln data (eq 4)			Pr → Tb data (eq 3)	Dy → Yb data (eq 3)	
nucleus	$(A_2{}^0G + A_2{}^2G')$	A	R^2	$(A_2{}^0G + A_2{}^2G')$	A	R^2	$(A_2{}^0G + A_2{}^2G')$	$(A_2{}^0G + A_2{}^2G')$	G_{calcd}[a]
C_o	0.212	0.464	0.938	0.138	0.667	0.967	0.103	0.220	0.159
C_α	0.045	−0.745	0.449	0.119	−0.974	0.986	0.154	0.045	0.061
C_β	0.038	0.054	0.996	0.039	0.053	0.996	0.035	0.040	0.034
C_γ	0.018	−0.003	0.965	0.022	−0.010	0.699	0.022	0.019	0.018
C_δ	0.017	0.002	0.970	0.019	−0.004	0.511	0.020	0.017	0.016
H_α	0.054	0.028	0.992	0.059	0.010	0.542	0.055	0.056	0.056
H_{β_1}	0.023	0.0003	0.983	0.044	−0.015	0.254	0.025	0.023	0.024
H_{β_2}	0.036	0.005	0.990	0.036	−0.016	0.699	0.034	0.037	0.036
H_γ	0.011	0.003	0.982	0.012	−0.005	0.683	0.011	0.012	0.012
H_δ	0.009	0.002	0.968	0.010	−0.004	0.654	0.009	0.010	0.009

[a] Calculated by using the axial symmetry model with the lanthanide cation position as determined from the relaxation data.

at each nucleus. This value is then assumed contant for the remaining ions and the contact shift at each nucleus is determined by the product $A\langle Sz\rangle$ using the value of $\langle Sz\rangle$ for each lanthanide ion. The pseudocontact or dipolar shift is found by the difference between Δ_{obsd} and $A\langle Sz\rangle$. An analysis of the pseudocontact shift at each carbon in Table I by this method indicates that $(A_2{}^0G + A_2{}^2G')$ is proportional to D for all ten lanthanide ions at C_β, C_γ, and C_δ but not at C_o or C_α. The observed linearity for the C_β, C_γ, and C_δ data suggests that A is indeed a constant and the separation procedure is valid at these nuclei. This does not provide information about axial symmetry vs. nonaxial symmetry at these nuclei but does indicate that the complexes are isostructural. The reasons for the nonlinearity between $(A_2{}^0G + A_2{}^2G')$ and D for the C_o and C_α data are uncertain but since A must also be a constant at these nuclei, the deviations could arise from a nonaxial component $(A_2{}^2G')$ to these shifts that is lanthanide ion dependent.

Methods[12] A_1 and A_2 do not rely upon knowing the Gd^{3+} shifts but rather fit the entire data set at each nucleus to one of the linear forms of eq 2. The results of a linear least-squares analysis of the data by using eq 3 and 4 are compared in Table II. Reuben and Elgavish[6] have argued that nuclei whose LIS are dominated by pseudocontact interactions should be analyzed by eq 3 while those dominated by contact contributions should be analyzed by eq 4. When this criterion is used, it is clear that the C_γ, C_δ, and all of the ^{1}H shifts are dominated by pseudocontact interactions and the separation is best achieved by using eq 3. The C_o, C_α, and C_β shifts contain significant contributions from the pseudocontact and contact terms for several of the ions and hence both equations should yield similar separation results. This is observed for C_β but once again, the C_o and C_α shift do not seem to fit the model. The C_o data fit both linear equations well but yield differing values for $(A_2{}^0G + A_2{}^2G')$ and A. The Reuben and Elgavish criterion[6] would dictate that the C_α shifts are dominated by the contact term and hence the separation is most properly achieved by using eq 4.

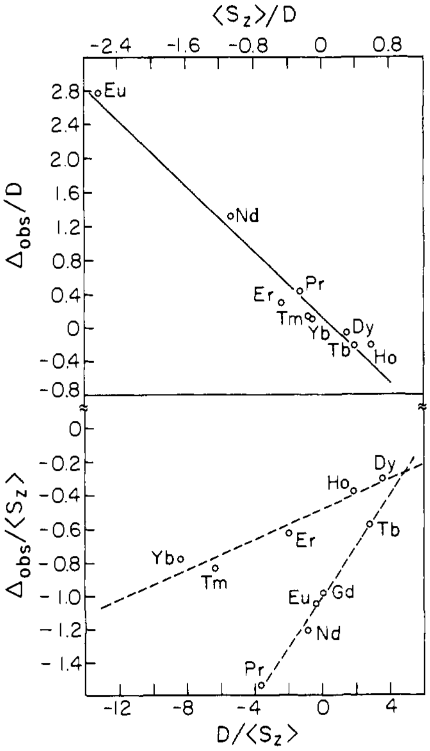

Figure 1. Plots of the C_α lanthanide-induced-shift data in L-proline according to eq 3 (lower) and eq 4 (upper).

Plots of the C_α LIS data according to eq 3 and 4 are compared in Figure 1. The Δ_{obsd}/D vs. $\langle Sz\rangle/D$ plot (eq 4) shows a reasonably linear curve through the entire data set (R^2=0.986) while

Table III. Lanthanide-Effected Longitudinal Relaxation Rates ($1/T_{1P}$, s^{-1}) and Distance Ratios for L-Proline (1 M)

		^{13}C								
Ln^{3+}	concn, mM	C_0	C_α	C_β	C_γ	C_δ	r_{C_α}/r_{C_0}	r_{C_β}/r_{C_0}	r_{C_γ}/r_{C_0}	r_{C_δ}/r_{C_0}
Pr	200.0	2.26	0.58	0.23	0.09	0.09	1.25	1.46	1.71	1.70
Nd	144.0	1.31	0.25	0.12	0.03	0.08	1.32	1.50	1.88	1.60
Gd	2.0	36.11	4.76	1.85	1.00	1.09	1.40	1.64	1.82	1.79
Tb	50.0	19.93	3.15	1.13	0.61	0.53	1.36	1.61	1.79	1.83
Dy	40.0	15.28	2.00	1.06	0.38	0.41	1.40	1.56	1.85	1.83
Ho	22.5	5.30	0.76	0.30	0.18	0.18	1.38	1.61	1.75	1.76
Er	43.6	13.03	1.91	0.75	0.36	0.45	1.38	1.61	1.82	1.75
Tm	36.0	6.05	0.90	0.52	0.25	0.27	1.37	1.50	1.70	1.68
Yb	122.0	3.31	0.37	0.17	0.05	0.07	1.44	1.64	2.03	1.90
						mean	1.37 ± 0.05	1.57 ± 0.07	1.82 ± 0.10	1.76 ± 0.09

Table IV. Lanthanide-Effected Longitudinal Relaxation Rates ($1/T_{1P}$, s^{-1}) and Distance Ratios for L-Proline (1 M)

		proton								
Ln^{3+}	concn, mM	H_α	H_{β_1}	H_{β_2}	H_γ[a]	H_δ[a]	$r_{H_{\beta_1}}/r_{H_\alpha}$	$r_{H_{\beta_2}}/r_{H_\alpha}$	$r_{H_\gamma}/r_{H_\alpha}$	$r_{H_\delta}/r_{H_\alpha}$
Pr	200.0	2.08		1.87	0.75	1.01		1.02	1.19	1.13
Nd	116.0	0.91	0.53	1.40	0.29	0.43	1.09	0.93	1.21	1.13
Gd	2.0	58.12	16.26		18.21	12.33	1.24		1.21	1.29
Tb	7.9	4.31	2.13	2.89	0.92	1.03	1.12	1.06	1.29	1.27
Dy	40.0	24.80	10.62	20.33	5.18	6.24	1.15	1.03	1.30	1.26
Ho	22.5	8.45	4.78	6.43	2.40	2.33	1.10	1.05	1.23	1.24
Er	43.6	17.28	8.00	10.37	5.55	5.24	1.14	1.09	1.21	1.22
Tm	63.0	20.63		24.37	6.60	(i) 11.21		0.97	1.21	(i) 1.11
						(ii) 1.04				(ii) 1.65
Yb	122.0	2.26		2.22	1.04	0.93		1.00	1.14	1.16
						mean	1.14 ± 0.05	1.02 ± 0.05	1.22 ± 0.05	1.21 ± 0.07

[a] Observed relaxation rates for two nonresolved protons. The distance ratios for the two resolved δ protons in the Tm^{3+} complex were not included in the calculation of the mean distance ratios.

the $\Delta_{obsd}/\langle Sz\rangle$ vs. $D/\langle Sz\rangle$ data (eq 3) appear to divide into two groups, one containing lanthanide cations from the first half of the series and another those from the last half of the lanthanide series. The latter plot suggests there is a unique slope ($A_2{}^0G + A_2{}^2G'$) and intercept (A) for each subgroup of lanthanide cations and indeed we have ascribed similar results with L-alanine to a structural change near the center of the lanthanide ion series.[4] Elgavish and Reuben[5] have used relaxation data to show that sarcosine forms isostructural complexes with all of the paramagnetic lanthanide cations yet plots of the sarcosine C_α shift data[14] according to eq 3 show two lines containing the same two subsets of lanthanide cations. It is unlikely that the deviation of the sarcosine, alanine, or proline C_α data from the model described by eq 3 arises from random scatter of the data due to experimental uncertainties in the LIS since the plots for all three ligands show the exact same trends. Clearly, the differences in the two subsets of lanthanide cations is extenuated when the data are plotted in the form of eq 3 because of large differences in the ($A_2{}^0G + A_2{}^2G'$) values (as reflected by the two slopes) within the two subsets. These subsets are less apparent in the Δ_{obsd}/D vs. $\langle Sz\rangle/D$ plots (eq 4) because the slopes in this plot (A) are quite similar whereas the intercept ($A_2{}^0G + A_2{}^2G'$) differences, although large, are not as obvious. Least-squares analysis of the separate Pr → Tb and Dy → Yb C_α data indicates that the data within each subset fit eq 3 and 4 equally well. A comparison of the ($A_2{}^0G + A_2{}^2G'$) values obtained by subdividing the lanthanides into two sets is found in Table II. Similar values are observed for each nucleus within the two subsets except for the carboxyl and C_α carbons. The ($A_2{}^0G + A_2{}^2G'$) values for the latter two nuclei are similar to those found for alanine within these same two lanthanide subsets.[4]

Relaxation Measurements. The paramagnetic contributions to the spin-lattice relaxation rates of all ^{13}C and ^1H nuclei in L-proline for nine different lanthanide cations are presented in Tables III and IV. The tabulated value for each nucleus is the observed relaxation rate in a 1 M proline solution in the presence of the indicated amount of paramagnetic cation minus the relaxation rate of this same nucleus in the presence of an equal concentration of diamagnetic lanthanum. As $T_{1P}{}^{-1}$ has been shown to arise from dipolar interactions in sarcosine–lanthanide complexes,[5] the measured relaxation rate of each nucleus in proline is inversely proportional to the sixth power of the distance between that nucleus and the paramagnetic cation and hence should be quite sensitive to any structural changes in the proline–lanthanide complexes along the series. The distance ratios calculated from the relaxation rates for each lanthanide cation indicate that proline forms isostructural complexes with all of the lanthanides examined. The mean relaxation data were used in a computer search for the location of the paramagnetic cation which gave the best overall agreement based upon R factors[15] between the observed and calculated relaxation rates for all proline nuclei. The correlation time which governs these relaxation rates was assumed constant for all nuclei in a given lanthanide–proline complex as has been found recently for Mn^{2+}–proline complexes.[16] Two sets of coordinates were used for the proline atoms in the search procedure: one taken from the crystal structure of proline[17] representing an envelope conformation with C_γ in an endo position relative to the carboxyl group and a second planar structure with all C–C and C–N bond lengths set at 1.5 Å, all C–H bond lengths at 1.1 Å, and all H–C–H angles at 110°. This planar structure is expected to closely approximate the averaged dynamic structure of proline in aqueous solutions.[18] The calculated relaxation rates for the two H_γ protons and the two H_δ protons were averaged and weighted 10% relative to the remaining nuclei since these proton pairs remain unresolved and only one experimental relaxation rate could be assigned to each pair. A well-defined minimum (as measured by the R factors) was observed for both proline structures with the paramagnetic cation located 3.8 ± 0.1 Å from the carboxyl carbon and 20° off the C_0–C_α bond axis. A somewhat

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better agreement was found by using planar coordinates for the proline ring ($R = 2.35\%$) than by using the crystal coordinates ($R = 3.16\%$). In both models, the largest deviation between observed and calculated relaxation rates was found for the β, γ, and δ protons and the γ carbon and these are the atoms that experience the most motion in the various proline ring conformation interconversions.[18]

Structural Considerations from LIS Data. A separation of pseudocontact and contact shifts by using the model described by eq 3 and 4 can be performed without making any assumptions of axial or nonaxial symmetry for the lanthanide–proline complex. As the $(A_2{}^0G + A_2{}^2G')$ values for C_β, C_γ, C_δ, and the five protons were similar regardless of whether the data are subdivided into two groups of lanthanides or included as one group, these were used in a computer search procedure to test for effective axial symmetry in these complexes. The lanthanide cation position was fixed as required by the relaxation data and the direction of the principal symmetry axis (from which θ is measured) was varied to obtain the best agreement between calculated $(3\cos^2\theta - 1)/r^3$ ratios and experimental $(A_2{}^0G + A_2{}^2G')$ ratios as detected by a minimum in the R agreement factor. The G_{calcd} values given in Table II represent the best-fit agreement for the C_β, C_γ, C_δ, and proton data for the planar proline ring structure. The agreement factor was very sensitive to the direction of the lanthanide principal symmetry axis and a minimum was found for an axis 20° from the Ln–C_o vector, a direction corresponding to the closest approach of one of the carboxyl oxygens to the lanthanide cation. The agreement between the observed $(A_2{}^0G + A_2{}^2G')$ values and the G_{calcd} values for the eight nuclei used in the search is excellent. G values were also calculated for the C_o and C_α carbons by using the proportionality constant determined from the other eight nuclei. These values do not agree with any of the $(A_2{}^0G + A_2{}^2G')$ values determined by the various fits as summarized in Table II.

Discussion

The successful use of lanthanide-induced NMR shifts to obtain dynamic structural information about lanthanide complexes in solution depends upon an accurate separation of contact and pseudocontact shifts from experimentally measured values. Reilley et al.[12] first applied eq 2 to separate contact and pseudocontact contributions to the ^{17}O shifts in water. The application of this method to the separation of contact and pseudocontact components to the ^{13}C and ^{1}H LIS of L-alanine[4] led to the conclusion that this amino acid binds differently to cations from the first half of the lanthanide series than to cations from the last half of the series. Elgavish and Reuben[5] challenged this conclusion and recommended the use of relaxation data to detect any possible structural changes along the lanthanide series. They have examined the conformity of the LIS in four different ligands to the model described by eq 3 and 4 and concluded that if the shift data for a given nucleus fit either linear equation with a high degree of confidence (as measured by correlation coefficients), those shifts conform to the axially symmetric model. Conversely, if a poor correlation is found for both equations then the dipolar shifts must have a nonaxial term.

The data presented above clearly shows that these conclusions may not in many cases be correct. There is only one reason why the LIS data for a given nucleus should conform well to one of the two linear forms (eq 3 or 4) and not to the other, i.e., when the contact shift for a given nucleus is small or negligible in comparison to the pseudocontact shift. For these situations, a good correlation to eq 3 should be found with a near-zero intercept while a poor correlation to eq 4 is expected. This is exemplified by the C_γ, C_δ, and proton LIS in L-proline. When the shifts for a nucleus contain both pseudocontact and contact components, the data must conform well to both eq 3 and 4 and similar values of $(A_2{}^0G + A_2{}^2G')$ and A must be obtained in the solution of either equation. The LIS data for C_β is an example of an excellent fit to the model. The C_o data correlates reasonably well to both equations ($R^2 = 0.938$ and 0.967) yet significantly different values of $(A_2{}^0G + A_2{}^2G')$ and A are observed in the two solutions. Therefore, this data set does not fit the model described by eq 2. Similarly, the C_α data give an excellent correlation to eq 4 ($R^2 = 0.986$), a very poor correlation to eq 3 ($R^2 = 0.449$), and very different values of $(A_2{}^0G + A_2{}^2G')$ and A for the two solutions. The plots in Figure 1 show quite clearly that the C_α shifts do not conform to the model, at least for the entire cation series. When the data are divided into two groups of cations, each subgroup fits the model well as evidenced by the excellent correlation to both eq 3 and 4. Why are different values for $(A_2{}^0G + A_2{}^2G')$ and A observed for the two subgroups of lanthanide cations only for the C_o and C_α shift data? The excellent agreement between the $(A_2{}^0G + A_2{}^2G')$ values for both subgroups of cations and the G_{calcd} values based upon the axial symmetry model for the last eight nuclei in Table II indicates that (a) the nonaxial term $(A_2{}^2G')$ makes a negligible contribution to the pseudocontact shift at these nuclei, (b) the crystal field coefficient, $A_2{}^0$, is constant along the lanthanide series for these complexes, and (c) the lanthanide–proline complexes are isostructural within the two subgroups. The observation that the $(A_2{}^0G + A_2{}^2G')$ values for the C_o and C_α nuclei are different from the expected axial dipolar shifts (G_{calcd}) suggests that the nonaxial term may make a significant contribution to the measured pseudocontact shift at these two nuclei. Reuben and Elgavish[6] have pointed out that the nonaxial term $(A_2{}^2G')$ cannot be averaged out by intramolecular rotational motions for those nuclei lying along the principal symmetry axis. Hence, rapid rotation about the C_o–C_α bond can result in effective axial symmetry for all nuclei in the molecule except C_o and C_α. The data in the last three columns of Table II indicate the C_o and C_α shifts contain a nonaxial pseudocontact component within both subgroups of cations and this component is different for the heavy vs. light lanthanide cations.

It is tempting to speculate that at least part of the deviation from the model given by eq 2 seen at C_o and C_α results from small changes in the hyperfine coupling constant, A, along the lanthanide ion series. These two nuclei being closest to the lanthanide coordination site will experience the largest contact shift contributions to their shifts, and consequently, any significant changes in orbital overlap between the ligand and the light vs. heavy lanthanide cations would be extenuated at these two nuclei. Clearly, the extent of this contribution to the nonconformity of the C_o and C_α cannot be determined without additional electron spin density information in these lanthanide complexes.

The relaxation data show quite clearly that L-proline forms isostructural complexes with all ten lanthanide cations examined in this study. The pseudocontact shifts for all nuclei further removed from the lanthanide coordination site than C_α conform well to the axial symmetry model with the principal symmetry axis intersecting a single oxygen atom 2.9 ± 0.1 Å from the lanthanide cation. This monodentate Ln–O bond distance is in reasonable agreement with these same bond distances found in solid carboxylate complexes[19] and similar to the structure proposed for the Tm^{3+}–indoyl-3-acetate and Tm^{3+}–tryptophan complexes.[3] Our results may also be compared with a previous hydroxyproline LIS study.[20] In this study, five proton shift ratios obtained by using two lanthanide cations and five proton distance ratios determined from gadolinium relaxation rates were used to assign the proton resonances and probe the five-membered-ring conformation of hydroxyproline. The lanthanide ion was forced to lie along the C_o–C_α axis and a Ln–O bond distance of 2.4 Å was found in their best agreement structure. This distance is considerably shorter than the value we determined from our data with L-proline. The reasons for this difference are not clear but the Ln–hydroxyproline structure may be better defined because (a) all of the protons are resolved in this structure and hence provide more detailed distance information than in our study and (b) no separation of contact and pseudocontact shift components was necessary for the proton LIS used in hydroxyproline. Another recent L-proline LIS study has also been reported[13] but a comparison of their conclusions with ours is not worthwhile since their

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system was underdetermined with only four useful proton LIS and no relaxation-rate measurements.

The data presented in this work point out the difficulties in attempting to separate contact and pseudocontact components to measured lanthanide-induced shifts. The problems appear to be compounded at nuclei near the ligation site and, in light of the results presented here, several of the previously determined structures of lanthanide–carboxylates and lanthanide–amino acids should be reexamined. The trends in the LIS data we reported earlier for L-alanine are very similar to the results presented in this work for L-proline. It seems likely that inadequate separation procedures for the contact and pseudocontact shifts at C_0 and C_α in alanine led to an incorrect prediction of different lanthanide–alanine structures for cations from the first and last half of the lanthanide series. Structural differences were also noted for 3-indoylacetate[2] and tryptophan[3] binding to lanthanide cations from the first vs. last part of the series. Plots of $\Delta_{\mathrm{obsd}}/\langle Sz\rangle$ vs. $D/\langle Sz\rangle$ for the α-CH_2 and indole H-2 proton lanthanide induced shifts of 3-indoylacetate[2] clearly show the same subdivision of lanthanide cations as observed here for proline. This also suggests that the pseudocontact shifts in this ligand system contain nonaxial components (as previously pointed out by Reuben and Elgavish[6]) and these nonaxial terms are different for the light vs. heavy lanthanide cations.

Elgavish and Reuben have used relaxation data to show that sarcosine forms isostructural complexes with nine different lanthanide cations.[5] An examination of the plots for the C_α shifts (Figure 6 in ref 14) shows the same trends as reported here for proline. Their failure to recognize the nonaxial contribution to the C_0 and C_α shifts undoubtedly complicated their structural analysis and led them to conclude that the lanthanide cation does not bind to the carboxyl group in a unique position but rather averages between a monodentate and bidentate coordination position. This conclusion should be reevaluated in view of the results presented above.

In summary, we have shown that the separation of contact- and pseudocontact-shift contributions to observed LIS at nuclei near the lanthanide cation binding site in small molecules may often be complicated by nonaxial dipolar contributions to the pseudocontact shifts, changes in hyperfine coupling constants along the lanthanide series, or perhaps a combination of these effects. Relaxation rate data must be used to detect metal–ligand structural alterations along the cation series.[5] A high correlation of LIS data to eq 3 does not assure conformity of the data to the axial symmetry model but merely indicates that the quantity $(A_2^0G + A_2^2G')$ is lanthanide independent.

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Registry No. L-Proline, 147-85-3; Pr(3+), 22541-14-6; Nd(3+), 14913-52-1; Eu(3+), 22541-18-0; Gd(3+), 22541-19-1; Tb(3+), 22541-20-4; Dy(3+), 22541-21-5; Ho(3+), 22541-22-6; Er(3+), 18472-30-5; Tm(3+), 22541-23-7; Yb(3+), 18923-27-8.

Aggregation of Lithium Phenolates in Weakly Polar Aprotic Solvents

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Abstract: The aggregation of lithium phenolate, 3,5-dimethylphenolate, 2,6-dimethylphenolate, and 2,6-di-*tert*-butylphenolate in dioxolane, dimethoxyethane, and pyridine has been investigated by a variety of methods including studies of vapor pressure barometry, ^{13}C chemical shifts, ^{7}Li nuclear quadrupole coupling constants, and ^{13}C spin–lattice relaxation times. The phenolates with no ortho substituents form tetramers under most conditions. In pyridine at low concentrations and temperature the tetramers coexist with dimers. Lithium 2,6-dimethylphenolate forms dimers under all conditions studied, and lithium 2,6-di-*tert*-butylphenolate exists as a monomer or an oligomer depending on conditions. Attempts to establish solvation numbers for the aggregates from solvent ^{13}C relaxation times have not been successful, and the reason for the failure, very fast solvent exchange, is discussed. The kinetics and thermodynamics of exchange between dimers and tetramers of lithium 3,5-dimethylphenolate in pyridine have been investigated, and the mechanism of interconversion has been shown to involve additional solvation of the tetramer prior to dissociation. The equilibrium 2 dimer $\rightleftarrows$ tetramer has $\Delta H = 7.1$ kcal mol^{-1} and $\Delta S = 34$ cal deg^{-1} mol^{-1}, the large entropy being due to release of solvent on formation of the tetramer.

It is established that counterions play an important role in the chemistry of enolate ions in weakly polar aprotic solvents[1] and that the structures of ion-pair aggregates must be known before the mechanisms of the reactions of enolates with electrophiles can be completely understood.[2,3] Although X-ray crystallography can provide accurate structures for aggregates in the solid state,[4,5] it is necessary to establish whether these structures prevail in solution. We have used NMR spectroscopy to probe the structures in solution of aggregates of lithioisobutyrophenone.[6,7] For some sets of conditions the existence of the tetrameric structure, which has been found for **1** ($R = OCBu^t{=}CH_2$; S = THF) in the solid state,[4] has been established. Under other conditions, however, the presence of the dimeric species **2** could be inferred. Although there appears to be no reason to doubt these conclusions, the possible conformational flexibility within the enolate moiety in the lithioisobutyrophenone aggregates did introduce some uncertainty into the interpretation of several of the NMR phenom-

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