Conformational changes of cholesteryl palmitoleate in the crystal structure at low temperature[1]

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Abstract At 123 K, crystals of cholesteryl *cis*-9-hexadecenoate (cholesteryl palmitoleate, $C_{45}H_{74}O_2$) are monoclinic, space group $P2_1$ with cell dimensions $a = 12.917(7)$, $b = 8.910(5)$, $c = 34.04(1)$ Å, $\beta = 94.95(7)°$ [$\lambda(CuK\alpha) = 1.5424$ Å] having two independent molecules (A and B) per unit cell. The crystal structure has been determined from 6178 reflections with $\sin\theta/\lambda \leq 0.56$ A^{-1}, of which 3406 gave $|F| > 3\sigma$. Structure refinement by alternating cycles of Fourier syntheses and block diagonal least squares gave $R = 0.24$ for all reflections, $R = 0.13$ for reflections $|F| > 3\sigma$. At 123 K, the crystal structure consists of closely packed layers very similar to those at 295 K. However, there are major conformational differences in the layer interface region, which affect the ester chain of molecule B and the C(17) tail of molecule A. Although the electron density is diffuse in this region, the B-chain, which is bent, appears to be ordered at 123 K and has a different conformation from the disordered B-chains at 295 K. The change in the A-tail, which is twisted at 123 K and extended at 295 K, is very similar to that which occurs in two of the molecules when anhydrous cholesterol undergoes phase transition. Measurements of the unit cell dimensions at twelve temperatures (295 K to 123 K) indicate that the major changes in the crystal structure of cholesteryl palmitoleate occur in a 10 K range near 173 K.—**Craven, B. M., and P. Sawzik.** Conformational changes of cholesteryl palmitoleate in the crystal structure at low temperature. *J. Lipid Res.* 1984. **25:** 857–864.

Supplementary key words chain conformation • unsaturated fatty acids • cholesteryl ester

The crystal structures of the long chain fatty acid esters of cholesterol are remarkable in forming layer structures in which the central region of the layers, composed largely of semi-rigid cholesteryl groups, is closely packed and nearly transposable from one crystal structure to another. These crystal structures are of interest in the interface region between layers where there is packing together of flexible fatty acid chains and the isoprenoid substituents at the cholesterol C(17) position. Because the fatty acid chains are irregularly packed, these structures might be considered as representative of arrangements occurring in noncrystalline lipid assemblies, such as the interior of biomembranes.

Due to their flexibility, particularly when they are unsaturated, chemically different fatty acid chains may adopt similar conformations in response to a similar crystal environment. Thus the oleate chain has one *cis*-ethylenic group while linolelaidate has two which are both *trans.* However, the crystal structures of cholesteryl oleate (1) and linolelaidate (2) are very similar. The two different alkenoate chains are kinked in a similar way so as to pass between the ring system of one cholesteryl and the tail of another.

Conversely, chemically identical chains may adopt quite different conformations because of differences in their crystal environment (see molecules A and B in **Fig. 1**). Furthermore, the same cholesteryl ester molecule may undergo major conformational change with a change in temperature, because the chain packing arrangement is sensitive to compression and shearing of the layer interface region.

Temperature-dependent conformational changes of this kind occur in the crystal structure of cholesteryl palmitoleate. Presently, we report the crystal structure determination at low temperature (123 K) and compare the results with the room temperature structure (295 K), previously determined by Sawzik and Craven (3).

EXPERIMENTAL

Cholesteryl palmitoleate, obtained from Serdary Research Laboratories, Inc., London, Ontario, was recrystallized slowly from acetone solution at 277 K. The crystals for low temperature X-ray study were monoclinic plates on (001), elongated on *b*, melting at 325 K. They were selected from the same batch as the crystal used for the

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Abbreviations: e.s.d., estimated standard deviation; m.s., mean square; r.m.s., root mean square.

[1] See NAPS document no. 04204 for 44 pages of supplementary material, consisting of tables of structure factors, anisotropic thermal parameters, bond lengths and angles and H-atom positional parameters. Order from NAPS c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163. Remit in advance in U.S. funds only $14.95 for photocopies or $4.00 for microfiche. Outside the U.S. and Canada, add postage of $4.50 for the first 20 pages and $1.00 for each 10 pages of material thereafter. $1.50 for microfiche postage.

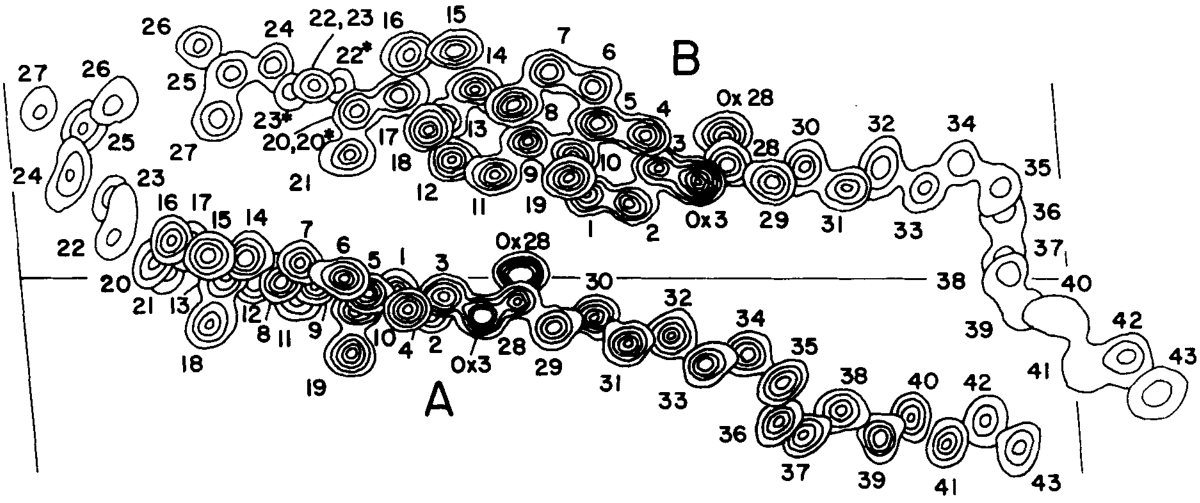

Fig. 1. Atomic nomenclature and electron density for the independent molecules A and B. The electron density was calculated in sections normal to *b* by Fourier synthesis including all reflections with $|F_o|$-coefficients. The contours are at levels 1, 2, 3 . . . eÅ^{-3}. The e.s.d. in the electron density is 0.01 eÅ^{-3}. A partial outline of the unit cell is shown.

room temperature study (3). The X-ray data were collected using an Enraf-Nonius CAD-4 diffractometer and CuKα graphite-monochromated radiation (λ = 1.5418 Å). Crystals were mounted with *b*-axes about 10° from the diffractometer Φ-axis. They were cooled in a stream of dry nitrogen gas provided by an Enraf-Nonius low temperature device. Although cooling was slow (~30K/hr; 5 K/hr near 173 K), many crystals fractured during cooling or during intensity data collection at 123 K. Crystal lattice parameters at twelve temperatures (293 K–123 K) were derived from least squares fitting of $\sin^2\theta$ values for 25 reflections ($18 \leq \theta \leq 27°$) measured at $\pm\theta$ at each temperature (see **Fig. 2**). Using $\theta/2\theta$ scans, intensities were measured for all reflections in an asymmetric quadrant of reciprocal space with $\sin\theta/\lambda \leq 0.56$ Å^{-1}. Beyond this limit (d_{min} = 0.89 Å) most reflections were unobservably weak. The intensity data accepted for processing consisted of 7181 reflections from three crystals, the largest being 0.5 × 0.3 × 0.1 mm. The variance in an integrated intensity was assumed to be $\sigma^2(I) = \sigma^2 + (0.02\ I)^2$, where σ^2 was the variance due to counting statistics. No corrections for X-ray absorption were applied. A least squares procedure (4) was used in scaling and averaging the structure amplitudes from the three crystals. This gave 6178 independent observations. The agreement for multiple observations from the same crystal was better than for reflections measured with more than one crystal. Thus, $R = \Sigma|\Delta F|/\Sigma\bar{F} = 0.01$ for the multiple observations, while the data from two crystals which yielded 5129 observations gave R = 0.04 for 330 reflections in common. Comparison of the third crystal with the other two gave R = 0.10 and 0.12. Possibly, the small differences in the intensity data from different crystals are real. They might arise because the details of the disorder in each crystal depend on the history of its growth and cooling. At this stage, continued efforts to improve the quality of the data were judged to be impractical.

The structure determination was carried out by means of alternating cycles of Fourier syntheses and block-diagonal least squares. Atomic scattering factors were taken from Cromer and Waber (5) and Stewart, Davidson, and Simpson (6). Fourier coefficients were $\omega\{2|F_o| - |F_c|\}$ with the weights $\omega = I_1(x)/I_0(x)$ where I_0 and I_1 are zero and first order modified Bessel functions of the first kind, as proposed by Sim (7), but with $x = 2|F_o||F_c|/\Delta^2$ where $\Delta = |F_o| - |F_c|$. For reflections with $|F_o| < \sigma(F)$, $\omega = 1$.

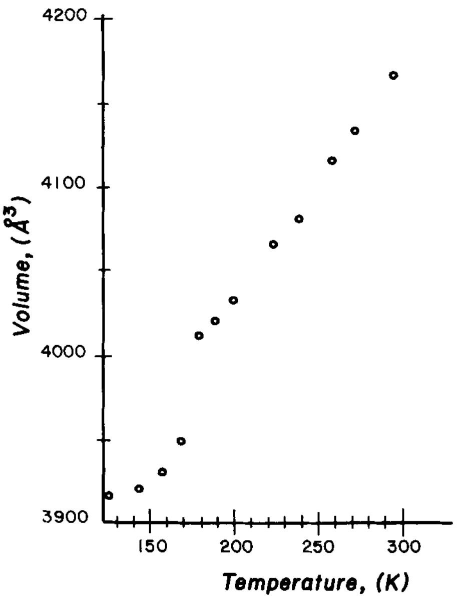

Fig. 2. Unit cell volume as a function of temperature.

In the least squares refinement, the residual $\Sigma w\Delta^2$ was minimized where $w = \sigma^{-2}(F)$. Damping factors 0.5 were applied to all parameter shifts.

The structure determination began using only the 3406 reflections with $|F_o| > 3\sigma$ and assuming the room temperature atomic positional parameters (3). After every few cycles, H-atom positions were calculated at standard bond lengths and angles. The H-atoms were assumed to have isotropic temperature factors $B = B_{eq} + 3.0$ Å^2 where B_{eq} was for the C-atom in the C–H bond. The H-atom parameters were not refined by least squares. The initial refinement of positional and anisotropic thermal parameters for all C- and O-atoms was concluded with $R = \Sigma|\Delta|/\Sigma F_o = 0.13$, $R_w = \{\Sigma w\Delta^2/\Sigma wF_o^2\}^{1/2} = 0.10$ and $s = \{\Sigma w\Delta^2/(n - m)\} = 3.90$. However, the results were considered to be unsatisfactory. Temperature factors for several atoms in the steroid framework were non-positive definite or nearly so. Although most bond lengths and angles were in agreement with accepted values, the range was large (1.3 to 1.8 Å for C–C). Most of these deficiencies involved the ester chain of molecule B or the isoprenoid tail of molecule A where the electron density distribution was diffuse.

It was considered that some improvement might come from using the complete set of 6178 observations (8) including those reflections with negative intensity.[2] For the many weak reflections, weights were assigned according to Rees (9). For reflections with $F_o^2 \leq 0$, the structure amplitudes were taken to be zero and for those with $F_o^2 \leq \sigma(F^2)$, the variances were taken to be $\sigma^2(F) = \sigma(F^2)/4$. In the continued refinement, it was soon found that the isoprenoid tail for molecule B was disordered with nearly resolved double peaks for both C(22) and C(23). The estimated weights for the two conformers were 0.6 and 0.4. These weights and also the positional parameters for the disordered atoms were assigned fixed values in the subsequent refinement. After refinement with the complete data set, there were only small differences in the electron density in the regions of the B ester chain and the A isoprenoid tail. As with partial data, the hydrocarbon chains could be traced readily, but the peaks were diffuse and poorly resolved (Fig. 1). In the central region of the B chain, atoms C(34) to C(40) were assigned fixed positions consistent with standard bond lengths and angles and only their isotropic thermal parameters were included as least squares variables. In the A-tail, atoms C(22) and C(23) were treated in the same way. The least squares refinement was slowly convergent, giving final values $R = 0.24$, $R_w = 0.14$, $s = 2.67$ for all reflections, and $R = 0.13$, $R_w = 0.12$ for the reflections with $F_o > 3\sigma(F)$. The largest parameter changes in the final cycle were 0.5 σ. Atomic parameters are in **Table 1.** Although the refinement using the complete data gave no decrease in R-values for the stronger reflections, there was a considerable improvement in bond lengths and bond angles. All temperature factors became positive definite.

RESULTS AND DISCUSSION

The molecular structure

Because of limitations in the X-ray data which are due to the large m.s. amplitudes of thermal motion and disorder, bond lengths and angles have not been accurately determined.[3] In the cholesteryl ring system where the precision is greatest, e.s.d.'s in bond lengths and angles are in the range 0.013 to 0.020 Å and 0.8 to 1.2°, respectively. In the rings, the C–C bond lengths between tetrahedral C-atoms range from 1.48(2) to 1.62(2) Å. The C(5)=C(6) distances are 1.31(2) and 1.32(1) Å. Corresponding bond lengths in the two cholesteryl moieties agree satisfactorily, the most significant difference being for C(4)–C(5) [1.49(1) Å in A; 1.57(2) Å in B]. From the torsion angles (**Table 2**) it is seen that the atoms of the ethylenic groups at C(5)=C(6) are coplanar within experimental error. The torsion angles also indicate that there are small conformational differences in the ring systems of the two molecules, particularly at the B/C ring junction. Thus torsion angles C(7)–C(8)–C(9)–C(10) are 62(1)° in molecule A and 50(1)° in B. These differences are attributed to differences in the crystal packing arrangement of molecules A and B (**Fig. 3**).

In molecule A and in the major conformer of molecule B, the isoprenoid tails at C(17) are twisted with a *gauche* conformation ($\tau = 81(2), 60°$; see Table 2) at the bond C(20)–C(22). In molecule B, the major conformer has a second twist ($\tau = -60°$) at the bond C(23)–C(24) so that the terminal isobutyl groups of both B-conformers are closely overlapping (**Fig. 4**). In the crystal structures of cholesteryl esters, the isoprenoid tails are usually almost fully extended, as presently observed for the minor conformer for molecule B. However, a variety of other tail conformations may occur, as in the two crystal forms of cholesterol (11, 12) and in cholesterol monohydrate (13).

Within the ester chains, the C-atoms of the *cis*-ethylenic group are nearly coplanar, having torsion angles 2(2) and 7° at C(36)=C(37) in molecules A and B. Carbon atoms are also nearly coplanar in the chain sections consisting of atoms C(29) through C(34) where r.m.s. atomic displacements from the best least squares plane are 0.02 Å

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[2] None of the integrated intensities were significantly negative in terms of their e.s.d.'s.

[3] The most accurate bond lengths and angles available for a cholesteryl ester are those of the acetate, determined at 123 K (10).

TABLE 1. Fractional coordinates ($\times 10^4$) and m.s. amplitudes ($Å^2 \times 10^3$) of thermal motion for carbon and oxygen atoms[a]

Atom	Molecule A				Molecule B			
	x	y	z	$\langle u^2\rangle^{b}$	x	y	z	$\langle u^2\rangle^{b}$
C(1)	−5365 (9)	−2149 (13)	−1472 (4)	80 (9)	−2765 (8)	−750 (12)	454 (3)	62 (7)
C(2)	−5813 (8)	−1677 (12)	−1101 (4)	68 (7)	−3096 (8)	−564 (13)	866 (4)	85 (8)
C(3)	−5469 (7)	−40 (12)	−991 (4)	71 (8)	−2153 (8)	−748 (11)	1163 (4)	67 (7)
C(4)	−5782 (8)	1026 (11)	−1335 (3)	55 (7)	−1341 (7)	447 (12)	1092 (4)	71 (8)
C(5)	−5438 (7)	476 (11)	−1716 (3)	57 (7)	−1050 (7)	334 (11)	654 (3)	53 (6)
C(6)	−4956 (8)	1404 (13)	−1936 (3)	65 (8)	−45 (7)	346 (11)	606 (4)	65 (7)
C(7)	−4609 (9)	971 (15)	−2339 (4)	81 (9)	340 (7)	269 (11)	212 (4)	71 (7)
C(8)	−5106 (8)	−456 (14)	−2517 (4)	75 (9)	−490 (7)	527 (11)	−150 (4)	67 (7)
C(9)	−5075 (9)	−1596 (11)	−2184 (3)	70 (8)	−1514 (7)	−160 (11)	−64 (3)	57 (6)
C(10)	−5699 (8)	−1129 (12)	−1836 (4)	76 (8)	−1908 (7)	300 (11)	344 (4)	66 (7)
C(11)	−5447 (12)	−3132 (16)	−2358 (5)	124 (12)	−2328 (7)	−165 (11)	−409 (4)	67 (7)
C(12)	−4912 (13)	−3707 (15)	−2721 (5)	139 (13)	−1935 (7)	−653 (13)	−800 (4)	68 (7)
C(13)	−4922 (11)	−2501 (14)	−3039 (5)	117 (12)	−947 (7)	123 (11)	−892 (4)	62 (7)
C(14)	−4477 (9)	−1005 (14)	−2851 (4)	85 (9)	−154 (7)	−51 (10)	−536 (4)	67 (7)
C(15)	−4382 (9)	−29 (15)	−3198 (4)	91 (10)	866 (7)	513 (13)	−687 (4)	84 (9)
C(16)	−4099 (11)	−1098 (16)	−3544 (4)	100 (10)	785 (8)	21 (15)	−1142 (4)	96 (10)
C(17)	−4202 (11)	−2728 (17)	−3386 (4)	113 (12)	−348 (7)	−501 (14)	−1251 (4)	85 (9)
C(18)	−6095 (12)	−2351 (15)	−3248 (4)	117 (12)	−1170 (8)	1805 (12)	−979 (4)	70 (8)
C(19)	−6901 (9)	−1259 (12)	−1942 (4)	72 (9)	−2394 (7)	1959 (12)	312 (4)	66 (7)
C(21)	−4541 (25)	−5452 (19)	−3530 (7)	319 (28)	−1845 (8)	−614 (15)	−1759 (4)	82 (10)
C(20)	−4466 (16)	−3912 (19)	−3690 (5)	175 (25)	−700	−30	−1692	59 (4)
C(22)	−4000	−3910	−4100	278 (14)	−70	−630	−2043	85 (8)
C(23)	−2930	−4550	−4060	238 (11)	−70	−2290	−2064	81 (7)
C(20)*[c]					−720	−570	−1649	58 (8)
C(22)*[c]					−10	−1610	−1803	76 (10)
C(23)*[c]					−160	−1570	−2287	68 (9)
C(24)	−2329 (19)	−4511 (19)	−4437 (4)	208 (30)	479 (9)	−2812 (14)	−2438 (3)	71 (8)
C(25)	−1281 (15)	−5470 (22)	−4331 (5)	160 (27)	274 (9)	−2527 (16)	−2858 (4)	96 (11)
C(26)	−562 (18)	−4965 (21)	−3989 (7)	229 (23)	924 (11)	−3366 (26)	−3127 (4)	144 (15)
C(27)	−665 (22)	−5400 (34)	−4702 (6)	266 (40)	−836 (12)	−2794 (18)	−3009 (5)	122 (13)
O(3)	−5972 (5)	468 (8)	−652 (2)	54 (4)	−2527 (6)	−556 (8)	1544 (2)	77 (5)
O(28)	−4903 (4)	−963 (8)	−254 (2)	63 (8)	−1338 (7)	−2185 (12)	1810 (3)	117 (8)
C(28)	−5621 (7)	−46 (12)	−300 (3)	60 (7)	−2088 (9)	−1387 (16)	1858 (4)	94 (10)
C(29)	−6230 (7)	498 (12)	36 (3)	51 (7)	−2548 (10)	−1332 (15)	2246 (4)	89 (10)
C(30)	−5969 (7)	−227 (12)	434 (3)	62 (7)	−2095 (11)	−2172 (14)	2579 (4)	91 (10)
C(31)	−6686 (8)	280 (14)	731 (3)	68 (8)	−2661 (10)	−2068 (17)	2955 (5)	115 (12)
C(32)	−6461 (8)	−427 (13)	1143 (4)	70 (8)	−2164 (16)	−2929 (15)	3309 (4)	144 (16)
C(33)	−7201 (9)	69 (12)	1452 (4)	82 (9)	−2707 (12)	−2897 (22)	3687 (4)	137 (14)
C(34)	−6960 (9)	−531 (13)	1846 (4)	79 (8)	−1980	−3550	4080	197 (9)
C(35)	−7649 (9)	−111 (14)	2192 (4)	100 (10)	−2620	−3400	4460	210 (10)
C(36)	−8666 (11)	−883 (16)	2124 (5)	123 (13)	−3230	−4750	4500	443 (25)
C(37)	−9006 (10)	−1977 (17)	2348 (4)	91 (10)	−4250	−4750	4480	357 (18)
C(38)	−8455 (10)	−2582 (16)	2708 (4)	98 (11)	−4880	−3340	4460	184 (9)
C(39)	−9067 (11)	−3010 (15)	3053 (3)	89 (9)	−5990	−3800	4550	317 (18)
C(40)	−8512 (12)	−3725 (18)	3399 (4)	106 (11)	−6060	−3790	4970	264 (14)
C(41)	−9220 (11)	−4280 (15)	3724 (4)	108 (11)	−7258 (22)	−3820 (38)	5107 (8)	274 (14)
C(42)	−8682 (16)	−5108 (19)	4102 (5)	161 (15)	−6979 (12)	−3749 (21)	5541 (5)	138 (14)
C(43)	−9386 (16)	−5420 (22)	4417 (4)	166 (18)	−7951 (17)	−3600 (21)	5782 (7)	220 (22)

[a] Estimated standard deviations are given in parentheses.

[b] $\langle u^2\rangle = (U_{11} + U_{22} + U_{33} + 2U_{13}\cos\beta)/3$ where U_{ij} are the atomic anisotropic thermal parameters.

[c] Atoms C(20), C(22), C(23) in molecule B are disordered. These atoms when marked with asterisks have site factors 0.40, and those without have site factors 0.6.

in A and 0.12 Å in B and in the section consisting of atoms C(40) through C(43) where displacements are 0.09 Å in A and 0.07 Å in B. From the angle between the best least squares lines through these sections, the bend in each ester chain is 18° for A and 42° for B. The twist between chain segments, given by the dihedral angle between their best least squares planes, is 29° for A and 32° for B. The kink between chain segments, given by the perpendicular separation of the skewed best least squares lines is 1.9 Å for A and 1.6 Å for B. Although there are striking differences in conformation near the C=C bond (Figs. 3, 4), the resultant effect on the overall shape of the chains is similar except that the A-chain is almost fully extended while the B-chain is bent. Three of the four C–C bonds in the α-position adjacent to the C=C bond have the preferred (+)- or (−)-skewed con-

TABLE 2. Torsion angles (°)[a]

Atoms	Mol. A	Mol. B	Atoms	Mol. A	Mol. B
C(10)−C(1)−C(2)−C(3)	−59 (1)	−56 (1)	C(17)−C(13)−C(14)−C(15)	46 (1)	44 (1)
C(1)−C(2)−C(3)−C(4)	56 (1)	61 (1)	C(4)−C(5)−C(10)−C(9)	−167 (1)	−168 (1)
C(2)−C(3)−C(4)−C(5)	−51 (1)	−56 (1)	C(1)−C(10)−C(5)=C(6)	133 (1)	139 (1)
C(3)−C(4)−C(5)−C(10)	51 (1)	50 (1)	C(7)−C(8)−C(9)−C(11)	−173 (1)	−172 (1)
C(4)−C(5)−C(10)−C(1)	−49 (1)	−44 (1)	C(10)−C(9)−C(8)−C(14)	−180 (1)	177 (1)
C(5)−C(10)−C(1)−C(2)	54 (1)	48 (1)	C(12)−C(13)−C(14)−C(15)	172 (1)	170 (1)
C(10)−C(5)=C(6)−C(7)	0 (2)	−2 (2)	C(8)−C(14)−C(13)−C(17)	176 (1)	177 (1)
C(5)=C(6)−C(7)−C(8)	16 (2)	13 (2)	C(4)−C(5)−C(10)−C(19)	70 (1)	73 (1)
C(6)−C(7)−C(8)−C(9)	−44 (1)	−36 (1)	C(8)−C(14)−C(13)−C(18)	63 (1)	63 (1)
C(7)−C(8)−C(9)−C(10)	62 (1)	50 (1)	C(13)−C(17)−C(20)−C(21)	−58 (2)	−61
C(8)−C(9)−C(10)−C(5)	−48 (1)	−41 (1)	C(13)−C(17)−C(20)*−C(21)		−24
C(9)−C(10)−C(5)=C(6)	15 (1)	15 (1)	C(13)−C(17)−C(20)−C(22)	162 (1)	178
C(8)−C(9)−C(11)−C(12)	51 (2)	45 (1)	C(13)−C(17)−C(20)*−C(22)*		−163
C(9)−C(11)−C(12)−C(13)	−51 (2)	−48 (1)	C(17)−C(20)−C(22)−C(23)	81 (2)	60
C(11)−C(12)−C(13)−C(14)	51 (2)	52 (1)	C(17)−C(20)*−C(22)*−C(23)*		−168
C(12)−C(13)−C(14)−C(8)	−59 (1)	−57 (1)	C(20)−C(22)−C(23)−C(24)	−175 (1)	175
C(13)−C(14)−C(8)−C(9)	61 (1)	54 (1)	C(20)*−C(22)*−C(23)*−C(24)		172
C(14)−C(8)−C(9)−C(11)	−55 (1)	−46 (1)	C(22)−C(23)−C(24)−C(25)	−172	−60
C(13)−C(14)−C(15)−C(16)	−33 (1)	−34 (1)	C(22)*−C(23)*−C(24)−C(25)		−178
C(14)−C(15)−C(16)−C(17)	8 (1)	11 (1)	C(23)−C(24)−C(25)−C(26)	−59 (2)	−174
C(15)−C(16)−C(17)−C(13)	20 (1)	15 (1)	C(23)*−C(24)−C(25)−C(26)		169
C(16)−C(17)−C(13)−C(14)	−40 (1)	−36 (1)	C(23)−C(24)−C(25)−C(27)	−178 (1)	−50
C(4)−C(5)=C(6)−C(7)	−178 (1)	−179 (2)			
C(23)*−C(24)−C(25)−C(27)		−66			
C(2)−C(3)−O(3)−C(28)	76 (1)	145 (1)			
C(4)−C(3)−O(3)−C(28)	−164 (1)	−96 (1)			
C(3)−O(3)−C(28)−C(29)	−177 (1)	−173 (1)			
C(3)−O(3)−C(28)=O(28)	−2 (1)	5 (2)			
O(3)−C(28)−C(29)−C(30)	171 (1)	−179 (1)			
C(28)−C(29)−C(30)−C(31)	−174 (1)	−178 (1)			
C(29)−C(30)−C(31)−C(32)	180 (1)	−179 (1)			
C(30)−C(31)−C(32)−C(33)	−179 (1)	−179 (1)			
C(31)−C(32)−C(33)−C(34)	−177 (1)	−167			
C(32)−C(33)−C(34)−C(35)	180 (1)	178			
C(33)−C(34)−C(35)−C(36)	72 (1)	89			
C(34)−C(35)−C(36)=C(37)	113 (2)	−117			
C(35)−C(36)=C(37)−C(38)	2 (2)	−7			
C(36)=C(37)−C(38)−C(39)	142 (1)	−165			
C(37)−C(38)−C(39)−C(40)	175 (1)	87			
C(38)−C(39)−C(40)−C(41)	−174 (1)	165			
C(39)−C(40)−C(41)−C(42)	178 (1)	−178			
C(40)−C(41)−C(42)−C(43)	173 (1)	175			

[a] Atoms C(20), C(22), C(23) in molecule B are disordered. These atoms when designated with asterisks have site factors 0.4 and those without have site factors 0.6.

formation (14, 15), the other being *trans.* As shown in Fig. 4, the chain dislocations extend to the β-bonds, with three of the four having a twisted conformation.

The molecular packing at 123 K and 295 K

The crystal structure consists of layers (Fig. 3 and **Fig. 5**). Within these layers, the closely-packed cholesteryl ring systems, the isoprenoid tail of molecule B, and much of the ester chain of molecule A[4] form an arrangement that is similar in cholesteryl palmitoleate at 123 K and 295 K, and in other cholesteryl ester crystal structures. These include the alkanoate esters with chains C_9 through C_{12} (16–21), and the unsaturated nervonate ester (22). The variable region in these structures is formed by the ester chain of molecule B and the tail of molecule A, which project into the interface between layers.

For one of these esters, cholesteryl dodecanoate (laurate), the crystal structure has been determined at two temperatures (298 K and 198 K) by Sawzik and Craven (19, 20) and also at room temperature by Dahlen (21). The room temperature structures differ in that Dahlen reported disordering near the end of the B-chain, whereas Sawzik and Craven found no convincing evidence of disorder, although the electron density in this region was diffuse. These structural differences may be due to dif-

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[4] The shortest C···C intermolecular distances are between atoms within layers. In cholesteryl palmitoleate at 123 K, these are between C(37) in molecule A and C(20) in B (3.52 Å), and C(36) in molecule A and C(23) in B (3.59 Å).

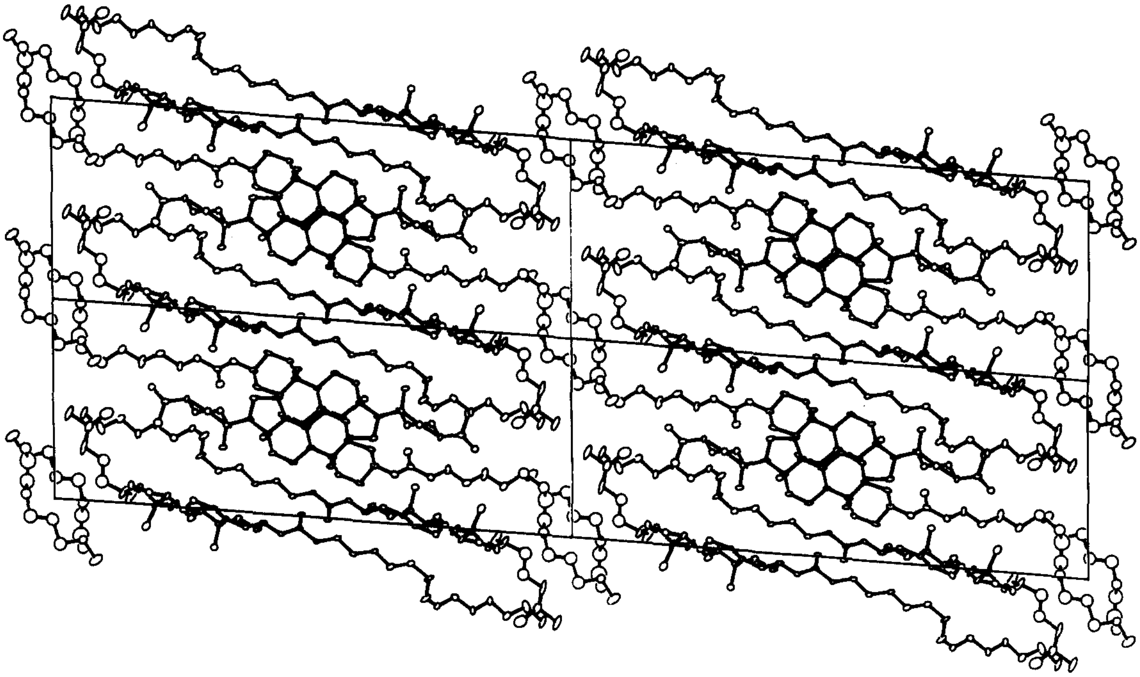

Fig. 3. The crystal structure of cholesteryl palmitoleate at 123 K in projection down the *b*-axis. The monolayers extend up and down the page and normal to it with interface regions at the center and borders of the diagram. Thermal motion is represented as the envelope with 10% probability of enclosing each atom center.

ferences in the conditions during crystal growth. On cooling to 198 K, all atoms are well-ordered. The B-chain adopts a conformation somewhat different from any of those at room temperature.[5]

For cholesteryl palmitoleate, similar effects are observed. In the crystal structure at 295 K (3), the B-chain is disordered with respect to conformers B1 and B2 (Fig. 5). Cooling to 123 K results in major conformational changes, both in the B-chain and the A-tail. However, the electron density remains diffuse in these regions (Fig.

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[5] See Fig. 2 in reference 20.

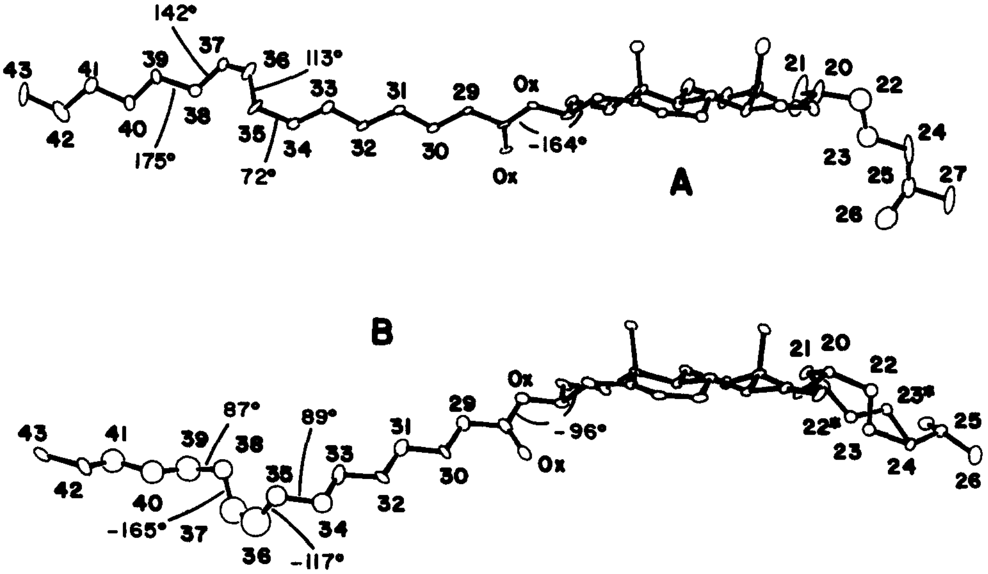

Fig. 4. Molecular structures viewed with the steroid ring system of molecules A and B in the same orientation. Selected torsion angles are shown for the ester linkage and near the ethylenic groups at C(36)=(37). Other torsion angles are in Table 2.

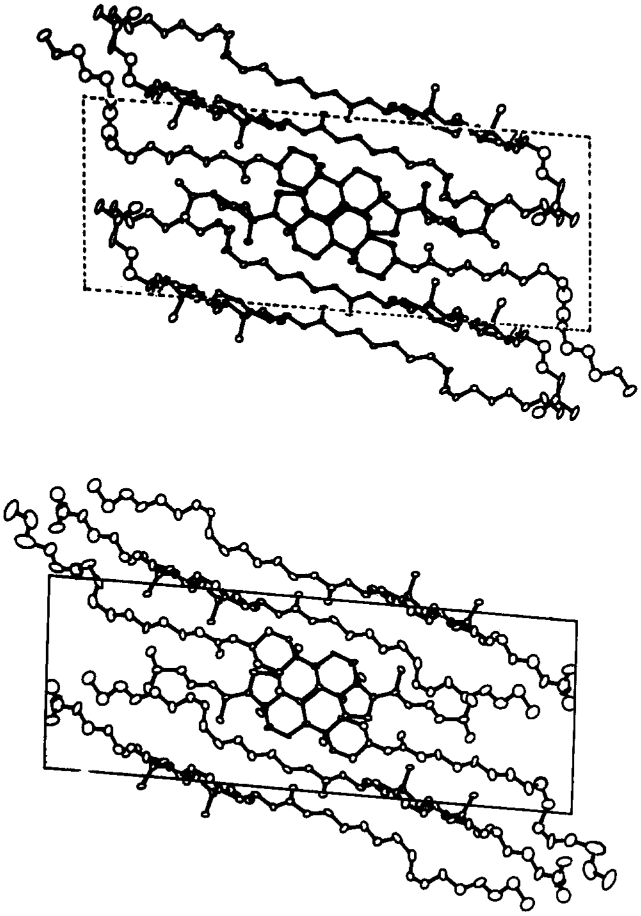

Fig. 5. Comparison of the crystal structures of cholesteryl palmitoleate at 123 K (above) and 295 K (below). Thermal motion is represented as the envelope with 10% probability of enclosing each atom center. The most important differences are in the isoprenoid tail of molecule A and the ester chain of molecule B. At 295 K, the B-chain is disordered. For clarity, only conformer B1 is shown at the top left and B2 at the bottom right corners of the unit cell.

1). A reasonable description of the structure comes from assuming that the B-chain and A-tail are ordered and that the diffuse electron density is due to atomic thermal vibrations with large m.s. amplitudes, $\langle u^2 \rangle$. For atoms within the layer, values of $\langle u^2 \rangle$ range from 0.05 to 0.12 Å^2 (Table 1), with moderate increases at the free end of the A-chain (0.17 Å^2) and B-tail (0.14 Å^2). Values are considerably greater for atoms of the layer interface region, with the largest (0.44, 0.36 Å^2) occurring at the ethylenic group in the B-chain. The variation in $\langle u^2 \rangle$ for the atoms of the B-chain is consistent with the barrier for torsional motion being lower at the bonds adjacent to the C=C bond (23, 24).

Factors other than thermal motion which might contribute to the diffuseness of the electron density are disorder and the occurrence of small structural variations in the three crystals used for the data collection (see Experimental). Although more than one conformer of the B-chain and A-tail may be present, they are not distinguishable in the observed electron density.

When the crystal is cooled from 295 K to 123 K, the changes in conformation of the B-chain and A-tail are associated with a decrease of 6.4% in the unit cell volume, from 4175(3) to 3903(5) Å^3. The course of these changes has not been followed in detail. No thermodynamic data are available in this temperature range. However, the discontinuity in cell volume which can be seen in Fig. 2 indicates that the crystal structure changes most rapidly in a 10 K range near 173 K, where there may be an abrupt phase transition. Each of the unit cell dimensions decreases uniformly from 295 K [$a = 12.873(7)$, $b = 9.173(4)$, $c = 35.42(1)$, Å, $\beta = 93.47(1)°$] to 178 K [$a = 12.74(1)$, $b = 9.002(6)$, $c = 35.12(2)$ Å, $\beta = 95.04(7)°$]. On further cooling to 168 K [$a = 12.90(1)$, $b = 8.96(1)$, $c = 34.30(2)$ Å, $\beta = 94.97(7)°$], there is a significant decrease in c (2.4%) and an increase in a (1.2%), while the cell volume decreases by 1.6%. If the structures shown in Fig. 5 are overlaid, with a suitable rotation to fit the cholesteryl groups, it appears that the net effect of crystal cooling is a compression and shearing of the interface region between layers.

At 123 K, the isoprenoid tail of molecule A is in a twisted conformation and is tucked between the ends of the ester chains of adjacent A-molecules. At 295 K, with more space available, the A-tail is almost fully extended. This is a conformation which would be energetically more favorable for an isolated cholesterol molecule. It is interesting that very similar conformational changes occur in the transition from the low (25°C) to the high (37°C) temperature crystal form of cholesterol. Hsu and Nordman (12) emphasize the importance of these changes (affecting molecules C and G) for understanding the mechanism of the transition.■

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