Carbohydrate Research, 174 (1988) 121–131
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

THE STRUCTURE OF THE COMPLEX FORMED FROM D-GALACTURONATE IONS AND CATIONS IN SOLUTION*

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(Received June 13th, 1987; accepted for publication, July 21st, 1987)

ABSTRACT

Complex formation between galacturonate and glucuronate anions and lanthanide cations has been investigated by the method of lanthanide-induced n.m.r. shifts. Several model compounds have also been studied. It is concluded that the cations are coordinated to the α anomers of these uronate anions at O-5 and one of the carboxylate oxygen atoms; in the β anomers, O-5 is not involved in complex formation.

INTRODUCTION

The calcium complexes of acidic polysaccharides are of great biological and industrial importance. In particular, the physical properties of alginic acid in solution change dramatically on addition of calcium ions. In an attempt to explain the reason for these changes, Smidsrød *et al.*[1] and, independently, Angyal[2] suggested the formation of a complex in which O-4, O-5, and O-6 of an α-L-guluronic acid residue and O-2 and O-3 of a contiguous L-guluronic acid residue in the polymer chain are coordinated to a calcium ion (O-4 of the first residue is O-1 of the second one). The existence of such a pentadentate structure has never been proven, and it is not certain that any glycuronic acid forms a complex at O-4, O-5, and O-6. A recent crystal structure determination[3] shows that, in the sodium salt of α-L-guluronic acid, one cation is close to O-1, O-2, and O-3 while another one is close to O-4, O-5, and O-6; however, the Na–O-2 and Na–O-5 distances are larger (2.85 and 2.72 Å) than the others and it is not certain that they represent coordinate bonds.

The same geometrical arrangement of O-4, O-5, and O-6 occurs in D-galacturonic acid, the monomer of pectic acid, and it has been suggested[4] that here, also, tridentate complexing occurs with these three oxygen atoms. In the crystals of the calcium–sodium and strontium–sodium salts of D-galacturonic acid, there is no coordination between O-4 and the cations[4,5], but it was proposed that, in solution, such tridentate complexing would occur, owing to its entropic advantage. This

*Dedicated to Professor Hans Paulsen.

0008-6215/88/$ 03.50

proposal was made mainly to explain why D-galacturonate ions form stronger complexes with cations than do D-glucuronate ions[6,7]. Anthonsen *et al.*[8] also suggested that, in solutions of galacturonates, O-4 is involved in coordination, in order to explain that the downfield shift of the signal of H-1 on addition of europium salts is much larger in the n.m.r. spectrum of α-D-galacturonate than in the spectrum of α-D-glucuronate. Kohn and Hirsch[9] suggested tridentate complexing for the Cu^{2+} salt of D-galacturonic acid because methyl α-D-galactopyranosiduronate forms a stronger complex then does methyl 4-deoxy-α-D-*xylo*-hexopyranosiduronate. On the other hand, Aruga[10] concluded, from a calorimetric study, that Cu^{2+} forms a bidentate complex with D-galaturonate and also with D-glucuronate.

From a detailed analysis of the lanthanide-induced shifts in the spectra of methyl α-D-galactopyranosiduronate, Anthonsen *et al.*[11,12] concluded that the cation is coordinated to O-4, O-5, and O-6. However, the distance calculated for Ln^{3+}–O-4 is so large (3.0 Å) that it probably does not indicate coordination.

More recently, Izumi[13] carried out a detailed analysis of the lanthanide-induced shift and the spin–lattice relaxation times in the ^{13}C-n.m.r. spectra of sodium D-galacturonate and D-glucuronate, and from these data calculated the location of the lanthanide cation within the complexes. In the α anomers, the cation was found to be close to O-5 and to one O-6, but in the β anomers it is not close to O-5 and is coordinated only to one (or both) of the carboxylate oxygen atoms. It appears that in the α, and only in the α, anomers electron-transfer from O-1 assists complex formation at O-5. This view accords with the fact that the α anomers form stronger complexes with cations than do the β anomers[6,7]. The actual values calculated by Izumi for the Ln^{3+}–O distances are too large (*e.g.*, 3.11 Å for Ln–O-5 in the *α-galacto* isomer), but the ratios of these distances show that in each of these complexes the cation is too far from O-4 for coordination to occur.

At the time Izumi's work was carried out, we investigated the lanthanide-induced changes in the ^1H-n.m.r. spectrum of sodium D-galacturonate. In view of the uncertainty still surrounding this subject, the results are now reported. The lanthanide-induced changes were found to be complex and, to understand them better, several model compounds were first investigated.

Model compounds. — Complex formation between hydroxycarboxylic acids and cations has been extensively studied, particularly in the crystalline state[14]. The undissociated acids do not form complexes, only their anions. The calcium affinities of α-hydroxycarboxylates are much higher than those of the unsubstituted carboxylates[15,16]. α-Hydroxycarboxylate anions generally form bidentate complexes involving the hydroxyl group and one of the carboxylate oxygen atoms, the conformation being such that the α-oxygen atom lies nearly in the plane of the carboxylate group[4,17]. A β-hydroxyl group participates only to a minor extent in complex formation[16]. When both an α- and a β-hydroxyl group are present, tridentate complexing involving both may or may not occur. Thus, Taga *et al.*[18] deduced from the lanthanide-induced shifts (LIS) that tridentate complexing occurs on O-1, O-2, and O-3 with DL-glycerate, D-gluconate, and lactobionate anions; the shift data are,

however, insufficient to prove those structures without making some initial assumptions. Gd^{3+}-induced relaxation rates suggest only a minor participation of the β-hydroxyl group in the coordination of glycerate[16]. It is interesting to point out that, in the crystals of calcium lactobionate, such tridentate complexing was found[19] but not in the crystals of calcium DL-glycerate[20] or potassium D-gluconate[21]; it occurs in the crystals of calcium and strontium D-arabinonate[22], but not in those of several other similar salts[23–25]. Each of these compounds is acyclic and has a free hydroxyl group in the α-position; they are not strictly comparable with galacturonic acid.

There is little information available on the complexing behaviour of carboxylic acids which have an alkoxyl group as a substituent; hence, 2- and 3-ethoxypropionic acids and 4-ethoxybutyric acid were studied as model compounds by determining the LIS in their ^{1}H-n.m.r. spectra[26]. Our LIS studies were only qualitative. Quantitative evaluation of the shifts (that is, their use for determining the precise position of the cation in the complex) is not readily applicable to simple monosaccharide derivatives. Not only the three coordinates of the cation but also the direction of the principal magnetic axis have to be determined; this requires at least six independent LIS values[26]. The spectra of the uronic acids contain only five proton signals, and those of our model compounds even fewer. The method has been used to determine the structure of sugar–cation complexes, but arbitrary assumptions were made about the direction of the principal magnetic axis[18].

The stoicheiometry of the hydroxyacid complexes is not simple. When the acid is in excess, a 1:3 complex is usually found; with an excess of cations, 1:1 complexes are common and 2:1 complexes can also be formed. Taga *et al.*[18], in their study of glycerate and glucuronate complexes, found mainly 1:1 complexes with the free acids and 1:2 complexes with their anions. Anthonsen *et al.*[11] found 1:3 complex

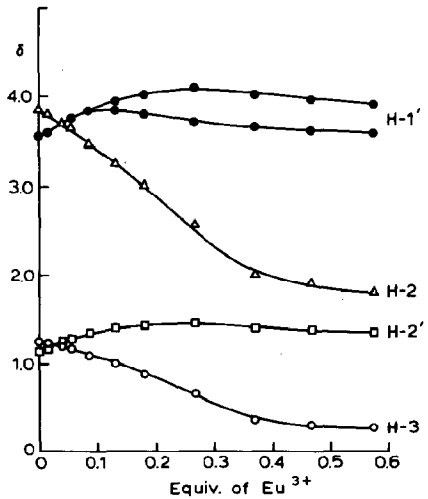


Fig. 1. LIS in the ^{1}H-n.m.r. spectrum of sodium 2-ethoxypropionate: H-2 (△), H-3 (○), H-1' (●), and H-2' (□), on addition of europium nitrate.

TABLE I

LIS (Hz)[a], WITH 0.03 EQUIV. OF CATION, IN THE ^{1}H-N.M.R. SPECTRUM OF SODIUM 2-ETHOXYPROPIONATE (~1.5M)

Cation	*H-2*	*H-3*	*H-1'*	*H-2'*
La^{3+}[b]	9	4	7	1.5
Pr^{3+}	15	8	−14[c]	−8[d]
Nd^{3+}	7.5	3	−5.5	−2
Eu^{3+}	−6	−2.5	7[e]	3.5[f]
Dy^{3+}	98	48	−204	−80
Ho^{3+}	−2	−5.5	−71	−33
Tm^{3+}	17[g]	23	88	47
Yb^{3+}	9[h]	10[i]	52	24

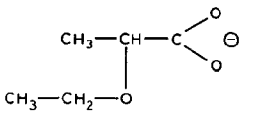
[a]Positive values indicate downfield shifts. [b]0.45 equiv. [c]Maximum LIS (−57 Hz) at 0.24 equiv. of Pr^{3+}, then becoming smaller. [d]Maximum LIS (−35 Hz) at 0.24 equiv. of Pr^{3+}, then becoming smaller. [e]Maximum LIS (27 Hz) at 0.22 equiv. of Eu^{3+}, then becoming smaller. [f]Maximum LIS (16 Hz) at 0.27 equiv. of Eu^{3+}, then becoming smaller. [g]Maximum LIS (19 Hz) at 0.04 equiv. of Tm^{3+}, then becoming smaller. [h]Maximum LIS (11 Hz) at 0.045 equiv. of Yb^{3+}; with more than 0.1 equiv., the LIS becomes negative. [i]Maximum LIS (27 Hz) at 0.17 equiv. of Yb^{3+}; with more than 0.5 equiv., the LIS becomes negative.

formation with methyl α-D-galactopyranosiduronate, but only 1:1 complexes were found by Jacques *et al.*[7] for calcium glucuronate and galacturonate in very dilute solution (10mM). Gould and Rankin[6] have also stated that they did not detect complexes of higher order than 1:1.

The lanthanide shift method involves the gradual addition of small amounts of lanthanide ions, and hence a low cation–substrate ratio; in most cases, the initial formation of 1:3 complexes can be assumed. It was, however, reported that methyl β-D-galactopyranosiduronate forms only 1:2 complexes[11].

Increasing amounts of lanthanide salts were added to solutions of sodium 2-ethoxypropionate (**1**) and the ^{1}H-n.m.r. spectra were recorded. Typical LIS obtained by the addition of europium nitrate are shown in Fig. 1. The two hydrogen atoms of the methylene group have chemical shifts very close to each other; hence, we list and show only their average value as H-1'. (However, on addition of lanthanide ions, these signals do separate and, after the addition of ~0.4 equiv. of Pr^{3+}, one of them is found at lower field than originally, though the initial LIS changes are upfield). The signals of H-2 and H-3 move upfield on addition of Eu^{3+}; those of H-1' and H-2' initially move downfield, then turn and move towards their original shift. The LIS caused by Nd^{3+} are similar but in the opposite direction; those caused by Pr^{3+} are also in the opposite direction but larger. The full curves are not reproduced here, but Table I shows the LIS after the addition of 0.03 equiv. of the cation; in most cases, the curves are close to straight lines up to this stage. Reversal in the direction of the LIS is indicated by footnotes.

LIS of positive and negative values within the spectrum of one compound are often caused by the occurrence of contact interactions[27]. However, the initial



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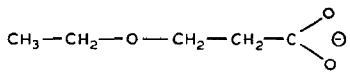

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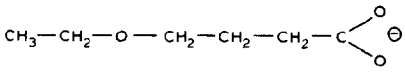

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proportion of the LIS for Pr^{3+}, Nd^{3+}, and Eu^{3+} is fairly constant. The ratio of shifts for these cations is close to that of the theoretical pseudoconstant shifts (−11.0, −4.2, and +4.0, respectively) calculated by Bleaney[28]. A diagram constructed according to the method of Reuben and Elgavish[29] produced lines passing close to the origin. These tests indicate the absence of substantial contact shifts. With the other lanthanides, the LIS of H-1′ and H-2′ are also roughly proportional to the Bleaney constants, but those of H-2 and H-3 are smaller with Dy^{3+} and are in the opposite direction with Ho^{3+}, Tm^{3+}, and Yb^{3+}, at least initially.

The conformation of ethoxypropionic acid may change on complex formation, but the effect of this change on the LIS is small, as shown by the relatively small LIS caused by much higher concentrations of La^{3+} ions (Table I). The effect of the conformational change was therefore neglected.

Fig. 1 shows that more than one complex is present; since their structures are not known, they are described as first and second complexes. Their structures must be similar because the LIS of H-2 and H-3 with Pr^{3+}, with Nd^{3+}, and with Eu^{3+} are the same for both, as are the LIS of H-1′ and H-2′ with Tm^{3+} and with Yb^{3+}; there is no noticeable break in any of these LIS curves. The large LIS of H-1′ and H-2′ indicate that O-2 is involved in complex formation; 2-ethoxypropionate, like other anions with an oxygen atom in the α-position, forms a bidentate complex with O-2 and one of the carboxylate oxygen atoms. The second complex is probably a 1:1 complex. When 1 and 2 equiv. of $LaCl_3$ were added to solutions of 2-ethoxypropionate containing 0.1 equiv. of a paramagnetic lanthanide ion, the LIS corresponded to the second complex; with an excess of cation, a 1:1 complex would be preponderant. The first complex could be a 2:1 or a 3:1 complex (or both). The mode of attachment of the cation would be the same in each case, but the direction of the magnetic axis could be affected by replacing one or two hydroxyacid anions by water molecules.

Since there are no contact shifts, the variation of the direction of the LIS must be due to geometrical factors. According to the theory of pseudocontact shift mechanism[30], the pseudocontact shifts are proportional to $(3\cos^2\theta - 1)/r^3$, where θ is the angle subtended by the principal magnetic axis and the vector connecting the cation to the observed nucleus, and r is the length of this vector. This expression becomes 0 when $\theta = 54.7°$ and negative when it is $>54.7°$.

TABLE II

LIS (Hz)[a], WITH 0.03 EQUIV. OF CATION, IN THE ^{1}H-N.M.R. SPECTRA OF SODIUM 3-ETHOXYPROPIONATE (**2**) AND 4-ETHOXYBUTYRATE (**3**) (~1M)

Compound	*Cation*	*H-2*	*H-3*	*H-4*	*H-1'*	*H-2'*
2	Pr^{3+}	34	17		4	0.4
	Eu^{3+}	−9	−6		−2	−0.2
	Yb^{3+}	−59	−26		−3	+3
3	Pr^{3+}	25	13.5	7	1.5	0.3
	Eu^{3+}	−10.5	−6	−4	−2	0
	Yb^{3+}	~−40	~−19	~−5	0	0

[a]Positive values indicate downfield shifts.

It is suggested that, in the lanthanide–ethoxypropionate complex, the principal magnetic axis is approximately parallel to the O-1–O-2 vector. In the first complex, H-2 and H-3 would then be located beyond the 54.7° angle, and H-1′ and H-2′ at a smaller angle. Change into the second complex would involve some dipping of the axis towards O-2. This change would only slightly affect H-2 and H-3 (θ between 70 and 90°), but changes in the angle of H-1′ and H-2′, which is close to 54.7°, could result in a large change of LIS and even of its sign. As the lanthanide ions become smaller, and their distance from the oxygen atoms decreases, the magnetic axis would move closer to H-1′ and H-2′ (the LIS of which then increase), but H-2 and H-3 may move closer to the 54.7° angle, and may ultimately appear on its other side. While the structures of the complexes remain the same, change of cation involves change of geometry because bond angles and length vary. Similar variations of LIS with the size of the lanthanide ion have been reported in other instances[31].

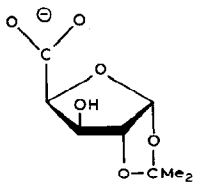
Addition of Gd^{3+} to solutions of 2-ethoxypropionate causes about equal broadening of the signals of H-2 and H-1′, indicating that these protons are equidistant from the cation. This fact supports the proposed structure. The signals of H-3 and H-2′ broaden only slightly.

Elgavish and Reuben[32] carried out similar measurements on methoxyacetic acid. Their LIS values are similar to those reported here, but the reported signs are opposite.

By contrast, the LIS of 3-ethoxypropionate (**2**) and 4-ethoxybutyrate (**3**) are, for each cation, in the same direction and decrease along the chain from the carboxylate group (Table II). There is no change in the direction of the LIS in any instance. Again, proportionality of the LIS, and construction of a Reuben diagram, indicate that contact shifts are insignificant. All signals move upfield on addition of Eu^{3+} and downfield on addition of Pr^{3+}. This finding shows that the ether oxygen atom is not involved in complex formation; the two carbonyl oxygen atoms are coordinated to the cation (as in carboxylate ions which have no other oxygen atoms) and the magnetic axis is parallel to the O–O vector.

The next model compounds investigated were the potassium salts of 1,2-*O*-isopropylidene-α-D-xylo- and -ribo-furanuronic acids (**4** and **5**), in order to observe whether the hydroxyl group on C-3 takes part in complex formation. In contrast to the pyranoses, HO-3 on the furanose ring, if *trans* to the carboxylate group, cannot approach it sufficiently to allow both of them to be coordinated to a cation. Moreover, the two fused five-membered rings make this system rather rigid, assuring the same conformation for the two epimers.

The behaviour of these compounds (Table III) on addition of lanthanide ions is similar to the bidentate behaviour of 2-ethoxypropionate: H-4 and H-3 correspond to H-2 and H-3 of the acyclic compound, and H-1 and H-2 to H-1′ and H-2′, respectively. Europium causes a downfield shift of H-1 and H-2 (and also of the methyl groups), and an upfield shift of H-4 and H-3. However, in all cases, the LIS of H-1 are greater than any others; in contrast to the ethoxy group in the aliphatic anion, which can rotate freely, C-1 and C-2 are constrained by the ring and, consequently, H-1 is held closer to the cation. The similarity of the LIS to those of the model compound, and the large LIS of H-1, indicate that the lanthanides are coordinated to the carboxyl group and to O-4, the ring oxygen atom. The cation must be above the ring because the carboxyl group is thus located.



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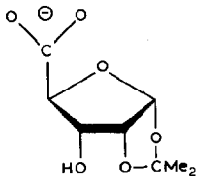

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Again, the spectra disclose the presence of more than one complex. The signals of H-1 and H-2 do not show any discontinuity, but those of H-4 and H-3 display irregular behaviour and even change from upfield to downfield or *vice versa* (see Table III).

TABLE III

LIS (Hz)[a], WITH 0.05 EQUIV. OF CATION, IN THE ^{1}H-N.M.R. SPECTRA OF POTASSIUM 1,2-*O*-ISOPROPYLIDENE-D-XYLOFURANURONATE (**4**) AND -RIBOFURANURONATE (**5**) (~0.3M)

Compound	*Cation*	*H-1*	*H-2*	*H-3*	*H-4*	*Me*
4	La^{3+}[b]	18	10	13	26	2,3
	Pr^{3+}	−157	−33	15	5	−20, −22
	Nd^{3+}	−79	−16	7	0	−11, −13
	Eu^{3+}	113	26	−7.5	2[c]	15,18
	Yb^{3+}	322	94	3[d]	29	38,44
5	La^{3+}[b]	5.5	3.5	12.5	13	1.5,2
	Pr^{3+}	−41	−7	14	15	−5, −5
	Eu^{3+}	30	6	−1	0[e]	5,7

[a]Positive values indicate downfield shifts. [b]3.0 equiv. [c]Maximum LIS (2.5 Hz) at 0.03 equiv. of Eu^{3+}, then moves upfield. [d]Maximum LIS (3 Hz) at 0.05 equiv. of Yb^{3+}, then moves upfield. [e]Moves upfield after the addition of 0.25 equiv. of Eu^{3+}.

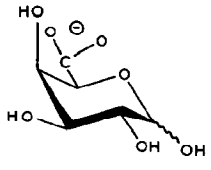
The question is then: is O-3 of the *xylo* isomer coordinated to the lanthanide cation? Because the LIS of H-3 are small and irregular, they do not provide an answer; apparently, both H-3 and H-4 lie close to the critical 54.7° angle. There are, however, two relevant differences in the behaviour of the two uronic acids. The *xylo* isomer forms a stronger complex, as shown by the LIS *versus* equiv. cation curves. With the *xylo* epimer, these flatten after the addition of about 0.5 equiv. of cation; with the *ribo* isomer, the change of LIS is still strong in this region. For example, with Pr^{3+} cations, the LIS of H-1 after the addition of 0.5 equiv. is 90% of that after the addition of 1.0 equiv.; for the *ribo* isomer, this figure is only 73%. Since the two epimers differ only in the configuration of C-3, this must be the reason for the stronger complexing ability of the *xylo* isomer. It is tempting to conclude, therefore, that O-3 is complexed to the cation, thereby increasing the stability of the complex.

The LIS of H-1 and H-2 are much greater for the *xylo* than for the *ribo* epimer. This finding again suggests participation of O-3 in complex formation because it would bring the cation closer to these hydrogen atoms.

Several facts, however, suggest that O-3 is not coordinated to the cation. The ratio (~5:1) of the LIS of H-1 and H-2 is the same for both isomers; coordination in the *xylo* isomer of O-3 would bring the cation into a position about equidistant from H-1 and H-2, and the ratio would be close to unity. One would also expect that coordination of O-3 should result in a very large LIS value for H-3; this is not the case.

There may be another explanation for the stronger complexing ability of the *xylo* isomer. The best conformation for complex formation, in which O-4 lies in the plane of the carboxylate anion[4,17,33], is favourable for the *xylo* isomer, whereas, for the *ribo* isomer, there is an interaction of O-3 and one of the carboxylate oxygen atoms. [As the coupling constants indicate, both isomers are predominantly in the $^3T_2$(D) conformation; the coupling constants do not change on complex formation.] In order to relieve this interaction in the *ribo* isomer, the carboxyl group will rotate and the cation will be further to the side, rather than "over the top", of the furanose ring, and further away from H-1 and H-2, thus inducing a smaller LIS. This arrangement being less stable, complexing will be weaker.

Galacturonic and glucuronic acids. — The ^{1}H-n.m.r. spectrum of sodium D-galacturonate (**6**) is well resolved[7,34] and the LIS of every signal could readily be observed. The signals in the ^{1}H-n.m.r. spectrum of sodium D-glucuronate (**7**) overlap extensively[7], even at 270 MHz; gradual addition of Pr^{3+}, however, separates them and the LIS of every signal can then be determined, thus demonstrating again that LIS can be useful for analysing n.m.r. spectra, even when high-field instruments are available. Addition of Eu^{3+}, which shifts the signals in the opposite direction, does not separate all of them, and the LIS of three proton signals could not be observed. The LIS of α-D-galactopyranuronate are similar to the values reported for sodium (methyl α-D-galactopyranosid)uronate[11].



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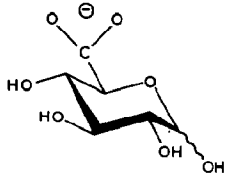

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TABLE IV

LIS (Hz)[a] WITH 0.1 EQUIV. OF CATION, IN THE ^{1}H-N.M.R. SPECTRA OF SODIUM D-GALACTURONATE (**6**) AND D-GLUCURONATE (**7**) (~0.65M)

Compound	*Cation*	*H-1α*	*H-2α*	*H-3α*	*H-4α*	*H-5α*	*H-1β*	*H-2β*	*H-3β*	*H-4β*	*H-5β*
6	Pr^{3+}	~ −720	−350	−95	68	39	−16	−26	8	55	50
	Eu^{3+}	421	191	65	−40	14	4	12	4	−34	−11
	Yb^{3+}[b]	297	153	61	−6	54	−1	2	−3	−18	−15
7	Pr^{3+}	−154	−53	−15	28	44	5	2	2	28	42
	Eu^{3+}	104	33	16		−19	−2			−21	−32

[a]Positive values indicate downfield shifts. [b]0.02 equiv.; with larger amounts of Yb^{3+}, the lines become very broad.

The behaviour observed for the α anomers of these uronic acids is similar to that of the model compounds (Table IV). The signals of H-1, H-2, and H-3 are shifted markedly, and those of H-4 and H-5 slightly and mostly in the opposite direction. These results show that the cation is coordinated to O-5. Potassium (methyl α-D-mannopyranosid)uronate behaves similarly: on addition of Eu^{3+}, the signals of H-1, H-2, and H-3, and of the methyl group, are shifted downfield, that of H-1 to the greatest extent, and those of H-4 and H-5 are shifted upfield.

On the other hand, the LIS for H-1, H-2, and H-3 of the β anomers are very small, particularly that of H-1; this confirms Izumi's conclusion[13] that O-5 is not coordinated to the cation in the β anomers*.

These data do not show decisively whether O-4 in α-D-galacturonic acid is coordinated to the cation. However, a large LIS value would be expected for H-4 if O-4 were linked to the cation; the LIS is, in fact, proportionately similar to the value found for α-D-glucuronate and for the β anomers. Moreover, the proportion of the LIS values for H-1, H-2, and H-3 is similar for the galacturonate and the glucuronate; this would be a most unlikely result if one complex were bidentate and the other tridentate. The similarity of the behaviour of α-D-galacturonate to that of the model compounds suggests that it also forms a bidentate complex. On the whole, our results appear to confirm Izumi's conclusion[13] that O-4 does not take part in complex formation.

*Cu^{2+} ions complex with hyaluronic acid, a polysaccharide containing alternate β-D-glucuronic acid residues, at one of the carboxylate oxygen atoms and O-4 (erroneously denoted as O-1 by the authors) of glucuronate[35]; the ring oxygen atom is not coordinated to the cation.

What then is the explanation for the stronger complexing of galacturonate compared with glucuronate? It should be noted that the formation constant of the complex with Cu^{2+} is about the same for galacturonate (K 16.5) and for 2-ethoxyacetate (16.3), but smaller for glucuronate (13.0)[10]. It is therefore not so much the axial hydroxyl group of galacturonate which increases the extent of complex formation but the equatorial one of glucuronate which decreases it. In the most favourable conformation for complex formation, in which O-5 is in the plane of the carboxylate group, there is a 1,3-parallel interaction of O-4 and the other carboxylate oxygen atom in the glucuronate; this would decrease the stability of the complex. However, the presence of the axial hydroxyl group on C-4 may in itself enhance complexation by forcing the carboxylate group into the conformation in which it is coplanar with O-5. This situation may explain the stronger complexing of methyl α-D-galactopyranosiduronate with Cu^{2+} compared to its 4-deoxy derivative[9]; Cu^{2+}, being much smaller than Ca^{2+} or the lanthanide ions, is unlikely to form a tridentate complex. The stronger complexing ability of galacturonate, compared to that of glucuronate, and its greater LIS values, can therefore be explained without invoking complex formation at O-4.

A completely different mechanism of complex formation has been proposed by Cook *et al.*[36] for complex formation between Cu^{2+} and D-glucuronic acid, based on the ability of Cu^{2+} to catalyse mutarotation. This mechanism is not applicable to calcium or lanthanide ions which have no such ability.

EXPERIMENTAL

The ^{1}H-n.m.r. spectra were recorded with a JEOL-4H-100S spectrometer operating at 25°, for solutions in deuterium oxide. Chemical shifts were measured from the methyl signal of sodium 4,4-dimethyl-4-silapentane-1-sulphonate as internal standard. It was ascertained, by comparison with the signal (δ 3.19) of tetramethylammonium iodide, that lanthanide ions have no substantial effect on the chemical shift of this standard.

N.m.r. data: sodium 2-ethoxypropionate: δ 3.83 (q, $J_{2,3}$ 7.0 Hz, H-2), 3.54 (2 q, $J_{1',2'}$ 7.1 Hz, H-1'), 1.26 (d, H-3), 1.18 (t, H-2'). Sodium 3-ethoxypropionate: δ 3.70 (t, $J_{2,3}$ 6.7 Hz, H-3), 3.58 (q, $J_{1',2'}$ 7.2 Hz, H-1'), 2.42 (t, H-2), 1.15 (t, H-2'). Sodium 4-ethoxybutyrate: δ 3.48 (t, $J_{3,4}$ 6.1 Hz, H-4), 3.46 (q, $J_{1',2'}$ 7.0 Hz, H-1'), 2.43 (quint, $J_{2,3}$ 7.4 Hz, H-3), 1.88 (t, H-2), 1.17 (t, H-2'). Potassium 1,2-*O*-isopropylidene-α-D-xyluronate: δ 6.07 (d, $J_{1,2}$ 3.7 Hz, H-1), 4.68 (d, $J_{2,3}$ <0.5 Hz, H-2), 4.63 (d, $J_{3,4}$ 3.0 Hz, H-4), 4.40 (d, H-3), 1.50, 1.15 (s, 2 Me). Potassium 1,2-*O*-isopropylidene-α-D-riburonate: δ 5.92 (d, $J_{1,2}$ 3.7 Hz, H-1), 4.74 (dd, $J_{2,3}$ 4.4 Hz, H-2), 4.20 (d, $J_{3,4}$ 9.2 Hz, H-4), 4.04 (dd, H-3), 1.57, 1.38 (s, 2 Me).

The spectrum of sodium D-galacturonate has been fully described[7]. The spectrum of sodium D-glucuronate has only been tentatively assigned[7], and not all of the assignments proved to be correct; LIS by Pr^{3+} allows unequivocal assignments: δ 5.24 (H-1α), 4.64 (H-1β), 4.08 (H-5α), 3.73 (H-3α + H-5β), 3.57 (H-2α), 3.50

(H-4α + H-3β + H-4β), 3.28 (H-2β). Potassium (methyl α-D-manno-pyranosid)uronate: δ 4.80 (d, $J_{1,2}$ 1.8 Hz, H-1), 3.86 (dd, $J_{2,3}$ 3.2 Hz, H-2), 3.80 (m, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-3,4,5), 3.42 (s, Me); LIS (Eu^{3+} 0.185 equiv.) H-1 1.56, H-2 0.34, H-3 0.13, H-4 −0.40, H-5 −0.50, Me 0.40 p.p.m.

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