Crystal Structure of Cholesteryl Nonanoate

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Cholesteryl nonanoate is monoclinic, space group $P2_1$, with lattice parameters a = 27.24(1), b = 9.183(3), c = 13.96(2) Å, β = 91.52(2)°, and Z = 4 [two independent molecules (A) and (B) in the unit cell]. The crystal structure was determined by Patterson rotation and translation methods, from the X-ray intensities of 2 496 reflections measured by diffractometer and refined by block-diagonal least-squares to R 0.10. Molecules (A) and (B) have almost fully extended conformations, but differ at the ends of the C(17) chains, in the rotations at the ester bonds, and in the nonanoate chains. The molecules are in antiparallel array forming monolayers with thickness d_{100} 27.2 Å, and having molecular long axes tilted at *ca.* 61° with respect to the layer interface. In the interface region, atoms are almost in the liquid state. The crystal structure is unusual in that the nonanoate chains pack with cholesteryl tetracyclic systems and not with each other. Arrangements of this kind are presumed to exist when cholesterol is incorporated within biological membranes.

THE fatty-acid esters of cholesterol are of interest because of their liquid crystalline phases and because they are an important storage form for lipids in biological systems. The crystal structures of some of these esters are being determined [1] because the molecular

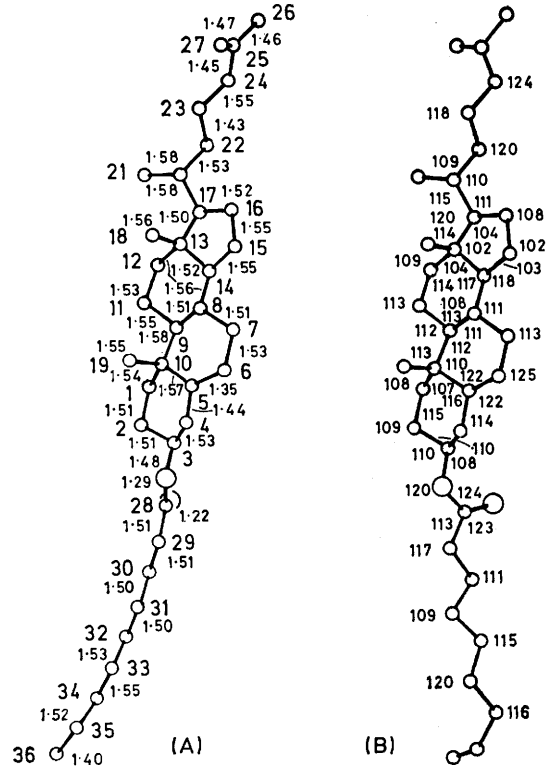

FIGURE 1 Atomic numbering system, mean bond lengths (Å), and angles (°) for cholesteryl nonanoate. Molecules (A) and (B) are shown in their observed conformations with their tetracyclic systems in the same orientation

arrangements might be relevant to the structures of lipid aggregates with less order. These include the liquid crystalline phases of cholesteryl esters,[2] arterial fatty deposits,[3] the lipid region of the low density lipoprotein transport particles,[4] and biological membranes with incorporated cholesterol.[5]

In the ascending homologous series of n-alkanoate esters, cholesteryl nonanoate (Figure 1) is the first which forms a liquid crystalline smectic or layer-type phase.[6] A reversible crystal to smectic phase transformation has been reported.[7] However, there seems to be present agreement [6,8] that on heating cholesteryl nonanoate, the transformations are crystal to cholesteric (77.8 °C), to isotropic liquid (92.0 °C), and that the metastable smectic phase appears only on cooling the cholesteric phase (72.5 °C). Cholesteryl nonanoate has only one crystalline phase. The structure, which is presently reported, is quite different from that of cholesteryl myristate.[1]

EXPERIMENTAL

Cholesteryl nonanoate (Sigma Chemical Co.) was recrystallized as monoclinic platelets by slow evaporation (3 months) of an isopropanol solution at 4 °C. From the chirality of the molecule, and from the diffraction symmetry ($2/m$) and systematic absences ($0k0$ with k odd) observed in X-ray precession photographs, the space group must be $P2_1$. All other X-ray data were obtained at room temperature by use of a computer-controlled four-circle diffractometer and graphite-monochromated Cu-K_α radiation. A crystal measuring 0.20 × 0.50 × 0.05 mm along the a, b, and c directions was mounted with a^* along the diffractometer Φ-axis. Crystal lattice parameters were obtained by a least-squares fit to the diffractometer angles for eight reflections measured at $+\theta$ and $-\theta$. There is disagreement with previously reported values.[9]

Crystal Data.—$C_{36}H_{62}O_2$, M = 526.9, a = 27.24(1), b = 9.183(3), c = 13.96(2) Å, β = 91.95(2)°, U = 3 490(5) Å^3, D_c = 1.002, Z = 4(2 molecules in the asymmetric unit), D_m = 1.005(5) g cm^{-3} (flotation in aqueous sucrose solution). Cu-K_α radiation, λ = 1.541 8 Å; μ(Cu-K_α) = 4.1 cm^{-1}. Space group $P2_1$.

Integrated X-ray intensities were measured by θ—2θ scans for all nonsymmetry-related reflections with $\sin\theta/\lambda < 0.59$ Å^{-1}. Of 6 334 reflections, 2 496 had $I > 2\sigma(I)$, where the variance in the integrated intensity is given by $\sigma^2(I) = \sigma^2 + (0.02I)^2$ and σ^2 is the variance due to counting statistics. Only the latter reflections were used in the structure determination and refinement. Intensities were not corrected for X-ray extinction or absorption.

The crystal structure was determined by Patterson rotation and translation methods using the procedure in ref. 1. In the rotation map obtained from the comparison

of the observed Patterson function with that of an artificial 20-atom steroid fragment, there were three large peaks, with relative magnitudes 55, 51, and 43. The two largest peaks required the plane of the steroid tetracyclic system to lie almost parallel to the crystal (010) plane, with orientations differing by 180° rotation about the molecular long axis. These two orientations have many intramolecular vectors in common. The orientation from the largest rotation peak was used to calculate a translation map for determining the vector between two-fold screw-related fragments. The largest peak at $y = 1/2$ gave the correct translation. The ring systems of these molecules [hereafter called (B)] lie close to the two-fold screw axis which runs through the centre of the unit cell. The packing arrangement for molecule (B) in cholesteryl nonanoate (Figure 2) is

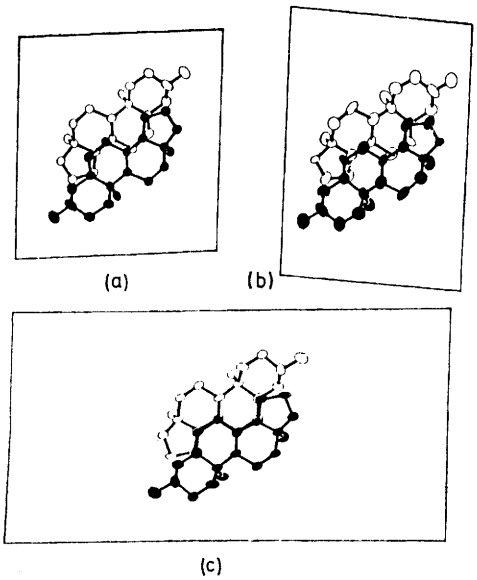

FIGURE 2 Projections showing a similar packing arrangement for tetracyclic systems in the crystal structures of three cholesteryl esters. The view is along the *b* direction, parallel to two-fold screw axes. A symmetry axis passes through the centre of each of the outlined unit cells. (a) Cholesteryl octanoate, $b = 9.20$ Å (ref. 10), (b) cholesteryl oleate, $b = 9.13$ Å (ref. 11), and (c) cholesteryl nonanoate (molecule B), $b = 9.18$ Å

very similar to that which was found in cholesteryl octanoate [10] and cholesteryl oleate [11], and was first observed in polymorph B of cholesteryl iodide.[12]

The second-largest rotation peak for cholesteryl nonanoate was expected to give the orientation of the tetracyclic system of molecule (A). Supposedly, this fragment would also be close to a screw axis, forming a stacking arrangement similar to molecule (B), but with the opposite sense along the *b* axis. However, this model was inconsistent with the translation maps. Eventually, it was concluded that the second-largest rotation peak was spurious, just as in the case of the octanoate and oleate structure determinations.[10,11]

The third rotation function peak was found to give the correct orientation for the (A)-fragment. Thus the molecular long axes for both (A) and (B) are almost parallel to the crystal [10$\bar{1}$] axis, but the plane of the ring system for molecule (A) is tilted almost perpendicular to that of (B). In the section $y = 1/2$ of the translation map for seeking symmetry-related (A)-fragments, the required peak was only 60% of the largest peak. However, it attracted attention because of its isolation from the higher spurious peaks which occurred in rows parallel to the [10$\bar{1}$] axis. Confidence in this assignment came from the map which was calculated in order to find the translation between the (A) and (B) fragments. The largest peak gave a trial structure for both tetracyclic systems in which there were no interfragment C · · · C distances <3.6 Å. Structure factors calculated for this model gave R 0.47. All remaining carbon and oxygen atoms were found during three cycles of Fourier refinement, after which R was 0.30.

Structure refinement was by block-diagonal least-squares, the function minimized being $\Sigma w\Delta^2$, where $w = 1/\sigma^2(F_o)$. In the final stages, it was assumed that $\sigma^2(F_o) = 4.0 + 0.01\ F_o^2$. Atomic form factors were taken from ref. 13 for carbon and oxygen, and from ref. 14 for hydrogen. Damping factors of 0.5 and 0.2 were applied to the changes in atomic positional and thermal parameters, so as to give a satisfactory convergence rate. When R was 0.24, anisotropic thermal parameters were introduced for carbon and oxygen atoms, and hydrogen atoms were included with parameters calculated from the carbon skeleton. Standard C–H bond lengths (1.00 Å) and bond angles were assumed and isotropic temperature factors for hydrogen atoms were assigned with values corresponding to those of the bonded carbon atoms. Hydrogen atom parameters were not refined. Convergence with this model was obtained at R 0.12. In a Fourier synthesis of electron density, peaks due to atoms at both ends of the molecule were found to be diffuse and not well resolved. With atomic positions from the least-squares refinement, four C–C bonds in these regions of the structure were very short (1.30—1.38 Å). Similar effects were observed in related structures which were determined at room temperature,[1,15] but not in cholesteryl acetate at −150 °C.[16] These are attributed to the ends of the molecule having a mobility approaching that of the liquid state. In cholesteryl nonanoate, atoms C(24)—(26) in both molecules and C(35) and C(36) in molecule (B) were given positions assigned by inspection of the electron-density map. For these atoms, only the anisotropic temperature factors were subsequently refined. The refinement finally converged with R 0.10. Atomic parameters with estimated standard deviations are in Table 1.*

RESULTS

Molecular Structure.—Bond lengths and angles (Figure 1) have not been accurately determined because of the large atomic thermal vibrations in the crystal structure. Excluding values for the three atoms at each end of the molecule, the root-mean-square differences between observed and mean values are 0.02 Å for the bond lengths and 1.4° for bond angles. These error estimates are close to the standard deviations from the least-squares refinement. None of the bond lengths and angles in cholesteryl nonanoate are considered to differ significantly from those obtained in the accurate crystal structure determination of cholesteryl acetate at −150 °C.[16]

There are considerable differences in the conformations of molecules (A) and (B) (Figure 1) but not in the tetracyclic system. When the atoms C(1)—C(19) are superposed and a best least-squares fit obtained [17] the root-mean-square

* Tables of anisotropic temperature factors and of observed and calculated structure amplitudes are listed in Supplementary Publication No. SUP 22513 (12 pp., 1 microfiche). See Notice to Authors No. 7 in *J.C.S. Perkin II*, 1978, Index issue.

displacement between corresponding atoms is 0.061 Å. Ring-puckering co-ordinates [18] and the superposability of the ring system have been calculated for twelve molecules including cholesteryl nonanoate.[16] The differences are small, from which it is concluded that the cholesterol framework is relatively rigid. Within experimental error, atoms in the ethylenic group C(4)—(7) and C(10) and in the ester linkage C(28), C(29), O, and O(3) are coplanar in each molecule.

The chains at C(17) have conformations which are almost fully extended (Table 2). There are differences involving the last four atoms [C(24)—(27)], but these are ill-defined because the electron density is so diffuse in this region. The last four atoms appear to be almost coplanar in molecule (A) (Figure 1) perhaps as a result of disordering of C(25) with respect to positions on each side of the plane through atoms C(24), C(26), and C(27).

The major difference in the overall shape of the molecules (Figure 1) can be attributed to the twist of the nonanoate chains about the C(3)–O(3) ester bond. The torsion angle C(2)–C(3)–O(3)–C(28) is 82° in molecule (A) and 136° in molecule (B). The nonanoate chains are well extended, but the atoms of each carbon spine are non-planar. There is a nearly regular curvature in the (A)-chain, giving rise to torsion angles along the chain which differ from 180° and alternative in sign. In the sequence of bonds from C(28)–C(29) through to C(34)–C(35), the torsion angles are 164, −176, 175, −170, 179, −172, and 168°. There is no such regularity in the (B)-chain where the corresponding angles are 177, −169, −171, 175, 174, −170, and 71°.

TABLE 1

Atomic positional parameters (fractional co-ordinates $\times 10^4$), with estimated standard deviations in parentheses; where none is quoted, atoms were positioned by inspection of electron density maps

	Molecule (A)		
Atom	*x*	*y*	*z*
C(1)	3 602(5)	4 522(17)	22(9)
C(2)	4 026(5)	4 125(18)	−595(9)
C(3)	4 209(5)	2 626(19)	−352(9)
C(4)	3 802(5)	1 546(17)	−437(2)
C(5)	3 376(5)	1 924(16)	98(8)
C(6)	3 185(6)	955(19)	725(12)
C(7)	2 728(6)	1 235(19)	1 293(11)
C(8)	2 453(4)	2 581(18)	969(8)
C(9)	2 799(5)	3 818(16)	788(8)
C(10)	3 161(4)	3 437(16)	−38(8)
C(11)	2 529(5)	5 267(20)	595(9)
C(12)	2 134(6)	5 591(22)	1 342(11)
C(13)	1 770(4)	4 393(20)	1 502(9)
C(14)	2 093(6)	3 067(21)	1 732(10)
C(15)	1 701(6)	1 967(24)	2 070(12)
C(16)	1 370(6)	2 981(29)	2 632(12)
C(17)	1 475(5)	4 526(24)	2 386(10)
C(18)	1 440(6)	4 240(25)	565(11)
C(19)	2 911(5)	3 547(20)	−1 061(8)
C(20)	991(6)	5 495(34)	2 371(12)
C(21)	1 088(8)	7 111(36)	2 066(16)
C(22)	750(6)	5 416(34)	3 344(13)
C(23)	244(10)	5 783(45)	3 399(19)
C(24)	24	5 586	4 374
C(25)	−482	5 444	4 589
C(26)	−648	5 479	5 599
C(27)	−709	4 631	3 775
C(28)	5 024(6)	2 619(20)	−880(9)
C(29)	5 393(5)	2 035(21)	−1 589(10)
C(30)	5 883(5)	2 776(18)	−1 636(10)
C(31)	6 193(6)	2 170(19)	−2 434(11)
C(32)	6 666(4)	2 932(18)	−2 544(10)
C(33)	6 945(6)	2 510(22)	−3 396(11)
C(34)	7 427(5)	3 290(24)	−3 586(13)
C(35)	7 660(7)	2 967(7)	−4 511(20)
C(36)	8 049(10)	3 755(38)	−4 893(25)
O	5 161(4)	3 539(19)	−267(8)
O(3)	4 586(3)	2 199(13)	−1 018(6)

TABLE 1 (*Continued*)

	Molecule (B)		
Atom	*x*	*y*	*z*
C(1)	5 310(6)	3 306(17)	2 302(9)
C(2)	5 765(5)	3 053(16)	1 735(9)
C(3)	6 205(5)	3 146(17)	2 432(11)
C(4)	6 152(5)	1 945(19)	3 223(9)
C(5)	5 709(5)	2 086(16)	3 745(10)
C(6)	5 696(5)	2 156(16)	4 692(8)
C(7)	5 234(4)	2 303(15)	5 274(8)
C(8)	4 772(4)	2 042(14)	4 671(8)
C(9)	4 788(5)	2 789(15)	3 703(8)
C(10)	5 215(4)	2 183(15)	3 073(7)
C(11)	4 288(5)	2 735(19)	3 135(9)
C(12)	3 865(5)	3 297(18)	3 693(8)
C(13)	3 816(5)	2 394(15)	4 692(8)
C(14)	4 306(5)	2 597(13)	5 193(7)
C(15)	4 226(5)	2 005(17)	6 212(8)
C(16)	3 688(5)	2 519(20)	6 390(8)
C(17)	3 482(5)	3 050(15)	5 407(8)
C(18)	3 702(6)	774(18)	4 452(18)
C(19)	5 103(6)	670(16)	2 659(9)
C(20)	2 921(5)	2 731(18)	5 307(9)
C(21)	2 658(6)	3 154(25)	4 313(10)
C(22)	2 646(6)	3 558(26)	6 075(10)
C(23)	2 133(7)	3 261(38)	6 205(15)
C(24)	1 868	4 043	7 044
C(25)	1 331	4 056	7 013
C(26)	1 127	4 862	6 213
C(27)	1 051	4 529	7 885
C(28)	7 059(6)	3 702(21)	2 086(14)
C(29)	7 452(7)	3 332(27)	1 406(17)
C(30)	7 912(7)	4 283(29)	1 486(17)
C(31)	8 225(10)	4 023(33)	677(26)
C(32)	8 693(12)	4 856(39)	965(18)
C(33)	9 133(8)	4 698(37)	288(18)
C(34)	9 619(15)	5 606(58)	459(30)
C(35)	9 977	5 509	−373
C(36)	9 896	6 165	−1 271
O	7 070(5)	4 624(22)	2 685(14)
O(3)	6 673(4)	2 868(13)	1 919(8)

TABLE 2

Torsion angles (°)

	(A)	(B)
C(13)–C(17)–C(20)–C(21)	−57	−57
C(13)–C(17)–C(20)–C(22)	178	−177
C(17)–C(20)–C(22)–C(23)	−159	−171
C(20)–C(22)–C(23)–C(24)	176	176
C(22)–C(23)–C(24)–C(25)	−160	163
C(23)–C(24)–C(25)–C(26)	−171	−65
C(23)–C(24)–C(25)–C(27)	35	168
C(2)–C(3)–O(3)–C(28)	82	136
C(3)–O(3)–C(28)–C(29)	177	−173
O(3)–C(28)–C(29)–C(30)	164	177

Molecular Packing.—In the crystal structure of cholesteryl nonanoate (Figure 3), the molecules are packed to form layers which are parallel to the crystal planes (100) and have a thickness of one unit cell (d_{100} 27.2 Å). There is penetration up to *ca.* 2 Å of molecules from opposite sides of the layer interface, but the interface is recognisable as the region where the atoms appear to be highly mobile and virtually liquid. In Figure 4, a slice through the crystal structure is projected along a direction close to the molecular long axes. The slice goes through the centre of one layer, through an interface region, and continues into the next layer. This shows the gradual transition between strongly contrasted regions of close packing within the layers, and loose packing between them.

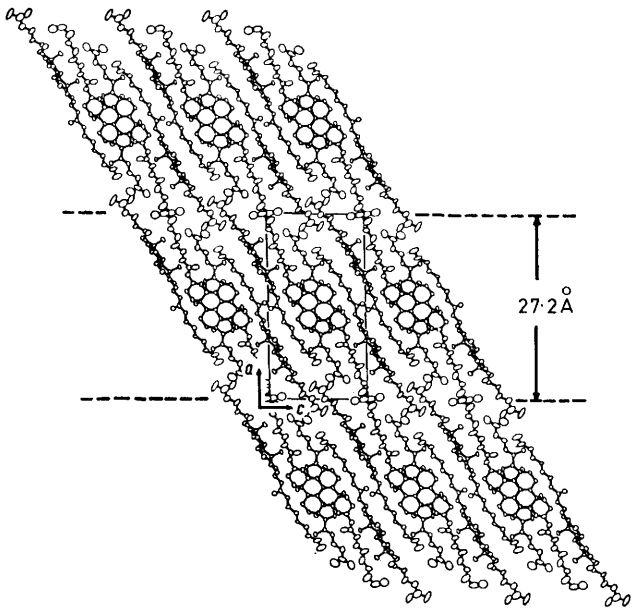

FIGURE 3 The crystal structure of cholesteryl nonanoate in projection down the *b* axis. Atoms are shown as 25% probability ellipsoids

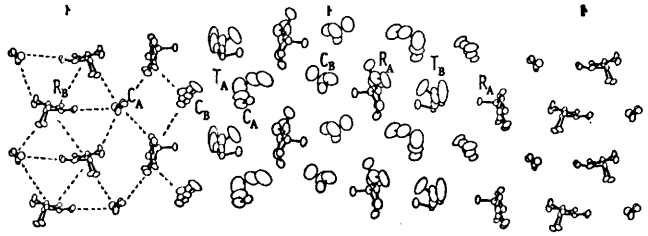

FIGURE 4 A slice through the crystal structure in projection down the crystal [10$\bar{1}$] direction which is close to the molecular long axes. The crystal *b* axis goes from the bottom to the top with the positions of screw axes marked at the top. Sections of each molecule are labelled: R for the tetracyclic ring system; T for the tail consisting of atoms C(20)—(27); C for the ester alkyl-chain. Atoms are shown as 25% probability ellipsoids. Dashed lines indicate multiple intermolecular C · · · C distances <4.5 Å. Fragments R_B at the far left correspond to the molecules at the centre of the unit cell which is outlined in Figure 3. Recurring fragments R_B at the far right then correspond to molecules in the cell translated $a + 3c$ from the cell outlined in Figure 3

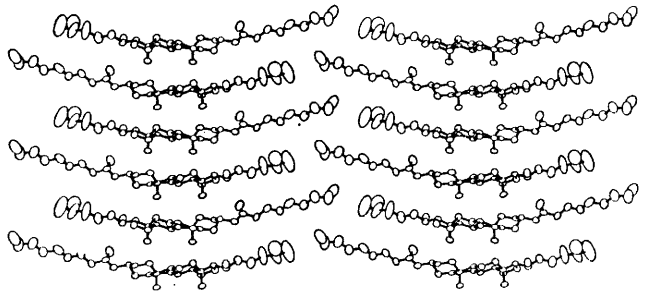

FIGURE 5 A partial crystal structure consisting of the (B)-molecules in two monolayers. The *b* direction is from the bottom to the top, with two-fold screw axes passing through the centre of each monolayer. Atoms are shown as 25% probability ellipsoids

Because the molecules are bowed, the directions of the long molecular axes are imprecise. However, for both molecules these directions are approximately parallel to the [10$\bar{1}$] crystal axis, which makes an angle 61.3° with respect to the (100) crystal face.

Projections of the partial crystal structure (*Figures* 2c and 5) show the close-packing of the tetracyclic systems for antiparallel (B)-molecules. The projecting C(18) and C(19) methyl groups have an interlocking effect which might help to make this packing arrangement important in other related structures (Figure 2).[10,11] Also of interest in Figure 5 is the end-to-end arrangement of (B)-molecules between adjacent layers of the structure. The gauche conformation at the end of the nonanoate chain (Figures 1 and 3) is necessary to avoid close contact with C(27) of a (B)-molecule in the next layer.

For the (A)-molecules (Figure 6) nearest neighbours are antiparallel with nonanoate chains opposite cholesteryl ring systems. There are sixteen intermolecular C · · · C

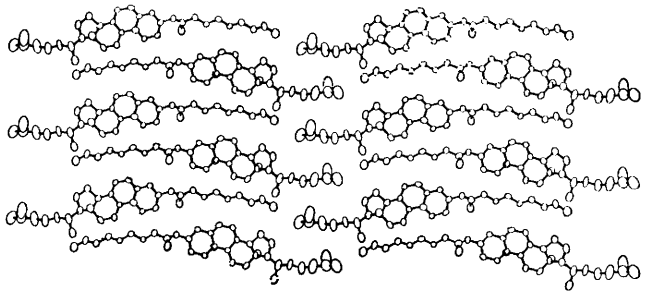

FIGURE 6 A partial crystal structure consisting of the (A)-molecules, but otherwise as in Figure 5

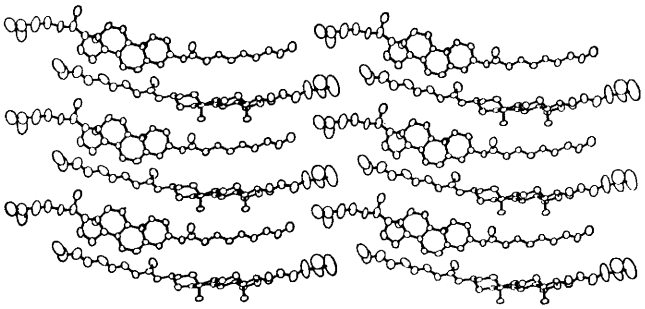

FIGURE 7 A partial crystal structure consisting of both (A)- and (B)-molecules, viewed in the same direction as Figures 5 and 6

distances <4.5 Å between nonanoate and cholesteryl ring atoms of (A) molecules. Between nearest-neighbour (A)- and (B)-molecules (Figure 7) there is also a close association between nonanoate chains and cholesteryl rings, with twenty intermolecular distances <4.5 Å between (A)-nonanoate and (B)-cholesteryl atoms, and fifteen distances between (B)-nonanoate and (A)-cholesteryl atoms.

It is emphasized that the nonanoate chains in this crystal structure do not pack with each other.

DISCUSSION

Cholesteryl Ester Crystal Structures.—The crystal structures of cholesteryl nonanoate and myristate are quite different. Cholesteryl myristate [1] forms bilayers

in which myristate chains pack regularly with each other at the centre of the bilayer and cholesteryls pack with each other in the outer regions. Henceforth, we refer to these cholesteryl ester structure types as the monolayer type (I) (nonanoate) and the bilayer (myristate). Crystal-structure determinations of cholesteryl decanoate [19] and laurate [20] show that these contain type (I) monolayers. The crystal structure of cholesteryl 17-bromoheptadecanoate [21] is of the bilayer type. However, from the available crystal-lattice parameters and space groups,[9,22] none of the n-alkanoate esters with chains longer than C_{12} belong to the monolayer structure type. Likewise none of the esters with chains shorter than C_{14} have the bilayer structure.

The type I monolayer structure has not been observed for esters with chain lengths less than C_9. In the crystal structure of cholesteryl octanoate,[10] there are monolayers consisting of antiparallel molecules, but the molecular long axes have a more severe tilt (28°) with respect to the layer interface than in type I monolayers (*e.g.* 61° for cholesteryl nonanoate). Unlike type I monolayers, there is deep penetration of chains from neighbouring layers. Also, the planes through the tetracyclic systems of all molecules are nearly parallel. Layers of this kind will henceforth be called type II monolayers. Cholesteryl oleate [11] has a type II monolayer structure. From the lattice parameters and space groups,[9] it appears that type II monolayers are also present in the crystal structures of C_6 and C_7 esters. The change in structure type from monolayers of type II to type I, which occurs between the C_8 and C_9 esters, coincides with a discontinuity in thermodynamic properties at this point in the homologous series.[6]

In summary, the crystal structures of the cholesteryl n-alkanoate esters with short-chain acids (C_6 to C_8) have monolayer type II structures, those with medium length chains (C_9 to C_{12}) have monolayer type I structures, and those with chains C_{14} or longer have the bilayer-type structure. This preliminary classification does not take into account the esters with chains shorter than C_6, the crystal polymorphism which has been reported for C_6, C_{11}, C_{12}, and C_{16},[23] or the undetermined orthorhombic crystal structure of the C_{16} ester.[9]

In the crystal structures of most long-chain lipids, the chains pack regularly according to various subcell arrangements.[24] In the crystal structures of cholesteryl esters, those containing bilayers can be incorporated in such a scheme, but not those containing monolayers. The latter are of particular interest because of irregularities in chain packing and conformation. Such crystal structures would appear to be more closely related to the arrangement of lipids in less-ordered aggregations. In the case of cholesteryl nonanoate, the structure (Figures 3 and 4) provides a model for phospholipid chains surrounding cholesterol molecules which have been incorporated in a biomembrane.

Structures of the Liquid Crystalline Phases.—Coates and Gray [25] have shown that the smectic and cholesteric liquid crystalline phases of cholesteryl nonanoate are separately miscible with the corresponding phases of both the myristate and palmitate esters. From binary miscibility studies with other smectogens, they also concluded that this is the smectic A phase in the Sackmann and Demus classification.[26] However, a study of light scattering by cholesteryl nonanoate [27] indicates that the smectic phase may be of type C. The smectic phases are believed to consist of a layered arrangement of molecules with disordering within the layers. The preferred direction of the molecular long axes is orthogonal to the layers in the smectic A phase, and tilted in the smectic C.

The *X*-ray diffraction patterns of the smectic and cholesteric phase of each cholesteryl ester are similar, consisting of a sharp intense inner ring and a diffuse weak outer ring.[28,29] The scattering angle for the inner ring decreases with increasing ester chain-length. Two different structures have been suggested, both of which are consistent with the *X*-ray data.

Wendorff and Price [29] interpret the inner ring as scattering from a regular stacking of smectic layers containing molecules in antiparallel array. The layer *d* spacings (27 for nonanoate, and 33 Å for myristate) are *ca.* 4 Å shorter than the repeat distance for an end-to-end arrangement of extended molecules. The shorter *d* spacings could be due either to molecular tilting, as in a smectic C structure, or to interpenetration between layers of a smectic A structure. Wendorff and Price suggest that the structure type is smectic A, to be consistent with the uniaxial behaviour of this phase in polarized light.

The inner diffraction ring is also consistent with an antiparallel arrangement of pairs of extended molecules having their ester chains side by side. Craven and DeTitta [1] showed this by calculation of the scattered *X*-ray intensity from pairs of molecules, one taken from each side of a myristate crystal bilayer. It is suggested that the *X*-ray scattering comes from a short-range ordering of molecules, rather than a long-range ordering to form layers. A structural model of this kind is suited to the cholesteric liquid crystalline phase, in which it is supposed that molecular layers are disrupted, but a localized alignment of molecular long axes is retained. Crystals of cholesteryl nonanoate transform directly to the cholesteric phase on heating.[6,8] Although the inner diffraction ring for the cholesteric phase may arise from paired molecules with antiparallel nonanoate chains side by side, this structural sub-unit is not preformed as part of the crystal structure.

For cholesteryl nonanoate, a smectic phase consisting of layers of tilted molecules (*d* 27 Å) might have a structure closely related to the crystal monolayers (d_{100} 27.2 Å, Figure 3). According to the miscibility studies,[25] the myristate smectic phase would have a similar layer structure (*d* 33 Å). However, this would require considerable reorganization in the transformation to the myristate crystal bilayers (*d* 50.7 Å). These structural

relationships appear to be inconsistent with the nature of the transformation from the smectic to the crystalline phase, which is reversible for the myristate but irreversible for the nonanoate.[6,8,23]

Proposals concerning structures for the liquid crystalline phases of the cholesteryl esters have merits in explaining various aspects of the physical data, including the *X*-ray diffraction patterns, but as yet, none is convincing.

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