

NMR-Studies of the Interaction of Metal Ions with Poly(1,4-hexuronates). I. Chelation of Europium Ions by D-Galacturonic Acid

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, ^bNorwegian Institute of Seaweed Research, N-7034 Trondheim-NTH, Norway

Calcium ions play an essential role in maintaining the supramolecular structure of many biologically-important polyuronate gels, but it is still not known how they are bound to the polymer chains. The idea that they can be bound by chelation has been disputed^{1,2} because of the strong dependence of the binding upon the electrostatic charge-density of the chains. However, accumulated evidence for high selectivities in the binding of pairs of metal ions of the same charge, taken from both the divalent³⁻⁵ and the trivalent⁶ series (among the lanthanides, europium ions are particularly strongly bound), has indicated that the ions must be bound to more than one point in the chain. From this and other evidence a binding site in polyguluronate consisting of a carboxy group and four closely-situated oxygen atoms in a dimer unit has recently been proposed.⁷

The effect of adding europium nitrate to 10% (w/v) solutions in deuterium oxide of D-galacturonic acid and sodium D-galacturonate, and of the corresponding substances in the D-glucose series, has now been studied by NMR spectroscopy. The chemical shifts of H-1 in the α -anomer, which are most drastically affected, as a function of the amount of added europium nitrate, are given in Figs. 1 and 2. Since galactose and glucose are C-4 epimeric, the marked differences between the two series indicate that O-4 is involved in the binding of europium to galacturonic acid. Moreover, the pronounced effect in going from the neutral sugar to the uronic acid and the sodium uronate indicates that the carboxy group must also take part in the binding. From an examination of molecular models, it is therefore suggested that the equatorial carboxy group, the axial hydroxy group

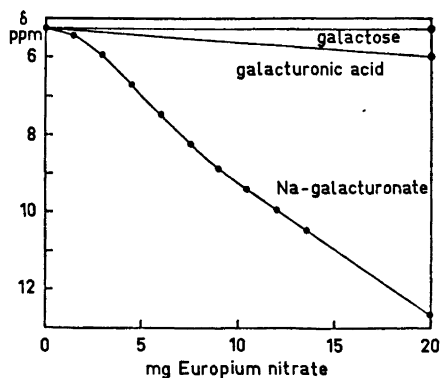


Fig. 1. The chemical shifts for the H-1 protons in the α -anomers in the D-galactose series, occurring upon addition of europium nitrate to 0.3 ml of 10% solutions of the sugars in D₂O.

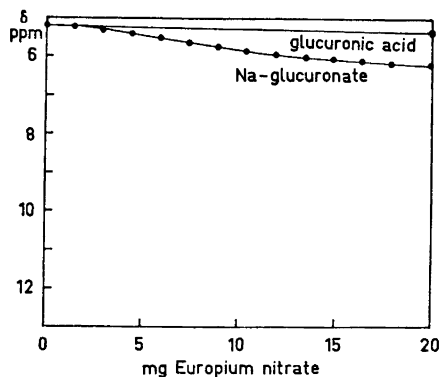


Fig. 2. As in Fig. 1, but for the glucose series.

on C-4, and the ring oxygen constitute the binding site in galacturonic acid (Fig. 3a). This triangular arrangement of the oxygens involved seems to explain in a satisfactory way the particularly large downfield shift of the H-1 proton.

From NMR-studies, Angyal and Davies¹⁰ have recently proposed that neutral sugars containing an *ax-eq-ax*-sequence of vicinal hydroxy groups on six-membered rings form complexes with calcium and other mono- and divalent metal ions of similar size. We have studied the influence of the trivalent europium on the NMR-spectrum of methyl glycosides containing the *ax-eq*-

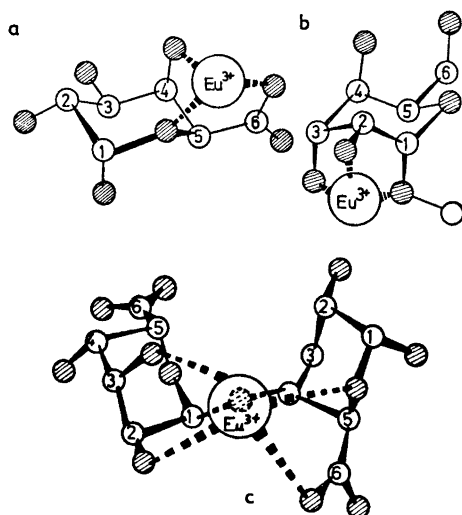


Fig. 3. Proposed binding sites for europium ions. Only carbon and oxygen atoms are drawn and the europium ions are not drawn to scale. (a) α -D-Galacturonic acid in the 4C_1 -conformation.⁸ Eu^{3+} above the plane of the paper. (b) α -D-Gulose in the 4C_1 -conformation.⁸ Eu^{3+} below the plane. (c) Dimer unit in poly-(α -1,4-L-guluronic acid), (1C_4 - 1C_4): Eu^{3+} above the plane.

ax-sequence and have observed a dramatic change in the spectrum of methyl- α -D-guloside, indicating a strong binding of europium ions (unpublished results). This is not surprising, since the crystallographic radii of Ca^{2+} (0.99 Å)¹¹ and Eu^{3+} (0.95 Å)¹² are very similar. In agreement with Angyal and Davies, there was little or no effect on methyl glycosides that did not contain the *ax-eq-ax* arrangement (e.g. methyl α -D-mannoside). As indicated in Fig. 3b, the binding site now proposed for D-galacturonic acid is very similar in size and shape to that of the guloside.

The binding site proposed earlier⁷ for Ca^{2+} in polyguluronic acid is composed of two very similar binding sites, situated near to each other, and forming a cavity between the sugar rings (Fig 3c). The present results, therefore, support the earlier hypothesis. The binding site in Fig. 3a also occurs in pectic acid and thus offers a possibility for partly explaining its very selective binding of calcium ions.^{3,13}

Further studies are in progress.

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