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The crystal structure of cycloinulohexaose produced from inulin by cycloinulo-oligosaccharide fructanotransferase

Masami Sawada*, Takanori Tanaka, Yoshio Takai, Terukiyo Hanafusa,
Material Analysis Centre, The Institute of Scientific and Industrial Research, Osaka University, Mihogaoka, Ibaraki, Osaka 567 (Japan)

Tomohiko Taniguchi,
Department of Sciences and Arts (Information Science), Osaka Kyoiku University, Ikeda, Osaka 563 (Japan)

Mishio Kawamura, and Takao Uchiyama*
Department of Biology, Osaka Kyoiku University, Tennoji-ku, Osaka 543 (Japan)

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ABSTRACT

The crystal of cycloinulohexaose trihydrate, $C_{36}H_{60}O_{30}\cdot 3H_2O$, is trigonal, space group $R3$, with unit-cell dimensions $a = 24.688$ (17), $c = 6.477$ (3) Å for a hexagonal cell, $Z = 3$. The molecule, which consists of six (2→1)-linked β-D-fructofuranose residues, has C_3 symmetry. The conformations of two D-fructofuranosyl moieties in an asymmetric unit are ${}^4T_3$ with P = 348.1° and $\tau_m = 38.9°$ for F1, and ${}^4T_3$ with P = 350.5° and $\tau_m = 41.2°$ for F2. The conformations of –O–CH_2–C–O– in the 18-crown-6 ring are *gauche*⁻ for O-1–C-1–C-2–O-1′ (+52.3°) and *trans* for O-1′–C-1′–C-2′–O-1 (+163.4°).

INTRODUCTION

Cycloinulohexaose, produced[1] from inulin by the extracellular cycloinulo-oligosaccharide fructanotransferase of *Bacillus circulans* OKUMZ 31B, is a (2→1)-linked cyclohexaose of β-D-fructofuranose. The structure has been determined by enzymic, spectroscopic, and chromatographic analyses[2]. The conformation is of interest in relation to that of α-cyclodextrin (cyclomaltohexaose), which has six (1→4)-linked α-D-glucopyranose residues, each in the ${}^4C_1$ conformation, and a cone-shaped cavity[3]. We now report details† of the crystal structure of cycloinulohexaose, $C_{36}H_{60}O_{30}$, and on the conformation of the central 18-crown-6 moiety[4].

EXPERIMENTAL

The cycloinulohexaose[2] had m.p. 231–233° (dec.), $[\alpha]_D^{20}$ −65° (*c* 1.08, water), and crystallisation from aqueous methanol at room temperature gave colorless, prismatic crystals. The crystallographic and experimental data are summarised in Table I.

Data were collected with a RIGAKU AFC-5FOS automated four-circle diffrac-

* Authors for correspondence.
† For a preliminary communication, see ref. 1.

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TABLE I

Crystallographic and experimental data

Formula	$C_{36}H_{60}O_{30}\cdot 3H_2O$
Molecular weight	1026.9
For hexagonal axes	
a (Å)	24.688 (17)
c (Å)	6.477 (3)
Crystal system	Trigonal
Space group	$R3$
Z (molecules/cell)	3
Volume (Å^3)	3419.0 (4.3)
D_{calc} (g/cm^3)	1.50
Crystal size (mm)	0.3 × 0.3 × 0.3
Radiation	graphite-monochromated MoK_α ($\lambda = 0.7107$Å)
Reflections used ($F_o > 3\sigma F$)	1698
μ (cm^{-1})	1.44
R	0.059

tometer at room temperature. The dimensions of the cell were determined by least-squares calculations with 2θ values of 25 reflections ($28° < 2\theta < 30°$). Intensity data of reflections with $2\theta \leqslant 55°$ were collected by the use of a θ–2θ scanning mode, with a scanning rate of 12°/min in 2θ. A scanning range of $\Delta\ (2\theta)° = 1.5 + 0.5\tan\theta$ was employed for each peak. No corrections for X-ray absorption were carried out.

The structure was solved by a direct method (MULTAN78)[5] and refined by a block-diagonal least-squares program (RASA-MBLS) with anisotropic temperature factors for carbon and oxygen atoms. Of the 22 hydrogen atoms in an asymmetric unit, 18 were located through difference synthesis and their positional parameters were refined with isotropic temperature factors. The remaining four hydrogen atoms (hydroxyl hydrogens) were not revealed and were not included in the computation. The final atomic positional parameters are given in Table II*. Bond lengths and angles, and torsion angles are given in Tables III and IV, respectively.

Computations were carried out on an ACOS-850 computer at the Crystallographic Research Centre, Institute for Protein Research, Osaka University, and on a FACOM S-3500 superminicomputer (in TASMAC) at the Material Analyses Centre, the Institute of Scientific and Industrial Research, Osaka University.

DISCUSSION

The inulobiosyl unit, 1-*O*-β-D-fructofuranosyl($^4T_3$)-β-D-fructofuranose($^4T_3$) of cycloinulohexaose is shown in Fig. 1 together with the numbering system, and PLUTO

* Tables (8 pages) of the anisotropic thermal parameters for non-hydrogen atoms and the observed and calculated structure factors (F_o–F_c Table) are 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/464/ *Carbohydr. Res.*, 217 (1991) 7–17

TABLE II

Atomic positional parameters ($\times 10^4$ for non-H atoms and $\times 10^3$ for H atoms)

Atom	x	y	z	B_{eq}[a]
O-1	551 (2)	1320 (2)	1565 (5)	2.1
O-2	1729 (2)	1617 (2)	−2222 (5)	2.2
O-3	1923 (2)	2216 (2)	2998 (6)	3.1
O-4	2984 (2)	3005 (2)	−100 (6)	3.0
O-6	2840 (2)	1432 (3)	−2089 (9)	4.6
C-1	816 (2)	1433 (3)	−468 (7)	2.0
C-2	1481 (2)	1575 (2)	−178 (7)	1.7
C-3	1919 (2)	2204 (2)	791 (7)	1.9
C-4	2547 (2)	2358 (2)	−74 (7)	2.0
C-5	2375 (3)	2112 (3)	−2297 (8)	2.3
C-6	2781 (3)	1885 (4)	−3240 (10)	3.9
O-1′	−1121 (2)	414 (2)	983 (6)	2.0
O-2′	−124 (2)	1718 (2)	1174 (6)	2.4
O-3′	−66 (2)	687 (2)	5011 (6)	3.1
O-4′	−184 (2)	1816 (3)	6686 (6)	3.8
O-6′	974 (3)	2883 (3)	2129 (9)	5.0
C-1′	−497 (2)	636 (3)	375 (8)	2.0
C-2′	−75 (2)	1192 (3)	1697 (8)	1.9
C-3′	−233 (3)	1093 (3)	4043 (8)	2.2
C-4′	80 (3)	1766 (3)	4764 (8)	2.5
C-5′	−80 (3)	2082 (3)	3016 (9)	2.7
C-6′	362 (4)	2763 (3)	2627 (12)	4.0
O-10	3727 (4)	1376 (5)	1131 (9)	7.8
				B_{iso}
H-61	262 (5)	170 (5)	−472 (14)	4.5
H-62	329 (4)	227 (4)	−309 (14)	3.3
H-5	243 (4)	250 (4)	−301 (11)	1.9
H-4	273 (3)	213 (3)	59 (11)	1.4
H-3C	177 (3)	254 (3)	29 (11)	1.1
H-11	84 (3)	186 (3)	−109 (11)	1.6
H-12	61 (4)	103 (4)	−113 (12)	1.9
H-60	244 (4)	106 (4)	−227 (14)	2.8
H-30	144 (4)	199 (4)	317 (13)	3.4
H-11′	−38 (3)	29 (3)	63 (10)	0.5
H-12′	−45 (4)	78 (4)	−102 (12)	2.5
H-61′	36 (5)	305 (5)	380 (15)	4.3
H-62′	19 (4)	298 (4)	133 (12)	2.5
H-5′	−53 (4)	206 (4)	329 (11)	1.8
H-4′	56 (3)	194 (3)	470 (11)	1.4
H-3C′	−70 (3)	88 (3)	429 (11)	1.1
H-3O′	37 (4)	74 (4)	470 (12)	2.7
H-101	341 (4)	149 (4)	111 (12)	2.5

[a] $B_{eq} = (4/3)\,(a^2B_{11} + b^2B_{22} + c^2B_{33} + abB_{12}\cos\gamma)$.

TABLE III

Bond lengths[a] and angles[a]

Atoms	*Length (Å)*	*Atoms*	*Length (Å)*
C-1–O-1	1.435	C-1′–O-1′	1.409
C-2–O-1′	1.410	C-2′–O-1	1.417
C-2–O-2	1.441	C-2′–O-2′	1.405
C-3–O-3	1.430	C-3′–O-3′	1.410
C-4–O-4	1.410	C-4′–O-4′	1.438
C-5–O-2	1.445	C-5′–O-2′	1.464
C-6–O-6	1.410	C-6′–O-6′	1.424
C-1–C-2	1.509	C-1′–C-2′	1.509
C-2–C-3	1.514	C-2′–C-3′	1.557
C-3–C-4	1.509	C-3′–C-4′	1.514
C-4–C-5	1.538	C-4′–C-5′	1.534
C-5–C-6	1.501	C-5′–C-6′	1.499
	Angle (°)		*Angle (°)*
C-2′–O-1–C-1	116.1	C-2–O-1′–C-1′	115.2
O-1–C-1–C-2	105.6	O-1′–C-1′–C-2′	108.6
C-1–C-2–O-1′	113.9	C-1′–C-2′–O-1	110.4
C-1–C-2–O-2	106.1	C-1′–C-2′–O-2′	111.5
C-1–C-2–C-3	115.2	C-1′–C-2′–C-3′	114.0
O-1′–C-2–C-3	107.0	O-1–C-2′–C-3′	104.7
O-1′–C-2–O-2	110.0	O-1–C-2′–O-2′	111.6
C-2–C-3–O-3	115.5	C-2′–C-3′–O-3′	114.0
C-2–C-3–C-4	102.8	C-2′–C-3′–C-4′	100.3
O-3–C-3–C-4	111.7	O-3′–C-3′–C-4′	118.3
C-3–C-4–O-4	113.5	C-3′–C-4′–O-4′	110.9
C-3–C-4–C-5	101.6	C-3′–C-4′–C-5′	102.2
O-4–C-4–C-5	109.9	O-4′–C-4′–C-5′	110.7
C-4–C-5–O-2	105.9	C-4′–C-5′–O-2′	104.4
C-4–C-5–C-6	115.0	C-4′–C-5′–C-6′	117.8
O-2–C-5–C-6	110.9	O-2′–C-5′–C-6′	109.9
C-5–C-6–O-6	115.8	C-5′–C-6′–O-6′	111.9
C-2–O-2–C-5	109.9	C-2′–O-2′–C-5′	110.9
O-2–C-2–C-3	104.4	O-2′–C-2′–C-3′	104.5

[a] The e.s.d. values for the bond lengths and angles are in the ranges 0.012–0.008 Å and 0.8–0.5°, respectively.

drawings of the whole molecule are presented in Fig. 2. A crystallographic three-fold axis passes through the centre of the molecule.

The two D-fructofuranose residues (F1 and F2 in Fig. 1) have almost the same $^4T_3$ conformations[6] (Fig. 3) with the exception of the torsional angle O-1–C-1–C-2–C-3 (−71.7° and +45.9°, respectively). In F1, C-4 is displaced by 0.118 Å on the *endo* side of the plane through C-2, C-5, and O-2, and C-3 is displaced by 0.494 Å on the *exo* side. This structure corresponds[6] to a pseudorotation phase angle P = 348.1° and a maximum torsional angle τ_m = 38.9°. The furanose ring in F2 has similar puckering with P = 350.5° and τ_m = 41.2°. This type of $^4T_3$ puckering has been observed often in fructofuranose rings in oligosaccharides[7].

TABLE IV

Torsion angles[a]

Atoms	*Angle (°)*	*Atoms*	*Angle (°)*
(*a*) D-*Fructose F1*		(*b*) D-*Fructose F2*	
O-1–C-1–C-2–O-1′	52.3	O-1′–C-1′–C-2′–O-1	163.4
O-1–C-1–C-2–O-2	173.4	O-1′–C-1′–C-2′–O-2′	−72.0
O-1–C-1–C-2–C-3	−71.7	O-1′–C-1′–C-2′–C-3′	45.9
C-1′–O-1′–C-2–C-1	48.3	C-1–O-1–C-2′–C-1′	57.1
C-1′–O-1′–C-2–C-3	176.6	C-1–O-1–C-2′–C-3′	−179.8
C-1′–O-1′–C-2–O-2	−70.6	C-1–O-1–C-2′–O-2′	−67.4
C-1–C-2–C-3–O-3	86.0	C-1′–C-2′–C-3′–O-3′	72.8
C-1–C-2–C-3–C-4	−152.1	C-1′–C-2′–C-3′–C-4′	−159.7
O-1′–C-2–C-3–O-3	−41.7	O-1–C-2′–C-3′–O-3′	−47.9
O-1′–C-2–C-3–C-4	80.2	O-1–C-2′–C-3′–C-4′	79.6
O-2–C-2–C-3–O-3	−158.2	O-2′–C-2′–C-3′–O-3′	−165.3
O-2–C-2–C-3–C-4	−36.3	O-2′–C-2′–C-3′–C-4′	−37.8
C-2–C-3–C-4–C-5	38.0	C-2′–C-3′–C-4′–C-5′	40.7
C-2–C-3–C-4–O-4	155.9	C-2′–C-3′–C-4′–O-4′	158.6
O-3–C-3–C-4–C-5	162.4	O-3′–C-3′–C-4′–C-5′	165.2
O-3–C-3–C-4–O-4	−79.7	O-3′–C-3′–C-4′–O-4′	−76.8
C-3–C-4–C-5–O-2	−26.8	C-3′–C-4′–C-5′–O-2′	−30.3
C-3–C-4–C-5–C-6	−149.7	C-3′–C-4′–C-5′–C-6′	−152.4
O-4–C-4–C-5–O-2	−147.2	O-4′–C-4′–C-5′–O-2′	−148.5
O-4–C-4–C-5–C-6	89.9	O-4′–C-4′–C-5′–C-6′	89.4
C-4–C-5–C-6–O-6	53.7	C-4′–C-5′–C-6′–O-6′	59.5
O-2–C-5–C-6–O-6	−66.4	O-2′–C-5′–C-6′–O-6′	−59.8
C-2′–O-1–C-1–C-2	178.3	C-2–O-1′–C-1′–C-2′	177.5
C-2–O-2–C-5–C-4	4.6	C-2′–O-2′–C-5′–C-4′	6.4

[a] The torsion angle A-1–A-2–A-3–A-4 is viewed along A-2–A-3, with clockwise rotation of A-4 against A-1 taken to be positive. The e.s.d. is ~0.7°.

Newman projections along an exocyclic C-1–C-2 bond are shown in Fig. 4. The orientations of the O-1–C-1 bond in F1 are *gauche*$^+$ (+52.3°), *gauche*$^-$ (−71.7°), and *trans* (+173.4°) to the C-2–O-1′, C-2–C-3, and C-2–O-2 bonds, respectively. The corresponding orientations of the O-1′–C-1′ bond in F2 are *trans* (+163.4°), *gauche*$^+$ (+45.9°), and *gauche*$^-$ (−72.0°) to the C-2′–O-1, C-2′–C-3′, and C-2′–O-2′ bonds, respectively (see Table IV).

The β configuration is reflected by the torsion angles of C-1–C-2–C-3–C-4 and O-1′–C-2–C-3–C-4 (see Table IV), namely, −152.1° and +80.2°, respectively, for F1, and −159.7° and +79.6°, respectively, for F2.

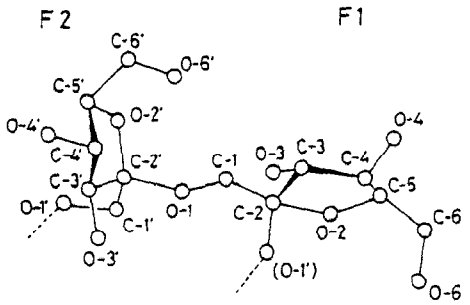

Fig. 1. A perspective view of the inulobiosyl unit in cycloinulohexaose, with numbering.

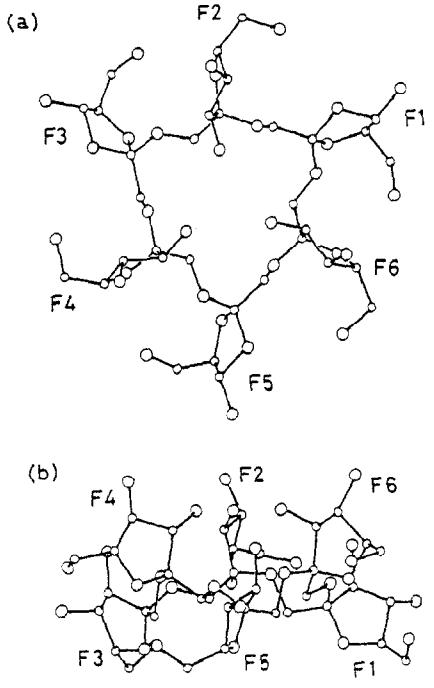

Fig. 2. The PLUTO drawing of cycloinulohexaose (hydrogen atoms are omitted for simplicity): (*a*) top view, (*b*) side view. Circles of C and O atoms are drawn arbitrarily.

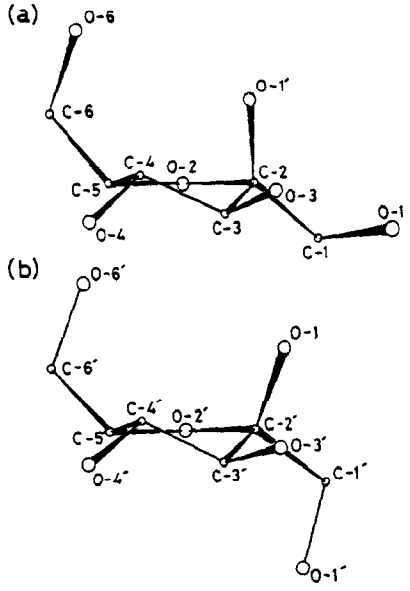

Fig. 3. Conformations of the two β-D-fructofuranose moieties in Fig. 1: (*a*) F1, (*b*) F2.

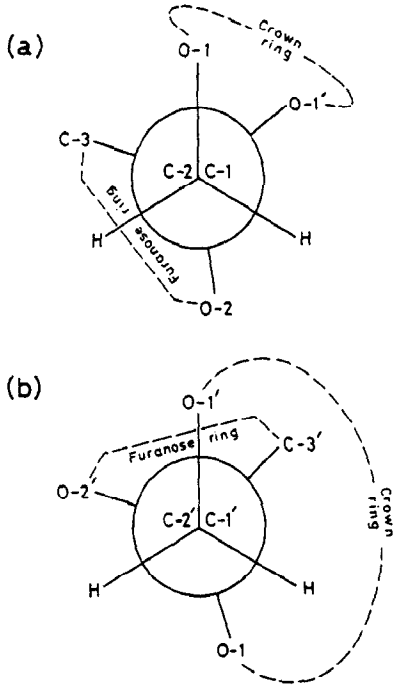

Fig. 4. Newman projections along the C-1–C-2 bond: (*a*) F1, (*b*) F2 (see Fig. 1).

The orientations of the O-6–C-6 bond in F1 are *gauche*$^+$ (+53.7°) to the C-5–C-4 bond, and *gauche*$^-$ (−66.4°) to the C-5–O-2 bond; the C-6–O-6 bond is located just above the furanose ring. The situation in F2 is similar to that in F1, and the corresponding values are *gauche*$^+$ (+59.5°) and *gauche*$^-$ (−59.8°). Therefore, O-6 is near to the neighbouring furanose ring in the same molecule. The distance between O-6 in F2 and O-3 in F1 is 3.520 Å, and that between O-6 in F1 and the ring oxygen O-2 in F6 is 3.607 Å (Fig. 2).

The lengths of the C–C and C–O bonds are in the range 1.49–1.56 Å and 1.40–1.46 Å, respectively (see Table III). The length of the C-2′–O-2′ bond in F2 (1.405 Å) is shorter than that of the C-5′–O-2′ bond (1.464 Å). The length of the C-2′–C-3′ bond in F2 (1.557 Å) is longer than that of the C-2–C-3 bond in F1 (1.514 Å). Most of the bond lengths accord with the values found in other oligosaccharides (1.49–1.55 Å for C–C and 1.39–1.46 Å for C–O).

Partial PLUTO drawings of the 18-crown-6 moiety in cycloinulohexaose are shown in Fig. 5. The side-view (Fig. 5b) of the 18-crown-6 skeleton shows that the six oxygens deviate alternately up and down by 0.19 Å from the mean plane. The conformation of O-1 and O-1′ around the C-1–C-2 bond is *gauche* (g) in F1 and *trans* (t) in F2. Consequently, the cycloinulohexaose molecule has a gtgtgt arrangement of the six –O–C–CH_2–O– units (*cf.* gtggtg for 18-crown-6[8,9] and gggggg for its K^+ ion complex[10,11]), which is one of the new types for uncomplexed 18-crown-6 derivatives[12–15].

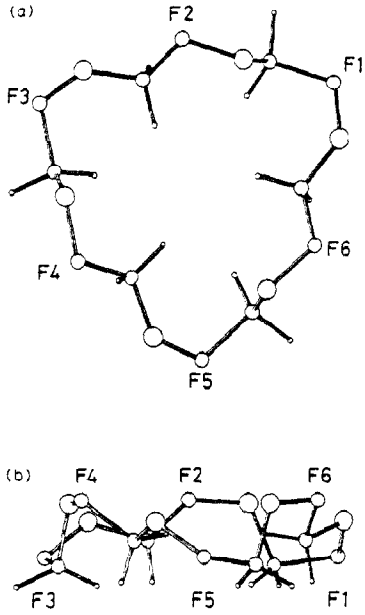

Fig. 5. Partial PLUTO drawings of the 18-crown-6 moiety of cycloinulohexaose (methylene hydrogens are included): (*a*) top view, (*b*) side view. Circles of C, O, and H atoms are drawn arbitrarily.

The distance between neighbouring oxygens in the 18-crown-6 moiety is 2.733 Å for the *gauche* O-1′–C-2–C-1–O-1 and 3.599 Å for the *trans* O-1–C-2′–C-1′–O-1′. The lengths of the C–C and C–O bonds in the 18-crown-6 moiety (Table III) are almost the same as those in the uncomplexed 18-crown-6 compound[8,9].

Fig. 2 shows that the six fructofuranose rings in cycloinulohexaose are arranged in spiro fashion around the 18-crown-6 skeleton. The furanose rings in F2, F4, and F6

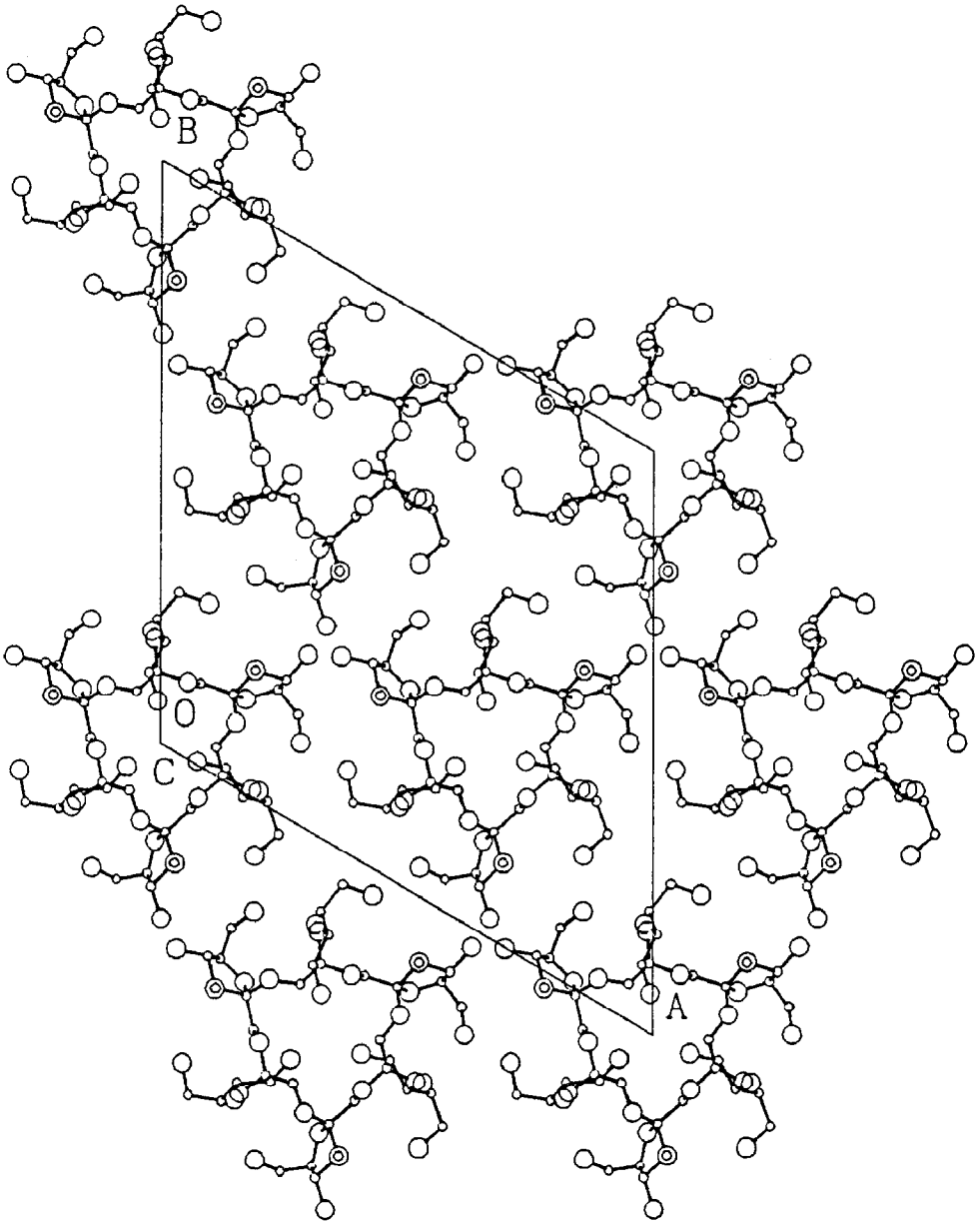

Fig. 6. Molecular packing of cycloinulohexaose viewed along *c* axis (hydrogens are excluded).

TABLE V

Selected distances for oxygen atoms in hydroxyl groups and the water molecule

Atoms O-A O-B	*Symmetry operation*[a]	*Distance* (Å)
O-4····O-4	$-y+2/3, x-y+1/3, z+1/3$	2.602
O-4····O-4	$-x+y+1/3, -x+2/3, z-1/3$	2.602
O-6-H-60····O-4′	$-x+y, -x, z-1$	2.834
O-6····O-6′	$-y+1/3, x-y+1/3, z-2/3$	2.826
O-4′····O-10	$-x+y+1/3, -x+2/3, z+2/3$	2.917
O-6′····O-10	$-x+y+1/3, -x+2/3, z-1/3$	2.809
O-3′-H-30′····O-3′	$-x+y, -x, z$	3.086

[a] The symmetry operation was performed on the oxygen atoms O-B. The e.s.d. values for O····O are 0.010–0.014 Å.

are aligned up towards the mean plane of the 18-crown-6 moiety, and the furanose rings in F1, F3, and F5 are aligned down towards the mean plane. Therefore, the C-3′–O-3′ bonds in F2, F4, and F6 block the upper side of the 18-crown-6 ring. The distance between O-3 in F2 and O-3 in F4 is 3.086 Å, which is slightly longer than the sum of the van der Waals radii. Three such oxygens can cap completely the upper side of the 18-crown-6 ring (see Fig. 2).

α-Cyclodextrin has a lipophilic cone-shaped cavity[3], whereas cycloinulohexaose has an 18-crown-6 skeleton with a bowl-shaped structure. The lower side of the molecule is lipophilic due to the methylene groups of the *gauche* O–C–CH_2–O units. A molecular-packing mode viewed along the *c* axis is shown in Fig. 6.

The distances between the hydroxyl oxygens and the water molecule are listed in Table V. There are two types of hydrogen bond, namely, (*a*) an endless chain that involves O-4····O-4 around a three-fold screw axis along *c*, and (*b*) an O-4′····O-6····O-6′····O-10····O-4′ sequence around a three-fold screw axis along *c*. Water molecules in the crystal are responsible for the intermolecular hydrogen bonding.

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