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Calcium Binding to Carbohydrates: Crystal Structure of Calcium Ascorbate Dihydrate

BY RICHARD A. HEARN AND CHARLES E. BUGG

Institute of Dental Research and Department of Biochemistry, University of Alabama in Birmingham, University of Alabama Medical Center, University Station, Birmingham, Alabama 35294, U.S.A.

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X-ray diffraction data were used to determine the crystal structure of calcium L-ascorbate dihydrate. Crystals of $Ca^{2+}(C_6H_7O_6^-)_2.2H_2O$ are monoclinic, space group $P2_1$, with $a=8{\cdot}842$ (3), $b=15{\cdot}777$ (9), $c=6{\cdot}364$ (3) Å, $\beta=115{\cdot}88$ (3)°, $Z=2$, ϱ(obs) = 1·76 and ϱ(calc) = 1·771 g cm^{-3}. Intensity data for 1321 reflections were collected with an automated diffractometer by use of nickel-filtered copper radiation. A trial structure was obtained by the heavy-atom method and refined by least-squares calculations to $R=0{\cdot}038$. The calcium ion is bound to two water molecules and three ascorbate anions. One ascorbate ion chelates the calcium ion through the two hydroxyl groups of its glycerol side chain; a second ascorbate anion chelates the calcium by using its pair of glycerol-hydroxyl groups in concert with the ionized oxygen atom; and a third one is coordinated to the calcium through the carbonyl oxygen atom of the lactone moiety. The eight oxygen atoms that are coordinated to the calcium ion form a slightly distorted square antiprism, with Ca–O distances ranging from 2·415 to 2·530 Å. The two ascorbate anions assume different conformations about the C–C bond of the glycerol side chain.

Introduction

We are currently examining the crystal structures of a series of hydrated calcium–carbohydrate complexes (Bugg & Cook, 1972; Bugg, 1973; Cook & Bugg, 1973*a*, *b*, 1974) and salts (Cook & Bugg, 1973*c*) in an effort to clarify the structural factors that control calcium interactions with carbohydrates in aqueous and biological systems. The calcium-binding properties of ascorbic acid (vitamin C) in aqueous solution have been examined in some detail (Forsberg, Johansson, Ulmgren & Wahlberg, 1973; Ulmgren & Wahlberg, 1973*a*, *b*). In this paper we describe calcium interactions with the ascorbate ions in the crystal structure of calcium ascorbate dihydrate. A preliminary account of this work has been presented (Hearn & Bugg, 1973).

Experimental

Clear prisms of calcium ascorbate dihydrate were grown by evaporating an aqueous solution that contained an approximately equimolar mixture of calcium L-ascorbate and calcium bromide. Weissenberg and oscillation photographs showed that the crystals are monoclinic; the space group is $P2_1$, as indicated by the systematic absence of reflections $0k0$ with k odd. A crystal fragment with approximate dimensions of 0·4, 0·3 and 0·2 mm was mounted on a Picker FACS-1 diffractometer with its c 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 1 ° min^{-1}, and the background was counted for 20 s at each terminus of the scans. Measurements were made for each of the 1321 independent reflections with $2\theta < 128°$. Three reflections (200, 00$\bar{2}$, 060) that were monitored periodically showed no significant intensity changes during the data-collection period. The intensity values were assigned variances, $\sigma^2(I)$, according to the statistics of the scan and background counts plus an additional 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). The intensities and their standard deviations were scaled by means of a Wilson (1942) plot.

Unit-cell parameters were determined before and after intensity data were collected. The initial parameters were calculated by a least-squares analysis of the

Table 1. *Crystal data*

Stoichiometry	$(C_6O_6H_7^-)_2Ca^{2+}(H_2O)_2$
Z	2
Space group	$P2_1$
a	8·842 (3) Å
b	15·777 (9)
c	6·364 (3)
β	115·88 (3)°
ϱ (calculated)	1·771 g cm^{-3}
ϱ (observed)	1·76 g cm^{-3}
μ	40·4 cm^{-1}

The unit-cell parameters were measured at 25 ± 3 °C. The reported standard deviations are three times those obtained from the least-squares analysis. The density was measured by flotation in a mixture of benzene and ethylene dibromide. The unit cell which corresponds to that of Hvoslef & Kjellevold (1974) and has cell parameters of $a=6{\cdot}364$ (3), $b=15{\cdot}777$ (9), $c=8{\cdot}340$ (3) Å, and $\beta=107{\cdot}48$ (3)°, can be obtained from the one above by the transformation matrix

$$\begin{pmatrix} 0 & 0 & \bar{1} \\ 0 & 1 & 0 \\ 1 & 0 & 1 \end{pmatrix}.$$

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

Values have been multiplied by 10^4. Temperature factors are in the form $T=\exp(-\beta_{11}h^2-\beta_{22}k^2-\beta_{33}l^2-2\beta_{12}hk-2\beta_{13}hl-2\beta_{23}kl)$. Final value of the isotropic extinction parameter is $g=0{\cdot}080$ (6).

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
Ca	2129 (1)	2500	2397 (1)	76 (1)	14 (1)	116 (2)	1 (1)	42 (1)	2 (1)
Ascorbate ion *A*									
C(1)	6464 (5)	4273 (3)	1700 (8)	69 (7)	17 (2)	149 (13)	3 (3)	50 (8)	3 (4)
C(2)	5080 (5)	4546 (3)	−347 (7)	73 (7)	16 (2)	113 (12)	−1 (3)	38 (8)	−9 (4)
C(3)	4913 (5)	5404 (3)	−269 (8)	61 (6)	16 (2)	110 (12)	−7 (3)	39 (8)	−7 (4)
C(4)	6179 (5)	5692 (3)	2121 (8)	50 (6)	14 (2)	141 (13)	−2 (2)	29 (8)	−3 (4)
C(5)	5375 (5)	5933 (3)	3720 (7)	63 (6)	15 (2)	116 (13)	−1 (3)	22 (7)	−5 (4)
C(6)	4362 (5)	6748 (3)	3055 (7)	72 (7)	19 (2)	129 (12)	2 (3)	27 (8)	−9 (4)
O(1)	7079 (4)	3558 (2)	2288 (5)	87 (6)	18 (2)	202 (11)	12 (2)	60 (6)	7 (3)
O(2)	4096 (4)	3964 (2)	−1987 (5)	125 (6)	11 (1)	110 (9)	−3 (2)	33 (6)	−8 (3)
O(3)	3880 (4)	5916 (2)	−1767 (5)	96 (5)	14 (1)	140 (10)	−3 (2)	21 (6)	1 (3)
O(4)	7189 (4)	4939 (2)	3167 (5)	69 (5)	16 (1)	154 (9)	2 (2)	17 (5)	−7 (3)
O(5)	6650 (4)	6055 (2)	6059 (5)	86 (5)	17 (1)	122 (9)	4 (2)	16 (6)	−2 (3)
O(6)	5497 (4)	7449 (3)	3678 (5)	114 (5)	17 (1)	170 (9)	−8 (2)	35 (6)	0 (4)
Ascorbate ion *B*									
C(1)	−53 (5)	5783 (3)	920 (7)	54 (6)	13 (2)	113 (13)	−5 (3)	31 (7)	1 (4)
C(2)	876 (5)	5290 (3)	2926 (7)	56 (6)	13 (2)	92 (11)	−4 (3)	24 (7)	−3 (4)
C(3)	1538 (5)	4600 (3)	2313 (7)	47 (6)	15 (2)	112 (11)	−2 (3)	23 (7)	2 (4)
C(4)	1052 (5)	4685 (3)	−280 (7)	58 (6)	13 (2)	129 (12)	4 (3)	44 (7)	5 (4)
C(5)	198 (5)	3935 (3)	−1901 (7)	68 (6)	16 (2)	100 (12)	7 (3)	36 (7)	6 (4)
C(6)	−1282 (6)	3559 (3)	−1647 (8)	67 (7)	21 (2)	137 (13)	−6 (3)	20 (8)	0 (5)
O(1)	−824 (4)	6459 (2)	683 (5)	97 (5)	16 (1)	128 (9)	11 (2)	46 (6)	0 (3)
O(2)	962 (4)	5512 (2)	5063 (5)	84 (5)	23 (2)	88 (8)	3 (2)	36 (5)	−5 (3)
O(3)	2520 (4)	4008 (2)	3572 (5)	96 (5)	12 (1)	94 (8)	10 (2)	20 (5)	2 (3)
O(4)	−61 (4)	5411 (2)	−1040 (5)	95 (5)	16 (1)	80 (8)	12 (2)	41 (5)	8 (3)
O(5)	1408 (3)	3271 (2)	−1381 (5)	80 (5)	13 (1)	144 (9)	5 (2)	60 (6)	3 (3)
O(6)	−697 (4)	3091 (2)	465 (5)	83 (5)	27 (2)	175 (10)	8 (2)	71 (6)	23 (3)
Water									
O(*W*1)	4511 (4)	2409 (2)	1508 (6)	99 (5)	17 (2)	268 (11)	4 (6)	88 (6)	9 (4)
O(*W*2)	9431 (4)	7106 (3)	5405 (6)	130 (6)	28 (2)	159 (10)	8 (2)	86 (6)	−3 (3)

Table 3. *Hydrogen-atom parameters and their standard deviations*

Positional parameters have been multiplied by 10^3.

	x	y	z	B(Å^2)
Ascorbate ion *A*				
H(C4)	697 (5)	605 (3)	206 (7)	1 (1)
H(C5)	464 (6)	539 (4)	379 (9)	2 (1)
H(C61)	354 (5)	675 (3)	136 (7)	2 (1)
H(C62)	368 (6)	675 (3)	393 (8)	2 (1)
H(O2)	347 (8)	416 (5)	−363 (11)	6 (1)
H(O5)	731 (7)	573 (4)	634 (9)	3 (1)
H(O6)	539 (10)	761 (6)	302 (13)	8 (2)
Ascorbate ion *B*				
H(C4)	204 (6)	478 (4)	−044 (9)	3 (1)
H(C5)	−021 (6)	405 (4)	−360 (9)	3 (1)
H(C61)	−193 (6)	322 (4)	−309 (9)	3 (1)
H(C62)	−218 (8)	403 (5)	−159 (12)	5 (2)
H(O2)	189 (6)	562 (4)	608 (8)	4 (1)
H(O5)	218 (9)	337 (6)	−129 (13)	8 (2)
H(O6)	−109 (8)	308 (6)	121 (13)	6 (2)
Water				
H(O*W*1)	531 (12)	287 (9)	184 (18)	10 (3)
H(O*W*1)′	480 (8)	211 (5)	165 (12)	6 (2)
H(O*W*2)	988 (9)	675 (5)	565 (12)	6 (2)
H(O*W*2)′	917 (8)	728 (5)	423 (13)	6 (2)

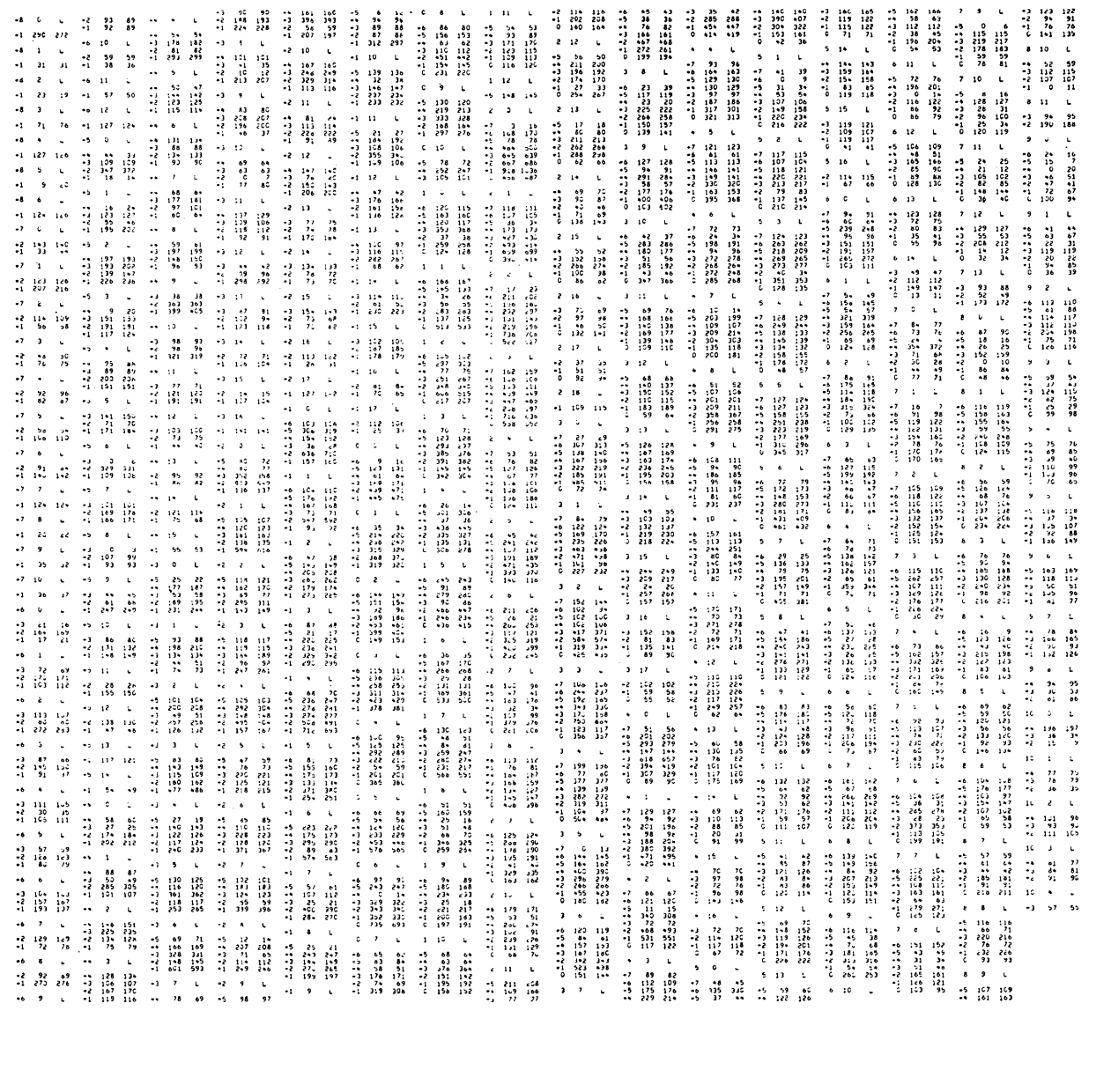
diffractometer angular settings for 12 low-angle reflections (Cu $K\bar{\alpha}$, $\lambda=1{\cdot}5418$ Å). The final cell parameters were obtained by a least-squares analysis of 2θ values for 14 high-angle reflections (Cu $K\alpha_1$, $\lambda=1{\cdot}54051$ Å) measured with the diffractometer. The initial and final cell parameters were not significantly different. Final cell parameters are listed in Table 1 along with other crystal data.

A suitable trial structure was obtained by the heavy-atom method as follows: coordinates for the calcium ion were determined from a sharpened, three-dimensional Patterson map; coordinates for the eight oxygen atoms in the calcium coordination shell and for the five atoms of the central ring from one ascorbate ion were determined from a Fourier map calculated by using phase angles derived from the calcium ion; the remaining nonhydrogen atoms were located in a Fourier map that was calculated by using phase angles derived from the previously located atoms. The trial structure was refined by using a modified version of the full-matrix least-squares program *ORFLS* (Busing, Martin & Levy, 1962; Busing, 1971). The quantity minimized was $\sum w(F_o^2-F_c^2/k^2)^2$, where k is a scale factor and the weight w is equal to $1/\sigma^2(F_o^2)$. Scattering factors for the nonhydrogen atoms (Ca^{2+}, O, C)

were from *International Tables for X-ray Crystallography* (1962), and anomalous dispersion correction factors (real and imaginary components) for these atoms were from Cromer & Liberman (1970). Hydrogen atom scattering factors were from Stewart, Davidson & Simpson (1965). All hydrogen atoms were located in difference Fourier maps that were calculated during the latter stages of refinement. We assumed that the ascorbate ions were in the *l*-form and we made no effort to refine the enantiomeric structure. Final cycles of refinement included all positional parameters, along with anisotropic temperature factors for the heavy atoms, isotropic temperature factors for the hydrogen atoms, and Zachariasen's (1963) isotropic extinction factor g [as formulated by Coppens & Hamilton (1970)]. Because of the limited core storage capacity of the computer it was impracticable to refine all parameters simultaneously; consequently, the parameters were distributed in two blocks, with the parameters for each of the ascorbate ions in separate blocks. Each block also contained parameters for the calcium ion and the water molecules, plus the scale factor and extinction parameter. The blocks of parameters were refined successively in alternate cycles. The final R index ($\sum||F_o|-|F_c||/\sum|F_o|$) for all reflections is 0·038; 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 2·16. During the final cycle of refinement no parameter shifted more than one-fourth of its standard deviation. A final difference Fourier map showed

Table 4. *Observed and calculated structure factors*

From left to right, the columns contain values of l, 10 F_o and 10 F_c.



several peaks and troughs of magnitudes ranging up to 0·4 e $Å^{-3}$ in the immediate vicinity of the calcium ion. No other peaks or troughs exceeded 0·3 e $Å^{-3}$ in magnitude.

Results and discussion

Table 2 lists the final heavy-atom parameters and their standard deviations. Table 3 gives the hydrogen-atom parameters and their standard deviations. In the positional coordinates estimated errors are about 0·001 Å for the calcium ion, 0·003–0·005 Å for the oxygen and carbon atoms, and about 0·07 Å for the hydrogen atoms. Observed and calculated structure factors are listed in Table 4.

Calcium coordination

Fig. 2 depicts the environment of the calcium ion, which is coordinated to three ascorbate ions, and to the two water molecules. One ascorbate ion, anion *A*, chelates the calcium ion through its O(5) and O(6) hydroxyl groups; a second, anion *B*, chelates the calcium ion by using atoms O(3), O(5), and O(6); and the third, a symmetry-equivalent anion *B*, is coordinated to the calcium through the single oxygen atom O(1). Therefore, the calcium ion is surrounded by a shell composed of eight oxygen atoms: two from water molecules and six from ascorbate ions. The eight oxygen atoms assume a distorted square-antiprism arrangement, with calcium–oxygen distances ranging from 2·415 Å to 2·530 Å. The calcium–ascorbate interactions and the geometry of the calcium coordination polyhedron are closely related to those found in other calcium–carbohydrate salts and complexes (Bugg & Cook, 1973; Bugg, 1973; Cook & Bugg, 1973*c*).

Solution studies have demonstrated that, in water, calcium ions also interact with ascorbate ions (Forsberg *et al.*, 1973; Ulmgren & Wahlberg, 1973*a*, *b*). In acidic aqueous solutions ($1 < p\mathrm{H} < 7$), these interactions lead primarily to the formation of discrete calcium–ascorbate ion pairs. A particularly suitable region for binding calcium ions is provided by the tridentate chelation site that is composed of atoms O(3), O(5), and O(6). This is the most likely site for forming the 1:1 calcium complexes that occur in aqueous solution. In alkaline aqueous solutions ($7 < p\mathrm{H} < 13$) calcium and ascorbate ions aggregate to form large complexes of varying compositions. As depicted in Fig. 1, the calcium ions in the crystal structure of calcium ascorbate dihydrate link ascorbate ions and water molecules together, resulting in an extensive array held together by calcium bridges. Presumably, similar interactions account for the calcium ascorbate aggregates that are formed in alkaline solutions.

Hydrogen bonding

Fig. 1 depicts the crystal packing and hydrogen-bonding schemes, and Table 5 lists distances and angles for hydrogen bonds. The ten hydrogen atoms that are covalently attached to oxygen atoms all participate in hydrogen bonding. The strong interaction between crystallographically independent ascorbate ions is an interesting feature of the hydrogen-bonding scheme. As shown in Fig. 1 and described in Table 5, ascorbate ions *A* and *B* are joined by two short O(2)–H···O(3) hydrogen bonds, with donor–acceptor distances of 2·548 and 2·566 Å, respectively. Ascorbate anions form dimers in aqueous solutions of calcium ascorbate (Forsberg *et al.*, 1973); possibly these solution interactions can be attributed to strong O(2)–H···O(3) hydrogen bonds like those in the crystal structure of calcium ascorbate dihydrate.

The ascorbate anions

Fig. 3 shows the conformations of the two ascorbate anions, along with the heavy-atom thermal ellipsoids. As in the crystal structure of sodium ascorbate (Hvoslef, 1969), the proton attached to O(3) of the free acid is the one that is lost upon salt formation. The two ascorbate ions assume different conformations about

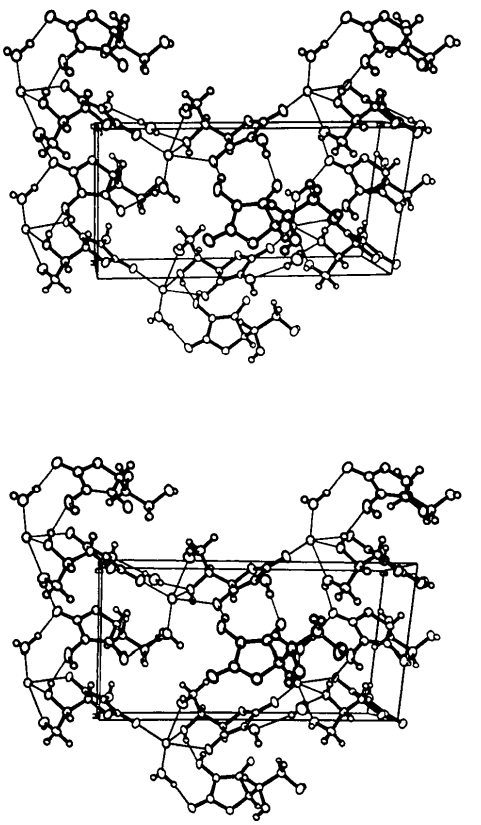


Fig. 1. Stereo drawing showing the crystal packing as viewed down the *c* axis. Heavy lines represent covalent bonds, and the thin lines represent hydrogen bonds and calcium–oxygen contacts. The nonhydrogen atoms are represented by thermal ellipsoids, which are scaled to include 50% probability. [This and Figs. 2–4, were prepared by the program *ORTEP* (Johnson, 1965)].

the C(4)–C(5) bonds: the torsion angle O(5)–C(5)–C(4)–O(4) is 56° for ascorbate ion *A*, as contrasted to 170° for ascorbate ion *B*. The other torsion angles are in general agreement for the two ascorbate anions. In both ions, O(5) is situated *gauche* to O(6), and the torsion angle O(5)–C(5)–C(6)–O(6) assumes a value of 47°. This *gauche* conformation about C(5)–C(6) allows the O(5) and O(6) hydroxyl groups to chelate the calcium ion. In the crystal structure of sodium ascorbate, where the sodium ion is chelated to the O(5)–O(6) pair of hydroxyl groups, O(5) is also situated *gauche* to O(6), but with a slightly larger O(5)–C(5)–C(6)–O(6) torsion angle of 70°. A considerably different conformation about C(5)–C(6) is found for both the crystallographically independent molecules in the crystal structure of ascorbic acid, (Hvoslef, 1969) where the O(5) and O(6) hydroxyl groups are *trans* and the O(5)–C(5)–C(6)–O(6) torsion angle is 171°. The conformations about C(4)–C(5) in sodium ascorbate and for the two molecules in ascorbic acid are nearly the same as that for ascorbate anion *A* in the calcium salt. Thus, the overall conformation of ascorbate anion *A* is similar to that of the ascorbate ion in the crystal structure of sodium ascorbate. The difference between the conformation of ascorbate anion *B* and that of sodium ascorbate is probably due to differences in the metal-binding interactions. Since it has been shown that calcium interactions can affect the conformations of carbohydrates (Bugg & Cook, 1972), the differences between calcium ascorbate and sodium ascorbate are not surprising.

Bond lengths and angles involving only nonhydrogen atoms are listed in Tables 6 and 7, respectively. The C–H bond lengths range from 0·91 to 1·10 Å with an

Table 5. *Hydrogen-bond distances and angles*

Atoms of ascorbate ions *A* and *B* are designated by the respective letter. The average estimated standard deviations are 0·06 Å for donor–acceptor distances, 0·06 Å for hydrogen–acceptor distances, and 7° for donor–hydrogen–acceptor angles.

Donor *D*	Hydrogen H	Acceptor *A*	$D\cdots A$ (Å)	$H\cdots A$ (Å)	$\angle D-H\cdots A$ (°)
O(2*A*)	H(O2*A*)	O(3B^{I})	2·548	1·62	153
O(5*A*)	H(O5*A*)	O(4B^{II})	2·864	2·25	142
O(6*A*)	H(O6*A*)	O(2A^{III})	2·709	2·34	141
O(2*B*)	H(O2*B*)	O(3A^{IV})	2·566	1·76	177
O(5*B*)	H(O5*B*)	O(2*A*)	2·792	2·15	160
O(6*B*)	H(O6*B*)	O(1A^{V})	2·784	2·15	150
O(*W*1)	H(O*W*1)	O(1*A*)	2·775	1·82	167
O(*W*1)	H(O*W*1)′	O(3A^{IV})	2·720	2·20	171
O(*W*2)	H(O*W*2)	O(2B^{VI})	2·909	2·28	159
O(*W*2)	H(O*W*2)′	O(5B^{III})	2·971	2·27	159

Symmetry codes

I	$x, y, z-1$;	IV	$x, y, z+1$
II	$x+1, y, z+1$;	V	$x-1, y, z$
III	$1-x, y+\frac{1}{2}, \bar{z}$;	VI	$x+1, y, z$

Table 6. *Bond lengths involving only nonhydrogen atoms*

Estimated standard deviations are 0·006 Å.

	Ascorbate *A*	Ascorbate *B*		Ascorbate *A*	Ascorbate *B*
C(1)–O(1)	1·237 Å	1·238 Å	C(3)–C(4)	1·511 Å	1·519 Å
C(1)–O(4)	1·365	1·376	C(4)–O(4)	1·461	1·449
C(1)–C(2)	1·409	1·412	C(4)–C(5)	1·523	1·534
C(2)–O(2)	1·376	1·374	C(5)–O(5)	1·434	1·429
C(2)–C(3)	1·366	1·370	C(5)–C(6)	1·518	1·508
C(3)–O(3)	1·278	1·289	C(6)–O(6)	1·429	1·418

Table 7. *Bond angles involving only nonhydrogen atoms*

Estimated standard deviations are about 0·3°.

	Ascorbate *A*	Ascorbate *B*		Ascorbate *A*	Ascorbate *B*
O(1)–C(1)–C(2)	130·4	131·1	C(3)–C(4)–O(4)	104·5	105·1
O(1)–C(1)–O(4)	118·8	118·2	C(3)–C(4)–C(5)	113·0	119·0
C(2)–C(1)–O(4)	110·9	110·8	C(5)–C(4)–O(4)	105·7	108·8
O(2)–C(2)–C(1)	120·2	120·8	C(4)–C(5)–C(6)	114·7	115·2
C(3)–C(2)–C(1)	109·5	109·4	O(5)–C(5)–C(4)	110·0	108·1
O(2)–C(2)–C(3)	130·2	129·7	O(5)–C(5)–C(6)	106·0	106·5
C(2)–C(3)–O(4)	106·9	106·9	C(5)–C(6)–O(6)	108·7	109·4
O(3)–C(3)–C(2)	130·8	130·9	C(1)–O(4)–C(4)	107·7	107·5
O(3)–C(3)–C(4)	122·3	122·2			

average value of 1·00 Å, and average standard deviations of 0·06 Å, and the O–H bond lengths range from 0·46 to 1·00 Å with an average value of 0·73 Å and average standard deviations of 0·06 Å. Corresponding values for ascorbate anions *A* and *B* are in agreement, with the exception of a few significant differences in bond angles, all of which may be attributable to differences in conformation. The largest difference is in the C(3)–C(4)–C(5) angle which is 6° larger for ascorbate ion *B* than for ascorbate ion *A*. In addition, there is a difference of 3° in the O(4)–C(4)–C(5) angles. For the most part, the bond lengths and angles are in agreement with those found for sodium ascorbate. The largest difference in bond lengths involves the C(5)–O(5) bond, which is 0·025 Å longer for ascorbate ion *A* and 0·020 Å longer for ascorbate ion *B* than for the sodium salt. In bond angles for the calcium and sodium salts, the principal differences involve atoms of the side chains. It is likely that these differences are directly due to conformational differences. Deviations from least-squares planes through the five-membered rings of the ascorbate ions are listed in Table 8. In both ascorbate ions, the central ring is significantly nonplanar, and the extraring substituents deviate from the best ring-planes by amounts ranging up to 0·16 Å.

Table 8. *Deviations from least-squares planes through the five ring atoms of the ascorbate anions*

	Ascorbate *A*	Ascorbate *B*
C(1)*	−0·007 Å	−0·024 Å
C(2)*	−0·024	−0·004
C(3)*	0·043	0·027
C(4)*	−0·045	−0·040
O(4)*	0·033	0·040
O(1)	−0·007	−0·077
O(2)	−0·162	0·027
O(3)	0·100	−0·007

* Atoms included in the calculation of the least-squares planes.

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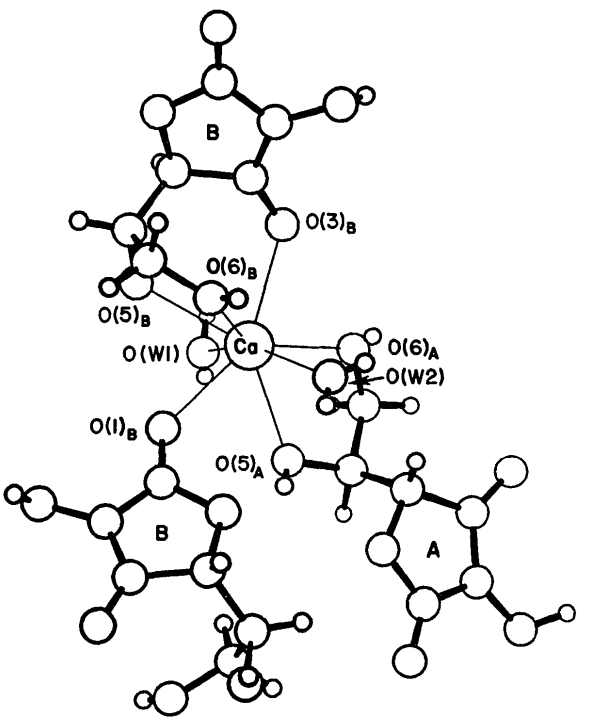

Fig. 2. Environment of the calcium ion. Atoms from ascorbate ions *A* and *B* are represented by the respective letter. O(*W*1) and O(*W*2) are oxygen atoms from water molecules.

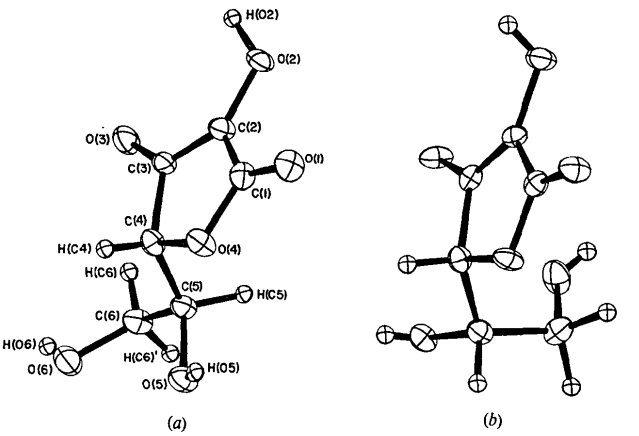

Fig. 3. Conformation of (*a*) ascorbate ion *A* and (*b*) ascorbate ion *B*. Nonhydrogen atoms are represented by thermal ellipsoids which are defined by the principal axes of thermal vibration and are scaled to include 50% probability. The hydrogen atoms are represented by spheres of 0·1 Å radius.

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The Crystal Structure of Calcium Ascorbate Dihydrate

BY J. HVOSLEF AND K. E. KJELLEVOLD

Department of Chemistry, University of Oslo, Blindern, Oslo 3, Norway

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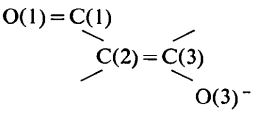
The crystal structure of calcium L-ascorbate dihydrate $Ca^{2+}(C_6H_7O_6^-)_2.2H_2O$ has been determined by X-ray diffraction, on an automatic diffractometer with Mo $K\alpha$ radiation. The space group is $P2_1$ with $a=8{\cdot}335$ (2), $b=15{\cdot}787$ (3), $c=6{\cdot}360$ (2) Å and $\beta=107{\cdot}48$ (1)°. The parameters were refined to $R=0{\cdot}036$ for 2283 observed reflexions. The average standard deviation in bond lengths is 0·0035 Å for the non-hydrogen atoms. Eight oxygen atoms surround the calcium ion at distances ranging from 2·409 to 2·520 Å, and form a distorted square antiprism. The independent ascorbate anions (*A* and *B*) form stacks which are connected to neighbouring stacks through interactions with calcium ions and water molecules. These anions form tightly bound pairs by short hydrogen bonds between their enediol oxygen atoms, but they are also bonded to each other by normal hydrogen bonds. The geometry of the lactone rings is partly determined by the side chains, which assume different orientations in *A* and *B*. Resonance stabilization of the O(1)=C(1)–C(2)=C(3)–O(3)$^-$ group is corroborated.

Introduction

The structural determination of sodium ascorbate (Hvoslef, 1969) revealed significant conformational and bonding changes in comparison with ascorbic acid (Hvoslef, 1968). Studies of infrared and Raman spectra (Hvoslef & Klæboe, 1971) of solids and aqueous solutions as well as circular dichroism measurements (Kresheck, 1968) confirmed these findings. The present study of calcium L-ascorbate dihydrate, $Ca(C_6H_7O_6)_2.2H_2O$, was primarily intended to elucidate the conformation of the ascorbate anion under packing conditions different from those in the sodium salt. It was also desirable to verify the bond lengths observed in the conjugated



system of the lactone ring. In this respect the choice of the calcium salt was favourable because it permitted two independent ascorbate anions to be determined simultaneously.

Particular interest is, however, connected with the coordination of calcium ions to sugar molecules, and C. E. Bugg and R. A. Hearn at the University of Alabama have undertaken a simultaneous investigation of this compound in order to obtain additional information of such interactions (see preceding paper).

Experimental

Crystals of calcium L-ascorbate dihydrate $Ca^{2+}(C_6H_7O_6^-)_2.2H_2O$ were grown according to the procedure given by Merrill & Ruskin (1947), but satisfactory X-ray data were only obtained from a specimen which had been cut from a large crystal with well developed faces. This fragment was pyramidal with a basal area of 0·034 × 0·024 cm and a height of 0·020 cm.

The crystal was checked on oscillation and Weissenberg diagrams, and the space group was determined as $P2_1$ from systematic absences ($k=2n+1$ absent in $0k0$) and from the fact that the molecules are optically active. X-ray data were collected on an automatic Picker diffractometer operating in the ω–2θ mode. The scan speed was 1 ° min^{-1} and background counts were 20 s on each side of the Bragg peak. Three reflexions