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The Crystal Structure of D-*iso*-Ascorbic Acid

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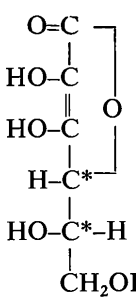
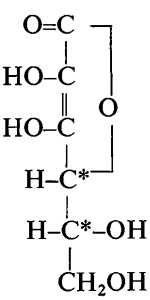
The structure of D-*iso*-ascorbic acid, $C_6H_8O_6$, has been determined from three-dimensional Cu $K\alpha$ ($\lambda = 1{\cdot}5418$ Å) X-ray data using the noncentrosymmetric direct method. There are two molecules in a unit cell of dimensions $a = 5{\cdot}165$ (4), $b = 14{\cdot}504$ (10), $c = 4{\cdot}724$ (4) Å, and $\beta = 99{\cdot}50$ (1)°. Space group is $P2_1$, $D_m = 1{\cdot}654$, $D_x = 1{\cdot}668$ g.cm^{-3}. The structure was refined by anisotropic full-matrix least-squares methods to an R value of 0·037. As in ascorbic acid, the major acyclic side chain adopts a conformation such that O(5) and C(6) are far from O(3). The molecules, which are arranged head-to-tail along **b**, are linked together by a zigzag chain of hydrogen bonds between hydroxyls; the three repeating links run roughly along **b**, **a**, and **c**, and a single hydrogen bond runs to the carbonyl oxygen atom along **c**. The ring oxygen atom is not hydrogen bonded.

Introduction

D-*iso*-Ascorbic acid, also known as *arabo*-ascorbic acid and erythrobic acid, is a stereoisomer of L-ascorbic acid (vitamin C) with inversion at C(5), one of the two asymmetric carbon atoms.



L-ascorbic acid



D-*iso*-ascorbic acid

The biosynthesis of L-ascorbic acid is known (Isherwood, Chen & Mapson, 1953) to proceed by two pathways involving complete inversion of hexoses. One starts with D-glucose and leads to L-gulono-γ-lactone *via* D-glucuronolactone. The other passes through a series of intermediates from D-galactose to L-galactono-γ-lactone. The crystal structures of most of these compounds are known.

Similarly, D-*iso*-ascorbic acid has been found (Isherwood, Chen & Mapson, 1954) to be synthesized *in vivo* from D-mannono-γ-lactone (rats) or from D-altrono-γ-lactone (cress, mung beans, and peas).

All these precursors share the characteristics that the hydroxyl on C(2) has the L configuration, and the hydroxyl on C(4) has the D configuration. Other lactones that do not comply with these conditions are not converted to ascorbic acids in either the plant or animal.

Ascorbic acid plays a role in several important biochemical processes, such as collagen and serotonin syntheses. In the gross organism, lack of ascorbic acid results in the complex of symptoms known as scurvy. In order for an isomer or homolog of ascorbic acid to have antiscorbutic activity, it must possess: a hydroxyl with a D configuration at C(4), a side chain, a hydroxyl at C(5), and all the hydroxyl groups must be

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unsubstituted (Rosenberg, 1942). D-*iso*-Ascorbic acid, among a few other compounds, fulfills these minimum requirements although its potency is at most $\frac{1}{20}$ that of L-ascorbic acid (*cf.* Dalmer & Mall, 1933). The effectiveness of *iso*-ascorbic acid, which is evidently related to the range of reactions in which Vitamin C participates, is disputed in the literature (Pelletier & Goden, 1969; Pelletier, 1969; Rivers, Huang & Dodds, 1963; Fabianek & Herp, 1967; Reiff & Free, 1959). The specificity of the various enzymes catalyzing these reactions must depend on different conformational, and/or configurational, features of the ascorbic-acid isomer. Consequently, the authors undertook a crystallographic structure analysis to provide data on the molecular shape for comparison with that of ascorbic acid (Hvoslef, 1968). Resultant information would then permit active sites of the enzymes involved in ascorbic acid utilization to be mapped, and it would aid in investigating the possible participation of ascorbic acid in membrane transport.

Crystal data

Prismatic crystals were obtained by recrystallization, from a methanol solution, of material supplied by Mann Research Laboratories, Inc. The crystal density was measured at 25°C by flotation in a mixture of cyclohexane and bromobenzene. Cell parameters given below were measured, using Cu $K\alpha$ radiation (λ = 1·5418 Å), on a four-circle automatic diffractometer.

iso-Ascorbic acid, $C_6H_8O_6$, M.W. 176·12.

Space group $P2_1$; $0k0$ systematically absent for $k=2n+1$.
$a=5{\cdot}165$ (4), $b=14{\cdot}504$ (10), $c=4{\cdot}724$ (4) Å.
$\beta=99{\cdot}50$ (10)°.
$Z=2$.
$D_m=1{\cdot}654$, $D_x=1{\cdot}668$ g.cm^{-3}.
$\mu_{\mathrm{Cu}\,K\alpha}=13{\cdot}7$ cm^{-1}.

Experimental

Both photographic and diffractometer data were collected. The first set of data was recorded along the *a* and *c* axes on multiple-film equi-inclination Weissenberg photographs, using Ni-filtered Cu $K\alpha$ radiation. A second set was later collected with a Picker four-angle automatic diffractometer, using a crystal cut to 0·3 × 0·3 × 0·3 mm. A total of 620 independent reflections was measured, using Ni-filtered Cu $K\alpha$ radiation and a θ–2θ variable scanning mode, with 2θ values below 130°. Background measurements were made at each end of the peak. Eleven reflections with $I_H < 1{\cdot}5\sigma(I_H)$ were assumed to be unobservably weak; they were assigned intensities of $\sigma(I_H)/2$, thereby giving $|F_H|=\sigma(F_H)$. Structure amplitudes were calculated from intensities with a program written by Shiono (1969). No corrections were made for possible sources of sys-

Table 1. *Atomic positional and thermal parameters for* D-*isoascorbic acid*

Temperature factor expression: $\exp[-(h^2\beta_{11}+k^2\beta_{22}+l^2\beta_{33}+2hk\beta_{12}+2hl\beta_{13}+2kl\beta_{23})]$.
Numbers in parentheses are e.s.d.'s in the least significant places.

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O(1)	−0·0786 (5)	0·4315 (3)	0·0305 (6)	0·0179 (12)	0·0021 (1)	0·0254 (12)	0·0010 (3)	−0·0062 (8)	−0·0006 (3)
O(2)	0·3025 (6)	0·4854 (3)	0·5444 (6)	0·0172 (12)	0·0025 (1)	0·0326 (14)	0·0012 (3)	−0·0024 (9)	−0·0044 (4)
O(3)	0·7201 (5)	0·3335 (3)	0·5747 (6)	0·0134 (10)	0·0021 (1)	0·0243 (12)	0·0012 (2)	−0·0052 (8)	−0·0011 (3)
O(4)	0·1857 (5)	0·3100 (3)	0·0051 (5)	0·0198 (11)	0·0016 (1)	0·0172 (11)	0·0006 (3)	−0·0079 (8)	−0·0011 (3)
O(5)	0·4306 (6)	0·1144 (3)	0·0627 (6)	0·0322 (14)	0·0010 (1)	0·0211 (14)	0·0017 (3)	0·0011 (10)	0·0008 (3)
O(6)	0·2192 (6)	0·0793 (3)	0·5296 (7)	0·0272 (13)	0·0028 (1)	0·0238 (15)	−0·0026 (4)	−0·0024 (10)	−0·0013 (4)
C(1)	0·1177 (7)	0·3901 (3)	0·1264 (8)	0·0171 (15)	0·0015 (1)	0·0190 (16)	0·0002 (3)	−0·0010 (11)	0·0004 (4)
C(2)	0·3141 (7)	0·4124 (3)	0·3727 (8)	0·0149 (13)	0·0017 (1)	0·0180 (15)	−0·0001 (4)	0·0003 (11)	−0·0021 (4)
C(3)	0·4981 (7)	0·3472 (3)	0·3923 (7)	0·0130 (13)	0·0021 (1)	0·0170 (15)	−0·0013 (4)	−0·0017 (10)	0·0002 (4)
C(4)	0·4282 (7)	0·2759 (0)†	0·1642 (7)	0·0137 (14)	0·0015 (1)	0·0176 (16)	0·0004 (3)	−0·0013 (12)	−0·0008 (4)
C(5)	0·3970 (7)	0·1786 (3)	0·2802 (8)	0·0148 (13)	0·0015 (1)	0·0166 (15)	0·0001 (3)	−0·0018 (10)	−0·0007 (4)
C(6)	0·1350 (8)	0·1652 (4)	0·3824 (9)	0·0178 (15)	0·0026 (2)	0·0291 (20)	−0·0000 (5)	0·0015 (14)	0·0009 (6)
HO(2)	0·156 (14)	0·510 (5)	0·516 (13)						
HO(3)	0·742 (12)	0·380 (5)	0·714 (13)						
HO(5)	0·471 (13)	0·071 (5)	0·141 (14)						
HO(6)	0·179 (12)	0·091 (4)	0·678 (16)						
HC(4)	0·573 (11)	0·278 (4)	0·011 (12)						
HC(5)	0·536 (11)	0·168 (4)	0·453 (13)						
HC(6)	0·089 (11)	0·218 (4)	0·489 (13)						
H′C(6)	0·011 (11)	0·163 (4)	0·215 (13)						

† Fixed parameter (polar space group).
An isotropic temperature factor $B=2{\cdot}0$ Å^2 was used for all H atoms.

tematic error such as X-ray absorption, extinction, or multiple diffraction.

Determination and refinement of the structure

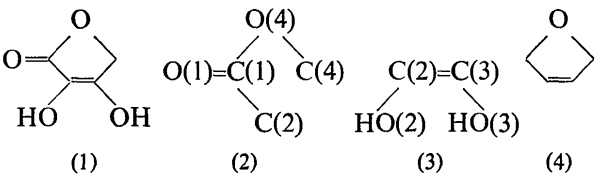
The structure was determined, from film intensities, by the noncentrosymmetric direct method (Karle & Karle, 1966). Three linearly independent reflections were used to define the origin, and two symbols were assigned to general reflections with high values of E which had many interaction pairs. Starting phases for tangent refinement were derived by use of symbolic addition. Ten cycles of tangent refinement using the Hall (1967) program, followed by an E synthesis, revealed a partial structure from which a new set of starting phases was chosen. A few more cycles of tangent refinement, followed by an E synthesis and difference map, revealed the structure. The structure was refined with diffractometer data, using full-matrix least-squares methods (Shiono, 1966) and a constant weighting scheme. Scattering factors used for carbon and oxygen were those of Cromer & Waber (1965) and for hydrogen they were those of Stewart, Davidson & Simpson (1965). Isotropic refinement converged at $R=0{\cdot}12$.* Anisotropic refinement resulted in an R of 0·06. The difference synthesis showed the eight hydrogen atoms which were refined isotropically. The refinement gave $R=0{\cdot}052$. A final least-squares cycle was calculated omitting 11 low-order strong reflections (marked with asterisks in the structure factor table) with high $\Delta F/\sigma$. The resulting R value was 0·037. Positional and temperature parameters are shown in Table 1; measured and calculated structure factors are listed in Table 2.†

* $R=\sum||F_{meas}|-|F_{calc}||/\sum|F_{meas}|$

Discussion of the structure

Molecular geometry

Fig. 1 shows the D-*iso*-ascorbic acid molecule whic consists of a lactone ring and an acyclic side chain. The ring system (1) which comprises the lactone group (2), C(2) C(1) O(1) O(4) C(4), and the enediol group (3), O(2) C(2) C(3) O(3), is roughly planar, with a root-mean-square deviation from the plane of 0·016 Å in *iso*-ascorbic acid (hereafter referred to as I) compared



(1) (2) (3) (4)

to the r.m.s. deviations in the two independent ascorbic acid molecules A and B (Hvoslef, 1968), which are 0·016 and 0·025 Å, respectively. All these are signifi-

† Ten collected reflections using the highest attenuator had $F_c/F_o\sim1{\cdot}3$, so a least-squares cycle with all data was calculated assigning a second scale-factor parameter to these reflections. The resulting R-value was 0·038 with negligible changes in atomic parameters.

Table 2. *Observed and calculated structure factors*

Columns are: index k, $10F_{obs}$, $10F_{calc}$. (Asterisks indicate reflection omitted from final least-squares calculation, + indicates unobserved reflections.)

cantly nonplanar. The lactone group (2) in I is planar to within 0·004 Å, whereas in *A* and *B* it is significantly non-planar, forming alternative zigzag chains. The enediol group (3) is planar to within 0·02 Å in all three molecules; the best five-atom plane including (3) contains C(1) in I within 0·002 Å, but contains C(4) in *A* within 0·002 Å and in *B* within 0·005 Å. The ring (4), C(1) C(2) C(4) O(4), is planar in I and *A* but non planar in *B* (r.m.s. deviations 0·008, 0·005, and 0·022 Å, respectively). This difference is correlated with the conformation around C(4)–C(5), discussed later.

Distances and angles in the ring (Fig. 1) are not significantly different from those found in ascorbic acid, except for the C(2)–O(2) bond length which is 0·02 Å shorter than in ascorbic acid. However, angles in the side chains of the two compounds differ markedly from each other. These differences, which may be related to the conformational difference discussed later, are not clearly understood, but they may affect the relative acidities of the protolytic hydrogen atoms in the diastereomers.

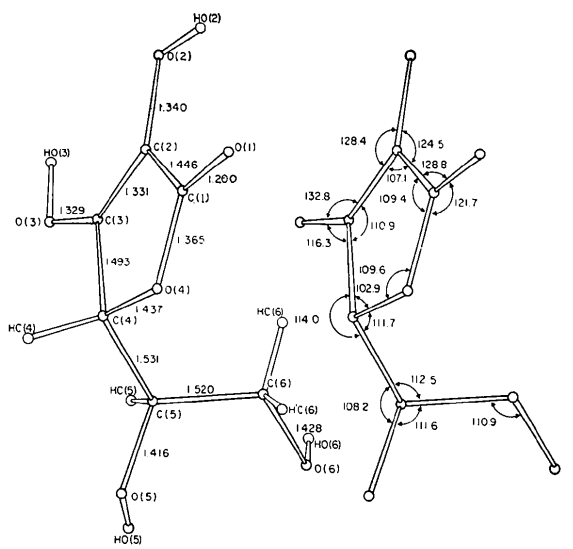


Fig. 1. Bond distances and angles in D-*iso*-ascorbic acid Average e.s.d. is 0·005 Å for the bonds and 0·3° for the angles. Average O–H distance is 0·77 Å ranging between 0·71 and 0·94 Å. Average C–H distance is 1·00 Å, ranging between 0·92 and 1·11 Å.

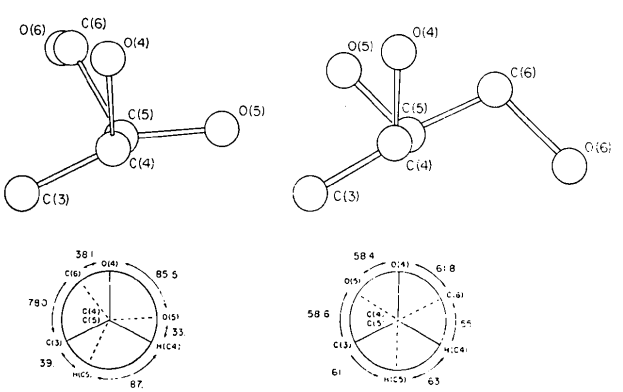


Fig. 2. Side chain of *iso*-ascorbic acid. and ascorbic acid. Corresponding dihedral angles are shown below. Those given for ascorbic acid (right) are averages for the two molecules.

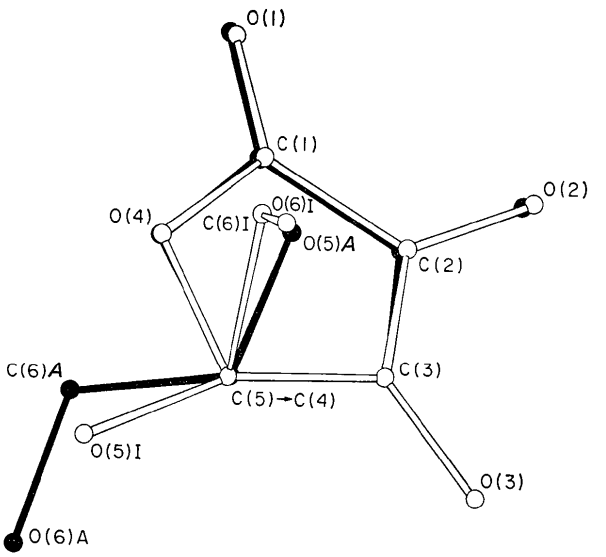


Fig. 3. Superposition of *iso*-ascorbic acid (I)over ascorbic acid (*A*), projected down C(4)–C(5). Black molecule is ascorbic acid.

Conformation of the side chain

None of the atoms of the long side chain is coplanar with the ring. However, the four atoms C(4), C(5), C(6), O(6) form an extended, roughly planar zigzag in which each atom is about 0·06 Å from the plane. The dihedral angle between this plane and that of the ring is 102·4°.

The most striking result of this study is the major conformational difference arising from a minor configurational difference in the side chain. In both L-ascorbic and D-*iso*-ascorbic acid, atoms O(5) and C(6) adopt a conformation to maximize their distance from O(3), so as to minimize unfavorable 1,3 vicinal (*peri*) interactions (Jeffrey & Kim, 1970). Thus, for both molecules this requires H(C5) to be antiparallel to O(4) (Fig. 2). As shown in Fig. 3, which depicts the two structures superimposed, O(5) in one molecule nearly coincides with C(6) in the other. Angle C(4)–C(5)–C(6) in *iso*-ascorbic acid is essentially the same as angle C(4)–C(5)–O(5) in ascorbic acid (112°). However, angle C(4)–C(5)–C(6) in *iso*-ascorbic acid is smaller than that in ascorbic acid.

The positions of the O(6) atoms in these two molecules are necessarily different. In both ascorbic acid and *iso*-ascorbic acid, O(6) is in the staggered conformation that takes it farthest from the ring. Corresponding torsion angles are 171·4 and 171·2° in *A* and *B* and

70·7° in I, and although O(6) is, in principle, free to rotate around the C(5)–C(6) bond, it is reasonable to suppose that these are the most favorable conformations for the molecules.

The staggering of the conformation around C(4)–C(5) (Fig. 2) in ascorbic acid is nearly ideal (*i.e.*, torsion angles are nearly 60°) contrasted with the highly distorted staggering in *iso*-ascorbic acid, which may be due to both an attraction between the electronegative O(5) and the H(C4) and repulsion between H(C4) and H(C5), as well as the orienting influence of the hydrogen-bonding scheme. The conformation around this bond is correlated with the closest nonbonded distance to the ring oxygen O(4). In I, this distance is 2·79 Å for C(6)–O(4), in *A* it is 2·80 Å for O(5)–O(4), and in *B* it is 2·95 Å for O(5)–O(4). Respective dihedral angles are ~38, ~51, and 66°. The shorter distance ~2·8 Å in I and *A* corresponds to the greater planarity of ring (4) in these two molecules.

Hydrogen bonding

Molecules in the crystal are held together by a zigzag hydrogen-bonding scheme (Fig. 4): $\cdots O(5c) \underset{B}{\rightarrow} O(2b) \underset{A}{\rightarrow} O(6a) \underset{C}{\rightarrow} O(5c)\cdots$, which links the ring of one molecule to the chain of another.† A single hydrogen bond, $O(3b) \underset{D}{\rightarrow} O(1)$, roughly parallels the *c* axis. Distances and angles associated with hydrogen bonding are given in Table 3. It is remarkable that the sequence of atoms in this hydrogen-bonding system duplicates one of the two schemes found in ascorbic acid: $\cdots O(5) \rightarrow O(2') \rightarrow O(6)^* \rightarrow O(5')\cdots$. This suggests the possibility that another crystalline modification of *iso*-ascorbic acid may exist, duplicating the second alternative: $\cdots O(5)^* \rightarrow O(6) \rightarrow O(2') \rightarrow O(5')^*\cdots$. In fact, the *B* and *K* forms of mannitol (Berman, Jeffrey & Rosenstein, 1968; Kim, Jeffrey & Rosenstein, 1968) are related by just such an opposite sense in their hydrogen-bonding scheme.

Table 3. *Hydrogen bonding in* D-*iso-ascorbic acid*

i	*j*	*k*	*d*(*ik*)	*d*(*jk*)	<(*ijk*)
O(2)—H	$\underset{A}{\rightarrow}$	O(6*a*)	2·584 Å	176· Å	170·7°
O(5*c*)–H	$\underset{B}{\rightarrow}$	O(2*b*)	2·827	2·13	157·4
O(6a)–H	$\underset{C}{\rightarrow}$	O(5*c*)	2·777	2·07	162·4
O(3b)–H	$\underset{D}{\rightarrow}$	O(1)	2·643	1·77	151·0

Symmetry operation:

a	$\bar{x}, \frac{1}{2}+y, 1-z$
b	$-1+x, y, -1+z$
c	$\bar{x}, \frac{1}{2}+y, \bar{z}$

† Symmetry code is given in Table 3. The letters refer to the axes along which the bonds are mainly oriented.

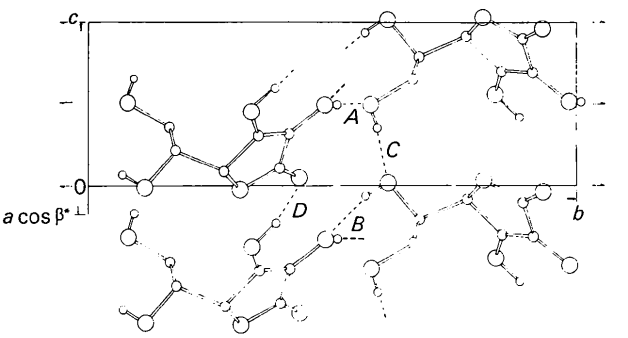


Fig. 4. Hydrogen bonding in the crystal structure of D-*iso*-ascorbic acid. View down the a^* axis.

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