JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS, Vol. 10, No. 9, September 2008, p. 2255 - 2260

Invited Paper

Structural elucidation of chemically modified cyclodextrins and their inclusion complexes for medicine applications

M. R. CAIRA*, W. T. MHLONGO, J. LI
Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa

Structural properties of selected derivatised cyclodextrins (CDs), gleaned from single crystal X-ray studies, are described with reference to the effects on molecular conformation and inclusion ability accompanying their formation from the corresponding native CDs. Species of interest include several acylated and methylated CDs as well as their inclusion complexes with drug guest molecules. As a representative example of an inclusion complex in this category for which full structural and thermal characterization has recently been achieved, that between permethylated α-CD and the antihypertensive metoprolol is presented. This inclusion complex has 2:1 host-guest stoichiometry that features novel 'bimodal' guest inclusion.

(Received February 25, 2008; accepted August 14, 2008)

Keywords: Cyclodextrin, Inclusion compounds, X-ray crystal structure

1. Introduction

The use of cyclodextrins (CDs) in the manufacture of advanced materials intended for application in the pharmaceutical and other fields (e.g. agricultural, chemical, cosmetics, food, adhesives, polymers) is well established [1]. In the case of pharmaceuticals, their inclusion within the hydrophobic cavities of CD molecules generally enhances their aqueous solubility and chemical stability, and frequently contributes to their improved performance and easier handling through e.g. reduction of adverse side effects, improvement in patient compliance through elimination of undesirable taste/odour of the active compound, and conversion of drugs that are liquids at room temperature into more manageable solid inclusion complexes [2, 3].

In addition to the native host compounds, α-, β- and γ-CD, containing 6, 7 and 8 α-1,4-linked D-glucopyranose rings respectively (Fig. 1), numerous CD derivatives have also been exploited in drug delivery [2]. These include derivatives obtained by alkylation, hydroxyalkylation, acylation, and sulfation, or sulfoalkylation, of the free hydroxyl groups of the parent molecules [4].

Derivatisation accordingly modifies the chemical properties, solubilities and inclusion abilities of these molecules, enabling a wider choice in the selection of a host molecule for a specific application. Thus, the highly soluble and less toxic hydroxyalkylated species are suitable for parenteral drug formulations, whereas the less soluble, hydrophobic CD derivatives such as peracetylated CDs are used to control the release rate of water-soluble drugs [5]. In this introduction, we describe some of the structural features of acylated and methylated CDs revealed by recent X-ray studies as a prelude to presenting a detailed account of the recent structural and thermal characterization of an inclusion complex of a fully methylated CD.

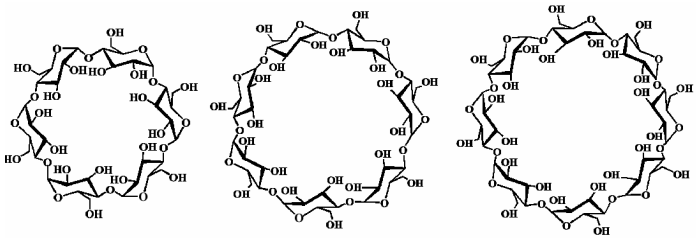

Fig. 1. Structures of native CDs: from left to right, α-, β- and γ-CD.

A native host molecule such as β-CD normally maintains a relatively 'round' shape in crystals owing to O2-H···O3' hydrogen bonding between contiguous rings comprising the macrocycle. The seven glucose rings consequently tilt to almost equal extents with respect to the relatively planar array of O4 glycosidic oxygen atoms.

Derivatisation (e.g. permethylation, peracetylation) may alter the molecular conformation profoundly by eliminating intramolecular hydrogen bonding, introducing repulsive interactions between sterically bulky substituents on contiguous glucose rings, and by 'self-inclusion' of such substituents. In the latter case, the extent of inclusion of substituents in the CD cavity may be sufficient to preclude complexation with a guest molecule.

The effect of peracylation of β-CD on its molecular structure has been investigated by single crystal X-ray analysis [6]. Crystals of heptakis(2,3,6-tri-*O*-acetyl)-β-CD, heptakis(2,3,6-tri-*O*-propanoyl)-β-CD, and heptakis(2,3,6-tri-*O*-butanoyl)-β-CD were found to contain elliptically distorted molecules with non-planar, boat-shaped structures. Relief of steric interactions between adjacent acyl chains in these molecules results in severe tilting of the glucose units relative to the glycosidic O4 plane. Furthermore, there is significant self-inclusion of acyl residues in these derivatised molecules. This suggests that guest inclusion, such as occurs in the native host molecules, is unlikely to take place either in the solid state or in solution. This was subsequently confirmed by circular dichroism studies, none of the test molecules (molsidomine, *m*-iodophenol, or 2,7-dihydroxynaphthalene) showing any tendency for inclusion in the CD cavities in solution.

An X-ray study of the structure of fully acetylated γ-CD (TAγCD, Fig. 2) likewise showed extensive macrocyclic distortion, in this case accompanied by self-inclusion of three acetyl residues [7]. A unique feature of the structure is the partitioning by these residues of the cavity region of the molecule into two distinct sub-cavities that accommodate water and ethanol molecules. The constancy of this unusual type of solvent 'bi-inclusion' was evidenced by powder X-ray diffraction experiments on a series of isostructural solvates of TAγCD containing small molecules such as acetone, *n*-propanol and ethanol.

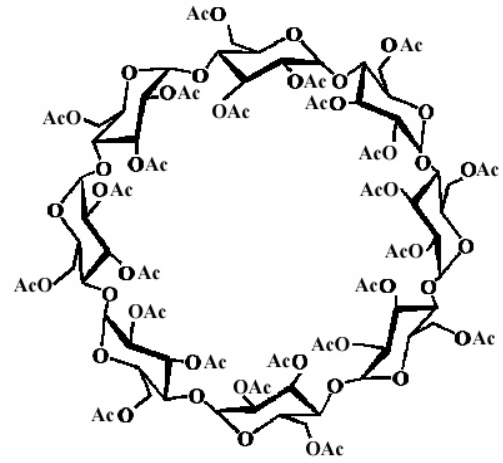

Fig. 2. Chemical structure of peracetylated γ-CD.

Despite the lack of classical inclusion ability, the acylated CDs are nonetheless employed in slow-release drug delivery. In the intimate presence of these CDs, hydrophilic drugs evidently adopt the insolubility of the host compounds, the rate of drug release diminishing with increasing hydrophobicity of the host molecules [6].

Partially and fully methylated CDs have been extensively studied owing to their significantly higher aqueous solubilities relative to the parent compounds, as well as their ability to include highly hydrophobic drugs [2]. Two well-defined, crystallisable derivatives, namely heptakis(2,6-di-*O*-methyl)-β-CD and heptakis(2,3,6-tri-*O*-methyl)-β-CD ('DIMEB' and 'TRIMEB' respectively) have been considered for potential use in pharmaceutical applications, and Harata has reviewed X-ray studies of their complexes with numerous organic guest molecules, including drugs [8]. In the last decade, on-going X-ray studies have revealed novel features associated with these molecules such as new crystalline modifications of the uncomplexed hosts and unusual crystal packing modes of their inclusion compounds [9].

Permethylated α-CD [hexakis(2,3,6-tri-*O*-methyl)-α-CD, or 'TRIMEA', Fig. 3] forms inclusion complexes that crystallize in two packing arrangements, namely 'channel' and 'layer' types [8]. In the former, the host molecules are aligned 'head-to-tail' while in the latter the macrocyclic rings are arranged with alternating orientations within a plane. As regards chiral recognition, TRIMEA is known to form 1:1 inclusion complexes with optically active guests. The TRIMEA complexes with (*R*)- and (*S*) –mandelic acids, for example, crystallize in the channel- and layer-type packing arrangements respectively, the guests being included in somewhat different modes, interpreted on the basis of an induced fit by the relatively flexible TRIMEA molecule [10].

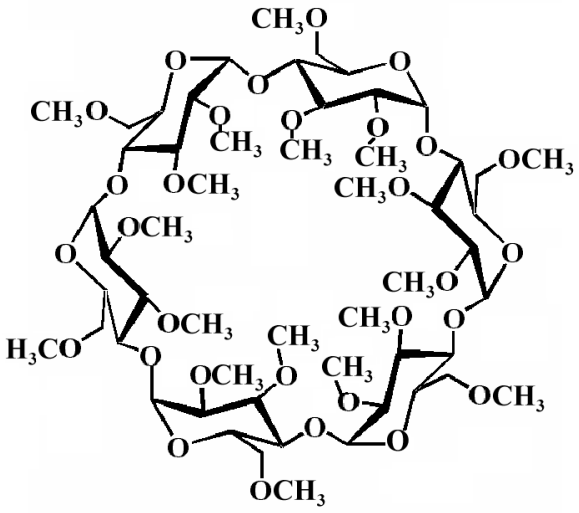

Fig. 3. Chemical structure of permethylated α-CD.

This host compound was one of several employed recently in our laboratory as a complexing partner for the antihypertensive drug metoprolol (1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-2-propanol], Fig. 4). The latter, in the form of the racemic free base, was found to form an inclusion complex with TRIMEA, with the unusual 2:1 host-guest stoichiometry. The preparation, thermal analysis and X-ray structure determination of this complex are reported here for the first time. This type of study has become obligatory for

CD inclusion complexes that have potential medicinal applications. Apart from the goal of extending the solid-state chemistry of the widely used drug metoprolol, one aim of its inclusion in TRIMEA was to compare its molecular conformation in the complexed state with that observed in crystals of the free base and the tartrate salt, whose X-ray structures we reported recently [11].

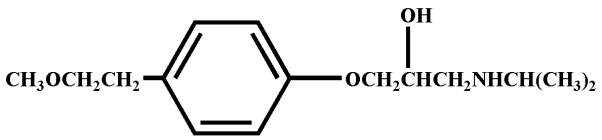

Fig. 4. Chemical structure of metoprolol.

2. Experimental

2.1 Materials

A sample of TRIMEA was purchased from Cyclolab (Budapest, Hungary) and was used as received. Metoprolol tartrate was purchased from Sigma Chemical Company (St. Louis, Missouri, USA) and was converted to the free base by addition of excess 1.0 M NaOH solution. The free base was extracted from the aqueous phase with dichloromethane and recovered as a solid after solvent evaporation under ambient conditions. Characterization of the solid was described elsewhere [11].

2.2 Complex preparation and preliminary characterization

To a saturated aqueous solution of TRIMEA at 0 °C, an equimolar amount of metoprolol free base was added with stirring. The resulting solution was filtered and incubated at approximately 60 °C. After one week, colourless crystals of the inclusion complex appeared. The complex formula was determined as $(TRIMEA)_2$·(metoprolol)·H_2O from a combination of thermogravimetry (0.72 ± 0.02% mass loss, n = 3, see below) and CHN elemental analyses which gave %C 54.25, %H 7.78, %N 0.74 [calculated for $(C_{54}H_{96}O_{30})_2$·$(C_{15}H_{25}NO_3)$·H_2O: %C 54.00, %H 8.07, %N 0.51].

Hot stage microscopy of the crystals was performed using a Linkam TH MS600 instrument and a heating rate of 10 K min^{-1} with the crystals immersed in silicone oil. Thermogravimetry (TGA) was performed using a Mettler Toledo STAR^e thermal analysis system (TGA/SDTA 851^e, Version 6.10), with samples in alumina crucibles. Differential scanning calorimetry (DSC) traces were recorded on a Perkin Elmer DSC7 instrument. Nitrogen purges (30 mL min^{-1}) were used for both TGA and DSC analyses.

2.3 X-ray structural analysis of the complex $(TRIMEA)_2$·(metoprolol)·H_2O

Intensity data were collected on a Nonius Kappa CCD diffractometer with graphite-monochromated MoKα-radiation (λ = 0.71073 Å) with the crystal cooled to 113 ± 2 K in a stream of nitrogen to optimise diffraction quality. Standard data-processing programmes were employed [see ref. 11] and the structure was solved in the monoclinic space group $P2_1$ using program SHELXD [12]. The asymmetric unit was seen to comprise two host molecules and a single molecule of metoprolol. Full-matrix least-squares refinement against F^2 with program SHELXL-97 [13] followed, with all atoms of the host molecules vibrating isotropically, except for the methoxyl carbons and the O2, O3 and O6 atoms, which refined anisotropically. All non-H atoms of the metoprolol molecule were treated anisotropically. H atoms were included in idealised positions in a riding model with isotropic thermal displacement parameters in the range 1.2-1.5 times those of their parent atoms. The single water molecule in the asymmetric unit was located outside the cavity of one of the host molecules.

3. Results and discussion

Owing to the low water content of the crystal, dehydration was practically indiscernible from HSM analysis. The latter showed only slight discoloration of the crystals at ~170 °C followed by melting, commencing at 180 °C. However, the TGA trace between 30 and 100 °C yielded the mass loss recorded in section 2.2, enabling accurate determination of the water content, namely one water molecule per $(TRIMEA)_2$·(metoprolol) complex unit. Owing to the hydrophobicity of methylated CDs, the extent of hydration of its inclusion crystals is typically much lower than what is observed for native CDs.

The melting endotherm in the DSC trace of the inclusion complex has an onset temperature of 180 °C. As the guest metoprolol is a low-melting solid (melting onset 48 °C [11]), its inclusion in TRIMEA increases its thermal stability considerably. The DSC trace of the inclusion complex is complicated by the presence of a very small endotherm at 170 °C (attributed to a phase transition of the anhydrous complex prior to melting) and a small exotherm at 185 °C (assumed to be due to the onset of complex decomposition).

Crystal data for the inclusion complex are listed in Table 1. The unit cell parameters differ from those of any previously reported TRIMEA inclusion complexes, which prevented routine structure solution by isomorphous replacement. Furthermore, the 2:1 host-guest stoichiometry established by chemical analysis and confirmed crystallographically indicated the possibility of an atypical guest inclusion mode and/or different crystal packing arrangement for the complex.

The structure of the complex unit is shown in Fig. 5. It consists of two TRIMEA host molecules (A, B), a single molecule of metoprolol and a water molecule (OW1). The methylglucose units of the independent CD(A) and CD(B) host molecules are denoted A1-A6 and B1-B6 respectively

(large numerals in Fig. 5). Of primary interest is the unusual bimodal inclusion of the guest molecule. The phenyl ring is located at the interface of the head-to-tail dimeric unit CD(A)/CD(B). The shorter methoxyethyl side chain (C16→C19) is included in the cavity of CD(A) from the host secondary side while the longer side chain (O7→C15) is included in the cavity of CD(B) from the host primary side.

Table 1. Crystal data and refinement parameters

Formula	$(C_{54}H_{96}O_{30})_2 \cdot (C_{15}H_{25}NO_3) \cdot H_2O$
M_r/g mol^{-1}	2736.0
Crystal system	Monoclinic
Space group	$P2_1$
a/Å	13.5228(2)
b/Å	23.4723(3)
c/Å	22.5602(4)
β/°	98.063(1)
V/ Å^3	7090.1(2)
Z	2
F(000)	2952
Crystal size/mm^3	0.10 x 0.30 x 0.40
θ-range/°	2-26
Index ranges	h:-16, 16; k:-28, 28; l:-27, 27
Measured data	44646
Unique data	25708
Reflections with $I>2\sigma(I)$	19635
R_{int}	0.0483
LS Parameters	1369
Mean Δ/σ	< 0.001
R_1	0.1144
wR_2	0.3301
S	1.037
$\Delta\rho_{min, max}$/e Å^{-3}	-0.56, 1.46

Bifurcated hydrogen bonds (thin lines in Fig. 5) link the hydroxyl group to methoxyl O6 atoms of host B. These bonds are O10-H···O6B1 and O10-H···O6B6, with respective O···O distances 3.01(1) and 2.94(1) Å. There are additional stabilising host-guest interactions in the form of C-H···O hydrogen bonds and C-H···π interactions. The water molecule occupies an interstitial space and hydrogen bonds to oxygen atoms of contiguous rings 3 and 4 of CD(B) (OW1···O6B3 2.79(1) Å, OW1···O5B4 2.80(1) Å).

It should be noted although racemic metoprolol was used in the inclusion experiment, there is evidence for only one enantiomer in the crystal selected, namely that with (*S*)-configuration at the chiral centre C9. Further experiments are necessary to ascertain the extent of host selectivity towards the individual enantiomers of metoprolol.

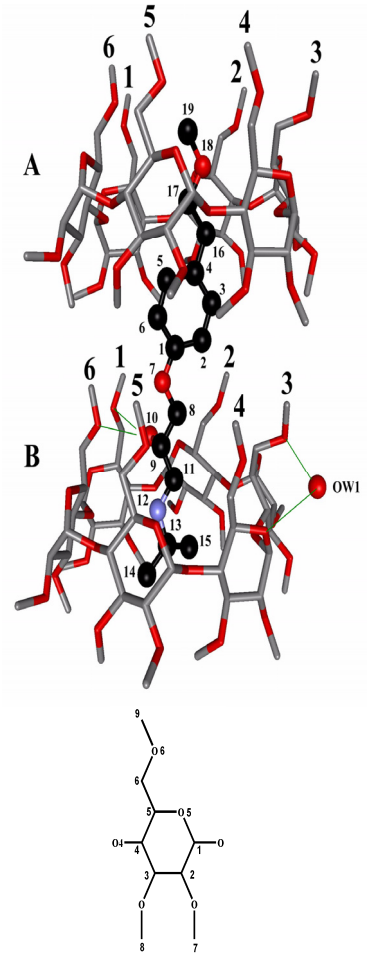

Fig. 5. Perspective view of the structure of the 2:1 TRIMEA-metoprolol inclusion complex. The inset above shows the host atomic numbering scheme. H atoms are omitted for clarity.

Fig. 6 shows the conformations of the metoprolol molecule occurring in (a) the crystal structure of the uncomplexed free base [11], and (b) the TRIMEA inclusion complex. While some folding of the side chains in the uncomplexed conformer (a) permits it to adopt a close-packed structure in its crystal, inclusion within the TRIMEA dimer involves a conformer (b) which is significantly more extended, with the planes of the staggered side chains aligned with the phenyl group.

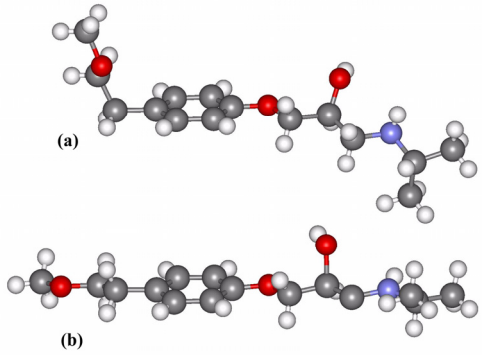

Fig. 6. Conformers of the metoprolol molecule occurring in (a) the crystal structure of the free base, and (b) the inclusion complex with TRIMEA.

The host conformations may be accurately defined with reference to several standard parameters, some of which are cited here. The ω parameter, relating to the primary side of the molecule and defined as the torsion angle O5-C5-C6-O6, effectively indicates the direction of the C6-O6 bond relative to the centre of the cavity. For example, the value ω = -60° [(-)-*gauche*] indicates that the C6-O6 bond vector points away from the cavity centre. In host CD(A), such a conformation is adopted in residues A2 and A3 only, all the remaining residues showing the (+)-*gauche* orientation (Fig. 5). Analogously, in host molecule CD(B), whose overall conformation is very similar to that of CD(A), the two residues B2 and B3 have ω around -60°, whereas all other residues have ω around +60°. A more obvious feature of the host conformations is the uniform extension of the chains C5-C6-O6-C9, resulting in the terminal bonds O6-C9 being aligned roughly parallel to the axes of the macrocycles.

All of the glucopyranose rings adopt the normal $^4C_1$ conformation. Both hosts maintain relatively 'round' structures (i.e. there is little elliptical distortion). Due to steric interactions between methoxyl groups on the secondary sides of contiguous rings, the glucopyranose units adopt varying degrees of tilt relative to the mean O4-polygon. Table 2 lists several relevant parameters with their definitions.

Table 2.

Methylglucose unit	[a] r/Å	[b] d/Å	[c] τ_1/°
A1	4.16	0.099(4)	-1.4(2)
A2	4.38	-0.238(4)	23.5(2)
A3	4.18	0.138(4)	21.7(2)
A4	4.17	0.103(4)	0.7(2)
A5	4.39	-0.237(4)	19.6(2)
A6	4.19	0.134(4)	21.5(2)
B1	4.15	0.084(4)	0.1(2)
B2	4.39	-0.238(4)	22.8(2)
B3	4.23	0.145(4)	20.9(2)
B4	4.16	0.105(4)	-4.0(2)
B5	4.39	-0.254(4)	24.6(2)
B6	4.22	0.158(4)	19.1(2)

[a] Distance between the O4 atom of the residue and the centroid of the O4-polygon.

[b] displacement of each O4 atom from the LS plane through the O4-polygon

[c] Angle between the O4-plane and the plane through C1, C2, C3, C4, C5, O5 of each ring. A positive value indicates that the primary side of the glucose unit is closer to the axis of the macrocycle than the secondary side.

The small differences between parameters for corresponding residues A*n* and B*n* confirm that CD(A) and CD(B) adopt very similar conformations, but such differences as do exist must arise because of the chemically and sterically different guest residues that occupy each cavity. There is thus evidence of the proposed 'induced fit' on guest inclusion by the TRIMEA molecule [8].

CD inclusion may involve complete or partial insertion of the guest molecule within the CD cavity. In the case of the above complex, the cavity provided by the dimeric host motif appears to be 'tailor-made' for efficient encapsulation of the entire guest molecule. This is evident from the space-filling representations in Fig. 7, where all atoms, including hydrogen, are shown.

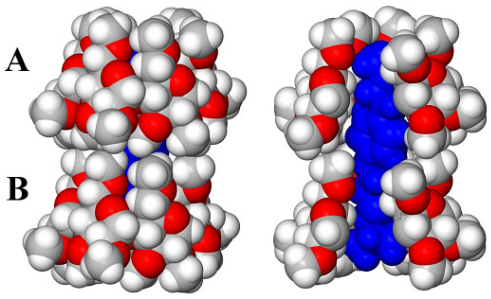

Fig. 7. Left: space-filling model of the 2:1 complex unit. Right: cutaway view showing the fit of the metoprolol molecule (in blue) within the dimeric cavity.

The small guest-induced differences in the conformations of the two host molecules CD(A) and CD(B), rendering them crystallographically non-equivalent, is significant in the context of crystal packing of the complex units. In Fig. 8, the 2:1 complex unit is compared with two units of a representative TRIMEA complex with 1:1 host-guest stoichiometry and 'channel-type' packing (the *p*-iodoaniline complex, CSD refcode BEYLOG [9]). The latter complex crystallizes in the monoclinic space group $P2_1$ with a = 11.440(4), b = 23.674(6), c = 13.531(3) Å, β = 91.90(2)° and Z = 2. The two complex units shown are related by translation along the a-axis. Comparison with the data in Table 1 for the metoprolol complex shows that the relationship between two sets of lattice parameters is: $a' \approx c$, $b' \approx b$, $c' \approx 2a$, where the primed symbols refer to the metoprolol complex. Thus, the arrangement of two crystallographically non-equivalent host molecules in the metoprolol complex gives rise to a periodicity c' ~ 22.56 Å, which is close to double that of the periodicity a ~ 11.44 Å in BEYLOG.

.

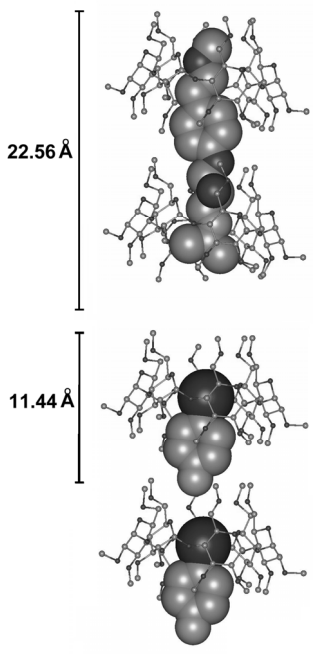

Fig. 8. A 2:1 TRIMEA-metoprolol complex unit (top) and two identical 1:1 TRIMEA-iodoaniline complex units (bottom), viewed from a common crystallographic direction.

There is evidently a fairly high degree of isostructurality between the CD(A)/CD(B) dimer of the metoprolol complex and the two translated TRIMEA molecules in the *p*-iodoaniline complex

However, significant differences in the extended crystal packings occur, the β angles of the two unit cells differing by ~6°. In the *p*-iodoaniline complex, the 1:1 complex units stack directly above one another, generating continuous channels that accommodate the guest molecules, whereas the 2:1 TRIMEA-metoprolol units are offset laterally, and consequently overlap when viewed down the axis of the macrocycle. This offset, indicated in Fig. 9, shows that for complex units related by translation along the *c*-axis, the abutting host faces are not aligned as they would be in the 'channel-packing' arrangement.

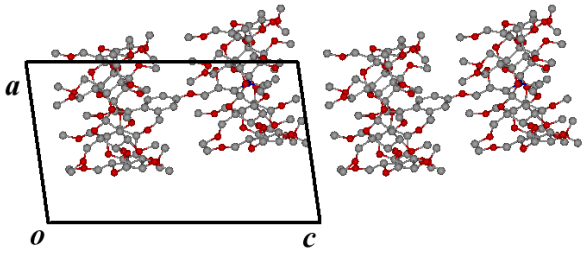

Fig. 9. Projection down [010] of the TRIMEA-metoprolol inclusion complex crystal, showing channel discontinuity.

The novel 2:1 stoichiometry observed for the TRIMEA-metoprolol complex is clearly associated with the relatively long substituents on the guest phenyl ring and their adoption of extended conformations. Inclusion complexes of TRIMEA for which single crystal X-ray data have previously been reported typically contain relatively small molecules such as benzaldehyde, *p*-iodoaniline and mandelic acid, with resultant 1:1 stoichiometry, which persists even for larger guests such as 1,7-dioxaspiro(5.5)undecane [14]. As an indication of the potential for yet more variety in the crystal chemistry of TRIMEA, we recently obtained preliminary evidence of guest encapsulation by this host that involves 2:1 host-guest stoichiometry based on a 'head-to-head' host dimer (as opposed to the 'head-to-tail' dimer reported here). This will be investigated further.

Novel guest inclusion modes and packing arrangements of CD inclusion complexes, such as that reported here, are of significant practical interest, especially in the context of routine, but unequivocal, complex identification by PXRD methods based on the availability of a library of reference PXRD patterns [15].

4. Conclusions

The significance of derivatised cyclodextrins, in particular acylated and methylated species, has been outlined in the context of their inclusion of medicinally active compounds. Their molecular conformations and inclusion abilities may be markedly different from those of the parent compounds as a result of intramolecular C-H···O hydrogen bonding, as well as steric repulsions and/or 'self-inclusion' involving bulky substituents appended to the rings.

Relevant structural features of the crystal chemistry of permethylated α-CD (TRIMEA) inclusion complexes were highlighted with reference to the novel 'bimodal' inclusion of the antihypertensive metoprolol and comparison with previously reported complexes. The origin of different host-guest stoichiometries and the possible effect this factor may have on crystal packing were described.

Acknowledgements

MRC wishes to thank the University of Cape Town and the NRF (Pretoria) for research support.

References

[1] E. M. Martin Del Valle, Process Biochem. **39**, 1033 (2004).
[2] K. H. Frömming, J. Szejtli, Cyclodextrins in Pharmacy. Topics in inclusion science. Dordrecht: Kluwer Academic Publishers, 1994.
[3] K. Uekama, F. Hirayama, T. Irie, Chem. Rev. **98**, 2045 (1998).
[4] H. Hashimoto, *In* New trends in Cyclodextrins and derivatives, Ed. D. Duchene, Editions de Santé, Paris, p.99, 1991.
[5] G. P. Bettinetti, M. Sorrenti, S. Rossi, F. Ferrari, P. Mura, M. T. Faucci, J. Pharm. Biomed. Anal. **12**, 1173 (2002).
[6] M. Anibarro, K. Gessler, I. Uson, G. M. Sheldrick, K. Harata, K. Uekama, F. Hirayama, Y. Abe and W. Saenger, J. Am. Chem. Soc., **123**, 11854 (2001).
[7] M. R Caira, G. Bettinetti, M. Sorrenti, L. Catenacci, D. Cruickshank and K. Davies. Chem. Commun., 1221 (2007).
[8] K. Harata, Chem. Rev., **98**, 1803 (1998).
[9] Cambridge Structural Database and Cambridge Structural Database System, ConQuest Version 1.9, 2007, Cambridge Crystallographic Data Centre, University Chemical Laboratory, Cambridge, England.
[10] K. Harata, K. Uekama, M. Odagiri, F. Hirayama, Bull. Chem. Soc. Jpn. **60**, 497 (1987).
[11] C. Ionescu, M. R. Caira, M. Bojita, L. R. Nassimbeni, W. T. Mhlongo, Farmacia (Bucharest, Romania), **54**, 9 (2006).
[12] T. R. Schneider, G. M. Sheldrick, Acta Crystallogr., Sect. D., **58**, 1772 (2002).
[13] G. M. Sheldrick, SHELXL-97, Program for Crystal Structure Refinement, University of Göttingen, Göttingen, Germany, 1997.
[14] S. Makedonopoulou, K. Yannakopoulou, D. Mentzafos, V. Lamzin, A. Popov, I. M. Mavridis, Acta Crystallogr., Sect. B:Struct.Sci., **57**, 399 (2001).
[15] M. R. Caira, Rev. Roum. Chim., **46**, 371 (2001).

*Corresponding author: Mino.Caira@uct.ac.za