

NMR-Studies of the Interaction of Metal Ions with Poly(1,4-hexuronates). II. The Binding of Europium Ions to Sodium Methyl α -D-Galactopyranosiduronate

THORLEIF ANTHONSEN,^a
BJØRN LARSEN^b and
OLAV SMIDSRØD^b

^aOrganic Chemistry Laboratories, Norwegian Institute of Technology, University of Trondheim, N-7034 Trondheim-NTH, Norway and ^bInstitute of Marine Biochemistry, University of Trondheim, 7034 Trondheim-NTH, Norway

Metal ions play an essential part in determining the physical and biological properties of many polyuronides, and we have recently¹ started an NMR-study in which lanthanide ions are used as probes for determining possible binding sites. The

effect of Eu^{3+} on sodium D-galacturonate was shown to be markedly greater than on sodium D-glucuronate. This difference between the two C_4 -epimeric sugars and other evidence, suggested that the binding site in D-galacturonic acid consisted of the carboxy group, together with O_4 and O_6 . In an attempt to locate the Eu^{3+} ion more exactly we have now determined the induced shifts in sodium methyl α -D-galacturonate. The 60 MHz PMR-spectrum is given in Fig. 1a.

After the addition of europium nitrate to an equivalent ratio of 3.6, the resonances are shifted as shown in Fig. 1b. The assignments were confirmed by double resonance experiments and all coupling constants are in satisfactory agreement with earlier observations.² They indicate that no change in conformation (${}^4\text{C}_1$) occurs during the addition of europium nitrate. The shift curves (Fig. 2) show that the resonance peaks are shifted both upfield and downfield. Even at high europium-to-sugar ratios, the magnitude of the shift still depends on the europium ion concentration.

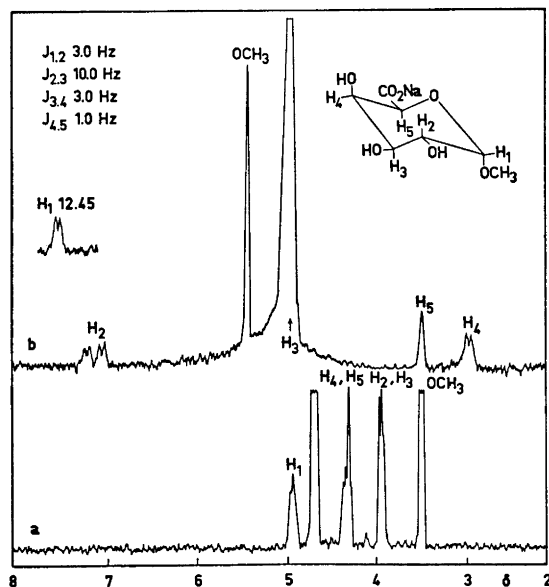


Fig. 1. The 60 MHz PMR-spectrum of sodium methyl α -D-galacturonate in D_2O before (a) and after (b) the addition of europium nitrate (equivalent ratio $[\text{Eu}]/[\text{S}] = 3.6$). The shifts are measured relative to sodium 3-(trimethylsilyl)propanesulfonate.

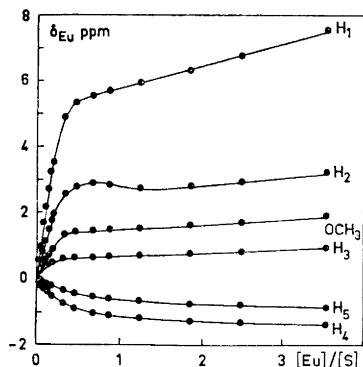


Fig. 2. The Eu^{3+} induced shift (δ_{Eu}) for the various protons in sodium methyl α -D-galacturonate as a function of the equivalent ratio $[\text{Eu}]/[\text{S}]$. ($[\text{S}] = 0.335 \text{ M.}$)

Assuming axial symmetry of the Eu^{3+} -uronic-acid complex, we have calculated the relative shifts of protons $\text{H}_1 - \text{H}_5$ (space coordinates for the various atoms were taken from an X-ray study of methyl α -D-galacturonic acid methyl ester³) according to the equation⁴

$$\Delta\nu/\nu_0 = K(3 \cos^2\phi - 1)/r^3 \quad (\text{I})$$

The experimental shifts were calculated from the initial slopes of the shift curves (Fig. 2) assuming 3:1 (sugar: Eu^{3+}) stoichiometry. The calculated shifts were scaled to the experimental ones by varying the value of K in eqn. I to obtain the minimum mean square deviation (R -factor). The best fit (Table 1) was obtained when the distances from the Eu^{3+} ion to O_4 , O_5 , and C_6 were set at 2.9, 2.0, and 2.2 Å, respectively, with the magnetic axis

Table 1. Observed and calculated europium induced shifts assuming 3:1 complex.

	Obs.	Calc.
H_1	5.50	5.50
H_2	3.00	3.01
H_3	0.80	0.74
H_4	-0.93	-0.96
H_5	-0.43	-0.41
OMe	1.42	—

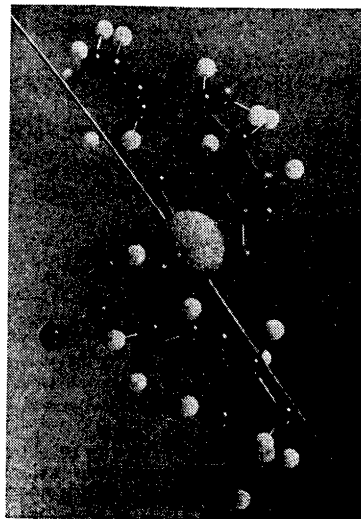


Fig. 3. Ball-stick-model where 2 of the three sugar molecules are arranged around the magnetic axis of Eu^{3+} according to the data. The third sugar ligand is omitted both for the sake of clarity in the figure and because of practical difficulties in building the 3:1 complex. The carboxy groups are rotated so as to give the smallest possible distance between one of the two oxygen atoms and the europium ion. The assumed position of the magnetic axis is indicated by the string through the center of the europium ion.

pointing towards H_1 (see Fig. 3). The calculated R -factor was 0.001.

Angyal⁵ has recently remarked that the direction and magnitude of the induced shifts in methyl β -D-hamamelopyranoside did not seem to be related to eqn. I. However, the agreement between calculated and observed shifts in Table 1 is remarkably good. This suggests that the method will prove to be of great value for evaluating binding sites in metal-sugar complexes.

Bleaney *et al.*⁶ have discussed the use of lanthanide-induced shifts (LIS) in aqueous solutions. They proposed that experimental tests for axial symmetry should be carried out and also that the LIS should be corrected for electrostatic shielding effects of the lanthanide ions. Such experiments are currently being carried out and will possibly lead to some small corrections in the positioning of the Eu^{3+} ion. However, the position of the europium ion that is indicated at present is reasonable from a

chemical viewpoint. The proximity of the europium ion to the carboxy group, the ring oxygen, and the axial O_4 is in agreement with our earlier proposal.¹ The essential part played by the carboxy group is clearly visible from the pH-dependence of the LIS of H_1 and H_2 in the α -anomer of D-galacturonic acid (Fig. 4). It is therefore

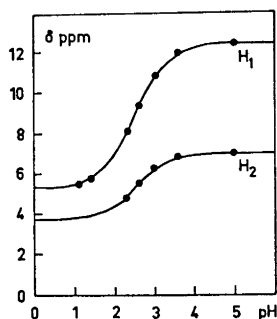


Fig. 4. The pH dependence of the Eu^{3+} induced shift (δ_{Eu}) of H_1 and H_2 in the α -anomer of sodium D-galacturonate (40.0 mg uronate and 21.7 mg europium nitrate in 0.3 ml D_2O).

very important to control the pH of the solution, and in the present experiments it was kept well above the critical value.

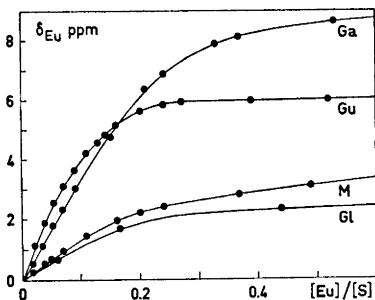


Fig. 5. The Eu^{3+} induced shift (δ_{Eu}) of H_1 in the α -anomers of sodium D-galacturonate (Ga), sodium L-gulonate (Gu), sodium D-mannuronate (M), and sodium D-glucuronate (GL) as a function of the equivalent ratio $[\text{Eu}]/[\text{S}]$. ($[\text{S}] \approx 0.4 \text{ M}$)

We have also studied the effect of Eu^{3+} ions on the NMR spectra of the sodium salts of D-galacturonic acid, D-glucuronic acid, D-mannuronic acid, and L-guluronic acid. The spectra of the anomeric mixtures are too complex for complete analysis at present. However, it is obvious that the H_1 proton of the α -anomer in all four cases is subject to the most dramatic downfield shift (Fig. 5). It is also clear that this proton is most strongly influenced in the spectra of D-galacturonic acid and L-guluronic acid. We conclude that the reason for this is the presence of the proposed binding site in these two acids. Moreover, α -L-guluronic acid also possesses the ax-eq-ax sequence of three hydroxy groups which offers⁷ an additional binding site.

A full analysis of the LIS curves (Fig. 2) in terms of the stoichiometry of the different possible complexes and the corresponding total shifts and equilibrium constants has not yet been carried out. This analysis and further studies in the field are in progress.

Acknowledgements. We are grateful to lic. techn. Johan Hjortås for X-ray diffraction data for methyl α -D-galacturonic acid methyl ester, to siv.ing. Hans Grasdalen for valuable discussions and for a grant from *Norges Tekniske Hogskoles fond* to T.A.

1. Anthonsen, T., Larsen, B. and Smidsrød, O. *Acta Chem. Scand.* **26** (1972) 2988.
2. Rees, D. A. and Wight, A. W. *J. Chem. Soc.* **1971** 1366.
3. Thanomkul, S. and Hjortås, J. *Private communication*.
4. Bleaney, B. J. *Magn. Resonance* **8** (1972) 91.
5. Angyal, S. J. *Carbohydr. Res.* **26** (1973) 271.
6. Bleaney, B., Dobson, C. M., Levine, B. A., Martin, R. B., Williams, R. J. P. and Xavier, A. V. *Chem. Commun.* **1972** 791.
7. Angyal, S. J. *Aust. J. Chem.* **25** (1972) 1957.

Received July 14, 1973.