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CALCIUM BINDING TO D-GLUCURONATE RESIDUES· CRYSTAL STRUCTURE OF A HYDRATED CALCIUM BROMIDE SALT OF D-GLUCURONIC ACID

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ABSTRACT

Three-dimensional X-ray diffraction data were used to determine the crystal structure of α-D-glucuronate $CaBr \cdot 3H_2O$, a model system for investigating the factors involved in the binding of calcium ions to D-glucuronate residues of oligo- and poly-saccharides Crystals of the salt are monoclinic, space group $P2_1$, having $a = 6\,410\,(1)$, $b = 10\,784\,(2)$, $c = 8\,879\,(1)$ Å, $\beta = 92\,07\,(1)°$, and $Z = 2$ Intensity data for 1082 reflections were measured with an automated diffractometer A trial structure, obtained by the heavy-atom method, was refined by least squares to $R = 0\,025$ The absolute configuration was confirmed by anomalous-dispersion effects An outstanding feature of the crystal packing is the interaction of D-glucuronate anions with calcium ions The calcium ion is coordinated to three symmetry-related D-glucuronate anions and to two water molecules. The D-glucuronate anion binds calcium cations through three chelation sites· one that involves a carboxyl-oxygen atom combined with O-5; one that includes the second carboxyl-oxygen atom acting in concert with O-4, and one composed of the O-1–O-2 pair of hydroxyl groups

INTRODUCTION

Calcium complexes of polysaccharides that contain D-glucuronate residues have been implicated in such biological processes as calcium storage[1], calcification[2–4], and calcium-dependent cell–cell adhesion[5–8] Little is known about the factors involved in calcium–D-glucuronate interactions, but it is generally assumed that the interactions are nonspecific and are governed by simple coulombic binding of the calcium cation to the anionic carboxyl groups of the D-glucuronate moieties[9] Contrary to this view, there is evidence that calcium interactions with glycosaminoglycans and with glycuronans display considerable stereospecificity, suggesting that these poly-

saccharides probably bind calcium through chelation sites composed of several ligands that act in concert[10–15]. Studies of calcium–glycuronan complexes indicate that hydroxyl groups combine with carboxyl groups at the calcium-binding sites[13–14]. Recent n m r.[16–18] and crystallographic[19–23] studies have shown that many simple carbohydrates also chelate calcium ions through sets of hydroxyl groups and at sites that involve carboxyl groups acting in concert with hydroxyl groups and other uncharged oxygen atoms[16]. Therefore, it is possible that calcium interactions with D-glucuronate residues may be somewhat stereospecific and may involve ligands other than the carboxyl group

In this paper, we describe the interactions of calcium ions with the D-glucuronate anions in the crystal structure of a hydrated calcium bromide salt of D-glucuronic acid

EXPERIMENTAL

Independent crystallographic studies of the salt were completed at the University of Alabama in Birmingham[24] and at the University of Montreal[25] As results of these two studies are in excellent agreement, only the experimental procedure and the results from one of these investigations will be described in this paper

Clear, triangular crystals (plates) of the salt were grown by evaporating an aqueous solution that contained an approximately equimolar mixture of sodium D-glucuronate and calcium bromide Weissenberg and oscillation photographs showed the crystals to be monoclinic, space group $P2_1$, as indicated by the systematic absence of reflections $0k0$ with k odd A crystal fragment having approximate dimensions of $0\,07 \times 0\,32 \times 0\,37$ mm was mounted on a Picker FACS-1 diffractometer with its b axis slightly inclined to the ϕ axis of the diffractometer Cell parameters for use in collecting intensity data were calculated by a least-squares analysis of the observed angular settings for twelve medium-angle reflections (CuK$\bar{\alpha}$, $\lambda = 1\,5418$ Å) Intensity data were collected with the diffractometer by use of a scintillation counter, nickel-filtered copper radiation, and a $\theta - 2\theta$ scanning technique The scanning speed was $1°\ \text{min}^{-1}$, and the background was counted for 20 sec at each terminus of the scans Measurements were made for the 1082 symmetry-independent reflections having $2\theta < 128°$. Three strong, medium-angle reflections, which were monitored periodically, showed no significant variations in intensity during the period of data collection Immediately after data collection, accurate values for the cell parameters were determined by a least-squares analysis of 2θ values for twelve high-angle reflections (CuKα_1, $\lambda = 1.54051$ Å), these cell parameters were not significantly different from those obtained prior to the measurement of intensities Crystal data are listed in Table I

Those reflections having scan counts below background level were given negative intensities and were retained in all subsequent calculations All intensities were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus a correctional term $(0\,03S)^2$, S being the scan counts The intensities and

TABLE I

CRYSTAL DATA

Stoichiometry	$C_6H_9O_7CaBr\ 3H_2O$
Z	2
Space group	$P2_1$
a	6 410 (1) Å
b	10 784 (2)
c	8 879 (1)
β	92 07 (1)°
ρ (calculated)	1 988 g cm^{-3}
ρ (observed)[a]	1 98 g cm^{-3}
μ (CuK$\bar{\alpha}$)	90 0 cm^{-1}

[a]The density was measured by flotation in tetrabromoethane–benzene A μ value of 101 4 cm^{-1}, which was calculated ignoring the water molecules, was used for calculating absorption corrections

their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied by using the computer program ORABS[26] Data were then scaled by means of a Wilson[27] plot

Coordinates for the bromide ion were determined from a sharpened, three-dimensional Patterson map, and those for the calcium ion from a three-dimensional Fourier map phased on the bromide ion The remaining nonhydrogen atoms were located in a Fourier map that was calculated by using phase angles derived from the two ions The trial structure was refined by using a modified version of the full matrix, least-squares program ORFLS[28,29] The quantity minimized was $\Sigma w(Fo^2-Fc^2/k^2)^2$, where k is a scale factor and the weight, w, is equal to $1/\sigma^2(Fo^2)$ Scattering factors for the nonhydrogen atoms (Ca^{2+}, Br^{1-}, C, and O) were obtained from the *International Tables for X-Ray Crystallography*[30], and hydrogen-atom scattering factors were those given by Stewart, Davidson, and Simpson[31] All hydrogen atoms were located in difference Fourier maps that were calculated during the latter stages of refinement

Real and imaginary anomalous-dispersion corrections were applied to the scattering factors of the nonhydrogen atoms, and both enantiomeric structures (D-glucuronate and L-glucuronate) were refined Included in the refinement for both enantiomers were all nonhydrogen-atom positional and anisotropic temperature-parameters, as well as Zachariasen's[33] isotropic extinction parameter g (as formulated by Coppens and Hamilton[34]) For the correct enantiomer (D-glucuronate), all hydrogen-atom positional parameters and isotropic temperature factors were also included, except for those of one water molecule (W1) that displayed excessive thermal motion Because of the limited core-storage capacity of the computer, it was impracticable to refine all parameters simultaneously, consequently the nonhydrogen-atom parameters were refined together, and the hydrogen atom parameters were refined in the alternate cycles For the incorrect enantiomer (L-glucuronate), the hydrogen atoms were assigned the isotropic temperature-factors of the atoms to which they are bonded and were included in the calculation of structure factors but not in the

TABLE II

FINAL HEAVY-ATOM PARAMETERS AND THEIR STANDARD DEVIATIONS[a]

Atom	*X*	*Y*	*Z*	β_{11}	B_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br	81100 (7)	76257 (0)	72276 (5)	1615 (13)	491 (5)	1021 (7)	179 (6)	−203 (6)	−57 (5)
Ca	75969 (10)	19317 (9)	85393 (8)	714 (17)	247 (7)	538 (9)	27 (9)	13 (9)	1 (7)
C-2	3774 (6)	3602 (4)	6683 (4)	84 (9)	37 (4)	44 (5)	13 (5)	4 (5)	0 (3)
C-3	3929 (6)	4910 (4)	7308 (4)	93 (10)	32 (4)	47 (4)	−5 (4)	−3 (5)	9 (3)
C-4	1899 (6)	5346 (4)	7977 (5)	93 (10)	27 (4)	60 (5)	−6 (5)	−6 (6)	−1 (3)
C-5	1158 (6)	4343 (4)	9046 (4)	84 (9)	24 (3)	53 (5)	1 (5)	−7 (6)	0 (3)
O-5	753 (4)	3252 (3)	8179 (3)	73 (6)	30 (2)	82 (4)	1 (4)	8 (4)	−18 (3)
C-1	2626 (6)	2696 (5)	7682 (4)	75 (8)	29 (3)	81 (5)	0 (5)	12 (5)	−1 (4)
O-1	3929 (4)	2381 (3)	8942 (3)	99 (6)	47 (3)	98 (4)	11 (4)	27 (4)	35 (3)
O-2	5788 (4)	3077 (3)	6507 (3)	101 (7)	42 (3)	69 (3)	12 (4)	30 (4)	6 (3)
O-3	4445 (5)	5689 (3)	6069 (3)	142 (7)	45 (3)	65 (4)	−11 (4)	5 (4)	14 (3)
O-4	2321 (5)	6492 (3)	8730 (3)	149 (7)	22 (3)	74 (4)	0 (3)	14 (4)	−2 (3)
C-6	−768 (6)	4665 (4)	9925 (4)	93 (9)	28 (4)	53 (4)	−2 (5)	−6 (6)	6 (4)
O-6′	−2301 (4)	3958 (3)	9833 (3)	93 (6)	33 (3)	77 (4)	−8 (4)	20 (4)	−10 (3)
O-6	−628 (5)	5630 (3)	10726 (3)	116 (7)	34 (3)	79 (4)	−10 (4)	29 (5)	−21 (3)
OW1	5323 (5)	−13 (3)	8180 (4)	132 (7)	51 (3)	106 (4)	9 (4)	27 (5)	1 (3)
OW2	1995 (6)	9271 (5)	5747 (4)	207 (10)	102 (5)	121 (5)	−1 (6)	18 (6)	−30 (4)
OW3	8646 (5)	1110 (4)	6192 (4)	162 (8)	67 (4)	80 (4)	32 (5)	−16 (5)	−28 (3)

[a]Values for bromide and calcium ions were multiplied by 10^5 and all others by 10^4 Temperature factors are in the form

$$T = \exp(-\beta_{11}h^2-\beta_{22}h^2-\beta_{33}l^2-2\beta_{12}hk-2\beta_{13}hl-2\beta_{23}kl)$$

Final value of the isotropic extinction-parameter is $g = 0\,004\,(2)$ The y coordinate of the bromide ion was not refined

least-squares refinement; all least-squares parameters were included in one matrix During the last cycle of refinement for each of the two models, no parameter shifted more than one-fourth of its standard deviation The final R index $(\Sigma||Fo|-|Fc||/\Sigma|Fo|)$ based on all reflections was 0 025 for the D-glucuronate enantiomer and 0 034 for L-glucuronate The goodness-of-fit, $\{\Sigma w(Fo^2-Fc^2/k^2)/(m-s)\}^{1/2}$, where m is the number of reflections used and s is the number of parameters refined, is 1.65 and 2 53 for the D- and L-glucuronate models, respectively In a final, difference Fourier map that was phased with the D-glucuronate enantiomer, there were several peaks and troughs having magnitudes as high as 0 8/Å^3 that occurred in the vicinities of the bromide and calcium ions, no other fluctuations exceeded 0 2 e/Å^3 in magnitude

RESULTS

Heavy-atom and hydrogen-atom parameters, together with their estimated standard deviations, are listed in Tables II and III respectively Estimated errors in positional parameters are about 0 001 Å for bromide and calcium ions, 0 004 Å for carbon and oxygen atoms, and 0 05 Å for hydrogen atoms A table of observed and calculated structure factors will be furnished by the authors upon request

TABLE III

FINAL HYDROGEN-ATOM PARAMETERS AND THEIR ESTIMATED STANDARD DEVIATIONS[a]

Atom	*X*	*Y*	*Z*	*B* (Å^2)
H-1	228 (6)	193 (5)	705 (4)	1 6 (0 8)
H-2	300 (5)	367 (4)	582 (4)	0 6 (0 7)
H-3	506 (8)	491 (6)	787 (7)	4 1 (1 3)
H-4	82 (7)	546 (5)	713 (5)	2 4 (0 9)
H-5	222 (5)	422 (4)	978 (4)	0 2 (0 6)
HO-1	335 (7)	191 (6)	930 (5)	2 9 (1 0)
HO-2	641 (7)	346 (5)	593 (5)	1 8 (0 8)
HO-3	526 (9)	611 (6)	649 (6)	4 8 (1 4)
HO-4	136 (11)	689 (9)	863 (7)	6 8 (1 8)
HOW1	610 (10)	−23 (8)	800 (7)	5 1 (1 4)
HOW1′	428 (9)	−25 (6)	859 (6)	3 7 (1 2)
HOW3	781 (6)	111 (4)	556 (4)	1 5 (0 8)
HOW3′	950 (10)	44 (8)	617 (7)	6 3 (1 8)
HOW2	134	856	630	3 9
HOW2′	272	963	630	3 9

[a]Positional parameters were multiplied by 10^3 Parameters for atoms H(OW2) and H(OW2′) were not refined The temperature factors for these two hydrogen atoms were taken from the last isotropic least-squares refinement of the oxygen atom, OW2

Fig 1 shows the crystal-packing and hydrogen-bonding schemes All hydrogen atoms that are covalently bonded to oxygen atoms participate in hydrogen bonding Hydrogen-bond distances and angles are listed in Table IV The calcium ion is surrounded by oxygen atoms, and the bromide ion is hydrogen bonded to hydroxyl groups and to water molecules The bromide ions are not directly coordinated to the

calcium ions; the closest contact between the two is 4 64 Å, which is about 1 70 Å longer than the sum of their ionic radii

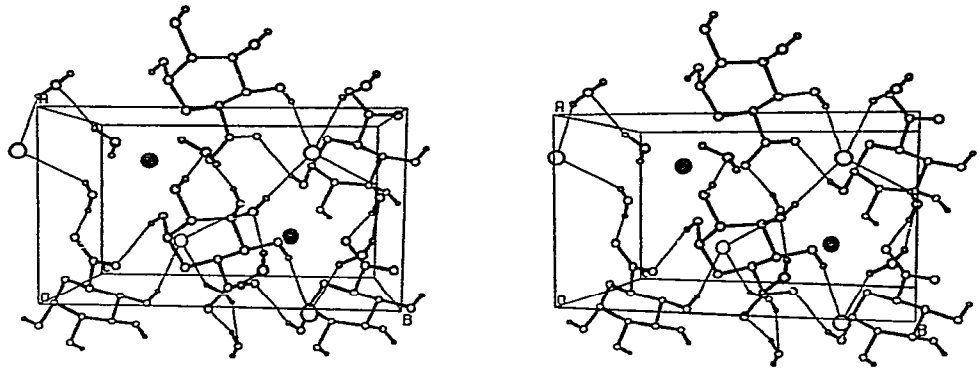


Fig 1. Stereo drawing of the crystal structure Heavy lines denote covalent bonds and thin lines denote hydrogen bonds (This drawing and those in Figs 2–4 were prepared with the program ORTEP[35])

TABLE IV

HYDROGEN-BOND DISTANCES AND ANGLES

Donor atom	*Hydrogen atom*	*Acceptor atom*	*Donor–acceptor distance* (Å)	*Hydrogen–acceptor distance* (Å)	*Donor–hydrogen acceptor angle* (°)
O-1	HO-1	O-6[a]	2 859	2 2	150
O-2	HO-2	OW2[b]	2 808	2 0	172
O-3	HO-3	Br[c]	3 281	2 5	167
O-4	HO-4	Br[d]	3 210	2 5	154
OW1	HOW1	Br[e]	3 241	2 7	146
OW1	HOW1′	O-6′[a]	2 888	2 1	162
OW2	HOW2	Br[d]	3 363	2 5	147
OW2	HOW2′	OW1[f]	3 079	2 3	159
OW3	HOW3	O-3[b]	2 806	2 1	168
OW3	HOW3′	OW2[g]	2 960	2 1	163

Symmetry codes [a] $-x, y-\frac{1}{2}, 2-z$ [b] $1-x, y-\frac{1}{2}, 1-z$ [c] x, y, z [d] $x-1, y, z$ [e] $x, y-1, z$ [f] $x, y+1, z$ [g] $x+1, y-1, z$

Fig 2 shows the environment of the calcium ion, which is coordinated to two water molecules and to three symmetry-related D-glucuronate ions One of the D-glucuronate ions chelates the calcium ion through the O-1–O-2 pair of hydroxyl groups, the second through hydroxyl-oxygen atom O-4 combined with carboxyl-oxygen atom O-6, and the third through carboxyl-oxygen atom O-6′ acting in concert with O-5, the ring-oxygen atom. The calcium ion is thus surrounded by a coordination polyhedron composed of eight oxygen atoms two from water molecules, three from

hydroxyl groups, two from the carboxyl group, and the ring-oxygen atom Fig 3 shows that these eight oxygen atoms form a slightly distorted, square-antiprism polyhedron around the calcium ion. The calcium–oxygen distances range from 2 38–2 57 Å This coordination-geometry is similar to that found in a number of other calcium–carbohydrate salts[19] and complexes[20,21]

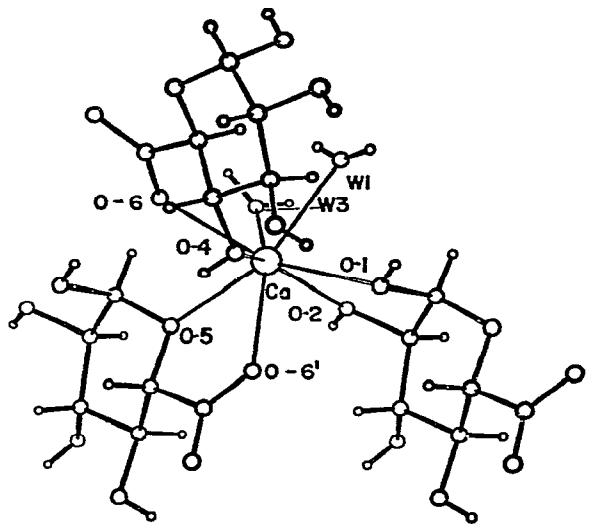


Fig 2 Environment of the calcium ion, which is coordinated to three D-glucuronate anions and to two water molecules W1 and W3 correspond to oxygen atoms of water molecules

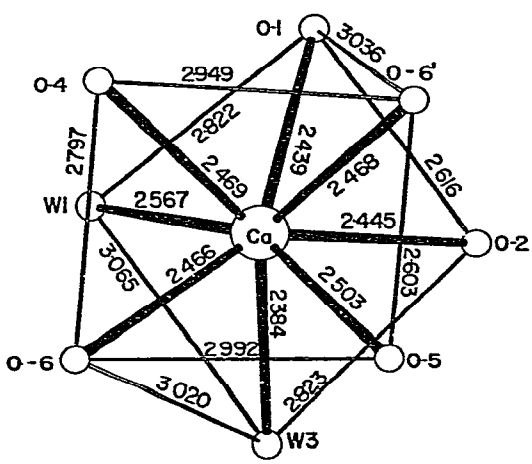
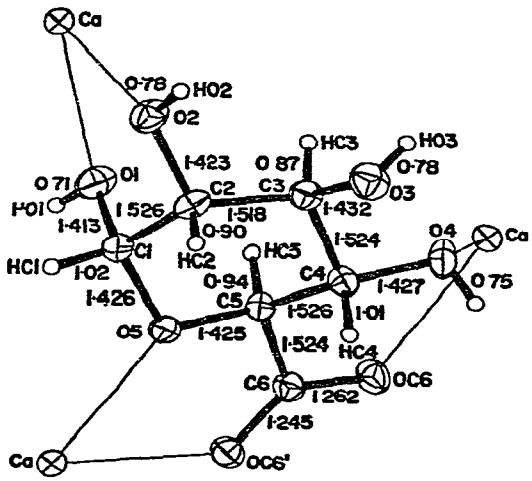
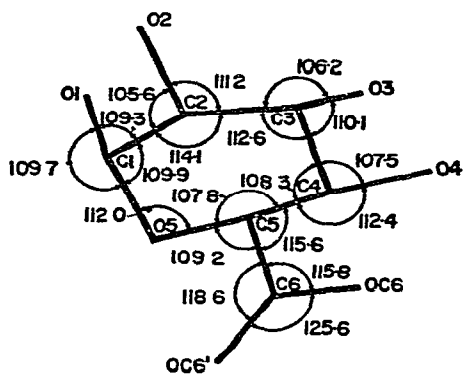


Fig 3 Geometry of the calcium-ion coordination shell All oxygen–oxygen contacts shorter than 3 10 Å are shown, together with the calcium–oxygen distance Estimated standard deviations are about 0 004 Å for Ca–O distances and 0 006 Å for O–O contacts W1 and W3 correspond to oxygen atoms of water molecules



(a)



(b)

Fig 4 In (*a*) nonhydrogen atoms are represented by thermal ellipsoids that are scaled to include 50% probability Hydrogen atoms are represented by spheres of 0 07 Å radius In (*b*) angles are given in degrees Estimated standard deviations in bond lengths are about 0 006 Å for those bond lengths involving only nonhydrogen atoms, 0 05 Å for those bond lengths involving hydrogen atoms, and 0 3° for bond angles

Fig 4 shows the D-glucuronate conformation, calcium-binding sites, thermal ellipsoids, bond lengths, and those bond angles that involve only nonhydrogen atoms Conformational torsion angles are listed in Table V

DISCUSSION

As depicted in Fig 4, the D-glucuronate anion provides three calcium-chelation sites, all of which involve uncharged oxygen atoms (hydroxyl groups and the ring-oxygen atom) Two of these sites also use oxygen atoms from the carboxyl group, but even at these sites, the interactions are not exclusively attributable to simple coulombic interactions between the calcium cation and the anionic oxygen atoms As the calcium

TABLE V

CONFORMATIONAL TORSION-ANGLES INVOLVING ONLY NONHYDROGEN ATOMS OF THE D-GLUCURONATE ANION[a]

Angle	*Magnitude (degrees)*	*Angle*	*Magnitude (degrees)*
O-5–C-1–C-2–C-3	45 8	O-6–C-6–C-5–C-4	−57 3
C-1–C-2–C-3–C-4	−42 4	O-6 –C-6–C-5–O-5	2 9
C-2–C-3–C-4–C-5	49 5	O-1–C-1–O-5–C-5	59 3
C-3–C-4–C-5–O-5	−62 4	O-1–C-1–C-2–C-3	−74 5
C-4–C-5–O-5–C-1	70 6	O-2–C-2–C-1–C-5	168 3
O-1–C-1–C-2–O-2	47 9	O-2–C-2–C-3–C-4	−161 7
O-2–C-2–C-3–O-3	77 9	O-3–C-3–C-2–C-1	−162 8
O-3–C-3–C-4–O-4	−70 7	O-3–C-3–C-4–C-5	167 7
O-4–C-4–C-5–C-6	56 6	O-4–C-4–C-5–O-5	179 1
O-6–C-6–C-5–O-5	−179 0	O-4–C-4–C-3–C-2	171 1
O-6′–C-6–C-5–C-4	124 6		

[a]Estimated standard deviations are about 0 4° The signs of the angles correspond to the notation of Klyne and Prelog[36]

ions are bound at these sites by uncharged oxygen atoms (O-4 and O-5) acting in concert with the carboxyl group, the resultant calcium complex must satisfy the overall geometry of the chelation sites, rather than merely assuming a conformation that permits suitable electrostatic contacts between the calcium cations and the carboxyl group The O-1 and O-4 atoms of D-glucuronate residues in oligo- and polysaccharides are generally involved in glycosidic linkages However, as acetal-oxygen atoms (such as atom O-5 of the D-glucuronate anion) are suitable ligands for binding calcium ions, it is reasonable to assume that all three of the chelation sites involved in the calcium bromide–D-glucuronate crystal structure may also participate in the binding of calcium ions to D-glucuronate residues of oligo- and polysaccharides

It is noteworthy that the glucuronate ion crystallizes as the α-anomer in this calcium salt, since the β-anomer predominates in aqueous solution[37] and is found in the crystal structures of the potassium and rubidium salts of D-glucuronic acid[38] It has been shown that calcium ions have different affinities for the α- and β-anomers of certain sugars in aqueous solution[16], and so it is possible that calcium interactions affect the favored anomeric form of the D-glucuronate anion It appears that calcium interactions also exert a large effect on the conformation of the D-glucuronate anion The carboxyl group is rotated about 30° from the position found for the D-glucuronate moieties in the crystal structures of potassium β-D-glucuronate[38] and an aldotriouronic acid[39], resulting in a conformation where O-5 is nearly in the plane of the carboxyl group This conformation appears to be stabilized by the calcium interactions, as it leads to simultaneous chelation of calcium ions by the O-4–O-6 and the O-5–O-6′ sites of the D-glucuronate anion Comparison of the α-D-glucuronate conformation with that in the crystal structure of an aldotriouronic acid indicates that the calcium interactions also affect the internal torsion-angles within the sugar ring

The corresponding torsion-angles differ by amounts ranging from 5 to 15°, with an overall root-mean-square difference of 11 8°. These conformational changes are similar to those found in other crystal structures of calcium–carbohydrate salts and complexes[19–23,40].

An interesting feature of the crystal structure is the role that calcium ions play in crosslinking D-glucuronate anions The calcium ion is chelated by three, symmetry-related D-glucuronate ions (Fig 2) and the calcium-coordination polyhedron is completed by water molecules Thus the crystal structure consists of hydrated calcium D-glucuronate bridges This same feature occurs in all calcium–carbohydrate crystal structures examined It has been consistently found that the calcium ions are coordinated to two or more carbohydrate residues, and usually to several water molecules, thus forming hydrated carbohydrate–calcium–carbohydrate bridges As in the calcium bromide–D-glucuronate structure, the bridges have only limited geometrical freedom, as they must satisfy the coordination geometry of the calcium ion These crystallographic findings suggest that calcium–carbohydrate interactions can provide an effective, stereospecific mechanism for linking carbohydrate chains together Such linkages may be of importance in biological adhesion and agglutination processes that are calcium-dependent and involve glycosaminoglycans or other saccharides composed of D-glucuronic acid[5–8].

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