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Calcium Binding to Carbohydrates: Crystal Structure of a Hydrated Calcium Bromide Salt of Lactobionic Acid

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The crystal structure of a hydrated calcium bromide salt of lactobionic acid [4-(β-D-galactosido)-D-gluconic acid] was determined by use of three-dimensional X-ray diffractometer data. Crystals of the salt are orthorhombic, space group $P2_12_12_1$, with $a=16{\cdot}662$ (3), $b=15{\cdot}075$ (1), and $c=8{\cdot}255$ (1) Å. There are four $C_{12}H_{21}O_{12}.CaBr.4H_2O$ formula units in the unit cell. The structure, solved by the heavy-atom method, was refined by least squares to $R=0{\cdot}058$, and the absolute configuration was confirmed by anomalous dispersion effects. The calcium ion binds to three water molecules and to the gluconate moieties of two lactobionate ions. One gluconate residue is coordinated to the calcium ion through a carboxyl oxygen atom and through O(2′) and O(3′), the two hydroxyl oxygen atoms located at the α and β positions adjacent to the carboxyl group. The second gluconate residue is coordinated through O(5′) and O(6′), the oxygen atoms of terminal hydroxyl groups. The eight oxygen atoms in the calcium coordination shell assume a distorted square-antiprism geometrical arrangement.

Introduction

Calcium ions bind to both anionic (Farber, Schubert & Schuster, 1957; Bowness, 1968; Herring, Andrews & Chipperfield, 1971; Smith & Lindenbaum, 1971) and uncharged (Rendleman, 1966) carbohydrates in aqueous solution, and calcium–carbohydrate interactions have been implicated in a variety of biological processes (Charley & Saltman, 1963; Manery, 1966; Williams, 1970; Pricer & Ashwell, 1971; Moore, 1971). We are currently investigating the crystal structures of calcium–carbohydrate salts and complexes to obtain information about the structural factors controlling calcium binding to carbohydrates. In this paper we describe the crystal structure of a hydrated calcium bromide salt of lactobionic acid [4-(β-D-galactosido)-D-gluconic acid] (Fig. 1).

Lactobionic acid, which is derived by oxidation of the glucose unit of lactose, contains a galactose moiety and a gluconic acid residue. Like the anions of other hydroxycarboxylic acids, the gluconate ion binds calcium in aqueous solution. Recent work in our laboratory has shown that galactose moieties chelate calcium ions in the solid state (Bugg & Cook, 1972). Therefore, two distinct sites of the lactobionate ion might be involved in calcium binding. We determined the crystal structure of this salt to examine the environment of the calcium ion.

Experimental

Clear, prismatic crystals of the salt were grown by evaporating an aqueous solution that contained an approximately equimolar mixture of calcium lactobionate and calcium bromide. Weissenberg and oscillation photographs showed that the crystals are orthorhombic; the space group is $P2_12_12_1$, as indicated by the systematic absence of reflections $h00$ with h odd, $0k0$ with k odd, and $00l$ with l odd. A crystal fragment with approximate dimensions 0·25, 0·25 and 0·10 mm was mounted on a Picker FACS-1 diffractometer with its b axis slightly inclined to the φ axis of the diffractometer. Intensity data were collected with the diffractometer, by use of a scintillation counter, nickel-filtered copper radiation, and a θ–2θ scanning technique. The scanning speed was 0·5°/min., and the background was counted for 40 sec at each terminus of the scans. Measurements were made for each of the 1981 independent reflections with $2\theta \leq 128°$. The 800, 040 and 004 reflections were chosen as standards and were monitored periodically. Considerable crystal decomposition occurred during the data collection process, as evidenced by the finding that the intensities of the standard reflections decreased by 25, 7 and 14%, respectively.

Unit-cell parameters were determined before and after we collected intensity data. The initial parameters were calculated by a least-squares analysis of 2θ values for 12 high-angle reflections (Cu $K\alpha_1$, $\lambda=1{\cdot}54051$ Å); the final cell parameters were obtained by a least-squares analysis of 2θ values for 15 high-angle reflections (Cu $K\alpha_1$). Both sets of unit-cell parameters, along with other crystal data, are listed in Table 1. The unit-cell parameters that we obtained after data collection were slightly, but significantly, larger than those measured initially. All subsequent calculations, including the calculations of bond lengths and angles, are based on the unit-cell parameters that were obtained after we had collected intensity data.

The intensity values were scaled by a least-squares procedure which is similar to that described by Ibers (1970). In this case, the intensities of the standard re-

Table 1. *Cell parameters*

Stoichiometry	$C_{12}H_{21}O_{12}.CaBr.4H_2O$	
Z	4	
Space group	$P2_12_12_1$	
ϱ (calculated)	1·75 g cm^{-3}	
ϱ (observed)*	1·74 (1) g cm^{-3}	
μ	57·5 cm^{-1}	
Cell dimensions	Before data collection	After data collection
a	16·657 (1) Å	16·662 (3) Å
b	15·066 (2)	15·075 (1)
c	8·232 (1)	8·255 (1)

* The density was measured by flotation in a benzene–carbon tetrachloride mixture.

flections were used to calculate scale factors as a linear function of crystal exposure time. The intensities were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus a correctional term $(0{\cdot}03S)^2$, S being the scan counts. The intensities and their variances were corrected for Lorentz and polarization factors, and absorption corrections were applied by using the computer program *ORABS* (Wehe, Busing & Levy, 1962). Finally, the data were scaled by means of a Wilson (1942) plot.

We arrived at a suitable trial structure by the heavy-atom method as follows: coordinates for the bromide ion were determined from a sharpened, three-dimensional Patterson map; coordinates for the calcium ion were determined from a sum-function superposition of sharpened Patterson maps translated to the bromide ion position; and the remaining non-hydrogen 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

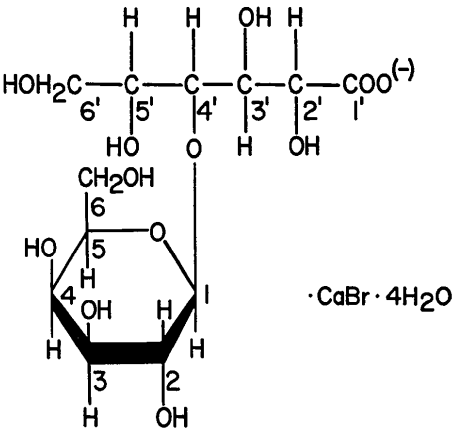


Fig. 1. Structural formula of the hydrated calcium bromide salt of lactobionic acid.

Table 2. *Final heavy-atom parameters and their standard deviations*

The values have been multiplied by 10^4. Temperature factors are in the form

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

The final value of the isotropic extinction parameter is $g=0{\cdot}001$ (4). $W1$, $W2$, $W3$ and $W4$ signify the oxygen atoms of the four water molecules.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Br	2336 (1)	1705 (1)	88 (2)	32 (1)	46 (1)	217 (2)	4 (1)	−15 (1)	−8 (1)
Ca	214 (1)	3838 (1)	259 (2)	24 (1)	10 (1)	47 (2)	0 (1)	−2 (1)	1 (1)
C(1)	1862 (5)	7012 (5)	−844 (9)	23 (3)	20 (3)	44 (10)	−2 (2)	−12 (4)	2 (5)
O(1)	1590 (3)	6557 (3)	−2220 (6)	17 (2)	14 (2)	60 (7)	−2 (2)	−6 (3)	−4 (3)
C(2)	2749 (4)	6803 (5)	−669 (9)	22 (3)	17 (3)	67 (11)	4 (3)	−9 (4)	−7 (5)
O(2)	2834 (4)	5895 (3)	−110 (7)	42 (2)	14 (2)	86 (9)	3 (2)	−19 (4)	8 (4)
C(3)	3180 (4)	7401 (5)	527 (9)	20 (3)	23 (3)	45 (10)	−2 (2)	−8 (4)	−5 (5)
O(3)	4018 (3)	7266 (3)	523 (6)	24 (2)	23 (2)	50 (7)	−1 (2)	−8 (3)	4 (4)
C(4)	2961 (5)	8371 (5)	296 (10)	23 (3)	23 (3)	73 (11)	−3 (3)	−8 (5)	−3 (6)
O(4)	3330 (4)	8728 (4)	−1079 (7)	24 (2)	23 (3)	113 (10)	−6 (2)	−2 (4)	10 (4)
C(5)	2026 (4)	8449 (5)	216 (10)	18 (3)	24 (3)	81 (11)	−2 (2)	−11 (5)	−17 (6)
O(5)	1753 (3)	7927 (3)	−1126 (6)	22 (2)	14 (2)	67 (8)	−1 (2)	−6 (3)	−4 (4)
C(6)	1740 (5)	9373 (5)	−62 (14)	29 (3)	17 (3)	173 (18)	0 (3)	−17 (7)	−12 (7)
O(6)	897 (4)	9431 (4)	−156 (8)	28 (2)	30 (3)	107 (10)	8 (2)	−7 (4)	−23 (5)
C(1′)	−68 (5)	6949 (5)	−6910 (8)	25 (3)	12 (3)	47 (10)	1 (2)	−6 (4)	−7 (5)
O(Cl′)	238 (5)	6361 (4)	−7773 (6)	62 (4)	21 (3)	39 (8)	2 (3)	14 (5)	−8 (4)
O′(Cl′)	−308 (4)	7695 (3)	−7373 (6)	36 (2)	13 (2)	50 (7)	2 (2)	−11 (4)	1 (3)
C(2′)	−149 (4)	6728 (4)	−5060 (8)	18 (2)	14 (3)	31 (9)	0 (2)	−2 (4)	1 (4)
O(2′)	−635 (3)	7356 (3)	−4271 (6)	18 (2)	12 (2)	55 (7)	−2 (2)	2 (3)	−9 (3)
C(3′)	678 (4)	6822 (5)	−4298 (8)	21 (3)	14 (3)	38 (10)	−1 (2)	−5 (4)	5 (4)
O(3′)	863 (3)	7739 (3)	−4491 (7)	18 (2)	9 (2)	102 (9)	−5 (2)	−11 (3)	22 (3)
C(4′)	735 (4)	6560 (4)	−2516 (8)	20 (3)	6 (2)	50 (10)	0 (2)	−4 (4)	−8 (4)
C(5′)	385 (4)	5644 (4)	−2112 (8)	22 (3)	8 (3)	23 (9)	−4 (2)	2 (4)	2 (4)
O(5′)	502 (3)	5422 (3)	−456 (6)	36 (2)	5 (2)	33 (7)	0 (2)	−2 (3)	−3 (3)
C(6′)	766 (5)	4908 (4)	−3071 (8)	24 (3)	7 (3)	44 (10)	−4 (2)	1 (4)	2 (4)
O(6′)	383 (3)	4084 (3)	−2624 (6)	27 (2)	9 (2)	43 (7)	−1 (2)	5 (3)	0 (3)
$W1$	1682 (4)	3868 (4)	250 (8)	28 (2)	29 (3)	140 (11)	−2 (2)	−4 (5)	8 (5)
$W2$	276 (5)	4603 (4)	2762 (6)	58 (4)	16 (2)	48 (8)	−4 (2)	9 (5)	−9 (4)
$W3$	−1092 (4)	4555 (4)	295 (13)	23 (2)	22 (3)	420 (24)	9 (2)	−6 (7)	−36 (7)
$W4$	2039 (4)	5535 (5)	2812 (9)	34 (3)	63 (4)	131 (12)	−2 (3)	−7 (5)	24 (6)

the full-matrix least-squares program *ORFLS* (Busing, Martin & Levy, 1962). The quantity minimized was $\sum w(F_o^2-F_c^2/k^2)^2$, where k is a scale factor and weight w is equal to $1/\sigma^2(F_o^2)$. Scattering factors for the non-hydrogen atoms were from *International Tables for X-ray Crystallography* (1962), and hydrogen atom scattering factors were from Stewart, Davidson & Simpson (1965). Coordinates for those hydrogen atoms bonded to carbon atoms were calculated by assuming tetrahedral coordination around the carbon atoms and C–H bond distances of 0·9 Å. All those hydrogen atoms bonded to oxygen atoms were located in difference Fourier maps that were calculated during the latter stages of refinement. The hydrogen atoms were assigned an isotropic temperature factor (3·0 Å^2) and were included in the calculation of structure factors but not in the least-squares refinement. The heavy-atom positional parameters and anisotropic temperature factors, as well as Zachariasen's (1963) isotropic extinction parameter g (as formulated by Coppens & Hamilton, 1970) were included in the refinement. Because of the limited core storage capacity of the computer it was impracticable to refine all parameters simultaneously; consequently, the parameters were refined in alternating cycles, with about half the parameters included in each cycle. As the refinement proceeded, coordinates of hydrogen atoms attached to oxygen atoms were improved by the use of difference Fourier maps.

The final R index ($\sum||F_o|-|F_c||/\sum|F_o|$) including all reflections is 0·058. The goodness-of-fit $\{[\sum w(F_o^2-F_c^2/k^2)^2/(m-s)]^{1/2}$, where m is the number of reflections used and s is the number of parameters refined$\}$ is 3·94. During the last cycle of refinement no parameter shifted more than one-fifth of its estimated standard deviation. A final three-dimensional difference Fourier map showed several peaks and troughs with magnitudes ranging from 0·9 to 1·1 e Å^{-3} in the immediate vicinities of the calcium and bromide ions. There were no other peaks or troughs in excess of 0·6 e Å^{-3} in this map. In a partial difference Fourier map, which was calculated with the hydrogen atoms omitted from the calculated structure factors, the electron densities at the hydrogen-atom positions had an average value of 0·80 e Å^{-3}.

During the refinement, both real and imaginary components of the anomalous dispersion correction factors were applied to the atomic scattering factors for bromide, calcium, and oxygen. The correction factors used were those from *International Tables for X-ray*

Table 3. *Hydrogen atom parameters*

The positional parameters, which were determined from difference Fourier maps, are multiplied by 10^3. All hydrogen atoms were assigned an isotropic temperature factor of 3·0 Å^2.

	x	y	z
H(C1)	160	685	7
H(C2)	299	688	−166
H(O2)	295	570	−110
H(C3)	303	724	154
H(O3)	420	710	− 30
H(C4)	315	870	115
H(O4)	305	890	−175
H(C5)	180	825	115
H(C6)	196	957	−102
H′(C6)	192	973	73
H(O6)	60	910	−113
H(C2′)	− 34	617	−491
H(O2′)	−108	720	−435
H(C3′)	103	650	−486
H(O3′)	140	780	−440
H(C4′)	49	697	−188
H(C5′)	− 15	564	−234
H(O5′)	50	585	10
H(C6′)	129	488	−284
H′(C6′)	70	500	−414
H(O6′)	65	370	−300
H(*W*1)	200	340	0
H′(*W*1)	180	443	− 15
H(*W*2)	− 20	440	333
H′(*W*2)	16	520	280
H(*W*3)	−130	510	12
H′(*W*3)	−150	420	0
H(*W*4)	160	540	265
H′(*W*4)	240	550	170

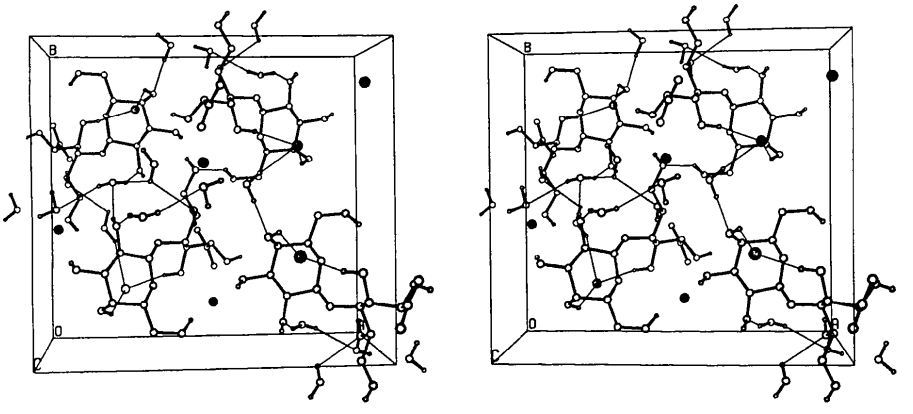


Fig. 2. Stereo drawing showing the crystal packing as viewed down the c axis. The bromide ions are shown as dotted circles, and the calcium ions are depicted by solid black circles. Only those hydrogen atoms that are bonded to oxygen atoms are shown. Heavy lines represent covalent bonds and the narrower lines represent hydrogen bonds. [This drawing, as well as those shown in Figs. 3–6, was prepared by using the program *ORTEP* (Johnson, 1965)].

Crystallography (1962). After refining the correct enantiomer (D-lactobionate), the coordinates were inverted and the incorrect enantiomer (L-lactobionate) was refined. The incorrect enantiomer refined to only $R = 0{\cdot}079$ and goodness-of-fit $= 4{\cdot}93$. By use of the R-value ratio test (Hamilton, 1964, 1965) a comparison of the refinements of the correct and incorrect enantiomers indicates that the D-lactobionate absolute configuration is correct, with a probable error of less than 0·5%.

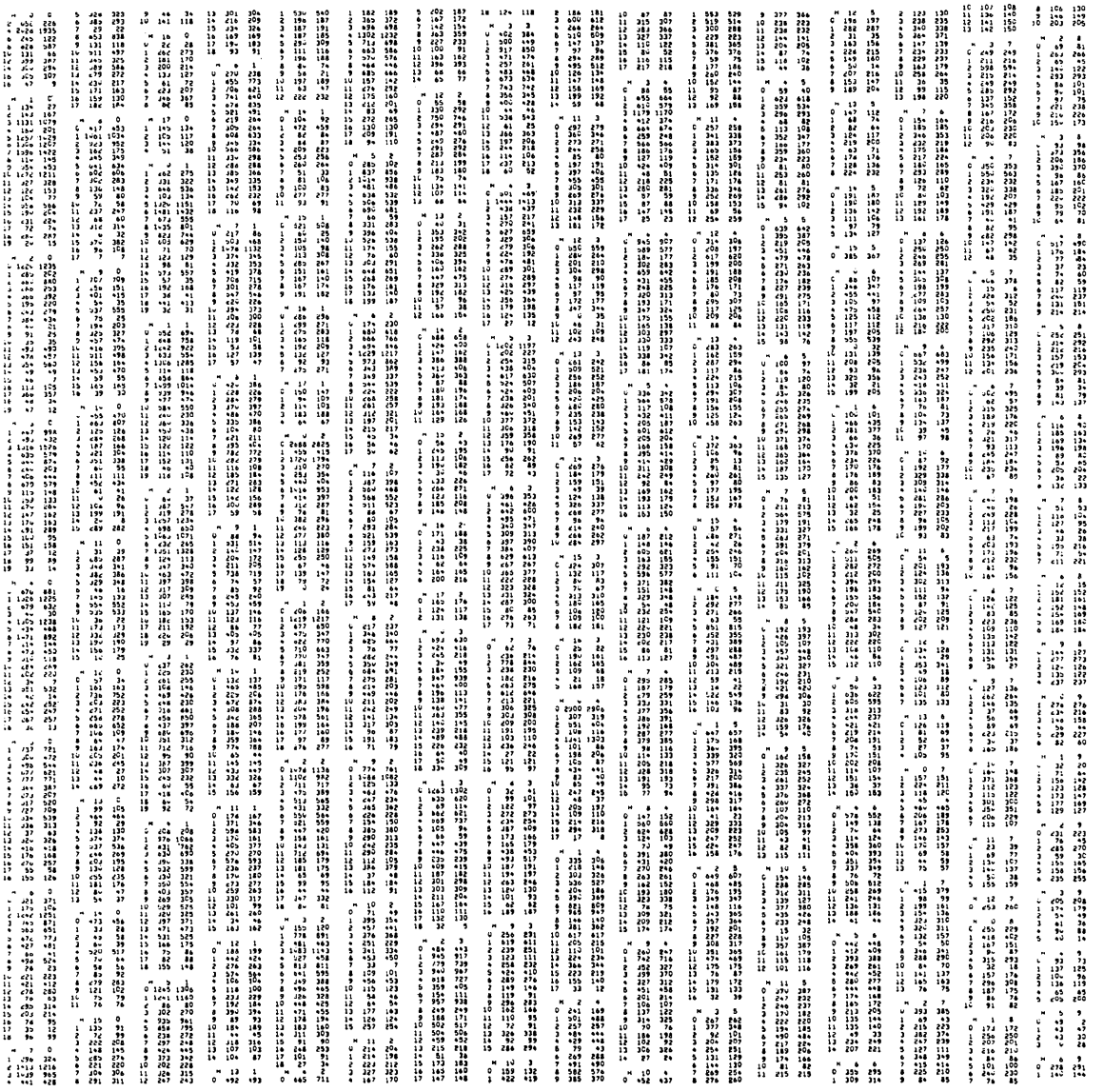
Results

Table 2 lists the final heavy-atom parameters and their estimated standard deviations. Table 3 gives the hydrogen atom parameters. The estimated errors in positional coordinates are about 0·001 Å for the bromide and calcium ions and 0·004–0·011 Å for carbon and oxygen atoms. Observed and calculated structure factors are listed in Table 4.

The crystal packing is shown in Fig. 2; distances and angles for possible hydrogen bonds are listed in Table 5. All the hydrogen atoms that are bonded to oxygen atoms are in contact with suitable hydrogen-bond acceptors, but it appears that several of these contacts are much longer than one would expect for normal hydrogen bonding. The calcium ion is surrounded by a shell of oxygen atoms, and the bromide ion is hydrogen-bonded to water molecules and to

Table 4. *Observed and calculated structure factors*

From left to right, the columns contain values of h, $10F_o$, $10|F_c|$.



hydroxyl groups. The closest bromide–calcium contact is 4·78 Å, a distance 1·75 Å longer than the sum of the bromide and calcium ionic radii.

Fig. 3 shows the environment of the calcium ion, which is coordinated to three water molecules and to the gluconate moieties of two lactobionate ions. One binds to the calcium ion through atoms O(5′) and O(6′), and the other binds through atoms O(2′), O(3′) and O′(Cl′). The calcium ion is thus surrounded by a shell composed of eight oxygen atoms: three from water molecules, four from hydroxyl groups, and one from a carboxyl group. The stereochemistry of the calcium-ion coordination shell is shown in more detail in Fig. 4. The eight oxygen atoms assume a distorted, square-antiprism arrangement, with calcium–oxygen distances ranging from 2·368 to 2·524 Å.

Fig. 5 shows the lactobionate conformation, atomic thermal ellipsoids, and those bond lengths that involve only non-hydrogen atoms. The O–H bond distances have an average value of 0·8 Å and range from 0·5 to 1·1 Å. Bond angles involving only non-hydrogen atoms are listed in Table 6 and conformational torsion angles are given in Table 7. The conformation of the galactose moiety is in agreement with that found in the crystal structure of α-lactose monohydrate (Fries, Rao & Sundaralingam, 1971; Beevers & Hansen, 1971), which was discussed in some detail by Fries *et al.* (1971); the root-mean-square difference between intra-ring tor-

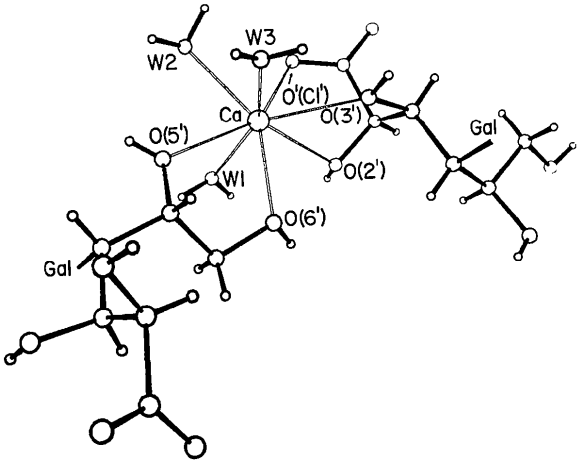


Fig. 3. Environment of the calcium ion. The galactose moieties are not shown.

Table 6. *Bond angles involving heavy atoms of the lactobionate anion*

The estimated standard deviations are about 0·5°.

C(1)–C(2)–C(3)	113·5°	C(1′)–C(2′)–C(3′)	107·8°
C(2)–C(3)–C(4)	112·1	C(2′)–C(3′)–C(4′)	115·6
C(3)–C(4)–C(5)	108·5	C(3′)–C(4′)–C(5′)	114·7
C(4)–C(5)–O(5)	107·9	C(4′)–C(5′)–O(5′)	111·6
C(5)–O(5)–C(1)	111·6		
O(5)–C(1)–C(2)	110·2		
		O(Cl′)–C(1′)–O′(Cl′)	126·5
O(1)–C(1)–O(5)	107·6	O(Cl′)–C(1′)–C(2′)	116·2
O(1)–C(1)–C(2)	106·8	O′(Cl′)-C(1′)–C(2′)	117·4
O(2)–C(2)–C(1)	108·7	O(2′)—C(2′)–C(1′)	111·0
O(2)–C(2)–C(3)	107·9	O(2′)—C(2′)–C(3′)	105·5
O(3)–C(3)–C(2)	112·4	O(3′)—C(3′)–C(2′)	103·9
O(3)–C(3)–C(4)	112·1	O(3′)—C(3′)–C(4′)	110·2
O(4)–C(4)–C(3)	111·5	O(1) —C(4′)–C(3′)	103·0
O(4)–C(4)–C(5)	112·0	O(1) —C(4′)–C(5′)	109·5
O(5)–C(5)–C(6)	107·0	O(5′)—C(5′)–C(6′)	106·0
C(4)–C(5)–C(6)	113·3	C(4′)—C(5′)–C(6′)	112·9
C(5)–C(6)–O(6)	112·6	C(5′)—C(6′)–O(6′)	108·3
C(1)–O(1)–C(4′)	117·0		

Table 5. *Distances and angles for possible hydrogen-bonded contacts*

Donor atom	Hydrogen atom	Acceptor atom		Distances (Å) Donor–acceptor	Hydrogen–acceptor	Donor–hydrogen–acceptor angle (°)
O(2)	H(O2)	*W*4	*b*	2·763	2·1	130
O(3)	H(O3)	O′(Cl′)	*e*	2·833	2·1	150
O(4)	H(O4)	Br	*b*	3·415	2·8	130
O(6)	H(O6)	O(6′)	*d*	2·862	1·9	140
O(2′)	H(O2′)	Br	*d*	3·073	2·3	170
O(3′)	H(O3′)	Br	*b*	3·135	2·3	160
O(5′)	H(O5′)	O(Cl′)	*c*	2·665	2·0	150
O(6′)	H(O6′)	O(3)	*b*	2·735	2·0	160
*W*1	H(*W*1)	Br	*a*	3·440	2·6	150
*W*1	H′(*W*1)	O(5′)	*a*	3·114	2·6	110
*W*1	H′(*W*1)	*W*4	*b*	3·065	2·6	110
*W*2	H(*W*2)	O(6)	*f*	2·792	1·9	150
*W*2	H′(*W*2)	O(Cl′)	*c*	2·689	1·8	160
*W*3	H(*W*3)	O(4)	*g*	2·836	2·0	150
*W*3	H′(*W*3)	Br	*h*	3·251	2·4	170
*W*4	H(*W*4)	*W*2	*a*	3·256	2·5	160
*W*4	H′(*W*4)	O(2)	*a*	2·805	1·8	160

Symmetry codes

a:	x	y	z	*e*:	$x+\frac{1}{2}$	$-y+\frac{3}{2}$	$-z-1$
b:	$-x+\frac{1}{2}$	$-y+1$	$z-\frac{1}{2}$	*f*:	$-x$	$y-\frac{1}{2}$	$-z+\frac{1}{2}$
c:	x	y	$z+1$	*g*:	$x-\frac{1}{2}$	$-y+\frac{3}{2}$	$-z$
d:	$-x$	$y+\frac{1}{2}$	$-z-\frac{1}{2}$	*h*:	$x-\frac{1}{2}$	$-y+\frac{1}{2}$	$-z$

sion angles of the galactose moiety in this structure and those in the structure of lactose monohydrate is 4·1°. The conformation of the gluconate moiety is considerably different from that in the crystal structures of potassium gluconate and rubidium gluconate (Littleton, 1953), where the gluconate ion is almost fully extended and its 6 carbon atoms lie approximately in a common plane.

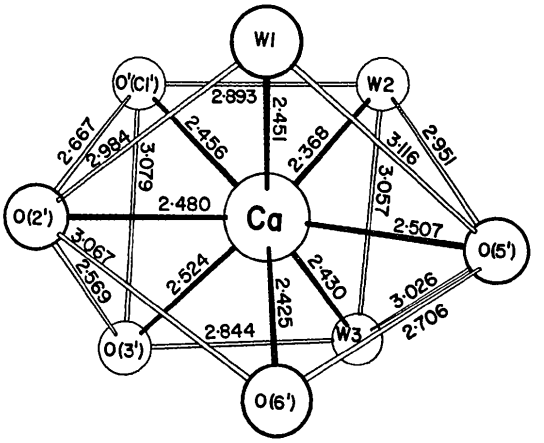


Fig. 4. Stereochemistry of the calcium ion coordination shell. The calcium–oxygen distances, as well as all oxygen–oxygen contacts shorter than 3·25 Å are shown. *W*1, *W*2, and *W*3 are oxygen atoms of water molecules.

Discussion

It is noteworthy that the lactobionate ions are coordinated to the calcium ions through their gluconate residues, with no direct galactose–calcium interactions. Since earlier work in this laboratory had shown that galactose binds calcium ions (Bugg & Cook, 1972), we had expected that the galactose moiety of the lactobionate ion might be participating in the calcium coordination shell. However, the observed calcium interactions with the gluconate moiety are consistent with findings from other studies which demonstrate that gluconate ion bindscalcium in aqueous solution (Cannan & Kibrick, 1938; Prescott, Shaw, Billello & Cragwall, 1953; Mehltretter, Alexander & Rist, 1953).

As shown in Figs. 3 and 4, the gluconate residue contributes one carboxyl oxygen atom and all four of its hydroxyl groups to the calcium ion coordination shell. Thus hydroxyl–calcium interactions are of major

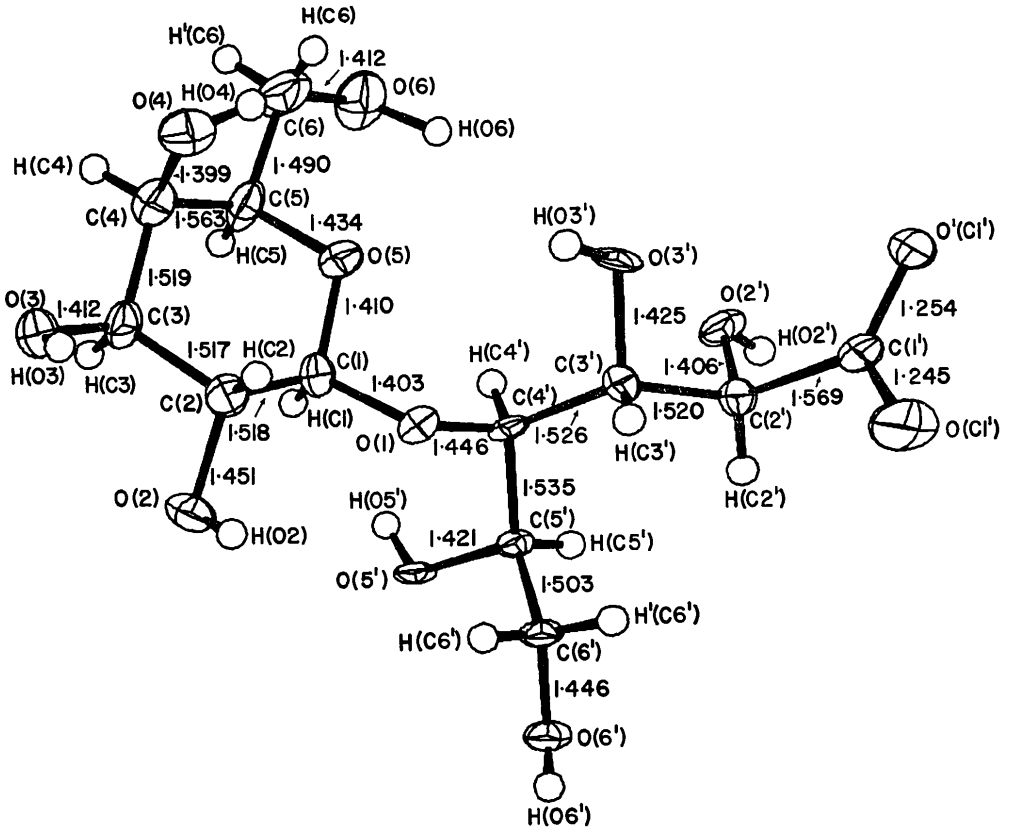


Fig. 5. Conformation of the lactobionate ion. The nonhydrogen atoms are represented by thermal ellipsoids defined by the principal axes of thermal vibration and scaled to include 60% probability. The hydrogen atoms are represented by spheres of 0·1 Å radius. Bond lengths involving nonhydrogen atoms are given: the estimated standard deviations in bond lengths are 0·008–0·01 Å.

importance in the chelation of calcium by the gluconate ion. It has been demonstrated that in aqueous solution, certain hydroxy–carboxylic acids display a much higher affinity for calcium ions than do simple carboxylic acids (Davies, 1938; Greenwald, 1938; Cannan & Kibrick, 1938). Calcium binding is particularly enhanced when a hydroxyl group is added to the alpha carbon atom of carboxylic acids. For example, the dissociation constant of calcium acetate in aqueous solution is approximately 40 times greater than that of calcium glycollate (Davies, 1938; Cannan & Kibrick, 1938). Additional hydroxyl groups further enhance the calcium binding capabilities of carboxylic acids, as evidenced by the finding that the dissociation constant

Table 7. *Conformational torsion angles involving heavy atoms of the lactobionate anion*

A positive torsion angle is defined for a view along the middle bond as a clockwise twist of the bond furthest from the viewer with respect to that which is nearest. The estimated standard deviations are about 0·7°.

Galactose moiety	
O(5)–C(1)–C(2)–C(3)	50·3°
O(1)–C(1)–C(2)–O(2)	− 73·0
C(1)–C(2)–C(3)–C(4)	− 45·4
O(2)–C(2)–C(3)–O(3)	66·6
C(2)–C(3)–C(4)–C(5)	49·2
O(3)–C(3)–C(4)–O(4)	53·0
C(3)–C(4)–C(5)–O(5)	− 59·9
O(4)–C(4)–C(5)–C(6)	− 54·7
C(4)–C(5)–O(5)–C(1)	68·6
C(5)–O(5)–C(1)–C(2)	− 63·1
C(4)–C(5)–C(6)–O(6)	179·1
O(5)–C(5)–C(6)–O(6)	60·3
Gluconate moiety	
O(Cl′) –C(1′)–C(2′)–C(3′)	76·3°
O(Cl′) –C(1′)–C(2′)–O(2′)	−168·6
O′(Cl′)–C(1′)–C(2′)–C(3′)	−103·1
O′(Cl′)–C(1′)–C(2′)–O(2′)	12·0
C(1′) —C(2′)–C(3′)–C(4′)	−175·3
O(2′) —C(2′)–C(3′)–O(3′)	− 54·7
C(2′) —C(3′)–C(4′)–C(5′)	50·8
O(3′) —C(3′)–C(4′)–O(1)	− 72·9
C(3′) —C(4′)–C(5′)–C(6′)	57·2
O(1)—C(4′)–C(5′)–O(5′)	61·2
C(4′) —C(5′)–C(6′)–O(6′)	−178·8
O(5′) —C(5′)–C(6′)–O(6′)	58·7
Bridge bonds	
O(5)–C(1)–O(1) –C(4′)	− 69·6°
C(2)–C(1)–O(1) –C(4′)	172·1
C(1)–O(1)–C(4′)–C(3′)	131·2
C(1)–O(1)–C(4′)–C(5′)	−106·2

Table 7 (*cont.*)

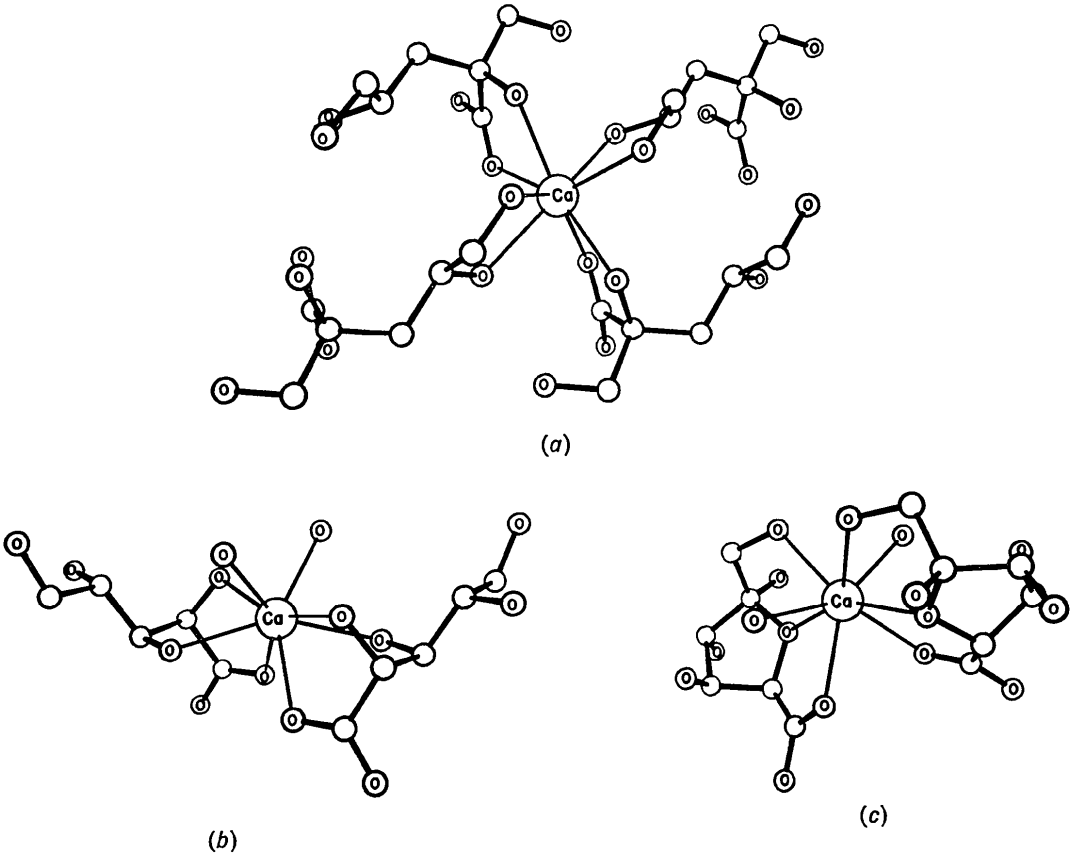


Fig. 6. Environments of the calcium ions in the crystal structures of (*a*) calcium β-D-glucoisosaccharate, (*b*) calcium arabonate pentahydrate, and (*c*) calcium 5-keto-D-gluconate dihydrate. Hydrogen atoms are not shown.

of calcium gluconate is even smaller than that of calcium glycollate (Cannan & Kibrick, 1938). It appears, therefore, that hydroxyl–calcium interactions also contribute to the calcium binding properties of the gluconate ion and the anions of other hydroxycarboxylic acids in aqueous systems.

Calcium–hydroxyl interactions are important in the crystal structures of calcium salts of other sugar acids. Fig. 6 shows the environments of the calcium ions in the crystal structures of calcium α-D-glucoisosaccharate (Norrestam, Werner & Von Glehn, 1968), of calcium arabonate pentahydrate (Furberg & Hellend, 1962) and of calcium 5-keto-D-gluconate dihydrate (Balchin & Carlisle, 1965). As in the lactobionate salt, the calcium ions are coordinated to hydroxyl groups as well as to the carboxyl groups. Calcium–hydroxyl interactions also occur in the crystal structures of the calcium salt of garcinia acid (Glusker, Minkin & Casciato, 1971), the calcium bromide complexes of lactose, galactose, myo-inositol (Bugg & Cook, 1972), and the calcium chloride complex of β-D-mannofuranose (Craig, Stephenson & Stevens, 1972).

The geometry of the calcium ion coordination shell is closely related to that found in the crystal structures of other calcium salts and complexes. Though capable of displaying coordination numbers ranging from six to nine, calcium ions bound exclusively to oxygen atoms generally display eightfold coordination and possess coordination shells of distorted square-antiprism geometry. In all of the calcium–carbohydrate salts and complexes examined to date, including the three salts depicted in Fig. 6, the calcium ions are coordinated to eight oxygen atoms, and the coordination shells can best be described as distorted square-antiprism polyhedra. The affinities of carbohydrates and hydroxycarboxylic acids for calcium ions are apparently related to the spatial arrangement of hydroxyl groups. The strongest binding results from sets of oxygen atoms that possess the proper spacing and geometry for substituting in the calcium coordination shell (Angyal & Davies, 1971). It is reasonable to expect that calcium binding to polysaccharide matrices of biological systems probably occurs preferentially at sites that possess sets of hydroxyl, carboxyl, and sulfate groups with the geometrical arrangement of oxygen atoms required for calcium coordination. Hence, it is possible that calcium–carbohydrate interactions may be sufficiently stereospecific to participate in the control of such biological processes as calcification and calcium-dependent cell–cell adhesion.

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