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The crystal structure and physicochemical properties of L-ascorbic acid 2-glucoside

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ABSTRACT

The stable L-ascorbic acid glucoside produced by the action of the cyclomaltodextrin glucanotransferase (CGTase, EC 2.4.1.19) from *Bacillus stearothermophilus* was crystallized from an aqueous solution. Determination of the molecular structure by single crystal X-ray analysis showed the compound to be 2-*O*-α-D-glucopyranosyl-L-ascorbic acid (AA-2G). The crystals are orthorhombic, space group $P2_12_12_1$, with unit-cell dimensions $a = 11.929$ Å, $b = 24.351$ Å, and $c = 4.864$ Å. The D-glucopyranose residue has the $^4C_1$ conformation. These conclusions are in good agreement with those based on the ^{13}C-NMR spectrum. The general physicochemical properties of crystalline AA-2G are reported.

INTRODUCTION

Two kinds of L-ascorbic acid glucosides can be enzymically synthesized by transglucosylation using α-glucosidases [1–5]. The D-glucopyranosyl moiety of maltose is site-specifically transferred to the C-2 hydroxyl group of L-ascorbic acid to give 2-*O*-α-D-glucopyranosyl-L-ascorbic acid (AA-2G) by the α-glucosidases from rat intestine and rice seed [1–3]. A different glucoside of L-ascorbic acid, 6-*O*-α-D-glucopyranosyl-L-ascorbic acid (AA-6G), is synthesized by the action of α-glucosidase from *Aspergillus niger* [4,5]. As with L-ascorbic acid, AA-6G has a strong reducing activity. In contrast, AA-2G shows no reducing activity; it is highly stable against oxidation and heat treatment [6]. In recent studies, we have found cyclomaltodextrin glucanotransferase (CGTase, EC 2.4.1.19) from *Bacillus stearothermophilus* highly active in the formation of AA-2G, compared with other transglycosylation enzymes. Conditions suitable for the mass production of AA-2G were

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established [7,8]. Since the structural assignment of AA-2G was made on the basis of spectral and chemical data alone, we considered it desirable to confirm it by single crystal X-ray analysis. In the present study, we obtained crystals of AA-2G from aqueous solution, and we now describe the X-ray crystallographic structure and physicochemical properties of this stable L-ascorbic acid glucoside.

EXPERIMENTAL

Preparation of AA-2G.—AA-2G having a purity of 97% on HPLC analysis was prepared according to the method of Aga et al. [7] Cations were eliminated using an ion-exchange resin in the H^+ form. Subsequently, an aqueous solution of AA-2G was brought to a concentration of 70% (w/w) and stored at room temperature, whereupon colorless crystals formed. These were washed with methanol, dried in vacuo, and used for investigations of the physicochemical properties.

General methods.—The melting point was determined with a Yamato MP-21 melting point apparatus. The heat of fusion (ΔH_f) was determined with a Rigaku DSC-8230 differential scanning calorimeter. The ^{13}C-NMR spectrum was recorded with a JEOL GSX-400 spectrometer for a solution in D_2O containing sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate (TSP) as internal standard. For

TABLE I

Crystal data and structure determination and refinement data for L-ascorbic acid 2-glucoside

Crystal data	
Formula	$C_{12}H_{18}O_{11}$
Mol wt	338.26
Crystal system	Orthorhombic
Cell dimensions (Å)	*a* 11.929(1)
	b 24.351(2)
	c 4.864(1)
Space group	$P2_12_12_1$
Z (molecules/cell)	4
D_{calcd} (g/cm^3)	1.590
D_{obsd} (g/cm^3)	1.583
Structure determination and refinement data	
Cu K_α radiation	$\lambda = 1.5418$ Å
Instrument	AFC-5R (RIGAKU)
$2\theta_{max}$	120°
Range of *hkl*	$0 \leq h \leq 14$, $0 \leq k \leq 28$, $0 \leq l \leq 6$
Corrections for absorption	no correction
Number of measured reflections	1354
Number of observed reflections, $I > 3\sigma$	1266
Final refinement values R, R_w	0.079, 0.098
S = Error in an observation of unit weight	0.97
Final shift/error maximum	0.37
Function minimized	$\Sigma w(\Delta F)^2$, where $w = 1$ if $\lvert F_0 \rvert \leq 10$
	$w = (10/\lvert F_0 \rvert)^2$ if $\lvert F_0 \rvert > 10$

field desorption MS a Hitachi M-80 mass spectrometer was used. Optical rotations were measured with a Yanaco automatic polarimeter OR-50. The solubility was determined by measuring the water content of a saturated solution using the

TABLE II

Fractional atomic coordinates and equivalent isotropic thermal parameters for L-ascorbic acid 2-glucoside [a]

Atom	x	y	z	B_{eq}
GC-1	$10970(5)\times10^{-4}$	$6089(2)\times10^{-4}$	$7026(12)\times10^{-4}$	1.7(3)
GC-2	11224(5)	5506(2)	6239(13)	1.9(3)
GC-3	10502(5)	5295(2)	3892(13)	1.5(2)
GC-4	9267(5)	5366(2)	4559(13)	1.0(3)
GC-5	9054(4)	5976(2)	5250(14)	1.7(3)
GC-6	7863(5)	6105(3)	6087(19)	3.6(3)
GO-2	12385(3)	5463(2)	5585(10)	2.1(2)
GO-3	10718(3)	4730(2)	3388(10)	2.5(2)
GO-4	8597(4)	5234(2)	2231(11)	1.1(2)
GO-5	9750(3)	6134(2)	7530(9)	1.8(2)
GO-6	7412(4)	5752(2)	8159(11)	2.4(2)
AC-1	11697(4)	7306(2)	7056(14)	1.3(3)
AC-2	11091(4)	6973(2)	5073(12)	1.4(2)
AC-3	10457(4)	7308(2)	3538(13)	1.4(3)
AC-4	10609(4)	7888(2)	4469(12)	1.4(2)
AC-5	9548(5)	8174(2)	5511(13)	1.5(2)
AC-6	9815(5)	8735(2)	6634(16)	2.0(3)
AO-1	12360(4)	7163(2)	8797(10)	2.0(2)
AO-2	11251(3)	6425(2)	4722(9)	1.8(2)
AO-3	9797(4)	7145(2)	1493(10)	1.0(2)
AO-4	11408(3)	7844(1)	6725(9)	0.9(2)
AO-5	9064(3)	7827(2)	7566(11)	3.3(2)
AO-6	8792(4)	8954(2)	7765(12)	2.2(2)
GH-1	$1132(11)\times10^{-3}$	$622(5)\times10^{-3}$	$894(34)\times10^{-3}$	
GH-2	1107(10)	524(5)	804(35)	
GH-3	1071(10)	554(5)	205(31)	
GH-4	905(11)	509(5)	629(32)	
GH-5	924(10)	620(5)	334(35)	
GH-6A	733(10)	608(5)	425(35)	
GH-6B	784(10)	653(5)	686(28)	
GH(O-2)	1254(10)	579(5)	468(31)	
GH(O-3)	1138(11)	474(5)	263(35)	
GH(O-4)	831(10)	491(5)	273(31)	
GH(O-6)	800(12)	570(5)	931(33)	
AH-4	1088(10)	814(5)	271(33)	
AH-5	895(11)	824(5)	382(33)	
AH-6A	1046(10)	870(5)	825(33)	
AH-6B	1013(11)	900(5)	498(33)	
AH(O-3)	946(11)	743(5)	92(33)	
AH(O-5)	856(12)	804(5)	828(31)	
AH(O-6)	899(11)	923(5)	861(34)	

[a] Each coordinate is followed by its least-squares standard deviation in parentheses. $B_{eq} = 4/3(\Sigma_i\Sigma_j B_{ij}a_i a_j)$.

routine quartz-sand method. Reducing activity was measured by the indophenol (DCIP) method [9].

Physicochemical properties.—AA-2G had mp 158.5–159.5°; $[\alpha]_D^{20}$ +190° (*c* 5.0, H_2O) at pH 2, +246° at pH 7.1; UV: λ_{max} 238 nm at pH 2.0, 260 nm at pH 7.0; ^{13}C-NMR: 63.1 (GC-6), 65.1 (AC-6), 71.9 (AC-5, GC-4), 74.1 (GC-5), 75.5 (GC-2), 75.9 (GC-3), 80.0 (AC-4), 101.9 (GC-1), 119.8 (AC-2), 169.6 (AC-3), and 176.3 (AC-1); FD-MS: *m/z* 339 $(M + H)^+$.

Anal. Calcd for $C_{12}H_{18}O_{11}$ (338.27): C, 42.61; H, 5.36. Found: C, 42.65; H, 5.37.

The compound has only one ionization constant (p*K* 3.0) because of the substitution of AO-2-H by the D-glucopyranosyl residue. The heat of fusion, ΔH_f, was found to be 38.5 kJ/mol, and the solubility in water is 125 g per 100 g at 25°. AA-2G shows no reducing power.

X-ray crystallographic analysis.—A colorless and pillared crystal of AA-2G with the dimensions 0.25 × 0.35 × 0.70 mm was used for X-ray analysis. The details of the data collection and structure refinement are given in Table I. Twenty reflections within the range $44 < 2\theta < 46°$ were used to determine the lattice parameters. The structure was solved by SHELX-86 (ref. 10), and refined by a block-diagonal least-squares program (HBLS-V) using anisotropic temperature factors for nonhydrogen atoms and isotropic temperature factors for hydrogen atoms. The positional parameters of all atoms are shown in Table II *.

RESULTS AND DISCUSSION

Molecular structure.—The molecular structure (ORTEP) of AA-2G obtained from X-ray crystallographic analysis is shown in Fig. 1, with the atomic notation indicated. The D-glucose residue has the $^4C_1$ pyranose conformation and is in α-(1 → 2) linkage with L-ascorbic acid. The bond lengths involving carbon atoms are given in Table III. The C–C bond lengths of the D-glucose residue are in the range 1.511–1.544 Å (mean 1.522 Å), and the C–O bond lengths are in the range 1.406–1.447 Å (mean 1.427 Å). The endocyclic C–O bond lengths of the α-D-glucose moiety are unequal, as reported for many pyranose compounds [11,12]. The glycosidic bond lengths in disaccharides are usually shorter than the other C–O bonds, whereas the glucosidic bond length of AA-2G (GC-1–AO-2) is the longest. The C–C–C angles of the D-glucose residue are in the range 108.4–114.5° (mean 111.2°) and the C–C–O angles are in the range 105.3–114.7° (mean 109.7°). These values fall into the normal range observed for D-glucopyranose residues in several disaccharides. The angle in the bridge, GC-1–AO-2–AC-2, is 114.7°, in good agreement with the usual values for α-glucosidically linked disaccharides.

* Tables of observed and calculated structure factors, anisotropic thermal parameters, and bond distances, valence angles, and torsional angles have been deposited with, and can be obtained from, Elsevier Science Publishers B.V., BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/000/*Carbohydr. Res.*, 232 (1992) 197–205.

TABLE III

Bond lengths for L-ascorbic acid 2-glucoside

Bond	Length (Å) [a]	Bond	Length (Å) [a]
GC-1–GC-2	1.518(9)	AC-1–AC-2	1.453(9)
GC-2–GC-3	1.519(9)	AC-2–AC-3	1.339(8)
GC-3–GC-4	1.518(9)	AC-3–AC-4	1.493(9)
GC-4–GC-5	1.544(9)	AC-4–AC-5	1.531(9)
GC-5–GC-6	1.511(11)	AC-5–AC-6	1.504(10)
GC-1–AO-2	1.447(7)	AC-1–AO-1	1.209(8)
GC-1–GO-5	1.406(8)	AC-1–AO-4	1.364(8)
GC-2–GO-2	1.425(8)	AC-2–AO-2	1.359(7)
GC-3–GO-3	1.420(8)	AC-3–AO-3	1.329(8)
GC-4–GO-4	1.422(8)	AC-4–AO-4	1.458(8)
GC-5–GO-5	1.438(8)	AC-5–AO-5	1.431(8)
GC-6–GO-6	1.430(11)	AC-6–AO-6	1.441(10)

[a] Standard deviations are given in parentheses.

TABLE IV

Endo- and selected exo-cyclic torsional angles in L-ascorbic acid 2-glucoside

Angle	L-Ascorbic acid 2-glucoside (°) [b]	L-Ascorbic acid [a]	
		Molecule A (°)	Molecule B (°)
Endocyclic			
AC-1–AC-2–AC-3–AC-4	−1.2(5)	−1.3	−2.4
AC-2–AC-3–AC-4–AO-4	0.0(4)	0.8	4.8
AC-3–AC-4–AO-4–AC-1	2.6(4)	0.1	−5.4
AC-4–AO-4–AC-1–AC-2	−1.0(4)	−0.9	4.3
AO-4–AC-1–AC-2–AC-3	−0.8(4)	1.4	−1.2
GO-5–GC-1–GC-2–GC-3	52.3(3)		
GC-1–GC-2–GC-3–GC-4	−52.6(3)		
GC-2–GC-3–GC-4–GC-5	55.3(3)		
GC-3–GC-4–GC-5–GO-5	−58.4(2)		
GC-4–GC-5–GO-5–GC-1	61.5(3)		
GC-5–GO-5–GC-1–GC-2	−58.0(3)		
Exocyclic			
AO-4–AC-4–AC-5–AC-6	58.5(4)		
AO-4–AC-5–AC-5–AO-5	−54.1(2)		
AC-4–AC-5–AC-6–AO-6	−176.2(5)		
AO-5–AC-5–AC-6–AO-6	−55.9(3)		
GC-1–AO-2–AC-2–AC-1	67.1(3)		
GC-1–AO-2–AC-2–AC-3	−120.1(4)		
GC-4–GC-5–GC-6–GO-6	49.6(5)		
GO-5–GC-5–GC-6–GO-6	−71.2(2)		
GO-5–GC-1–AO-2–AC-2	62.3(2)		
GC-2–GC-1–AO-2–AC-2	−176.9(4)		

[a] Data from ref. 16. [b] Standard deviations are given in parentheses.

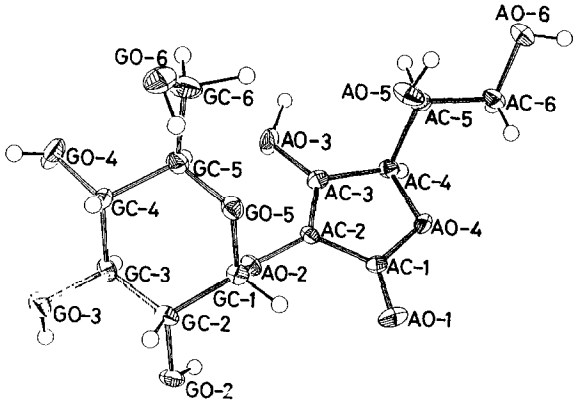

Fig. 1. Molecular structure of L-ascorbic acid 2-glucoside.

The endo- and exo-cyclic torsional angles are given in Table IV. The endocyclic torsional angles vary from 52.3° to 61.5° (mean 56.4°), within the normal range for the chair conformation of glucopyranose [11–15]. The conformation of the GC-5–GC-6 bond in the D-glucosyl residue of AA-2G is *gauche–gauche*, while that in the D-glucosyl residue of α- and β-maltose is *gauche–trans* [16,17].

TABLE V

Distances of individual atoms from the best planes through parts of the ascorbic acid ring system

Atom	L-Ascorbic acid 2-glucoside (Å)	L-Ascorbic acid	
		Molecule A (Å)	Molecule B (Å)
Lactone group			
AC-1	0.0009	0.0110	0.0008
AC-2	−0.0028	0.0025	−0.0139
AO-1	0.0033	−0.0127	0.0192
AO-4	−0.0068	0.0113	−0.0346
AC-4	0.0055	−0.0122	0.0285
Enediol group			
AO-2	0.0303	−0.0008	−0.0034
AC-2	−0.0430	0.0014	0.0047
AC-3	−0.0089	−0.0004	0.0008
AC-4	0.0215	−0.0004	−0.0024
AO-3	0.0001	0.0002	0.0002
Ring			
AC-1	0.0051	−0.0064	0.0164
AC-2	−0.0040	0.0077	0.0047
AC-3	0.0015	−0.0060	−0.0215
AC-4	0.0016	0.0019	0.0297
AO-4	−0.0042	0.0028	−0.0292

The L-ascorbic acid residue is composed of a planar five-membered ring and a side chain, and there is a large variation in the C–O bond lengths of this residue, especially in the ring. Such a phenomenon is usually observed in lactone groups, because of their potential for resonance. Comparison of the endocyclic torsional

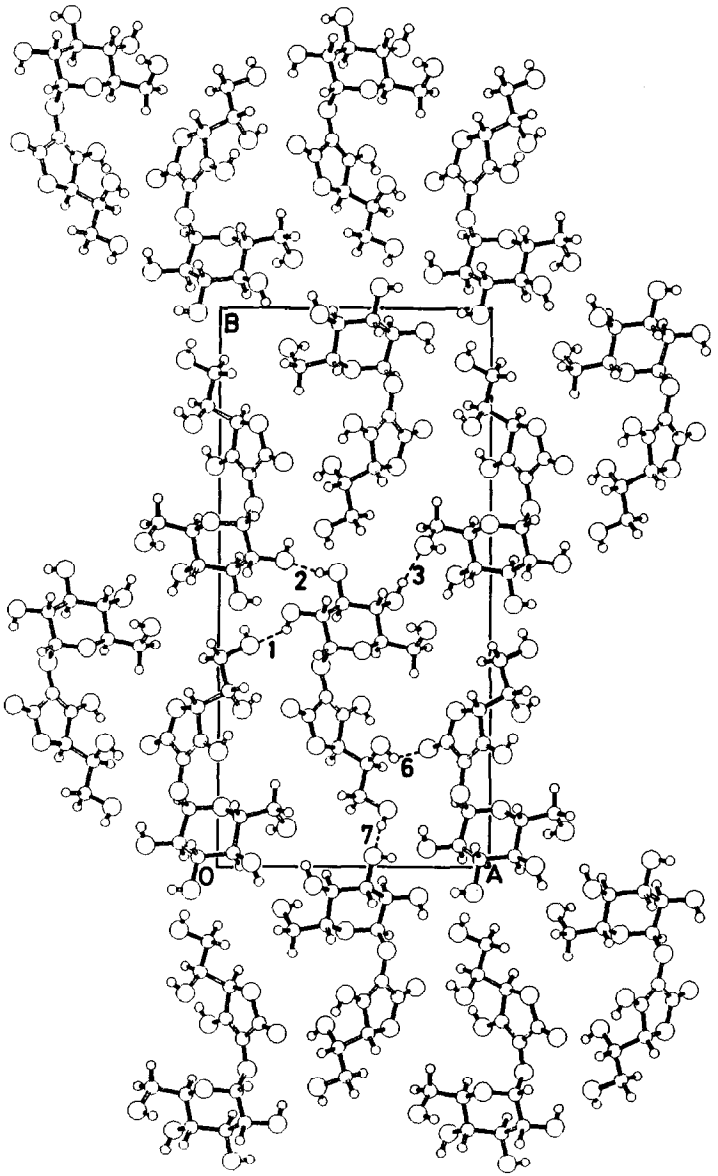

Fig. 2. Molecular packing viewed along the *c* axis. Hydrogen bonds, numbered according to Table VI, are shown by broken lines.

TABLE VI

Hydrogen bond distances and angles in crystals of L-ascorbic acid 2-glucoside

Number	Location	H···O (Å)	O···O (Å)	O–H···O (°)	Data set
1	GO-2–H(GO-2)···AO-6	2.01	2.73	134.3	$x+1/2, -y+3/2, -z+1$
2	GO-3–H(GO-3)···GO-2	1.85	2.68	150.6	$-x+5/2, -y+1, z-1/2$
3	GO-4–H(GO-4)···GO-6	1.83	2.72	169.7	$-x+3/2, -y+1, z-1/2$
4	GO-6–H(GO-6)···GO-4	1.95	2.73	145.0	$x, y, z+1$
5	AO-3–H(AO-3)···AO-5	1.95	2.68	143.0	$x, y, z-1$
6	AO-5–H(AO-5)···AO-1	2.08	2.70	128.1	$x-1/2, -y+3/2, -z+2$
7	AO-6–H(AO-6)···GO-3	1.93	2.72	160.2	$-x+2, y+1/2, -z+3/2$

angles of the L-ascorbic acid residue with the corresponding values for L-ascorbic acid (Table IV) shows the endocyclic distortions are minute, and the endocyclic torsional angles are little influenced by the glucosylation of the C-2 hydroxyl group. Comparison of the planarity of the lactone and enediol groups reveals that in AA-2G the enediol group is nonplanar, whereas the planarities of the lactone group and ring atoms are better than those of L-ascorbic acid (Table V). The marked distortion of the enediol group is caused by the binding of α-D-glucose to AO-2.

The molecular packing is shown in Fig. 2, and the intermolecular hydrogen bonds are listed in Table VI. The molecules are oriented perpendicularly to the *c* axis. All seven hydroxyl groups are involved in hydrogen-bond networks, and act as donors. Although the hydrogen on O-6 of α-D-glucopyranose residues is generally directed outward, that of AA-2G projects inward. This is caused by bonding with GO-4 as acceptor.

L-Ascorbic acid has two ionization constants, one for the dissociation of the C-3 hydroxyl group (pK 4.1) and one for the C-2 hydroxyl group (pK 11.8). The glucosylation of the 2-hydroxyl causes a shift of the pK of the C-3 hydroxyl from 4.1 to 3.0, and the disappearance of reducing activity.

It is well known that the UV spectra of L-ascorbic acid derivatives vary with pH, and that λ_{max} values may be shifted by substitution at O-2 or O-3 (refs. 17–20). The λ_{max} of AA-2G is shorter than that of L-ascorbic acid, as expected for a 2-*O*-substituted derivative.

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