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CRYSTAL STRUCTURE OF TRISODIUM β-D-FRUCTOFURANOSE 1,6-DIPHOSPHATE OCTAHYDRATE*

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ABSTRACT

Trisodium β-D-fructose 1,6-diphosphate octahydrate crystallises in the monoclinic space group $P2_1$ with unit-cell dimensions $a = 13.289(2)$, $b = 11.643(3)$, $c = 7.092(1)$ Å, and $\beta = 102.32(2)°$. The unit cell contains two symmetry-related molecules. The structure has been determined by direct methods, and refined to an R value of 0.035 and an R_w value of 0.049. The puckering of the furanose ring is C-3-*exo*, corresponding to an E_3 conformation slightly distorted towards $^4T_3$. The sodium atoms are hexaco-ordinated. The crystal packing involves alternating charged layers and a network of hydrogen bonds which links the molecules belonging to the same layer and to adjacent layers.

INTRODUCTION

The biological importance of D-fructose 1,6-diphosphate[1] is well established[2], but it is not clear how the regulatory effect is exerted. D-Fructose 1,6-diphosphate exists as α- and β-furanose forms and an acyclic keto-form. ^{13}C-N.m.r. studies[3,4] showed that, in solution, several isomers of D-fructose 1,6-diphosphate are present in different concentrations, which have different affinities and reactivities (anomeric specificity) for enzymic catalytic and allosteric sites. The anomeric specificity could be the basis of its regulatory action of carbohydrate metabolism[5] and this has stimulated the pharmacological use of its salts, especially those with alkaline or alkaline earth metals[6]. The trisodium salt is of interest from a structural point of view since β-D-fructose 1,6-diphosphate (FDP) shows only two dissociation steps (pK 1.48 and 6.32).

We now report on the crystal structure of trisodium FDP and the manner of the co-ordination of the sodium ions. The cell parameters (Table I) and the number

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*Preliminary data presented at the Fourth Yugoslav–Italian Crystallographic Conference, Bled, Yugoslavia, May 31st–June 3rd, 1982.

TABLE I

CRYSTAL DATA FOR TRISODIUM β-D-FRUCTOSE 1,6-DIPHOSPHATE OCTAHYDRATE

Formula	$Na_3H(PO_4)_2C_6H_{10}O_6 \cdot 8\,H_2O$
Mol wt.	550.205
Monoclinic	
a	13.289(2) Å
b	11.643(3) Å
c	7.092(1) Å
β	102.32(2)°
Volume	1072,0(4) Å^3
Space group	$P2_1$
Z	2
D_c	1.70 g.cm^{-3}
Radiation MoKα	0.71069 Å
μ	3.66 cm^{-1}
Crystal size	0.20 × 0.25 × 0.25 mm

of molecules of water of crystallisation were different from those reported[7] by Clegg and Goaman.

EXPERIMENTAL

Trisodium FDP was provided by Biomedica Foscama. Platelet-like crystals, suitable for X-ray diffraction study, were obtained from aqueous ethanol at pH 6.2. The three-dimensional crystal data were collected on a $P2_1$ Syntex four-circle diffractometer by θ–2θ scan, using graphite-monochromated MoKα radiation: 3080 independent reflections were collected ($2\theta \leq 57°$), and 2159 reflections with $I \geq 3\sigma(I)$ were used in structure elucidation. The unit-cell dimensions were obtained by a least-squares refinement of 15 high-angle reflections ($28.0 \leq 2\theta \leq 38.3$) and are given in Table I together with other crystallographic data.

The structure was determined by direct methods, using MULTAN-78[8]. The best set of phases was used in the calculation of an E-map in which all of the atoms of FDP were recognised. The remaining water molecules and the three sodium ions were obtained by Fourier syntheses. Subsequent Fourier-difference maps revealed the positions of all the H atoms. All non-hydrogen atoms were refined by several block-diagonal (9 × 9) least-squares calculations. The hydrogen atoms were included in the last refinement with a fixed B value equal to that of the carrier atom. The final R and R_w values were 0.035 and 0.049, respectively. The positional and thermal parameters with relative standard deviations for all atoms are listed in Table II*.

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*Lists of the observed and calculated structure factors, the anisotropic thermal parameters, and Tables V, VI, and VII 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/331/*Carbohydr. Res.*, 147 (1986) 183–190.

TABLE II

FRACTIONAL CO-ORDINATES AND U_{eq} VALUES (E.S.D 'S IN PARENTHESES)

Atom	*x/a*	*y/b*	*z/c*	U_{eq}
O-11	0.2963(3)	0.5935(4)	0.7169(5)	0.039(2)
O-12	0.2549(3)	0.7844(4)	0.8296(5)	0.040(2)
O-13	0.3039(2)	0.7649(4)	0.5002(5)	0.031(2)
P-1	0.2519(1)	0.7177(1)	0.6507(2)	0.021(0)
O-1	0.1359(2)	0.6939(3)	0.5405(4)	0.023(1)
C-1	0.0685(3)	0.6236(4)	0.6239(6)	0.019(2)
C-2	0.0078(3)	0.5452(4)	0.4681(5)	0.014(2)
O-2	−0.0685(2)	0.4890(3)	0.5426(5)	0.022(1)
O-5	−0.0364(2)	0.6152(3)	0.3059(4)	0.018(1)
C-3	0.0720(3)	0.4559(4)	0.3868(5)	0.016(2)
O-3	0.0896(3)	0.3600(3)	0.5102(5)	0.024(1)
C-4	0.0057(3)	0.4340(4)	0.1855(6)	0.020(2)
O-4	0.0598(3)	0.3934(4)	0.0447(5)	0.034(2)
C-5	−0.0393(3)	0.5548(4)	0.1275(6)	0.016(2)
C-6	−0.1476(3)	0.5496(4)	0.0115(6)	0.022(2)
O-6	−0.1832(2)	0.6644(3)	−0.0308(4)	0.021(1)
P-2	−0.2495(1)	0.6985(1)	−0.2441(1)	0.014(0)
O-21	−0.3511(2)	0.6341(3)	−0.2740(5)	0.025(1)
O-22	−0.1869(2)	0.6655(3)	−0.3906(4)	0.023(1)
O-23	−0.2599(2)	0.8279(3)	−0.2283(4)	0.021(1)
Na-1	0.5333(2)	0.5545(2)	0.1695(3)	0.030(1)
W-1	0.5045(2)	0.6984(4)	−0.0755(5)	0.033(2)
W-2	0.4863(3)	0.6987(4)	0.3863(5)	0.034(2)
W-3	0.6861(3)	0.6874(4)	0.2450(4)	0.034(2)
Na-2	0.5530(1)	0.8313(2)	0.1790(3)	0.027(1)
W-4	0.6195(3)	0.9428(3)	0.4701(5)	0.030(2)
W-5	0.6245(3)	0.9548(4)	−0.0321(5)	0.032(2)
W-6	0.4177(3)	0.9624(3)	0.5367(5)	0.030(2)
W-7	0.3969(3)	0.9337(3)	0.0467(5)	0.029(2)
Na-3	0.0182(2)	0.8145(2)	0.2711(3)	0.027(1)
W-8	0.1593(3)	0.7688(5)	0.1419(6)	0.049(2)
H-11	0.319(6)	0.558(8)	0.637(12)	0.039
H-1	0.105(5)	0.569(7)	0.734(10)	0.020
H-12	−0.113(5)	0.544(7)	0.577(11)	0.024
H-2	0.016(5)	0.675(6)	0.668(10)	0.020
H-13	0.128(5)	0.311(7)	0.469(10)	0.024
H-3	0.137(5)	0.492(7)	0.369(10)	0.024
H-14	0.116(6)	0.368(7)	0.096(11)	0.032
H-4	−0.050(5)	0.373(7)	0.195(10)	0.020
H-5	0.007(6)	0.602(6)	0.063(10)	0.020
H-6	−0.186(5)	0.509(7)	0.108(10)	0.023
H-7	−0.151(6)	0.510(7)	−0.988(10)	0.023
HW-11	0.549(6)	0.673(7)	−0.156(11)	0.032
HW-12	0.466(6)	0.692(8)	−0.105(10)	0.032
HW-21	0.537(5)	0.694(8)	0.519(11)	0.035
HW-22	0.427(6)	0.719(8)	0.404(11)	0.035
HW-31	0.722(6)	0.682(8)	0.340(11)	0.032
HW-32	0.726(6)	0.694(8)	0.176(10)	0.032
HW-41	0.643(6)	0.889(7)	0.544(11)	0.028
HW-42	0.565(6)	0.949(7)	0.484(11)	0.028
HW-51	0.665(6)	0.990(8)	0.028(12)	0.033

TABLE II *(continued)*

Atom	*x/a*	*y/b*	*z/c*	U_{eq}
HW-52	0.669(6)	0.920(8)	−0.079(11)	0.033
HW-61	0.393(6)	1.009(7)	0.465(11)	0.032
HW-62	0.378(6)	0.897(8)	0.529(11)	0.032
HW-71	0.360(6)	0.889(8)	−0.011(11)	0.029
HW-72	0.364(6)	0.967(8)	0.105(11)	0.029
HW-81	0.188(6)	0.770(8)	0.037(12)	0.044
HW-82	0.215(6)	0.775(8)	0.219(12)	0.044

RESULTS AND DISCUSSION

The molecular structure of the FDP anion and the numbering of the atoms are shown in Fig. 1. The bond lengths and angles involving C, O, and P are given in Table III, and those involving H atoms are reported in Table V.

The bond lengths and angles of the fructofuranose moiety were similar to those for other carbohydrate molecules containing fructofuranosyl groups[9–18].

The conformation of the FDP anion is described by torsion angles reported in Table IV. The endocyclic dihedral angles are in the range of values observed in many structures containing a fructofuranosyl moiety[9]. In particular, the conformation of the furanose ring of FDP closely resembles that of one fructose residue in 1-kestose[11]. The furanose ring has a C-3-*exo* puckering. In terms of the conformational descriptors ($P = -16.7°$ and $\varphi = 37.6°$), an E_3 conformation slightly distorted towards $^4T_3$ (ref. 19) can be assigned. The atoms C-2, O-5, C-5, and C-4

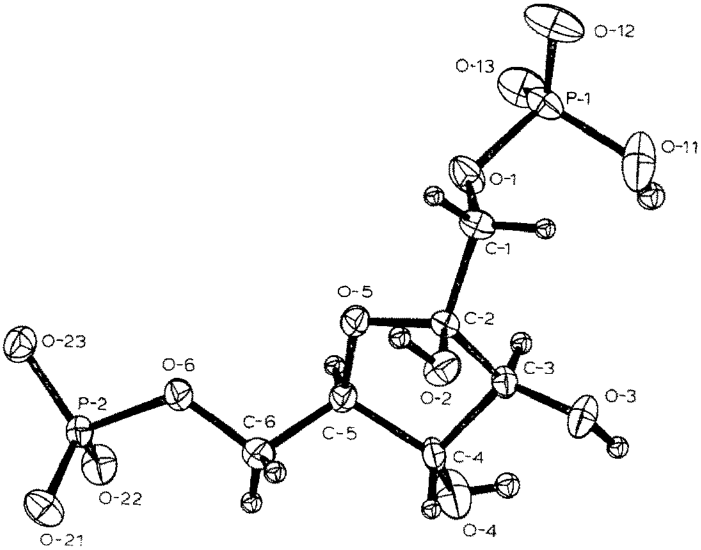

Fig. 1. Structure of FDP anion with atom-numbering scheme; ellipsoids are drawn to enclose 60% probability density.

TABLE III

BOND DISTANCES (Å) AND ANGLES (°) (WITH E.S.D IN PARENTHESES) INVOLVING NON-HYDROGEN ATOMS

P-1–O-1	1.597(3)	C-2–C-3	1.533(5)
P-1–O-11	1.594(5)	C-2–O-2	1.401(5)
P-1–O-12	1.480(4)	C-2–O-5	1.430(5)
P-1–O-13	1.495(3)	C-3–C-4	1.531(6)
P-2–O-6	1.629(4)	C-3–O-3	1.407(5)
P-2–O-21	1.519(4)	C-4–C-5	1.549(6)
P-2–O-22	1.513(3)	C-4–O-4	1.429(5)
P-2–O-23	1.519(4)	C-5–C-6	1.499(7)
C-1–O-1	1.431(5)	C-5–O-5	1.441(5)
C-1–C-2	1.524(6)	C-5–O-6	1.429(6)

O-1–P-1–O-11	104.1(.2)	C-1–C-2–O-5	107.8(.3)
O-1–P-1–O-12	111.0(.2)	O-2–C-2–C-3	109.3(.3)
O-1–P-1–O-13	105.0(.2)	O-2–C-2–O-5	111.3(.3)
O-11–P-1–O-12	106.4(.2)	C-3–C-2–O-5	104.5(.3)
O-11–P-1–O-13	110.2(.2)	C-2–C-3–C-4	102.0(.3)
O-12–P-1–O-13	119.1(.2)	C-2–C-3–O-3	109.6(.3)
O-6–P-2–O-21	107.3(.2)	O-3–C-3–C-4	115.7(.4)
O-6–P-2–O-22	107.9(.2)	C-3–C-4–C-5	102.0(.4)
O-6–P-2–O-23	102.2(.2)	C-3–C-4–O-4	115.6(.4)
O-21–P-2–O-22	112.4(.2)	O-4–C-4–C-5	109.7(.3)
O-21–P-2–O-23	114.1(.2)	C-2–O-5–C-5	110.9(.3)
O-22–P-2–O-23	112.0(.2)	C-4–C-5–O-5	105.9(.3)
P-1–O-1–C-1	121.4(.3)	O-5–C-5–C-6	110.1(.3)
O-1–C-1–C-2	108.9(.3)	C-4–C-5–C-6	112.4(.4)
C-1–C-2–C-3	115.3(.3)	C-5–C-6–O-6	108.3(.4)
C-1–C-2–O-2	108.7(.3)	P-2–O-6–C-6	120.8(.3)

define a nearly perfect plane, with C-3 displaced out of plane by 0.584 Å on the opposite side of C-6. The distances of C-3 and C-4 from the plane passing through C-2, O-5, and C-5 are 0.559 and 0.033 Å, respectively. The arrangement of the C-1–O-1 bond with respect to the C-2–O-5 and C-2–C-3 ring bonds is *gauche–gauche*. This conformation is similar to that preferred by the sequence O-1–C-1–C-2–O-5 in structures containing the ketofuranose group. The C-6–O-6 bond adopts the *trans–gauche* conformation in relation to the C-4–C-5 endocyclic bond.

The two phosphate groups of FDP are in a staggered conformation with respect to the ester bonds, and the dihedral angles about C-1–O-1 and C-6–O-6 correspond to an intermediate *trans–gauche*$^-$ conformation in the former and to an intermediate *trans–gauche*$^+$ in the latter. All P–O bond lengths are within normal ranges for phosphate groups in compounds of this type. The location of H-11 near O-11, in addition to the pattern of the bond lengths and angles of the phosphate groups, indicates P-1–O-11 to be a P–OH bond type, and the negative charge is distributed between the other two oxygens. The two negative charges are uniformly spread over the three oxygen atoms of the P-2 phosphate group. The three sodium ions have an octahedral co-ordination with Na–O distances between 2.301 and

TABLE IV

TORSIONAL ANGLES (°) WITH E S D IN PARENTHESES

Endocyclic	
C-5–O-5–C-2–C-3	22.1(.4)
O-5–C-2–C-3–C-4	−36.3(.4)
C-2–C-3–C-4–C-5	36.0(.4)
C-3–C-4–C-5–O-5	−23.9(.4)
C-4–C-5–O-5–C-2	1.3(.4)
Exocyclic	
O-5–C-2–C-1–O-1	−50.6(.4)
C-3–C-2–C-1–O-1	65.6(.4)
O-5–C-5–C-6–O-6	60.8(.4)
C-4–C-5–C-6–O-6	178.6(.3)
O-3–C-3–C-4–O-4	−86.0(.5)
O-1–C-1–C-2–O-2	−171.4(.3)
O-2–C-2–C-3–O-3	−40.2(.4)
O-4–C-4–C-5–C-6	92.7(.4)
P-1–O-1–C-1–C-2	−137.6(.3)
P-2–O-6–C-6–C-5	137.9(.3)
Phosphate groups	
C-1–O-1–P-1–O-11	49.0(.4)
C-1–O-1–P-1–O-12	−65.1(.4)
C-1–O-1–P-1–O-13	164.9(.4)
C-6–O-6–P-2–O-21	65.5(.4)
C-6–O-6–P-2–O-22	−56.0(.4)
C-6–O-6–P-2–O-23	−174.2(.3)

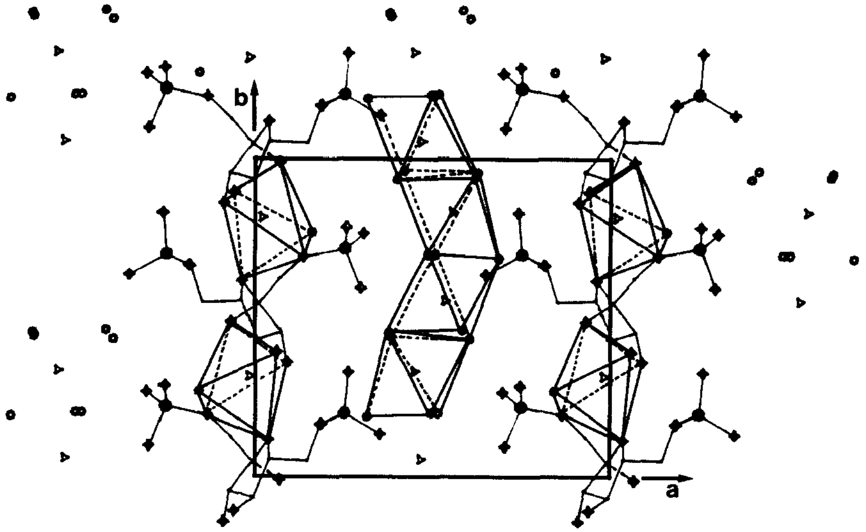

Fig. 2. View of crystal packing of FDP parallel to the *b* axis; ○, water molecules; △, sodium ions.

2.606 Å (Table VI). Na-1 and Na-2 co-ordinate only water molecules, whereas Na-3 is surrounded by oxygens of three FDP molecules and by one water molecule; no phosphate oxygens are involved in Na(+) linkages. The seven water molecules surrounding Na-1 and Na-2 form two nearly regular polyhedra. Through a screw-axis parallel to *b*, they generate infinitely extended chains of polyhedra sharing alternatively faces and edges (Fig. 2). The somewhat distorted Na-3 polyhedron helps to hold the FDP molecules together.

The molecular packing, projected down the $c = c^* \sin\beta$ axis, is shown in Fig. 2. The crystal structure is made up of alternating charged layers extending on the planes $x = 0.0$ and $x = 0.5$. The negatively charged layer is formed by the FDP anions, the Na-3 cations, and the water molecules W-8. The structure of this layer is stabilised by interactions of FDP moieties and Na-3 ions, and hydrogen bonds. The net charge is spread over the two faces of the layer. The positively charged layer is formed by lattice translation along the *c* axis of the infinite chains of the co-ordination polyhedra of Na-1 and Na-2 cations. The interlayer forces are mainly due to ion–ion, ion–dipole, and dipole–dipole interactions, and to a network of hydrogen bonds between the oxygen atoms of FDP and the seven water molecules surrounding the Na-1 and Na-2 cations.

In the asymmetric unit of the crystal, there are 20 oxygen atoms. Assuming the Van der Waals radius $R_0 = 1.60$ Å, there are 28 short contacts ranging between 2.4 and 3.2 Å (ref. 20); they are all listed in Table VII. Fig. 3 shows crystal packing

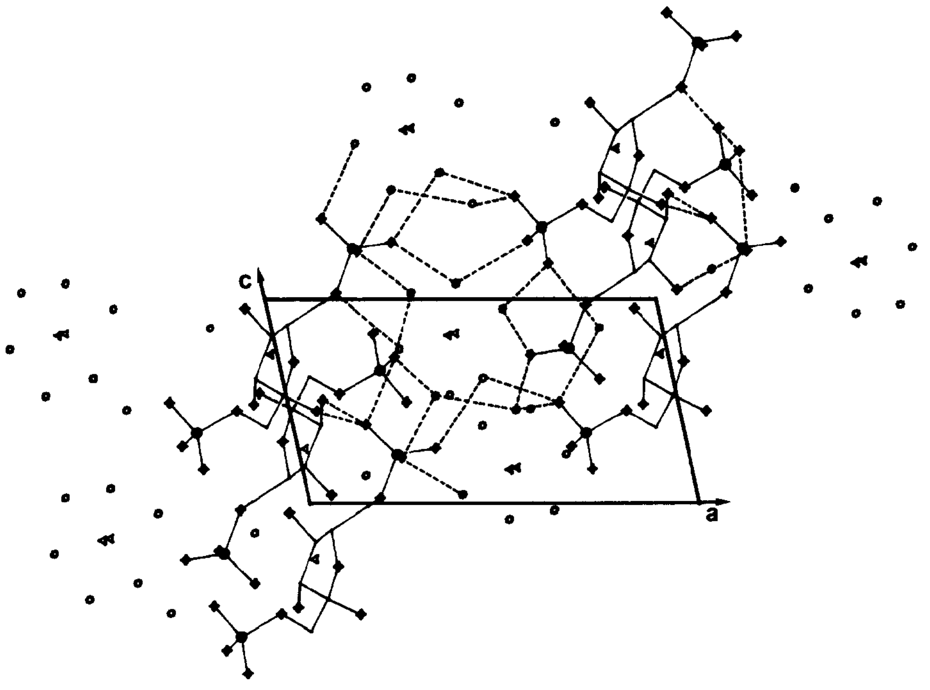

Fig. 3. Crystal packing of FDP projected along the *b* axis; ○, water molecules; △, sodium ions.

projected along the *b* axis with the hydrogen-bond scheme. Among these short distances, as all the hydrogen atoms were localised in the ΔF synthesis, 21 hydrogen bonds are recognisable, of which 20 are two-centered and only one is three-centered. Some short contacts, as reported in Table VII, might be due to dipole–dipole interactions[21–23].

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