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A SIDE CHAIN OF DIASTEREOMERIC ILOPROST PROTRUDES FROM THE CAGE IN THE COMPLEX WITH CYCLOMALTOHEPTAOSE (β-CYCLODEXTRIN): CRYSTAL STRUCTURE OF (β-CYCLODEXTRIN)$_2$ ILOPROST·20.5 H_2O

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ABSTRACT

A diastereomeric mixture of iloprost (16*R*:16*S* = 55:45) was co-crystallized with cyclomaltoheptaose (β-cyclodextrin, βCD) from aqueous solution as the complex (β-cyclodextrin)$_2$·iloprost·20.5 H_2O. The crystals are triclinic *P*1; a = 15.474(10), b = 15.446(10), c = 18.081(10) Å, α = 99.40(3); β = 112.99(3), γ = 103.10(3)°. The βCD forms dimers with the O-2,3 sides hydrogen-bonded to each other. The dimers are arranged in layers in the *ab* plane and adjacent layers are shifted laterally so that the cavities of the dimers are closed by adjacent βCD molecules in a brick-type cage structure. The iloprost is disordered and identified only by a number of peaks of low electron density too close to be covalently bonded. The distribution of these peaks indicates that iloprost is partly included in the cavity of the dimer and that one of its side chains protrudes into the space between the dimers.

INTRODUCTION

Cyclomalto-oligosaccharides (cyclodextrins, CDs) can accommodate guest molecules of suitable size in their central cavities and thereby form inclusion complexes which can be obtained in crystalline form. There have been numerous[1,2] spectroscopic, kinetic, crystallographic, and other physical and chemical investigations aimed at understanding the inclusion phenomena[1,2].

CDs can direct the stereoselectivity of certain reactions, improve the physical properties of drugs and pharmaceuticals, and dramatically reduce the sensitivity of some of them towards air. These findings led to the need for large quantities of CDs, and βCD is now available commercially.

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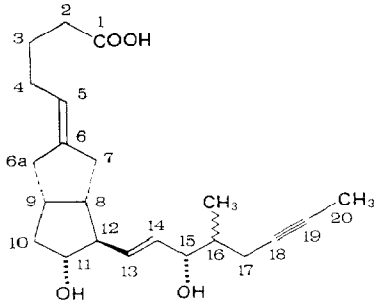

Fig. 1. Structure and numbering of the atoms in iloprost. A mixture of C-16 diasteromers was co-crystallized with βCD.

One application of βCD is the inclusion of iloprost (Fig. 1), which is a member of the prostacyclin family. Iloprost is a viscous, oily liquid mixture of two diastereomers and is stable under ambient conditions[3,4]. In order to improve its handling[5], it was prepared as a solid inclusion complex with βCD, the crystal structure of which is now reported. The presence of the diastereomers gave rise to difficulties in the interpretation of the electron density maps, but the general location of the iloprost molecule could be determined.

EXPERIMENTAL

When concentrated aqueous solutions of βCD and iloprost are mixed, a white powder precipitates. As indicated by n.m.r. measurements on a solution in methyl sulfoxide and by thermogravimetric analysis, the powder contains the individual components βCD–iloprost–water in the ratios 2:1:10, and iloprost occurs as an *RS*-mixture with a 16*R*:16*S* ratio of 55:45 (ref. 6).

Single crystals suitable for X-ray diffraction experiments were obtained by slow cooling of a hot, concentrated aqueous solution. Most of the crystals were twinned, but a single crystal (0.5 × 0.2 × 0.2 mm) was isolated and sealed in a glass capillary together with some mother liquor and used for all X-ray studies. The space group is triclinic, *P*1, and the unit-cell dimensions, as determined by least-squares refinement based on the angular positions of 15 reflections, are a = 15.474(10), b = 15.446(10), c = 18.081(10) Å; α = 99.40(3), β = 112.99(3), γ = 103.10(3)°; V_{cell} = 3719 Å^3, ρ_{calc} = 1.339 g/cm^3, $F(000)$ = 1500, μ = 8.52 cm^{-1}.

The X-ray intensities of 8950 independent reflections to $2\theta_{max}$ = 120° or a nominal resolution of 0.9 Å were measured on a STOE four circle diffractometer employing Ni-filtered CuK_α radiation and an $\omega/2\theta$ scan mode with stationary background measurements on both sites of each scan. The X-ray intensities were corrected for background, Lorentz, and polarization effects, and 3204 reflections with $F_o \leq 1\sigma(F)_o$ were regarded as unobserved and excluded from the refinement.

Structure determination and refinement. — The volume of the unit cell suggested that it contained two βCD molecules and therefore one iloprost molecule

and a number of water molecules. The initial structure solution was obtained with the Patterson search programme PATSEE[7], using atomic co-ordinates of a single βCD molecule from the βCD · 11 H_2O complex[8] as the input fragment for the rotation and following translation search. The best solution of this procedure was used for phasing and partial structure expansion with SHELX-86[9], and revealed the second βCD molecule in the unit cell. Several cycles of consecutive least-squares refinement and difference Fourier maps gradually revealed 22 atomic positions that had separations from hydroxyl oxygens of βCD molecules and from each other which are characteristic of hydrogen-bonding interactions. These peaks were identified therefore as the sites of water oxygens. All but three water positions were fully occupied, the occupation factors summing to a total of 20.5.

Extensive disorder was observed in the cavity of one βCD molecule and above its narrower rim formed by the HO-6 groups. This is the only region not occupied by βCD and water molecules, and is suggestive of the location of the iloprost molecule. In this region of the difference Fourier map, numerous peaks of low electron density were found which had too short separations to be directly bonded to each other. Several attempts to identify fragments of the iloprost molecule failed, although the central part of the molecule, which is formed by a rigid group of 12 non-hydrogen atoms, is so characteristic that it should be an outstanding feature in difference Fourier maps. Therefore, it appears that the iloprost molecule was disordered crystallographically over several positions and that an average of overlapping atomic sites was observed in a region which corresponded to the iloprost molecule and was not interpretable in unambiguous terms.

Refinement employing anisotropic temperature factors for the βCD molecules and the ordered water oxygen atoms converged at $R = 13.5\%$ if the described disordered region representing the iloprost molecule was excluded. The inclusion of the 40 strongest peaks in that region to the refinement decreased the R-factor to $R = 10.5\%$.

RESULTS AND DISCUSSION

Atomic parameters of the (βCD)$_2$ · iloprost · 20.5 H_2O complex have been deposited with the Cambridge Crystallographic Data Base. The molecular structure and packing arrangement are illustrated in Figs. 2–5.

Structure of the βCD molecules. — The unit cell contains one βCD dimer (Fig. 2) in an arrangement in which the rims formed by HO-2,3 of the βCD molecules face each other and form a regular pattern of well defined intra- and inter-molecular distances that are indicative of hydrogen bonding (Fig. 4). The ring of intramolecular hydrogen bonds between HO-2 and HO-3 of adjacent glucose residues within each βCD has been observed in all published βCD crystal structures.

The O-4 atoms of both molecules form a near-regular heptagon, giving the macrocycle a circular cross-section (Fig. 3). All C-6–O-6 bonds, which, in principle,

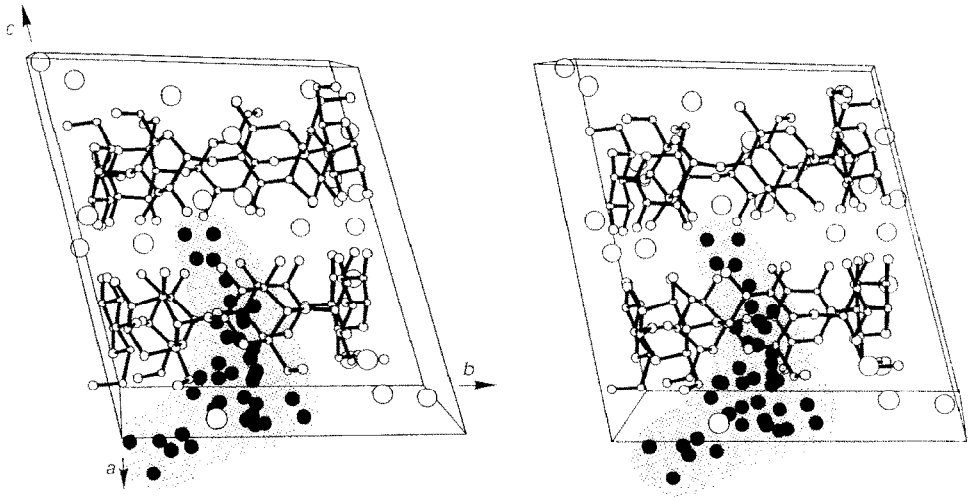

Fig. 2. Stereoview of the crystal unit cell, looking along the *a*-axis: ○, water molecules; ●, strongest electron density peaks in the region of disorder (shaded area).

may have two orientations, point away from the axis of the dimer, corresponding to the (−)-*gauche* conformation for the torsion angles O-5–C-5–C-6–O-6.

Packing of the dimers. — The general molecular packing scheme is displayed in Fig. 5 showing a view along the unit cell *b*-axis.

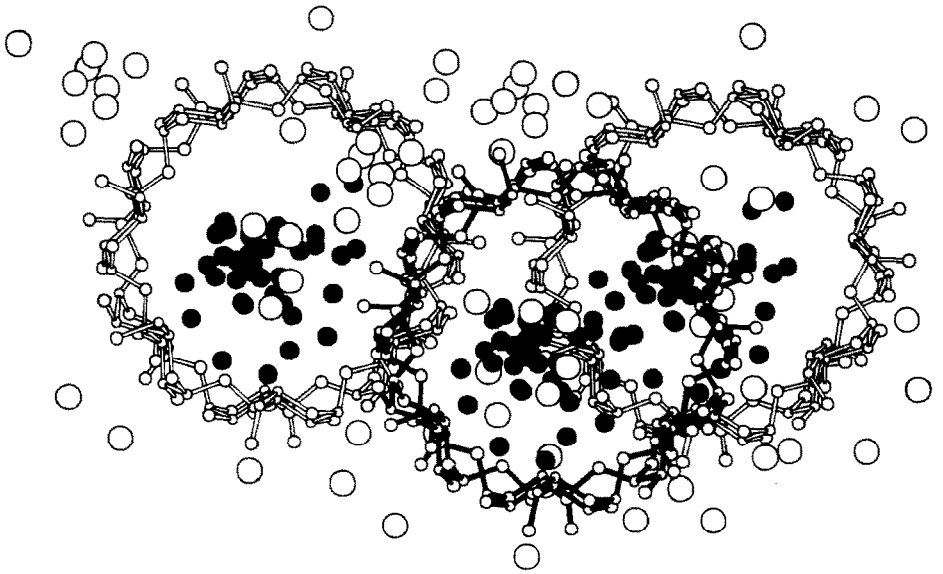

Fig. 3. View along the molecular axis of βCD, showing three dimers. The rims of the molecules in adjacent layers partially close each other's cavities. Dimers are in position x, y, z, $x - 1, y, z$ (open bonds), and $x, y, z + 1$ (solid bonds). The dimer drawn with solid bonds is placed "above" the two others.

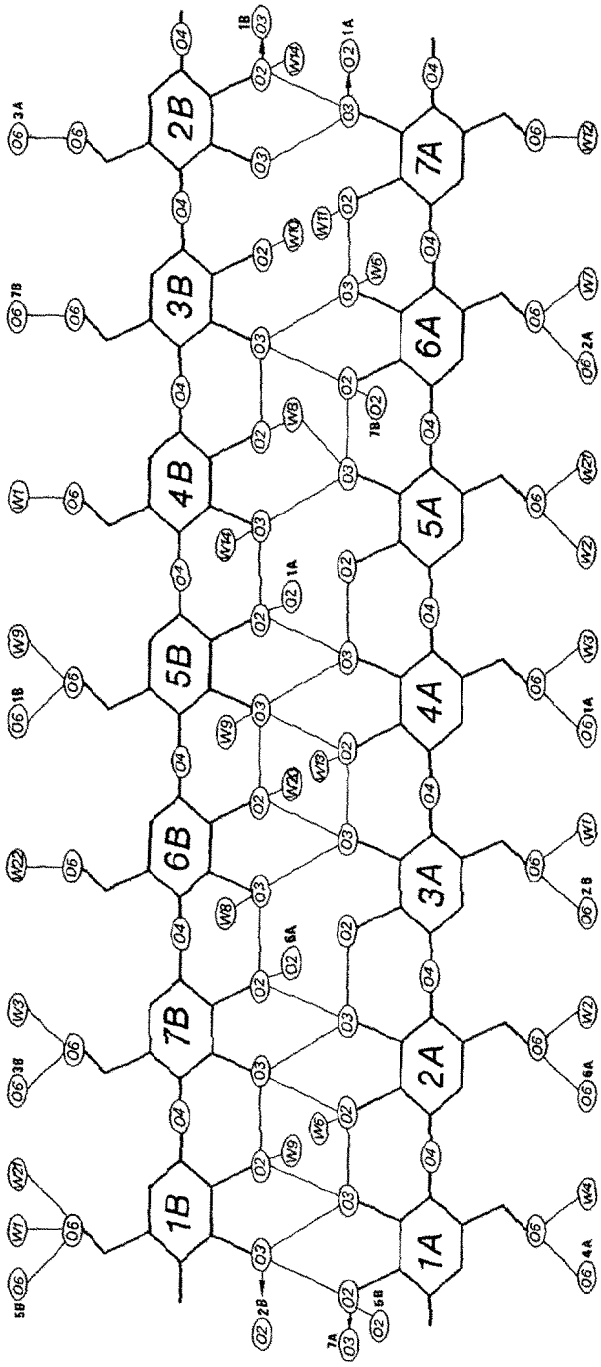

Fig. 4. Schematic projection of a βCD-dimer, showing the possible hydrogen bonds (thin lines): O · · · O distances <3.1 Å are indicated; ⊗, water molecules.

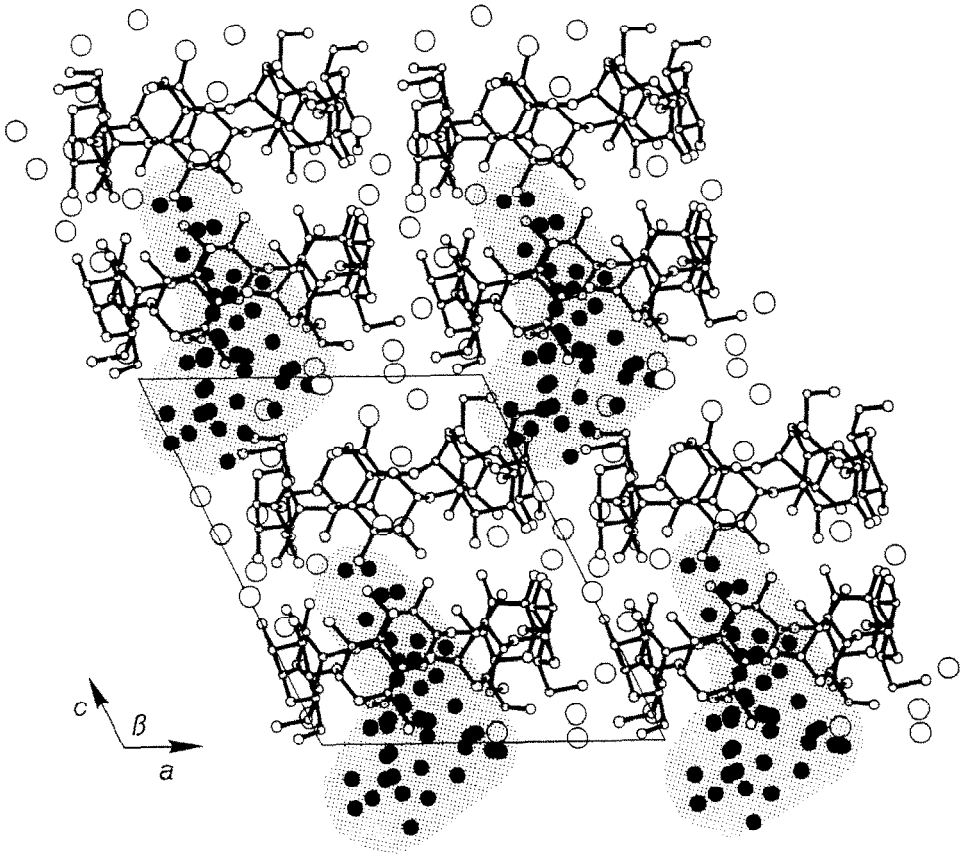

Fig. 5. General view of the structure along the *b* axis to show the brick-type arrangement of βCD dimers; symbols as in Fig. 2.

The axis of the βCD dimer is approximately perpendicular to the crystallographic *ab* plane, which results in layers of dimers in which each dimer has close contacts with four neighbours. Because of the large angle β of 112.99°, adjacent βCD layers in the *ab* plane are shifted laterally with respect to each other. This shift gives rise to a brick-type cage crystal structure[10] in which the central cavity of one dimer is closed on both ends (formed by the O-6-rims) by the adjacent molecules "above" and "below" the dimer (Fig. 4). Nevertheless, there is a relatively large axial distance of the adjacent dimers, so that they do not close each other's cavities completely.

This arrangement appears to be a more general packing pattern in βCD inclusion complexes; an isomorphous packing is observed in the βCD · 1-propanol hydrate complex[11,12] where, however, a different setting of the unit cell was chosen, so that a direct comparison with the present crystal structure cannot be made.

The guest molecule iloprost. — The region of extensive disorder in the crystal structure is drawn shaded in Figs. 2 and 5. It has the shape and size expected for an

iloprost molecule, and it is concluded that there is one iloprost enclosed in the cavity provided by a βCD dimer, in agreement with the molar ratio of this complex determined by n.m.r. spectroscopy. As evident from Fig. 2, the bulk of the iloprost, which we associate with the central bicyclic system, is located in the cavity, and an extension, probably one of its side chains, protrudes into the space between the βCD dimers.

It is unfortunate that the guest molecule iloprost is not directly "seen" in the crystal structure of this complex. The disorder may be due to the fact that the iloprost was a mixture of diastereomers and we should expect to see the C-17/20 chain (see Fig. 1) in two positions at approximately half occupancy if the molecules are in the same location. Also, this kind of packing might be intrinsically prone to have the guest molecule disordered.

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