The Hexagonal Phase of the 3:2:1 Canal Complex between Deoxycholic Acid, Ethanol and Water: An Inclusion Compound with Hydrophilic Channels

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The hexagonal phase of the 3:2:1 canal complex between deoxycholic acid (DCA), ethanol (ETH) and water (W) crystallizes in space group $P6_5$, with $a = 15 \cdot 115$ (14), $c = 18 \cdot 676$ (15) Å, $Z = 6$. The structure was refined to a final R of 0·08. The host molecules are arranged in spirals around the 6_5 axes and are held together mainly by hydrogen bonds which are also formed with the guest molecules. Potential-energy calculations allowed us to find a satisfactory model for the ethanol molecules with their ethyl groups in non-equivalent crystallographic positions. The DCA:ETH:W ratio was confirmed by gas-chromatographic measurements. The ethanol and water molecules fit into canals, parallel to c, with hydrophilic interior surfaces and are arranged according to the repeating sequence ETH–ETH–W. The side-chain conformation of DCA is nearly extended. It differs from the one found in the orthorhombic phase, but is similar to that presented by cholic acid in an addition compound with ETH. The correlation between the side-chain and ring D conformations, found previously in some cholanic acids, is confirmed. The hexagonal phase may be used as a host lattice in which polar monomers can give rise to inclusion polymerization by, for example, γ- or X-radiations.

Introduction

$3\alpha,12\alpha$-Dihydroxy-5β-cholan-24-oic acid (deoxycholic acid, DCA) is a bile acid showing the unusual property of forming canal complexes with a wide variety of organic compounds, including aliphatic and aromatic hydrocarbons, fatty acids, alcohols, esters, ethers, phenols, azo dyes, alkaloids, camphor, methyl orange, β-carotene and cholesterol (Sobotka, 1934; Fieser & Fieser, 1959; Herndon, 1967). The Na salt of DCA possesses the ability to associate with several biological systems as a particular class of histones from chromatin (Hadler, Smart & Bonner, 1971), phospholipids (Philippot, 1971), proteins (Smart & Bonner, 1971; Visser, Robinson & Tanford, 1975) and sequential polypeptides (Corsi, D'Alagni & Giglio, 1976). Moreover, the bile salt molecules form micelles in aqueous solution (Small, 1971).

DCA has a hydrophobic side with three protruding methyl groups and a polar side with one carboxyl and two hydroxyl groups (Fig. 1): this is responsible for its strong chemical activity. The molecular packing of DCA in the orthorhombic phase was studied by Craven & De Titta (1972) in the acetic acid/choleic acid complex and by Candeloro De Sanctis, Giglio, Pavel & Quagliata (1972) in the phenanthrene/choleic acid and p-diiodobenzene/choleic acid complexes. These structures are characterized by channels covered inside with non-polar groups and the interactions between host and guest molecules are of a hydrophobic type.
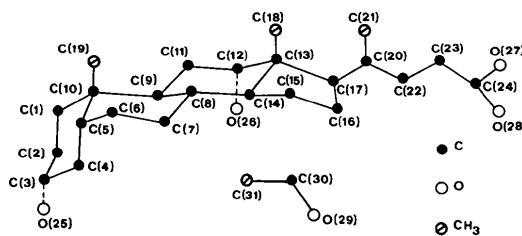
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Fig. 1. Atomic numbering of DCA and ETH molecules.

DCA gives rise to two other crystal systems, tetragonal (Bonamico & Giacomello, 1962) and hexagonal, as well as to a helical macromolecule (Rich & Blow, 1958; Blow & Rich, 1960). To acquire physicochemical information about the behaviour of this bile acid and its Na salt in solution and in the solid state we have solved the crystal structure of the hexagonal 3:2:1 complex (DCAETH) between DCA, ethanol and water.

Experimental

Colourless hexagonal prisms of DCAETH were grown by slow evaporation from ethanol or from acetone/ethanol at room temperature. The crystals are elongated along the hexagonal axis. Crystal data are collected in Table 1.

Differential scanning calorimetry showed that the crystals lose ethanol within a range of temperature centred at 104 °C by an endothermic process and subsequently melt at 177 °C after reorganization.

The composition of DCAETH was checked by gaschromatography, X-ray and density measurements. The observed density $(1 \cdot 16$ g cm$^{-3})$ was obtained by flotation in cyclohexane and carbon tetrachloride. However, this method is inadequate to determine the DCA:ETH:W ratio, owing to the small contribution of one guest molecule to the density.

The ratio was found to be 3:2:1 by gas chromatography. The amount of ETH was determined with a Hewlett–Packard model 7620 A apparatus equipped with a Hewlett–Packard 3380 A integrator and a 6 ft × $\frac{1}{8}$ in i.d. column packed with Chromosorb WHP (80–100 mesh) coated with 6% Dexsil 300 GC. The analysis was carried out at 60 °C.

For water a Hewlett–Packard model 5700 A apparatus with a 6 ft × $\frac{1}{8}$ in i.d. column packed with Chromosorb 101 (80–100 mesh) was used at 90 °C.

The space group is $P6_1$ or $P6_5$, the 001 reflexions being absent for $l \neq 6n$.

A crystal $0 \cdot 70 \times 0 \cdot 32 \times 0 \cdot 28$ mm was mounted on a Picker diffractometer with a graphite monochromator and the lattice parameters were determined with Cu $K\alpha$ radiation (λ for $K\alpha_1 = 1 \cdot 54050$ Å).

Intensities were collected on the same diffractometer by the θ–2θ scanning method. 2287 independent reflexions with $I > 0$, corresponding to 81% of the

Table 1. *Crystal data of DCAETH*

$3\alpha,12\alpha$-Dihydroxy-5β-cholan-24-oic acid, $C_{24}H_{40}O_4$, FW 392·58
Ethanol, C_2H_6O, FW 46·07
Water, H_2O, FW 18·02
Hexagonal, space group $P6_5$
$Z = 6$, $F(000) = 1420$
$a = 15 \cdot 115$ (14), $c = 18 \cdot 676$ (15) Å
$V = 3695 \cdot 2$ Å^3, λ(Cu $K\alpha$) $= 1 \cdot 5418$ Å
$D_m = 1 \cdot 16$, $D_x = 1 \cdot 16$ g cm^{-3}
μ(Cu $K\alpha$) $= 6 \cdot 3$ cm^{-1}

whole Cu $K\alpha$ sphere, were recorded up to $2\theta = 160°$. Each reflexion was scanned at a rate of $2°$ min^{-1} over a range of $1 \cdot 8°$, with a dispersion factor allowing for α_1–α_2 splitting. Background counts were taken for 10 s at each side of a reflexion. Three standard reflexions monitored periodically after every 30 reflexions showed only the deviations from the mean predicted by counting statistics. Lorentz and polarization factors were applied taking into account the monochromator crystal (Azaroff, 1955). No correction was made for absorption.

Structure solution and refinement

The structure was solved by *MULTAN* (Germain, Main & Woolfson, 1971) from 300 reflexions with $|E| \geq 1 \cdot 42$.

From an E map, computed from one of the twelve best sets of phases, all the non-hydrogen atoms, except C(21) of DCA and the atoms of ETH and W, were recognized. The space group was determined as $P6_5$. Structure factor calculations, performed with the DCA atoms, including C(21) generated at the expected position, and subsequent Fourier syntheses allowed us to locate only one more peak, O(29), which later proved to be the O atoms of ETH and W probably occupying approximately crystallographically equivalent positions. Thus it was decided to refine the DCA molecule and O(29) by isotropic and anisotropic block-diagonal least squares with all the observed reflexions and programs of Domenicano, Spagna & Vaciago (1969).

Scattering factors for C and O were taken from Cromer & Mann (1968) and for H from Hanson, Herman, Lea & Skillman (1964). The function minimized was $\sum w(|F_o| - |F_c|)^2$, with $w = (a + |F_o| + b|F_o|^2)^{-1}$.

The H atoms were generated with C—H $= 1 \cdot 08$ Å and H—C—C $= 109 \cdot 5°$. Those of the methyl groups were taken staggered with respect to the atoms linked to C(10), C(13) and C(20). The H atoms bonded to the tertiary C atoms make two H—C—C angles of $109 \cdot 5°$, the third being defined by the non-regular tetrahedral hybridization of the C atoms. All these H atoms were included in the last cycles, whereas those of the hydroxyl groups were neglected; an isotropic B of 5 Å^2 was kept fixed together with the positional parameters.

R was 0·09. A difference synthesis showed many peaks lower than $3\sigma(\rho)$ and only one of approximately $5\sigma(\rho)$, corresponding to 0·5 e Å^{-3}. This peak, 1·47 Å from O(29), could be attributed to the methylene C(30) of ETH, since it occupies one corner of the tetrahedron around O(29), the other two sites of which are identified by O(26) of the asymmetric units at (x,y,z) and $(x - y, x, z - \frac{1}{6})$. The O(29)$\cdots$O(26) distances were 2·7 Å and agreed with the hydrogen-bond
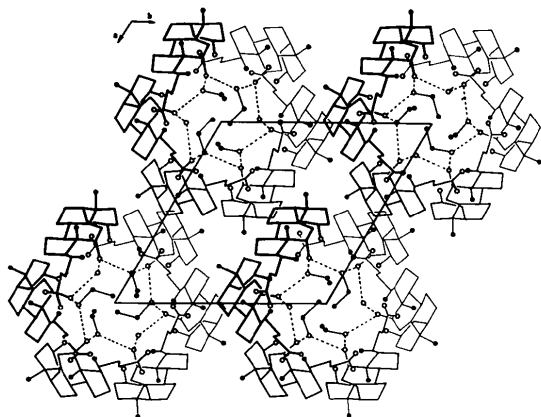
Fig. 2. A view of the DCAETH crystal structure along c. The broken lines indicate the hydrogen bonds among the DCA and ETH molecules. The black circles represent methyl groups and the methylene C atom of ETH; open circles refer to O atoms.

Fig. 3. Bond lengths (Å) and angles (°) of ETH.

Table 2. *The coefficients of the potential functions*

The energy is in kcal per atom pair if the interatomic distance is in Å.

Interaction	$a(\times 10^{-3})$	b	c	d
H···H	6·6	4·080	49·2	0
H···C	44·8	2·040	125·0	6
H···O	42·0	2·040	132·7	6
H···CH₃	49·1	3·705	380·5	0
C···C	301·2	0·000	327·2	12
C···O	278·7	0·000	342·3	12
C···CH₃	291·1	1·665	981·1	6
O···O	259·0	0·000	358·0	12
O···CH₃	272·7	1·665	1026·3	6
CH₃···CH₃	273·9	3·329	2942·0	0

formation. A scheme of the DCAETH crystal packing, which accounts for the actual stoichiometry of the canal complex and corresponds to the model proposed by us (see later), is shown in Fig. 2.

In order to locate the methyl C atom of ETH it was decided to resort to potential-energy calculations, assuming the atomic coordinates so far obtained, which are practically coincident with the final ones (Table 3). The coefficients of the potentials, expressed in the generalized form: $V(r) = a \exp(-br)/r^d - cr^{-6}$ are given in Table 2. The methyl group was treated as one atom. The geometry of ETH, adopted for the heavy atoms, was derived from Jönsson (1976), whereas the H atoms were generated according to the above rules. Bond distances and angles are given in Fig. 3. The calculations were performed with angular increments of 5° and a cut-off distance of 7·5 Å. All the orientations of ETH were explored rotating about three axes passing through C(30) and translating the molecule so that O(29) occupied the experimental position. The DCA molecules were not moved.

At first a 1:1 DCA:ETH ratio was investigated. The energy results clearly ruled out this model and indicated
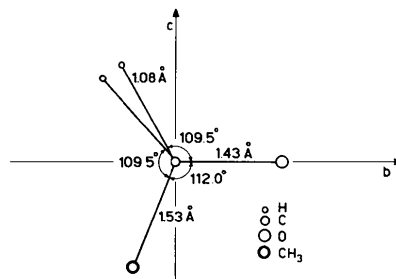
that some vacancies must occur among the ETH molecules. Moreover, it emerged that the methyl groups of two successive ETH molecules cannot be generated by the 6_5 axis, otherwise short contacts would occur, and that C(30) must be placed in the region of the highest peak of the difference synthesis in order to save the O(29)···O(26) hydrogen-bond system and to avoid unrealistic approaches with DCA and adjacent ETH molecules. Hence we undertook the analysis of sequences of the type (ETH)$_n$W, where n represents the number of ETH molecules between two water molecules and (ETH)$_n$W is the repeating unit. Only one degree of freedom, φ, the clockwise rotation angle viewed along O(29)—C(30), was taken into account for each ETH. Since the symmetry operator 6_5 cannot be applied to the generation of the methyl groups, the number of independent degrees of freedom was n. However, it was soon plain that the methyl group and the H atoms of C(30) cannot undergo free rotation.

To save computing time a previous run was made as a function of φ for only one ETH, including the DCA molecules and O(29) and C(30) of the other ETH and W molecules. The starting position, $\varphi = 0°$, corresponds to the atomic coordinates $x = 0·142, y = 0·059$, $z = 0·116$ for the methyl C atom of ETH.

The results, shown in Fig. 4, clearly indicate the occurrence of two sharp minima centred at -5 and $-95°$. Therefore φ was explored within a range of 40° in these regions.

The models with $3 \leq n \leq 5$ were discarded owing to some unrealistic contacts. The model with $n = 2$ provides the most favoured packing when the two adjacent ethanol molecules, ETH$_1$ and ETH$_2$, have $\varphi_1 = -5$ and $\varphi_2 = -105°$, ETH$_2$ being translated by $c/6$ with respect to ETH$_1$. These findings were supported by gas-chromatographic measurements (DCA:ETH:W = 6:3·9:1·9) establishing the validity of the (ETH)$_2$W model. The sequence with $n = 1$ (DCA:ETH:W = 2:1:1) is energetically less stable; nevertheless, it cannot be excluded that a small percentage of (ETH)$_2$W triplets lack one ETH, thus giving such sequences as ···ETH–ETH–W–ETH–ETH–W–ETH–W–ETH–ETH–W···, or sequences with other less probable kinds of defects (for example doublets of ETH separated by two W).
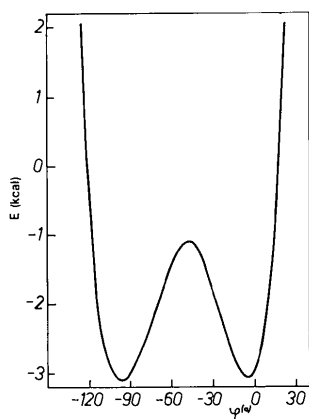
Fig. 4. Potential-energy curve due to the rotation φ of only one ETH. The methyl groups and the methylene H atoms of the other ETH molecules were omitted from the calculation.

Fig. 5. Bond distances (Å) of DCA with e.s.d.'s in parentheses.

Fig. 6. Bond angles (°) of DCA with e.s.d.'s in parentheses.

Table 3. *Fractional coordinates* ($\times 10^4$) *with e.s.d.'s in parentheses*

	x	y	z
C(1)	5156 (5)	2572 (5)	1918 (4)
C(2)	4283 (5)	1966 (5)	1400 (3)
C(3)	4376 (5)	2606 (5)	760 (3)
C(4)	4358 (4)	3578 (4)	987 (3)
C(5)	5265 (4)	4221 (4)	1502 (3)
C(6)	5280 (4)	5213 (4)	1696 (4)
C(7)	4431 (4)	5034 (4)	2220 (3)
C(8)	4443 (4)	4441 (4)	2887 (3)
C(9)	4369 (3)	3427 (4)	2678 (3)
C(10)	5262 (4)	3595 (4)	2172 (3)
C(11)	4241 (5)	2753 (5)	3321 (3)
C(12)	3425 (4)	2637 (4)	3849 (3)
C(13)	3545 (3)	3658 (4)	4092 (3)
C(14)	3578 (3)	4240 (3)	3391 (3)
C(15)	3503 (5)	5139 (4)	3669 (4)
C(16)	2849 (5)	4756 (4)	4351 (4)
C(17)	2667 (3)	3667 (4)	4495 (3)
C(18)	4552 (4)	4242 (6)	4511 (4)
C(19)	6292 (4)	4190 (6)	2561 (4)
C(20)	2507 (4)	3383 (5)	5283 (3)
C(21)	2401 (11)	2373 (10)	5423 (5)
C(22)	1592 (4)	3424 (6)	5590 (3)
C(23)	1575 (5)	3449 (7)	6405 (4)
C(24)	584 (5)	3179 (5)	6744 (3)
O(25)	3534 (4)	2027 (4)	274 (*)
O(26)	2421 (3)	2028 (3)	3528 (3)
O(27)	−237 (3)	2582 (4)	6370 (3)
O(28)	513 (4)	3466 (5)	7330 (3)
O(29)*	1634	1669	2199
C(30)*	1439	693	1977
C'(31)*	1330	560	1160
C"(31)*	320	−130	2080

* Coordinates kept fixed during the refinement.

coordinates for C'(31) and C"(31) are those derived from the potential-energy calculations at $\varphi_1 = -5°$ and $\varphi_2 = -105°$ respectively. These coordinates fit positive regions of the difference synthesis.

Some least-squares cycles showed that the parameters of O(29) and C(30) converge, while those of C'(31) and C"(31) do not. Thus the atomic coordinates, corresponding to the $(ETH)_2W$ triplet minimum, found in the energy calculations, were assumed and kept fixed during the refinement together with the C'(31) and C"(31) thermal parameters. The H atoms were generated and included in the last cycles. The final R and R_w were 0·08 and 0·13 respectively. These values, rather high, and the incomplete refinement reflect, in our opinion, the complexity of the structure and the disorder. The final atomic coordinates are reported in Table 3.*

The bond lengths and angles of DCA are given in Figs. 5 and 6.

At this stage the refinement of the structure was completed by introducing O(29) and C(30) with occupancy factors of 1 and 0·67 respectively and two methyl C atoms, C'(31) and C"(31), weighted 0·33, all with isotropic temperature factors. The starting atomic
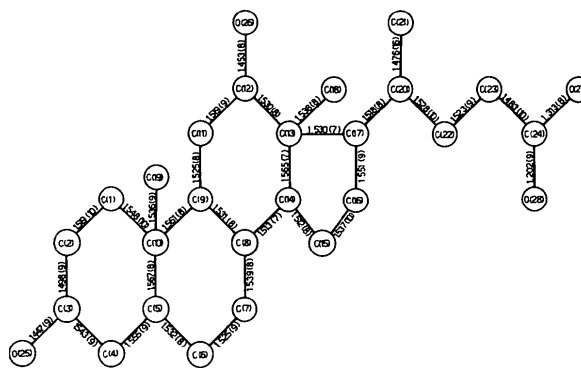
Discussion

The DCA bond distances and angles show good agreement with those of similar structures, one of which, $3\alpha,6\alpha$-dihydroxy-5β-cholan-24-oic acid, was solved with a high degree of accuracy (Hall, Maslen & Cooper, 1974). The most interesting feature of the DCA geometry is the side chain and ring D conformation.

The relevant dihedral angles are listed in Table 4 according to the convention of Klyne & Prelog (1960). Giglio & Quagliata (1975) computed the van der Waals energy of DCA as a function of four internal rotation angles defining the side-chain conformation. The results indicated a considerable conformational flexibility since several minima appeared in the potential-energy surface. All the cholanic acids structures, including DCAETH, show side-chain conformations populating three of the deepest minima as far as the C(13)—C(17)—C(20)—C(21), C(17)—C(20)—C(22)—C(23) and C(20)—C(22)—C(23)—C(24) rotation angles are concerned.

The side-chain C atoms of DCA in the hexagonal phase adopt a nearly extended conformation, very similar to that of cholic acid (Johnson & Schaefer, 1972), although in DCAETH the deviation from the *trans* form is more noticeable.

The main difference between the DCA molecules in the orthorhombic and hexagonal phases consists in the value of the C(17)—C(20)—C(22)—C(23) torsion angle, which characterizes a *gauche* and a *trans* state respectively. The nearly constant value of about $-60°$ of the C(13)—C(17)—C(20)—C(21) dihedral angle is confirmed once more, in agreement with the van der Waals energy calculations. In fact, the C(21) methyl group and the H atoms linked to C(20) and C(22) are hindered from rotating around C(17)—C(20), owing to short contacts with the C(18) methyl group, one H atom of C(16) and that of C(12).

Thus the large widening of the C(13)—C(17)—C(20) angle is caused by the repulsive interactions of the C(21) methyl group with that of C(18) and with the H atom of C(12).

Another point of interest concerns the C(22)—C(23)—C(24)—O(27) torsion angle of 25°, which lies on a maximum van der Waals energy zone as for the

acetic acid/choleic acid and phenanthrene/choleic acid complexes. This value differs considerably from those found for the other cholanic acids (Giglio & Quagliata, 1975) and further supports the ability of DCA to form various hydrogen-bonding schemes, which seem to be the driving force in the association of the DCA molecules both in the solid state and in solution (Sugihara & Tanaka, 1976). The corresponding energy gain largely counterbalances the higher van der Waals contribution.

Ring D is suitably described by Δ, the phase angle of pseudorotation, and φ_m, the maximum angle of torsion (Altona, Geise & Romers, 1968).

The values reported in Table 4 for DCAETH are near those of cholic acid, which approaches a β-envelope symmetry and presents satisfactory intramolecular van der Waals interactions among the side-chain and ring D atoms.

Cholic acid and DCA in the hexagonal phase assume very similar geometries, thus confirming the influence of the side chain on the ring D conformation, so that an extended side chain induces the C(13) envelope, whereas the folded one in the *gauche* state, characterizing the orthorhombic phases of DCA, seems to be coupled with the half-chair form (for a more exhaustive discussion see Giglio & Quagliata, 1975).

The molecular packing is shown in Fig. 2 where the ethanol molecules are arranged according to the sequence $(ETH)_2W$ with the same crystallographically equivalent position in different canals randomly occupied by ETH_1, ETH_2 or W. The DCA molecules forming one canal are held together mainly by

Table 4. *Torsion angles* (°) *for the side chain and ring D of DCA together with* Δ *and* φ_m

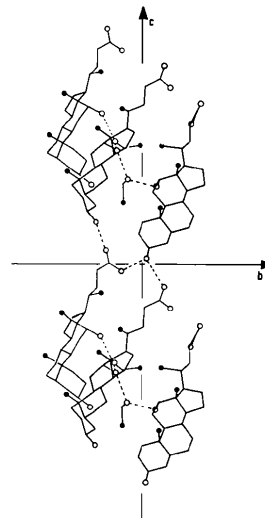|			
C(13)—C(17)—C(20)—C(22)	180	C(13)—C(14)	45·0
C(17)—C(20)—C(22)—C(23)	−164	C(14)—C(15)	−31·2
C(20)—C(22)—C(23)—C(24)	−161	C(15)—C(16)	5·7
C(13)—C(17)—C(20)—C(21)	−57	C(16)—C(17)	22·2
C(16)—C(17)—C(20)—C(21)	−177	C(13)—C(17)	−40·7
C(21)—C(20)—C(22)—C(23)	72	Δ	23·7
C(22)—C(23)—C(24)—O(27)	25	φ_m	46·0
C(22)—C(23)—C(24)—O(28)	−155		

Fig. 7. Part of the DCAETH packing viewed along the normal to the *bc* plane. The broken lines represent hydrogen bonds. The filled and open circles represent methyl groups and O atoms respectively.

hydrogen bonds (Fig. 7), where two triplets of DCA molecules, related by a translation along c, are shown. Molecules at z, $\frac{1}{6} + z$ and $\frac{2}{6} + z$ form a triplet. Each O atom is engaged in the hydrogen-bonding scheme: O(25) with O(27) of the asymmetric unit at $(y - x, \bar{x}, z - \frac{2}{3})$ and O(28) of that at $(y, y - x, z - \frac{5}{6})$, acting as acceptor and donor respectively; O(26) with two O(29). The corresponding O$\cdots$O distances are respectively 2·59, 2·79, 2·71 and 2·69 Å and the C—O$\cdots$O angles are in the range 110–136°. However, these values must be treated with caution owing to the unlikely crystallographic equivalence of the ETH and W O atoms, so that the O(29) coordinates represent an average position.

The spirals of DCA molecules interact by van der Waals forces. The canals around the 6_5 axes are covered with hydrophilic surfaces.

The cavities have sections perpendicular to c of an approximately circular shape (Fig. 2) and diameters of about 4·0 Å. Thus, only small or thread-like molecules can be accommodated.

In this connexion the ability of DCA to allow radiation-induced canal polymerization in the ortho-rhombic phase (Miyata & Takemoto, 1975, 1976, 1977; Audisio & Silvani, 1976) when the guest molecules are monomers should be mentioned.

Inclusion polymerization by γ- or X-radiations can be utilized in the solid state to prepare stereoregular polymers and copolymers, usually with high molecular weight, which grow into the canals, the crystalline lattice behaving as an organized solvent. Moreover, asymmetric polymerizations have been performed, DCA being a chiral molecule. However, DCA in the orthorhombic phase gives rise to hydrophobic interactions with the guest molecules, so that only non-polar monomers have been occluded. DCAETH, on the contrary, presents a host lattice which can receive polar monomers and, therefore, new kinds of polymers and copolymers could be obtained.

A parallel study is in progress on a similar canal complex between DCA, dimethyl sulphoxide and water, having the same cell parameters as DCAETH and a similar intensity distribution, in order to investigate the possible interactions and arrangements of guest molecules of different sizes and natures within the cavities.

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