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Inclusion of parabens in β-cyclodextrin: A solution NMR and X-ray structural investigation

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A parallel study was conducted of the inclusion of alkyl parabens (guests) in the host β-cyclodextrin (β-CD). ^{1}H NMR data indicated an insertion of the guest phenyl ring into the β-CD cavity. The stoichiometry of each complex was 1:1, as determined by a continuous variation method that utilises the chemical shifts of the host protons. These chemical shifts were additionally used to determine the association constant yielding K values of 1631, 938, 460 and 2022 M^{-1} at 298 K for the methyl-, ethyl-, propyl- and butyl paraben solution state complexes, respectively. NOE experiments conducted on the methyl paraben solution complex indicated that the phenolic group of the guest was located at the secondary rim of the cyclodextrin cavity. Solid state structure analyses of the methyl and propyl paraben β-CD complexes were performed. Both complexes crystallised at ambient temperature in the space group $C2$, $Z = 4$ with a host to guest ratio of 1:1. Additionally, a second crystal structure between methyl paraben and β-CD is reported. This complex crystallised at 7°C in the space group $P1$, $Z = 2$ with a 1:1 host–guest stoichiometry.

Keywords: β-cyclodextrin; parabens; association constant; ^{1}H NMR; X-ray structure

Introduction

The alkyl esters of 4-hydroxybenzoic acid or parabens (Figure 1) are widely used as typical preservatives for food products, cosmetics and pharmaceutical formulations. Parabens are active over a wide pH range, have a wide range of antimicrobial activity, are safe to use (i.e. relatively non-irritating, non-sensitising and of low toxicity) and are most effective against yeasts and moulds (*1*). In general, the antimicrobial activity, antiseptic action and clinical safety of parabens increase as the chain length of the ester group increases. However, practical use of parabens with longer alkyl chains has been limited because of their low solubility in water, and therefore sodium salts are frequently used in formulation. An alternative approach to increasing their solubility is cyclodextrin complexation. This report describes the solid state inclusion of parabens in γ-cyclodextrin as well as a parallel study of the inclusion of parabens in β-cyclodextrin, both in solution and in solid state using NMR and X-ray techniques, respectively.

Results and discussion

Solution NMR

The inclusion of the paraben guest in the β-CD host molecule is shown by changes in the chemical shifts of some of the guest and host protons in comparison with the chemical shifts of the same protons in the free components. The partial 300 MHz NMR spectra of the pure β-CD and of the equilibrium mixtures containing the β-CD-paraben complexes are displayed in Figure 2. Distinct peaks are not observed for a bound and a free form, implying that complexation is a dynamic process, the included molecule undergoing fast exchange (relative to the NMR timescale) between the free and bound states. The stoichiometries of the complexes were determined using continuous variation method by following the changes in the chemical shifts of the host protons, which showed the greatest variations *viz.* H3, H5 and H6. For each β-CD-paraben complex, the Job plots (Figure 3) have an almost symmetric appearance and show a maximum at $r = 0.5$, indicating the existence of a complex with 1:1 stoichiometry within the range of concentrations investigated. The association constant for a 1:1 complex was calculated using a C^{++} program (*2*). Details of the procedure employed by the program have been outlined by Bogdan et al. (*3*). In each case, the observed chemical shift changes for the H3, H5 and H6 protons of β-CD were evaluated as a function of β-CD concentration, according to Equation (1) (see Materials and methods). The overall association constants (K) obtained from this procedure are listed in Table 1, along with loss functions (E), correlation factors (R) and complexation-induced shifts ($\Delta\delta_c$). The results indicate that $\Delta\delta$ (H5) $>$ $\Delta\delta$ (H3), demonstrating that the primary

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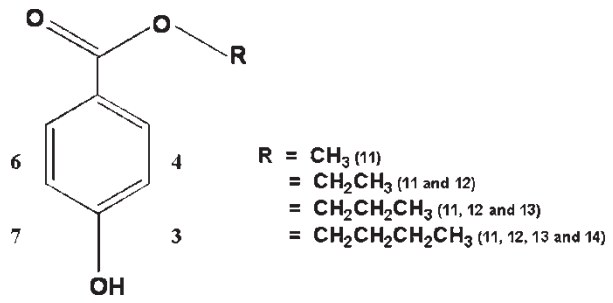

Figure 1. Chemical structure of methyl-, ethyl-, propyl- and butyl paraben. Numerals correspond to proton positions referred to in the NMR study.

side of the β-CD cavity is involved in complex formation. In addition, these results show that K generally decreases as the alkyl chain length increases. However, the β-CD-butyl paraben complex is inconsistent with this trend and the calculated K value suggests that this guest has the tightest fit within the CD cavity. This result implies that not only the hydrophobic effect but also some other factors play a significant role in determining the formation of these complexes. For each β-CD-paraben mixture, an increase in the concentration of the CD caused a downfield shift of the alkyl chain and ester moiety signals and upfield shifts of the aromatic ring protons (Table 2). This study represents a significant advance on previous NMR studies on the same four complexes, for only a single concentration of each paraben was previously considered (*4*). The shielding of the aromatic protons was interpreted as a consequence of their inclusion in the CD (*5*), while the deshielding of the alkyl protons indicated that this part of the guest lies outside the cavity. The guest position is consistent with the upfield shifts of the β-CD cavity protons, as the β-CD protons experience anisotropic shielding attributed to the inclusion of the guest aromatic ring (*6–14*). In the β-CD-propyl paraben and β-CD-butyl paraben complexes, $\Delta\delta$ (H3, H7) $>$ $\Delta\delta$ (H4, H6), suggesting that the guest hydroxyl groups are located at the primary rim. In the β-CD-methyl paraben complex, $\Delta\delta$ (H3, H7) $<$ $\Delta\delta$ (H4, H6), suggesting that phenol moiety is located at the secondary rim. In the β-CD-ethyl paraben complex $\Delta\delta$ (H3,H7) $\sim$ $\Delta\delta$ (H4, H6), i.e. both the aromatic protons show an approximately equal interaction with the CD and hence the orientation of the guest cannot be unequivocally determined. A clearer geometrical relationship between the host CD and the guest in the inclusion complex could be obtained by measuring the ^{1}H spectral enhancement due to the homonuclear Overhauser effect (NOE). These experiments confirmed the orientation of the methyl paraben guest within the cavity. The NOE experiments of the ethyl-, propyl- and butyl paraben complexes showed that there was no interaction between the host and guest, suggesting that the intermolecular interactions between the two species were greater than 4 Å.

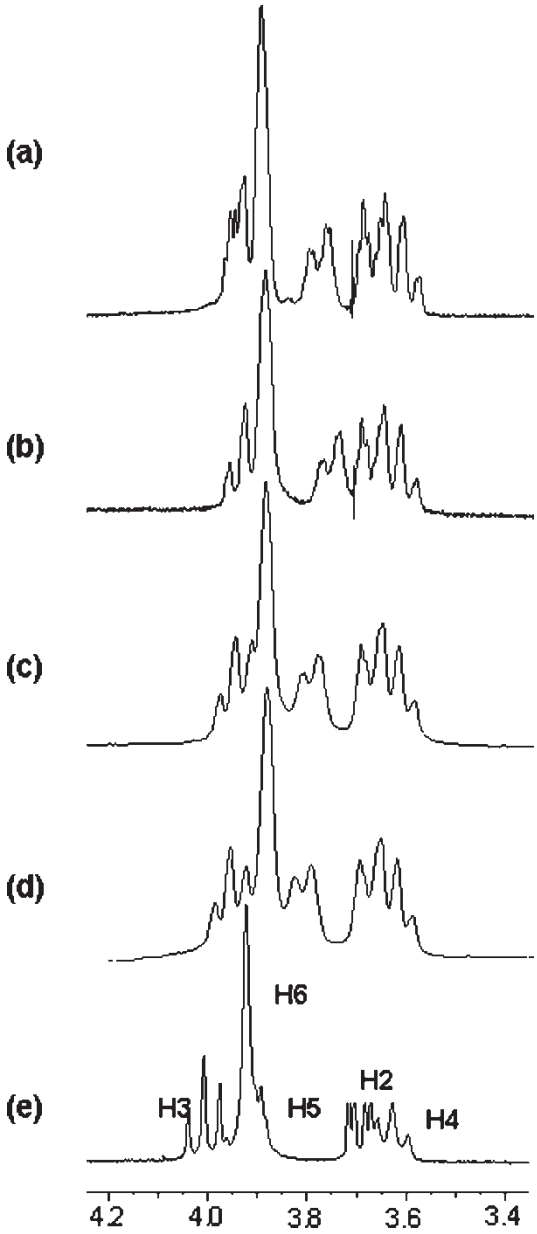

Figure 2. Partial 300 MHz spectra of (a) 5 mM methyl paraben and 5 mM β-CD (b) 2.5 mM ethyl paraben and 2.5 mM β-CD (c) 0.75 mM propyl paraben and 0.75 mM β-CD (d) 0.35 mM butyl paraben and 0.35 mM β-CD and (e) pure β-CD at 298 K.

Solid state structures

Solid state inclusion complexes of γ- and β-CD as hosts with each paraben were prepared. In the case of γ-CD, attempted complexation by kneading led to crystalline powders whose PXRD patterns matched those of the

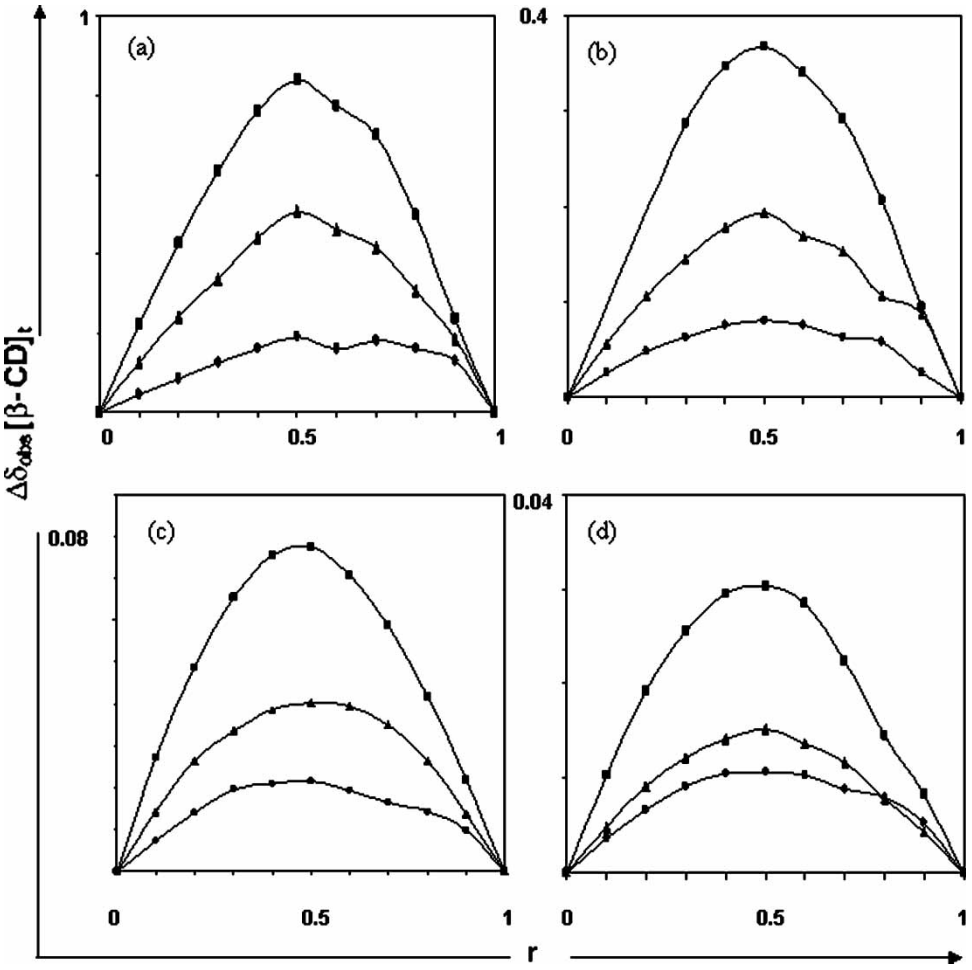

Figure 3. Job plots for protons of β-CD [-▲- H3; -■- H5 (top); -•- H6 (lower)] in the (a) 10 mM β-CD-methyl paraben complex, (b) 5 mM β-CD-ethyl paraben complex, (c) 1.5 mM β-CD-propyl paraben complex and (d) 0.7 mM β-CD-butyl paraben complex.

physical mixtures of the components. However, inclusion complexes with H:G stoichiometry 1:2 were obtained by co-precipitation method. The PXRD patterns of these complexes matched the reference pattern (*15*) for the unique tetragonal family of γ-CD inclusion complexes with $a \sim 23.8$, $c \sim 23.2$ Å crystallising in space group $P42_12$. Repeated attempts to grow suitable crystals for single crystal X-ray analysis were unsuccessful. This report will therefore focus on results obtained with β-CD. A preliminary account of the two methyl paraben complex structures has been reported in a recent communication, which included the method of preparation, unit cell data and data collection details (*16*). A survey of the available cyclodextrin crystal structures (*17*) revealed that this is the first instance of structurally different inclusion complexes of a cyclodextrin with a common organic guest crystallising in different space groups. Two *n*-propanol solvates of β-CD crystallising in the space group *P*1 were described in an earlier report (*18*).

Complex formation in the solid state of methyl-, ethyl-, propyl- and butyl parabens with β-CD *via* kneading and co-precipitation methods was confirmed

Table 1. *K*, *E*, *R* and $\Delta\delta_c$ of the β-CD complexes at 298 K.

	β-CD-methyl paraben	β-CD-ethyl paraben	β-CD-propyl paraben	β-CD-butyl paraben
K (M^{-1})	1631	938	460	2022
E	5.198×10^{-4}	1.177×10^{-4}	1.459×10^{-4}	1.378×10^{-4}
R	0.9980	0.9991	0.9988	0.9984
$\Delta\delta_c$ (H3) (ppm)	0.1363	0.1421	0.2550	0.1298
$\Delta\delta_c$ (H5) (ppm)	0.2369	0.2768	0.4820	0.2687
$\Delta\delta_c$ (H6) (ppm)	0.0488	0.0629	0.1327	0.0975

Table 2. Chemical shifts (ppm) of the parabens in the free (experimental values) and complexed states ($\Delta\delta_c = \delta_c - \delta_{free}$).

Parabens	Protons	δ_{free}	δ_c	$\Delta\delta_c$
Methyl paraben	3 and 7	7.986	7.992	0.006
	4 and 6	6.999	7.027	0.028
	11	3.934	3.910	−0.024
Ethyl paraben	3 and 7	8.003	8.040	0.037
	4 and 6	7.004	7.048	0.044
	11	4.400	4.361	−0.039
	12	1.412	1.380	−0.032
Propyl paraben	3 and 7	8.004	8.130	0.126
	4 and 6	6.890	6.942	0.052
	11	4.312	4.214	−0.098
	12	1.818	1.791	−0.027
	13	1.027	0.995	−0.032
Butyl paraben	3 and 7	7.994	8.106	0.112
	4 and 6	6.990	7.032	0.042
	11	4.376	4.317	−0.059
	12	1.796	1.767	−0.029
	13	1.498	1.489	−0.009
	14	0.989	0.944	−0.045

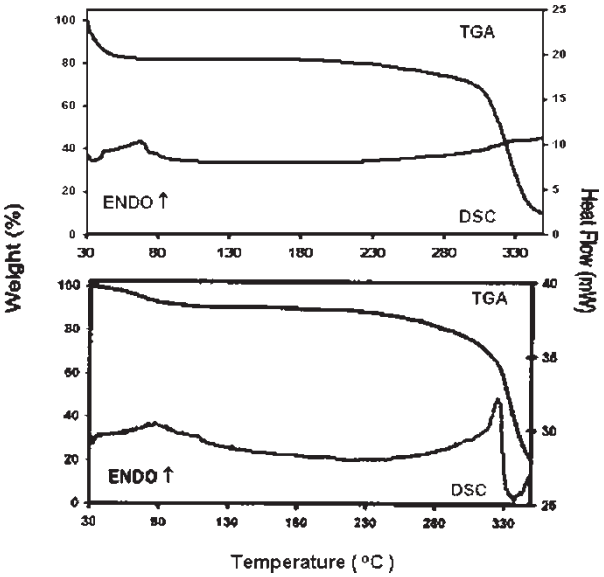

Figure 4. TGA and DSC traces of **1** (lower) and **3** (upper).

by various methods (*19*) and indicated that these four complexes are isostructural to each other (*15*). Crystalline complexes were obtained from slow cooling and slow evaporation of hot aqueous solutions (~45°C) of CD and guest in 1:1 molar ratios at an ambient temperature. Suitable single crystals of the β-CD-methyl paraben (**1**) and β-CD-propyl paraben (**2**) complexes were obtained after 1–2 weeks. The structural analysis of **2** is reported here. Structures for the ethyl- and butyl paraben complexes are not included, as suitable single crystals of these complexes could not be obtained, despite repeated efforts to grow them. In addition to the above-mentioned complexes, a second complex, grown at 7°C, of β-CD and methyl paraben is discussed here (**3**).

*Thermal analysis of **1** and **3***

The two complexes have different degrees of hydration, which becomes apparent when comparing the thermal analysis data. The crystals of both the complexes are colourless at ambient temperature. Crystals of **1** develop cracks and begin to dehydrate upon removal of their mother liquor at room temperature, discolouring at 194°C on the hot stage. Crystals of **3** appear to be more stable than **1** as dehydration on the hot stage begins at 39°C only, with the material turning brown at 251°C. Thermogravimetric and differential calorimetry results confirm these findings (Figure 4). This behaviour can be reconciled with the crystal structures.

Overall description of the structures

Crystallographic data for complex **2** are listed in Table 3. Analogous data for **1** and **3** were published earlier (*16*). Complexes **1** and **2** crystallise in the monoclinic space group *C*2 with a single β-CD molecule, its associated guest and water molecules comprising the asymmetric unit of the structure. Complex **3** crystallises in the triclinic space group *P*1 with two crystallographically independent β-CD molecules, two guest molecules and 28 water molecules comprising the asymmetric unit. Each complex has a 1:1 host–guest stoichiometric ratio, as determined by UV spectrophotometry and elemental analysis.

Geometries of the host molecules

The geometrical data for the β-CD molecule/s of complexes **1**–**3** are listed in Table 4. (e.s.d.s are in the range 0.005–0.019 Å for distances and 0.1°–1.5° for angles.) The seven glucosidic residues have been assigned the Gn notation in **1** and **2**, whereas in **3** the two CDs have been referred to as CD(A) and CD(B) with the glucose units numbered 1–7. There is no significant difference in the conformations of the two independent β-CD molecules, A and B in **3**, and the geometric data for this complex closely resemble those for **1** and **2**. In each of the complexes, all the D-glucose units adopt $^4C_1$ chair conformation and the orientations of the majority of primary hydroxyl groups are associated with (−) *gauche* conformation. Exceptions occur for residues G7 as well as G3 and G6 (disordered sites) in the complexes **1** and **2**. For **3**, exceptions occur for residues A6 as well as A3, A5 and B5 (disordered sites). For all three complexes the O(4) heptagon has a high degree of planarity and shows a seven-fold symmetry based on the O(4)···O(4′) distances and O(4)···O(4′)···O(4″) angles. The O(4) angles do not

Table 3. Crystal data and structure refinement for complex **2**.

	2
Molecular formula	$C_{42}H_{70}O_{35}{\cdot}C_{10}H_{12}O_3{\cdot}7.0H_2O$
Formula weight	1441.3
Crystal system	Monoclinic
Space group	*C*2
a (Å)	19.137(2)
b (Å)	24.534(1)
c (Å)	15.793(1)
α (°)	90.0
β (°)	109.52(2)
γ (°)	90.0
Volume/*Z*	6988.8 (2)Å^3/4
Density (calculated) (g cm^{-3})	1.370
Temperature of collection (K)	173(2)
Radiation/wavelength (Å)	0.71069
Crystal dimensions (mm)	0.25 × 0.24 × 0.14
θ-range for collection (°)	2–24
Index ranges	$-20 \le h \le 16$, $-26 \le k \le 27$, $-11 \le l \le 17$
Reflections collected	9174
Independent reflections	6733
Number of parameters	512
R_{int}	0.0411
Goodness of fit	1.096
R_1 [$I > 2\sigma(I)$]	0.1465
wR_2	0.3575
Largest diffraction peak and hole	0.83 and −0.46 e.Å^{-3}

Table 4. Geometrical parameters for the β-cyclodextrin molecules.

Residue	D[a] (Å)	ϕ[b] (°)	d[c] (°)	α[d] (Å)	D_3[e] (Å)	τ[f] (°)
Complex ***1***						
G1	4.50	132	2.3	0.02	2.78	1.0
G2	4.29	129	−4.5	0.10	2.76	7.4
G3	4.39	124	−0.1	−0.06	2.82	8.9
G4	4.42	131	4.7	−0.06	2.90	3.9
G5	4.41	130	−2.2	0.09	2.83	8.0
G6	4.31	127	−3.1	0.02	2.78	7.0
G7	4.33	126	3.2	−0.10	2.84	5.5
Complex ***2***						
G1	4.56	133	3.3	0.01	2.81	0.2
G2	4.33	129	−5.4	0.10	2.76	3.9
G3	4.44	124	0.3	−0.08	2.85	6.7
G4	4.51	130	5.9	−0.06	2.85	3.6
G5	4.43	130	−4.5	0.13	2.85	6.5
G6	4.39	127	−1.1	−0.01	2.80	6.5
Complex ***3***						
A1	4.28	130	2.3	−0.03	2.83	11.9
A2	4.39	126	−0.9	0.01	2.83	4.4
A3	4.31	127	0.3	0	2.84	8.4
A4	4.47	131	−0.2	0	2.81	3.3
A5	4.33	130	−0.7	−0.01	2.73	3.4
A6	4.29	123	2.4	−0.01	2.76	4.1
A7	4.50	133	−3.4	0.04	2.79	2.2
B1	4.30	126	1.3	−0.01	2.79	4.8
B2	4.46	131	−3.1	−0.02	2.79	3.3
B3	4.23	129	3.3	0.04	2.82	8.8
B4	4.40	128	−1.7	−0.04	2.77	2.2
B5	4.33	129	0.0	0	2.82	9.2
B6	4.39	128	0.3	0.01	2.76	2.5
B7	4.37	130	−0.2	0	2.76	3.4

[a] Glycosidic O4*n*···O4(n + 1) distance.
[b] O4(n − 1)···O4*n*···O4(n + 1) angle.
[c] O4(n − 1) ··O4*n*···O4(n + 1)···O4(n + 2) torsion angle.
[d] Deviation of atoms O4*n* from the least-squares planes (mean e.s.d. 0.007).
[e] Inter-ring hydrogen bond O(2*n*)···O(3n − 1) distances (mean e.s.d. 0.010).
[f] Tilt angle between the mean O(4) plane and the mean plane through the six pyranose ring atoms, namely C(1), C(2), C(3), C(4), C(5) and O(5) of each glucose unit (mean e.s.d. 0.2°).

differ significantly from 129°, the angle of the regular heptagon, denoting that the cavity has not become distorted due to the inclusion of the guests. In addition, the high degree of planarity is illustrated in the small deviation of the O(4) atoms from the mean O(4) plane. However, it was noticed that the O(4) heptagon in **3** was more symmetrical and less distorted than those in **1** and **2**. These differences were clearly expressed in the smaller deviations of the O(4) atoms from the mean O(4) plane and O(4) torsion angles. This suggests that the constraints that the two-fold symmetry places on the guest, in the space group *C*2, lead to a less favourable fit of the guest within the cavity and consequently a slightly more distorted β-CD macrocyclic structure.

Guest conformation and mode of inclusion

The mode of inclusion of the propyl paraben molecule in **2** could not be determined due to the disordered nature of this guest within the CD cavity. The modes of guest inclusion are analogous for complexes **1** and **3**; the phenyl rings are centralised within their respective host molecules, the guest hydroxyl groups being located at the primary rim and the ester moieties occupying the dimer interface. Figure 5 shows the orientation of the methyl paraben guest within the β-CD cavity, as well as illustrating how the guest is totally included within the CD dimer cavity. This is achieved by tilting of the guest molecules within the β-CD cavity. The average tilt angles for the phenyl ring relative to the O(4) mean planes of the CD are 78° and 66° for **1** and **3**, respectively. Hence, the guest included in the *C*2 complex has a greater tilt angle than the guest included in the *P*1 complex. The orientation of the guest can be attributed to the stabilisation afforded by hydrogen bonding between the guest hydroxyl group and other 'extra-cavity' hydrophilic centres. In **1** there is direct hydrogen bonding between the guest hydroxyl oxygen atoms and host O(6) hydroxyl oxygen atoms that are in the (+)-*gauche* conformation. In **3** this hydrogen bonding involves water molecules that bridge the guest hydroxyl

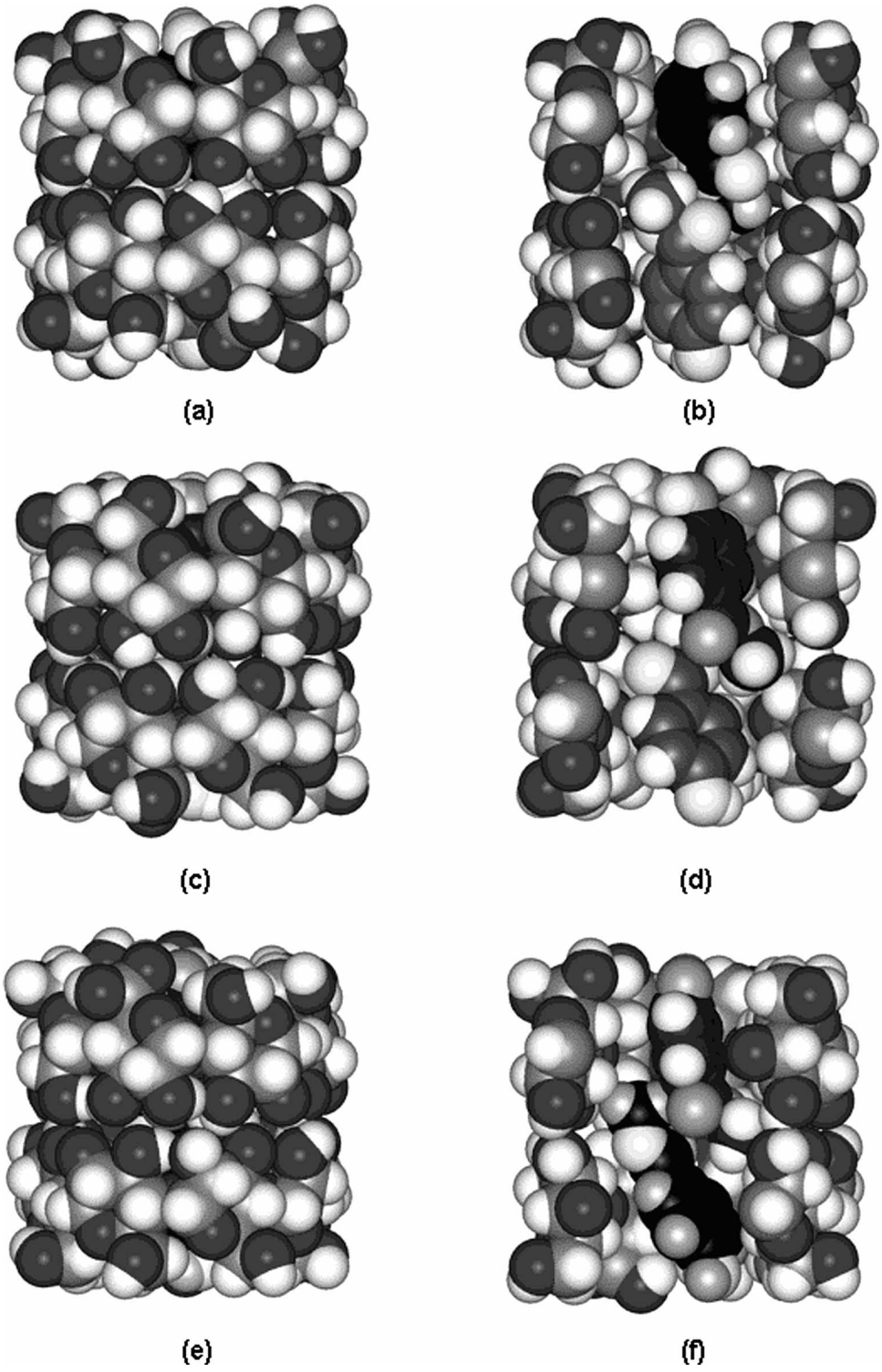

Figure 5. Space-filling diagram of **1** (a) side view (b) sectioned view of the same orientation, showing guests A and B[§] [the C_2-related counterpart of molecule B of the asymmetric unit] and **3** (c) side view and (d) sectioned view of the same disordered model; (e) and (f) correspond to the second disordered model.

group and host primary hydroxyl groups. Solvation of the polar group has long been recognised as an important factor in determining the orientation, positioning and stability of the guest in the β-CD cavity (*20*).

Hydrogen bonding interactions

In all three complexes the host molecules are stabilised by seven intramolecular O(2)···O(3′) hydrogen bonds and these bonds contribute to the rigidity and highly symmetrical conformation of the cyclodextrin. Dimer formation was observed through the association of the two β-CD monomers by hydrogen bonding of the secondary O(3) hydroxyl oxygen atoms. The mean distances of these contacts are 2.85(1), 2.86(2) and 2.81(3)Å for complexes **1**–**3**, respectively. In addition, the primary hydroxyl groups form hydrogen bonds to adjacent dimers of the same layer, either directly or *via* water-mediated interactions. The O(2)···O(2) and O(6)···O(6) contacts, which are present in all three complexes, are the only direct CD-to-CD intra-layer interactions. In addition, to these intra-layer interactions, inter-layer interactions were found between the primary –OH groups in the *C*2 complexes. In these complexes, the minor position of the disordered primary hydroxyl group on residue G3 forms a hydrogen bond to its symmetry-related hydroxyl group on an adjacent dimer in the next layer of the channel. The relative weakness of this bond, due to the low site-occupancies of 0.19 and 0.37 in **1** and **2**, respectively, may explain in part the instability of the crystal structure. This inter-layer interaction was not observed in **3** because of the distortion in the linearity of the dimeric columns. In addition to these host–host interactions, some of these primary and secondary hydroxyl groups are linked to parallel CD columns *via* hydrogen bonding to the water channel. It was observed that **3** contains more O(6)···water and water···water contacts than the *C*2 structures.

Crystal packing

The dimeric units in **1** and **2** pack in the channel mode, which is characterised by close-packed layers of dimers stacked in alignment to produce channels (Figure 6). The guests therefore find themselves shielded from the water environment and interact only with the host O(6) hydroxyl groups. The water molecules find themselves in a channel-type packing, which will facilitate the dehydration process, explaining the relative instability of **1** compared with **3**. The dimeric complex units in **3** pack in the intermediate packing mode, which is characterised by close-packed layers of dimers that stack with a lateral displacement of 6.2 Å (*21*). This shift puts the seven-fold axis of the dimer near the rim of the dimer below it and results in the guest molecules being located in a nearly 'cage-like' environment. The primary hydroxylic sides are therefore partially closed by the β-CD atoms of adjacent layers. The intermediate packing is more open than the channel-type packing and permits the entrance of water molecules between the primary faces of the dimers (Figure 7).

Final remarks on complexes 1 and 3

In summary, the observed differences between **1** and **3** are that (i) the *P*1 complex exhibits a more symmetrical macrocycle than the *C*2 complex, (ii) the tilt angles of the guests in the *C*2 complex are greater than those of the guests included in the *P*1 complex, (iii) the *P*1 complex is more extensively hydrated than the *C*2 complex and (iv) there are differences in the interactions of the guest with the host and water molecules. With regard to the latter observation, when crystals of the methyl paraben inclusion complexes are grown at room temperature, they display channel-packing, which allows the guest to hydrogen bond to the CD primary hydroxyl groups and minimise contact with the waters of crystallisation. In contrast, when the crystals are grown at low temperature, the inclusion complex crystallises in the

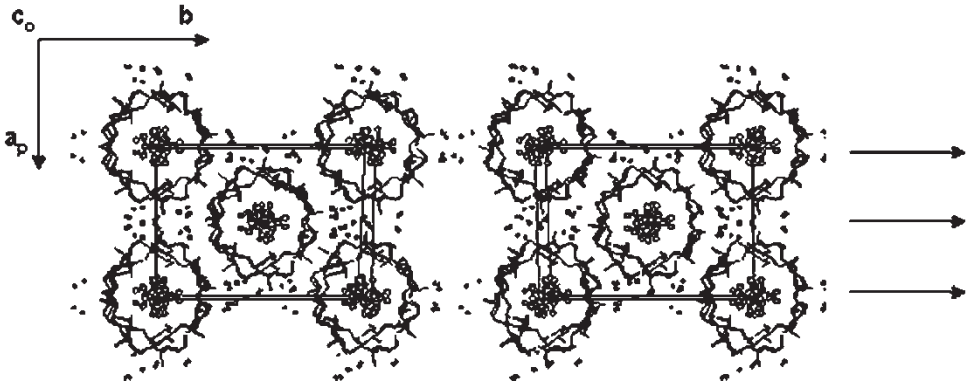

Figure 6. Stereo packing diagram of **1** (*c*-axis projection).

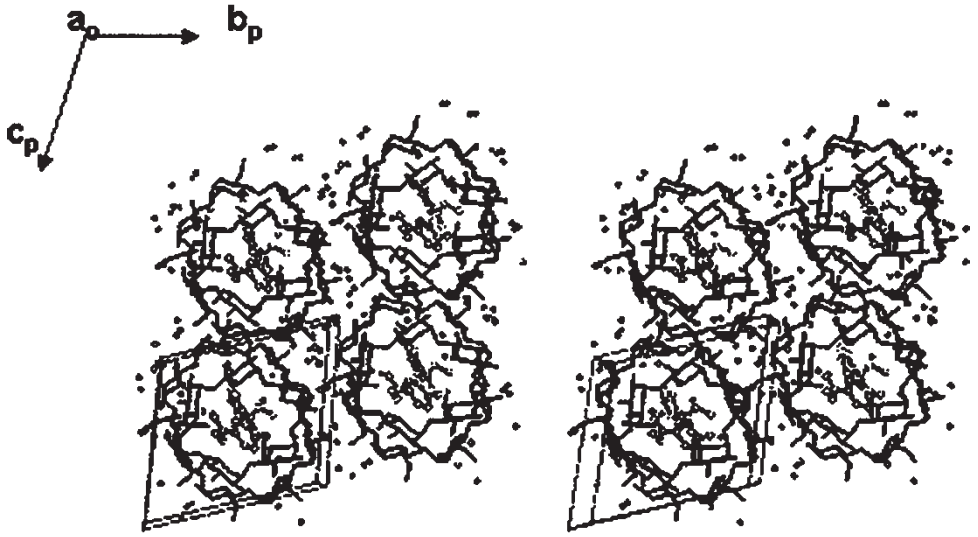

Figure 7. Stereo packing diagram of **3** with guests C and D (*a*-axis projection).

intermediate type packing, allowing the guest to interact with the hydrophilic water environment. Hence, a possible explanation for this tendency for crystallisation in different modifications is that the hydroxyl group of the guest forms a strong interaction with the water environment, favouring the intermediate packing over the channel. If this interaction is purely electrostatic, then the spatial fit of the guest to the cavity becomes important. If the guest does not provide a tight spatial fit (as suggested by the disorder observed in the guest position within the cavity), then the interaction with the water molecules will be destabilised and channel type packing will form. At low temperature the degree of thermal motion of the guest is reduced and the hydration shell is more ordered, thereby preventing the channel-type of packing from forming and thus favouring intermediate packing mode. It can therefore be concluded that the formation of multiple forms of CD inclusion complexes is rare, but systematic variations in crystallisation conditions could increase the chances of its occurrence. This was predicted earlier (*15*).

Materials and methods

Materials

β-Cyclodextrin was obtained from Cyclolab (Budapest, Hungary) and was used as received. The guest compounds methyl-, ethyl-, propyl- and butyl parabens were purchased from Sigma Chemical Company (St. Louis, Missouri, USA). The D_2O (deuterium content 99.7%) was obtained from the Institute of Cryogenics and Isotope Separation (Rm. Vălcea, Romania). The D_2O (deuterium content 99.9%) was additionally obtained from Aldrich Chemical Company, Inc, USA.

NMR experiments

Proton NMR spectroscopy was performed at 300 MHz using a Varian-Gemini spectrometer. ^{1}H NMR spectra were recorded in D_2O solution at 298 ± 0.5 K. Typical conditions were as follows: 32 K data points, sweep width 4500 Hz giving a digital resolution of 0.28 Hz/point. The pulse width was 13 μs (90°) and the spectra were collected by co-addition of 32 or 64 scans. Homonuclear Overhauser effect (NOE) difference experiments were performed at 300 MHz with a Varian-Mercury 300 spectrometer with a 3-second mixing time.

β-CD and the paraben under investigation were prepared in D_2O as two individual equimolar stock solutions. The concentrations of the stock solutions were determined by the solubility of each paraben in water and were 10.0, 5.0, 1.5 and 0.7 mM for the methyl-, ethyl-, propyl- and butyl paraben solutions, respectively. The stoichiometry of the complex was determined by continuous variation method. The equimolar stock solutions of the β-CD and paraben under investigation were mixed to a constant volume at varying proportions, so that a complete range ($0 < r < 1$) of ratios $r = [X]/([H]_t + [G]_t)$ was sampled. This maintained the total concentration of each sample solution constant. In the preceding expression, [X] is equivalent to the concentration of the host (β-CD) or of the guest (paraben) for the sample and $[H]_t$ and $[G]_t$ are the total concentrations of the host and the guest, respectively. From the NMR data, the quantity $\Delta\delta_{obs}[X]$ was obtained by subtracting the observed shift value for a given sample from the chemical shift of the free X. $\Delta\delta_{obs}[X]$ was then plotted against r and the maximum of the curve corresponded to the stoichiometry. For the determination

of the association constant (K), the same set of samples as those for the determination of stoichiometry were used. The data were evaluated according to Equation (1)

$$\Delta\delta_{\mathrm{obs}}^{[\mathrm{x}]} = \frac{\Delta\delta_{\mathrm{c}}^{[\mathrm{x}]}}{2[\mathrm{x}]}\left[\mathrm{M} + (1/K) - \left\{\left(\mathrm{M} + (1/K)\right)^2 - 4[\mathrm{G}]_{\mathrm{t}}[\mathrm{H}]_{\mathrm{t}}\right\}^{1/2}\right] \quad (1)$$

in which $\Delta\delta_{\mathrm{c}}[\mathrm{X}]$ represents the chemical shift difference (for a given proton) between the free molecule and the pure complex and $M = [\mathrm{G}]_{\mathrm{t}} + [\mathrm{H}]_{\mathrm{t}}$. This equation involves no approximations and correlates the total concentration of the guest and host molecules with the observed difference in chemical shift, $\Delta\delta_{\mathrm{obs}}[\mathrm{X}]$.

X-ray diffraction

The procedures for data collection, structure solution and refinement of **1** and **3** have been reported earlier (*16*). Intensity data for **2** were collected on a Nonius Kappa CCD diffractometer from crystals coated with Paratone N oil (Exxon Chemical Co., TX, USA). Data-collection (COLLECT software (*22*)) involved a combination of ϕ- and ω-scans of 1.0°, each with a crystal to detector distance of 40 mm. The program DENZO-SMN (*23*) was used for unit cell refinement and data reduction. The structure was solved using the isomorphous replacement method and refined by full-matrix least-squares against F^2 (SHELXL-97 (*24*)). Two of the β-CD primary O atoms were disordered over two positions. All the host oxygen atoms were refined anisotropically, except the disordered primary hydroxyl oxygen atoms and three other primary hydroxyl oxygen atoms. The remaining atoms were refined isotropically. The mode of inclusion of the propyl paraben guest could not be determined due to the disordered nature of the guest within the CD cavity. In the final refinement, 14 low-angle reflections were omitted as their observed intensities were significantly less than their calculated values due to beam-stop truncation.

The CIF file for **2** has been deposited at the Cambridge Crystallographic Data Centre (CCDC 668997).

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