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Structure of the complex of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin with (2,4-dichlorophenoxy)acetic acid☆

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Abstract

The structure of the complex formed by heptakis(2,6-di-*O*-methyl)-β-cyclodextrin and (2,4-dichlorophenoxy)acetic acid was studied by X-ray diffraction. The dichlorophenyl moiety of the guest molecule was found outside the host hydrophobic cavity in the primary methoxy groups region whereas the oxyacetic acid chain penetrates the cavity from the primary face. The host molecules stacks along the *a* crystal axis forming a column. In the space between three successive hosts of the column, a guest molecule is accommodated.

Keywords: Inclusion compound; Crystal structure; Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin; (2,4-Dichlorophenoxy)acetic acid; Screw column

1. Introduction

(2,4-Dichlorophenoxy)acetic acid (2,4-D, **2**), a synthetic compound causing many responses common to natural auxins, is classified as a plant growth regulator and not as a hormone, since it is not produced by the plants.[2] It is the first selective herbicide discovered and, as it is chemically very stable, it has been widely used commercially. Although it exhibits auxin activity at low concentrations, as all the phenoxyacetic acids, it becomes phytotoxic at relatively higher concentrations and thus it was used as a defoliant in the war in Vietnam.[3] Heptakis(2,6-di-*O*-methyl)cyclomaltoheptaose (DMβCD, **1**), a chemically modified cyclomaltoheptaose (β-cyclodextrin, β-CD) derivative arising by methylation of the 2 and 6 hydroxyl groups, exhibits an increased solubility in water and organic solvents,[4] compared to the native β-CD, enabling to use it as a carrier for poorly soluble molecules in hydrophobic solvents or in water.[5,6] The DMβCD molecule is conformationally similar to native β-CD as there still exist the hydrogen bonds between the O-2 methoxy and O-3 hydroxy groups of adjacent glucose units, keeping the macrocycle rigid. However, steric hindrance due to methylation of the O-2 hydroxy groups does not allow for a dimerization, as in the majority of the crystalline β-CD complexes. The methylation extends the depth of the hydrophobic torus, estimated to be 10–11 Å compared with 7.8 Å for the native β-CD, enabling it to form complexes with larger substrates.[7] In spite of this ability of the host, the guest molecules are not found inside the DMβCD macrocycle cavity in four reported structures. In three complexes, those of *p*-iodophenol/DMβCD, *p*-nitrophenol/DMβCD[8] and 2-naphthoic acid/DMβCD,[9] the guest was found outside the cavity in the region of the primary methoxy groups, while in carmofur (1-hexylcarbamoyl-5-fluorouracil)/DMβCD[10] it was located in the area of the secondary rim of CD. In three others, those of 1,7-dioxaspiro[5.5]undecane/DMβCD,[11] adamantanol/DMβCD[7,12] and acetic acid/DMβCD[13] complexes, the guest was located inside the cavity. In a continuing effort to study inclusion compounds of plant growth regulators in cyclodextrins, we prepared the complex of 2,4-D/DMβCD, the crystal structure of which is now presented.

☆ Part III in the series: Inclusion compounds of plant growth regulators in cyclodextrins. For Part II, see: Ref. 1.

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2. Experimental

Crystallization.—2,4-D (obtained from Fluka) and DMβCD (purchased from Cyclolab) was dissolved in water (concentrations 0.02 M) at a 1:1 host:guest mole ratio. The resulting mixture was heated at 50 °C and left at this temperature for a period of three days, at the end of which colorless crystals of the title complex suitable for X-ray data collection had formed.

X-ray data collection.—Final lattice parameters, determined by 32 reflections, are given in Table 1 along with other information on data collection and structure refinement. Data collection was done on a crystal, sealed in a Linderman glass capillary, on a Syntex $P2_1$ four-circle diffractometer upgraded by Crystal Logic, attached to a Rigaku rotating anode generator, using a graphite monochromatized Cu Kα radiation. Preliminary data collection has indicated the crystal to be orthorhombic. As a consequence, one quarter of the sphere was collected giving 8941 (8245 unique) reflections with $2\theta_{max} < 100°$. The scan mode was θ–2θ. Three standard reflections monitored every 97 reflections showed an overall decay of 14.2% of the intensity. Lorenz, polarization and decay corrections were applied to the intensity data.

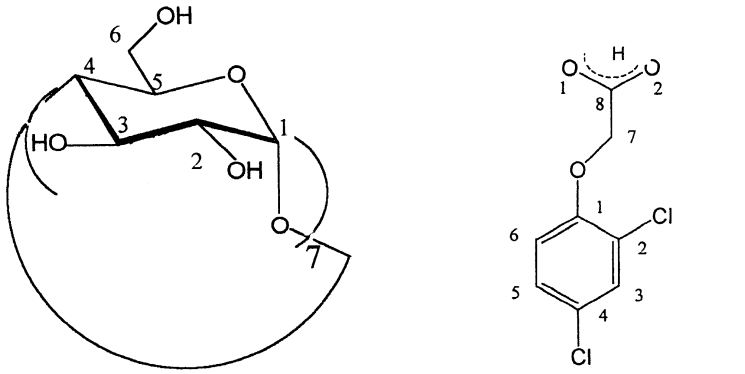
Table 1
Crystal data and structure refinement for 2,4-D/DMβCD

Empirical formula	$C_{64}H_{91}O_{35}\cdot C_8H_6O_3Cl_2\cdot (H_2O)_{0.35}$
Formula weight	1557.38
Temperature (K)	293(2)
Wavelength, λ Cu Kα (Å)	1.54180
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Unit cell dimensions (Å)	$a = 14.886(7)$ $b = 18.980(4)$ $c = 28.515(6)$
Volume ($Å^{-3}$)	8057(4)
Z	4
D_{calcd} (Mg/m^{-3})	1.284
Absorption coefficient (mm^{-1})	1.486
$F(000)$	3316
Crystal size (mm)	$0.3 \times 0.4 \times 0.5$
θ Range for data collection (°)	2.80–50.01
Limiting indices (°)	$-14 \le h \le 0$ $-18 \le k \le 5$ $-28 \le l \le 28$
Reflections collected/unique	8941/8245 [R(int) = 0.0414]
Completeness to θ	50.01, 99.7%
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	8245/0/888
Goodness-of-fit on F^2	1.057
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0753$, $wR_2 = 0.1900$
R indices (all data)	$R_1 = 0.1479$, $wR_2 = 0.2414$
Absolute structure parameter	0.97 (6)
Extinction coefficient	0.00076 (11)
Largest diff. peak and hole (e $Å^{-3}$)	0.242 and −0.191

Structure solution and refinement.—The structure was solved by molecular replacement using the skeleton atom coordinates of the DMβCD molecule of the *p*-nitrophenol/DMβCD complex.[8] Sequential difference electron density maps ($\Delta\rho$) revealed the positions of the remaining non-hydrogen atoms of the host, all the atoms of the guest and the oxygen atom of one water molecule. The refinement, based on F^2, proceeded by using the SHELXL-97 program.[14] Hydrogen atoms linked to carbon atoms of the host and guest molecules were used at calculated positions with C–H distance 0.98 Å for the tertiary, 0.97 Å for the secondary, 0.96 Å for the primary and 0.93 Å for the benzyl H-atoms, while their thermal parameters have been set to $1.2 \times U_{iso}$ of the isotropic thermal parameter of the corresponding C atom. All the non-hydrogen atoms of the host molecule, the chlorine atoms of the guest and the oxygen atom of the water molecule were refined anisotropically. Extinction correction was applied, and 19 reflection intensities exhibiting poor agreement were given zero weight during the final refinement cycles. The refinement converged at $R = 0.0753$ and 0.1479 for observed ($I_o > 2.0\sigma(I_o)$) and all reflections (Table 1), respectively. An atomic numbering of the host and guest molecules is given in Scheme 1. A top and side views of the complex are given in Fig. 1. C-*mn* and O-*mn* denoting the *m*th atom within the *n*th glucosidic residue of the host.

3. Results and discussion

Molecular geometry and conformation of DMβCD.—Two methyl groups, the primary C-91 and the secondary C-72, and one methoxy group, the O-65–C-95, are disordered over two sites, the occupancies of their major sites being 0.69, 0.66 and 0.54, respectively. The DMβCD atoms exhibit an increased thermal movement, about the same as the atoms of the guest molecule. This is not unusual, as it is observed also in the complex of DMβCD with 1,7-dioxaspiro-[5.5]undecane[11] and the anhydrous DMβCD.[15] The thermal parameters of C-93 are particularly high but attempts to find more than one atomic sites failed. Four methoxy groups have a gauche–gauche orientation pointing outwards of the cavity, see the torsion angles (Table 2). The O-63–C-63 and O-64–C-64 and both sites of the disordered O-65–C-65 groups exhibit a gauche–trans orientation pointing towards the DMβCD cavity. Thus the opening is limited at the primary side of the CD cavity. The methyl groups of



β-CD (2,4-dichlorophenoxy)acetic acid

1 **2**

Scheme 1. An atomic numbering of the host and guest molecules.

the secondary side are oriented away from the torus, an arrangement favorable for the intramolecular O-3n–H···O-2(n + 1) hydrogen bonds[12] varying between 2.754 and 2.972 Å and giving the rigid shape to the truncated cone of the host. The distances of the glycosidic O-4n atoms from their mean plane range between − 0.173(6) and 0.134(5) Å, their values being significantly greater than the observed in β-CD.[1,16] The O-4n···O-4(n + 1) distances vary between 4.36 and 4.44 Å, the O-4(n − 1)···O-4n···O-4(n + 1) angles range from 126.3 to 132.2°, while the distances of the approximate center K of the O-4n heptagon vary between 4.91 and 5.19 Å and the O-4n···K···O-4(n + 1) angles range from 50.2 to 52.5°. These values indicate that although the O-4n atoms mean plane of the DMβCD molecule is more puckered than in the native β-CD, its shape is nearly round. The tilt angles of glycopyranose residues, defined as the angles between the O-4n mean plane and the individual mean planes formed by the O-4(n − 1), C-1n, C-4n and O-4n atoms, range between 1.2 (9) and 23.5 (7)°, being all positive as in the *p*-iodophenol and *p*-nitrophenol/DMβCD isomorphous complexes[8] and in two DMβCD-hydrates.[17] Therefore, the glucose units are tilted towards the approximate sevenfold molecular axis.

The guest molecules.—The main feature of the structure of the 2,4-D/DMβCD complex is that the dichlorophenyl group of the 2,4-D molecule is located outside the hydrophobic cavity at the primary side of the host and the hydrophilic moiety, the oxyacetic group, penetrates into the cavity from the primary face (Fig. 1). This arrangement may be attributed to the difficulty of the aromatic ring, substituted at 2 and 4 positions by chlorine atoms, to fit into the rigid cavity and, therefore, staying in the space between three host molecules. This arrangement, common to all three crystal structures that are isomorphous to the title complex, is not quite well understood.[8,9] An explanation is given by Harata pretending that, because of the many methyl groups of the hydrophobic cavity of the DMβCD molecule, unlike that of the native β-CD, a guest molecule can favorably be accommodated in the interstitial site when its shape and size are well suited to the space.[18] Besides, the guest molecules of all these complexes have a common feature: they consist of a planar ring with hydrophilic moieties bonded to it. Note that the adamantol molecule, having a ball shape, is quite included inside the DMβCD cavity.[7,11] The characterization of these molecules, not inserting inside the DMβCD cavity, as 'guests' is valid only if we accept that a guest must not be necessarily fully trapped inside the cavity to be designated as such.

A hydrogen bond is formed between the O-1 atom of the carboxyl group of the guest and the water molecule (2.78 Å), found inside the cavity and having the low occupancy factor of 0.35, but between the host and guest atoms only van der Waals contacts exist. In the *p*-iodophenol and the *p*-nitrophenol/DMβCD complexes,[8] H-bonds are observed between hosts and guests of the same asymmetric unit while in the 2-naphthoic acid/DMβCD complex, where the carboxyl group is pointing away from the host molecule, such a H-bond is formed between the guest and an adjacent host molecule.[9] The molecular geometry of 2,4-D is nearly the expected, the values of the bond lengths and angles not differing significantly from the generally accepted. The dichlorophenyl group, the chlorine atoms and the ether oxygen atom lie on a plane within 0.038 (17) Å; the carboxyl group is nearly perpendicular to this plane forming an angle of 75 (1)° with it.

The molecular packing.—The complexes are stacked along the *a* crystal axis and the O-4n best planes of the host molecule forms an angle of 25.4° with the *bc* plane. The O-4n best planes of two adjacent DMβCD

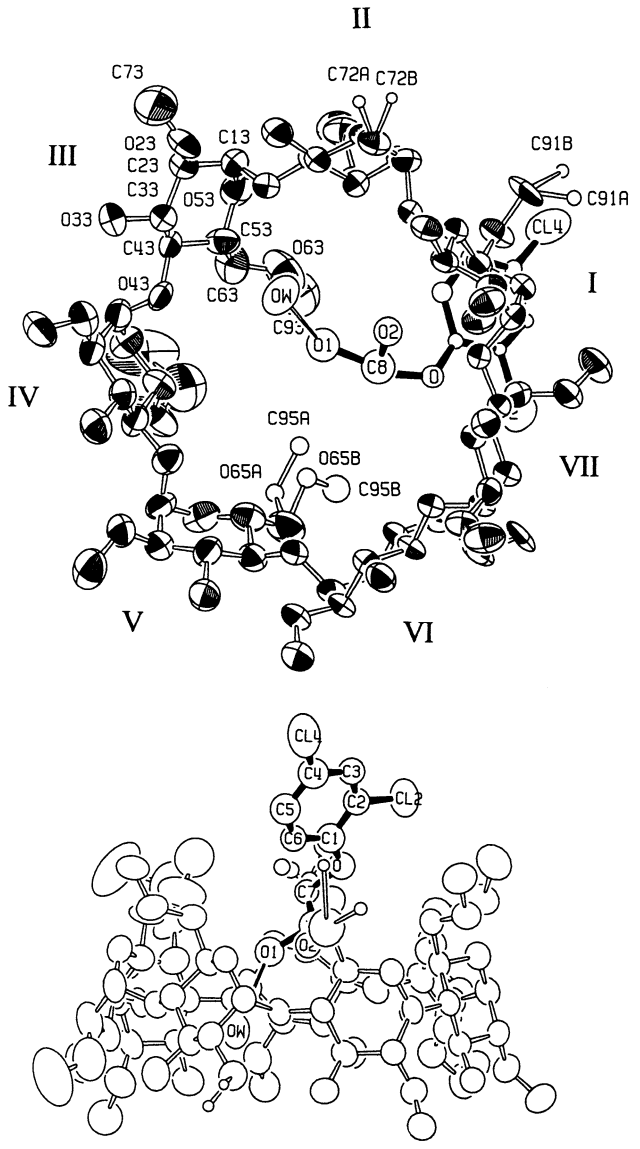


Fig. 1. A front- and side-view of the complex. C-*mn* and O-*mn* denote the *m*th atom within the *n*th glycoside residue.

molecules of the same stack form an angle of 50.8°, two primary methoxy groups of one DMβCD molecule entering into the cavity of the other (Fig. 2). As a result the DMβCD cavity is closed from the secondary side, the host and guest molecules forming a compact column. The molecular packing consists of such columns linked by the *b* and *c* screw axis through van der Waals contacts, and it is characterized as 'cage type',[8,9] but it may be designated also as a 'screw column', as the host molecules of these compact columns are linked by a screw axis.

This molecular packing is found also in the carmofur/DMβCD complex[10] and the anhydrous DMβCD structure.[15] In the former complex, the fluorouracil moiety of the guest molecule, disordered over two sites, is located outside the cavity, in the region of the secondary side of the host. The long alkyl chain of the hexyl group of the major site penetrates into the host cavity, ending in the primary methoxy groups region, while this of the minor site was found completely outside the cavity. The orientation of the guest molecule is quite different from the observed one in the title complex, but at any rate the fluorouracil moiety does not enter in the cavity. The molecular packing of the complexes of DMβCD with guests having an aromatic or planar moiety is a 'screw column'. To our knowledge, only three DMβCD structures have been found till now not having a 'screw column' molecular packing: the adamantanol/DMβCD complex where the hosts are stacked along a column,[7,12] the 1,7-dioxaspiro[5.5]undecane/DMβCD complex with hosts forming layers[11] and the acetic acid/DMβCD complex being of herringbone-type.[13] In all those structures, the guest molecules are entrapped in the cavity and an aromatic moiety is in any case not involved. The water forms weak hydrogen bonds with the O-64 (2.94 Å) and O-65A (3.03 Å) atoms of the host. In the 'screw column' structures[8–10] the water molecules are also located inside the cavity.

Table 2
Selected torsion angles (°) for 2,4-D/DMβCD complex

Torsion angles	Site	$n=1$	$n=2$	$n=3$	$n=4$	$n=5$	$n=6$	$n=7$
C-3n–C-2n–O	A	−97.8 (11)	−97.1 (14)	−111.0 (19)	−92.0 (14)	−97.1 (13)	−99.2 (13)	−96.9 (12)
-2n–C-7n	B		−136.7 (19)					
C-1n–C-2n–O	A	142.2 (10)	142.1 (13)	125.3 (19)	143.9 (12)	140.3 (12)	140.3 (11)	142.3 (10)
-2n–C-7n	B		102.5 (19)					
C-4n–C-5n–C	A	48.7 (13)	46.2 (14)	−160.9 (13)	−170.8 (16)	170.3 (16)	49.4 (12)	51.7 (11)
-6n–O-6n	B					−141.7 (18)		
O-5n–C-5n–C	A	−68.4 (11)	−74.9 (12)	76.6 (16)	77.5 (18)	50.1 (19)	−72.8 (11)	−66.9 (10)
-6n–O-6n	B					98.1 (19)		
C-5n–C-6n–O	A	88.9 (18)	−172.5 (11)	173 (2)	−104.1 (19)	84 (3)	−171.8 (11)	−175.7 (10)
-6n–C-9n	B	126 (2)				176 (4)		

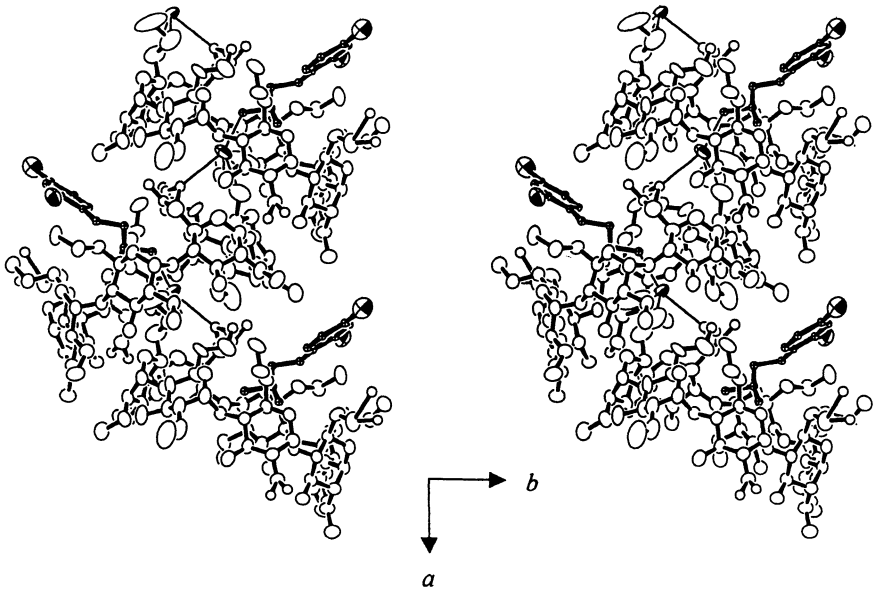


Fig. 2. A stereo diagram of the channel of the complex. The directions of the axis are indicated.

4. Supplementary material

Full crystallographic details, excluding structure factors, have been deposited with the Cambridge Crystallographic Data Center, deposition No CCDC 178967. These data may be obtained, on request, from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. Tel.: +44-1223-336408; fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk.

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References

1. Kokkinou A.; Yannakopoulou K.; Mavridis I. M.; Mentzafos D. *Carbohydr. Res.* **2001**, *332*, 85–94.
2. Salisbury F. B.; Ross C. W. *Plant Physiology*; Wadsworth: Belmont, CA, 1992; pp 361–372.
3. Devlin R.; Witham F. *Plant Physiology*; Willard Grant Press: Boston, 1983; pp 354–356.
4. Uekama K.; Irie T. In *Cyclodextrins and their Industrial Uses*; Duchene D., Ed.; Editions de Santé: Paris, 1987; pp 395–439.
5. Szejtli J. *J. Incl. Phenom.* **1983**, *1*, 135–150.
6. Szejtli J. *J. Incl. Phenom.* **1992**, *14*, 25–36.
7. Stezowski J. J.; Czugler M.; Eckle E. In *Adv. Incl. Sci.*; Szejtli J., Ed.; Reider Editions: Dordrecht, 1981; pp 151–161.
8. Harata K. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 1939–1944.
9. (a) Harata K. *Chem. Commun.* **1999**, 191–192;
(b) Harata K. *J. Chem. Soc., Chem. Commun.* **1993**, 546–547.
10. Harata K.; Hirayama F.; Uekama K.; Tsoucaris G. *Chem. Lett.* **1988**, 1585–1588.
11. Rysanek N.; Le Bas G.; Villain F.; Tsoucaris G. *Acta Crystallogr.* **1992**, *C48*, 1466–1471.
12. Czugler M.; Eckle E; Stezowski J. J. *J. Chem. Soc., Chem. Commun.* **1981**, 1291–1292.
13. Selkti M.; Navaza A.; Villain F.; Charpin P.; De Rango C. *J. Incl. Phenom.* **1977**, *27*, 1–12.
14. Sheldrick, G. M. SHELXL-97, Program for the refinement of Crystal Structures, University of Göttingen, Germany, 1993
15. Steiner T.; Saenger W. *Carbohydr. Res.* **1995**, *275*, 73–82.
16. Kokkinou A.; Makedonopoulou S.; Mentzafos D. *Carbohydr. Res.* **2000**, *328*, 135–140.
17. Stezowski J.; Parker W.; Hilgenkamp S.; Gdaniec M. *J. Am. Chem. Soc.* **2001**, *123*, 3919–3926.
18. Harata K. *Chem. Rev.* **1998**, *98*, 1803–1827.