Chemistry and Physics of Lipids 23 (1979) 179–188

THE CRYSTAL STRUCTURE OF CHOLESTERYL DODECANOATE: CO-PACKING OF STEROID SKELETA AND HYDROCARBON CHAINS

BIRGITTA DAHLEN
Department of Structural Chemistry, Faculty of Medicine, University of Göteborg, Fack S-400 33 Göteborg (Sweden)

Received March 22nd, 1978 accepted August 15th, 1978

The crystal structure of cholesteryl dodecanoate has been determined. The compound shows a co-packing of cholesterol skeleta and hydrocarbon chains. There are two molecules in the asymmetric unit both almost fully extended. The hydrocarbon chain axes are however somewhat bent in order to get a good close-packing side by side with the rigid cholesterol skeleta. The two non-symmetry related skeleta show different packing surroundings. One skeleton packs with both hydrocarbon chains and other skeleta while the other skeleton is completely surrounded by hydrocarbon chains. The latter packing is of particular interest as it is considered to indicate important packing principles in biological lipid bilayers.

I. Introduction

Esters of cholesterol with long-chain hydrocarbon fatty acids have been studied by X-ray diffraction methods as this might show how cholesterol and hydrocarbon chains can pack side by side – an important feature of the architecture of biological membranes. Crystal cell dimensions for different cholesteryl esters have been reported by Schulze [1], Abrahamsson and Selin [2], Wendorff and Price [3] and Barnard and Lydon [4] and it is evident from these data that cholesteryl esters may exist in several crystal forms in the solid state.

The crystals of cholesteryl dodecanoate used for this structure determination were of the same phase as that reported by Abrahamsson and Selin [2]. The cell dimensions also coincide within standard deviations with those given by Barnard and Lydon [4] when transformed to a corresponding unit cell.

Earlier the three-dimensional structures have been determined for cholesteryl tetradecanoate [5] and cholesteryl 17-bromoheptadecanoate [6,7]. In both compounds cholesterol and hydrocarbon chains pack in separate regions, and only indirect information on the chain-cholesterol arrangement in lipid bilayers can be deduced. The present structure, however, shows a co-packing of cholesterol and hydrocarbon chains.

II. Materials and methods

Cholesteryl laurate was synthesized from dodecanoic acid chloride and cholesterol in the presence of pyridine. Crystals were obtained from an acetic acid-ethyl acetate solution.

A. Crystal data

Molecular formula	$C_{39}H_{68}O_2$
Unit cell:	
monoclinic	$a = 12.995(8)$, $b = 9.013(8)$
	$c = 34.25(3)$ Å, $\beta = 110.97(5)^\circ$
V	3745.4 Å^3
Z	4
Mol. wt.	568.98
D_c	1.009 g · cm^{-3}
Spacegroup	$P2_1$
λ	1.54051 Å
Crystal dimensions	0.1 × 0.4 × 0.8 mm

Intensity data were collected on a Packer FACS-1 automatic diffractometer with the Vanderbilt disc-oriented program system [8]. Graphite monochromated CuKα radiation was used to measure all reflexions up to $2\theta = 100^\circ$. The reflexions were scanned in 10 steps of 2 s each with a total scan width of 2.0°. 10 s stationary background counts were taken on both sides of the peak. 4157 independent reflexions were recorded of which 1741 were less than 3σ (I) and considered as unobserved. Corrections were made for the Lorentz and polarization factors, but not for absorption.

III. Structure determination and refinement

The structure was solved with the Patterson rotation and translation function program ROTRAN [9]. The atomic coordinates from 21 atoms in the steroid skeleton of cholesteryl benzoate (Dahlén - unpublished data) were used to match the Patterson function of cholesteryl dodecanoate. The two cholesterol skeleta could thus be located and the remaining nonhydrogen atoms were obtained by conventional electron and difference density syntheses. The structure was refined by full-matrix methods. The 4 outermost carbon atoms in the hydrocarbon chain of molecule A seem to be disordered. When first located in the difference map the peaks which most corresponded to a straight chain were chosen. During the refinement the temperature factors increased too much and from further difference maps an

alternative chain end was found. The two chain ends were included in the refinement with a population parameter of 0.5 for all 8 atoms which from the refinement seemed to be reasonable. It should be emphasized that the positional parameters for the atoms in the disordered part are uncertain. Their thermal parameters are large which may indicate an even more complex disorder which, however, it was not possible to determine. The hydrogen atoms were included at their calculated positions with a C–H distance of 1.0 Å and with the same isotropic temperature factors as the carbon atoms to which they were bonded. Hydrogen atoms in the disordered hydrocarbon chain part were omitted. The hydrogen atoms were never refined. Anisotropic temperature factors were applied during the last cycles of the refinement. The structure refined to a final R-value of 0.078. The average shift over error was then 0.08.

The weighting scheme used during the final stage of the refinement was $w = 1/[1 + \{(|F_o| - 12)/10\}^2]$. Atomic shattering factors were taken from Cromer and Mann [10] except for hydrogen atoms where those of Stewart, Davidson and Simpson [11] were used.

The calculations were made on a DEC10 computer using the X-ray System of Crystallographic Programs [12]. System modifications for DEC10 were made by Dr. Steve Ernst, University of Pittsburgh and by Dr. Robert Pearson at this Department. The NIH display programs [13] have been used for all drawings. Interface routines between XRAY72 and the display programs have been written by Prof. S. Abrahamsson.

IV. Description of the structure

The final atomic parameters for the non-hydrogen atoms are given in Table 1. (A table of the anisotropic temperature factors and a list of the observed and calculated structure factors can be obtained from this Department.) The atomic numbering and interatomic distances and angles are given in Figs. 1 and 2. All intramolecular distances and angles are normal within standard deviations. The cholesterol side chains are well defined in the structure which has not been the case in a number of earlier determined cholesterol containing structures (refs. 5,6,14 and I, Pascher and S Sundell, to be published). In these the bulky steroid skeleta pack in separate layers and leave the side chains with too much space resulting in high thermal parameters and/or disorder. Both molecules pack almost fully extended in the c-axis direction. In the hydrocarbon chains all torsion angles are close to 180° except for the disordered chain end of molecule A. This chain end is in a low density packing region of other hydrocarbon chains and cholesterol side chains. The hydrocarbon chain of molecule B on the other hand is in packing contact with the rigid skeleta and consequently better ordered. The two chain axes are bent to allow a good molecular packing side by side with the rigid steroid skeleta. The bend of chain B is due to the fact that after passing the cholesterol skeleton it can bend towards the hydrocarbon

Table 1

Fractional atomic coordinates ($\times 10^4$) for the non-hydrogen atoms.

Estimated standard deviations are given in parentheses

Molecule A	X	Y	Z	Molecule B	X	Y	Z
O (1)	8728 (6)	5222 (–)	1643 (2)	O (1)	6461 (5)	11010 (–)	700 (2)
O (2)	9952 (12)	3550 (18)	1934 (3)	O (2)	5063 (5)	9646 (11)	274 (2)
C (1)	7646 (8)	4837 (12)	487 (3)	C (1)	6382 (8)	8498 (14)	1567 (3)
C (2)	7645 (9)	5064 (12)	927 (3)	C (2)	6577 (9)	8971 (13)	1172 (4)
C (3)	8768 (10)	5002 (12)	1235 (3)	C (3)	6218 (8)	10537 (14)	1053 (3)
C (4)	9522 (9)	6223 (12)	1153 (3)	C (4)	6782 (9)	11585 (13)	1407 (4)
C (5)	9514 (8)	6092 (11)	709 (3)	C (5)	6648 (9)	11134 (14)	1804 (4)
C (6)	10442 (7)	6062 (12)	636 (3)	C (6)	6322 (10)	12108 (14)	2021 (4)
C (7)	10532 (7)	5962 (12)	215 (3)	C (7)	6199 (10)	11815 (13)	2429 (4)
C (8)	9438 (7)	6188 (10)	–147 (3)	C (8)	6718 (8)	10358 (14)	2632 (3)
C (9)	8492 (7)	5429 (10)	–47 (3)	C (9)	6500 (8)	9146 (13)	2296 (3)
C (10)	8365 (7)	5961 (11)	356 (3)	C (10)	6945 (7)	9519 (12)	1948 (3)
C (11)	7411 (7)	5431 (13)	–433 (3)	C (11)	6863 (10)	7572 (14)	2490 (4)
C (12)	7509 (7)	4888 (13)	–830 (3)	C (12)	6437 (10)	7153 (14)	2835 (4)
C (13)	8411 (7)	5766 (10)	–939 (3)	C (13)	6763 (10)	8386 (18)	3185 (4)
C (14)	9460 (7)	5629 (11)	–552 (3)	C (14)	6293 (9)	9856 (16)	2964 (4)
C (15)	10372 (8)	6212 (13)	–714 (3)	C (15)	6365 (11)	10867 (19)	3327 (4)
C (16)	9986 (7)	5696 (12)	–1164 (3)	C (16)	6198 (13)	9898 (24)	3662 (4)
C (17)	8782 (8)	5106 (12)	–1282 (3)	C (17)	6172 (10)	8293 (20)	3506 (4)
C (18)	8045 (8)	7438 (13)	–1041 (3)	C (18)	8008 (10)	8431 (22)	3411 (4)

C (19)	7826 (8)	7535 (12)	306 (3)	C (19)	8204 (9)	9341 (14)	2092 (4)
C (20)	8077 (8)	5381 (12)	−1745 (3)	C (20)	6552 (13)	7167 (24)	3878 (5)
C (21)	6874 (9)	4962 (14)	−1862 (3)	C (21)	6611 (15)	5583 (25)	3733 (5)
C (22)	8592 (9)	4539 (16)	−2021 (4)	C (22)	5817 (13)	7323 (28)	4145 (5)
C (23)	8123 (11)	4861 (21)	−2484 (4)	C (23)	6366 (20)	6537 (30)	4576 (6)
C (24)	8632 (10)	3973 (21)	−2742 (5)	C (24)	5608 (18)	6906 (35)	4832 (6)
C (25)	8249 (12)	4333 (26)	−3186 (5)	C (25)	6041 (21)	6378 (54)	5251 (7)
C (26)	8942 (14)	3609 (40)	−3392 (6)	C (26)	5171 (21)	6588 (43)	5447 (7)
C (27)	7079 (15)	4080 (30)	−3401 (5)	C (27)	7016 (24)	6297 (78)	5465 (8)
C (28)	9329 (12)	4450 (16)	1960 (4)	C (28)	5810 (7)	10481 (14)	322 (3)
C (29)	9134 (12)	4796 (16)	2351 (4)	C (29)	6174 (8)	11122 (14)	−17 (3)
C (30)	9528 (12)	3686 (19)	2703 (4)	C (30)	5625 (8)	10394 (13)	−446 (4)
C (31)	9318 (12)	4050 (18)	3083 (4)	C (31)	6097 (8)	10970 (13)	−760 (3)
C (32)	9650 (13)	2943 (23)	3428 (5)	C (32)	5658 (8)	10203 (13)	−1182 (3)
C (33)	9425 (17)	3320 (27)	3807 (6)	C (33)	6211 (9)	10685 (14)	−1480 (3)
C (34)	9764 (21)	2241 (33)	4177 (8)	C (34)	5873 (9)	9873 (13)	−1888 (4)
C (35)	9305 (43)	2665 (48)	4540 (11)	C (35)	6561 (11)	10243 (18)	−2150 (4)
C (36, I)	9142 (51)	1478 (127)	4693 (26)	C (36)	6266 (11)	9288 (16)	−2552 (4)
C (36, II)	9758 (65)	1762 (102)	4898 (14)	C (37)	6855 (15)	9734 (24)	−2841 (5)
C (37, I)	8836 (111)	2074 (138)	5039 (25)	C (38)	6591 (17)	8780 (27)	−3215 (6)
C (37, II)	9269 (91)	2091 (134)	5306 (18)	C (39)	7094 (23)	9149 (35)	−3512 (7)
C (38, I)	8504 (90)	720 (95)	5181 (37)				
C (38, II)	8277 (57)	2206 (111)	5164 (19)				
C (39, I)	8553 (42)	1947 (88)	5575 (17)				
C (39, II)	7731 (39)	1241 (72)	5493 (14)				

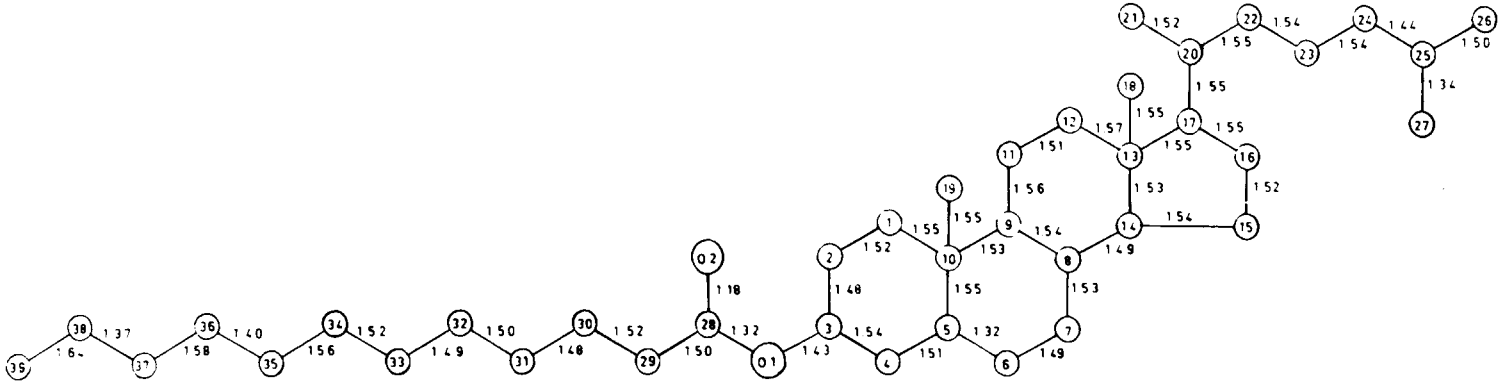

Fig. 1. Bond distances representing average values of the individual bonds of the two molecules in the asymmetric unit. The estimated standard deviations are 0.01–0.03 Å and considering these there are no significant differences of the steroid skeleta.

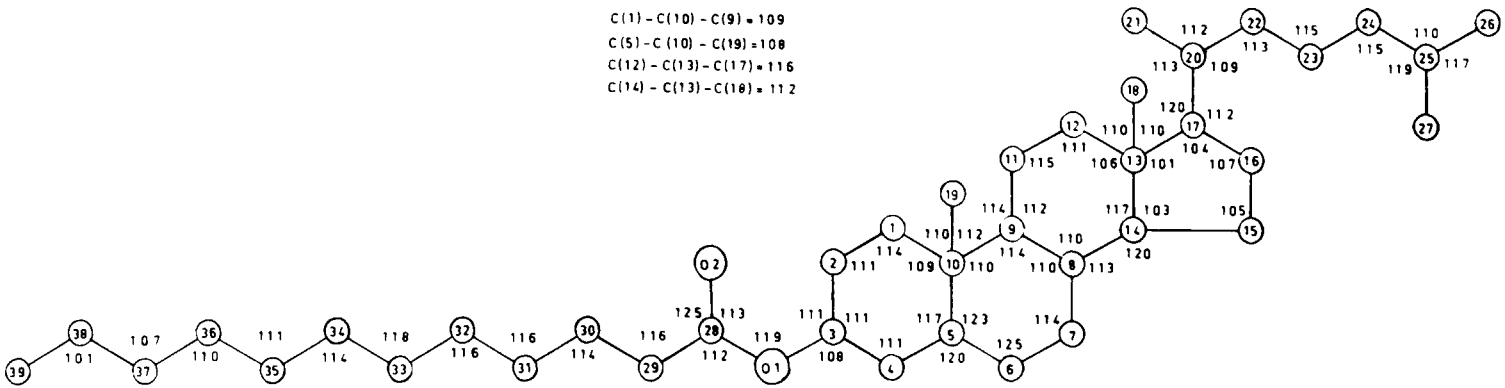

Fig. 2. Bond angles averaged for the two molecules in the asymmetric unit. The estimated standard deviations are 1–2°. There are no significant differences of the steroid skeleta.

chain of an A-molecule. However, even so the chain end of B does not come closer to chain end A than 4.28 Å (C(32)A–C(39)B). The bend of chain A is necessary to avoid the chain end coming too close to the projecting methyl groups of a B-molecule.

The ester bonds continue in the zig-zag chain directions. The torsion angle O(1) – C(28) – C(29) – C(30) is 163 and 170° in molecule A and B respectively. A similar *trans*-conformation has been observed in cholesteryl myristate where these torsion angles are 173 and –161 respectively for the two independent molecules while in cholesteryl 17-bromoheptadecanoate one molecule shows a *trans*-conformation (169°) and the other has a *gauche* conformation (–88°).

In order to obtain the best packing conditions the hydrocarbon chains may have to rotate differently around the ester bond with respect to the ring system. The conformation around the C(3) – O(1) bond thus differs in all cholesterol esters studied so far. (Table 2.)

The intricate packing of cholesteryl dodecanoate molecules is shown in Fig. 3. The two independent molecules are oriented with their cholesterol skeleta planes approximately perpendicular to each other. They are arranged head to tail and the only close contacts between their skeleta are C(2) (A) – C(2) (B) (3.98 Å) and C(2) A – C(4) B (3.89 Å) where C(2)B and C(4)B belong to different molecules separated one unit cell along *b*.

The two independent molecules are differently located in relation to the 2-fold screw axes which lead to quite different packing surroundings for the A and B molecules respectively. The cholesterol skeleta of the A-molecules pack closely along the 2-fold screw-axis in a way similar to form B of cholesteryl iodide [15]. Symmetry related A-molecules are translated so that the contours of their skeleta follow each other. The planes of the skeleta are inclined to each other so that each skeleton is in contact with both the skeleton and the projecting methyl groups of

Table 2

The conformation around the C (3) – O (1) bond

Compound	Torsion angle	
	C (2) – C (3) – O (1) – C (28)	C (4) – C (3) – O (1) – C (28)
Cholesteryl dodecanoate		
molecule A	139°	–99°
molecule B	75	–163
Cholesteryl tetradecanoate		
molecule A	86	–149
molecule B	130	–109
Cholesteryl 17-bromoheptadecanoate		
molecule A	87	–155
molecule B	102	–136

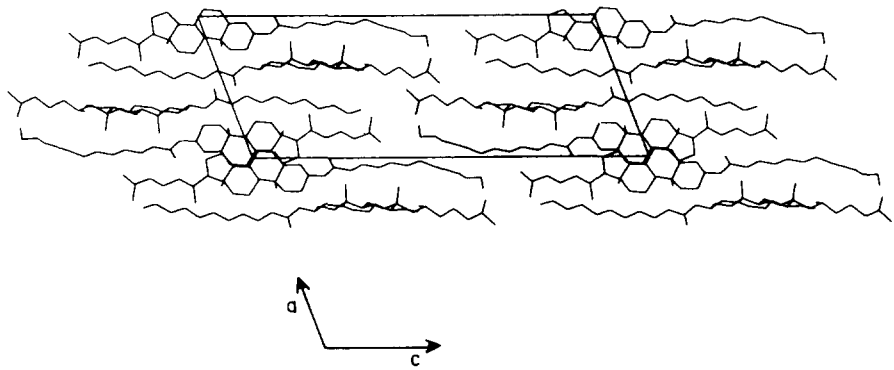

Fig. 3. Molecular packing of cholesteryl dodecanoate seen along the *b*-axis direction. Only one alternative chain end of molecule A is shown.

the adjacent molecule. The closest methyl-skeleton contact is 3.81 Å (C(18) (A) – C(6) (A)) and the shortest skeleton-skeleton contact is 4.06 Å (C(1) (A) – C(15) (A)). The cholesterol skeleta of the A-molecules are also in contact with hydrocarbon chains of B-molecules. The most important contacts here range from 3.71 Å to 4.10 Å (see Fig. 4). This packing can be considered as a pleated sheet arrangement in which rows of cholesterol and hydrocarbon chains alternate.

The steroid skeleta of B-molecules are almost hexagonally surrounded by hydrocarbon chains and cholesterol side chains (Fig. 5). The shortest contacts are to the carbon chains of two symmetry related B-molecules along the *b*-axis (3.69 Å and

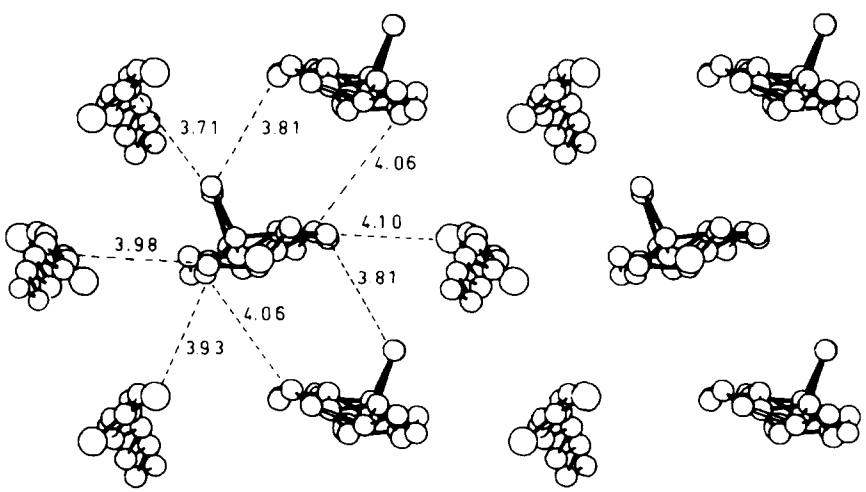

Fig. 4. Surrounding of the skeleta of A-molecules as seen in the direction of maximum extension of the skeleta.

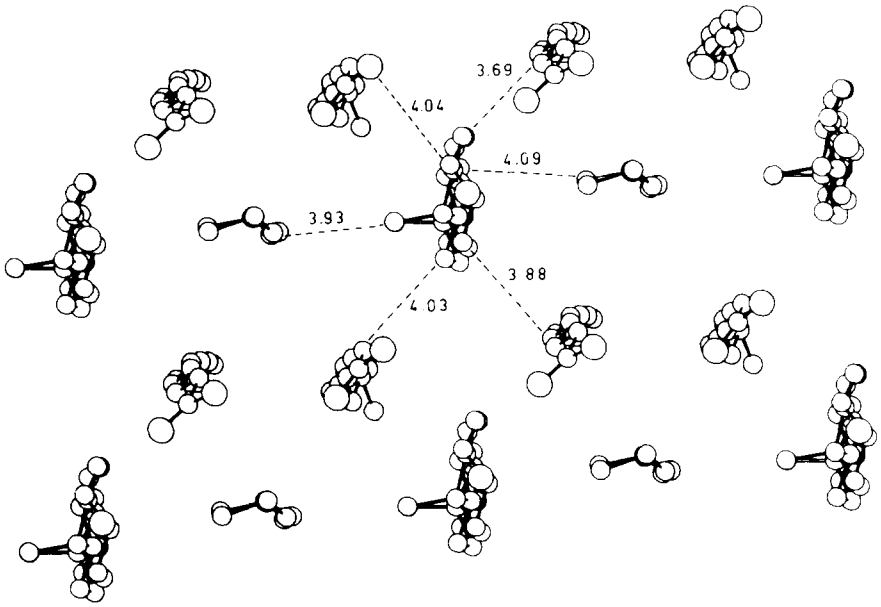

Fig. 5. Surrounding of the skeleta of B-molecules as seen in the direction of maximum extension of the skeleta.

3.88 Å). The skeleton is also in packing contact with the carbon chains of two A-molecules along the *b*-axis with closest contacts of 4.03 and 4.04 Å. One side chain of an A-molecule is in close contact with the projecting methyl groups while the other side chain translated one unit cell along the *a*-axis is in contact with the smooth side of the cholesterol skeleton.

The calculated density for the title compound is 1.009 g · cm^{-3}. The corresponding value for cholesteryl tetradecanoate is 1.006 g · cm^{-3} and for cholesteryl hexadecanoate, isomorphous with the tetradecanoate 1.006 g · cm^{-3}. Cholesterol and hydrocarbon chains can thus peak together just as effectively as when they are arranged in separate regions in a crystal structure.

In biological membranes the cholesterol skeleta are not arranged head to tail as in this crystal phase. The structure is, however, important in showing that cholesterol skeleta and hydrocarbon chains do form a close packing side by side. Details in this packing are no doubt to be found in biological lipid bilayers. Of special interest here is the cross section area containing B-skeleta (Fig. 5) where the skeleta are only surrounded by chains (i.e., ester chains and cholesterol side chains). These form in fact a carbon chain matrix, which resembles the common orthorhombic chain packing O⊥ [16] though the chain axes separation is, of course, larger due to the incorporation of cholesterol.

Acknowledgements

I thank Professor S. Abrahamsson for valuable comments. Grants in support of this Department were obtained from the Swedish Medical Research Council, the Swedish Board for Technical Development, The Wallenberg Foundation and the U.S. Public Health Service (G.M.-11653).

References

[1] G.E.R. Schulze, Z. Phys. Chem. A 171 (1935) 436
[2] S. Abrahamsson and K. Selin, Acta Cryst. 16 (1963) A58
[3] J.H. Wendorff and F.P. Price, Mol. Cryst. Liquid Cryst. 22 (1973) 85
[4] J.A.W. Barnard and J.E. Lydon, Mol. Cryst. Liquid Cryst. 26 (1974) 285
[5] B.M. Craven and G.T. DeTitta, J. Chem. Soc. Perkin Trans. (1976) 814
[6] S. Abrahamsson and B. Dahlén, J. Chem. Soc. Chem. Commun. (1976) 117
[7] S. Abrahamsson and B. Dahlén, Chem. Phys. Lipids 20 (1977) 43
[8] P.G. Lenhert, J. Appl. Cryst. 8 (1975) 568
[9] B.M. Craven, ROTRAN Computer Programs Technical Report, Crystallography Department, University of Pittsburgh (1975)
[10] D.T. Cromer and J.B. Mann, Acta Cryst. A 24 (1968) 321
[11] R.F. Stewart, E.R. Davidson and W.T. Simpson, J. Chem. Phys. 42 (1965) 3175
[12] J.H. Stewart, G.J. Kruger, H.L. Ammon, G. Dickinson and S.R. Hall, Technical Report TR-192. Univ. of Maryland, Computer Science Center (1972)
[13] CHEM. Crystal Structure Search Retrieval, DCRT, National Institutes of Health, Bethesda 1974
[14] I. Pascher and S. Sundell, Acta Chem. Scand. A31 (1977) 767
[15] C.H. Carlisle and D. Crowfoot, Proc. Roy Soc. 184A (1945) 64
[16] S. Abrahamsson, B. Dahlén, H. Löfgren and I. Pascher, Progr. Chem. Fats Other Lipids 16 (1978) 125