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Inclusion compounds of plant growth regulators in cyclodextrins. V. 4-Chlorophenoxyacetic acid encapsulated in β-cyclodextrin and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin

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The crystal structures of 4-chlorophenoxyacetic acid (4CPA) included in β-cyclodextrin (β-CD) and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMβCD) have been studied by X-ray diffraction. The 4CPA/β-CD complex crystallizes as a head-to-head dimer in the space group *C*2 in the *Tetrad* packing mode. The packing modes of some β-CD dimeric complexes, having unique stackings, are also discussed. The 4CPA/TMβCD inclusion complex crystallizes in the space group $P2_1$ and its asymmetric unit contains two crystallographically independent complexes, complex *A* and complex *B*, exhibiting different conformations. The host molecule of complex *A* is significantly distorted, as a glucosidic residue rotated about the O4′—C1 and C4—O4 bonds forms an aperture where the guest molecule is accommodated. The phenyl moiety of the guest molecule of complex *B* is nearly perpendicular to the mean plane of the O4*n* atoms. The conformations of the guest molecules of the two complexes are similar. The crystal packing consists of antiparallel columns as in the majority of the TMβCD complexes published so far.

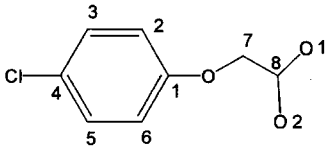
1. Introduction

Cyclodextrins (CDs) are cyclic oligosacharides comprising six, seven or eight α-(1–4)-linked D-glucoses, called α-, β- or γ-CD, respectively, having a central hydrophobic cavity where a plethora of molecules, with appropriate shapes and sizes can be encapsulated. CDs are soluble in water and have environmentally compatible chemical properties (Yannakopoulou & Mavridis, 2004). Therefore, they can be used in plant cell biotechnology as solubilizers of the relatively insoluble precursors for bioconversions by plant cells or enzymes, and they probably protect agents and reactant carriers (van Uden *et al.*, 1994). Besides, they may be used to achieve the slow release of substances from their respective CD complexes to sustain their action. As the cost of CD production decreases, they have become increasingly important. β-CD, which is the cheapest among its homologues, has received particular attention.

The permethylated CDs, resulting from substitution of the hydroxyl groups by methoxy groups, are able to include several molecules that cannot be encapsulated inside the cavity of the native CDs as they have a deeper and more flexible hydrophobic cavity. Their complexes exhibit an increased aqueous solubility compared with the corresponding complexes of native CDs and they protect the included guests from hydrolysis both in solution and in the solid state (Caira *et al.*, 1994). Hence, the permethylated CDs are currently used as carriers of drugs, natural products, flavors, cosmetics *etc.*

TMβCD, also being the cheapest among its homologues, has received increased attention.

Auxins are plant hormones producing a growth response and, therefore, they are characterized as plant growth regulators (Davies, 1995). In addition to the naturally occurring auxins, more than 200 synthetic ones, with effects on plant growth at low concentrations, are commercially available. They are also used as herbicides or pesticides at higher concentrations (Hance & Holly, 1990). Several hundred commercial products, containing such substances, are used in various forms, concentrations and combinations. Chlorophenoxyacetic acids are synthetic substances mimicking the action of auxins. 4-Chlorophenoxyacetic acid (4CPA) [see (I)] is used to improve the fruit setting in tomatoes, to inhibit sprout formation in mung beans, to achieve fruit thinning in peaches (Roberts, 1998), to improve the settings of the vine fruit and enhance the cluster but not berry weight (Delgado & Martin, 2002), or to increase the weight and number of the fruits of pepinos (Ercan & Akilli, 1996). Moreover, genotoxic evaluation in the wing spot test in *Drosphila melanogaster*, which assesses somatic mutation and recombination events, indicates that 4CPA does not induce any significant increase in the frequency of evaluated spots (Kaya *et al.*, 1999). The Environmental Protection Agency of the USA has decided that 4CPA is generally of low acute and subchronic toxicity, although it is a severe eye irritant, and does not exhibit mutagenic properties (WPA-738-F-97-001, 1997). Therefore, 4CPA is considered to be a sufficiently safe plant growth regulator, exhibiting only slightly toxic qualities, and it is fairly widely used in the food industry. However, the inclusion compounds of 4CPA in CDs have not received any attention.



(I)

As part of a systematic investigation of the inclusion compounds of plant growth regulators in CDs we report here the crystal structures of the complexes of 4CPA encapsulated in β-CD (4CPA/β-CD) and in TMβCD (4CPA/TMβCD). Studies concerning the effects of these complexes on plant growth are in progress.

2. Experimental

2.1. Crystallization

Aqueous solutions of 4CPA (concentration = 0.018 *M*) and β-CD (concentration = 0.02 *M*) were mixed and the mixture was stirred for 2 h. The resulting precipitate was redissolved at 343 K and left to cool to room temperature over a period of two weeks. At the end of this period, colorless transparent crystals of 4CPA/β-CD formed in the form of multiple platelets stacked together. A single crystal suitable for X-ray data collection was obtained by the careful separation of one such platelet.

Crystals of 4CPA/TMβCD were obtained by mixing aqueous solutions of 4CPA and TMβCD at a 1:1 host:guest mole ratio (concentrations = 0.02 *M*). The mixture was stirred for 30 min at 318 K and then maintained at 323 K for 3 d. Colorless transparent crystals of the complex, suitable for X-ray data collection, were obtained. 4CPA and TMβCD were purchased from Aldrich, and β-CD from Fluka.

2.2. X-ray data collection

The final lattice parameters of 4CPA/β-CD and 4CPA/TMβCD are summarized in Table 1 along with information on data collection and refinement.[1]

The X-ray data of 4CPA/β-CD were collected at room temperature using Cu $K\alpha$ radiation (λ = 1.5418 Å) by the θ–2θ scanning method on a Syntex $P2_1$ diffractometer upgraded by Crystal Logic (Strouse, 2002), equipped with a Rigaku rotating anode and graphite monochromator. The single crystal chosen was sealed in a glass capillary with some mother liquor. Three standard reflections monitored every 97 reflections showed an overall decay of 9.67% of the intensity. Lorenz, polarization and decay corrections were applied to the intensity data.

Low-temperature and high-resolution X-ray data of 4CPA/TMβCD were collected using the synchrotron-radiation light source at the EMBL X13 beamline at the DORIS storage ring, DESY, Hamburg, using a marCCD 165 detector. From a batch of crystals kept under oil, one crystal was picked with a cryoloop and flash cooled under the N_2 stream to 100 K. Two data sets were collected:

(i) a high-resolution set (0.74 Å) of 250 frames with rotation $\Delta\varphi = 1^\circ$ and

(ii) a low-resolution set (2.00 Å) of 125 frames with rotation $\Delta\varphi = 1^\circ$.

The programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) were used for data processing and scaling. The data collected contained $h, k, +/-l$ reflections. Although $h, -k, +/-l$ reflections are not equivalent due to the anomalous contribution, they were not collected because it was not considered necessary as the correct absolute configuration of TMβCD was known. The presence of the $-k$ reflections would have increased the accuracy slightly, primarily because of the counting statistics, but their absence does not introduce any systematic error into the structural parameters.

2.3. Structure solution and refinement

The structure of 4CPA/β-CD was solved by molecular replacement using the atom coordinates of the skeleton of the host molecule of the 4,7-dimethylcoumarin/β-CD structure (Brett *et al.*, 2000). Subsequent $\Delta\rho$ maps revealed the positions of the remaining non-H atoms of the host, the guest and the O atoms of the water molecules. The refinement, based on F^2, was performed using *SHELXL*97 (Sheldrick, 1993).

[1] Supplementary data for this paper are available from the IUCr electronic archives (Reference: NS5002). Services for accessing these data are described at the back of the journal.

Table 1
Experimental details.

	4-CPA/β-CD	4CPA/TMβCD
Crystal data		
Chemical formula	$C_{50}H_{77}ClO_{55.25}$	$C_{141.50}H_{238}Cl_2O_{80}$
M_r	1597.57	3290.22
Cell setting, space group	Monoclinic, $C2$	Monoclinic, $P2_1$
a, b, c (Å)	18.998 (9), 24.820 (10), 16.662 (7)	10.926 (2), 25.284 (5), 29.954 (6)
β (°)	105.29 (3)	92.86 (3)
V (Å^3)	7578 (6)	8265 (3)
Z	4	2
D_x (Mg m^{-3})	1.400	1.322
Radiation type	Cu $K\alpha$	Synchrotron
No. of reflections for cell parameters	30	150
θ range (°)	2.75–58.38	1.54–32.79
μ (mm^{-1})	1.46	0.14
Temperature (K)	293 (2)	100 (2)
Crystal form, colour	Plate, colourless	Rod, colourless
Crystal size (mm)	0.3 × 0.2 × 0.05	0.5 × 0.4 × 0.2
Data collection		
Diffractometer	Syntex P21	marCCD165
Data collection method	θ–2θ	Single oscillation
Absorption correction	None	None
No. of measured, independent and observed reflections	5632, 5472, 3104	20 382, 20 382, 19 350
Criterion for observed reflections	$I > 2\sigma(I)$	$I > 2\sigma(I)$
R_{int}	0.116	0.000
θ_{max} (°)	58.4	32.8
Range of h, k, l	$-4 \Rightarrow h \Rightarrow 20$ $-4 \Rightarrow k \Rightarrow 27$ $-18 \Rightarrow l \Rightarrow 17$	$0 \Rightarrow h \Rightarrow 13$ $0 \Rightarrow k \Rightarrow 34$ $-40 \Rightarrow l \Rightarrow 40$
Intensity decay (%)	9.67	None
Refinement		
Refinement on	F^2	F^2
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.123, 0.399, 1.46	0.078, 0.231, 1.15
No. of reflections	5472	20 382
No. of parameters	778	2594
H-atom treatment	Constrained refinement	Independent refinement
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.2P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$	$w = 1/[\sigma^2(F_o^2) + (0.2P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.174	0.913
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.70, −0.53	0.94, −0.67
Extinction method	None	*SHELXL*
Extinction coefficient	–	0.0201 (13)

Computer programs used: *SHELXS*97 (Sheldrick, 1993), *DIRDIF*99 (Beurskens *et al.*, 1998).

Calculated positions for H atoms linked to C atoms of the β-CD molecule were used with C—H distances of 0.98 Å for the secondary and 0.97 Å for the tertiary H atoms. The displacement parameters of these H atoms were 1.2 × U_{iso} of the isotropic displacement parameter of the corresponding C atom. The guest molecule was found to be disordered over two sites and its atomic positions were modelled by fitting them on the maxima of the electron density map, using the molecular graphics program *O* (Jones & Kjedgaad, 1995). The phenyl rings of the guest molecules of both sites were forced to form an ideal hexagon (C—C distances 1.39 Å, angles 120°). The occupation factors of the two sites of the guest molecule were refined, the sum of their values having been kept equal to 1, thus implying a host:guest stoichiometry of 1:1 for the complex. The small number of observations, leading to a small ratio of observed reflections-to-parameters (final reflections/parameters ratio = 7.0, Table 1), has not permitted an anisotropic refinement for all the non-H atoms. Thus, only the non-disordered O atoms, the C1*n* and C4*n* atoms, and the O atoms of some water molecules were refined anisotropically. The 35 reflections exhibiting poor agreement or being negative were given zero weight during the final refinement cycles. The refinement converged to R_1 = 0.1231 and R_2 = 0.2033 for the observed and all reflections, respectively. High values of R are frequently observed in the cyclodextrin structures because of:

(i) the frequent disorder of the guest, the water molecules and some of the hydroxyl groups of the host, and

(ii) the small but significant flexibilities of both the glycosidic links and the glucose rings of the host.

As a result the observed data are limited in number and do not allow for the anisotropic treatment of all the non-H atoms. The case of the 4CPA/β-CD crystals was not an exception.

The structure of 4CPA/TMβCD has been solved by a Patterson-vector search method and Fourier recycling with *DIRDIF*99 (Beurskens *et al.*, 1998), using the coordinates of the macrocycle of the indole-3 butyric acid/TMβCD complex (Tsorteki *et al.*, 2004). Subsequent difference electron density maps ($\Delta\rho$) revealed the positions of the remaining non-H atoms of the host, guest and water molecules, and the majority of the H atoms. Refinement of the occupancy factors of the guest molecules of both complexes converged to 1.0, implying that the host:guest stoichiometry is 1:1. Anisotropic refinement for all the non-H and non-disordered atoms has also been carried out and an extinction correction has been applied [extinction coefficient 0.0201 (13)]. Three reflections exhibiting poor agreement were given zero weight during the final refinement cycles. The refinement was based on F^2 using block-diagonal least-squares and proceeded with the *SHELXL*97 program (Sheldrick, 1993). Two blocks were used, each including one complex. The refinement converged to R_1 = 0.0783 and R_2 = 0.0799 for observed and all reflections.

3. Results and discussion

3.1. 4CPA/β-CD

3.1.1. Description of the structure. The numbering scheme of the host molecule is given in Fig. 1 (*ORTEP*III; Burnett & Johnson, 1996). C*mn* and O*mn* denote the *m*th C and O atoms within the *n*th glucosidic residue. The complex crystallizes in the space group *C*2. Two complexes related by the *b* axis form a dimer *via* the usual O3*n*···O3′(8 − *n*) hydrogen bonds (*D*···*A* distances range from 2.795 to 2.859 Å, mean value 2.819 Å; *C*—*D*···*A* angles range from 114.5 to 119.0°, mean value 116.7°). The lattice parameters differ slightly from those of the other structures crystallizing in the space group *C*2 according to the *Channel* packing mode (CH; see Mentzafos *et al.*, 1991), but they are similar to those of the 4,7-dimethylcoumarin/β-CD complex (Brett *et al.*, 2000). The volume of the asymmetric unit, 1895 Å^3, is greater than the mean value, 1760 Å^3, of the volume of the structures crystallizing in the CH mode. As the structure was solved by molecular replacement of the coordinates of 4,7-dimethylcoumarin/β-CD, which has been assigned a different distinct mode (*Tetrad* mode), the 4CPA/β-CD complex is isostructural to it and crystallizes not according to the CH packing mode, but according to the *Tetrad* (TT) mode

The crystal packing consists of layers stacking along the *c* axis, a common characteristic of all the complexes crystallizing in the space group *C*2. The distance between the centres of the O4*n* heptagons of the hosts in two adjacent layers is 15.12 Å. These heptagons form an angle of 9.54° with the *ab* crystal plane, which is approximately equal to that observed in the CH mode. However, their orientation towards the *ab* crystal plane was opposite that observed in CH structures (Figs. 2 and 3). Therefore, the angle between the normal to the O4*n* mean plane and the *c* axis is 24.83°, whereas in the CH packing mode it is 9.9°. The relative shifting of two dimers of the adjacent layers in 4CPA/β-CD is 7.00 Å, which is much greater than the mean value of 2.7 Å observed in CH packing modes, but close to the value of 6.87 Å found in 4,7-dimethylcoumarin/β-CD. As this shifting is even greater than the value of 6.0 Å observed in the *Intermediate* packing modes (IM), it is reasonable to consider TT to be a new packing mode.

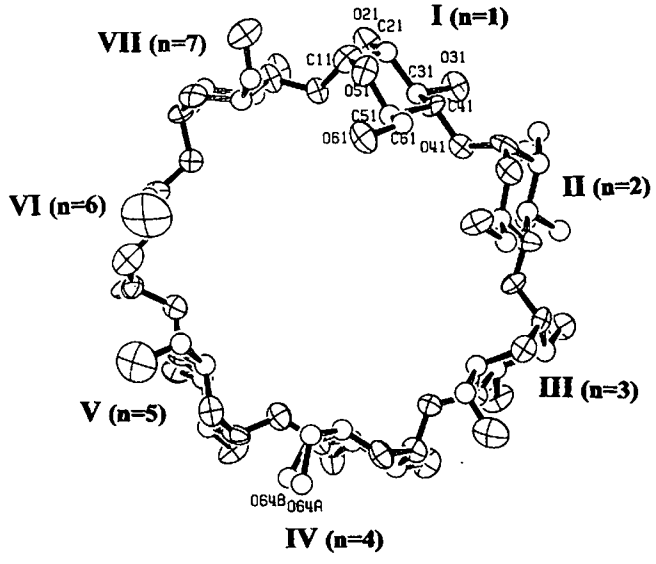


Figure 1
A side view of the 4CPA/β-CD complex. C*mn* and O*mn* denote the *m*th atom within the *n*th glycoside residue.

3.1.2. Packing modes of the β-CD dimers. The great majority of the β-CD dimeric structures crystallize according to the four packing modes (Mentzafos *et al.*, 1991) CH, IM, *Screw-Channel* (SC) and *Chessboard* (CB). As well as the two structures crystallizing in the TT packing mode, some dimeric structures with different stacking have recently been published (Table 2). Two dimeric structures, with *p*-hydroxybenzaldehyde (Braga *et al.*, 2002) or benzoic acid (Aree & Chaichit, 2003) as guest molecules, crystallize in the space group *P*1. Their unit-cell axes are similar to those of the dimeric β-CD structures of CH (*P*1) or IM packing modes, but their unit-cell angles are different. Attempts to reduce these unit cells to the corresponding CH (*P*1), IM or another packing mode with higher symmetry failed. Layers of the *p*-hydroxybenzaldeyde/β-CD complex stack along the *c* axis and the two consecutive layers shift by only 1.14 Å. This is much less than the 2.7 Å observed in CH packing modes (Fig. 4); the mean planes of the host dimer form angles of 11.1 and 9.8° with respect to the *ab* plane. The lateral shift of the two adjacent layers of the benzoic acid/β-CD complex, stacked along the *c* axis, is 6.49 Å. This value is about the average shift observed in the IM and TT packing modes (mean values 6.0 Å and 6.93 Å). The mean planes of the O4*n* atoms form angles of 2.6° and 2.8° with the *ab* plane (Fig. 5). Therefore, hydroxybenzaldeyde/β-CD tends to form an ideal channel (Fig. 4), whereas the packing mode of benzoic acid/β-CD is somewhat between IM and TT (Fig. 5). As a consequence, these two structures are not isostructural to any other or to each other and have unique packing modes which have never been observed before. Apart from these structures, the β-CD dimeric complexes of paroxetine (Caira, de Vries & Nassimbeni, 2003) and adamantanone (Sanchez-Ruiz *et al.*, 1999) crystallize in the space group $P2_1$ with the packing mode SC and show a displacement of the contiguous layers of *ca* 4 Å (Caira, de Vries & Nassimbeni, 2003; see Table 2). This is much greater than the 2.6 Å observed in the other structures of the SC packing modes. These two complexes are not isostructural to the other SC structures, although they have the same space group and unit-cell parameters.

Finally, the neohesperidin-dihydrochalcone/β-CD complex (Malpezzi *et al.*, 2004) crystallizes in the space group $C222_1$ and has quite different cell dimensions (Table 2) compared with those of the other $C222_1$ structures reported so far, which pack according to the CB packing mode. In this structure the dimers also stack along the *c* axis and their O4*n* mean planes form angles of 7.77° with the *ab* plane. However, the packing mode is not CB. Instead, the dimers form a 'screw column' and the projection of the centers of the O4*n* heptagon on the *ab* plane have a distance of *ca* 4.4 Å, which is half the 8.7 Å observed in the other $C222_1$ structures. The hydrophobic isovanilin moiety of the guest molecule is included inside the

hydrophobic dimeric cavity, while the rest of the guest molecule is accommodated at the interface of the dimers of the column.

Four dimeric β-CD structures with more than one dimer in their asymmetric units have also been reported:

(i) The 1,2-bis(4-aminophenyl)ethane/β-CD complex (Giastas *et al.*, 2003), which crystallizes in the space group $C2$. The c axis is 33.315 Å, which is double the length of those observed in the other $C2$ structures, because of the existence of two types of β-CD dimeric complexes with different guest conformations. Its crystal mode resembles the SC mode, but the screw channel is developed along the c screw axis and the adjacent dimers are crystallographicaly independent. The angles of the approximately sevenfold axes of the two dimers with the c axis are 23 and 3°, respectively, and their lateral displacement is *ca* 2.45 Å.

(ii) The (barbital)$_4$/β-CD complex (Nakanishi *et al.*, 1984), which crystallizes in the space group $P1$ with an a axis of 34.341 Å. The two dimers included in the unit cell form a tetrameric channel, with the tetramers stacking according to the IM packing mode.

(iii) Similarly, in the (*S*)-(−)-methyl-*p*-tolyl sulfoxide/β-CD complex (Fujiwara *et al.*, 1988), which crystallizes in the space group $P2_1$, there are two dimers per asymmetric unit which also form tetrameric channels. The length of the b axis, 65.04 Å, is approximately double the mean value of the c axis of the β-CD dimeric structures which crystallize in the space group $C222_1$ and stack according to the CB packing mode.

(iv) Finally, the cyclizine/β-CD complex (Caira *et al.*, 2001), with similar cell dimensions and the same space group ($P2_1$) as the former complex, seems to be isostructural with it. Its crystal packing also consists of tetramers having a unique host:guest stoichiometry of 4:3, owing to the different stoichiometry of the two dimers of the asymmetric unit as one dimer includes one guest molecule and the other includes two guests.

3.1.3. Conformation of the host molecule. All the pyranose residues have the usual $^4C_1$ conformation, as indicated by the Cremer–Pople parameters Q and θ (Cremer & Pople, 1975). The primary hydroxyl group O64 is disordered over two positions with the occupation factor of its major site being 0.54. Four primary hydroxyl groups have a *gauche–gauche* orientation pointing outside the cavity. The remaining three primary hydroxyl groups, which are attached to residues I, II and VI, have the *gauche–trans* orientation pointing to the interior of the cavity. The primary hydroxyl groups of residues I and II of 4,7-dimethylcoumarin/β-CD exhibit the same orientations. The O62 hydroxyl group forms hydrogen bonds

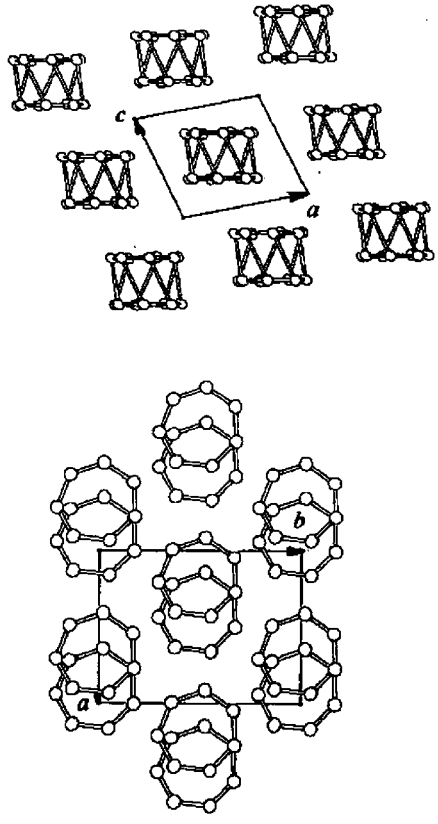


Figure 2
Projection of the β-CD dimers crystallizing according to the TT packing mode onto the *ab* plane and along the b axis. Each β-CD molecule is represented by its O4*n* heptagon.

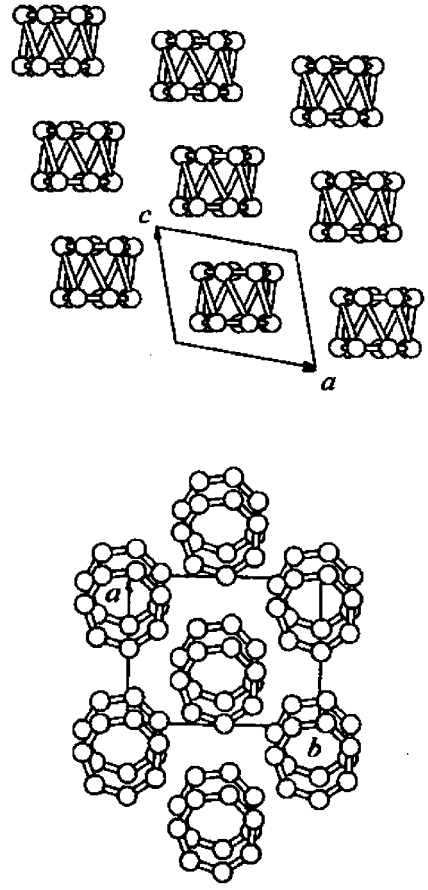


Figure 3
Projection of the β-CD dimers crystallizing according to the CH packing mode onto the *ab* plane and along the b axis. Each β-CD molecule is represented by its O4*n* heptagon.

with the O61 and O62 hydroxyl groups of an adjacent dimer (Table 3), as in 4,7-dimethylcoumarin/β-CD. This is a feature of the TT packing mode and the interdimeric hydrogen bonds explain the *gauche–trans* orientation of the hydroxyl groups. The *gauche–trans* orientation of the O66 hydroxyl group is due to the formation of hydrogen bonds with four water molecules lying in the interlayer space (Table 3).

The glucosidic O4*n* atoms lie on a plane to within 0.086 (8) Å. The deformation of the heptagon formed by these O atoms is limited, as indicated by the distances of the approximate centre (K) of the heptagon from these atoms (K$\cdots$O4*n*, range 4.92–5.23 Å), the O4*n*$\cdots$O4(n + 1) distances varying between 4.25 and 4.59 Å, and the corresponding angles O4*n*$\cdots$K$\cdots$O4(n + 1) (range 49.2–55.3°) and O4(n − 1)$\cdots$O4*n*$\cdots$O4(n + 1) (range 124.4–131.3°). The tilt angles of the glucopyranose residues vary between 4.4 and 16.5°, the maximum values being those of residues I and II which are attributed to the hydrogen bonds of their primary hydroxyls with those of an adjacent dimer. The torsion angles φ = O5(n + 1)—C1(n + 1)—O4*n*—C4*n* and ψ = C1(n + 1)—O4*n*—C4*n*—C3*n*, describing the relative orientation of the individual glucoses, range between 107.5–116.1° and 122.9–134.2°; the generally accepted ranges being 102–123° and 112–149° (Saenger & Steiner, 1998). Therefore, the distortion of the host molecule is limited.

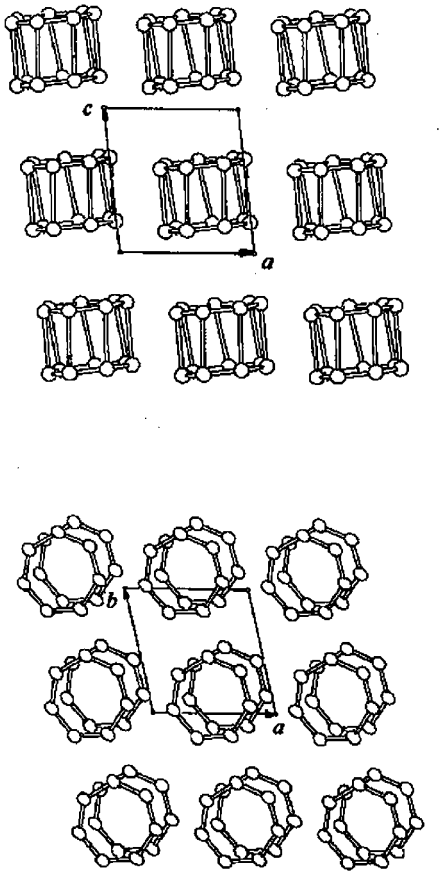


Figure 4
Projection of the *p*-hydrobenzaldehyde/β-CD complex onto the *ac* and *ab* planes. Each β-CD molecule is represented by its O4*n* heptagon.

3.1.4. The guest molecule. The guest molecule is disordered over two sites with occupation factors 0.64 (site *A*) and 0.36 (site *B*). The atomic displacement parameters of the molecule are generally small and similar to those of the host molecule, although the atoms of the phenyl groups have been forced to form an ideal hexagon. The C and the O1 atoms of the carboxyl group are common to both sites.

The phenyl group of the guest forms angles of 50.0 (5) (site *A*) or 60.2 (9)° (site *B*) with the mean plane of the heptagon of the O4*n* atoms. The angle between the phenyl groups of the two guest molecules inside the dimeric cavity is 15.3° (site *A*) or 9.1° (site *B*). The mutual distances between the atoms of these phenyl groups range between 3.83 and 4.52 Å (site *A*, mean value 4.17 Å) or 3.41 and 3.83 Å (site *B*, mean value 3.62 Å). These values suggest a π–π interaction between the guest molecules of site *B* and a weak interaction between those of site *A* (Fig. 6). The carboxyl group and the Cl atom are found in the primary and secondary rim regions of the host molecule, the whole guest being completely enclosed within the dimeric cavity (Fig. 7). The Cl atom is located near the secondary rim of the other host molecule of the dimer at a distance of 0.94 (site *A*) or 0.60 Å (site *B*) from the best plane of its O2*n* and O3*n* atoms. The carboxyl group is nearly perpendicular to the phenyl group, making an angle of 85 (2)° (site *A*) or 82 (3)° (site *B*). It has been found to be almost on the best plane of the C6*n* atoms, with the greater distance from it being that of the C atom which is common to both sites (0.277 Å).

3.1.5. The network of hydrogen bonds. The degree of hydration of 4CPA/β-CD is the same as that of the 4,7-dime-

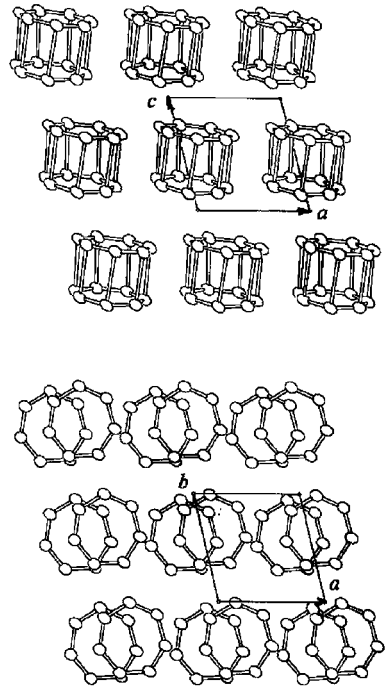


Figure 5
Projection of the benzoic acid/β-CD complex onto the *ac* and *ab* planes. Each β-CD molecule is represented by its O4*n* heptagon.

Table 2
Crystal data and packing modes of some dimeric β-CD structures.

Guest	a (Å) α (°)	b (Å) β (°)	c (Å) γ (°)	Space group	Packing mode	Lateral shifting of the dimers (Å)
4CPA[a]	18.998 (9)	24.820 (10) 105.29 (3)	16.662 (7)	$C2$	TT	7.00
4,7-Dimethylcoumarin[b]	19.514 (4)	24.024 (5) 104.49 (3)	16.414 (3)	$C2$	TT	6.86
p-Hydroxybenzaldehyde[c]	15.262 (2) 92.67 (1)	15.728 (1) 96.97 (1)	16.350 (1) 103.31 (1)	$P1$	Channel	1.14
Benzoic acid[d]	15.210 (1) 89.13 (1)	15.678(1) 74.64 (1)	15.687 (1) 76.40 (1)	$P1$	average of IM and TT	6.49
Paroxetin[e]	15.2262 (3)	31.4771 (1) 104.320 (1)	16.6739 (1)	$P2_1$	SC	4.0
Adamantanone[f]	15.428 (5)	32.545 (5) 103.56 (2)	15.437 (5)	$P2_1$	SC	4.0
Neohesperidin-dihydrochalcone[g]	15.125 (5)	30.523 (5)	41.332 (5)	$C222_1$	Screw column	4.4
1,2-Bis(4-aminophenyl)ethane[h]	19.319 (2)	24.19 (6) 103.92 (1)	33.315 (7)	$C2$	SC along the c axis	2.45
$(Barbital)_4$[i]	34.341 (5) 103.82 (1)	15.529 (2) 100.58 (2)	15.568 (2) 106.67 (1)	$P1$	Combination of CH and IM	
(*S*)-(−)-Methyl-*p*-tolyl sulfoxide[j]	15.495 (2)	65.04 (2) 102.60 (2)	15.471 (5)	$P2_1$	CB	
Cyclizine[k]	15.246 (1)	65.075 (5) 102.62 (1)	15.609 (1)	$P2_1$	CB	

References: (*a*) this work; (*b*) Brett *et al.* (2000); (*c*) Braga *et al.* (2002); (*d*) Aree & Chaichit (2003); (*e*) Caira, de Vries & Nassimbeni (2003); (*f*) Sanchez-Ruiz *et al.* (1999); (*g*) Malpezzi *et al.* (2004); (*h*) Giastas *et al.* (2003); (*i*) Nakanishi *et al.* (1984); (*j*) Fujiwara *et al.* (1988); (*k*) Caira *et al.* (2001).

thylcoumarin/β-CD complex. There are 17.2 water molecules distributed over 23 sites which form a network of hydrogen bonds with the hydroxyl groups of the β-CD. The water molecules govern the organization of the crystal structure and constitute two independent subnetworks related to the primary or secondary hydroxyl groups (Table 3). The O1 and O2*B* atoms of the guest are hydrogen bonded to water molecules which are found in the interdimeric area. O*W*O1, which is hydrogen bonded to O1, is also hydrogen bonded to the O61 hydroxyl group, thus forming a bridge between them. The water molecule O*W*61, which is hydrogen bonded to O61, is found inside the cavity near the primary rim of the host molecule, at a distance of 0.30 Å from the best plane of the C6*n* atoms. It is also hydrogen bonded with both sites of the disordered O64 hydroxyl group of an adjacent dimer and the O*W*65 water molecule (O*W*61$\cdots$O*W*65 distance = 2.575 Å). The complete immersion of the guest molecule inside the dimeric cavity and the formation of hydrogen bonds only *via* the water molecules located in the interdimeric space are also observed in the crystal structure of the *trans*-cinnamic acid/β-CD complex (Kokkinou *et al.*, 2000). The dimers of the same layer are linked by short hydrogen bonds which are formed between their O63 and O67 hydroxyl groups (Table 3).

3.2. 4CPA/TMβCD

3.2.1. Description of the structure. The crystal packing of 4CPA/TMβCD is unique. The complex crystallizes in the space group $P2_1$, like the inclusion compounds of indole-3-butyric acid, 2,4-dichlorophenoxyacetic acid (Tsorteki *et al.*, 2004) and 1-(*p*-bromophenyl) ethanol (Grandeury *et al.*, 2003) in TMβCD, but it has different unit-cell parameters (Table 4). Its crystal axes do not differ considerably from those of the TMβCD complexes with 1,7-dioxaspiro[5.5]undecane (Makedonopoulou *et al.*, 2001), methylcyclohexane (Rontoyianni *et al.*, 1998; Cardinael *et al.*, 2001) and L-menthol (Caira *et al.*, 1996; see also Table 4), crystallizing in the space group $P2_12_12_1$ and the volume of its asymmetric unit being 4133 Å^3, which is approximately double the volumes of the three complexes ranging between 2037 and 2144 Å^3. As a consequence, its asymmetric unit includes two crystallographically independent complexes, cited as complex *A* and complex *B*, with opposite orientations. This is the first time that such a structure has been observed. The existence of two complexes in the asymmetric unit was due to the different orientation of the guest inside the cavities, as described in detail below. Side views (Burnett & Johnson, 1996) of the two complexes are given in Figs. 8 (complex *A*) and 9 (complex *B*), along with the numbering scheme of the host molecules [C*mn*(*A* or *B*) and O*mn*(*A* or *B*) denote the *m*th atom within the *n*th glycosidic residues of complex *A* or complex *B*]. The mean planes of the heptagons of the O4*n* atoms of the two complexes are nearly perpendicular to the *ac* plane, forming angles of 74.7 (complex *A*) and 82.1° (complex *B*). The complexes form columns along the *a* axis which are inclined toward the *bc* plane. The mean planes of the O4*n* atoms form angles of 31.5 (complex *A*) and 24.7° (complex *B*) with the *bc* plane. The crystal packing consists of adjacent antiparallel and crystallographically independent columns (Fig. 10), while the adjacent columns of the crystal structures of the TMβCD complexes reported so far are linked by a twofold screw axis. There are 3.47 water molecules which are distributed over four sites and form hydrogen bonds with some atoms of the host or guest molecules (Table 3).

Table 3
Hydrogen bonds (Å, °).

4CPA/β-CD			
O61···O62^{i}	2.727	C61—O61···O62^{i}	108.8
		O61···O62—C62^{i}	109.9
O62···O62^{i}	2.712	C62—O62···O62	126.1
O63···O67^{ii}	2.751	C63—O63···O67^{ii}	127.8
		O63···O67—C67^{ii}	107.8
O25···O27^{iii}	2.701	C25—O25···O27^{iii}	123.3
		O25···O27—C27^{iii}	108.7
O21···OW21^{iv}	2.819	C21—O21···OW21^{iv}	114.9
O22···OW4^{iv}	2.778	C22—O22···OW4^{iv}	110.0
O23···OW23^{v}	2.872	C23—O23···OW23^{v}	98.8
O26···OW26^{vi}	2.516	C26—O26···OW26^{vi}	115.8
O34···OW4^{vii}	2.841	C34—O34···OW4^{vii}	109.5
O35···OW21^{vii}	2.693	C35—O35···OW21^{vii}	126.1
O36···OW9^{vi}	2.915	C36—O36···OW9^{vi}	114.9
O61···OW61^{i}	2.760	C61—O61···OW61^{i}	108.8
O61···OWO1^{vi}	2.847	C61—O61···OWO1^{vi}	131.0
O63···OW63^{viii}	2.707	C63—O63···OW63^{viii}	104.5
O63···OW7^{v}	2.704	C63—O63···OW7^{v}	129.3
O64*A*···OW64^{ix}	2.492	C64—O64*A*···OW64^{ix}	131.0
O64*A*···OW61^{ix}	2.954	C64—O64*A*···OW61^{ix} =	104.8
O64*B*···OW61^{ix}	2.738	C64—O64*B*···OW61^{ix}	120.8
O64*B*···OW64^{ix}	2.961	C64—O64*B*···OW64^{ix}	110.5
O65···OW65^{ix}	2.604	C65—O65···OW65^{ix}	113.0
O65···OW11^{iii}	2.810	C65—O65···OW11^{iii}	102.0
O66···OW13^{iv}	2.712	C66—O66···OW13^{iv}	117.5
O66···OW66^{vi}	2.815	C66—O66···OW66^{vi}	115.3
O66···OW11^{iii}	2.859	C66—O66···OW13^{iii}	123.8
O66···OW66^{x}	2.997	C66—O66···OW66^{x}	101.8
O67···OW67^{vi}	2.664	C67—O67···OW67^{vi}	117.9
O1···OWO1^{vi}	2.841	C—O1···OWO1^{vi}	123.0
O2*B*···OWO2^{xi}	2.499	C—O2*B*···OWO2^{xi}	119.8
4CPA/TMβCD			
OW1···OW2^{iv}	2.806		
OW1···O53*A*^{iv}	2.814	C13*A*—O53*A*···OW1^{iv}	103.7
		C53*A*—O53*A*···OW1^{iv}	134.9
OW1···O53*B*^{xii}	2.984	C13*B*—O53*B*^{xii}···OW1	113.3
		C53*B*—O53*B*^{xii}···OW1	123.1
OW2···O35*A*^{xiii}	2.728	C35*A*—O35*A*^{xiii}···OW2	114.6
		C85*A*—O35*A*^{xiii}···OW2	123.2
OW2···O1*A*^{xiv}	2.606	C*A*—O1*A*^{xiv}···OW2	114.4
OW3···O36*B*^{xv}	2.802	C36*B*—O36*B*^{xv}···OW3	108.2
		C86*B*—O36*B*^{xv}···OW3	138.5
OW3···O61*B*^{xvi}	2.822	C61*B*—O61*B*^{xvi}···OW3	127.3
		C91*B*—O61*B*^{xvi}....OW3	117.9
OW4···O34*B*^{xvii}	2.990	C34*B*—O34*B*^{xvii}···OW4	120.9
		C84*B*—O34*B*^{xvii}···OW4	108.3
O2*B*···O66*B*^{iv}	2.662	C*B*—O2*B*···O66*B*^{iv}	113.7
		O2*B*···O66*B*^{iv}—C66*B*	126.6
		O2*B*···O66*B*^{iv}—C96*B*	104.1

Symmetry codes: (i) $1-x, y, 2-z$; (ii) $-\frac{1}{2}+x, \frac{1}{2}+y, -z$; (iii) $\frac{3}{2}-x, \frac{1}{2}+y, 1-z$; (iv) x, y, z; (v) $\frac{1}{2}-x, \frac{1}{2}+y, 1-z$; (vi) $1-x, y, 1-z$; (vii) $\frac{1}{2}+x, \frac{1}{2}+y, -z$; (viii) $\frac{1}{2}+x, \frac{1}{2}+y, 1+z$; (ix) $\frac{1}{2}+x, \frac{1}{2}+y, z$; (x) $1+x, y, 1+z$; (xi) $\frac{1}{2}+x, \frac{1}{2}+y, 1-z$; (xii) $x, y, 1-z$; (xiii) $-x, \frac{1}{2}+y, 1-z$; (xiv) $1-x, -\frac{1}{2}+y, 1-z$; (xv) $1-x, \frac{1}{2}+y, 1-z$; (xvi) $x, y, -1-z$; (xvii) $-x, -\frac{1}{2}+y, 2-z$.

We are currently studying the X-ray data taken from another crystal of the inclusion compound of 4CPA with TMβCD (Tsorteki *et al.*, 2004), obtained from the same batch of 4CPA/TMβCD, which crystallized in the space group $P2_12_12_1$. This crystal has different cell dimensions, which are similar to those of the ethyl laurate/TMβCD complex (Mentzafos *et al.*, 1994; Table 4). Therefore, the inclusion compounds of 4CPA in TMβCD exhibit polymorphism (Bernstein *et al.*, 1999). The occurrence of polymorphism in crystals of the CD complexes is rare (Caira, 2001). Nevertheless, the crystal structure of the methylparaben/β-CD complex has recently been published. This complex crystallizes in two different space groups, *P*1 and *C*2 (Caira, de Vries, Nassimbeni & Jacewicz, 2003), and is the first CD complex to exhibit polymorphism.

3.2.2. Conformation of the host molecule. As the data collection was performed at low temperature (100 K), the displacement parameters were considered to be low for a CD structure. The displacement parameters of the atoms of complex *A* are higher than those of complex *B*. As the Cremer–Pople puckering parameters indicate (Cremer & Pople, 1975) all pyranose rings of both complexes have the usual $^4C_1$ conformation.

The conformations of the host molecules of complexes *A* and *B* exhibit significant differences. The TMβCD molecule of complex *A* appears more distorted than that of complex *B*, with this distortion being related to the orientation of the guest molecule inside the cavity. Residue VI of the host molecule of complex *A* is rotated about the C46*A*—O46*A* and C16*A*—O45*A* bonds, linking it with their vicinal residues. The mean plane of the C26*A*, C36*A*, C56*A* and O56*A* atoms forms an angle of 54.06 (13)° with the mean plane of the O4*n* atoms [the tilt angle of this residue is very high, 67.94 (15)°]. The chlorophenoxy moiety of the guest forms an angle of 39.19 (11)° with the mean plane of the O4*n* atom and 27.10 (13)° with the mean plance of C26*A*, C36*A*, C56*A* and O56*A* atoms. These angles indicate that the guest molecule 'raises' residue VI whilst attempting to exit to the inter-complex space (Fig. 8). Similar features have been observed in the indole-3-butyric acid/TMβCD and 2,4-dichlorophenoxy-

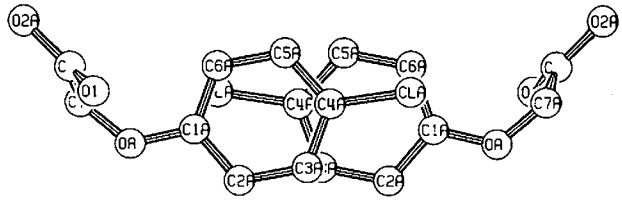


Figure 6
The two guest molecules of site *A* of the 4CPA/β-CD complex included inside the dimeric cavity.

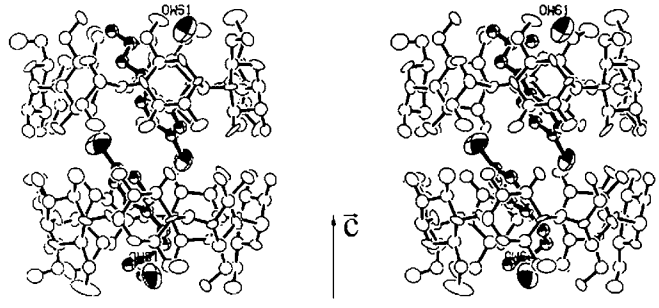


Figure 7
A stereo diagram of the dimer of the 4CPA/β-CD complex. The guest molecule of the major site *A* is shown.

Table 4
Cell dimensions and space groups of some TMβCD complexes.

Guest	a (Å)	b (Å) β (°)	c (Å)	Space group
(L)-Menthol[a]	11.060 (3)	26.138 (6)	29.669 (6)	$P2_12_12_1$
1,7-Dioxaspiro[5.5]undecane[b]	10.936 (7)	25.53 (2)	29.64 (4)	$P2_12_12_1$
Methylcyclohexane[c]	11.149 (2)	25.664 (2)	29.427 (5)	$P2_12_12_1$
Methylcyclohexane[d]	11.043 (4)	25.333 (4)	29.132 (2)	$P2_12_12_1$
Indole-3-butyric acid[e]	11.411 (7)	28.629 (7) 111.91 (2)	15.069 (4)	$P2_1$
2,4-Dichlorophenoxyacetic acid[e]	11.68 (2)	28.23 (5) 112.63 (7)	15.02 (3)	$P2_1$
1-(*p*-Bromophenyl)ethanol[f]	10.975 (8)	14.818 (9) 96.79 (3)	27.403 (17)	$P2_1$
Ethyl laurate[g]	14.796 (2)	22.444 (6)	27.720 (8)	$P2_12_12_1$
4CPA[h]	14.929 (10)	21.989 (14)	27.965 (18)	$P2_12_12_1$
4CPA[i]	10.926 (2)	25.284 (5) 92.86 (3)	29.954 (6)	$P2_1$

References: (*a*) Caira *et al.* (1996); (*b*) Makedonopoulou *et al.* (2001); (*c*) Rontoyianni *et al.* (1998); (*d*) Cardinael *et al.* (2001); (*e*) Tsorteki *et al.* (2004); (*f*) Grandeury *et al.* (2003); (*g*) Mentzafos *et al.* (1994); (*h*) Tsorteki *et al.*, unpublished results; (i) this work.

acetic acid/TMβCD structures (Tsorteki *et al.*, 2004), but their distortions were considerably smaller. Such a feature is not observed in complex *B*, where all the residues have similar tilt angles. Moreover, the orientation of the guest molecule is quite different, the chlorophenoxy moiety being almost perpendicular to the mean plane of the O4*n* atoms, thus forming an angle of 83.07° (Fig. 9).

The distances from the individual O4*n* glucosidic atoms to their mean plane range between −0.824 (2) and 0.511 (3) Å (complex *A*) or between −0.449 (2) and 0.501 (2) Å (complex *B*), indicating the deformation of the host, a characteristic also observed in other TMβCD structures (Tsorteki *et al.*, 2004). Although the range of O4$n\cdots$O4($n+1$) distances is limited (4.14–4.49 Å for complex *A* and 4.17–4.49 Å for complex *B*), the O4($n-1$)$\cdots$O4$n\cdots$O4($n+1$) angle range is significant (their values varying between 115.5 and 144.6° for complex *A* and 118.8 and 135.5° for complex *B*). In addition, the distances of the approximate K center of the heptagon from the individual O4*n* atoms range between 4.27 and 5.29 Å (complex *A*) or 4.64 and 5.35 Å (complex *B*). Therefore, both heptagons have an elliptical shape. The long axes of the ellipses are almost parallel to the lines passing from the O41 and O44 atoms.

Many methoxy and methyl groups of the host molecules are disordered. All the primary methoxy groups of complex A, except those of residue VII, are disordered over two or three sites. O63*A* is disordered over two sites, but we could not find two sites for the C93*A* methyl group bonded to it, although its displacement parameters are relatively high. In contrast, complex *B* only has two disordered primary methoxy groups. No unusual conformation of the primary chains has been found, the majority of them having the *gauche–gauche* (*gg*) conformation pointing outwards and the remainder having the *gauche–trans* (*gt*) conformation pointing inwards. Some secondary methoxy groups of both complexes are disordered, this disorder being more significant in complex *A*. Owing to the orientation of residue VI in complex *A*, both sites of its disordered primary methoxy group turn and close the primary aperture, producing the shape of a 'bowl'. The orientations of all the residues in complex *B* are such that the primary aperture is left open.

3.2.3. The guest molecule. The conformations of the guest molecules of both complexes are similar despite their different

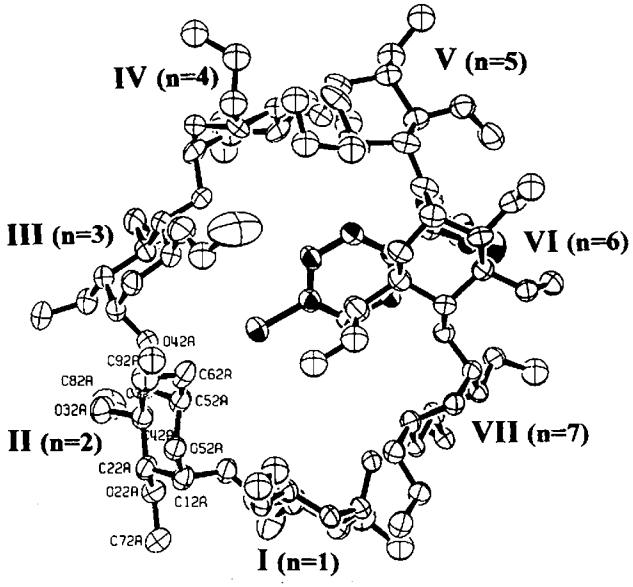


Figure 8
A side view of complex *A* of the 4CPA/TMβCD complex. C*mnA* and O*mnA* denote the *m*th atom within the *n*th glycoside residue.

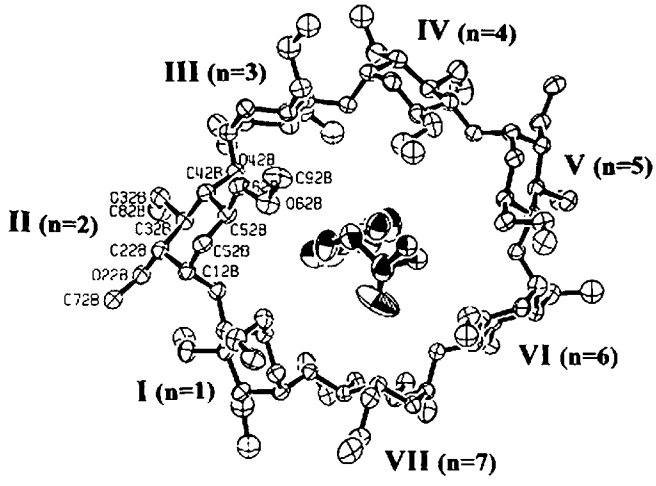


Figure 9
A side view of complex *B* of the 4CPA/TMβCD complex. C*mnB* and O*mnB* denote the *m*th atom within the *n*th glycoside residue.

orientations inside the cavities. The atoms of the guest molecules exhibit low thermal motion, the values of their displacement parameters being similar to those of the host molecules atoms. In both complexes the O2 atom of the carboxyl group of the guest molecule is disordered over two sites with the occupation factors of the major sites being 0.67 (complex *A*) or 0.75 (complex *B*). The chlorophenoxy moieties are planar within 0.018 (3) (complex *A*) and 0.043 (7) Å (complex *B*).

The Cl atom of complex *A* is found at a distance of 0.249 Å from the mean plane of the O2*nA* and O3*nA* atoms. The carboxyl group protrudes into the space existing between the adjacent host molecules within the column. This carboxyl group is nearly perpendicular to the mean plane of the chlorophenoxy moiety. The mean planes of the C*A*, O1*A*, O2*A* and C*A*, O1*A*, O2*C* atoms form angles of 88.6 (5) and 70.7 (5)° with the chlorophenoxy moiety and the C1*A*—O*A*—C7*A*—C*A* torsion angle is 73.2 (5)°. Both sites of the disordered O2*A* atom (O2*A* and O2*C*) turn towards the interior of the TMβCD cavity. The O1*A* atom forms a hydrogen bond with the O*W*2 water molecule, which is also hydrogen bonded with O35*A* of a vicinal complex *A* (Table 3).

The whole guest molecule of complex *B* is 'swallowed' inside the TMβCD cavity, not protruding out of it, and it is nearly perpendicular to the O4*n* mean plane. The carboxyl group and Cl atom of complex *B* have been found in the primary and secondary rims of the host molecule, respectively. The Cl atom is found at a distance of 1.84 Å from the mean plane of the O2*nB* and O3*nB* atoms. The carboxyl group is oriented towards the primary region of residue VI and the O1*B* atom is found at a distance of −0.070 Å from the mean plane of the C6*nB* atoms. The chlorophenoxy plane is almost parallel to the line linking the O41*B* and O44*B* atoms of the host molecule. The carboxyl group is nearly perpendicular to the mean plane of the chlorophenoxy moiety. The mean planes of the C*B*, O1*B*, O2*B* and C*B*, O1*B*, O2*D* atoms form angles of 79.1 (6) and 77.0 (7)° with the chlorophenoxy moiety and the C1*B*—O*B*—C7*B*—C*B* torsion angle is 69 (7)°, similar to that observed in complex *A*. The O2*B* atom forms a hydrogen bond with O66*B* of the host molecule of the same asymmetric unit, the corresponding methoxy group having the *gt* orientation pointing in towards the cavity (Table 3).

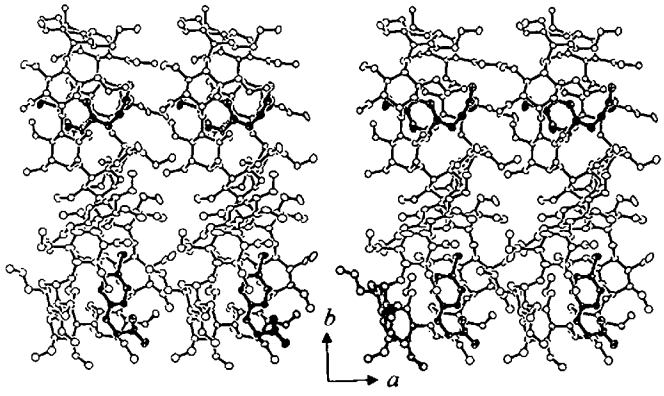


Figure 10
A stereoview of two adjacent columns of the 4CPA/TMβCD complexes formed by complex *A* (down) and complex *B* (up).

4. Concluding remarks

The crystal structures of the two inclusion compounds of 4CPA, in particular the TMβCD complex, are of great interest.

It has been suggested that the dimeric β-CD inclusion compounds stack according to four packing modes (Channel, Intermediate, Screw-Channel and Chessboard), which are characterized by different relative displacements of the adjacent dimeric layers (Mentzafos *et al.*, 1991). Since then some new packing modes have been reported. Additionally, for some crystal structures the space groups and cell dimensions indicate that they should be isostructural to one of the four above-mentioned packing modes, but they are not. Therefore, close correspondence of unit-cell dimensions is a necessary but not a sufficient condition for isostructurality (Giastas *et al.*, 2003). Thus, 4CPA/β-CD crystallizes in the space group *C*2, but rather than stacking according to the Channel packing mode it stacks according to the Tetrad packing mode, observed for the first time in the 4,7-dimethylcoumarin/β-CD complex (Brett *et al.*, 2000). In addition, the crystal structures of some β-CD dimeric complexes with unique packing have been published. In two complexes, *p*-hydroxybenzaldehyde/β-CD and benzoic acid/β-CD, a relative displacement of the adjacent dimeric layers of 1.1 and 6.5 Å is observed. In another complex (the neohesperin-dihydrochalcone/β-CD) a screw column is formed although a chessboard-packing mode was expected according to its space group. These findings indicate that it is not always possible to predict the crystal packing mode of a β-CD dimeric complex by its space group and unit-cell parameters.

4CPA/TMβCD is the first TMβCD structure with two complexes per asymmetric unit, owing to the different orientation of the guests inside the cavity of the host. Its crystal packing consists of adjacent antiparallel crystallographically independent columns, while the majority of the TMβCD complexes reported so far also crystallizes in the space group $P2_1$ and consist of adjacent antiparallel columns linked by a twofold screw axis. The guest molecule of complex *A* tends to be oriented parallel to the mean plane of the O4*n* atoms, while that of complex *B* is approximately perpendicular to it. As a result the cavity of complex *A* is excessively distorted and that of complex B tends to be a normal truncated cone. It is noted that the complexation process may induce these striking differences between the hosts of the two complexes, although the guest molecules retain the same conformation. Thus, the same guest having the same conformation does not inevitably cause a definite conformation of the host.

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Pinotsis for data collection of the 4CPA/TMβCD complex at the XXX beamline at Doris storage ring.

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