

# Crystal structure of form II of cholesteryl palmitelaidate at 295 K<sup>1</sup>

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**Abstract** Form II for cholesteryl palmitelaidate (*trans*-9-hexadecenoate) (C<sub>43</sub>H<sub>74</sub>O<sub>2</sub>) is monoclinic P2<sub>1</sub> with  $a = 12.745(3)$ ,  $b = 9.006(2)$ ,  $c = 18.153(4)$  Å,  $\beta = 96.63(2)^\circ$ ,  $Z = 2$ . The X-ray crystal structure of form II has been determined from 2506 reflections of which 2126 gave ( $F > 2\sigma$ ). The data up to  $\sin \theta/\lambda = 0.44 \text{ \AA}^{-1}$  ( $D_{\min} = 1.14 \text{ \AA}$ ) were measured with CuK $\alpha$  radiation from a sealed tube. These were supplemented up to  $\sin \theta/\lambda = 0.52 \text{ \AA}^{-1}$  ( $D_{\min} = 0.96 \text{ \AA}$ ) by measurements on the same crystal using a rotating anode X-ray source. The electron density was diffuse in the ester chain and the atoms of the cholesteryl tail were found to be disordered. The tail and the chain atoms were refined by restrained least squares methods to give  $R = 0.087$  and  $R_w = 0.10$  for reflections with  $F > 2\sigma$ . Crystal forms I and II represent two standard structure types already characterized for fatty acid esters of cholesterol. In form II, the ester chain is almost fully extended as is also the case for one of the two independent molecules (A) in form I. In form II, the chains pack loosely together for most of their length. M.s. amplitudes of thermal vibration for the chain C-atoms are almost uniform along the entire chain ( $\sim 0.25 \text{ \AA}^2$  at 295 K). In form I, the proximal part of the A chain is surrounded by rigid cholesteryl groups. In this region, C-atom m.s. amplitudes are much reduced ( $\sim 0.10 \text{ \AA}^2$ ) but they increase to about  $0.5 \text{ \AA}^2$  at the distal end of the chain where packing is very loose.— **Srivastava, R. C., and B. M. Craven.** Crystal structure of form II of cholesteryl palmitelaidate at 295 K. *J. Lipid Res.* 1989. 30: 893–898.

**Supplementary key words** unsaturated fatty acid • chain conformation • dynamical effects

The crystal structure determination of cholesteryl palmitelaidate was undertaken as a part of the program of systematic crystal structure determination of unsaturated fatty acid esters of cholesterol. The main aim of this study is to determine the conformation and packing of the unsaturated fatty acid chains. These structures could help in understanding molecular aggregation in less ordered lipid systems. Cholesteryl palmitelaidate is of particular interest because we have observed three different crystalline forms, all obtained from the same crystallization, but difficult to distinguish by optical inspection or optical microscopy. Cho and Craven (1) determined the crystal struc-

ture of form I and described a second form, discovered by P. Sawzik. More recently, we discovered the third isomorph now designated as form II, and presently report its crystal structure determination. These new results indicate that the form designated II by Cho and Craven (1) should be renamed III. In this way forms I and II correspond to well-known structure types for cholesteryl esters containing so-called monolayers of types I and II, respectively (2). The unit cell constants indicate that form III may be a new structure type for cholesteryl esters. X-ray data collection for form III is not yet complete.

## Experimental

Crystals of cholesteryl palmitelaidate form II were obtained from the same crystallization as forms I and III, by slow evaporation of petroleum ether solution at 273 K. All forms have blade-like habit, exhibiting {001} and were elongated on  $b$ . A crystal of dimensions  $0.20 \times 0.45 \times 0.06$  mm was mounted on an Enraf Nonius CAD-4 diffractometer using Ni-filtered CuK $\alpha$  radiation ( $\lambda = 1.5418 \text{ \AA}$ ) from a sealed tube operating at 0.9 Kw with the object of completing the intensity data collection for polymorph III (Cho and Craven's form II). However, the reflections could be indexed only on the basis of a new polymorph designated as form II. Unit cell dimensions were obtained by least squares fitting of  $\sin^2\theta$ -values for 25 reflections in the range  $19 < \theta < 27^\circ$  measured at  $\pm \omega$ . This gave the unit cell dimensions  $a = 12.745(3)$ ,  $b = 9.006(2)$ ,  $c = 10.153(3) \text{ \AA}$  and  $\beta = 96.63(2)^\circ$ .

It had been observed that the crystals in form I tended to fracture on cooling below 275 K. Because the availabili-

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ty of good crystals of form II was uncertain, the data were collected at room temperature (295 K). It was observed that at higher angles the reflection intensities became very weak. Between  $\sin \theta/\lambda = 0.44$  and  $0.52 \text{ \AA}^{-1}$  ( $D_{\min} = 1.14$  and  $0.96 \text{ \AA}$ ) out 993 reflections measured only 292 gave  $F > 2\sigma$ . It was therefore decided to measure the intensities of the higher angle reflections from the same crystal on an AFC5 Rigaku diffractometer equipped with a rotating Cu-anode source operating at 11 Kw. This resulted in 685 reflections giving  $F > 2\sigma$  in the same  $\sin \theta/\lambda$  range as mentioned above. The two data sets were merged resulting in the final intensity data consisting of 2506 reflections ( $D_{\min} = 0.96 \text{ \AA}$ ) of which 2126 gave  $F > 2\sigma$ . Variances were assumed to be  $\sigma^2(F) = \sigma^2(F^2)/4F^2$ , except for very weak reflections with  $F^2 < \sigma(F^2)$ , where  $\sigma^2(F) = \sigma(F^2)/4$  (3). The e.s.d.s from counting statistics were further modified to include a factor for instrumental instability such that  $\sigma^2(F_0) = \sigma^2 F + (0.025F)^2$ . Reflections with  $F^2 < 0$  were assigned  $|F_0| = 0$ .

The crystal structure was determined by making use of the close relationship with the structure of cholesteryl linolelaidate (4). The fractional coordinates of 28 atoms C1 through C24, C28, C29, O3, and O28 were transformed from their values in cholesteryl linolelaidate into the cholesteryl palmitelaidate cell assuming common directions for  $a$  and  $b$  axes. The initial R was 0.59 calculated with an overall  $B = 5 \text{ \AA}^2$  and the scattering factors of Cromer and Waber (5). The remaining atoms were located by successive weighted Fourier synthesis and block-diagonal least squares refinement. In Fourier syntheses, the coefficients were  $w\{2|F_0| - |F_c|\}$  with weights  $w = I_1(x)/I_0$ , where  $I_1$

and  $I_0$  are first and zero order modified Bessel functions of the first kind, and  $x = 2|F_0||F_c|/\Delta^2$ , where  $\Delta = |F_0| - |F_c|$ . The procedure was based on that of Sim (6). The electron density was diffuse in much of the ester chain as well as the cholesteryl tail, and a model based on standard bond lengths and angles was fitted. With the chain and the tail atoms fixed full matrix least squares refinement with anisotropic temperature factors and varying about 230 parameters at a time converged with  $R_w = 0.13$ . The weights in the refinement were  $w = 1/\sigma^2$ . Hydrogen atoms were fixed in calculated positions and were assigned isotropic  $B = B(\text{ext}) + B(\text{int})$ , where  $B(\text{ext})$  is the equivalent isotropic  $B$  of the C-atom to which the hydrogen is bonded, and the second term arises from the intramolecular thermal vibration of the hydrogen with an average value  $B(\text{int}) = 1 \text{ \AA}^2$  derived from a neutron diffraction study of cholesteryl acetate at 20 K (7). The hydrogen atom scattering factors have been taken from Stewart, Davidson, and Simpson (8).

A difference Fourier at this stage indicated disorder in the cholesteryl tail. The atoms C25, C26, and C27 were assigned two positions each with an occupancy of 0.5. However, when the atomic positions of the chain and the tail atoms were refined, unrealistic bond lengths and angles were obtained because of the low resolution in the electron density of this part of the structure. The chain and the tail atoms were refined by restrained least squares using Konnert-Hendrickson's program for protein refinements as modified by Furey and following the procedure described by Chang et al. (9). This program restricts the thermal parameters to be isotropic. Refinement resulted

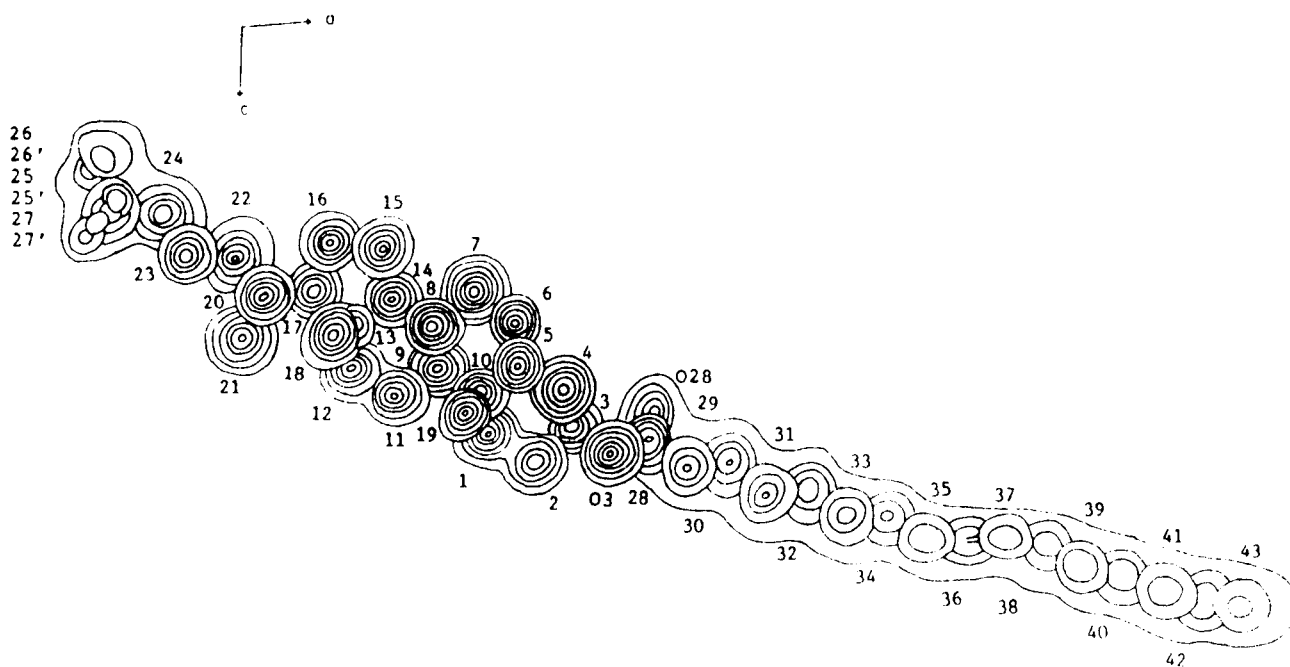


Fig. 1. The electron density and atomic nomenclature for cholesteryl palmitelaidate form II. Contour interval  $0.6e/\text{\AA}^3$ .

in reasonable geometry for the chain and tail atoms and converged with  $R = 0.13$ . When the occupancy factors were varied for the six atoms involved in disorder, the average value for C25, C26, and C27 was 0.56 and that of C25', C26', and C27' was 0.58. Subsequently, the occupancy factors for all these sites were assigned a fixed value 0.5. The coordinates of the chain and tail atoms resulting from this refinement were kept fixed in subsequent anisotropic least squares refinement using ORFLS as adapted by Shiono (10). This refinement converged with  $R = 0.087$ ,  $R_w = 0.10$  for reflections  $F_{obs} > 2\sigma$  and 0.105 and 0.102 for all reflections. A Fourier synthesis

TABLE 1. Atomic positional parameters ( $\times 10^4$ ) with estimated standard deviations (e.s.d.) in brackets. Parameters with zero e.s.d.s were not varied in anisotropic least squares refinement.

Atom	x/a	y/b	z/c
C1	5948 (5)	1562 (10)	7210 (3)
C2	6980 (5)	1857 (10)	7703 (3)
C3	7889 (5)	1810 (10)	7241 (4)
C4	7719 (5)	2969 (9)	6630 (3)
C5	6690 (4)	2786 (8)	6162 (3)
C6	6643 (4)	2758 (8)	5436 (3)
C7	5667 (4)	2648 (8)	4916 (3)
C8	4656 (4)	2887 (8)	5292 (2)
C9	4754 (4)	2103 (7)	6040 (3)
C10	5719 (4)	2673 (8)	6564 (3)
C11	3705 (4)	2112 (8)	6377 (3)
C12	2763 (4)	1599 (8)	5856 (3)
C13	2633 (4)	2536 (7)	5140 (2)
C14	3708 (4)	2353 (7)	4817 (2)
C15	3435 (4)	3028 (8)	4033 (3)
C16	2304 (4)	2543 (9)	3797 (3)
C17	1848 (4)	1940 (7)	4493 (3)
C18	2405 (4)	4125 (8)	5298 (3)
C19	5484 (4)	4188 (9)	6877 (3)
C20	675 (4)	2264 (8)	4477 (3)
C21	207 (5)	1753 (12)	5174 (3)
C22	31 (4)	1648 (9)	3793 (3)
C23	-1054 (5)	2211 (10)	3617 (3)
C24	-1646 (6)	1653 (11)	2914 (3)
C25	-2620 (0)	2631 (0)	2620 (0)
C25'	-2797 (0)	1781 (0)	2758 (0)
C26	-3008 (0)	2386 (0)	1809 (0)
C26'	-3333 (0)	1037 (0)	2063 (0)
C27	-3439 (0)	2530 (0)	3166 (0)
C27'	-3153 (0)	3380 (0)	2831 (0)
C28	9709 (7)	1575 (15)	7699 (5)
C29	10569 (0)	2306 (0)	8226 (0)
C30	11637 (0)	1709 (0)	8180 (0)
C31	12428 (0)	2301 (0)	8800 (0)
C32	13506 (0)	1752 (0)	8761 (0)
C33	14282 (0)	2494 (0)	9323 (0)
C34	15372 (0)	1898 (0)	9360 (0)
C35	16133 (0)	2676 (0)	9911 (0)
C36	17145 (0)	1831 (0)	9946 (0)
C37	18031 (0)	2491 (0)	9984 (0)
C38	19064 (0)	1639 (0)	10115 (0)
C39	19770 (0)	2260 (0)	10756 (0)
C40	20846 (0)	1564 (0)	10828 (0)
C41	21641 (0)	2419 (0)	11324 (0)
C42	22670 (0)	1617 (0)	11415 (0)
C43	23526 (0)	2698 (0)	11639 (0)
O28	9814 (5)	844 (13)	7182 (5)
O3	8816 (3)	2259 (8)	7746 (2)

TABLE 2. Selected bond torsion angles ( $^\circ$ ) in forms I and II

Atoms	Form II	Form I	
		Molecule A	Molecule B
C22 C23 C24 C25	-161 (2)	-122 (1)	-179 (1)
C22 C23 C24 C25'	163 (2)		
C23 C24 C25 C26	161 (2)	-180	-65 (2)
C23 C24 C25 C27	-66 (1)	-60	163 (2)
C23 C24 C25' C26'	-173 (2)		
C23 C24 C25' C27'	54 (1)		
O3 C28 C29 C30	-175 (2)	168 (1)	163
C34 C35 C36 = C37	138 (2)	-124 (2)	115
C35 C36 = C37 C38	172 (2)	-174 (2)	-174
C36 = C37 C38 C39	-126 (2)	113 (2)	-130
C2 C3 O3 C28	141 (1)	78 (1)	140

with  $|F_o|$  as coefficients gave the electron density distribution shown in Fig. 1. A final difference Fourier synthesis gave a maximum residual electron density  $0.31(5) \text{ e}/\text{\AA}^3$  near the disordered tail and the chain end. Final atomic coordinates are listed in Table 1.

This crystal structure determination lacks detail in the regions of the ester chain and cholesteryl tail which might well have been enhanced if the data had been collected at lower temperature and hence higher resolution. However, the measurement of almost 400 reflections with intensity above background, made possible by using the intense rotating anode X-ray source, compensated in part for the limitations inherent in the room temperature data set.

## RESULTS AND DISCUSSION

Bond lengths and angles agree well with those of cholesteryl palmitelaidate form I and other related structures (1, 11-15). The C-C single bond ranges from 1.47 to 1.56 (0.01)  $\text{\AA}$  in the tetracyclic ring system, 1.47 to 1.57(0.03)  $\text{\AA}$  in the cholesteryl tail and 1.47 to 1.52(0.03)  $\text{\AA}$  in the ester chain. The length of C=C double bond in the cholesterol ring is 1.31(0.01)  $\text{\AA}$  whereas that in the ester chain is 1.27(0.03)  $\text{\AA}$ . The effective steroid length (16) taken as C3-C16 distance is 8.94  $\text{\AA}$ . A measure of twist within the ring system is given by the torsion angle C19-C10-C13-C18, which is  $10.5^\circ$ . In related structures this torsion angle varies from  $7.9$  to  $18.0^\circ$  and the effective steroid length from 8.85 to 9.02  $\text{\AA}$  (2). The length of the palmitelaidate molecule, taken as the C26'-C43 distance (which is the greatest length) is 36.6  $\text{\AA}$ . In form I the greatest length C26-C43 is 34.3  $\text{\AA}$ . The rotation about the ester linkage, taken as the torsion angle C2-C3-O3-C28, has a value  $140.7^\circ$  whereas in form I this angle is  $78^\circ$  for molecule A and  $140^\circ$  for molecule B. This angle is important as it describes the relationship between the ester chain and the cholesterol moiety and hence the over-all shape of the molecule. Some important torsion angles for forms I (molecules A and B) and form II are listed for comparison in Table 2.

The C17 side chain has the extended conformation in form II as in form I. However, the isopropyl group in form II has orientational disordering giving rise to two positions for each of the atoms C25, C26, and C27. The positions C25 and C25' are related such that the torsion angles about the bond C23–C24 are  $161^\circ$  and  $-163^\circ$  (Table 2). The torsion angles C23–C24–C25–C27 and C23–C24–C25'–C27' are  $-66(1)^\circ$  and  $+54(1)^\circ$ . In ordered crystal structures containing cholesterol, side chain configurations similar to one or the other of these are strongly preferred. In an isolated molecule the two conformers would have similar energies so that it might be expected they would occur with almost equal frequency, as in the present disordered crystal structure. However, a survey of 69 crystal structures showed that (+) – gauche conformation at the C24–C25 bond is favoured 2:1 (17).

In form II the ester chain is almost fully extended (Fig. 2) as is also the case for one of the two independent molecules (A) in form I (1). Chain dislocations occur at the double bonds in all crystal structures of cholesteryl esters involving unsaturated fatty acids, regardless of whether the chains contain *cis* or *trans* double bonds. The dislocations occur because the preferred conformations are (+) – or (–) – skew for C–C bonds adjacent to a C=C bond, except in conjugated systems (18). In form I the

chain dislocations were described by considering two nearly coplanar chain backbone sections consisting of atoms C30 to C35 and C39 to C42. In form I maximum displacement of a C-atom from these planes was 0.07 and 0.05 Å for molecule A and 0.08 and 0.18 Å for molecule B, respectively. The dihedral angles between the planes were  $2.7^\circ$  and  $67.4^\circ$  for the molecules A and B, respectively. The corresponding displacements for form II are 0.06 and 0.09 Å and the dihedral angle is  $8.2^\circ$ . The bend in the chain is determined by the angle between the least squares lines through the atoms constituting the two coplanar sections and the kink at the double bond by the perpendicular separation between these lines. In form I the bend was  $171^\circ$  and  $149^\circ$  for molecules A and B, respectively, and the kinks 1.21 and 1.85 Å. In form II the bend and the kink in the chain are  $174.5^\circ$  and 1.04 Å, respectively. These detailed considerations confirm the similarity of the ester chain conformations in form II and in molecule A of form I.

The crystal structure of cholesteryl palmitelaidate form II is shown in Fig. 2. The molecules are arranged in monolayers parallel to the crystal planes (001) and all the molecular long axes are nearly parallel to the [301] direction. The smallest intermolecular C...C distance occurs between C26 and C36 (3.45 Å). Within the monolayers, in form

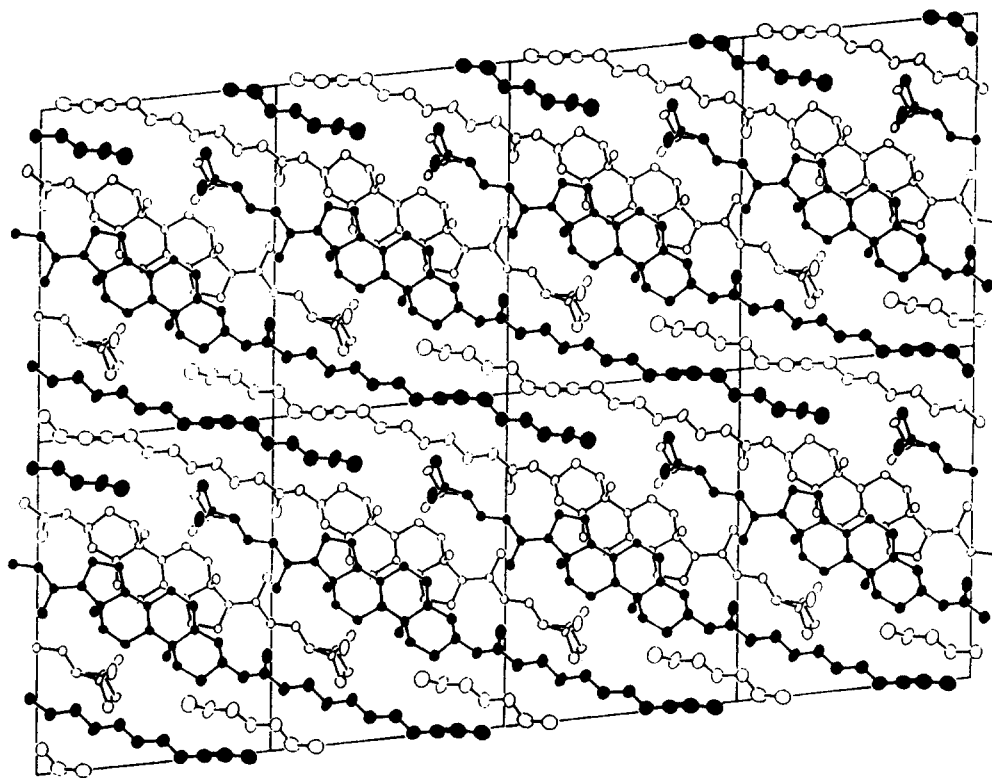


Fig. 2. The crystal structure of cholesteryl palmitelaidate form II. Crystal axis *a* is directed from left to right, *c* from top to bottom, and *b* into the page. Molecules with shaded atoms are close to  $y = 1/4$  (the disordered atoms C25', C26', and C27' have not been shaded) and the others are close to  $y = 3/4$ .

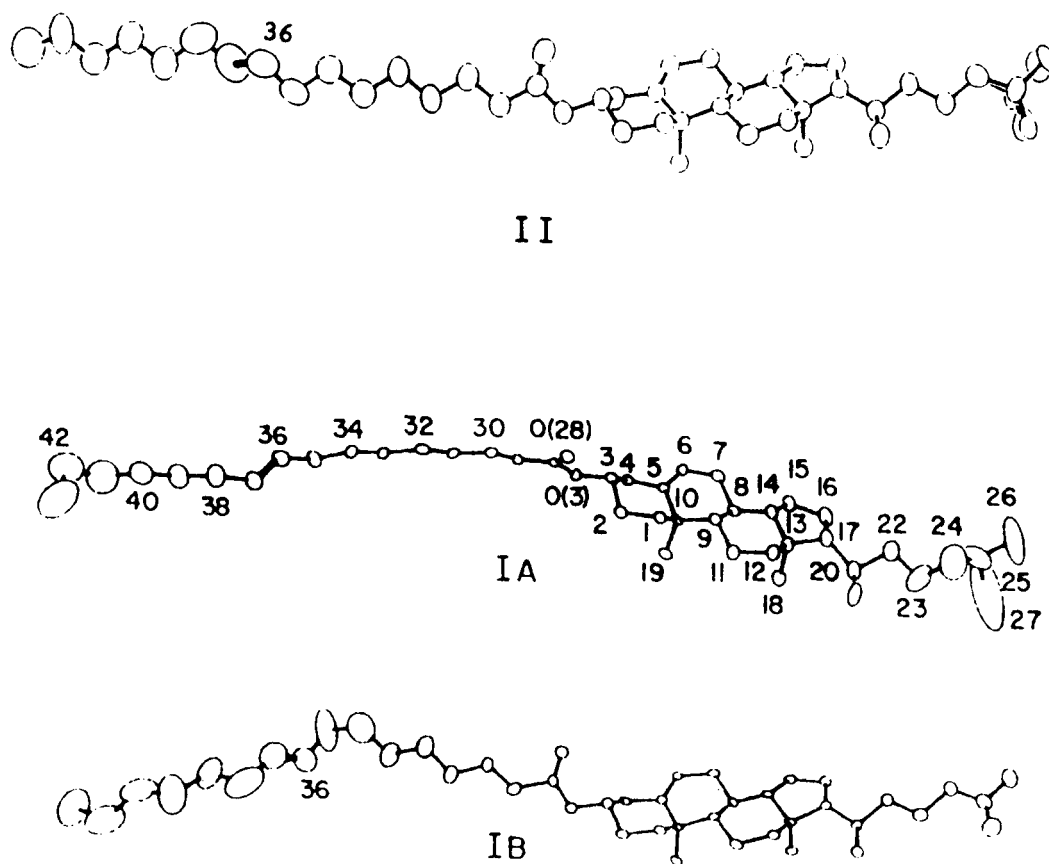


Fig. 3. Molecules from form II and form I (A and B) are shown with cholesteryl ring system in a similar orientation (25% probability ellipsoids).

II, the ester chains pack loosely together for most of their length. The effect of this packing arrangement becomes evident on reducing the anisotropic thermal parameters to equivalent isotropic vibrations and comparing their mean square amplitudes  $\langle U^2_{eq} \rangle$ , where  $\langle U^2_{eq} \rangle = 1/3$

TABLE 3. M.s. displacement ( $\text{\AA}^2$ ) thermal vibrations of the atoms of the ester chain in cholesteryl palmitelaidate form II and form I (molecule A).

Atoms	Form II	Form I (Molecule A)
C29	0.203 (7)	0.088 (8)
C30	0.208 (7)	0.095 (8)
C31	0.214 (7)	0.096 (8)
C32	0.202 (7)	0.107 (8)
C33	0.212 (7)	0.094 (8)
C34	0.236 (7)	0.101 (8)
C35	0.239 (7)	0.145 (11)
C36	0.236 (9)	0.166 (13)
C37	0.285 (12)	0.208 (15)
C38	0.296 (13)	0.231 (18)
C39	0.252 (10)	0.275 (20)
C40	0.253 (10)	0.310 (23)
C41	0.271 (11)	0.701 (74)
C42	0.280 (11)	0.506 (44)
C43	0.333 (15)	0.611 (57)

$[U_{22} + \{1/\sin^2\beta\} \{U_{11} + U_{33} + 2U_{13}\cos\beta\}]$ . As seen in Table 3 the m.s. amplitudes of vibration for C-atoms are almost uniform along the entire chain. The average value is  $0.25 \text{ \AA}^2$  (at 295 K).<sup>3</sup> In form I, the proximal part of the molecule A chain is surrounded by almost rigid cholesteryl groups. In this region (Table 3) the C-atom m.s. amplitudes are much reduced (about  $0.10 \text{ \AA}^2$  at 295K) but they increase to about  $0.5 \text{ \AA}^2$  at the distal end of the chain where the packing is very loose. For comparison, molecules of form I (A and B) and form II are shown in Fig. 3 with their cholesteryl ring systems in a similar orientation. Atoms are shown by ellipsoids (19) having 25% probability of enclosing the atom centers. The striking difference in dynamical behavior of these two ester chains, having very similar conformations but quite different crystal environments is suggestive of the effect which

<sup>3</sup>For m.s. amplitudes of this magnitude, the assumption that these atomic displacements represent only harmonic thermal vibrations is unlikely to be true. The observed electron density is the result of averaging the atomic positions over the unit cells in the crystal as well as with respect to time, and thus may include the effect of small local variations in chain conformation.

the introduction of cholesterol might have on the lipid chains within biological membranes. ■

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## REFERENCES

1. Cho, S.-I., and B. M. Craven. 1987. Commensurate molecules in isostructural crystals of cholesteryl *cis*- and *trans*-9-hexadecenoate. *J. Lipid Res.* **28**: 80-86.
2. Craven, B. M. 1986. Cholesterol crystal structures: Adducts and esters. In *Hand-Book of Lipid Research*, 4. D. M. Small, Editor. Plenum Press, New York. 149-182.
3. Rees, B. 1977. Assessment of accuracy. *Isr. J. Chem.* **16**: 180-186.
4. Craven, B. M., and P. Sawzik. 1983. Conformation and packing of unsaturated chains in cholesteryl linoleaidate at 123 K. *J. Lipid Res.* **24**: 784-789.
5. Cromer, D. T., and J. T. Waber. 1965. Scattering factors computed from relativistic Dirac-Slater wave functions. *Acta Crystallogr.* **18**: 104-109.
6. Sim, G. A. 1960. A note on heavy atom method. *Acta Crystallogr.* **13**: 511-512.
7. Craven, B. M. 1987. Studies of hydrogen atoms in organic molecules. *Trans. Am. Crystallogr. Assoc.* **23**: 71-81.
8. Stewart, R. F., E. R. Davidson, and W. T. Simpson. 1965. Coherent x-ray scattering for the hydrogen atoms in the hydrogen molecule. *J. Chem. Phys.* **42**: 3175-3187.
9. Chang, C. H., J. Pletcher, W. Furey, and M. Sax. 1980. The crystal structure of 5'-acetamido-3'-deoxythymidine chloroform solvate. *Acta Crystallogr.* **B26**: 2655-2660.
10. Shiono, R. 1971. Technical report no. 49, Department of Crystallography, University of Pittsburgh, Pittsburgh, PA USA.
11. Sawzik, P., and B. M. Craven. 1982. The crystal structure of cholesteryl palmitoleate at 295 K. *Acta Crystallogr.* **B38**: 1777-1781.
12. Guerina, N. G., and B. M. Craven. 1979. The crystal structure of cholesteryl nonanoate. *J. Chem. Soc. Perkin Trans. II.* **10**: 1414-1419.
13. Craven, B. M. 1979. Pseudosymmetry in cholesterol monohydrate. *Acta Crystallogr.* **B35**: 1123-1128.
14. Sawzik, P., and B. M. Craven. 1979. The crystal structure of cholesteryl laurate. *Acta Crystallogr.* **B35**: 789-791.
15. Pattabhi, V., and B. M. Craven. 1979. Crystal structure of cholesteryl decanoate. *J. Lipid Res.* **20**: 753-759.
16. Duax, W. L., and D. A. Norton. 1975. *Atlas of Steroid Structures*, 1. IFI/Plenum Data, New York.
17. Duax, W. L., J. F. Griffin, D. C. Rohrer, and C. M. Weeks. 1980. Conformational analysis of sterols: comparison of x-ray crystallographic observations with data from other sources. *Lipids.* **15**: 783-797.
18. Sundaralingam, M. 1972. Molecular structures and conformations of phospholipids and sphingomyelins. *Ann. N. Y. Acad. Sci.* **195**: 324-355.
19. Johnson, C. K. 1965. Oak Ridge thermal ellipsoid program. Report ORNL-3794, Oak Ridge National Laboratory, Tennessee.