NMR-Studies of the Interaction of Metal Ions with Poly-(1,4-hexuronates). III. Proton Magnetic Resonance Study of the Binding of Lanthanides to Methyl α-D-Gulopyranoside in Aqueous Solution

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The influence of some paramagnetic lanthanide ions on the PMR spectra of methyl α-D-gulo-pyranoside in aqueous solution has been studied. Analysis of the induced shift curves indicated that a 1:1-complex was formed. The induced shift ratios for Eu^{3+} and Pr^{3+} were not equal for all protons, thus suggesting significant contact shifts or a complex of non-axial symmetry. By carrying out an approximate correction for asymmetry, the best fit of calculated and observed shifts was obtained with the lanthanide ion located near the ax-eq-ax sequence of the three oxygen functions at C_1, C_2, and C_3.

The exploration of possible metal-ion binding-sites in sugar molecules has recently been intensified [1-4] with the main aim of obtaining a better understanding of the rather unique ion-binding properties of polyuronides.[5]

The effect of certain inorganic salts on the position of the equilibrium at the anomeric centre of a number of reducing sugars in aqueous solution has been studied.[2] It was suggested that pyranose rings possessing an ax-eq-ax sequence of three contiguous hydroxy groups offer a particularly good binding site.[2] LIS(lanthanide induced shift)-measurements [4] indicated that methyl α-D-galactopyranosiduronic acid provides a similar, triangularly-shaped binding site, composed of the axial hydroxy group at position 4, the equatorial carboxy group, and the ring oxygen. The induced shifts of both $Eu(NO_3)_3$ and $Pr(NO_3)_3$ could be accounted for remarkably well by a pseudo-contact mechanism assuming axial symmetry of the complex.[6]

These results suggests that the use of lanthanides as probes for evaluating binding sites in metal-sugar complexes has a great potential. However, in the general case the interpretation of the results may not be so simple as for α-D-galactopyranosiduronic acid. The recent LIS-results of Angyal [7] on methyl β-D-hamame-lopyranoside (containing the ax-eq-ax-sequence) seemed not to be related to the equation [8] for pseudocontact shifts in axially symmetrical complexes:

$$\Delta\nu/\nu_0 = K(3\cos^2\theta - 1)/r^3 \qquad (1)$$

This may suggest that the interpretation of LIS-data for neutral sugar derivatives may represent a more difficult problem than for uronic acid derivatives, and we will here present LIS-results on α-D-gulopyranoside, another neutral sugar derivative containing the ax-eq-ax sequence of two hydroxy groups and one OCH_3 group.

RESULTS AND DISCUSSION

The 60 MHz ^1H NMR-spectrum before and after the addition of $Eu(NO_3)_3$ to a molar ratio (Eu^{3+}/sugar) of 2.9 is given in Fig. 1. The assignments were confirmed by double-resonance experiments, and the size of the coupling constants indicates that no change in conformation ($^4C_1$) occurs during the addition of lanthanide. The shifts induced by $Eu(NO_3)_3$ and $Pr(NO_3)_3$ are given in Fig. 2. Use of $Pr(ClO_4)_3$
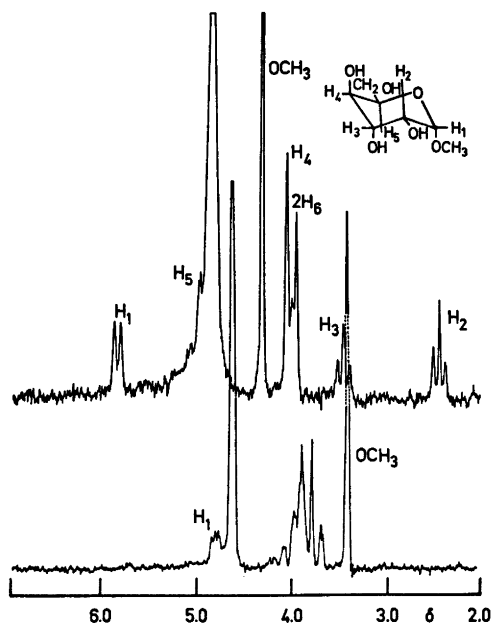
Fig. 1. The 60 MHz ^{1}H NMR-spectrum of methyl α-D-gulopyranoside in D_2O before (a) and after (b) the addition of europium nitrate to a molar ratio of 2.9 (Eu^3+/sugar). $J_{1,2} = J_{2,3} = J_{3,4} = 3.5$ Hz, $J_{4,5} = 1.0$ Hz and $J_{5,6} = 6.0$ Hz.

instead of Pr(NO$_3$)$_3$ yielded identical results. The initial slopes, and the ratios between the initial slopes for the two sets of experiments, are given in Table 1.

According to Bleaney *et al.*,[9] the ratios between pseudocontact shifts at different proton sites in a complex of axial symmetry should be

Table 1. Initial slopes, k, and slope ratios from Figs. 2 and 3.

Proton	k_{Eu} Hz	k_{Pr} Hz	k_{Pr}/k_{Eu}
H$_1$	57	−57	−1
H$_2$	−96	23.5	−0.24
H$_3$	−30	34	−1.13
H$_4$	∼0	1	—
H$_5$	54	−45.5	−0.84
H$_6$	12	−12	−1
O−CH$_3$	48	46	−0.95

independent of the lanthanide ion, provided the geometrical structure of the complex is retained. Table 1 shows that this is the case for all protons except H$_2$. The marked deviation for this proton may accordingly be due to (1) the presence of more than one type of complex in solution, the relative proportions of which may be different for different cations, (2) a large contact-contribution to the H$_2$-shift, (3) lack of axial symmetry in the complex, or (4) different geometry of the Eu^3+ and the Pr^3+-sugar complexes. These possibilities must, therefore, be considered separately.

(1). A test for stoichiometry proposed by Job and applied by Roth *et al.*[10] on LIS-problems has been used. The difference $(\delta_1 - \delta_2)$ between the induced shifts of the H$_1$- and the H$_2$-protons was measured at a series of substrate, [S]$_0$, and Eu(NO$_3$)$_3$-concentrations, [Eu]$_0$. In Fig. 3 the plot of [S]$_0$ $(\delta_1 - \delta_2)$, where $\delta_1 - \delta_2$ was measured at a constant ([S]$_0$ + [Eu]$_0$) = 0.5 (M), against [Eu]$_0$/([S]$_0$ + [Eu]$_0$), is given. The
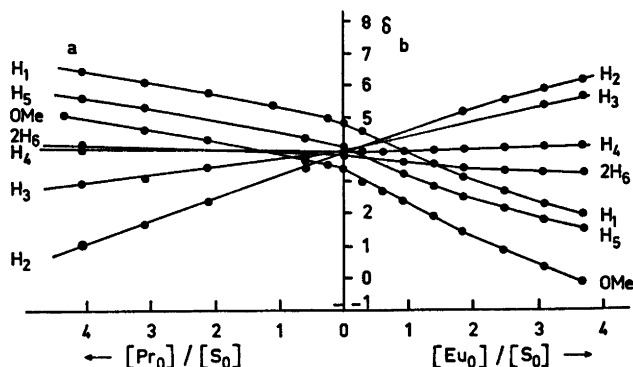

Fig. 2. a. Induced shifts, δ, relative to dioxane against the molar ratio of europium nitrate to substrate [Eu]$_0$/[S]$_0$ at [S]$_0$ = 0.39 M. b. Same data as in a for praseodymium nitrate. [S]$_0$ = 0.282 M.
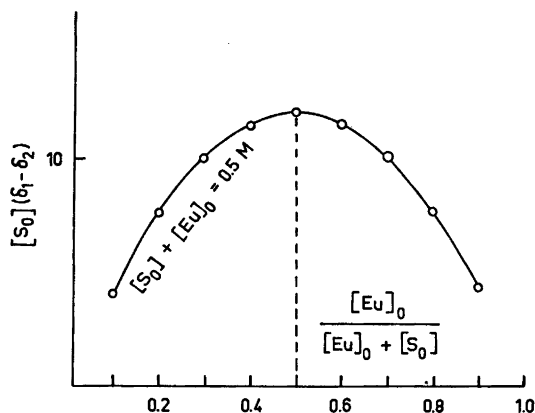
Fig. 3. Job's method used on the complex formation between α-methyl-D-gulopyranoside and europium nitrate.

maximum of the curve at $[Eu]_0/([S]_0 + [Eu]_0) = 0.5$ indicates, according to Roth et al.,[10] and in agreement with earlier suggestions,[1] that a complex of 1:1 stoichiometry is the dominating species. Initial slopes are, therefore, proportional to the bound shifts, Δ, and may be used in attempting to position the Ln-ion in the complex.

(2). An automatic search program,[6] applying eqn. (1), was used to find the best position of the Ln^{3+} ions and the magnetic axis for the two sets of data in Table 1, in order to establish whether contact-shift contributions appeared more probable for the Eu or the Pr-complexes. The results in Table 2 clearly indicate that both the lanthanide-ions are located at the $O_1(ax) - O_2(eq) - O_3(ax)$-site, but the data for the Pr-

complex yield distances which are chemically more reasonable within the accuracy of 0.3 Å suggested [13] for this type of analysis. The distance between the position of the Pr^{3+} and the Eu^{3+} ion was found to be 1.1 Å. It appears, therefore, that a significant contact contribution to the H_2-shift in the Eu-complex could explain the difference between the two sets of data. Because the tendency for contact-shift contributions [11,14] is larger for the Eu^{3+} than for the Pr^{3+} ion, this possibility cannot be ruled out. At present, it seems difficult to estimate the contact shift contribution for the various protons in methyl α-D-gulopyranoside. We therefore want to demonstrate that the observed LIS may be explained on the basis of an asymmetric complex.

(3). Since in a 1:1 Ln-sugar complex the ligand field is most probably less than axially symmetrical, it seems possible that some asymmetry in the magnetic properties of the complex could account for the results. The equation for pseudocontact shifts in complexes of rhombic magnetic symmetry is, according to Bleaney et al.,[9]

$$\frac{\Delta \nu}{\nu_0} = A \frac{3\cos^2\theta - 1}{r^3} + D \frac{\sin^2\theta \cos 2\phi}{r^3} \qquad (2)$$

The first term in eqn. (2) is seen to be equivalent to the axially symmetrical situation, and the task is, therefore, to see if it is possible to find a position of the axis of asymmetry giving a large value, for the second term in eqn. (2), for the H_2-proton relative to all the other protons.

The geometric part of the second term was

Table 2. Observed relative shifts and "best fit" calculation assuming axial symmetry of the complexes. The sign convention in the calculations is the same as that used previously.[6]

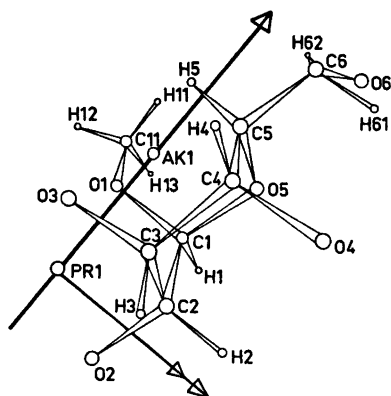| Proton	Eu-data Obs.	Calc.	Pr-data Obs.	Calc.
H_1	1.0	1.03	1.0	1.05
H_2	−1.68	−1.60	−0.41	−0.42
H_3	−0.53	−0.81	−0.60	−0.62
H_4	0.01	0.08	0.03	0.05
H_5	0.95	0.97	0.80	0.82
H_6	0.21	0.33	0.21	0.30
Distance to	O_1, O_2, O_3 (Å) 2.2, 1.5, 3.3		1.7, 1.9, 2.5	
Mean square deviation	1.7×10^{-2}		1.2×10^{-3}	

Fig. 4. Computer drawing of Pr^{3+}-sugar complex as obtained from the asymmetry calculations in Table 3. The symmetry axis is given by a single and the asymmetry axis by a double arrow. PR1: the praseodymium ion (not to scale); AK1: an auxilliary point introduced to define the direction of the symmetry axis.

data now give very similar positions of the Eu^{3+} and the Pr^{3+} ion. The distance between the two sites is 0.3 Å. Somewhat disturbing is the fact that the distance from the Ln^{3+} ion to O_3 is as high as 3 Å. However, taking into account that the present calculation must be regarded only as a first, crude correction for asymmetry, the results strongly suggest that both sets of data can be accounted for by eqn. (2), *i.e.* without assuming any contact shift contributions to the observed shifts.

(4). In the above treatment we have assumed that the Pr^{3+}- and the Eu^{3+}-sugar complexes have an equivalent geometry. Induced shifts have also been obtained for $Er(NO_3)_3$, $Yb(NO_3)_3$, and $Ho(NO_3)_3$ (unpublished results). The three sets of shift curves were, as expected, very similar (Ho^{3+} giving shifts of opposite signs compared to Er^{3+} and Yb^{3+}), but markedly different from the Eu and Pr data. Most interesting was a very large induced shift for the HDO-proton, which was observed in all three cases. The corresponding shift was very small in the case of Eu^{3+} and Pr^{3+}. It seems therefore that both the chemistry and the geometry may vary among the lanthanide-sugar complexes. The similarity of the Er^{3+}, Yb^{3+}, and Ho^{3+} results on one hand, and the Pr^{3+} and Eu^{3+} results on the other, suggests, however, that this change occurs in a discontinuous manner along the lanthanide series and that the LIS-approach is valuable when proper account is taken of the possibility of magnetic asymmetry in the complexes and contact shift contributions.

calculated by keeping the direction of the symmetry axis and the Ln position as indicated from the Eu^{3+}-data, and by rotating the asymmetry axis in small steps. For a certain direction of the asymmetry axis (see Fig. 4) the relative values 0.006, 1.0, 0.090, 0.020, 0.012, and 0.040 for the protons H_1, H_2, H_3, H_4, H_5, and H_6, respectively, were obtained. Very similar values were found for the Pr^{3+} site. By assuming that $D_{Pr}/D_{Eu} = -A_{Pr}/A_{Eu} = 1$, the two sets of data could be corrected, and the search procedure applying eqn. (1) could be carried out. The results in Table 3 show that the corrected

Table 3. Observed relative shifts corrected for asymmetry (see text) and "best-fit" calculation applying the equation for axial symmetry.

Proton	Eu-data Corrected	Calc.	Pr-data Corrected	Calc.
H_1	1.0	1.03	1.0	1.04
H_2	−1.01	−0.98	−1.05	−1.03
H_3	−0.45	−0.61	−0.64	−0.73
H_4	0.01	0.05	−0.03	−0.06
H_5	0.87	0.87	0.79	0.81
H_6	0.23	0.33	0.19	0.31
Distance to O_1, O_2, O_3 (Å)	1.8, 1.8, 3.2		1.7, 1.85, 3.0	
Mean square deviation	5.5×10^{-3}		3.2×10^{-3}	

Note added in proof. In a recent paper by Angyal; Greeves and Pickles (*J. Chem. Soc. Chem. Commun* (1974) 589) it has been demonstrated on the basis of LIS-experiments with *epi*-inositol and 1,6-anhydro-β-D-allopyranose that the contact interaction is greatest when the bonds connecting the proton and the cation form a planar zig-zag arrangement. A preliminary analysis of their data suggests that a similar, planar arrangement of $Ln^{3+} - C_2 - H_2$ in methyl α-D-gulopyranoside may cause a considerable contact shift contribution to the H_2-shift (see Table 1).

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