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Note

The crystal structure of di-D-fructose anhydride III, produced by inulin D-fructotransferase

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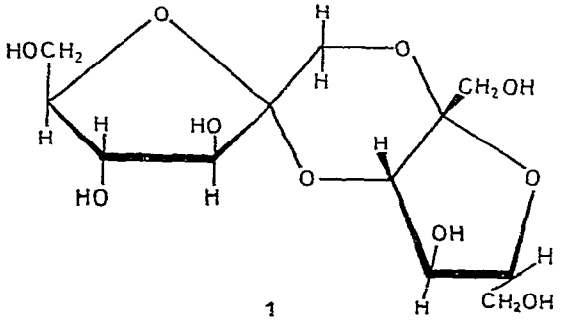
Di-D-fructose anhydride III has been isolated as a by-product of the acid hydrolysis of inulin[1], and identified as di-D-fructofuranose 2′,1:2,3′-dianhydride[2] (**1**). In later studies, Tanaka and Uchiyama[3–5] showed that inulin D-fructotransferase (EC 2.4.1.93) of *Arthrobacter ureafaciens* produces di-D-fructose anhydride III from inulin in high yield. Recently, the dianhydride was assigned the structure α-D-fructofuranose β-D-fructofuranose 2′,1:2,3′-dianhydride by a carbon-13 nuclear magnetic resonance study[6], but the molecular conformation was not established. We now report the X-ray structure of the dianhydride.



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EXPERIMENTAL

Difructose anhydride III was prepared from an inulin culture-medium of *A. ureafaciens*[7]. After two recrystallizations from 95% (v/v) ethanol, colorless prismatic crystals were obtained; m.p. 162°, $[\alpha]_D^{20}$ +136° (*c* 1.98, H_2O). The space group was determined from preliminary, Weissenberg photographs. Crystal data are: a = 16.438(2), b = 10.483(2), c = 8.0928(7) Å, space group $P2_12_12_1$, Z = 4. The measured and calculated crystal densities are D_m = 1.548 mg/m^3, and D_c = 1.544 mg/m^3. A

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crystal of size $0.25 \times 0.3 \times 0.4$ mm^3 was used for data collection on a Rigaku C-4R diffractometer, with graphite-monochromated MoKα radiation. Cell dimensions were determined by least-squares calculations with 2θ values of 22 reflections measured on the diffractometer. Intensity data of 2156 reflections with $2\theta \leqslant 58°$ were collected by use of the $\omega - 2\theta$ mode, with a scanning rate of $8°.\text{min}^{-1}$ in 2θ. The number of observed reflections, $F_0 > 2\sigma(F_0)$ is 2034. The data were corrected for Lorentz and polarization factors, but not for absorption.

The structure was solved with MULTAN78[8]. The E map revealed all the non-hydrogen atoms. After the block-diagonal, least-squares refinements[9] with anisotropic thermal parameters, all of the hydrogen atoms were located on a difference synthesis. The positional parameters for the hydrogen atoms were included in the refinements, with a fixed, isotropic, thermal parameter, $B = 2.5$ Å^2. The least-squares function refined is in the form $\Sigma w(|F_0| - k|F_c|)^2$. A weighting scheme of the type $w = 1 - \exp[-15s^2]$ with $s = \sin\theta/\lambda$ was used. The final R value was 0.063. The positional parameters are given in Table I*, the bond lengths and angles in Table II, and

TABLE I

POSITIONAL PARAMETERS ($\times 10^4$; FOR HYDROGEN ATOMS, $\times 10^3$)

Atom	*x*	*y*	*z*	*Atoms*	*x*	*y*	*z*
O-1	3296(2)	−323(3)	7242(4)	HO-3	253(4)	−368(6)	779(9)
O-2	3505(2)	−2059(3)	3458(4)	HO-4	180(4)	−474(6)	468(9)
O-3	2498(2)	−3146(3)	7090(4)	HO-6	309(4)	−246(6)	−108(9)
O-4	2142(2)	−4704(3)	3884(4)	HC-11	311(4)	−18(6)	471(8)
O-6	3250(2)	−2358(3)	−9(4)	HC-12	244(4)	−96(6)	597(9)
C-1	3000(2)	−796(3)	5721(6)	HC-3	334(4)	−397(6)	593(9)
C-2	3430(2)	−2035(3)	5203(5)	HC-4	199(4)	−284(6)	405(8)
C-3	2957(2)	−3261(3)	5632(5)	HC-5	340(4)	−381(6)	222(9)
C-4	2441(2)	−3446(3)	4086(5)	HC-61	230(4)	−329(6)	43(9)
C-5	3040(2)	−3089(3)	2740(5)	HC-62	235(4)	−187(6)	147(8)
C-6	2639(2)	−2628(4)	1207(5)	HO-1′	412(4)	−94(6)	1039(9)
O-1′	4436(2)	−624(3)	9915(5)	HO-4′	577(4)	−101(6)	335(9)
O-2′	4142(2)	1203(2)	6124(4)	HO-6′	469(4)	349(6)	433(9)
O-3′	4202(2)	−2118(2)	5979(4)	HC-11′	492(4)	72(6)	865(9)
O-4′	5752(2)	−548(3)	4232(4)	HC-12′	399(4)	106(6)	911(9)
O-6′	4329(2)	3147(3)	3635(5)	HC-3′	507(4)	−115(6)	708(8)
C-1′	4392(3)	416(4)	8831(5)	HC-4′	458(4)	−58(6)	385(8)
C-2′	4113(2)	74(3)	7107(5)	HC-5′	522(4)	150(6)	568(9)
C-3′	4632(2)	−936(3)	6189(5)	HC-61′	494(4)	169(6)	246(10)
C-4′	4915(2)	−292(4)	4589(5)	HC-62′	404(4)	146(6)	272(9)
C-5′	4779(2)	1135(3)	4911(5)				
C-6′	4515(3)	1826(4)	3369(6)				

*Lists of the anisotropic thermal parameters for non-hydrogen atoms, the least-squares best planes, and the observed and calculated structure factors are deposited with and can be obtained from: Elsevier Scientific Publishing Company, BBA Data Deposition, P.O. Box 1527, Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/222/*Carbohydr. Res.*, 107 (1982) 255–262.

TABLE II

BOND LENGTHS[a] AND ANGLES[a]

Atoms	*Length (Å)*	*Atoms*	*Length (Å)*
C-1–O-1	1.414	C-1′–O-1′	1.401
C-2–O-3′	1.417	C-2′–O-1	1.410
C-2–O-2	1.418	C-2′–O-2′	1.428
C-3–O-3	1.406	C-3′–O-3′	1.437
C-4–O-4	1.416	C-4′–O-4′	1.431
C-5–O-2	1.445	C-5′–O-2′	1.437
C-6–O-6	1.434	C-6′–O-6′	1.434
C-1–C-2	1.538	C-1′–C-2′	1.512
C-2–C-3	1.542	C-2′–C-3′	1.548
C-3–C-4	1.524	C-3′–C-4′	1.533
C-4–C-5	1.516	C-4′–C-5′	1.534
C-5–C-6	1.486	C-5′–C-6′	1.507
	Angle (degrees)		*Angle (degrees)*
O-1–C-1–C-2	112.0	O-1′–C-1′–C-2′	114.2
C-1–C-2–C-3	114.3	C-1′–C-2′–C-3′	116.0
C-1–C-2–O-2	109.0	C-1′–C-2′–O-2′	107.9
O-2–C-2–O-3′	111.3	O-2′–C-2′–O-1	108.6
O-2–C-2–C-3	104.7	O-2′–C-2′–C-3′	106.3
C-1–C-2–O-3′	110.0	C-1′–C-2′–O-1	106.7
C-3–C-2–O-3′	107.5	C-3′–C-2′–O-1	111.1
C-2–C-3–C-4	101.7	C-2′–C-3′–C-4′	105.8
C-2–C-3–O-3	112.8	C-2′–C-3′–O-3′	112.1
C-4–C-3–O-3	113.7	C-4′–C-3′–O-3′	115.4
C-3–C-4–C-5	101.4	C-3′–C-4′–C-5′	104.0
C-3–C-4–O-4	114.0	C-3′–C-4′–O-4′	112.3
C-5–C-4–O-4	111.9	C-5′–C-4′–O-4′	111.0
C-4–C-5–C-6	113.1	C-4′–C-5′–C-6′	111.7
C-4–C-5–O-2	103.8	C-4′–C-5′–O-2′	105.7
C-6–C-5–O-2	109.1	C-6′–C-5′–O-2′	109.4
C-5–C-6–O-6	109.0	C-5′–C-6′–O-6′	113.7
C-2–O-2–C-5	111.6	C-2′–O-2′–C-5′	111.3
C-1–O-1–C-2′	111.3	C-3′–O-3′–C-2	116.1

[a]The e.s.d. values for the bond lengths and angles are in the ranges of 0.005–0.006 Å and 0.3–0.4°, respectively.

the conformation angles in Table III. The computations were made on an ACOS-700 computer of the Crystallographic Research Center, Institute for Protein Research, Osaka University, and on an ACOS-900 computer of the Computation Center of Osaka University.

DISCUSSION

The molecular conformation of the dianhydride is shown in Fig. 1; in numbering the carbon and oxygen atoms, those of D-fructose 1 are unprimed, and those of D-

TABLE III

SELECTED CONFORMATION ANGLES[a]

Feature	*Angle (degrees)*	*Feature*	*Angle (degrees)*
(a) Ring of D-*fructose 1*		*(d) Other angles of interest*	
C-5–O-2–C-2–C-3	7.2	C-1–C-2–C-3–C-4	89.5
O-2–C-2–C-3–C-4	−29.4	C-1–C-2–C-3–O-3	−32.7
C-2–C-3–C-4–C-5	40.0	C-1–C-2–O-2–C-5	−115.5
C-3–C-4–C-5–O-2	−36.3	O-3′–C-2–C-3–C-4	−148.1
C-4–C-5–O-2–C-2	18.6	O-3′–C-2–C-3–O-3	89.8
		O-3′–C-2–O-2–C-5	123.0
(b) Ring of D-*fructose 2*		C-6–C-5–C-4–C-3	−154.5
C-5′–O-2′–C-2′–C-3′	−14.6	C-6–C-5–O-2–C-2	139.4
O-2′–C-2′–C-3′–C-4′	−2.5		
C-2′–C-3′–C-4′–C-5′	17.0	C-1′–C-2′–C-3′–C-4′	−122.4
C-3′–C-4′–C-5′–O-2′	−25.7	C-1′–C-2′–C-3′–O-3′	111.0
C-4′–C-5′–O-2′–C-2′	25.8	C-1′–C-2′–O-2′–C-5′	110.4
		O-1–C-2′–C-3′–C-4′	115.5
(c) 1,4-Dioxane ring		O-1–C-2′–O-2′–C-5′	−134.3
O-1–C-1–C-2–O-3′	−23.2	C-6′–C-5′–C-4′–C-3′	−144.5
C-1–C-2–O-3′–C-3′	−35.6	C-6′–C-5′–O-2′–C-2′	146.2
C-2–O-3′–C-3′–C-2′	54.2		
O-3′–C-3′–C-2′–O-1	−11.0	O-6–C-6–C-5–C-4	−177.9
C-3′–C-2′–O-1–C-1	−46.4	O-6–C-6–C-5–O-2	67.1
C-2′–O-1–C-1–C-2	66.3	O-1′–C-1′–C-2′–C-3′	−57.6
C-1–C-2·····C-3′–C-2′	15.3	O-1′–C-1′–C-2′–O-2′	−176.8
C-2–C-1·····C-2′–C-3′	15.9	O-1′–C-1′–C-2′–O-1	66.7
		O-6′–C-6′–C-5′–C-4′	176.1
		O-6′–C-6′–C-5′–O-2′	59.4

[a]The torsion angle A-1–A-2–A-3–A-4 is viewed along A-2–A-3, with clockwise rotation of A-1 to A-4 taken to be positive. The e.s.d. is ~0.5°.

fructose 2 are primed. As shown in Fig. 2, D-fructoses 1 and 2 are respectively the α and β anomer of D-fructofuranose. The conformations of the moieties in the molecule are as expected, with two puckered furanose rings and a 1,4-dioxane ring fused onto the furanose ring of D-fructose 2. The 1,4-dioxane ring also connects with the ring of D-fructose 1 at the anomeric carbon atom (C-2) in a spiro arrangement. The ring of D-fructose 1 has the $^4T_3$ conformation, with C-4 displaced by 0.469 Å on the endo side of the plane through atoms C-2, C-5, and O-2, and with C-3 displaced by 0.186 Å on the opposite side. The same type of puckering was found in the β-D-fructofuranose moieties of raffinose [*O*-α-D-galactopyranosyl-(1→6)-α-D-glucopyranosyl β-D-fructofuranoside][10] and melezitose [*O*-α-D-glucopyranosyl-(1→3)-β-D-fructofuranosyl α-D-glucopyranoside][11]. The D-fructose 2 moiety has the $^4T_5$ conformation, not previously observed in any structure of molecules having D-fructofuranosyl components. The C-5′ and C-4′ atoms are displaced by 0.338 and 0.064 Å respectively on the opposite sides of the plane through the atoms C-2′, C-3′ and O-2′.

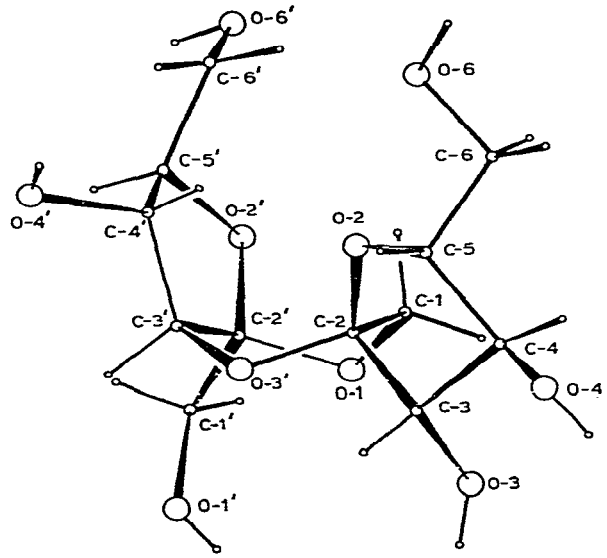

Fig. 1. A perspective view of the molecule of di-D-fructose anhydride III, with the atomic numbering.

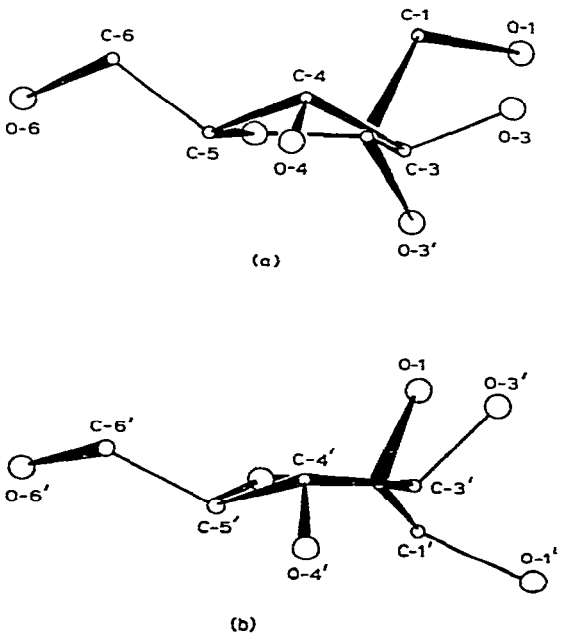

Fig. 2. Comparison of conformations of the two D-fructofuranose moieties, showing puckering and configurations. [(a) D-Fructose 1, and (b) D-fructose 2.]

The 1,4-dioxane ring has a skew conformation, as found in the dimer of dehydro-L-ascorbic acid[12]. The dihedral angle between the planes C-3′, O-3′, C-2 and C-1, O-1, C-2′ is 91.3°, slightly larger than the corresponding 85.1° angle in dehydro-L-ascorbic acid. The pseudo-torsion angles of C-1–C-2···C-3′–C-2′ and C-2–C-1···C-2′–C-3′ are 15.3 and 15.9°, respectively (see Table III). These angles are smaller than the corresponding angles of 19.7° in dehydro-L-ascorbic acid.

The anomeric C–O and exocyclic C–C bonds for D-fructoses 1 and 2 have the characteristic orientations of the α and β anomer of D-fructofuranose, respectively. The conformation angles of O-3′–C-2–C-3–C-4 and C-1–C-2–C-3–C-4 for D-fructose 1 are −148.1 and +89.5°, respectively (see Table III). The corresponding angles for D-fructose 2 are +115.5 and −122.4°, respectively. The primary alcohol groups about exocyclic C–C bonds have the most favored form of noneclipsing orientation. The

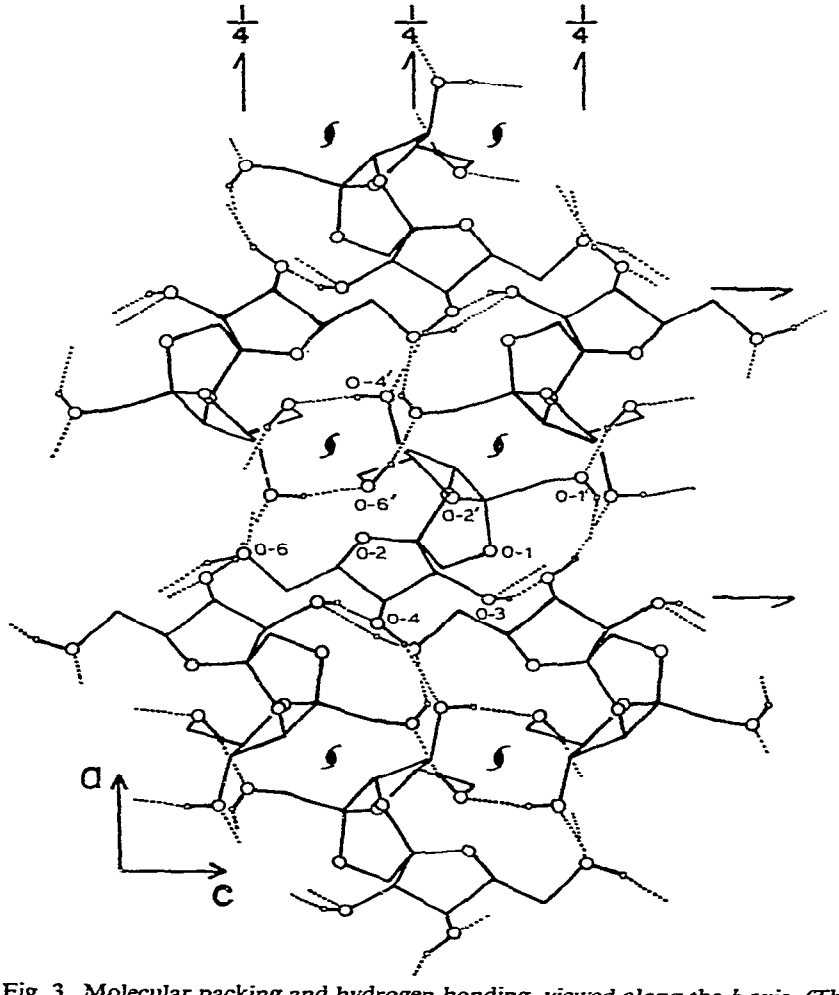

Fig. 3. Molecular packing and hydrogen bonding, viewed along the *b* axis. (The hydrogen atoms of the hydroxyl groups are shown as small, open circles.)

TABLE IV

HYDROGEN-BOND DISTANCES AND ANGLES

	O···O (Å)	O–H (Å)	H···O (Å)	O-H···O (degrees)	Symmetry operation[a]
O-6′–HO-6′···O-1′	2.676	0.89	1.82	161	656.4
O-1′–HO-1′···O-6	2.667	0.73	2.10	137	556.1
O-6–HO-6···O-3	2.779	0.91	1.91	158	554.1
O-3–HO-3···O-4	2.746	0.80	1.98	159	545.2
O-4–HO-4···O-4′	2.761	0.86	1.96	156	446.3
O-4′–HO-4′···O-6′	2.697	0.86	1.84	171	645.4

[a]The symmetry operation is performed on the acceptor oxygen-atoms. The first three digits specify the lattice translations. The last digit indicates one of the following symmetry operations: (1) x, y, z; (2) $1/2 - x, -y, 1/2 + z$; (3) $1/2 + x, 1/2 - y, -z$; (4) $-x, 1/2 + y, 1/2 - z$ (*e.g.*, 645.4 is **a** − **b** from 555.4). The e.s.d. values for O···O, O–H, H···O, and O–H··· are ~0.005, 0.07, and 0.07 Å, and 7°, respectively.

C-1′–O-1′ bond about the C-1′–C-2′ bond is *trans*, *gauche*$^+$ and *gauche*$^-$ to the C-2′–O-2′, C-1′–O-1 and C-2′–C-3′ bonds, respectively. The C-6–O-6 (or C-6′–O-6′) bond is *gauche*$^+$ to C-5–O-2 (or C-5′–O-2′) and *trans* to C-5–C-4 (or C-5′–C-4′).

The C–C and C–O bonds (see Table II) are in the range 1.486–1.548 and 1.406–1.445 Å, respectively, which agree well with the values found in D-fructofuranose moieties of the other oligosaccharides (1.489–1.554 Å for C–C and 1.390–1.463 Å for C–O). The shortest C-5–C-6 bond (1.486 Å) is 0.038 Å shorter than the mean C–C bond (1.524 Å). The shortening of this bond may be affected by the anomeric disproportion reported by Rohrer[13]. The C-5–O-2 bond is 0.027 Å longer than the C-2–O-2 bond. The same type of anomeric disproportion in D-fructose 2 is not significant; the difference between C-2′–O-2′ and C-5′–O-2′ is 0.009 Å, and the shortening of C-5′–C-6′ is 0.017 Å from the mean C–C bond.

The molecular packing and hydrogen-bond scheme are shown in Fig. 3, and the hydrogen-bond distances and angles are given in Table IV. The hydrogen bonding consists of infinite, helical chains extending along the two-fold screw-axes parallel to the *b* axis, consisting of O-6′-H (555.1) → O-1′-H (656.4) → O-6-H (655.4) → O-3-H (656.4) → O-4-H (546.3) → O-4′-H (555.1) → O-6′-H (645.4) →. These are all strong bonds, with O···O distances ranging from 2.667 to 2.779 Å. Pairs of hydroxyl groups take part in the two intermolecular hydrogen-bonds, one as a donor, and the other as an acceptor. The endocyclic atoms O-1, O-2, O-2′, and O-3′ are not included in the hydrogen-bond system.

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