All three Pro residues in these structures have C^{γ} *exo* with respect to the peptide chain (Fig. 2). In both Pro(1) residues the pyrrolidine rings have an 'envelope' conformation that approximates idealized mirror symmetry with the mirror passing through C(1)γ and between N(1) and C(1)α. In the Pro(2) residue the ring has a 'half chair' conformation that approximates twofold axial symmetry with the axis passing through N(2) and between C(2)β and C(2)γ. In the Aze(2) residue the four-membered azetidine ring is flat (r.m.s. out-of-plane deviation is 0·004 Å).

The overall conformations of the two molecules (Figs. 2 and 5) hardly differ at all. The φ and ψ conformation angles at the Aze(2) residue do not differ from those at the Pro(2) residue by any more than the corresponding differences between the two chemically equivalent Pro(1) residues. Thus, substitution of Aze for Pro in a protein should be expected to affect the protein structure mainly through side-group steric effects and not directly through effects on the conformation of the main polypeptide chain.

Dr Edward L. McGandy originally suggested this study, and he helped measure the diffraction data. The diffraction measurements were made in the Crystallography Department of the University of Pittsburgh while one of us (RHB) was an NIH postdoctoral trainee there during 1970–1972 supported by USPHS Grant No. GM-01728. Our work at Buffalo was supported by USPHS Grants Nos. AM-19856 and GM-19684.

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The Structure of the 2:1 Complex between the Bile Acid Deoxycholic Acid and (+)-Camphor

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Abstract

The 2:1 deoxycholic acid–camphor complex is orthorhombic, $P2_12_12$, with $a = 27{\cdot}353$ (3), $b = 13{\cdot}814$ (2), $c = 7{\cdot}233$ (1) Å, $Z = 4(C_{24}H_{40}O_4 \cdot \frac{1}{2}C_{10}H_{16}O)$/unit cell. The crystal structure was solved by direct methods and refined by least squares to $R = 0{\cdot}07$ for all 2933 measured X-ray reflections. As in other previously determined orthorhombic crystal complexes of this bile acid, the deoxycholic acid molecules form hydrogen-bonded bilayers stacked in an array which leaves hydrophobic channels between them. Filling the channels, which in this particular case are centered on crystallographic twofold axes and nearly cylindrical, are columns of camphor molecules distributed between two possible orientations related by the twofold axis. Comparison of this structure with other orthorhombic deoxycholic acid complexes shows that while the steroid bilayers are very similar in all cases, they are shifted parallel to each other in either or both of two directions, thus forming channels of differing sizes, shapes, and orientations, to accommodate guest molecules with optimal van der Waals guest–host interactions. Variations in geometric and/or electronic structure of guest molecules yield different complexes. The relations among the types so far observed are

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discussed with detailed illustrations and comparisons of four representative complexes: 1:1 deoxycholic acid: acetic acid, $P2_12_12_1$; 2:1 deoxycholic acid:camphor, $P2_12_12$; 2:1 deoxycholic acid:cyclohexanone, $P2_12_12_1$ but pseudo-$P2_12_12$; 3:1 deoxycholic acid:phenanthrene, $P2_12_12_1$.

Introduction

The bile acids are hydroxylated derivatives of the steroid 5β-cholan-24-oic acid. They play important physiological roles in the digestion and absorption of fats (Bloom & Fawcett, 1975). A crystalline compound first isolated from ox bile by Latschinoff (1885) was shown by Wieland & Sorge (1916) to be a stoichiometric 8:1 complex of the bile acid deoxycholic acid (DCA, 3α,12α-dihydroxy-5β-cholan-24-oic acid, $C_{24}H_{40}O_4$) with a fatty-acid mixture of palmitic and stearic acids. Subsequently, it was shown that such stoichiometric crystalline complexes, so-called 'choleic acids', were formed between a wide variety of organic compounds and DCA; a few such complexes have been described with cholic acid and apocholic acid [see reviews by Sobotka (1934), Fieser & Fieser (1959) and Herndon (1967), and references contained therein]. Certain human enteroliths (intestinal stones) have been found to consist chiefly of choleic acid complexes of DCA and fatty acids in a molecular ratio of 8:1 (Fowweather, 1949).

DCA also appears to interact strongly with organic molecules in solution, as evidenced by its ability to render soluble in aqueous basic solution an otherwise insoluble alkane (Huntress & Phillips, 1949) and to cause shifts in the absorption spectra of aromatic hydrocarbons (Fieser & Newman, 1935) and of various azo dyes (Cilento, 1951, 1952; Lautsch, Bandel & Broser, 1956; Angelescu & Nicolau, 1965). The importance of bile in the excretion of a variety of substances of endogenous and exogenous origin (Smith, 1973) suggests the possibility that DCA complexes may be involved in that process.

Because of the expectation that detailed study of the nature of the association between DCA and other organic molecules might help to understand the physiological roles of bile acids, and also because of the intrinsic crystallographic interest in these molecular complexes, some X-ray crystallographic studies of the choleic acids were carried out quite early. Only within the past decade, however, were complete accurate structure determinations made of these light-atom complexes. These determinations include the orthorhombic choleic acids 1:1 DCA:acetic acid (Craven & DeTitta, 1972), 2:1 DCA: *p*-diiodobenzene and 3:1 DCA:phenanthrene (Candeloro de Sanctis, Giglio, Pavel & Quagliata, 1972); 8:1:1 DCA:palmitic acid:ethanol (Coiro, D'Andrea & Giglio, 1980); 2:1 DCA:norbornadiene (D'Andrea, Fedeli, Giglio, Mazza & Pavel, 1981); 2:1 DCA:*X* (where *X* may be acetone, diethyl ketone, acetophenone, ethyl methyl ketone, and chloroacetone); 2:1 DCA:*Y* [where *Y* may be cyclohexanone, (*S*)-3-methylcyclohexanone, (*R*)-3-methylcyclohexanone]; and the 4:1 complex DCA: di-*tert*-butyl diperoxycarbonate (Tang, 1978; Friedman, Lahav, Leiserowitz, Popovitz-Biro, Tang & Zaretzkii, 1975; Lahav, Leiserowitz, Popovitz-Biro & Tang, 1978).

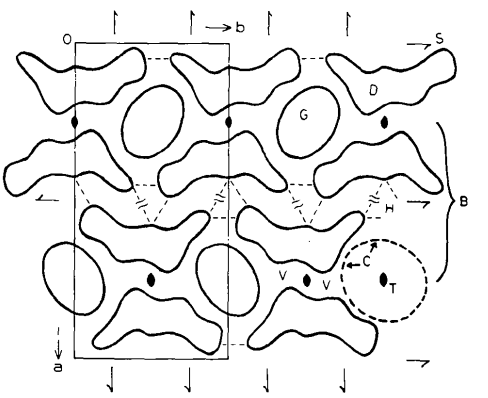

Fig. 1. Schematic structure of orthorhombic deoxycholic acid molecular complexes. (*B*) Bilayer of deoxycholic acid molecules; (*D*) deoxycholic acid; (*G*) guest molecule; (*H*) helical hydrogen-bonding scheme holding hydrophilic sides of deoxycholic acid in bilayer; (*V*) van der Waals contacts between hydrophobic sides of deoxycholic acid bilayers; (*C*) channel along *c* with hydrophobic walls for containing guest molecule; (*O*) origin of unit cell; (*S*) 2_1 at $z = 0$ in $P2_12_12$ or at $z = \frac{1}{4}$ in $P2_12_12_1$; (*T*) 2 in $P2_12_12$ or 2_1 in $P2_12_12_1$; both exact 2_1 and approximate 2 in $P2_12_12_1$ which are pseudo-$P2_12_12$.

These orthorhombic crystal structures all have the common structural pattern shown schematically in Fig. 1: bilayers of DCA molecules, with channels between successive bilayers into which guest molecules fit. The stacking of these bilayers in all the structures is not, however, identical, and shows that the guest molecules influence in small ways (*e.g.* shifts along *b*) or in more complex and significant ways (*e.g.* space-group changes $P2_12_12_1 \leftrightarrow P2_12_12$) the relative positions of successive DCA bilayers. These bilayer shifts cause variations in the size, shape, and orientation of the channels which accommodate particular guest molecules. The DCA:guest interactions in all the examples listed above are hydrophobic, and thus depend principally on the size and shape of the guest molecule. The latter can be grouped in three categories: (1) long ribbon-like molecules, such as palmitic acid, or molecules such as acetic acid which form narrow chains in the choleic acids; (2) planar aromatic molecules, such as phenanthrene or *p*-diiodobenzene; and (3) other small molecules such as acetone or cyclohexanone.

We decided, therefore, to investigate the choleic acid formed between DCA and camphor, which is an

essentially spherical molecule, quite different in size and shape from any guest molecule in a choleic acid of established structure.

Choleic acids have been reported with both 2:1 and 1:1 DCA:camphor ratios (Rheinboldt, König & Flume, 1929). The former, apparently the more stable complex, was used as a pharmaceutical preparation, 'Cadechol', to exploit, for camphor, the ability of DCA to render soluble and transport molecules otherwise insoluble in water. We decided to determine the nature and extent of the bilayer shifts necessary for DCA to accommodate camphor in a choleic acid, and to compare that arrangement with previously known structures, as part of a study to investigate the range and limits of size and shape of guest molecules which the type of structural organization found in these crystalline complexes can accommodate.

Experimental

Colorless lath-shaped crystals, elongated along c, were grown by slow evaporation at room temperature of an ethanolic solution containing a 2:1 molecular ratio of deoxycholic acid and (+)-camphor. When heated, the crystals transformed between about 308–333 K, probably from loss of camphor vapor, while remaining solid. They finally melted at 448 K, close to the melting point of pure deoxycholic acid. This behavior resembles that of the alkane-containing choleic acids (Huntress & Phillips, 1949). The crystals exhibited diffraction symmetry *mmm* and were thus orthorhombic; systematically absent reflections $h00$, $h = 2n + 1$, and $0k0$, $k = 2n + 1$, identified the space group as $P2_12_12$. Unit-cell parameters at 288 K were obtained from least-squares analysis of diffractometer-angle measurements for 15 reflections. The density $D_m = 1{\cdot}137$ (5) g cm^{-3}, measured by flotation in a CCl_4/hexanes mixture, indicated $Z = 4$; the asymmetric unit $C_{24}H_{40}O_4{\cdot}\frac{1}{2}(C_{10}H_{16}O)$ gives a calculated density $D_x = 1{\cdot}139$ g cm^{-3}.

Intensity data were collected at 288 K from a crystal of approximate dimensions $0{\cdot}15 \times 0{\cdot}25 \times 0{\cdot}40$ mm on a Syntex $P2_1$ diffractometer with Ni-filtered Cu X-radiation ($\lambda = 1{\cdot}54178$ Å), using a θ–2θ scan, up to $2\theta = 140°$ ($\sin\theta/\lambda = 0{\cdot}607$ Å^{-1}). Statistical fluctuations only were observed in the intensities of three standard reflections monitored every 100 reflections. Of the 2933 independent intensities measured, 2527 had $I > 3\sigma(I)$. Lorentz and polarization corrections were applied, but the absorption coefficient of 5·55 cm^{-1} for Cu $K\alpha$ X-rays was sufficiently small that absorption corrections for a crystal of the size used could be neglected. The data were put on an approximately absolute scale, a thermal parameter was estimated, and E values were calculated with the normalization program in *MULTAN* 78 (Main, Hull, Lessinger, Germain, Declercq & Woolfson, 1978). The use of spherical average molecular scattering factors (Debye, 1915) for both deoxycholic acid and camphor gave a nearly linear normalization plot, a considerable improvement over the ordinary Wilson plot obtained on the assumption of randomly positioned individual atoms. The difference between the two normalization techniques can be of critical importance when employing direct methods to solve crystal structures.

Structure determination and refinement

A Fourier synthesis of 300 E values with phases obtained from the direct-methods program in *MULTAN* 78 revealed all the C and O atoms of the deoxycholic acid molecule, but no atoms of camphor. The initial residual $R = 0{\cdot}26$ was lowered by least-squares refinement of the DCA C and O atom positions and the inclusion of H atoms in calculated positions to 0·19, but would not go lower. A weighted difference Fourier synthesis showed *one* large, roughly spherical peak ~7 Å in diameter on the twofold rotation axis in the space between the deoxycholic acid layers.

At this stage it was assumed that the camphor molecules were essentially spherically disordered, as they are in the crystal of (±)-camphor, which is face-centered cubic, $a \simeq 10{\cdot}1$ Å, $Z = 4$ (Finback, 1938), with each camphor molecule occupying a spherical space $10{\cdot}1(\sqrt{2}/2) = 7{\cdot}14$ Å in diameter. With the single peak in the Fourier map taken as the centroid of the molecule, scattering from camphor could thus be represented by the spherical average molecular scattering factor g calculated by the normalization program in *MULTAN* 78 using the Debye scattering formula

$$g^2 = \sum_{p=1}^{N}\sum_{q=1}^{N} f_p f_q \frac{\sin(4\pi r_{pq}\sin\theta/\lambda)}{(4\pi r_{pq}\sin\theta/\lambda)}$$

where N is the number of atoms, r_{pq} the distance between atoms p and q, and f_p the atomic scattering factor of atom p. An atomic model for camphor, including all H atoms, was derived from the crystal structure determination of *endo*-3,9,9-tribromocamphor (Phillips & Trotter, 1977). The spherically averaged scattering factor g, shown in Fig. 2, was represented in computations by the function $g = 75{\cdot}13 \times \exp(-195{\cdot}31\sin^2\theta/\lambda^2) + 9{\cdot}98\exp(-3{\cdot}67\sin^2\theta/\lambda^2) + 1{\cdot}95$, found by least-squares fitting. (This commonly used form, which fits the scattering factor quite well, is required by the particular refinement program we used.) Anisotropic refinement of all C and O atoms in DCA, plus camphor represented as a single giant 'atom', reduced R to 0·12; including the scattering from camphor was crucial for fitting many large, low-angle reflections.

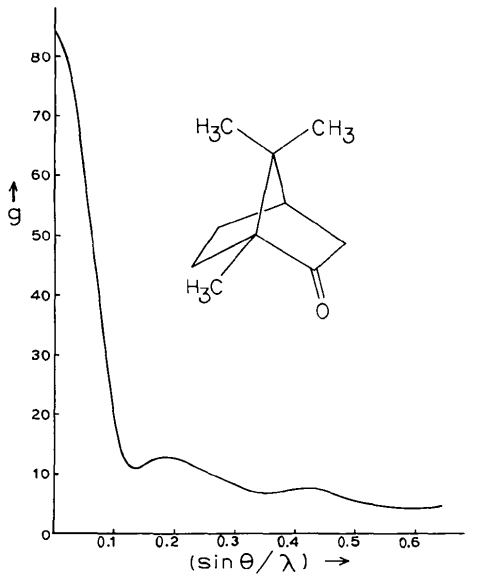

Fig. 2. Spherical average molecular scattering factor for (+)-camphor.

Removal of the camphor molecule from this scattering model gave $R = 0{\cdot}15$, and a weighted difference Fourier synthesis now showed several resolved peaks in the space from which the camphor had been removed. Successive refinements, difference syntheses, and construction of models allowed us finally to interpret the peaks as representing a (+)-camphor molecule with only twofold disorder, the minimum required by its location on the crystallographic twofold rotation axis.

Anisotropic refinement was continued with all C and O atoms of deoxycholic acid treated independently, and H atoms riding on the atoms to which they are attached. Camphor was refined as a rigid body because it is both disordered and subject to large thermal motions. The refinement slowly converged to a final conventional residual $R = \sum |\Delta F| / \sum |F_o| = 0{\cdot}0696$ and a weighted residual $R_w = (\sum w|\Delta F|^2 / \sum wF_o^2)^{1/2} = 0{\cdot}0883$ for all 2933 measured structure factors. A final difference map showed a maximum electron density of $0{\cdot}3$ e Å^{-3} and had no features.

Least-squares refinements and geometric calculations were performed using the *SHELX* program system (Sheldrick, 1978).

Results and discussion

Final atomic coordinates are listed in Table 1.* Bond distances and angles are shown in Figs. 3 and 4. All

* Lists of structure factors and anisotropic thermal parameters for C and O atoms have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 36483 (15 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

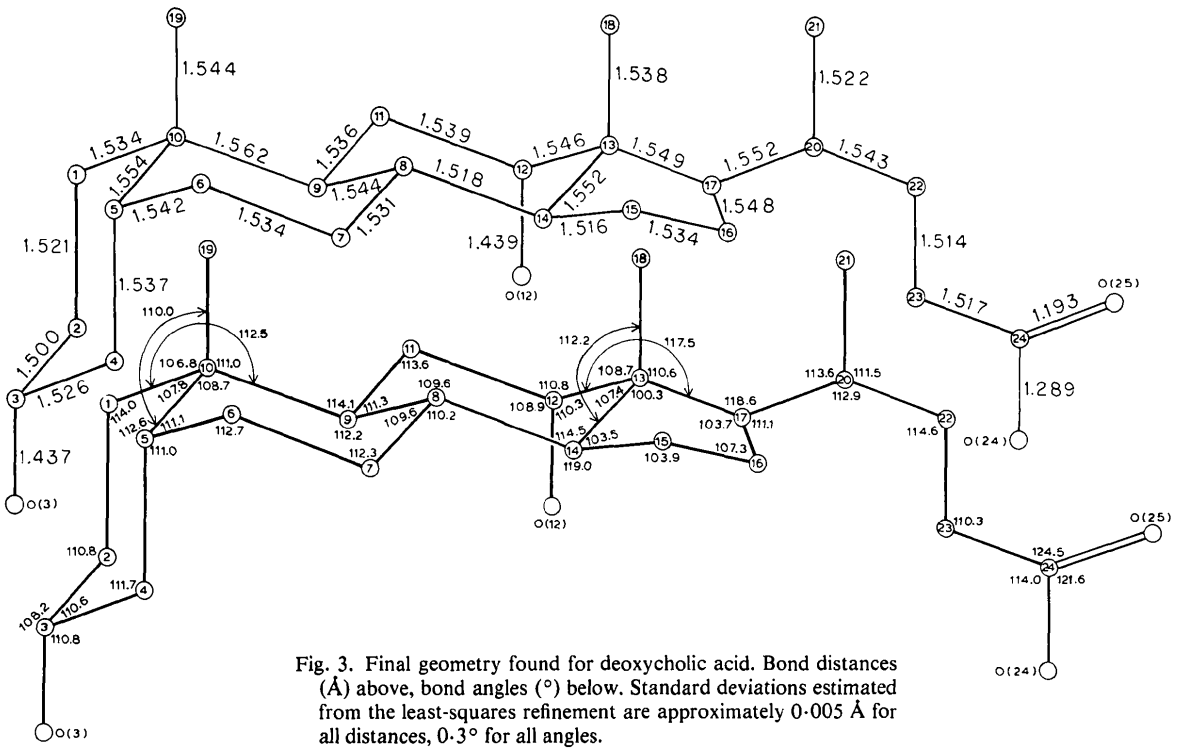

Fig. 3. Final geometry found for deoxycholic acid. Bond distances (Å) above, bond angles (°) below. Standard deviations estimated from the least-squares refinement are approximately 0·005 Å for all distances, 0·3° for all angles.

C–H bond distances were fixed at 1·08 Å, O–H bond distances at 1·00 Å, H–C–H angles at 109·5°.

The crystalline arrangement, which follows the scheme shown in Fig. 1, is similar but not identical to that in other known orthorhombic complexes of deoxycholic acid, with which it is compared in detail in Figs. 5 and 7. Bilayers of deoxycholic acid molecules extending parallel to the *bc* plane are held together by a helical hydrogen-bonding system, summarized below:

Donor	Acceptor	O···O distance
O(3)	O(25′)	2·732 (4) Å
O(24′)	O(12″)	2·655 (4)
O(12″)	O(3‴)	2·710 (4).

Table 1. *Final fractional atomic coordinates* (×10⁴), *and* U_{eq} [$=\frac{1}{3}(U_{11}+U_{22}+U_{33})$] *for* C *and* O *atoms*

Standard deviations estimated from the least-squares refinement are given in parentheses as deviations in the last significant figure. Camphor was refined as a rigid body. H atoms were kept at r(C–H) = 1·08 Å and r(C–O) = 1·00 Å; U_{iso}(H) = U_{eq} of the atom to which H is attached.

	x	y	z	U_{eq} (Å² ×10³)		x	y	z
Deoxycholic acid								
C(1)	−1313 (1)	1950 (3)	−6152 (6)	58 (2)	H(C4)	−1448	3068	−1275
C(2)	−1814 (1)	2303 (3)	−5544 (6)	60 (2)	H′(C4)	−1708	1937	−1829
C(3)	−1770 (1)	2999 (3)	−3962 (6)	60 (2)	H(C5)	−784	2782	−3409
O(3)	−2255 (1)	3280 (2)	−3409 (5)	80 (2)	H(C6)	−736	2184	−140
C(4)	−1496 (1)	2536 (3)	−2353 (6)	60 (2)	H′(C6)	−341	1586	−1694
C(5)	−991 (1)	2160 (2)	−2948 (6)	58 (2)	H(C7)	−1299	842	−179
C(6)	−718 (2)	1696 (3)	−1305 (6)	70 (2)	H′(C7)	−709	403	326
C(7)	−938 (2)	720 (3)	−730 (6)	66 (2)	H(C8)	−611	−171	−2815
C(8)	−975 (1)	11 (2)	−2352 (5)	53 (2)	H(C9)	−1615	701	−3429
C(9)	−1261 (1)	488 (2)	−3952 (5)	47 (2)	H(C19)	−271	890	−4418
C(10)	−1019 (1)	1452 (2)	−4614 (5)	52 (2)	H′(C19)	−335	1948	−5760
C(19)	−502 (1)	1267 (3)	−5394 (7)	71 (2)	H″(C19)	−546	829	−6621
C(11)	−1362 (1)	−238 (2)	−5513 (5)	52 (2)	H(C11)	−1598	105	−6517
C(12)	−1610 (1)	−1177 (2)	−4851 (5)	47 (2)	H′(C11)	−1019	−424	−6157
O(12)	−2092 (1)	−951 (2)	−4187 (4)	57 (1)	H(C12)	−1634	−1677	−5995
C(13)	−1305 (1)	−1666 (2)	−3310 (5)	49 (2)	H(O12)	−2333	−1235	−5073
C(18)	−816 (1)	−2003 (3)	−4136 (6)	59 (2)	H(C18)	−613	−2404	−3109
C(14)	−1234 (1)	−908 (2)	−1749 (5)	52 (2)	H′(C18)	−605	−1386	−4580
C(15)	−1020 (2)	−1497 (3)	−177 (6)	71 (2)	H″(C18)	−890	−2465	−5308
C(16)	−1284 (2)	−2474 (3)	−318 (6)	76 (2)	H(C14)	−1567	−567	−1288
C(17)	−1550 (1)	−2496 (2)	−2206 (5)	53 (2)	H(C15)	−630	−1587	−349
C(20)	−1548 (1)	−3530 (2)	−3043 (6)	57 (2)	H′(C15)	−1092	−1156	1139
C(21)	−1804 (2)	−3599 (3)	−4906 (6)	67 (2)	H(C16)	−1023	−3058	−240
C(22)	−1737 (1)	−4305 (3)	−1684 (6)	64 (2)	H′(C16)	−1546	−2544	791
C(23)	−2267 (2)	−4186 (3)	−1125 (7)	78 (3)	H(C17)	−1939	−2360	−2168
C(24)	−2413 (1)	−4975 (3)	226 (7)	69 (2)	H(C20)	−1167	−3684	−3300
O(24)	−2588 (1)	−4654 (2)	1762 (4)	74 (2)	H(C21)	−1608	−3137	−5856
O(25)	−2381 (2)	−5821 (2)	−78 (6)	116 (3)	H′(C21)	−2165	−3306	−4672
H(C1)	−1361	1440	−7268		H″(C21)	−1835	−4317	−5487
H′(C1)	−1106	2564	−6639		H(C22)	−1515	−4275	−449
H(C2)	−2031	1690	−5115		H′(C22)	−1696	−5006	−2328
H′(C2)	−1991	2661	−6691		H(C23)	−2315	−3488	−477
H(C3)	−1565	3630	−4384		H′(C23)	−2495	−4231	−2340
H(O3)	−2300	3608	−2188		H(O24)	−2708	−5143	2675
Camphor (occupancy 0·5 for each atom)								
C(1)	67 (3)	5166 (4)	756 (12)	213 (6)	H(C5)	701	5271	4422
C(2)	83 (3)	4114 (4)	117 (12)	201 (6)	H′(C5)	936	4258	3235
C(3)	114 (3)	3509 (4)	1854 (12)	117 (5)	H(C6)	856	5056	426
C(4)	145 (3)	4278 (4)	3436 (12)	191 (6)	H′(C6)	665	6100	1631
C(5)	643 (3)	4780 (4)	3282 (12)	229 (6)	H(C8)	−738	4021	1555
C(6)	599 (3)	5337 (4)	1423 (12)	172 (6)	H′(C8)	−970	5209	1744
C(7)	−228 (3)	5050 (4)	2621 (12)	164 (6)	H″(C8)	−894	4493	3743
C(8)	−745 (3)	4666 (4)	2400 (12)	340 (7)	H(C9)	162	6204	3915
C(9)	−212 (3)	5958 (4)	3797 (12)	574 (7)	H′(C9)	−354	5801	5157
C(10)	−133 (3)	5846 (4)	−745 (12)	576 (7)	H″(C9)	−430	6516	3158
O	93 (3)	3817 (4)	−1412 (12)	304 (6)	H(C10)	−136	6579	−227
H(C3)	−208	3062	2008		H′(C10)	−499	5631	−1109
H′(C3)	436	3056	1837		H″(C10)	99	5807	−1952
H(C4)	86	4005	4817					

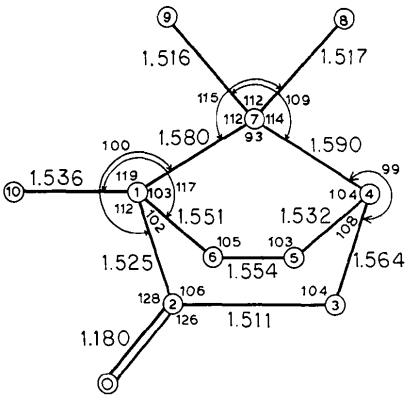

Fig. 4. Rigid-body geometry used in refinement for (+)-camphor, derived from Phillips & Trotter (1977). (Distances in Å, angles in deg.)

The two halves of the bilayer are related by a 2_1 screw axis parallel to *b*. The bilayers are stacked along the *a* direction by the twofold rotation axis. Between the hydrophobic surfaces of adjacent DCA bilayers there are channels of roughly circular cross-section, centered on twofold rotation axes, into which the columns of camphor molecules shown in Fig. 6 just fit. The crystal transformation on heating and the larger thermal parameters for camphor show that in this choleic acid the guest molecule is not bound as tightly in the crystal as is DCA. However, the fit of camphor into the channel is sufficiently good as to allow only two possible orientations, and examination of the stereo-drawing in Fig. 7 shows that each camphor molecule is held by van der Waals contacts with eight DCA molecules.

The remarkable capacity of deoxycholic acid for accommodating such a wide variety of guest molecules in the channels of these orthorhombic crystals does not result from any conformational changes in individual DCA molecules, but is possible because the same basic DCA bilayers can be shifted in major and in subtle ways relative to each other along *a* and/or *b* and/or *c* such that the combined host–guest crystal complex achives a stable configuration. Since the guest molecules are packed into hydrophobic channels, the accommodations seem to be those which maximize van der Waals attraction, that is, those which result in the closest packing in the crystal as a whole. The way this is done can best be appreciated by detailed pictorial comparison (Figs. 5 and 7) of the deoxycholic acid:camphor structure with some other representative complexes, of which we have chosen those listed in Table 2.

The dimensions *b* and *c*/*n* are very nearly constant; these represent the repeat distances in the two directions of DCA bilayer extension. Variations in *a* are much more pronounced, but these in fact are consequences of different relative positions of successive DCA bilayers either along *b* or along *c* or both. The packing diagrams in Fig. 5 show most clearly the shifts of the DCA bilayers along *b*, and the variations in channel size, shape, and orientations which most tightly fit and bind the particular included guest molecule. In the DCA:acetic acid choleic acid, hydrogen-bonded acetic acid molecules form narrow flat ribbons extended along *c*; the approximately rectangular channels here are very like those found in the complex containing palmitic acid. Phenanthrene is flatter and somewhat wider than acetic acid, and the approximately rectangular channels in which it lies are oriented perpendicular to those in the acetic acid complex. Cyclohexanone is more bulky in the directions normal to *c*, and the DCA bilayer shift along *b* is intermediate, between the extremes of the acetic acid and phenanthrene types of choleic acids. Camphor is bulkier still, being nearly spherical, ~7·2 Å in all dimensions, and it lies in an essentially cylindrical channel. (The channel is quite similar in the DCA:norbornadiene complex, which was not yet published when this study was begun.)

The shifts along *c* and more detailed views of the way different guest molecules fit in the channel can be seen in the stereoscopic drawings in Fig. 7. The height of one DCA molecule along *c* in all these structures is ~7·1–7·2 Å. In DCA:acetic acid, the *c* repeat distance holds two acetic acid molecules, related by a 2_1 screw axis, and opposite walls of the DCA channel are staggered, since they are also related by the same 2_1 axis. In DCA:camphor, the 7·2 Å space in the guest channel is filled by one essentially spherical camphor molecule, sitting in either of two possible orientations on a twofold rotation axis; the DCA channel walls are not staggered, but directly opposite, related by the twofold rotation axis, as the space group has changed from $P2_12_12_1$ in DCA:acetic acid to $P2_12_12$ in

Table 2. *Representative orthorhombic complexes of deoxycholic acid*

Guest	DCA:guest ratio	Space group	*a* (Å)	*b* (Å)	*c* (Å)	*n*
Acetic acid	1:1	$P2_12_12_1$	25·55	13·81	7·109	1
Camphor (disordered)	2:1	$P2_12_12$	27·353	13·814	7·233	1
Cyclohexanone	2:1	$P2_12_12_1$ (pseudo-$P2_12_12$)	26·990	13·354	14·141 (=2 × 7·0705)	2
Phenanthrene	3:1	$P2_12_12_1$	26·81	13·60	21·66 (=3 × 7·22)	3

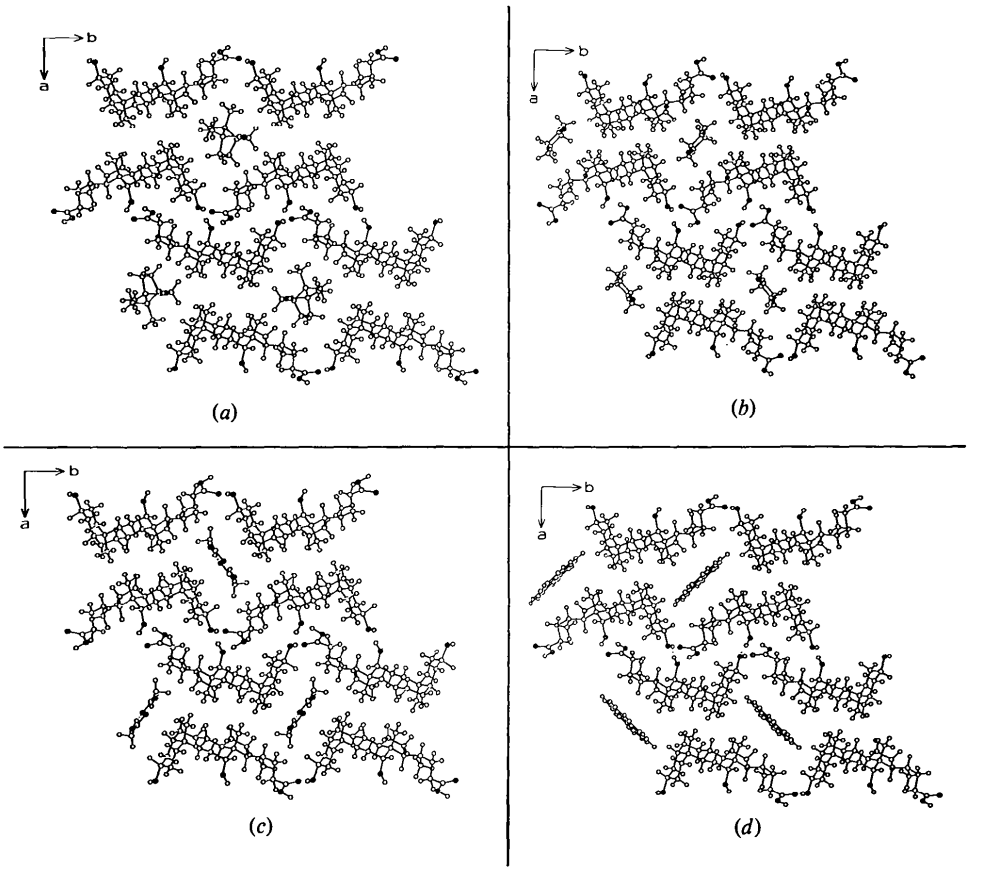

Fig. 5. Packing diagrams showing DCA bilayer arrangement and variations in guest channel position, shape, and orientation in: (*a*) 2:1 DCA:camphor, (*b*) 2:1 DCA:cyclohexanone, (*c*) 1:1 DCA:acetic acid, (*d*) 3:1 DCA:phenanthrene. The H atoms shown here were included, if necessary, in calculated positions, in order to illustrate better the hydrophobic intermolecular interactions.

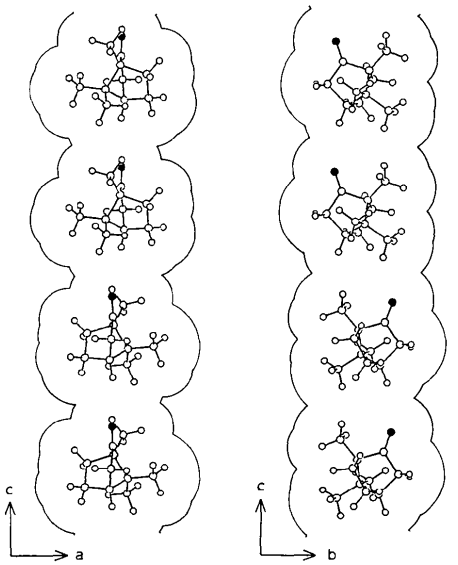

Fig. 6. Stacking of (+)-camphor along *c* in DCA:camphor, viewed along *b* and along *a*. Each of the two possible orientations is shown. The outlines, which are the projection of the van der Waals surface of the molecule, show that the stack of molecules is nearly a circular cylinder.

DCA:camphor. The DCA bilayers have shifted along *c* with respect to each other by $\pm c/4$, or $\sim 1{\cdot}8$ Å.

In DCA:phenanthrene the *c* axis is tripled, and two phenanthrene molecules related by a 2_1 screw axis occupy the 21·66 Å length. The DCA molecules in each wall of the channel are related essentially by a 7·22 Å translation (perhaps exact, perhaps approximate; the structure is not very accurately determined). Thus, opposing walls are staggered by essentially 3·6 Å, as in DCA:acetic acid [21·66/2 = 10·83 = $\frac{3}{2}$(7·22), and 7·22 Å along *c* is essentially a translation relating DCA molecules].

Cyclohexanone is best accommodated in a DCA channel formed by walls with two independent, slightly differently oriented DCA molecules related by an approximate 7·07 Å translation along *c*. Opposing walls of the DCA channel are related by an exact crystallographic 2_1 screw axis, but because of the approximate translation and the doubling of the *c* axis they are also related by an approximate twofold rotation axis rather than being staggered. The space group is strictly $P2_12_12_1$ with a 14·14 Å *c* axis, and approximately $P2_12_12$ with a 7·07 Å *c* axis. (The same pseudo-$P2_12_12$ arrangement, with a slight alteration in

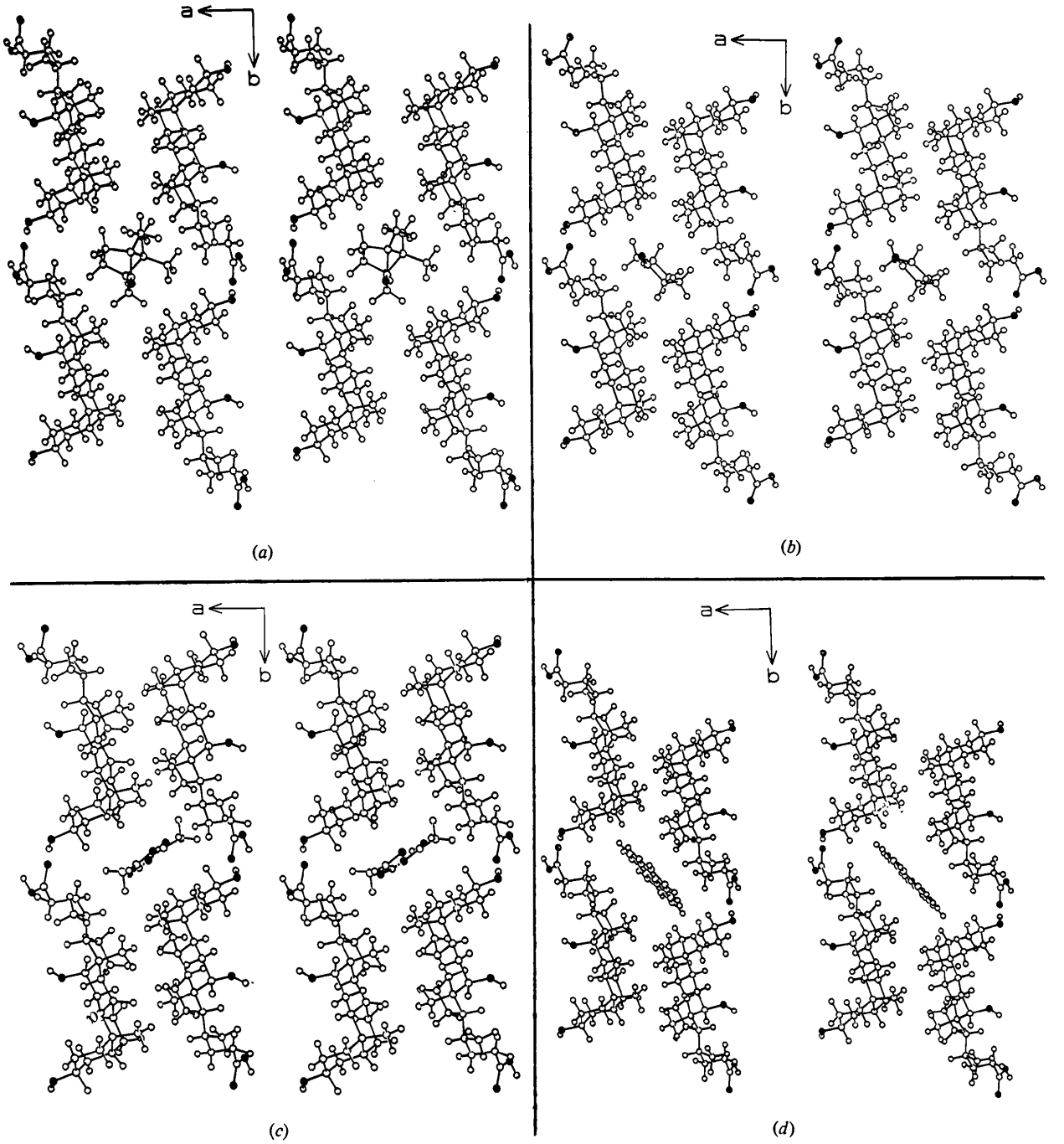

Fig. 7. Stereoscopic diagrams showing the packing of the guest molecule into a variety of channels between DCA bilayers in: (*a*) 2:1 DCA:camphor, $P2_12_12$, (*b*) 2:1 DCA:cyclohexanone, strictly $P2_12_12_1$ but nearly $P2_12_12$, as shown, (*c*) 1:1 DCA:acetic acid, $P2_12_12_1$, (*d*) 3:1 DCA:phenanthrene, $P2_12_12_1$.

channel shape, is found with norbornadiene as the guest molecule, even though norbornadiene is nearly spherical, like camphor. Norbornadiene is, however, slightly less bulky than camphor.) The DCA:camphor complex is the first completely determined orthorhombic structure of this type known rigorously to be in the space group $P2_12_12$. There is no evidence whatever on X-ray diffraction photographs of this crystal of any weak layer lines indicating doubling or tripling of the *c* axis.

These examples illustrate the subtle and flexible adjustments that the characteristic bilayer structure of

DCA makes in order to accommodate the variety of hydrophobic molecules with which the bilayers co-crystallize. Van der Waals energy calculations on the orthorhombic bilayer arrangement of DCA, assuming space group $P2_12_12_1$ and neglecting any guest molecules (Tang, 1978; Candeloro de Sanctis & Giglio, 1979), have found two local minima as a function of shifts along b; the relative positions and energies of these minima depend also on the cell parameter a, which is twice the distance between centers of successive bilayers. These calculations were not extended to the space group $P2_12_12$ which accommodates camphor, nor to the pseudo-$P2_12_12$ space group found to accommodate cyclohexanone. As Figs. 5 and 7 indicate, the bilayer shifts actually observed are both more varied and subtle than simple calculations imply, and must depend on van der Waals interactions between DCA and guest as well as on those between DCA bilayers. This is borne out by calculations including all interactions which were made on the DCA:palmitic acid:ethanol complex (Coiro, D'Andrea & Giglio, 1980).

There are two other known modes of host–guest crystallization with DCA: (1) a complex in space group $P6_5$, 3:2:1 DCA:ethanol:water (Candeloro de Sanctis, Coiro, Giglio, Pagliuca, Pavel & Quagliata, 1978) and 2:1:1 DCA:dimethyl sulfoxide:water (Candeloro de Sanctis, Giglio, Petri & Quagliata, 1979) in which helical tubes of DCA centered on the 6_5 axes enclose the hydrophilic molecules and the hydrophobic exteriors of the tubes are stacked parallel to each other; and (2) a tetragonal form, previously thought to include ethanol, but now known to be a hydrate, 2:3 DCA:water, space group $P4_12_12$, which has a somewhat higher packing density than the orthorhombic bilayer structures (Tang, 1978). Both DCA and sodium deoxycholate self-aggregate to form helical arrays in solution under appropriate conditions (Rich & Blow, 1958; Blow & Rich, 1960).

Future investigations to test the extent of the ability of DCA and other bile acids to interact strongly and specifically with molecules of a wider variety of sizes, shapes, and solubility properties than have hitherto been tried are certainly warranted.

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