CROATICA CHEMICA ACTA CCACAA **64** (3) 311–317 (1991)

ISSN 0011-1643
UDC 547.475.2
CCA-2003

Original Scientific Paper

Crystal and Molecular Structure of (4S, 5S)-6-Ammonium-6-deoxy-3-O-ascorbate

Biserka Kojić-Prodić, Željko Daniłović, Vitomir Puntarec, Sanja Tomić
Ruđer Bošković Institute, P.O.B. 1016, 41001 Zagreb, Croatia

and

Božidar Šušković
Research Institute PLIVA, Zagreb, Croatia

Received May 13, 1991

The antitumor activity of 6-bromo-6-deoxy-L-ascorbic acid (Osmak *et al.*, *Res. Exp. Med.* 190, (1990) 443) initiated research of other C6-derivatives and the title molecule, which has been structurally characterized by X-ray diffraction methods. Molecular dynamics and semiempirical approaches have been used to evaluate side chain conformations. In the crystalline state the molecule appears as a *zwitterion* formed by dissociation of the proton at O3. The γ-lactone ring exhibits a twisted $^1T_0$ conformation. The enediole and the lactone groups are not planar. Molecular packing is dominated by a three-dimensional hydrogen bond network *via* N–H···O and O–H···O interactions. The structure is refined to an $R = 0.052$ [$F_o > 4\sigma(F_o)$] using low temperature data [103(3) K]

INTRODUCTION

The examination of the structure and chemical reactivity relationships of L-ascorbic acid and its derivatives initiated new syntheses of the halogen[2] and amino derivatives of L-ascorbic acid.[3] Many aspects of the biological functions of vitamin C and its derivatives have offered a wide range of possibilities to study structure-activity relationships. For example, the antiscurvey activity of the D-isoascorbic acid is only 1/20 of the L-ascorbic activity.[4] In relation to this we determined the absolute configuration of 6-bromo-6-deoxy-L-ascorbic acid.[5]

Among the versatile biological activities[6,7] the cofactor action of L-ascorbic acid, for the enzyme prolyl hydroxylase in the synthesis of collagen, is of paramount significance. The most recent results have shown that the plasma concentration of vitamin C is inversely proportional to the risk of angina pectoris.[8] However, at present, the anticancer activity of this molecule and its derivatives attracts lot of interest.

Various types of antitumor activity of L-ascorbic acid[9] have been described. A new group of promising antitumor agents represented by the *cis*-diamineplatinum(II) com-

plex of vitamin C with the metal –C (π-bond) coordination has exhibited good activity *in vivo*.[10] It is also an example of a new type of coordination complexes of L-ascorbic acid. Examination of anticancer activity of halogen derivatives has shown that 6-bromo-6-deoxy derivative inhibits growth of mouse melanoma B 16 cells.[1]

The present work gives details of the crystal and molecular structure of the title compound and conformation analysis of a side chain.

EXPERIMENTAL

After many crystallization trials aimed at growing crystals suitable for X-ray diffraction, the experiment with 10 mg/ml in water solution precipitated with slow evaporation of 5 ml methanol yielded tiny crystals. Crystallization took over two weeks in the refrigerator at about 275 K.

The crystallographic data and details of data collection and refinement are listed in Table I. Data reduction was performed by Enraf-Nonius SDP/VAX package[11]; Lorentz and polarization effects were corrected. The structure was solved by direct methods using the program SHELX86.[12]

TABLE I

Crystal data and details of structure determination

a) Crystal Data	
Formula	$C_6H_9O_5N$
M_r	175.14
a (Å)	5.271(2)
b (Å)	9.695(10)
c (Å)	13.976(5)
V (Å^3)	714.3(9)
D_{calc} (g/cm^3)	1.629
Z	4
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Crystal size (mm)	0.6 x 0.12 x 0.1
Linear absorption coefficient (cm^{-1})(MoKα)	0.95
F (000)	368
b) Data Collection	
Diffractometer	Enraf-Nonius-CAD4F
Radiation	MoKα (λ = 0.71073 Å) graphite-monochromator
Temperature (K)	103(3)
θ_{min}, θ_{max} (°) for cell det.	5–16
No of reflections for cell det.	25
θ_{min}, θ_{max}	2–25
$\omega/2\theta$ scan(°)	$\Delta\omega$ = 0.80 + 0.35 tanθ
h k l limits	0 → 6; 0 → 11; 0 → 13
Reflections measured	732
Reflections observed with $I > 2\sigma(I)$	448
c) Refinement	
No of parameters	145
Quantity minimized, $\Sigma\omega\|\|F_o\|-\|F_c\|\|^2$	$w = k/(\sigma^2(F_o)+0.001\ F_o^2)$ k = 1.120
R, R_w	0.052, 0.052
Max. parameter shift. $(\Delta/\sigma)_{max}$	0.55 (C6,y)
Residual electron density, $(\Delta\rho)_{max}$,$(\Delta\rho)_{min}$(eA^{-3})	0.42, –0.33

TABLE II

Final atomic coordinates and equivalent isotropic thermal parameters ($\times \sqrt{10^4}$)

	x	y	z	UEQ/Å^2
O1	0.4213(13)	0.0224(7)	1.1404(4)	224(22)
O2	0.7972(11)	−0.1973(7)	1.0832(4)	143(18)
O3	1.0013(12)	−0.0894(6)	0.8952(4)	149(18)
O4	0.5274(11)	0.1229(6)	1.0032(4)	132(17)
O5	0.4434(12)	−0.0233(6)	0.8149(4)	144(18)
N1	0.3071(15)	0.2211(7)	0.7154(5)	176(25)
C1	0.5515(18)	0.0163(9)	1.0681(6)	145(27)
C2	0.7314(17)	−0.0803(9)	1.0334(7)	179(29)
C3	0.8342(20)	−0.0360(10)	0.9506(6)	161(31)
C4	0.7091(18)	0.1000(10)	0.9273(7)	158(31)
C5	0.5824(18)	0.1017(10)	0.8309(6)	148(28)
C6	0.4172(19)	0.2243(9)	0.8142(6)	150(32)

$$U_{eq} = 1/3 \sum_i \sum_j U_{ij} a_i^* a_j^* \vec{a}_i \vec{a}_j$$

Scattering factors were those included in SHELX77.[12] The hydrogen atoms were located from a difference Fourier map. The structure was refined by a full-matrix, least-squares method using the program SHELX77.[13] Interatomic distances, bond angles and torsion angles calculations, and hydrogen bond analysis were done using the program PLATON incorporated in the EUCLID PACKAGE.[14] Drawings were prepared by the ORTEP[15] and PLUTON program.[14]

Calculations were carried out on the MICROVAX II in the X-ray Laboratory of Rudjer Bošković Institute, Zagreb, Yugoslavia.

Final atomic coordinates and equivalent isotropic temperature factors are listed in Table II.*

MOLECULAR AND CRYSTAL STRUCTURE

Interatomic distances, bond and selected torsion angles are listed in Tables III and IV. The molecular structure with atom numbering is presented by means of the ORTEP plot (Figure 1). The diagram illustrating the packing of molecules in the crystal lattice *via* hydrogen bonds is given in Figure 2; hydrogen bond geometry is displayed in Table V.

Since the L-ascorbic acid is a precursor for the title compound, the (4*S*) configuration is assumed. There was no reason to expect inversion of the configuration at C4. Therefore, the known chirality of C4 was used as an internal standard for the absolute configuration at C5; thus, the absolute configuration is (4*S*, 5*S*). Crystallography of the ascorbates was reviewed by Hvoslef[16] in 1982. Cambridge Structural Database,[17] version 4 (1991) revealed 19 crystal structures of ascorbic acid and its derivatives. The structure of the title compound is the only example with *zwitterion*. The dissociation at C3 is detected in all salts and in the present structure. The only exception is the complex of *cis*-diamineplatinum(II) with L-ascorbic acid[10], where the coordination is *via* O5 and π-interaction of C2=C3. However, bivalent anions have not been isolated and characterized so far.

* Supplementary material (Final atomic coordinates of H-atoms, Anisotropic thermal parameters, and Observed and calculated structure factors) is available on request from the Redaction of *CCA*.

TABLE III

Bond distances (Å) and angles (degrees)

O1	– C1	1.223(11)	C1 – O4 – C4	107.8(7)
O2	– C2	1.375(11)	O1 – C1 – O4	117.0(8)
O3	– C3	1.282(11)	O4 – C1 – C2	109.3(7)
O4	– C1	1.381(10)	O1 – C1 – C2	133.6(8)
O4	– C4	1.446(11)	O2 – C2 – C1	122.7(8)
O5	– C5	1.434(11)	C1 – C2 – C3	110.6(8)
N1	– C6	1.498(11)	O2 – C2 – C3	126.6(8)
C1	– C2	1.418(13)	O3 – C3 – C2	131.8(9)
C2	– C3	1.348(13)	C2 – C3 – C4	106.7(8)
C3	– C4	1.510(14)	O3 – C3 – C4	121.5(8)
C4	– C5	1.503(13)	O4 – C4 – C3	105.4(7)
C5	– C6	1.492(13)	C3 – C4 – C5	113.4(8)
			O4 – C4 – C5	111.2(8)
			O5 – C5 – C4	110.9(8)
			C4 – C5 – C6	114.1(7)
			O5 – C5 – C6	110.5(8)
			N1 – C6 – C5	110.7(7)

TABLE IV

Selected torsion angles (degrees)

O4 — C1 — C2 — C3	3 (1)
C1 — C2 — C3 — C4	–1 (1)
C2 — C3 — C4 — O4	–1 (1)
C3 — C4 — O4 — C1	3.2(9)
C4 — O4 — C1 — C2	–4.0(9)
C2 — C3 — C4 — C5	–123.1(9)
C3 — C4 — C5 — C6	169.0(8)
C3 — C4 — C5 — O5	43 (1)
O5 — C5 — C6 — N1	–56.5(9)

TABLE V

Hydrogen bonds

D — H ... *A*	D..*A*(Å)	D—H(Å)	H..*A*(Å)	D—*A*...H	Symmetry operation on *A*
O2 — H2 ... O3	2.608(9)	1.050(99)	1.810(99)	129(1)	1/2+x–1, 1/2–y, –z
N1 — H12 ... O2	2.796(9)	1.080(11)	1.726(9)	170.3(7)	1/2–x+1, –y, 1/2+z–1
N1 — H13 ... O3	2.900(9)	1.080(10)	1.980(9)	141.0(7)	–x+1, 1/2+y, 1/2–z–1
N1 — H13 ... O5	2.837(9)	1.080(10)	2.344(10)	106.0(7)	–x+1, 1/2+y, 1/2–z–1
N1 — H11 ... O1	2.850(10)	1.080(10)	1.925(9)	141.5(7)	1/2–x, –y, 1/2+z–1
O5 — H51 ... O1	3.105(8)	1.030(99)	2.140(99)	155(1)	1/2–x, –y, 1/2+z–1

The γ-lactone ring is not planar. It exhibits a twisted $^1T_0$ conformation with the mean value of torsion angles of 2.4(9)° (Table IV). Both lactone (C1, C2, O1, C4, O4) and enediol groups are not planar. The mean deviation from the best least-squares plane of lactone group is 0.022(9)° and for enediol group it is 0.010(8)Å. The torsion angle O2–C2–C3–O3 (about double bond) is 4(2)°. These findings are in agreement with the observations of Kanters, Roelofsen, and Alblas[18] in the structure of sodium D-isoascorbate. The conjugated system O1=C1–C2=C3–O3 in the title compound showed

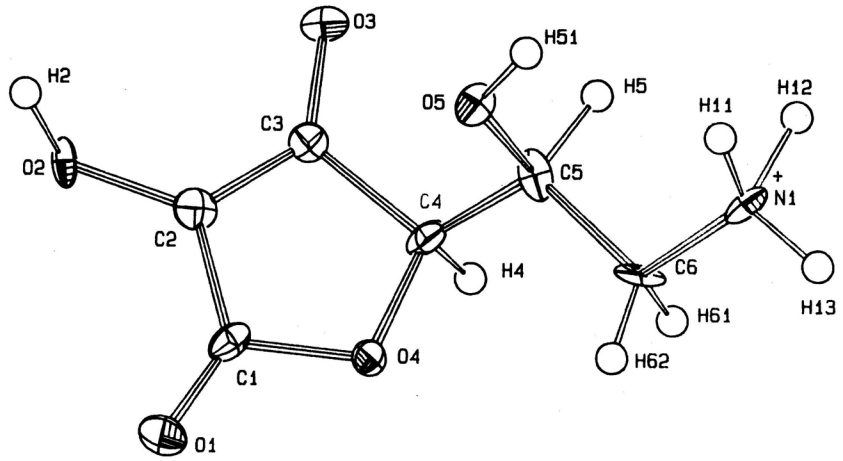

Figure 1. The ORTEP drawing of the molecule with atom numbering.

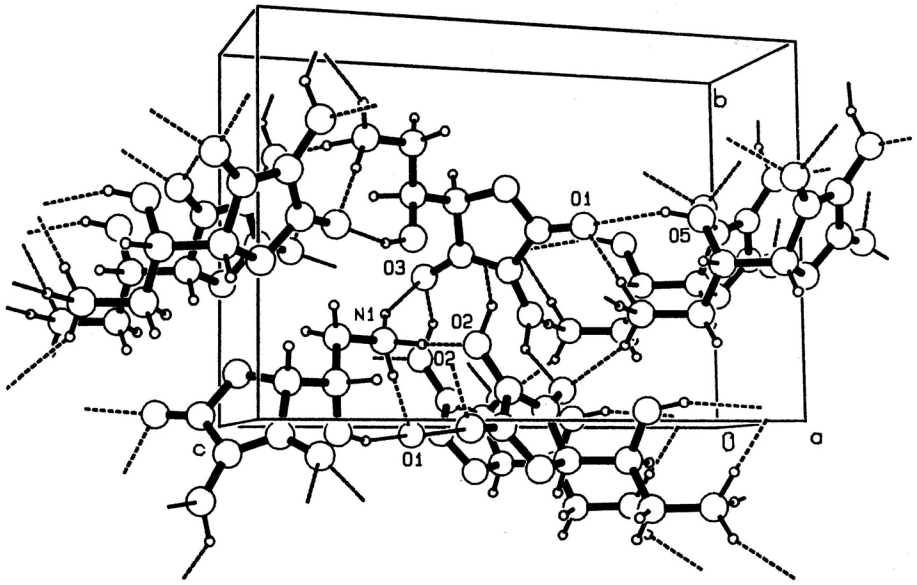

Figure 2. Molecular packing *via* hydrogen bonds.

shortening of the single and lengthening of the double bonds (Table III), as it was observed in other ascorbates.[16,18] The shortening of the C3–O3 bond to 1.283(11) Å, due ionization, agrees with the values observed in the structure of sodium ascorbate[19] (1.287 Å) and L-arginine L-ascorbate[20] (1.275(6) Å).

The opening of the O3–C3–C4 angle to the value of 121.5(8)° was detected in the structure of sodium L-ascorbate[19] (122.9°) and sodium D-isoascorbate[18] (121.7(4)) Å).

SIDE CHAIN CONFORMATION

The side chain backbone (C4, C5, C6, N1) is in the extended zigzag conformation with a mean deviation from the plane of 0.014(9) Å. The angle between the best least-squares planes through the γ-lactone ring and this plane is 128.6(5)°.

The conformation about C5–C6 bond with respect to the O5 hydroxyl group and bulky substituent at C6, being halogen (Cl, Br) or NH_3^+, is different from that observed in L-ascorbic acid. In both conformers of L-ascorbic acid the antiperiplanar conformation occurs. However, in 6-bromo-, one conformer of 6-chloro-, and 6-ammonium- derivatives, the (–)synclinal conformation was observed.[4]

The relative orientation of the side chain was studied by semiempirical (AMPAC/MOPAC),[22] molecular mechanics[23], and molecular dynamics[24] methods. Calculations were performed by using X-ray atomic coordinates and by those generated from stereochemical grounds (template). Both ways gave consistent results. For the analysis of a side chain conformation two torsion angles were selected (O5–C5–C6–N1; C4–C5–C6–N1). From the molecular dynamic studies two different conformers (Figure 3) were recognized: an extended one with the torsion angle C4–C5–C6–N1 = 177°) like in the crystalline state, 177.7(7)°) and a folded one with the value of torsion angle –69°. Molecular dynamics and molecular mechanics (in a gas phase) indicated that folded conformation is slightly energetically favoured with respect to the extended one. In favour of this conformation there is an intramolecular hydrogen bond N–H...O4 with the N...O distance about 2.8 Å. The *ab initio* study of ascorbic acid[21] (in a gas phase) by the STO-3G optimization also revealed the most stable conformer with three intramolecular hydrogen bonds. However, in the extended conformer, the separation is about 4.3 Å; the same distance is observed in the present crystal structure.

Theoretical studies of vitamin C and related systems were reported by Eckert-Maksić, Maksić, and Bischof (1987).[25]

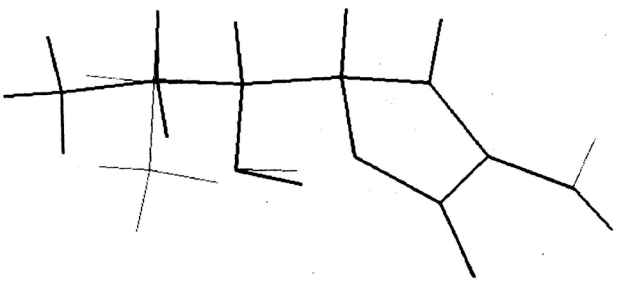

Figure 3. Overlap of the two stable conformations obtained by MNDO geometry optimization calculations.

CRYSTAL PACKING

Molecular packing (Figure 2) is dominated by the three-dimensional network of O–H...O and N–H...O hydrogen bonds (Table V). The hydroxyl groups O2 and O5 act as donors and acceptors. The ionized hydroxyl group O3 can be an acceptor only as well as the carbonyl O1 of a lactone group. Each of them is a double acceptor (Table V). The NH_3^+ group is a donor only. One of its protons, H13 is involved in a bifurcated hydrogen bond between O3 and O5. The endocyclic O4 is not included in hydrogen bonds at all.

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SAŽETAK

Kristalna i molekulska struktura (4S.5S)-6-amonium-6-deoksi-3-*O*-askorbata

B. Kojić-Prodić, Ž. Danilović, V. Puntarec, i S. Tomić

Antitumorska aktivnost 6-bromo-6-deoksi-L-askorbinske kiseline (Osmak *et al. Res. Exp. Med.* 190 (1990) 443) inicirala je istraživanje drugih C6-derivata, među kojima i spoja koji je predmet ovog rada. Metodom rendgenske strukturne analize utvrđena je njegova molekulska i kristalna struktura. Molekulska dinamika i semiempirijske metode korištene su za određivanje konformacija bočnog lanca. U kristalu molekula postoji u obliku dvostrukog iona, koji je nastao disocijacijom protona na položaju O3. γ-laktonski prsten ima izlomljenu konformaciju tipa ^1T_o. Enediolska i laktonska skupina nisu planarne. Molekulsko pakovanje ostvareno je sistemom trodimenzijskih vodikovih veza tipa N-H···O i O-H···O. Struktura je utočnjena do vrijednosti R = 0.052 [$F_o > 4\sigma(F_c)$] korištenjem podataka mjerenih na 103(3) K.