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X-ray Studies on Crystalline Complexes Involving Amino Acids. V. The Structure of L-Serine–L-Ascorbic Acid*

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Abstract

L-Serine–L-ascorbic acid, $C_3H_7NO_3.C_6H_8O_6$, a 1:1 complex between the amino acid serine and the vitamin ascorbic acid, crystallizes in the orthorhombic space group $P2_12_12_1$ with four formula units in a cell of dimensions a = 5·335 (3), b = 8·769 (2), c = 25·782 (5) Å. The structure was solved by direct methods and refined by full-matrix least squares to an R of 0·036 for 951 observed reflections. Both molecules are neutral in the structure. The conformation of the serine molecule is different from that observed in the crystal structures of L-serine, DL-serine and L-serine monohydrate. The enediol group in the ascorbic acid molecule is planar, whereas significant departures from planarity are observed in the lactone group. The conformation of this molecule is similar to that observed in arginine ascorbate. The unlike molecules aggregate into separate columns in the crystal structure. The columns are held together by hydrogen bonds. Among these, a pair of hydrogen bonds between the enediol group of ascorbic acid and the carboxylate group of serine provides a possible model for a specific interaction between ascorbic acid and a carboxylate ion.

Introduction

As part of a programme of X-ray studies on crystalline complexes involving amino acids, among themselves and with other biomolecules, we report the crystal structure of a 1:1 complex between the amino acid serine and the vitamin ascorbic acid. The crystal structure of another such complex, that between arginine and ascorbic acid, has already been reported (Sudhakar & Vijayan, 1980). Both of the molecules in arginine ascorbate are ionized and the complex is stabilized primarily through the interactions between the ascorbate anion and the positively charged guanidyl group of arginine. The side chain of serine is not easily ionizable and, hence, it was thought likely that the ascorbic acid would remain neutral in its complex with serine. Therefore, the X-ray analysis of this complex was undertaken in order to study, at the atomic resolution, the possible non-covalent interaction between neutral ascorbic acid and an amino acid.

Experimental

Transparent, plate-like crystals of the complex were grown from an aqueous solution of the components in molar proportion by slow evaporation in a nitrogen atmosphere. The unit-cell dimensions and the space group were determined from X-ray diffraction photographs. The former were subsequently refined on a four-circle diffractometer. The density was measured by flotation in a mixture of chloroform and carbon tetrachloride.

Crystal data

L-Serine–L-ascorbic acid, $C_3H_7NO_3.C_6H_8O_6$; orthorhombic, $P2_12_12_1$; a = 5·335 (3), b = 8·769 (2), c = 25·782 (5) Å; D_m = 1·56 (1), D_c = 1·55 Mg m^{-3}; Z = 4; μ(Mo $K\alpha$) = 0·1524 mm^{-1}.

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The intensity data were collected on a CAD-4 computer-controlled diffractometer from a specimen of dimensions 0·5 × 0·4 × 0·2 mm using graphite-monochromated molybdenum radiation up to a Bragg angle of 25°. Of the 1273 unique reflections in this range, 951 had $I > 2\sigma(I)$ and were subsequently used for structure determination and refinement. The data were corrected for Lorentz and polarization factors. The structure was solved using *MULTAN* (Germain, Main & Woolfson, 1971) and refined with a modified version of the full-matrix structure-factor least-squares program *LALS* written by Gantzel, Sparks and Trueblood for an IBM 360/44 computer. The heavy atoms and the hydrogen atoms, determined from a difference map and geometrical considerations, were given anisotropic and isotropic temperature factors, respectively. The refinement converged at $R = 0{\cdot}036$. The weighting scheme was of the form $1/(a + bF_o + cF_o^2)$, where $a = 2{\cdot}103$, $b = -0{\cdot}215$ and $c = 0{\cdot}010$. The scattering factors for the non-hydrogen atoms and the hydrogen atoms were taken from Cromer & Waber (1965) and Stewart, Davidson & Simpson (1965), respectively. The final positional parameters are given in Table 1.* The bond lengths and angles involving non-hydrogen atoms are shown in Fig. 1.

Table 1. *Final positional parameters of non-hydrogen atoms* (×10⁴) *and hydrogen atoms* (×10³)

Isotropic temperature factors are also listed. The *B* values of non-hydrogen atoms are the equivalent isotropic temperature factors calculated from anisotropic thermal parameters using Hamilton's (1959) formula. The estimated standard deviations are given in parentheses.

	x	*y*	*z*	*B* (Å²)
O(1)	3128 (5)	−279 (3)	1955 (1)	3·2 (1)
O(2)	3597 (6)	15 (3)	1103 (1)	3·6 (1)
C(1)	3827 (6)	444 (4)	1567 (1)	2·3 (1)
C(2)	5118 (7)	1970 (4)	1644 (1)	2·1 (1)
N(1)	4264 (6)	2713 (3)	2135 (1)	2·3 (1)
C(3)	7947 (7)	1729 (4)	1668 (2)	2·8 (1)
O(4)	9267 (5)	3111 (3)	1721 (1)	2·9 (1)
C(11)	6096 (7)	6301 (3)	1587 (1)	2·4 (1)
O(11)	4895 (5)	5945 (5)	1972 (1)	2·9 (1)
C(12)	8354 (7)	7189 (3)	1543 (1)	2·1 (1)
O(12)	9357 (6)	7769 (3)	1984 (1)	3·1 (1)
C(13)	8978 (7)	7234 (4)	1041 (1)	2·3 (1)
O(13)	10828 (6)	7866 (3)	771 (1)	3·5 (1)
C(14)	7027 (7)	6403 (4)	729 (1)	2·5 (1)
O(14)	5355 (5)	5792 (3)	1117 (1)	2·9 (1)
C(15)	8003 (8)	5127 (4)	386 (1)	2·7 (2)
O(15)	9303 (6)	4069 (3)	702 (1)	3·3 (1)
C(16	5873 (9)	4404 (4)	95 (1)	3·3 (2)
O(16)	6779 (7)	3316 (3)	−274 (1)	3·8 (2)
H(C2)	477 (7)	270 (4)	135 (1)	2 (1)
H1(C3)	839 (8)	105 (5)	198 (2)	3 (1)
H2(C3)	836 (9)	119 (5)	137 (2)	4 (1)
H1(N1)	264 (9)	292 (5)	212 (1)	2 (1)
H2(N1)	446 (7)	210 (5)	244 (2)	3 (1)
H3(N1)	522 (8)	364 (5)	221 (1)	3 (1)
H(O4)	929 (13)	347 (8)	147 (2)	8 (1)
H1(C16)	478 (7)	532 (5)	−10 (1)	2 (1)
H2(C16)	474 (8)	385 (5)	35 (2)	3 (1)
H(C15)	924 (10)	555 (5)	13 (2)	4 (1)
H(C14)	612 (8)	720 (5)	50 (1)	3 (1)
H(O12)	1074 (12)	815 (7)	193 (2)	6 (1)
H(O13)	1175 (16)	855 (8)	103 (3)	8 (2)
H(O16)	748 (11)	365 (6)	−47 (2)	3 (2)
H(O15)	1008 (10)	353 (6)	48 (2)	4 (1)

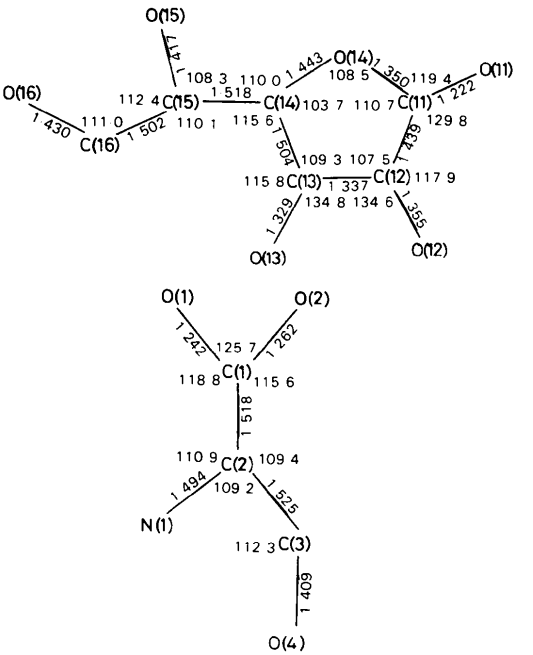

Fig. 1. Bond lengths (Å) and valency angles (°) involving non-hydrogen atoms. The estimated standard deviations for bond lengths and angles are 0·005 Å and 0·3°, respectively.

Discussion

The serine molecule

The neutral serine molecule in the structure is zwitterionic and has bond lengths and angles comparable to those in L-serine, DL-serine and L-serine monohydrate (Benedetti, Pedone & Sirigu, 1972; Frey, Lehmann, Koetzle & Hamilton, 1973). The conformation of serine in the present structure, which could be described by the dihedral angles $\psi^1 = -28$, $\psi^2 = 153$ and $\chi^1 = -61°$ (IUPAC–IUB Commission on Biochemical Nomenclature, 1970), is, however, different from that observed in the structures mentioned above. Steric considerations indicate that the side chain

* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34790 (8 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

of serine, with an oxygen atom at the γ position, can assume all the possible staggered conformations with equal facility (Bhat, Sasisekharan & Vijayan, 1979). However, the side-chain hydroxyl group is *gauche* to both the carboxylate and the amino groups ($\chi^1 \simeq 60°$) in L-serine, DL-serine and L-serine monohydrate. In the present structure, the hydroxyl group is *trans* to the carboxylate group and *gauche* to the amino group ($\chi^1 \simeq -60°$).

The ascorbic acid molecule

The ascorbic acid molecule in serine–ascorbic acid is neutral unlike that in the structure of arginine ascorbate. The only other instance in which neutral ascorbic acid molecules are found is in the crystal structure of ascorbic acid itself (Hvoslef, 1968). The bond lengths and angles of the molecule in the present structure are comparable to those observed in the structure of ascorbic acid. The enediol group, made up of C(12), O(12), C(13) and O(13), is planar whereas significant, though small, departures from planarity are observed in the lactone group [C(14), O(14), C(11), O(11) and C(12)], a situation similar to that found in the crystals of neutral ascorbic acid. It may be recalled that both these groups were planar in the ascorbate anion in arginine ascorbate (Sudhakar & Vijayan, 1980), the only structure which provides a description of the ascorbate anion unaffected by the geometrical constraints and other disturbances caused by the requirements of metal coordination. Thus, it would appear that while the enediol group is planar and the lactone group slightly non-planar in the neutral molecules, both these groups are planar in the anion when unaffected by the effects of metal coordination.

The torsional angles about C(14)–C(15) and C(15)–C(16), which define the conformation of the molecule, are shown in Fig. 2. As pointed out in the discussion of the structure of arginine ascorbate (Sudhakar & Vijayan, 1980), the sterically favoured arrangement about the C(14)–C(15) bond is the one in which O(15) is staggered between O(14) and C(13). The observed conformation corresponds to this arrangement. For this conformation about the C(14)–C(15) bond, only two of the three staggered

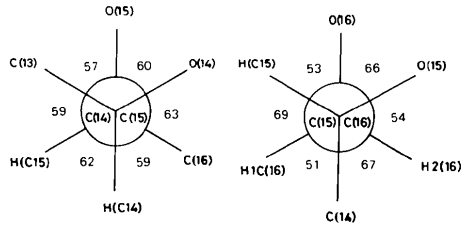

Fig. 2. Torsional angles (°) about C(14)–C(15) and C(15)–C(16) in the side chain of the ascorbic acid molecule.

Table 2. *Hydrogen-bond parameters*

A–H···B	A···B (Å)	∠H–A···B (°)
N(1)–H1(N1)···O(4)$^{(b)}$	2·893 (4)	19 (3)
N(1)–H2(N1)···O(11)$^{(e)}$	2·812 (4)	3 (2)
N(1)–H3(N1)···O(11)$^{(a)}$	2·885 (4)	32 (2)
O(4)–H(O4)···O(15)$^{(a)}$	2·756 (4)	9 (6)
O(12)–H(O12)···O(1)$^{(c)}$	2·642 (4)	18 (4)
O(13)–H(O13)···O(2)$^{(c)}$	2·542 (4)	21 (4)
O(15)–H(O15)···O(16)$^{(d)}$	2·709 (4)	20 (4)
O(16)–H(O16)···O(2)$^{(d)}$	2·766 (4)	13 (5)

Symmetry code

(*a*) x,y,z
(*b*) $x-1,y,z$
(*c*) $x+1,y+1,z$
(*d*) $\frac{1}{2}+x,\frac{1}{2}-y,-z$
(*e*) $-x+1,\frac{1}{2}+y-1,\frac{1}{2}-z$

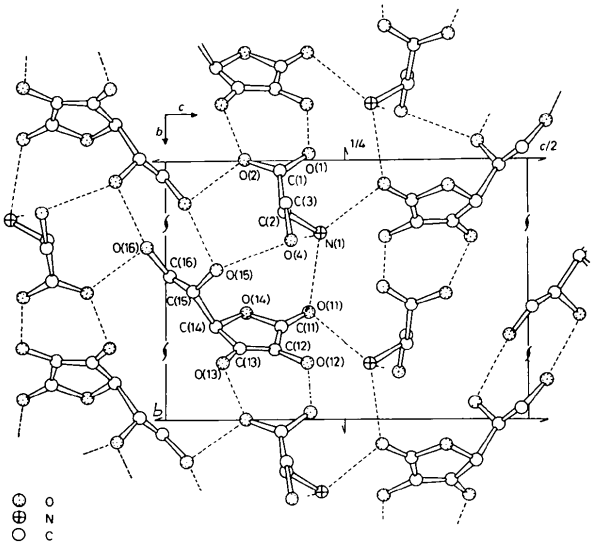

Fig. 3. Crystal structure of serine–ascorbic acid as viewed along the *a* axis. Broken lines indicate hydrogen bonds. The atoms N(1) and O(4) connected by a hydrogen bond belong to adjacent molecules related by an *a* translation.

arrangements about the C(15)–C(16) bond are sterically possible: O(16) could be staggered between either C(14) and H(C15) or O(15) and H(C15). In the present structure, O(16) is staggered between O(15) and H(C15) as in arginine ascorbate and one of the two crystallographically independent molecules in the crystals of ascorbic acid.

Crystal structure and hydrogen bonding

The crystal structure of serine–ascorbic acid is shown in Fig. 3. The parameters of the hydrogen bonds which stabilize the structure are given in Table 2. All the hydrogen atoms attached to nitrogen and oxygen atoms are made use of for hydrogen bonding. The hydroxyl oxygen atoms in the side chains of serine and ascorbic acid are involved in two hydrogen bonds each, in one as an acceptor and in the other as a donor. Each

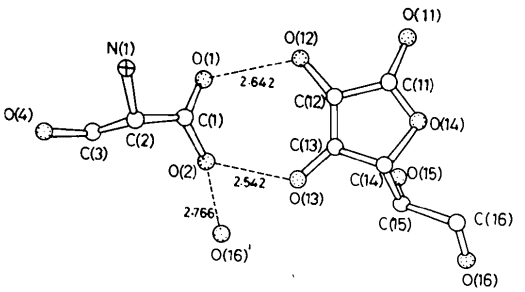

Fig. 4. The hydrogen-bonded interaction between the enediol group of ascorbic acid and the carboxylate group of serine. The primed atom belongs to a neighbouring molecule.

of the two oxygen atoms in the enediol group of the ascorbic acid molecule donates a proton to a hydrogen bond while the carboxyl group is an acceptor for two hydrogen bonds. One of the oxygen atoms in the carboxylate group of serine accepts two hydrogen bonds whereas the other accepts only one.

Unlike the structures of the crystalline complexes involving amino acids analysed so far (Bhat & Vijayan, 1976, 1977, 1978; Sudhakar & Vijayan, 1980), the unlike molecules do not aggregate into separate layers in the present structure. Instead, they aggregate separately into columns parallel to the *a* axis. The column of ascorbic acid molecules is in the form of a helix with a 2_1 screw as the axis. The column of serine molecules is generated by a periodic translation along the *a* axis. Two molecules from each unit cell are involved in each ascorbic acid column whereas only one molecule from each unit cell is involved in each serine column. There are, however, twice as many serine columns as ascorbic acid columns. Six serine columns surround each ascorbic acid column and each serine column is surrounded by three ascorbic acid columns. The ascorbic acid columns are stabilized by hydrogen bonds involving the side-chain hydroxyl groups whereas the adjacent molecules in the serine column are held together by a N—H···O hydrogen bond involving the α-amino group and the side-chain hydroxyl group. The separate columns of unlike molecules in the crystal structure are held together by a number of O—H···O and N—H···O hydrogen bonds. Among these, a pair of hydrogen bonds between the enediol group of ascorbic acid and the carboxylate group of serine, shown in Fig. 4, is worthy of special note. This is a possible model for a specific interaction between ascorbic acid and a carboxylate ion.

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