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Crystal structure of anhydrous hexakis-(2,3,6-tri-*O*-methyl)-cyclomaltohexaose (permethyl-α-cyclodextrin) grown from hot water and from cold NaCl solutions [1]

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Abstract

An X-ray diffraction study was carried out on anhydrous permethyl-α-cyclodextrin crystallized from aq solution at 40 °C, space group $P2_12_12_1$ with $a = 15.424(9)$, $b = 18.167(7)$, $c = 23.128(8)$ Å, vol = 6481(5) Å^3. The molecule is boat-shaped with the narrow end of the cyclodextrin cavity closed by two diametrically opposing glucose residues. These are strongly tilted towards the molecular axis, so that their primary methoxy groups form van der Waals contacts across the molecular opening. The remaining cavity volume is occupied by the methoxy group of a neighboring molecule (self-inclusion). From solutions containing 1 M NaCl, anhydrous permethyl-α-cyclodextrin crystallizes isomorphously at room temperature.

Keywords: Crystal structure; Trimethyl-α-cyclodextrin; α-Cyclodextrin

1. Introduction

The solubility of the methylated cyclodextrins (cyclomalto-oligosaccharides, CDs) in water is extraordinary because they are extremely soluble at room temperature, although their surface is hydrophobic in character [1,2]. The fully methylated cyclodextrins, which

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[1] Topography of Cyclodextrin Inclusion Complexes, Part 39. For part 38, see ref. [18]. For Part 37, see ref. [4].

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carry no hydroxyl groups, have higher solubilities in water than the native species which carry three hydroxyl groups per glucose (for example, the solubility in H_2O at 25 °C is 150 g/L for α-CD, and 200 g/L for permethyl-α-CD [2]). A second peculiarity is the negative temperature coefficient of the solubilities in water: if a cold aq solution is heated, the solubility falls to almost zero and crystals grow. When cooled again, the crystals re-dissolve.

Crystal structures are published for two methylated cyclodextrins crystallized from hot water: heptakis-(2,3,6-tri-*O*-methyl)-β-CD (permethyl-β-CD, TRIMEB) [3] and heptakis-(2,6-di-*O*-methyl)-β-CD (dimethyl-β-CD, DIMEB) [4]. TRIMEB crystallizes as a monohydrate; the molecular conformation is distorted in such a way that the volume of the molecular cavity is significantly reduced. This is associated with inversion of one glucose residue from $^4C_1$ to $^1C_4$ chair conformation (extreme glucose distortions have also been reported for inclusion complexes of TRIMEB [5]). The cavity is free of water, and one water molecule per TRIMEB is located as a space-filler between the macrocycles [3]. DIMEB crystallizes from hot water without any water of hydration [4]. In contast to TRIMEB, the DIMEB molecule is not distorted but adopts a round shape which is stabilized by systematic intramolecular O-3–H · · · O-2′ hydrogen bonds between neighboring glucose units. The molecular cavity is closed on one end by primary methoxy groups which are oriented towards the molecular axis. The remaining cavity volume is occupied by part of the C-6–O-6–Me rim of a neighboring molecule (''self-inclusion''). From cold water, only poorly ordered crystals of DIMEB have been obtained, and no full structure determination has as yet been reported.

To investigate whether the above behavior is systematic for the methylated cyclodextrins, we determined the crystal structure of hexakis-(2,3,6-tri-*O*-methyl)-α-CD (permethyl-α-CD, TRIMEA) crystallized from hot water and from cold water containing 1 M NaCl. An anhydrous crystal structure was found characterized by reduced cyclodextrin cavity volume and ''self-inclusion''.

2. Experimental

Crystallization.—Dilute aq solutions of TRIMEA (purchased from Cyclolab, Budapest) were prepared at room temperature, and heated to 40 °C. The initial concentrations were so low that this did not lead to spontaneous crystallization. As the water slowly evaporated, transparent colorless rod-shaped crystals appeared. The solution was kept at 40 °C until all water had evaporated. At 60 °C, crystals grow isomorphously, but show poorer diffraction quality.

X-ray diffraction experiments.—Crystals grown at 40 °C show excellent diffraction quality. Diffraction data were collected at room temperature on a 0.65 × 0.15 × 0.15 mm^3 specimen glued to a glass pin (Enraf-Nonius Turbo-CAD4 diffractometer on a FR571 rotating anode generator, Ni-filtered Cu $K\alpha$ radiation with $\lambda = 1.542$ Å). The space group is orthorhombic $P2_12_12_1$ with $a = 15.424(9)$, $b = 18.167(7)$, $c = 23.128(8)$ Å, vol = 6481(5) $Å^3$ (determined from the diffraction angles of 25 reflections), $Z = 4$, MW = 1225.3, $D_x = 1.26$ g cm^{-3}. Intensities of 5332 unique reflections were measured to a nominal resolution of $\lambda/2 \sin \theta_{max} = 0.89$ Å ($2\theta_{max} = 120°$, ω–2θ scan mode, no absorption correction).

Structure solution and refinement.—The structure was solved by direct methods (program SIR92 [6]) and refined by standard techniques (program SHELX76 [7] aided by computer graphics, program FRODO [8], function minimized $\Sigma w(|F_o| - |F_c|)^2$ with $w = 1.0$, H atoms calculated to ideal positions). No water site could be located, neither in the cavity nor in interstices. Refinement converged smoothly with $R = 0.059$ for 4616 reflections with $F_o > \sigma(F_o)$.

In the final model, the highest difference electron density peak corresponds to 0.41 electrons per $\mathring{A}^3$ and is close to atoms C-6^2 and O-6^2, indicating some minor disorder of the group C-6^2–O-6^2–Me. This disorder could not be resolved. The second highest difference electron density peak corresponds to 0.29 electrons per $\mathring{A}^3$; this is a typical value for fully refined crystal structures, indicating that there are no water sites which have been overlooked.

3. Results and discussion

General.—Fractional atomic coordinates and equivalent isotropic temperature factors are listed in Table 1. Atom labeling is as in our previous contributions (ref. [4] and references therein), e.g., C-2^3 means atom C-2 of glucose residue 3 of the oligosaccharide. The methyl C atoms attached to O-2^n, O-3^n and O-6^n are labeled C-7^n, C-8^n and C-9^n, respectively.

The calculated density $D_x = 1.26$ g cm^{-3} is typical for methylated cyclodextrins: 1.26 g cm^{-3} for TRIMEB monohydrate [3], 1.24 g cm^{-3} for DIMEB anhydrate [4], 1.21 g cm^{-3} for TRIMEB–4-biphenylacetic acid monohydrate [5]. Crystalline native cyclodextrins have appreciably higher densities, e.g., 1.49 g cm^{-3} for α-CD hexahydrate [9] and 1.45 g cm^{-3} for β-CD dodecahydrate [10].

Molecular conformation.—The TRIMEA molecule is shown in Fig. 1 in a projection on the least-squares (lsq) plane of the O-4 atoms, and in Fig. 2 in projections perpendicular to this plane. A selection of relevant geometrical parameters is given in Tables 2 and 3. The overall conformation of the molecule has almost two-fold symmetry, with opposing glucose residues in very similar configurations, Fig. 1A. Glucoses 1 and 4 are strongly tilted "inward" (tilt angles 38.3° and 33.7°, respectively), and their C-6–O-6–Me groups are oriented towards the cavity axis so that they form van der Waals contacts across the narrow end of the cyclodextrin cavity. A third glucose (residue 2) has its primary methoxy group also pointing "inward", forming van der Waals contacts with O-6–Me of residues 1 and 4. As is illustrated by a space-filling model shown in Fig. 1B, this completely closes the molecular cavity at its O-6 end. The overall shape is therefore not that of a torus, but that of a bowl (or that of a boat with the bows at glucose residues 3 and 6). This is similar to the conformations of TRIMEB [3] and DIMEB [4] crystallized from hot water, where the molecular cavities are also closed at the O-6 end by a "lid" of primary methoxy groups.

All glucose residues are in the normal $^4C_1$ chair conformation. The deviation from ideal chair geometry, which is measured by the ring puckering parameter θ [11], is in the normal range for all residues, with somewhat larger values only for residues 3 and 6 ($\theta = 13.8°$ and 17.1°, respectively). The tilt angles of the glucoses with respect to the

Table 1
Fractional atomic coordinates and equivalent isotropic temperature factors U_{eq}

Atom	x/a	y/b	z/c	U_{eq} (Å^2)
C-1^1	0.3543(4)	0.0082(3)	−0.1945(3)	0.062(6)
C-2^1	0.3609(4)	0.0698(3)	−0.2383(3)	0.061(6)
C-3^1	0.3644(4)	0.1440(3)	−0.2072(3)	0.060(6)
C-4^1	0.2911(4)	0.1524(3)	−0.1618(3)	0.061(6)
C-5^1	0.2799(4)	0.0821(3)	−0.1263(3)	0.064(6)
C-6^1	0.1935(5)	0.0834(4)	−0.0947(3)	0.087(8)
C-7^1	0.4222(6)	0.0295(5)	−0.3260(3)	0.117(9)
C-8^1	0.4335(6)	0.2290(4)	−0.2719(4)	0.111(9)
C-9^1	0.2313(7)	0.0379(7)	−0.0046(4)	0.18(1)
O-2^1	0.4350(3)	0.0628(3)	−0.2731(2)	0.078(5)
O-3^1	0.3535(4)	0.2033(2)	−0.2475(2)	0.082(5)
O-4^1	0.3189(3)	0.2095(2)	−0.1232(2)	0.065(4)
O-5^1	0.2788(3)	0.0176(2)	−0.1609(2)	0.068(5)
O-6^1	0.1866(5)	0.0224(3)	−0.0533(3)	0.132(8)
C-1^2	0.5912(4)	−0.1478(3)	−0.0842(2)	0.055(6)
C-2^2	0.6302(4)	−0.0966(3)	−0.1295(2)	0.054(6)
C-3^2	0.5715(4)	−0.0307(3)	−0.1400(2)	0.050(5)
C-4^2	0.4797(4)	−0.0553(3)	−0.1533(2)	0.055(6)
C-5^2	0.4472(4)	−0.1077(3)	−0.1059(3)	0.067(6)
C-6^2	0.3569(5)	−0.1456(5)	−0.1265(5)	0.148(9)
C-7^2	0.7806(5)	−0.1238(4)	−0.1155(3)	0.089(8)
C-8^2	0.6214(6)	0.0846(4)	−0.1749(3)	0.100(8)
C-9^2	0.2692(10)	−0.1707(7)	−0.0562(6)	0.22(1)
O-2^2	0.7131(3)	−0.0696(2)	−0.1140(2)	0.065(4)
O-3^2	0.6051(3)	0.0102(2)	−0.1871(2)	0.062(4)
O-4^2	0.4289(3)	0.0091(2)	−0.1577(2)	0.059(4)
O-5^2	0.5068(3)	−0.1686(2)	−0.1017(2)	0.065(4)
O-6^2	0.3351(6)	−0.1859(9)	−0.0846(6)	0.31(1)
C-1^3	0.5874(5)	−0.1382(3)	0.1437(3)	0.068(6)
C-2^3	0.6773(5)	−0.1333(4)	0.1178(3)	0.070(6)
C-3^3	0.6703(4)	−0.1043(3)	0.0562(2)	0.058(6)
C-4^3	0.6105(4)	−0.1528(3)	0.0211(2)	0.054(5)
C-5^3	0.5242(4)	−0.1725(3)	0.0513(2)	0.058(6)
C-6^3	0.4797(5)	−0.2384(4)	0.0257(3)	0.077(7)
C-7^3	0.7982(9)	−0.1204(7)	0.1771(6)	0.23(1)
C-8^3	0.7902(5)	−0.0325(4)	0.0231(4)	0.092(8)
C-9^3	0.5022(7)	−0.3586(5)	−0.0071(5)	0.16(1)
O-2^3	0.7323(3)	−0.0860(3)	0.1502(2)	0.086(5)
O-3^3	0.7527(3)	−0.1045(2)	0.0298(2)	0.065(4)
O-4^3	0.5909(3)	−0.1111(2)	−0.0306(2)	0.056(4)
O-5^3	0.5394(3)	−0.1915(2)	0.1119(2)	0.064(4)
O-6^3	0.5398(4)	−0.2969(2)	0.0211(2)	0.093(6)
C-1^4	0.3851(5)	0.0743(3)	0.2240(3)	0.065(6)
C-2^4	0.4656(5)	0.0529(3)	0.2577(3)	0.071(6)
C-3^4	0.5304(4)	0.0181(3)	0.2176(3)	0.064(6)
C-4^4	0.4906(4)	−0.0478(3)	0.1883(3)	0.060(6)
C-5^4	0.4019(5)	−0.0294(3)	0.1617(3)	0.066(6)
C-6^4	0.3509(5)	−0.1042(4)	0.1519(4)	0.091(8)
C-7^4	0.4629(7)	0.1427(5)	0.3340(3)	0.134(9)

Table 1 (continued)

Atom	x/a	y/b	z/c	U_{eq} (Å^2)
C-8^4	0.6791(5)	0.0395(5)	0.2413(4)	0.102(8)
C-9^4	0.2393(7)	−0.1569(5)	0.1035(5)	0.17(1)
O-2^4	0.5070(4)	0.1146(3)	0.2845(2)	0.095(6)
O-3^4	0.6050(3)	−0.0073(3)	0.2489(2)	0.078(5)
O-4^4	0.5480(3)	−0.0688(2)	0.1417(2)	0.061(4)
O-5^4	0.3475(3)	0.0099(2)	0.2010(2)	0.072(5)
O-6^4	0.2866(4)	−0.0872(3)	0.1141(3)	0.121(7)
C-1^5	0.3069(4)	0.3104(3)	0.0985(3)	0.064(6)
C-2^5	0.3808(4)	0.3231(3)	0.1408(3)	0.062(6)
C-3^5	0.4246(4)	0.2516(3)	0.1604(3)	0.060(6)
C-4^5	0.3598(4)	0.1926(3)	0.1752(3)	0.058(6)
C-5^5	0.2898(4)	0.1858(3)	0.1300(3)	0.063(6)
C-6^5	0.2193(5)	0.1319(4)	0.1453(3)	0.083(7)
C-7^5	0.3905(6)	0.3958(5)	−0.1956(3)	0.116(9)
C-8^5	0.5643(5)	0.2607(5)	0.2058(4)	0.106(9)
C-9^5	0.1229(6)	0.1963(5)	0.2072(4)	0.119(9)
O-2^5	0.4484(3)	0.3687(2)	0.1179(2)	0.077(5)
O-3^5	0.4744(3)	0.2684(2)	0.2110(2)	0.069(5)
O-4^5	0.4075(3)	0.1252(2)	0.1798(2)	0.061(4)
O-5^5	0.2499(3)	0.2568(3)	0.1209(2)	0.067(4)
O-6^5	0.1856(3)	0.1406(3)	0.2014(2)	0.090(6)
C-1^6	0.2730(4)	0.2765(3)	−0.1266(3)	0.063(6)
C-2^6	0.3335(4)	0.3394(3)	−0.1116(3)	0.066(6)
C-3^6	0.3708(4)	0.3309(3)	−0.0506(2)	0.057(6)
C-4^6	0.2985(4)	0.3197(3)	−0.0072(2)	0.057(6)
C-5^6	0.2239(4)	0.2690(4)	−0.0276(3)	0.064(6)
C-6^6	0.1399(5)	0.2829(5)	0.0043(3)	0.092(8)
C-7^6	0.4235(6)	0.4436(3)	0.1104(3)	0.093(8)
C-8^6	0.5066(5)	0.3914(5)	−0.0313(4)	0.103(8)
C-9^6	0.0472(9)	0.3800(8)	−0.0104(8)	0.32(1)
O-2^6	0.4049(3)	0.3455(3)	−0.1507(2)	0.074(5)
O-3^6	0.4157(3)	0.3958(2)	−0.0346(2)	0.065(4)
O-4^6	0.3398(3)	0.2897(2)	0.0434(2)	0.059(4)
O-5^6	0.2030(3)	0.2795(2)	−0.0884(2)	0.065(4)
O-6^6	0.1192(3)	0.3591(4)	0.0054(3)	0.117(7)

molecular axis range from −4.0° to +38.3°, Table 2, i.e. from a slight tilt of the O-6 side away from the molecular axis to a large tilt towards the axis. The span of tilt angles is large compared to native cyclodextrins or anhydrous DIMEB [4], but much smaller than for the highly distorted molecule in TRIMEB monohydrate, where the tilt angles range from −24.5° to +72.9° [3].

The geometry of the O-4 hexagon is shown in Fig. 3 in a projection on the O-4 lsq plane. The hexagon is relatively regular with the O-4^{n-1} ··· O-4^n ··· O-4^{n+1} angles in the narrow range 116.1–122.8° (compare TRIMEB monohydrate: 91.9–161.6°!). The difference between the shortest and longest diagonal, 8.09 and 8.69 Å, is only 0.60 Å, which is smaller than in some complexes of native α-CD (e.g., 8.05 and 8.91 Å with a difference of 0.86 Å in α-CD-benzaldehyde-hexahydrate [12]). The deviations of the

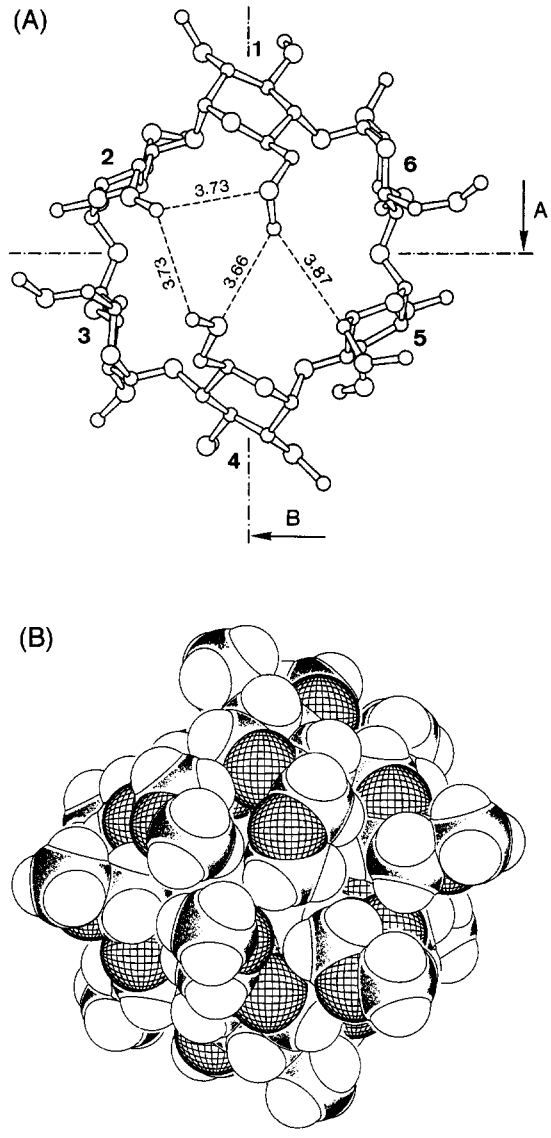


Fig. 1. The TRIMEA molecule in projection on the O-4 least-squares plane; the O-6–Me rim is towards the viewer. (A) Ball-and-stick model. Dashed lines show van der Waals contacts between O-6–Me groups (distances in Å). Dash-dotted lines indicate the projection planes of Fig. 2. (B) Space-filling model showing that the molecular cavity is completely closed at the O-6 side.

O-4 atoms from their lsq plane are ≤ 0.48 Å, which is large compared to native α-CD and its complexes (e.g., ≤ 0.14 Å in α-CD-hexahydrate [9]), but small compared to TRIMEB monohydrate, where one of the seven O-4 atoms deviates 1.09 Å from the O-4 lsq plane [3].

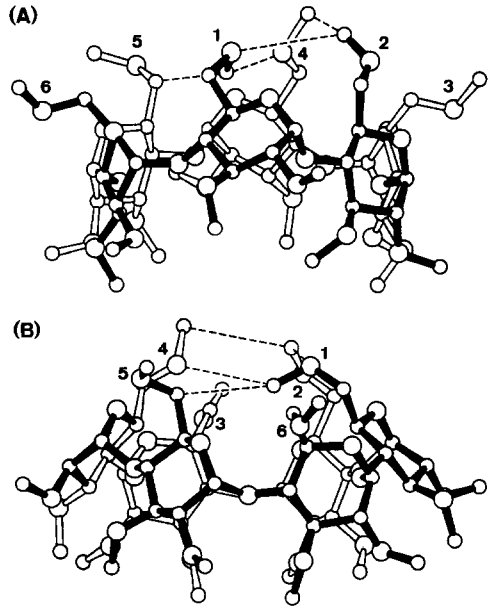


Fig. 2. The TRIMEA molecule in two projections perpendicular to the O-4 least-squares plane, as indicated in Fig. 1. Glucose residues are labeled at the O-6 atoms. Dashed lines show van der Waals contacts as in Fig. 1. (A) Projection plane through atoms O-4^3 and O-4^6. (B) Projection perpendicular to (A).

Table 2
Geometric parameters of the TRIMEA molecule

Parameter	Residue					
	1	2	3	4	5	6
Tilt angle [a] (deg)	38.3	4.6	1.2	33.7	15.4	−4.0
O-2 · · · O-3′ (Å)	3.429(6)	3.441(6)	3.332(7)	3.309(6)	3.598(6)	3.510(6)
C-4–O-4–C-1″ (deg)	116.0(5)	118.6(5)	118.6(4)	118.6(5)	119.0(5)	118.1(5)
QT [b] (Å)	0.539(6)	0.574(6)	0.560(7)	0.558(7)	0.532(6)	0.528(6)
θ_2 [c] (deg)	10.8(6)	3.6(6)	13.8(7)	9.8(7)	9.8(6)	17.1(8)

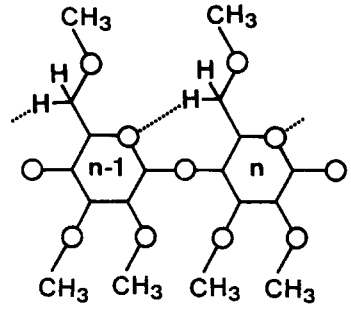
[a] Tilt angle of the least-squares (lsq) plane through C-1–C-2–C-3–C-4–C-5–O-5 with respect to the molecular axis. The molecular axis is defined as the normal to the lsq plane through the O-4 atoms; positive and negative signs denote tilt of the O-6 side towards and away from the molecular axis, respectively.
[b] Puckering amplitude [11].
[c] θ_2 measures the deviation from ideal chair conformation [11] (ideal value: $\theta_2 = 0°$).

Intramolecular C–H · · · O interactions.—In native and 2,6-dimethylated CDs, there are two types of intramolecular hydrogen bonds which stabilize the molecular conformation. As the dominant motif, O–H · · · O bonds are systematically formed between O-2 and O-3 of adjacent glucose residues. In addition, there are much weaker, but similarly systematic C–H · · · O interactions, C-6^n–H · · · O-5^{n-1} [4,13]. In permethylated CDs,

Table 3
Selection of torsion angles (deg)

	Residue					
	1	2	3	4	5	6
Secondary methoxy groups						
C-1–C-2–O-2–C-7	96.8(7)	70.5(6)	112.9(8)	74.1(7)	69.6(7)	94.8(7)
C-2–C-3–O-3–C-8	−89.5(7)	−124.4(6)	−106.9(6)	−104.7(6)	−115.2(6)	−109.3(6)
Primary methoxy groups						
O-5–C-5–C-6–O-6	66.6(7)	65.6(9)	−71.0(6)	78.1(7)	−73.3(7)	−73.6(7)
C-4–C-5–C-6–O-6	−171.2(6)	−178.7(8)	50.0(7)	−162.8(6)	48.5(8)	49.3(8)
C-5–C-6–O-6–C-9	78.7(9)	113(1)	−175.4(6)	178.9(6)	81.5(8)	128(1)
Interglucose linkage						
C-3–C-4–O-4–C-1′	109.7(6)	151.2(5)	137.1(5)	117.2(6)	140.8(5)	145.0(5)
C-4–O-4–C-1′–O-5′	91.6(6)	115.7(5)	97.7(6)	94.8(6)	110.9(6)	97.7(6)

the O–H · · · O interactions are deleted, whereas the weak C-6^n–H · · · O-5^{n-1} interactions persist, as shown below.



Even for the extremely distorted molecule of TRIMEB crystallized from hot water, Caira et al. [3] noted that these interactions occur systematically with H-6 · · · O-5

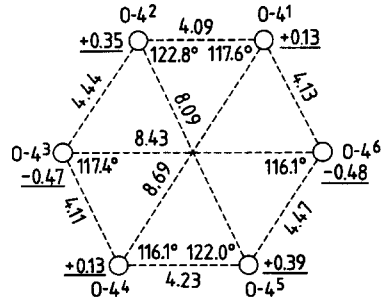


Fig. 3. Geometry of the hexagon of O-4 atoms (given in Å and degrees). Values for angles O-4^{n-1}–O-4^n–O-4^{n+1} and deviations from the O-4 least-squares plane are given at the O-4 atoms.

Table 4
Geometry of the intramolecular C-6^n–H · · · O-5^{n-1} contacts (for ideal H-positions with C–H = 1.09 Å; these are well defined by the C-5–C-6–O-6 bonds)

	Residue					
	1	2	3	4	5	6
C-6 · · · O-5^{n-1} (Å)	3.568(9)	3.30(1)	3.234(8)	3.438(9)	3.238(9)	3.220(9)
H-6 · · · O-5^{n-1} (Å)	2.66	2.33	2.34	2.45	2.41	2.34
Angle at H-6 (deg)	141	147	138	151	132	136

distances as short as 2.35 Å. In the present structure, C-6^n–H · · · O-5^{n-1} contacts are also found systematically between all neighboring glucoses, with H · · · O separations in the range 2.33–2.66 Å, as shown in Table 4. These are typical values for C–H · · · O hydrogen bonds [14].

Crystal packing.—In the crystal lattice, the TRIMEA molecules are stacked in a herringbone pattern, Fig. 4. The primary methoxy group O-6^6–Me intrudes into the cavity of a neighboring molecule (''self inclusion''). The isotropic displacement parameter of the terminal C atom C-9^6 of the included group, U_{eq} = 0.32 Å^2, is the largest in the crystal structure (Table 1) and indicates extensive thermal vibrations with a rms displacement from its center of almost 0.6 Å. The distance of C-9^6 of the included group and atom C-9^1 of the host, which is the central atom of the ''lid'' closing the narrow end of the cavity, is 5.11 Å. This is more than van der Waals separation, but still far too small to permit the insertion of a water molecule. The arrangement parallels that in anhydrous DIMEB, where the CD cavity is also closed on one end and filled by self-inclusion from the other end.

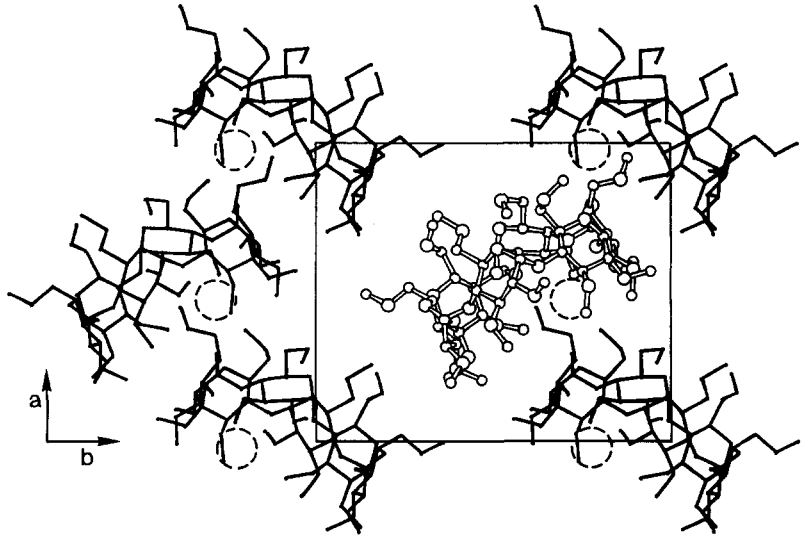


Fig. 4. Crystal packing in projection on the $a-b$ plane. For clarity, one molecule is drawn with open bonds. The location of a small void at the O-2–O-3 opening of the CD cavity is sketched by a dashed sphere.

The TRIMEA molecule has 30 ether–O atoms as potential hydrogen-bond acceptors, but only the C–H groups as potential donors. This suggests that there might be numerous intermolecular C–H · · · O interactions in the crystal structure, which are often formed when the number of acceptors exceeds the number of O–H or N–H donors [15]. They are abundant in carbohydrates [13] and were also found in anhydrous DIMEB [4]. These interactions, however, play only a minor role in the present crystal structure. This may be because the O atoms are sterically shielded by the attached Me groups, and not easily accessible for intermolecular contacts. Notably, in anhydrous DIMEB, the most frequent acceptors of intermolecular C–H · · · O interactions are the hydroxyl groups O-3–H [4]. In consequence, the crystal cohesion in anhydrous TRIMEA is almost entirely due to van der Waals contacts between C–H groups.

Is there a small void left in the cavity?.—Using the program PLATON [16], the crystal structure was searched for voids which are large enough to accommodate water molecules. Only one small void was found at the O-2–O-3 side of the TRIMEA cavity, sketched by dashed lines in Fig. 4. The center of the void ($x/a = 0.517$, $y/b = 0.222$, $z/c = -0.115$) has two O atoms in potential H-bonding distances (2.95 Å to O-2[6], 3.07 Å to O-4[1]), and in addition, there are seven contacts to H atoms bonded to C (2.41–2.84 Å). The distance to the terminal atom C-9[6] of the included methoxy group is only 3.07 Å; since this atom vibrates extensively, thereby reducing the effective size of the void, it is questionable whether a water molecule can in fact be accommodated. To test whether the void is empty or filled, the structure was re-refined with a water molecule included at this site. The water occupancy and displacement parameters were allowed to vary, leading to unrealistic values ($U = 1.04$ Å^2, occ. $= 0.20$) without improvement of the R-factor, indicating that the void is empty.

No voids were found between the macrocycles, indicating effective and dense packing. This is apparently facilitated by appropriate orientation of the flexible methoxy groups.

Crystallization from cold water containing NaCl.—For cyclodextrins, different crystal forms of the same complex are sometimes obtained under different crystallization conditions. This is also the case for DIMEB crystallized from an aq solution, where the anhydrous form is obtained from hot water [4], whereas from cold water at least two forms with larger unit cell volume can be grown, which contain a considerable amount of water of hydration [17]. TRIMEA failed to crystallize from pure cold water, but crystals were grown when the solvent contained NaCl. This can be taken as an analogy to the "salting out" in protein crystallization. Diluted aq solutions of TRIMEA containing about 1 M NaCl were left at room temperature, and as the water slowly evaporated, crystals of TRIMEA and of NaCl grew separately. Diffraction data were collected for such a TRIMEA crystal: space group $P2_12_12_1$, $a = 15.474(3)$, $b = 18.180(3)$, $c = 23.136(4)$ Å, vol $= 6509(2)$ Å^3. Since the unit cell constants are virtually the same as for the crystal grown from hot water, the coordinates from Table 1 were used as the starting model for structure solution. The refinement rapidly converged to $R = 0.058$ for 4822 reflections with $F_o > \sigma(F_o)$. The refined structure is isomorphous with that of the crystal grown from hot water, including details like the orientation of the methoxy groups. In this case also, the small void at the O-2–O-3 opening of the TRIMEA cavity does not accommodate a water molecule.

Conclusions.—This is the third crystal structure of a methylated cyclodextrin grown from hot water. These three structures have some common features (1) The crystal structures contain no or only one molecule of crystal water in the asymmetric unit. The CD cavities are free of water. (2) The molecules are bowl-shaped, i.e. the molecular opening is closed at the O-6 end. This largely reduces the cavity volume. In TRIMEB, this is associated with extreme structural distortions. The remaining cavity volume is filled by parts of neighboring CD molecules. (3) The crystal packing is dense, so that accommodation of interstitial water molecules is avoided. Unlike the native cyclodextrins, the methylated species can effectively prevent interstitial voids by suitable orientation of the flexible methoxy groups. (4) Crystal cohesion is primarily due to van der Waals interactions.

These similarities suggest that maximization of hydrophobic interactions is a dominant driving force in the crystallization of methylated CDs. The reason for the high solubility in cold water remains an unanswered question.

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