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A molecular modelling study of the distortion of α-cyclodextrin (cyclomaltohexaose) in complexes with guest molecules

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ABSTRACT

Analysis of the crystal structures of complexes of cyclomaltohexaose (α-cyclodextrin, αCD) by molecular modelling techniques showed the αCD molecules to be distorted by the guest compounds. The ratio of the largest to the smallest diameter of the hexagon formed by the glycosidic oxygen atoms varied from 1.00 for a symmetrical to 1.12 for a distorted αCD molecule. The distortion was correlated with the van der Waals volume of the guest compounds. A procedure, based on crystallographic data, has been developed for constructing an αCD molecule with a specific distortion and a reasonable geometry for use in modelling experiments. Energy calculations on the complex of αCD with *p*-hydroxybenzoic acid showed that the lowest energy of interaction involved a distorted αCD molecule. MM2 calculations showed that the internal energies of the conformations of αCD studied were equal to within 10 kJ/mol. The distortion of the αCD molecule is therefore an important factor in the formation of complexes.

INTRODUCTION

Cyclodextrins (cyclomalto-oligosaccharides, CDs) are shaped like a hollow truncated cone that contains a hydrophobic cavity in which guest compounds of the appropriate size can be included. Because CDs can form inclusion complexes, they have been applied in chromatography, catalysis, pharmacy, and the modelling of drug–receptor interactions. The physical properties and bioactivities of drugs can be influenced by the formation of complexes with CDs[1,2].

The forces involved in the formation of complexes with CDs have been ascribed variously to hydrogen bonding, van der Waals interactions, dipole–dipole interactions, and hydrophobic binding.

Two approaches have been used in molecular modelling experiments in order to calculate the energies of interaction. In the simplest approach, the CD molecule is regarded as a rigid molecule and the conformation taken from crystal structure data is used for the calculations. Although this approach is not very realistic, it may give reasonable results[3,4], especially when series of closely related guest compounds are

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studied. The assumption that the structure of the CD is similar within the series may be valid. The advantage of this approach is that results can be obtained with a minimum of computer time.

The second approach allows for flexibility in the CD molecule and the structure of the complex is optimised completely. This method is more realistic but requires large amounts of computer time, partly because of the problem in dealing with local minima[5–7].

Analyses of the crystal structures of complexes[8–18] of αCD (cyclomaltohexaose) and n.m.r. experiments on complexes in solutions[19] have shown that the αCD is distorted when complexes are formed, so that the guest compound fits tightly into the cavity. We have developed a procedure for constructing a distorted αCD molecule for use in molecular mechanics calculations and now describe its use with *p*-hydroxybenzoic acid as the guest compound.

EXPERIMENTAL

All crystal structures of αCD complexes were obtained from the Cambridge Structural Database[20,21]. Structures were optimised with the MM2 force field and the steepest-descent minimiser as available in MacroModel[22]. Since these optimisations were performed solely to relieve the structures of unusual strain, only 50 cycles were involved. This process resulted in structures with reasonable energy in which the desired distortion was still present. Complete optimisations could not be done because the modelled distortion of the CD molecule would have disappeared due to the absence of a guest compound. Energies of interaction E(inter) are defined as E(comp) − E(CD) − E(guest), where E(comp) is the total energy of the complex, E(CD) is the energy of the CD molecule, and E(guest) is the energy of the guest molecule. All structures were analysed using Chem-X[23]. The contour maps were calculated using the "calculate conformations" facility. This rigid-body approach was chosen in order to analyse the energies of interaction of a guest compound with different conformations (distortions) of the CD molecule. These calculations were preceded by a Gasteiger charge calculation and only intermolecular energies were calculated. The electrostatic cut-off distance was set to 50 Å. This large value was necessary because, at the default value of 12 Å, very strange fluctuations were found in the electrostatic energies of interaction when the guest was moved out of the cavity. The van der Waals volumes were calculated using a mesh value of 0.5 Å in Chem-X.

RESULTS AND DISCUSSION

Analysis of crystal structures. — A search in the Cambridge Structural Database gave 28 references for complexes of αCD. Complexes with ionic guests were not examined. Only 14 references (Table 1) contained data about guest compounds completely incorporated in the cavity of αCD molecules, and these data were used for the analysis of the complexes. Fig. 1 shows the structure of αCD with the atom numbering scheme and an explanation of the terms used.

TABLE I

αCD Complexes obtained from CSD

Complex	*Compound*	*Code*[20]	*Ref.*	*van der Waals volume (Å^3)*
1	*p*-Hydroxybenzoic acid	ACDHBA	8	97.8
2	*N,N*-Dimethylformamide	ACDMFM	9	60.0
3	*p*-Nitrophenol	ACDPNP	8	95.6
4	2-Pyrrolidone	ACDPRO	9	66.5
5	Benzaldehyde	BAJJAX	10	83.8
6	Water	BANXUJ	11	13.4[a]
7	*p*-Iodoaniline (1)	CDEXIA01	12	110.4
8	*p*-Iodoaniline (2)	CDEXIA10	13	110.4
9	Krypton (1)	CDEXKR10	14	24.1[a]
10	Methanol	CDEXME10	15	27.6[a]
11	*m*-Nitroaniline	CDNOAN	16	98.6
12	Krypton (2)	CYDXKR10	14	15.6[a]
13	Iodine	CDEXTI10	17	70.7[a]
14	*p*-Iodophenol	CHAIPL	18	106.8

[a] Corrected for the number of molecules found in the cavity.

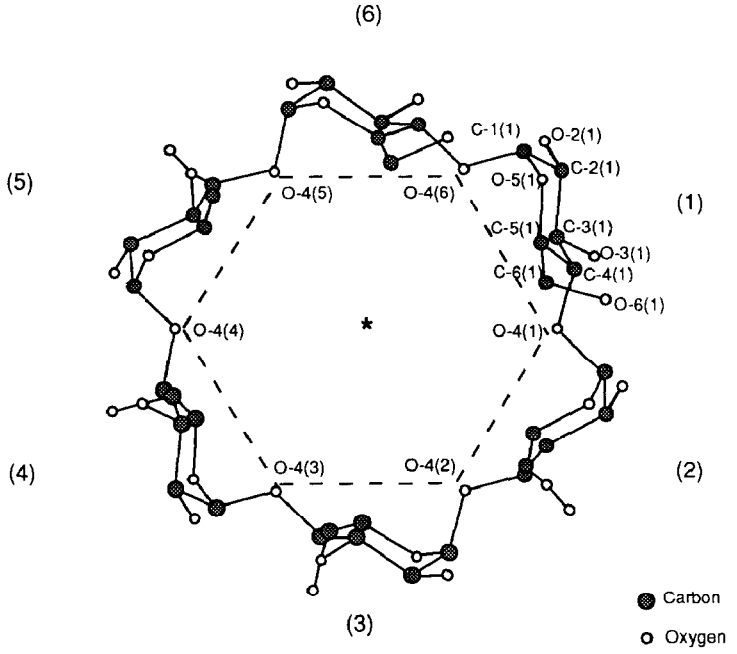


Fig. 1. Structure and atom numbering of αCD: the O-4 plane is the least-squares plane through O-4(1–6), the glucose plane is the least-squares plane through C-2, C-3, C-5, and O-5 of each glucose residue, centre dummy (*) is a dummy atom that is positioned in the centre of the O-4 plane, and the z-axis is the axis perpendicular to the O-4 plane and through the centre dummy. The $+z$-axis points away from the reader; diagonal O-4 distances are O-4(1)–O-4(4), O-4(2)–O-4(5), and O-4(3)–O-4(6).

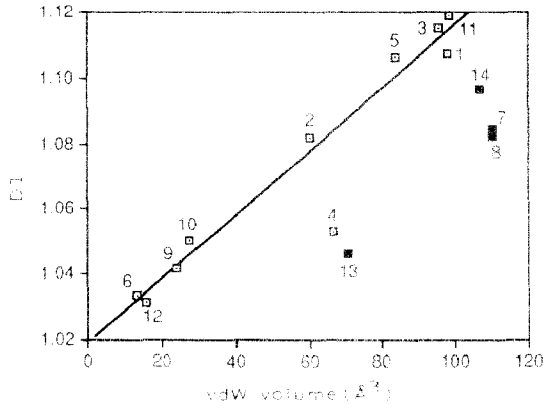


Fig. 2. Distortion (DI) of αCD versus van der Waals (vdW) volume of the guest compound. Numbers refer to the compounds in Table I: □, general compounds; ■, iodine-containing compounds.

Harata[8] reported a distortion of the αCD molecule in complexes with phenyl compounds. The O-4$_{(n)}$–O-4$_{(n+1)}$ distances parallel to the plane of the phenyl ring were longer than the remaining O-4$_{(n)}$–O-4$_{(n+1)}$ distances. This situation implies that O-4(1–6) do not form a regular hexagon (Fig. 1) but a hexagon that is stretched parallel to the plane of the phenyl ring. Thus, the diagonals in the hexagon are not equal. The distortion (DI) of an αCD molecule is defined by

$$DI = d\text{-max}/d\text{-min}, \qquad (1)$$

where *d*-max is the maximum and *d*-min is the minimum diagonal O-4–O-4 distance.

Fig. 2 shows the relation between DI and the van der Waals volume of the guest molecule. Large guest molecules cause a larger distortion of the αCD molecule. Four of the five outliers in this plot contain an iodine atom. Since iodine is a relatively large atom (diam. 4.3 Å), the diagonals in the αCD molecule cannot be shortened too much, so that *d*-min does not become small. Therefore, the distortion of αCD by these compounds is smaller than might be expected on the basis of their van der Waals volumes.

A model for building αCD molecules. – The above results led to the assumption that the distortion of an αCD molecule is an important factor in the formation of complexes. Hence, the distorted molecules were used as the starting conformations in calculations. However, an αCD molecule with a reasonably low energy and a certain distortion cannot easily be built from scratch due to its cyclic structure. Therefore, a simple mathematical model was derived in order to build up such an αCD molecule.

This model is based on the following observations, which were made during the analysis of crystal structures of αCD complexes. (*a*) The glycosidic angle has the value 118.7 ± 1.3 (n = 84) which, after energy minimisations with MacroModel, was adjusted to 118.5°. (*b*) The O-4$_{(n)}$–O-4$_{(n+1)}$ distance is correlated with the angles C-1$_{(n)}$–O-4$_{(n+1)}$–O-4$_{(n)}$ and C-4$_{(n)}$–O-4$_{(n)}$–O-4$_{(n+1)}$, which results in a relation between the angles C-1$_{(n)}$–O-4$_{(n+1)}$–O-4$_{(n)}$ and C-4$_{(n)}$–O-4$_{(n)}$–O-4$_{(n+1)}$ as shown in Fig. 3. (*c*) The glycosidic oxygen atoms O-4(1–6) are coplanar. (*d*) The distortion is determined by the length of

the $O\text{-}4_{(n)}$ $O\text{-}4_{(n+3)}$ diagonals. (*e*) A distorted αCD molecule consists of two glucose residues parallel to *d*-max, which have $O\text{-}4_{(n)}$–$O\text{-}4_{(n+1)}$ distances that are larger than for the remaining four glucose residues. (*f*) The orientation of the glucose residues can be defined by the angle between the glucose and O-4 planes (Fig. 1). This angle varies between 75° and 90° and has a mean value of 80.5° in αCD crystal structures. The spreading of this angle within an αCD molecule seems to be random, so that, in the model, the mean value was taken. (*g*) The distortion depends on the volume of the included compound.

The information in (*a*)–(*g*) was used to build an αCD molecule with two-fold symmetry and a reasonable geometry, consisting of two different glucose residues. These glucose residues with different $O\text{-}4_{(n)}$–$O\text{-}4_{(n+1)}$ distances were obtained from the CD complexes shown in Table I. In order to eliminate differences caused by crystal packing forces, only the glucose backbone of each unit was used. Hydrogens were added to the ring, using standard Chem-X values, and HO-2 and HO-3 were placed in such a way that hydrogen bonds could form between adjacent glucose residues. The C-6–O-6 side chain of each glucose residue was set to the *gauche–gauche* conformation (*i.e.*, pointing outwards).

Fig. 4 shows a schematic view of an αCD molecule that consists of six glucose residues, only two of which have a long $O\text{-}4_{(n)}$–$O\text{-}4_{(n+1)}$ distance. The following equations can be derived:

$$\alpha 1 + \beta 2 + \gamma 1 + \delta = 360 \quad (2)$$

$$\alpha 2 + \beta 1 + \gamma 2 + \delta = 360 \quad (3)$$

$$\alpha 2 + \beta 2 + \gamma 3 + \delta = 360 \quad (4)$$

$$\gamma 1 + \gamma 2 + \gamma 3 = 360 \quad (5)$$

$$\beta 2 = 0.77 * \alpha 2 - 12.6 \quad (6)$$

$$\delta = 118.5 \quad (7)$$

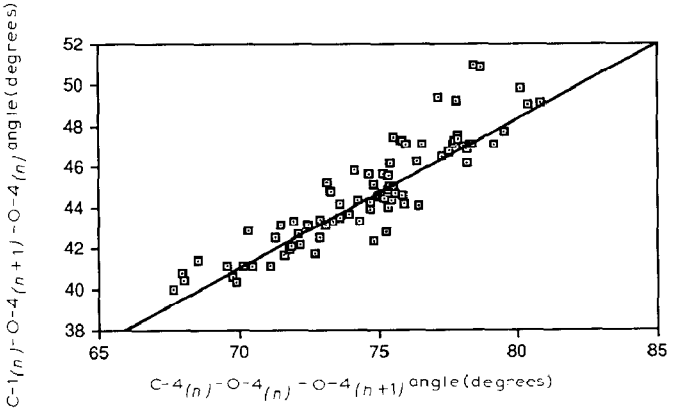


Fig. 3. Relation between the angles $C\text{-}1_{(n)}$–$O\text{-}4_{(n+1)}$–$O\text{-}4_{(n)}$ and $C\text{-}4_{(n)}$–$O\text{-}4_{(n)}$–$O\text{-}4_{(n+1)}$.

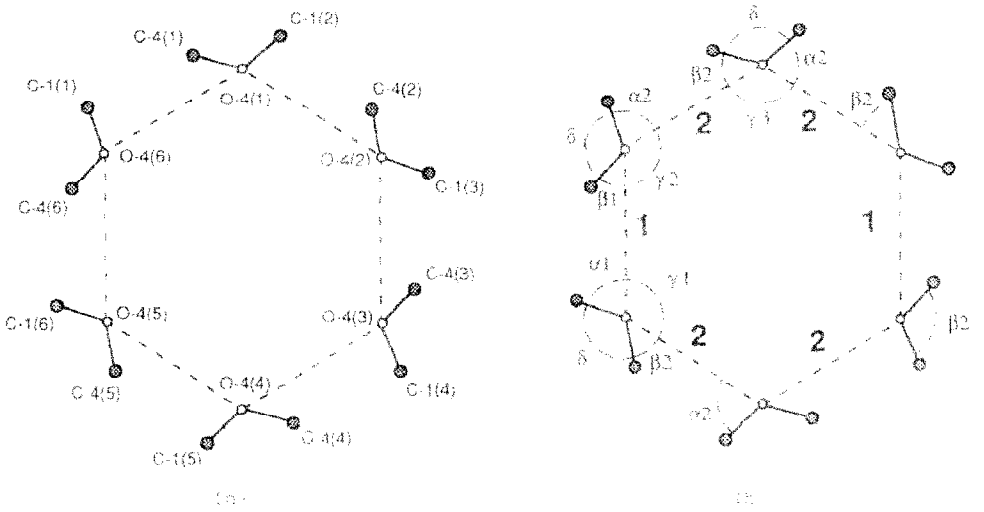


Fig. 4. Schematic view of an αCD molecule. (*a*) The hexagon formed by O-4(1)–O-4(6). (*b*) The hexagon with numbering scheme for relevant angles; 1 represents a large glucose residue, and 2 a smaller glucose residue.

Equations 2–4 arise from the fact that the bonds that make up these angles are coplanar, and equation 5 arises from the fact that the sum of the angles of a hexagon is 720°. Equation 6 describes the correlation between the angles C-$1_{(n)}$ O-$4_{(n-1)}$ O-$4_{(n)}$ and C-$4_{(n)}$ O-$4_{(n)}$ O-$4_{(n+1)}$ as shown in Fig. 3. Equation 7 is the mean glycosidic angle that is found in 14 crystal structures of αCD complexes.

One glucose residue with a desired O-$4_{(n)}$ O-$4_{(n+1)}$ distance is chosen. Since α1 and β1 are now known, the above set of equations can be solved. This means that, for a given glucose residue, there is only one other glucose residue that can be used to build an αCD structure with a reasonable geometry.

The set of equations only holds if all of the atoms in Fig. 4 are coplanar, as is the case when the tilt of each glucose residue is ~90°. However, in crystal structures, an average tilt of 80.5° was found. Therefore, the set of equations has to be changed slightly in order to allow for this different tilt. In Fig. 4, glucose residue 1 is situated between C-1(1) and C-4(1), and the planes of this glucose residue and O-4(1)–O-4(6) are perpendicular. In order to describe a different tilt, it is assumed that the glucose residue can be rotated 9.5° along the O-4(6)–O-4(1) axis. In fact, the plane that consists of O-4(1), C-4(1), C-1(1), and O-4(6) is rotated 9.5° along this axis. This results in the desired tilt of 80.5° of the plane of the glucose residue. If this process is carried out for each glucose residue, there will be a decrease in each glycosidic angle *o*. From matrix multiplications, it can be shown that this decrease is 1.5° if the tilt of the glucose residues is changed to 80.5°. Thus, if the angle *o* is set to 120.0° in equation 7, tilting of the plane of the glucose residues to 80.5° will result in proper glycosidic binding angles of 118.5°.

Energy calculations. Three αCD structures are described, namely, a 6-fold symmetrical αCD with a diameter of 8.31 Å (DI = 1 by definition), a slightly distorted αCD with a largest diameter of 8.55 Å (DI = 1.02), and a substantially distorted αCD

with a largest diameter of 9.0 Å (DI = 1.10). Since a simple model was used to build the αCD molecules, the modelled structures were optimised with the MM2 force field in MacroModel (see Experimental). Table II shows the resulting energies after 50 optimisation cycles. This process does not yield completely optimised structures because, with more than 50 cycles, the structures would have changed too much due to the absence of a guest molecule. The energies of the CD structures are equal to within 10 kJ/mol. Therefore, at room temperature, it should be possible for these different conformations to occur.

Table II also shows the energies when a complex is formed with *p*-hydroxybenzoic acid. The αCD molecule with the largest distortion yields an energy of interaction that is 12.5 kJ/mol lower than that observed using a symmetrical αCD. Therefore, these calculations confirm the assumption that the distortion of αCD molecules is important and that a simple model can be used to construct these αCD molecules.

The last two columns in Table II show results for a *p*-hydroxybenzoic acid molecule that is complexed in the reverse direction, *i.e.*, with the phenolic hydroxyl group pointing into the cavity. This situation does not occur in crystal structures and it is assumed generally that this mode of formation of complexes does not occur in solution either[24]. In an earlier study, in which molecular mechanics was used, it was claimed that the direction of inclusion could be predicted[3]. Table II shows that the energy of interaction for this mode of inclusion is less negative compared to that for the inclusion with the carboxylic group first. Therefore, it is tempting to conclude from these calculations that the model also predicts the direction of inclusion. However, these calculations include van der Waals and Coulombic interactions but exclude solvent interactions. As long as the guest compounds are incorporated in the same direction, it can be assumed that the contribution of this solvent interaction remains constant. For a reverse inclusion, this is almost certainly not true.

The results in Table II give limited information because they describe only one conformation of each complex. In order to obtain more information about the way

TABLE II

Energies of αCDs (kJ/mol) constructed according to the procedure described (the guest compound is *p*-hydroxybenzoic acid)

	Orientation of the guest[a]				
	COOH			*OH*	
	Distortion (DI)			*Distortion (DI)*	
	1.10	*1.02*	*1.00*	*1.10*	*1.02*
E(CD)	−562.1	−570.8	−565.5	−561.4	−567.5
E(guest)	−13.6	−14.0	−12.9	−13.9	−13.8
E(comp)	−641.0	−636.5	−631.2	−631.2	−625.0
E(inter)	−65.3	−51.7	−52.8	−55.9	−43.7

[a] The substituent designated is that included in the cavity.

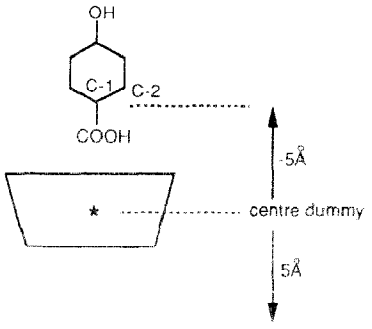
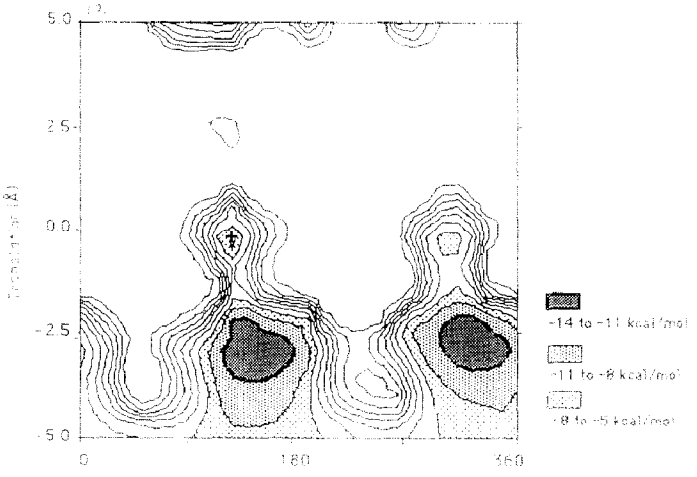


Fig. 5. Side-view of an αCD molecule with a guest compound at the entrance to the cavity. The distance between C-1 in the benzene ring and the centre dummy gives the extent of translation. The extent of rotation is given arbitrarily by the imaginary torsion angle C-2–C-1–centre dummy–O-4(1).

guest compounds are included in the αCD cavity, the structures were analysed using the "calculate conformations" facility as available in Chem-X. Thus, the guest molecule was moved 5 Å out of the cavity along the *z*-axis (Fig. 5). From here the guest molecule was moved back in steps of 0.25 Å, and within each step, it was rotated around the *z*-axis in steps of 15°. The energy of interaction was calculated for each orientation, and contour maps were obtained which showed an energy-profile for the guest–CD interaction. Fig. 6 shows the results for *p*-hydroxybenzoic acid with three conformations of the αCD molecule, namely, the crystal structure, a symmetrical αCD molecule, and a distorted αCD molecule.

The contour map obtained with the crystal structure (Fig. 6a) shows that the position of the guest is found in a local minimum. There is a small energy barrier between this local minimum and the global minimum that is positioned 2.5 Å towards the wider rim of the cavity. Such a barrier has also been reported[7] in a modelling study of βCD. Clearly, the van der Waals interaction is not the only force that determines the



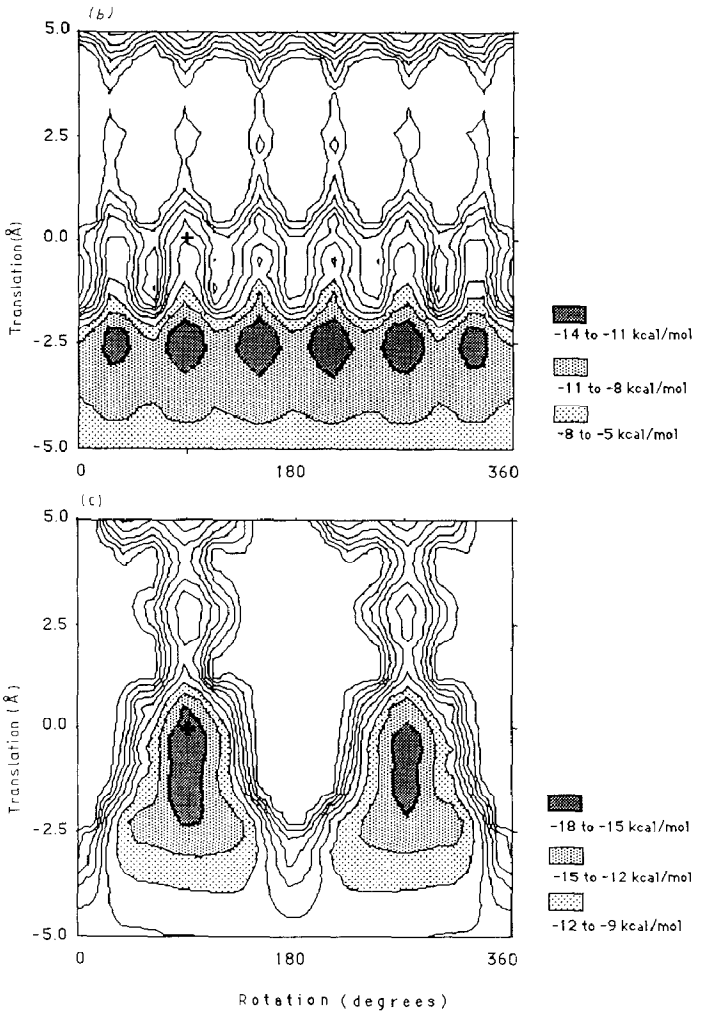


Fig. 6. Contour maps of the energy of interaction of *p*-hydroxybenzoic acid with αCD. The *y*-axis gives the depth of penetration of the guest molecule, and the *x*-axis gives the extent of rotation along the *z*-axis. Iso-energy lines are separated by 3 kcal/mol (~12.6 kJ/mol), and the low-energy regions are shaded. The position of the guest molecule as found in the crystal structure is marked with a cross. (*a*) Crystal structure, (*b*) symmetrical model, (*c*) distorted model (DI = 1.10).

depth to which a guest compound can enter the cavity. The contour map also shows a two-fold symmetry that is caused by the distortion of the CD molecule. It can be concluded that the guest molecule has no freedom of rotation within the cavity unless the αCD molecule changes its conformation. The guest compound can enter the cavity only at the wider rim of the αCD molecule. At the narrower rim, there is a large energy barrier that is not likely to be passed at room temperature.

The contour map obtained with a symmetrical cyclodextrin (Fig. 6b) has a six-fold symmetry, and the position of the guest, as found in crystal structures, does not

correspond to a minimum in the map. In fact, the guest compound cannot enter the cavity and remains at a distance of 2.5 Å from the centre. Therefore, a symmetrical CD molecule cannot be used to predict the position of a guest compound as found by experiment.

The contour map obtained with the distorted αCD molecule (Fig. 6c) shows more resemblance to the crystal structure. The two-fold symmetry is clear and the position of the guest compound in the crystal structure corresponds to the global minimum in the energy map. Because the model is simple, the map of the distorted αCD molecule shows some differences. The energy minimum is ~ 16 kJ/mol lower than that of the crystal structure, and the energy barrier at the narrower rim of the cavity is also lower. Nevertheless, it is concluded that this distorted structure is a better description of the crystal structure and that it is possible to predict the position of the guest with this αCD structure.

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