Acta Cryst. (1972). B28, 3656

A Study of the Crystal Packing of the 2:1 and 3:1 Canal Complexes Between Deoxycholic Acid and *p*-Diiodobenzene and Phenanthrene

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(*Received* 5 *August* 1972)

The crystal structures of the 2:1 and 3:1 canal complexes between deoxycholic acid and *p*-diiodobenzene and phenanthrene have been investigated. The crystals are orthorhombic, space group $P2_12_12_1$, with four and twelve molecules of deoxycholic acid in the unit cell. The cells constants are: $a=26{\cdot}59$, $b=13{\cdot}58$, $c=7{\cdot}17$ Å; and $a=26{\cdot}81$, $b=13{\cdot}60$, $c=21{\cdot}66$ Å for the *p*-di-iodobenzene and phenanthrene complexes respectively. Heavy-atom and minimum residual phasing techniques were used to solve the two very similar structures. The host molecules are held together mainly by hydrogen bonds and form bi-layers along the *b* and *c* axes, while the guest ones fit into canals, parallel to the *c* axis, with hydrophobic interior surfaces. The aromatic hydrocarbons interact in a pile and also form contacts with the deoxycholic acid molecules, as a result of van der Waals forces. The *p*-diiodobenzene molecules have two statistical orientations which to satisfy the screw axis along **c**. The phenanthrene molecules do not show any evidence of disorder.

Introduction

3α,12α-Dihydroxycholanic acid (deoxycholic acid, hereafter referred to as DCA) has a rigid cyclopentenophenanthrene nucleus, on one side of which are clustered three methyl groups and on the other, two hydroxyl groups (see Fig. 1). A short aliphatic chain protrudes from one end of the nucleus and terminates in a carboxyl group. Therefore DCA contains both one hydrophobic and one hydrophilic side and this property is mainly responsible for its remarkable chemical behaviour.

It has been discovered that this bile acid (Wieland & Sorge, 1916), together with apocholic and β-apocholic acids (Boedecker, 1920), forms well defined molecular compounds, termed choleic acids. These are obtained as crystalline solids in which the acholic constituents may be acids, aliphatic or aromatic hydrocarbons, alkaloids, alcohols, azo dyes, esters, ethers, phenols and many other molecules such as, for example, cholesterol, β-carotene and methyl orange (Sobotka, 1934; Fieser & Fieser, 1959; Herndon, 1967).

DCA plays a leading role in a wide variety of biological systems, being strongly reactive and giving rise to large aggregates stabilized by hydrophobic forces (Small, Penkett & Chapman, 1969).

Its sodium salt is used to solubilize biological membranes. Both proteins and phospholipids are solubilized and a close association is found between deoxycholate and phospholipids in a constant ratio of 13 to 1 mole respectively (Philippot, 1971). The type of binding has been studied by n.m.r. (Small, Penkett & Chapman, 1969) and the results obtained seem to indicate that the bile salt molecules interact hydrophobically among themselves and with the alkyl chains of the lecithin, thus forming a micelle. The outer groups of the micelles are hydrophilic and are in contact with the aqueous environment.

Similar mixed micelles occur with cholesterol (Small, Bourges & Dervichian, 1966; Bourges, Small & Dervichian, 1967) and their size changes as a function of the temperature, *p*H and of the urea and counterion concentration in the medium (Small, 1968). Furthermore it has been found that sodium deoxycholate causes a selective dissociation of a particular class of histones from chromatin (Hadler, Smart & Bonner, 1971) as well as the solubilization of many subcellular components. The results obtained by the above authors again seem to support a mechanism of interaction mainly due to hydrophobic forces rather than to electrostatic bonds.

It is worth while mentioning the pronounced hypercholesterolemic effect of DCA in the mouse (Howe, Bosshardt & Huff, 1960) and in the rat (Howe & Hutchison, 1962), as well as in a number of other species (Portman, 1962). This, together with the results of a study on the action of dietary bile acids on cholesterol metabolism, suggested that the hydroxyl group in position 12 is necessary for the hypercholesterolemic effect to occur.

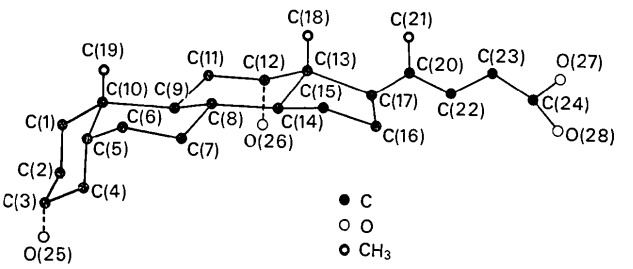

Fig. 1. The DCA molecule and its atomic numbering.

It is doubtful whether the DCA present in bile conjugated with glycine and taurine could be responsible for the dissolving power of bile with regard to the water-insoluble fats. In fact, the glycodesoxycholic acid does not form complexes with fatty acids, like DCA itself when its hydroxyl or carboxyl groups are masked by the introduction of organic radicals (Cortese & Bauman, 1936). However it is impossible at present to exclude some mechanism of micelle formation in such a type of solubilization.

Both sodium deoxycholate and DCA form a helical macromolecule in solution (Rich & Blow, 1958; Blow & Rich, 1960) under proper conditions. It is likely that hydrogen bonding is the driving force in this phenomenon and that the aggregation of DCA molecules in the polymer shows a crystalline internal structure.

Pioneering crystallographic and chemical studies on some fatty acid choleic acids have been carried out by Go & Kratky (1934), Giacomello & Kratky (1936), Kratky & Giacomello (1936), Caglioti & Giacomello (1939) and Giacomello & Bianchi (1943). Subsequent attempts to solve the crystal structure of the DCA orthorhombic phase were unsuccessful (Fischmeister, 1954; Bonamico & Giacomello, 1962).

In order to throw light on the peculiar behaviour of DCA and to understand the main forces that determine the formation of the choleic acids in the solid state and of the micelles in solution, we are carrying out a research programme which was begun in 1966 (Damiani, Giglio, Morosoff, Puliti & Rosen, 1967). Meanwhile we have become aware of a crystal structure determination of the DCA *p*-bromoanilide (Schaefer & Reed, 1972) and of the acetic acid choleic acid (Craven & De Titta, 1972). For the last compound no atomic coordinates have been published.

In this paper we report a structural study of the *p*-diiodobenzene and phenanthrene choleic acids (hereafter referred to as PDIB and PHEN respectively).

Experimental

PDIB and PHEN crystals were prepared by dissolving DCA and aromatic hydrocarbons in hot absolute alcohol (Fieser & Newman, 1935). Crops of small colourless crystals, which separated on slow cooling of the solution, have a prismatic shape elongated along the *c* axis. The melting points are 180 and 184 °C for PDIB and PHEN respectively. The compositions of the complexes were determined by optical rotation, ultraviolet spectroscopy, and X-ray and density measurements (Damiani, Giglio, Morosoff, Puliti & Rosen, 1967). The cell parameters are: $a=26{\cdot}59\pm0{\cdot}04$, $b=13{\cdot}58\pm0{\cdot}02$, $c=7{\cdot}17\pm0{\cdot}01$ Å for PDIB and $a=26{\cdot}81\pm0{\cdot}04$, $b=13{\cdot}60\pm0{\cdot}02$, $c=21{\cdot}66\pm0{\cdot}03$ Å for PHEN.

The theoretical densities, 1·41 (PDIB) and 1·14 (PHEN) g.cm^{-3}, calculated by assuming 2 molecules of *p*-diiodobenzene and $\frac{4}{3}$ of phenanthrene for 4 molecules of DCA, are in good agreement with the observed values, 1·42 and 1·15 g.cm^{-3}, measured by flotation in cyclohexane and carbon tetrachloride.

Oscillation, precession and Weissenberg photographs established the space group as $P2_12_12_1$, the $h00$, $0k0$ and $00l$ reflexions being absent for h, k and l odd. The two complexes differ in the length of the *c* axis, which is tripled for PHEN. In this case the layers with $l\neq3n$, due to the contribution of the phenanthrene molecules alone, have only a few reflexions and these are of very weak intensity. The intensities of 1162 independent reflexions, belonging to the $hk0$, $hk3$, $hk6$, $hk9$, $hk12$ and $hk15$ layers, were collected on multiple-film equi-inclination Weissenberg photographs, using Cu $K\alpha$ radiation. PDIB forms as very small crystals, nearly always twinned, which are decomposed by X-rays, with loss of iodine. Out of about fifty crystals tested only three were considered suitable for recording intensity data. Only 204 $hk0$ and $hk1$ independent reflexions were collected in the same way as for PHEN.

All the intensities for PDIB and PHEN were of poor quality: they were estimated visually and corrected for Lorentz, polarization and change of spot shape on upper layers. No absorption corrections were considered necessary.

Structure determination and refinement

A Patterson function was computed for PDIB by using (001) intensity data and the iodine atoms were located in projection. A Fourier synthesis was calculated in the *ab* plane with the phases derived from the positions of the iodine atoms. A model for DCA was built up with the same bond lengths and bond angles as those of desoxycholic acid *p*-bromoanilide (Schaefer & Reed, 1972) by means of a program written in our laboratory (Gavuzzo, Pagliuca, Pavel & Quagliata, 1972). The atoms C(23), C(24), O(27) and O(28) were generated in the conformation shown in the figure of the paper by Craven & De Titta (1972), which also corresponds to a minimum of the intramolecular energy map of DCA previously computed by us.

After some trials the approximate layout of DCA was found from the Fourier map. A minimum residual program (Damiani, Giglio, Liquori & Ripamonti, 1967) allowed us to refine the position of both DCA and *p*-diiodobenzene, by rotating and translating independently the two rigid molecules. A standard geometry had been chosen for the aromatic hydrocarbon, assuming all the angles 120° to be and the C–C and C–I bond distances 1·40 and 2·05 Å respectively. The best agreement index, calculated with an overall isotropic temperature factor of 5 Å^2 for all the observed reflexions, was 0·19. The fractional atomic coordinates of *p*-diiodobenzene are reported in Table 1.

At the same time the analysis of the crystal structure of PHEN was undertaken by locating the DCA molecule in the same position as in PDIB and by employing the minimum residual program. The minimum

Table 1. *Final fractional atomic coordinates of the p-di-iodobenzene molecule*

	x	*y*	*z*
C′(1)	0·2884	0·5393	0·3752
C′(2)	0·2543	0·4651	0·4239
C′(3)	0·2937	0·5675	0·1884
C′(4)	0·2306	0·4475	0·0990
C′(5)	0·2253	0·4193	0·2858
C′(6)	0·2647	0·5216	0·0503
I(1)	0·3308	0·6064	0·5773
I(2)	0·1882	0·3803	−0·1031

R was obtained at the same position as for PDIB. The subsequent introduction of a regular phenanthrene molecule (C–C = 1·40 Å, C–C–C = 120°) lowered the R value from 0·36 to 0·29 for the 118 reflexions with sin $\theta/\lambda \leq 0{\cdot}20$ Å^{-1}. These and the later calculations were performed for a cell with $c = 7{\cdot}22$ Å, taking into account one molecule of DCA and one of phenanthrene, with a weight of one third, as the asymmetric unit.

Several cycles of least-squares refinement were carried out employing unit weights and isotropic, as well as anisotropic thermal, parameters. The temperature factors of the phenanthrene atoms were assumed to be isotropic and set equal to 8·5 Å^2. The hydrocarbon molecule was not refined by least squares, but its position was improved by tests performed with the minimum residual search. The hydrogen atoms linked to the carbons of DCA were generated at the expected positions (C–H = 1·08 Å) and included in the last two cycles. The refinement was considered to be complete when the parameter shifts were about 10% of the estimated standard deviations. A final structure-factor calculation using all the observed data resulted in an R value and a weighted discrepancy index of 0·13 and 0·14 respectively.

The atomic scattering factors used for the heavy atoms were those given by Cromer & Mann (1968) and, for the hydrogen atoms, the values were taken from the scattering factor table of Hanson, Herman, Lea & Skillman (1964). A set of programs written for the UNIVAC 1108 computer by Domenicano, Spagna and Vaciago (1969), together with a versatile structure-factor least-squares program of Robert Carruthers, were employed in these calculations.

The final coordinates and anisotropic temperature factors of the DCA atoms, together with their estimated standard deviations in parentheses, are given in Tables 2 and 3. The atomic coordinates corresponding to the best arrangement found for the rigid phenanthrene molecule are reported in Table 4.

Table 2. *Final values of the fractional atomic coordinates* ($\times 10^4$) *of DCA in PHEN and their standard deviations* ($\times 10^4$) *in parentheses*

	x	*y*	*z*
C(1)	1166 (8)	1335 (14)	1343 (31)
C(2)	640 (8)	1641 (13)	1958 (30)
C(3)	682 (7)	2358 (13)	3512 (31)
C(4)	989 (8)	1918 (12)	5228 (34)
C(5)	1525 (7)	1625 (12)	4558 (33)
C(6)	1818 (7)	1190 (15)	6235 (37)
C(7)	1616 (7)	174 (14)	6808 (31)
C(8)	1609 (7)	−574 (13)	5133 (32)
C(9)	1284 (7)	−126 (13)	3528 (33)
C(10)	1491 (6)	897 (13)	2819 (32)
C(11)	1211 (7)	−899 (12)	1926 (27)
C(12)	977 (7)	−1887 (12)	2634 (34)
C(13)	1294 (7)	−2323 (14)	4306 (31)
C(14)	1352 (7)	−1535 (12)	5813 (28)
C(15)	1599 (8)	−2116 (16)	7351 (35)
C(16)	1325 (8)	−3127 (13)	7249 (31)
C(17)	1038 (7)	−3168 (12)	5531 (30)
C(18)	1814 (7)	−2663 (15)	3304 (35)
C(19)	2026 (7)	736 (14)	1874 (35)
C(20)	1051 (7)	−4236 (11)	4713 (33)
C(21)	776 (8)	−4329 (14)	2671 (34)
C(22)	839 (8)	−4978 (13)	6067 (34)
C(23)	295 (8)	−4791 (16)	6530 (37)
C(24)	99 (8)	−5596 (13)	7922 (37)
O(25)	173 (5)	2634 (10)	4249 (24)
O(26)	456 (4)	−1678 (8)	3324 (22)
O(27)	127 (9)	−6493 (12)	7569 (30)
O(28)	−85 (5)	−5321 (9)	9429 (24)

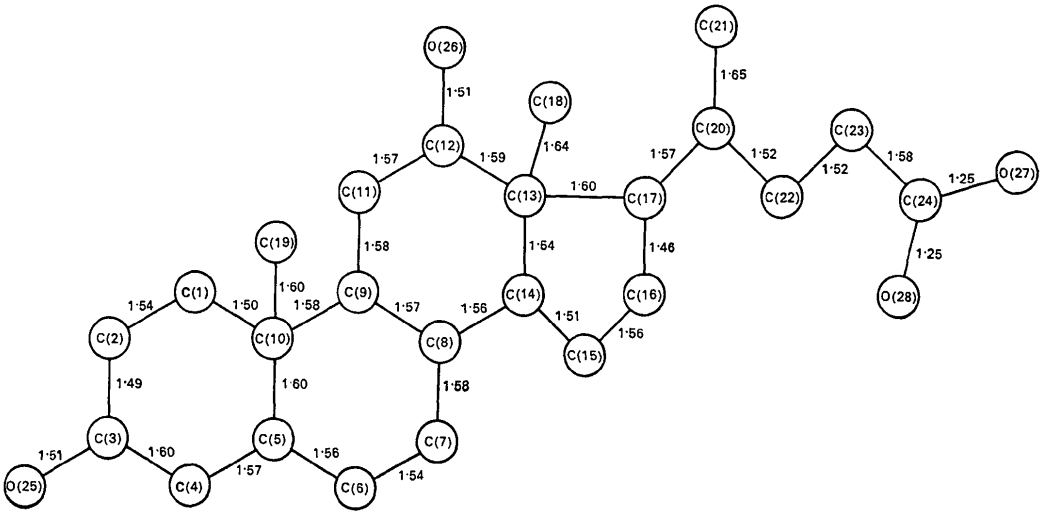

Fig. 2. Schematic drawing of DCA showing bond distances.

Table 3. *Thermal parameters* ($\times 10^4$) *for DCA in PHEN and their standard deviations* ($\times 10^4$)

The form of the temperature factor is:

$$\exp\left[-(b_{11}h^2+b_{12}hk+b_{13}hl+b_{22}k^2+b_{23}kl+b_{33}l^2)\right].$$

	b_{11}	b_{12}	b_{13}	b_{22}	b_{23}	b_{33}
C(1)	21 (4)	1 (13)	7 (28)	67 (14)	56 (51)	124 (55)
C(2)	22 (4)	−15 (12)	11 (27)	41 (11)	53 (52)	146 (56)
C(3)	16 (3)	5 (10)	−21 (27)	38 (10)	67 (51)	184 (59)
C(4)	21 (4)	−3 (10)	30 (30)	26 (10)	−95 (50)	256 (68)
C(5)	17 (4)	−16 (10)	−29 (29)	25 (9)	−54 (53)	298 (69)
C(6)	18 (4)	−8 (12)	−25 (31)	57 (13)	−120 (60)	301 (73)
C(7)	16 (4)	2 (11)	−21 (26)	55 (13)	−7 (52)	151 (62)
C(8)	14 (4)	3 (10)	−1 (26)	45 (11)	−57 (50)	191 (64)
C(9)	12 (3)	−5 (9)	15 (27)	43 (11)	23 (51)	221 (63)
C(10)	9 (3)	−8 (9)	44 (25)	45 (11)	−15 (54)	274 (66)
C(11)	16 (4)	−12 (10)	14 (23)	44 (11)	−80 (45)	87 (50)
C(12)	17 (4)	−3 (10)	30 (29)	33 (10)	−80 (52)	257 (67)
C(13)	14 (3)	−8 (11)	39 (27)	60 (13)	−85 (53)	175 (62)
C(14)	16 (4)	2 (10)	21 (24)	32 (10)	−33 (44)	132 (53)
C(15)	22 (4)	−11 (14)	−31 (33)	81 (16)	−7 (63)	237 (73)
C(16)	27 (5)	3 (12)	−9 (31)	41 (11)	−14 (50)	161 (59)
C(17)	21 (4)	−1 (10)	39 (26)	28 (10)	−20 (46)	139 (52)
C(18)	12 (3)	7 (11)	−4 (28)	70 (14)	80 (64)	284 (74)
C(19)	12 (3)	−11 (11)	42 (28)	60 (13)	67 (61)	314 (76)
C(20)	18 (4)	−4 (10)	34 (28)	24 (9)	39 (48)	234 (64)
C(21)	27 (5)	1 (12)	11 (33)	39 (12)	−65 (54)	220 (68)
C(22)	22 (4)	12 (11)	10 (28)	38 (12)	−30 (46)	175 (66)
C(23)	23 (5)	16 (14)	85 (34)	65 (14)	13 (65)	260 (78)
C(24)	21 (4)	6 (11)	26 (35)	31 (11)	33 (56)	351 (84)
O(25)	15 (2)	11 (8)	5 (20)	68 (9)	−60 (44)	374 (54)
O(26)	12 (2)	−2 (7)	37 (18)	43 (7)	1 (37)	281 (42)
O(27)	61 (6)	13 (15)	184 (35)	79 (12)	28 (51)	375 (59)
O(28)	19 (3)	14 (8)	70 (21)	46 (8)	−97 (39)	340 (50)

Table 4. *Fractional atomic coordinates of the phenanthrene molecule used in the calculations*

	x	y	z
C(29)	0·2551	0·5144	0·0911
C(30)	0·2449	0·4856	−0·0911
C(31)	0·2756	0·5721	0·4555
C(32)	0·2244	0·4279	−0·4555
C(33)	0·2907	0·5875	0·1257
C(34)	0·2702	0·5299	−0·2388
C(35)	0·2298	0·4701	0·2388
C(36)	0·2093	0·4125	−0·1257
C(37)	0·3009	0·6164	0·3079
C(38)	0·2600	0·5010	−0·4210
C(39)	0·2401	0·4990	0·4210
C(40)	0·1991	0·3836	−0·3079
C(41)	0·1943	0·3970	0·2042
C(42)	0·1840	0·3682	0·0220

At this stage the fractional coordinates of DCA listed in Table 2 were transformed for the PDIB unit cell so that the molecular geometry was unchanged. The minimum residual analysis was begun again and the lowest value of R was 0·18 for all the observed reflexions without any change in the atomic coordinates of Table 1 and in those derived from Table 2. A list of the observed and calculated structure factors is available from the authors on request.

Results and discussion

The intramolecular distances and angles of DCA are summarized in Figs. 2 and 3.

The poor quality of the intensity data and the low number of reflexions recorded have caused rather large standard deviations in the atomic coordinates. It is therefore impossible to gain detailed information about the molecular geometry. However, although some bond lengths show considerable deviations from the usual values, there is a general qualitative agreement.

The conformation of the DCA side chain deserves some attention. The internal rotation angles are reported in Table 5 and a rotation is taken as positive if, starting from the *cis* conformation which corresponds to 0°, the highest numbered atom is moved in the counterclockwise direction looking along the bond C(n)–C(k) with $n>k$. The intramolecular potential energy map of DCA, computed as a function of three rotational degrees of freedom around the C(17)–C(20), C(20)–C(22) and C(22)–C(23) bonds, neglecting the oxygen atoms of the carboxyl group, presents several minima that indicate a remarkable conformational flexibility of the side chain (our unpublished results). The first five angles of Table 5 correspond to an energy minimum but it is possible that DCA may adopt other conformations in solution, in the macromolecule or in the tetragonal (Bonamico & Giacomello, 1962) as well as in the hexagonal (our unpublished results) crystal phases, giving rise to different schemes of hydrogen bonding.

The PHEN complex does not show any evidence of disorder. The PDIB choleic acid contains *p*-di-

Table 5. *Internal rotation angles of the DCA side chain in PHEN*

C(13)–C(17)–C(20)–C(21)	−59°
C(16)–C(17)–C(20)–C(21)	175
C(17)–C(20)–C(22)–C(23)	62
C(21)–C(20)–C(22)–C(23)	−65
C(20)–C(22)–C(23)–C(24)	179
C(22)–C(23)–C(24)–O(27)	−56
C(22)–C(23)–C(24)–O(28)	124

iodobenzene molecules disposed in accordance with the $P2_12_12_1$ space group and therefore they must be oriented in two arrangements, *A* and *B* related by a screw axis parallel to the *c* axis, in order to satisfy the stoichiometry of the complex. The two arrangements may occur in the same canal in the sequence (···*AAAACBBBB*···), where *C* represents one defect or one vacancy, or in different canals as (···*AAAA*···) and (···*BBBB*···).

The canals have a section perpendicular to the *c* axis of approximate rectangular shape with edges of about 7·5 and 4·0 Å, and can be seen in Fig. 4 for PHEN. The packing of PDIB is not reported owing to the close similarity of the two structures.

The empty spaces are bounded by DCA molecules mainly linked by hydrogen bonds along the *b* and *c* axes (see Fig. 5) and by van der Waals interactions along the *a* axis. The oxygen atoms involved in the hydrogen bonding scheme extend along the *c* axis, the repetitive unit consisting of three bonds, about 2·7 Å long, between O(25)···O_1(27), O_1(28)···O_2(26) and O_2(26)···O_3(25), where the 1, 2 and 3 subscripts refer to the molecules at $(x, 1+y, z)$, $(\bar{x}, \frac{1}{2}+y, \frac{3}{2}-z)$ and $(x, y, 1+z)$ respectively.

The DCA double-layers, parallel to the *bc* plane and visible in Fig. 5, possess a translational degree of freedom along the *b* axis, which causes a change in the shape and dimensions of the canals and in the orientation of the guest molecules. This is inferred from inspection of the figure published in the paper of Craven & De Titta (1972) where the bi-layers are similar but shifted along the *b* axis and the included molecules are rotated about 80° around the *c* axis as compared with the PHEN and PDIB structures. Thus the ability of DCA to accommodate a wide variety of molecules of different size in orthorhombic crystals of very similar unit-cell parameters can be accounted for. The molecules which are included further stabilize the lattice energy increasing the number of contacts in the crystal and hence the melting point of the choleic acid with respect to the DCA alone.

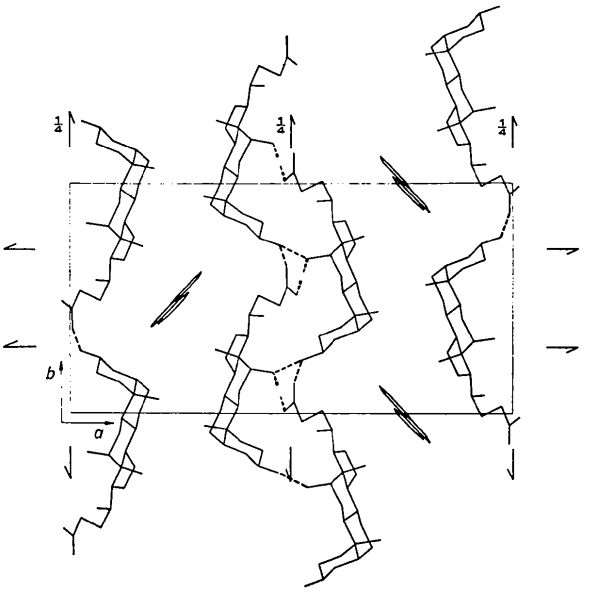

Fig. 4. Molecular packing in the PHEN crystal viewed along the *c* axis. The dashed lines indicate hydrogen bonding.

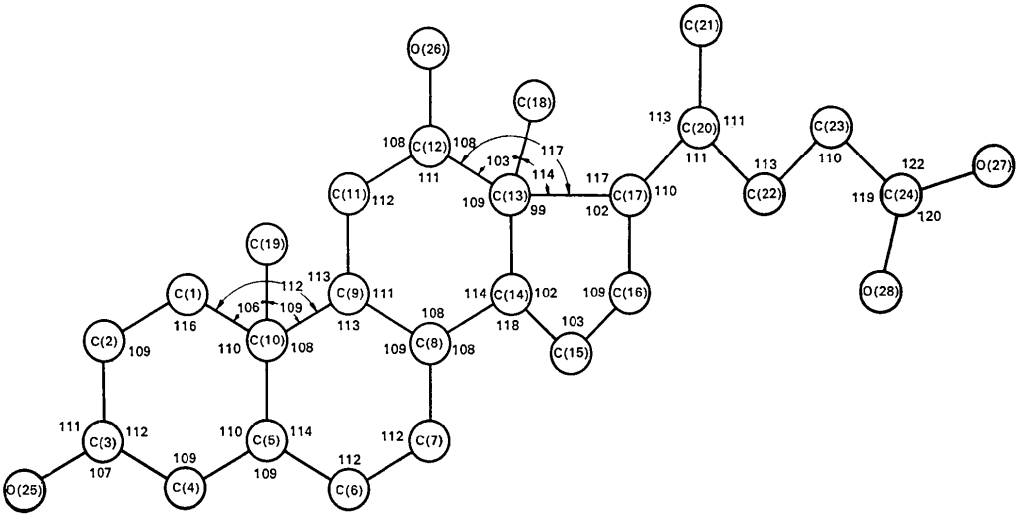

Fig. 3. Schematic drawing of DCA showing bond angles.

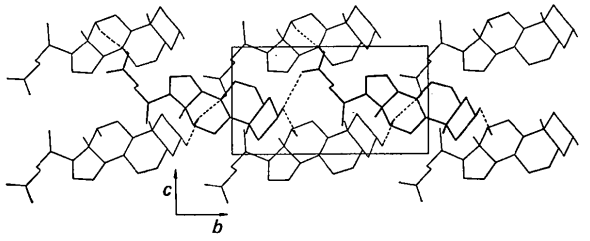

Fig. 5. Molecular packing of DCA molecules in the PHEN and PDIB crystals viewed along the *a* axis. The dashed lines show the hydrogen bonding scheme allowing the formation of hydrophobic bi-layers.

The phenanthrene molecule is strongly associated with two C(18) methyl groups belonging to the DCA molecules at $(x, 1+y, z)$ and $(\frac{1}{2}-x, \bar{y}, z-\frac{1}{2})$ and pointing approximately towards the centres of the two outermost rings. Four of the twelve approaches range from 3·5 to 3·6 Å, so that there are specific hydrophobic interactions.

A similar situation arises in PDIB. Among five contacts less than 4 Å involving iodine atoms, the two shortest are 3·7 and 3·8 Å long and bind the methyl groups C(21) of the molecule at $(\frac{1}{2}-x, \bar{y}, \frac{1}{2}+z)$ and C(18) of the molecule at $(\frac{1}{2}-x, \bar{y}, z-\frac{1}{2})$. Therefore it may be supposed that the main intermolecular attraction between DCA and the hydrocarbons is due to 'polarization bonding' involving the iodine atoms in PDIB and the π charge cloud in PHEN.

On the basis of these crystal structures it may be reasonably thought that DCA molecules give rise in solution to micelles, where the bi-layers of the bile acid, characterized by large periodic hydrophobic regions on both sides, are associated with one another through the guest molecules. However the hydrophobic pleated sheets show hydrophilic regions along lines surrounded by hydroxyl and carboxyl groups which behave as attraction centres with respect to the solvent molecules.

Further work is being carried out to provide both physicochemical information about the behaviour of this important biological compound in solution and the detailed crystal structures of the hexagonal, tetragonal and other orthorhombic phases, as well as the helical structure of the macromolecule.

We thank Dr R. Puliti for valuable help in collecting the data and Mr G. Iaquaniello for skilled technical assistance. The authors acknowledge the Consiglio Nazionale delle Ricerche for financial support.

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