with the theoretical predictions for dipolar shifts in axially symmetric isostructural complexes of ytterbium and praseodymium.[10]

The formation of hydroxo complexes of the $Ln(EDTA)^-$ chelates, previously inferred from the effect of pH on the induced shifts and broadenings of acetate, is confirmed by the pH profiles in the present work. Here, however, the cations associate with $Ln(EDTA)(OH)^{2-}$ and larger broadenings induced by gadolinium are observed as pH >8. The complications in the data interpretation that may arise from the presence of multiple equilibria can be avoided by working at pH <8.

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Aqueous Lanthanide Shift Reagents. 4. Interaction of Pr^{3+}, Nd^{3+}, and Eu^{3+} with Xylitol. Origin of Induced Shifts in Polyols

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Abstract: Xylitol interacts in aqueous solution with trivalent lanthanides to form 1:1 as well as 2:1 complexes. The apparent dissociation constants, evaluated from an analysis of the lanthanide-induced shifts, show that the stability of the complexes increases in the sequence Pr < Nd < Eu. Details on the method of data analysis and on the criteria for assessing the reliability of the resulting parameters are given. An interpretation of the induced shifts in this and related systems indicates that nuclei close to the site of lanthanide complexation experience large contact shifts.

The recent interest in studying the complexes between metal ions and carbohydrates is related to their possible involvement in a number of biological processes.[1,2] Angyal has shown that proton NMR methods can be applied to the study of such complexes in aqueous solution.[3] In particular it has been demonstrated that paramagnetic lanthanides induce appreciable shifts in the spectra of some aldoses,[4] alditols,[5] and cyclitols.[6,7] Lanthanides have been used to afford spectral resolution in a study of the interaction of xylitol with xylose isomerase[8] and to induce stereospecific relaxation rate enhancements in a number of mono- and disaccharides[9] and aldohexuronic acids.[10] Lanthanide-induced shifts in the spectra of methyl-α-D-gulopyranoside[11] and methyl-α-D-galactopyranosiduronate[12] have been treated as being of a purely dipolar origin and used to determine the structure of the complexes. However, results obtained for *epi*-inositol with a number of lanthanides indicate the presence of large contact contributions to the induced shifts.[6,7] The utilization of lanthanide-induced shifts for structure elucidation requires not only the evaluation of the dipolar shift contribution, but also the determination of the stoichiometry of the complex, in view of possible complications due to higher than 1:1 complexes. Predominantly 1:1 sugar-cation complexes are formed with D-lyoxose, D-ribose,[13] and methyl-α-D-gulopyranoside.[11] In the *cis*-inositol-calcium system a 2:1 complex has been detected in addition to the prevalent 1:1 complex.[14] While 1:1 stoichiometry has tacitly been assumed for alditols,[5,15] the formation of 2:1 complexes cannot be ruled out.

In an attempt to answer some of the questions regarding the stoichiometry of alditol-lanthanide complexes and the origin of their proton shifts, we have investigated the interaction of xylitol with Pr^{3+}, Nd^{3+}, and Eu^{3+}. The lanthanide-induced shifts were monitored in two types of titrations, in which the concentration of one of the components is held constant and that of the other varied. The two sets of data thus obtained are compatible only if 2:1 complexes are included in the analysis. An interpretation of lanthanide-induced shifts in this and related systems is also presented.

Experimental Section

Lanthanide chlorides, rather than the nitrates used by other workers,[5,15] were employed throughout this work.[16] Their solutions were prepared from the corresponding oxides (Research Chemicals, Phoenix, Ariz.) by treatment with analytical grade hydrochloric acid. Concentrations were determined by EDTA titrations using Arsenazo as the end-point indicator. Xylitol was obtained from Sigma Chemical Co., St. Louis, Mo., and used without further purification. Solutions were made up in D_2O containing ca. 0.1% v/v *tert*-butyl alcohol, which served as an internal reference for the chemical shift measurements. Proton NMR spectra were recorded on a Varian T-60 spectrometer at the ambient probe temperature of 39 ± 1 °C.

Results

At 60 MHz the proton spectrum of xylitol consists of a featureless band centered at 2.45 ppm from *tert*-butyl alcohol. The effects of successive additions of $EuCl_3$ are depicted in Figure 1. Already at low concentrations a well resolved and readily assignable spectrum is obtained. As seen in Figure 1, complex formation markedly alters the coupling constant $J_{2,3}$ from 4.5 Hz at the lowest $EuCl_3$ concentration to <2 Hz at the highest.

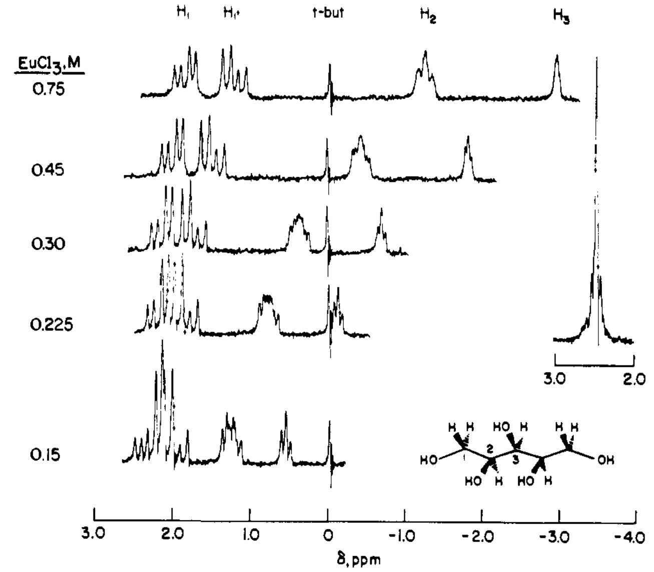

Figure 1. The 60-MHz spectra of 0.45 M xylitol in the presence of increasing concentrations of $EuCl_3$. The inset on the right is the spectrum in the absence of shift reagent.

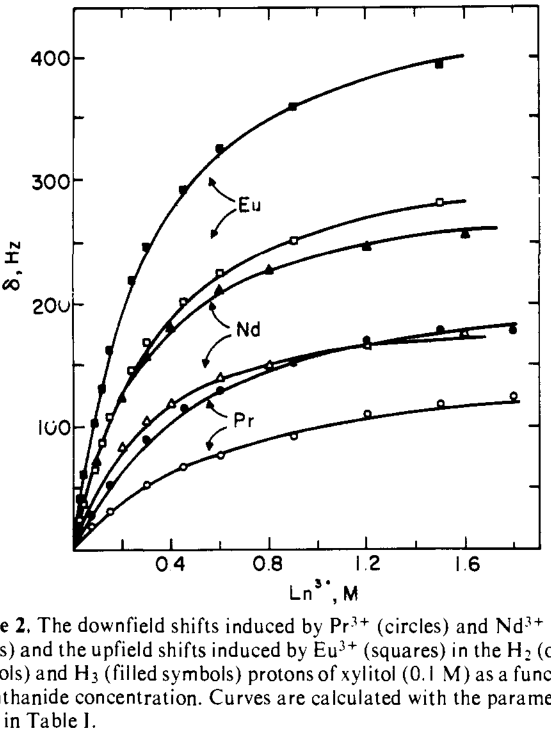

Figure 2. The downfield shifts induced by Pr^{3+} (circles) and Nd^{3+} (triangles) and the upfield shifts induced by Eu^{3+} (squares) in the H_2 (open symbols) and H_3 (filled symbols) protons of xylitol (0.1 M) as a function of lanthanide concentration. Curves are calculated with the parameters given in Table I.

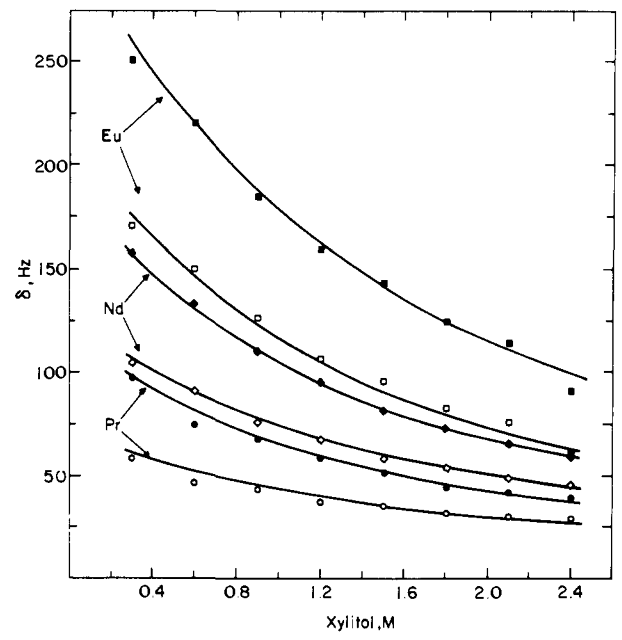

Figure 3. The downfield shifts induced by 0.45 M Pr^{3+} (circles) and 0.40 M Nd^{3+} (diamonds) and the upfield shifts induced by 0.45 M Eu^{3+} (squares) in the H_2 (open symbols) and H_3 (filled symbols) protons of xylitol as a function of its concentration. Curves are calculated with the parameters given in Table I.

For the determination of the dissociation constants the lanthanide-induced shifts in the H_2 and H_3 protons were measured in two types of titrations. In the type I titration, the xylitol concentration was held constant at 0.1 M and that of the lanthanide varied over a wide range. The results are shown in Figure 2. In the type II titration, the lanthanide concentration was held constant and that of xylitol varied. The results thus obtained are shown in Figure 3. An attempt to analyze the data assuming the formation of only 1:1 xylitol-lanthanide complexes showed that the results of the two titrations were incompatible. However, good simultaneous fits are obtained if the formation of 2:1 complexes is also assumed. Equilibria in shift reagent systems involving the stepwise formation of 2:1 adducts have been treated on several occasions.[17-19] The approach[20] used in the present work is somewhat different and includes an adaptation of the criteria outlined by Deranleau[21] and by Maier and Drago[22] for related cases.

Data Analysis. The observed shift δ is a sum of two contributions

$$\delta = [RS]\delta_1/[S_t] + 2[RS_2]\delta_2/[S_t] \quad (1)$$

where δ_1 and δ_2 are respectively the chemical shifts of the RS and RS_2 species, R being the lanthanide and S the substrate, xylitol in our case. The apparent dissociation constants are defined as

$$K_1 = [R][S]/[RS] \quad (2)$$

$$K_2 = [RS][S]/[RS_2] \quad (3)$$

Since the total concentrations, $[R_t]$ and $[S_t]$, are known it is convenient to express the free substrate concentration in the form of the cubic equation

$$[S]^3 + (2[R_t] - [S_t] + K_2)[S]^2 + K_2([R_t] - [S_t] + K_1)[S] - K_1K_2[S_t] = 0 \quad (4)$$

Equation 4 can be solved by the Newton-Raphson method and with the value of [S] the equilibrium concentrations of other species are easily calculated.[18] Equation 1 can be rearranged to the following form:

$$\frac{[RS]}{[S_t]\delta} = \frac{1}{\delta_1} - \frac{2[RS_2]}{[S_t]\delta}\frac{\delta_2}{\delta_1} \quad (5)$$

It is seen that a plot of $[RS]/[S_t]\delta$ vs. $2[RS_2]/[S_t]\delta$ should be a straight line confined by the axes with intercepts of $1/\delta_1$ on the ordinate and $1/\delta_2$ on the abscissa. The use of eq 5 for the data analysis has the same advantage as using the Scatchard plot; both are confined by the axes.[21] The following procedure is then followed. A set of K_1 and K_2 values is chosen and equilibrium concentrations are calculated. These are used with eq 5 to obtain (by linear least squares) the limiting shifts δ_1 and δ_2. With the latter, values of the chemical shift δ_c are calculated using eq 1 and compared with the observed δ values to compute the standard deviation. The values of K_1 and K_2 are varied over a wide range until a minimum in the standard deviation is obtained. The set of K_1, K_2, δ_1, and δ_2 parameters corre-

Table I. Apparent Dissociation Constants and Induced Shifts[a] for Lanthanide–Xylitol Complexes

	Pr^{3+}	Nd^{3+}	Eu^{3+}
K_1, M	0.50 ± 0.10	0.25 ± 0.01	0.26 ± 0.04
K_2, M	8 ± 4	2.0 ± 0.1	1.3 ± 0.3
$\delta_1(H_2)$, Hz	158 ± 8	202 ± 2	−340 ± 10
$\delta_1(H_3)$, Hz	232 ± 8	300 ± 3	−466 ± 15
$\delta_2(H_2)$, Hz	117 ± 80	187 ± 15	−189 ± 30
$\delta_2(H_3)$, Hz	121 ± 70	231 ± 20	−309 ± 40

[a] At 60 MHz, 39 °C, upfield shifts given as negative.

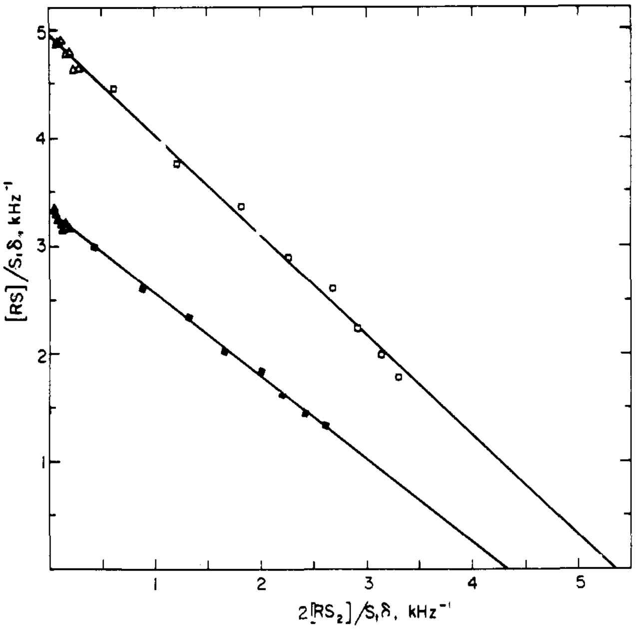

Figure 4. The data for the xylitol–Nd^{3+} system (cf. Figures 2 and 3) plotted according to eq 5. Equilibrium concentrations were calculated with the apparent dissociation constants given in Table I. The lines were calculated by linear least squares.

sponding to this minimum is considered to be the best one describing the data. A summary of the constants thus obtained is given in Table I. The smooth curves in Figures 2 and 3 were calculated with these constants. The plots according to eq 5 are illustrated in Figure 4 with the neodymium data. These plots may be regarded as proof for the presence of 2:1 complexes particularly in the type II titration. The reliability of the constants obtained from the fitting procedure was judged according to the following considerations.

In each NMR measurement only one value of the chemical shift δ is obtained, which contains contributions from both of the complexes of interest (cf. eq 1). Over different concentration ranges of $[R_t]$ and $[S_t]$, the relative contributions of the two complexes will change. The normalized relative contributions to the observed shift can be defined as

$$P_j = j[RS_j]\delta_j/[S_t]\delta \tag{6}$$

where $j = 1$ or 2. The reliability of the coupled pair of equilibrium parameters K_j and δ_j will be maximized if the data from which they were derived were taken under conditions where the corresponding P_j values were maximal, i.e., close to unity. In the present work, only for Nd^{3+} and Eu^{3+} did P_2 exceed 0.5. For Pr^{3+}, $P_2 < 0.27$ was obtained and therefore K_2 and δ_2 in this case are less reliable. The accuracy of the *individual* parameters is assessed according to the range of values encompassed by the saturation factors

$$s_j = [RS_j]/[R_t] \tag{7}$$

$$\sigma_j = j[RS_j]/[S_t] \tag{8}$$

For the case of 1:1 stoichiometry Deranleau has shown (cf. Figure 1 of ref 21) that the minimum relative error in K is obtained for s values between 0.2 and 0.8 and that the minimum relative error in the limiting shift is obtained for $\sigma \doteq 1$, with reasonable accuracy expected for σ values above 0.2.[21] These criteria are now extended to the case of 2:1 stoichiometry. For the accurate determination of δ_j the corresponding fraction of complexed substrate, σ_j, should cover the range above $\sigma_j \doteq 0.2$, with best results expected for $\sigma_j \doteq 1$. For the accurate determination of K_j, s_j should cover the range between 0.2 and 0.8. The uncertainties given in Table I were estimated from the standard deviations obtained in the fitting procedure using the relative errors computed by Deranleau (Figure 1 of ref 21).

Discussion

The formation of 2:1 xylitol–lanthanide complexes in addition to the 1:1 complexes has been demonstrated. The apparent dissociation constants show that the stability of the complexes increases along the series: Pr < Nd < Eu. The higher stability and the better resolution should make $EuCl_3$ the shift reagent of choice for this and related systems. The finding that $J_{2,3}$ decreases upon complex formation is in agreement with the results of Angyal et al.[5] and is in accord with their suggestion that xylitol assumes a threo-threo arrangement in its lanthanide complex. Referring to the induced shifts in the 1:1 complexes (cf. Table I) one finds that the ratio $\delta_1(H_3)/\delta_1(H_2)$ is nearly constant for the three lanthanides. Thus, it may appear, at first sight, that these are dipolar shifts in axially symmetric isostructural complexes. Also, the relative signs of the shifts are in accord with theoretical predictions for dipolar shifts in lanthanide complexes.[23] However, the relative magnitudes of the shifts do not follow the theoretical estimates. On the other hand, both the signs and the magnitudes seem to conform better with the expectations for contact shifts in lanthanide complexes.[24]

The total induced shift for a nucleus, i, in an axially symmetric complex at a given temperature can be expressed as a sum of the contact[24] and dipolar[23] contributions:

$$\delta_i = A_i\langle S_z\rangle + G_iD \tag{9}$$

where A_i is the hyperfine coupling constant, $\langle S_z\rangle$ is the projection of the total electron spin magnetization on the direction of the external magnetic field, G_i is the geometrical function $(3\cos^2\theta_i - 1)r_i^{3}$, and D is a constant for a given lanthanide. The relative values of $\langle S_z\rangle$ and of D have been tabulated by Golding and Halton[24] and by Bleaney et al.,[25] respectively. When data for a number of lanthanides are available, the following rearrangement of eq 9,

$$\delta_i/\langle S_z\rangle = A_i + G_i(D/\langle S_z\rangle) \tag{10}$$

has been found convenient for graphical interpretation.[26,27] A plot of $\delta_i/\langle S_z\rangle$ against $D/\langle S_z\rangle$ should be linear provided that both A_i (the intercept on the ordinate) and G_i (the slope) are constant along the lanthanide series. Deviations from the model should be clearly revealed in such plots. We have treated in this way our data as well as the shifts for the H_3 and H_6 protons of the related *epi*-inositol complexes.[6,7] The results are shown in Figure 5. A fairly good linear relationship with a very small intercept is obtained for the H_6 proton of *epi*-inositol. This means that the model holds well, i.e., the complexes are axially symmetric and isostructural. Large contact shifts are indicated for the proton shifts of xylitol. The points for the H_3 proton of *epi*-inositol exhibit great scatter. Clearly the model does not hold for this close to the site of complexation proton,

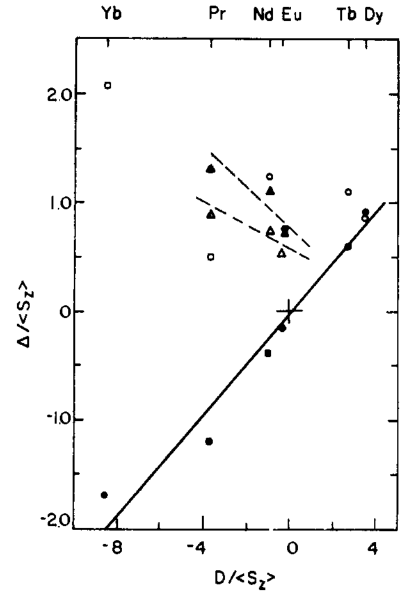

Figure 5. A plot according to eq 10 of data (shifts in ppm) for the H_2 (open triangles) and H_3 (filled triangles) protons of xylitol (cf. Table I) and the H_3 (open circles) and H_6 (filled circles) protons of *epi*-inositol.[6,7] The values of $\langle S_z \rangle$ and D were taken from ref 24 and 25, respectively.

probably because the apparent $A(H_3)$ is not constant along the lanthanide series. Deviations from this model have also been observed for nuclei close to the site of complexation in other lanthanide complexes and additional pathways for lanthanide-induced shifts have been suggested in order to explain them.[26,27] These pathways include nonbonding overlap between the lanthanide 4f orbitals and ligand orbitals and dipolar shifts arising from the unpaired spin density distributed over anisotropic ligand orbitals. More data, particularly for carbon-13, are needed in order to elucidate in greater detail the mechanism of lanthanide-induced shifts in polyols and related compounds. Lanthanide coordination to polyols and carbohydrates usually occurs at a site composed of three hydroxyl groups on consecutive carbon atoms.[3-7,13-15] Thus, a substantial number of the ligand nuclei are close to this site and are likely to experience large contact shifts. However, it is only the dipolar contribution that is of value in structural determinations and therefore suitable methods are needed for its proper evaluation. Meaningless structural fits are bound to result if the total induced shifts are treated as being of a purely dipolar origin. It is not surprising therefore that, for a carbohydrate derivative, lanthanide-oxygen distances ranging from 1.5 to 3.3 Å have been obtained from the interpretation of NMR data,[11] whereas x-ray structures of calcium-carbohydrate complexes show that the metal-oxygen distances fall in the rather narrow range of 2.38-2.55 Å.[2,28,29]

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