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The Crystal Structure of Sodium Cholesteryl Sulfate Dihydrate

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Sodium cholesteryl sulfate crystallizes as the dihydrate in space group $P1$ with $a=6.296$, $b=6.238$, $c=40.813$ Å, $\alpha=88.94$, $\beta=89.51$ and $\gamma=107.44°$. The unit cell contains two independent molecules with their main conformational difference in the side chain. The molecules are arranged tail to tail in a double layer. Three regions with different lateral interactions exist. In the polar part each sodium ion is roughly octahedrally surrounded by oxygen atoms, four belonging to sulfate groups and two belonging to water of crystallization. The steroid part is in van der Waals contact only with other steroid skeleta. In the third region only side chains interact. Due to the bulkiness of the steroid part the side chains are left with unusually large space which causes disorder and/or large thermal vibrations. The lateral arrangement of the steroid skeleta is of interest as it resembles in geometry and cross sectional area the hybrid packing modes of hydrocarbon chains.

The existence of cholesteryl sulfate in animal tissues and fluids has been known for several years. Minor amounts have been identified in adrenal glands,[1] in kidney, liver, plasma, urine and bile [2] and in blood and gallstone.[3] A relatively high concentration in faeces suggested a role as a metabolite of cholesterol.[2] Recently, however, cholesteryl sulfate was shown to be an abundant constituent of the brushborder membrane of intestine (K.-A. Karlsson, unpublished). Its occurrence in faeces therefore appears more likely to be due to the rapid turnover of these membranes.

This type of steroid sulfate has also been identified as one of the major lipid components in the starfish *Asterias rubens*.[4,5] The high amount of 1.3 mg per g dry weight in different tissues also here suggests a function as a membrane constituent.

In several vertebrate tissues an interesting correlation between the concentration of cerebroside sulfate (sulfatide) and the Na^+-K^+- ATPase activity was found suggesting this sulfolipid to be involved in the sodium transport across the plasma membrane.[6,7] Similarly, cholesteryl sulfate may participate in ion transport, especially as other sulfolipids have not been found in the starfish.

This marine organism has a vascular system for motion and progression. The fluid pressure in this hydraulic system is thought to be regulated by osmotic principles by means of a potassium pump.[8] The demand of a potassium pump and presence of cholesteryl sulfate support the hypothesis that sulfolipids may function as ion receptors in sodium-potassium transport across membranes.

It is therefore of interest to investigate the properties of these sulfolipids and their significance for membrane structure and function. The phase behaviour of cholesteryl sulfate and phosphate in pure form and in presence of water has been determined.[9] In the case of the phosphate a remarkable lameller long range order was observed in the aqueous gel phase. Further structural information on sulfolipids has been obtained by the X-ray analysis of sodium dodecyl sulfate.[10]

EXPERIMENTAL

Cholesteryl sulfate (cholesteryl-3β-sulfate) was synthesized according to Sobel *et al.*[11] and converted into its sodium salt on an ion exchange column. Crystals were obtained from a 1 % solution in 95 % ethanol as thin elongated plates.

A crystal with the dimensions $0.25 \times 0.54 \times 0.02$ mm was used for data collection on a Picker FACS I diffractometer.[12] The reflexions were measured by a $\theta - 2\theta$ step scan with 20 steps of 0.13° each. The counting time for each step was 2 s. Background counts of 10 s were taken at each side of the peak. 3439 independent reflexions in the 2θ range $3 - 100°$ were measured. 2582 reflexions with intensities greater than $2\sigma(I)$ were considered observed. For these reflexions corrections for Lorentz and polarization effects were made.

CRYSTAL DATA

Molecular formula: $C_{27}H_{45}SO_4Na.2H_2O$.
Crystal system: triclinic. Space group: $P1$.
Unit cell: $a = 6.296(11)$, $b = 6.238(14)$,
$c = 40.813(67)$ Å, $\alpha = 88.94(3)$,
$\beta = 89.51(2)$, $\gamma = 107.44(2)°$.
$V = 1529$ Å^3, $M = 524.74$, $Z = 2$, $D_c = 1.14$ g cm^{-3}, $\lambda = 1.5405$ Å (Cu$K\alpha$ radiation), $\mu = 13.8$ cm^{-1}.
Systematic absences: None.

Table 1. Fractional coordinates and thermal parameters ($\times 10^3$) with estimated standard deviations in parentheses. The isotropic and anisotropic thermal parameters are in the form $\exp -8\pi U(\sin^2\theta/\lambda^2)$ and $\exp -2\pi^2(h^2a^{*2}U_{11} + \ldots + 2klb^*c^*U_{23})$, respectively.

Atom	x	y	z	U or U11	U22	U33	U12	U13	U23
Na(1)	0.7039(17)	0.4426(18)	0.0322(3)	27(5)	46(6)	48(6)	20(5)	1(5)	-8(5)
Na(2)	0.2073(17)	0.4589(20)	0.9704(3)	26(6)	62(7)	50(7)	25(5)	-5(5)	-4(6)
O(1)	0.8865(37)	0.8270(34)	0.0454(7)	55(14)	39(13)	129(22)	22(11)	-19(14)	-16(13)
O(2)	0.5488(33)	0.0578(33)	0.0264(6)	45(13)	48(13)	83(17)	23(11)	3(12)	-9(12)
O(3)	0.3937(37)	0.8546(33)	0.9596(6)	66(14)	45(13)	80(16)	29(11)	-7(12)	-20(12)
O(4)	0.0538(35)	0.0760(32)	0.9782(6)	49(13)	29(12)	109(19)	2(10)	-8(13)	0(12)
Molecule A									
S(1)	0.2390(11)	0.4963(12)	0.0571(2)	18(3)	35(4)	39(4)	16(3)	-4(3)	0(3)
O(11)	0.3309(29)	0.5111(29)	0.0252(5)	27(10)	36(11)	58(12)	11(9)	-22(9)	10(9)
O(12)	0.0034(28)	0.3471(31)	0.0596(5)	20(10)	49(12)	53(13)	8(9)	0(9)	6(10)
O(13)	0.2692(29)	0.7058(31)	0.0715(5)	30(10)	50(12)	52(13)	18(9)	-5(9)	15(10)
O(14)	0.3878(29)	0.3709(33)	0.0771(4)	34(11)	67(14)	34(11)	33(10)	-1(8)	-11(10)
C(1)	0.2787(43)	0.2011(49)	0.1661(8)	23(16)	49(19)	68(23)	18(14)	8(15)	10(16)
C(2)	0.2349(51)	0.1749(60)	0.1285(8)	38(19)	79(25)	62(24)	24(18)	3(17)	1(19)
C(3)	0.3956(42)	0.3814(50)	0.1115(7)	22(15)	65(21)	45(19)	29(15)	-12(13)	7(16)
C(4)	0.6525(40)	0.3907(55)	0.1186(7)	8(14)	87(24)	40(19)	11(15)	5(12)	7(16)
C(5)	0.6851(40)	0.3903(47)	0.1555(7)	14(14)	47(18)	65(22)	23(13)	11(13)	10(15)
C(6)	0.8590(43)	0.5553(50)	0.1674(8)	22(15)	54(19)	65(23)	21(14)	-8(15)	-9(16)
C(7)	0.9205(41)	0.5680(48)	0.2029(7)	18(14)	55(19)	50(20)	14(14)	-1(13)	12(15)
C(8)	0.7998(37)	0.3432(40)	0.2232(7)	9(12)	24(15)	47(17)	-5(11)	15(12)	0(13)
C(9)	0.5484(42)	0.2606(52)	0.2135(7)	17(14)	67(21)	42(19)	13(14)	-8(13)	13(16)
C(10)	0.5292(36)	0.2103(43)	0.1768(7)	3(12)	40(17)	47(19)	0(11)	-5(12)	7(13)
C(11)	0.4180(47)	0.0725(52)	0.2365(8)	32(17)	55(21)	65(23)	6(16)	-3(16)	-2(17)
C(12)	0.4526(47)	0.1116(55)	0.2736(8)	29(16)	69(23)	57(22)	13(15)	9(15)	-17(17)
C(13)	0.7053(44)	0.1928(51)	0.2835(7)	32(16)	65(20)	28(16)	22(15)	-9(13)	-8(14)
C(14)	0.8117(45)	0.4057(54)	0.2599(7)	28(16)	68(21)	41(18)	10(15)	-4(14)	6(16)
C(15)	1.0428(49)	0.5002(55)	0.2747(8)	39(19)	59(21)	60(22)	-4(16)	-31(16)	-22(17)
C(16)	1.0037(57)	0.4591(63)	0.3119(9)	54(22)	85(27)	64(25)	18(20)	-26(18)	2(20)
C(17)	0.7538(59)	0.3172(65)	0.3189(9)	63(24)	88(28)	55(23)	5(21)	-24(19)	-24(20)
C(18)	0.5601(48)	-0.0330(54)	0.1698(9)	33(18)	62(22)	77(24)	21(16)	-15(16)	-14(18)
C(19)	0.8002(46)	-0.0067(47)	0.2792(7)	40(17)	42(18)	46(19)	15(15)	-5(15)	-1(15)
C(20)	0.7216(78)	0.1509(69)	0.3482(8)	125(37)	85(29)	34(20)	-37(26)	-14(22)	27(20)
C(21)	0.4868(86)	0.0037(90)	0.3528(9)	126(39)	153(45)	37(23)	-33(34)	-7(23)	58(26)
C(22)	0.7984(101)	0.3389(106)	0.3798(12)	154(51)	179(58)	73(33)	-22(43)	20(32)	77(36)
C(23)	0.8181(118)	0.1863(114)	0.4101(17)	155(23)					
C(24)	1.0282(285)	0.3600(312)	0.4337(47)	389(83)					
C(25)	0.9966(204)	0.1791(260)	0.4623(32)	274(50)					
C(26)	0.9407(305)	-0.0637(339)	0.4666(44)	408(88)					
C(27)	1.1462(385)	0.3423(363)	0.4903(54)	482(107)					

Table 1. Continued.

Atom	x	y	z	U or U11	U22	U33	U12	U13	U23
Molecule B									
S(1)	0.7487(11)	0.5286(12)	0.9460(2)	24(3)	35(4)	44(4)	18(3)	8(3)	-2(3)
O(11)	0.8380(33)	0.5263(33)	0.9784(6)	43(12)	48(13)	79(15)	22(10)	29(11)	-6(11)
O(12)	0.5272(27)	0.3823(30)	0.9420(5)	20(9)	42(12)	63(13)	13(9)	1(9)	-4(10)
O(13)	0.7749(30)	0.7498(33)	0.9336(5)	28(11)	60(14)	68(14)	14(10)	8(10)	-10(11)
O(14)	0.9169(26)	0.4364(30)	0.9249(4)	18(9)	57(12)	37(11)	25(9)	11(8)	-6(9)
C(1)	1.0644(48)	0.5388(45)	0.8354(7)	44(18)	28(16)	57(21)	7(14)	9(15)	5(14)
C(2)	1.0720(44)	0.5761(47)	0.8718(7)	35(17)	51(19)	30(17)	4(15)	-28(13)	-13(14)
C(3)	0.8800(39)	0.4147(43)	0.8907(7)	12(13)	28(15)	66(20)	18(12)	-2(13)	6(14)
C(4)	0.8594(49)	0.1613(52)	0.8821(8)	43(19)	58(22)	56(21)	23(17)	8(16)	16(17)
C(5)	0.8593(51)	0.1316(50)	0.8463(8)	45(19)	35(18)	61(21)	21(16)	7(16)	27(16)
C(6)	0.7013(56)	-0.0427(61)	0.8333(10)	43(20)	62(24)	97(30)	27(20)	7(19)	25(21)
C(7)	0.6744(49)	-0.1082(53)	0.7957(8)	37(19)	54(21)	66(24)	0(16)	7(17)	12(17)
C(8)	0.8863(42)	0.0224(48)	0.7769(7)	23(15)	47(18)	49(18)	21(13)	6(13)	3(14)
C(9)	0.9743(39)	0.2712(42)	0.7877(7)	16(14)	30(15)	49(18)	4(12)	1(12)	-14(13)
C(10)	1.0440(39)	0.2937(46)	0.8257(7)	9(13)	43(17)	56(19)	3(12)	5(13)	0(14)
C(11)	1.1777(55)	0.4102(54)	0.7641(8)	58(22)	53(21)	65(23)	16(17)	-3(18)	-10(18)
C(12)	1.1122(45)	0.3772(48)	0.7259(7)	32(16)	45(19)	49(19)	10(14)	-6(14)	-6(15)
C(13)	1.0367(50)	0.1347(49)	0.7174(8)	43(18)	41(19)	69(23)	13(15)	25(16)	-6(16)
C(14)	0.8283(45)	0.0172(55)	0.7406(7)	23(16)	78(24)	41(19)	7(16)	10(14)	-3(17)
C(15)	0.7269(46)	-0.2136(53)	0.7268(9)	25(17)	58(22)	72(25)	2(15)	5(16)	-32(19)
C(16)	0.7714(58)	-0.1779(72)	0.6910(13)	36(21)	89(32)	145(44)	2(21)	-7(24)	-54(29)
C(17)	0.9129(66)	0.0695(68)	0.6843(8)	86(28)	95(30)	34(20)	13(24)	2(18)	-25(19)
C(18)	1.2706(42)	0.2484(50)	0.8312(9)	11(14)	45(19)	99(26)	3(13)	-20(15)	-8(17)
C(19)	1.2267(55)	0.0138(69)	0.7217(9)	44(20)	115(32)	62(23)	40(21)	4(17)	-10(21)
C(20)	1.0683(70)	0.1114(74)	0.6521(10)	83(30)	93(32)	82(30)	-16(25)	-34(24)	-31(25)
C(21)	1.2052(90)	0.3585(101)	0.6487(9)	140(44)	183(54)	22(21)	-16(39)	31(24)	10(26)
C(22)	0.9073(121)	0.0561(102)	0.6207(9)	274(78)	173(54)	15(22)	-63(52)	11(32)	-50(27)
C(23)	0.7909(229)	0.2327(221)	0.5999(31)	306(56)					
C(24)	0.6465(191)	0.0415(187)	0.5813(27)	262(47)					
C(25)	0.6213(345)	0.2079(376)	0.5526(50)	433(106)					
C(26)	0.6893(271)	0.4394(317)	0.5461(39)	371(78)					
C(27)	0.4935(241)	-0.0082(229)	0.5278(33)	316(58)					

STRUCTURE DETERMINATION AND REFINEMENT

From a sharpened three-dimensional Patterson function the peak corresponding to the S – S vector was easily found. At a distance of about 1.4 Å from this peak there were eight peaks which could be separated into two groups. Each group contained four likely S – O vectors consistent with the known geometry of the sulfate group. The positions of atoms of the two sulfate groups thus determined were used as a first phasing model. In subsequent Fourier syntheses all non-hydrogen atoms except those belonging to the outer part of the side chains C(23) – C(27) in the two independent molecules were found. At this stage two cycles of block diagonal refinement with isotropic temperature factors were performed which reduced the R-value from 0.26 to 0.16. From a Fourier and a difference synthesis a reasonable suggestion for the location of the missing part of the side chains was obtained. The refinement now again continued with anisotropic temperature factors. The last located atoms of the side chains, however, were given isotropic temperature factors. After five cycles using the full matrix the refinement converged at $R = 0.118$ and $R_w = 0.129$. The weight assigned to each reflexion [13] was:

$$1/[1 + (F_o - 2.7F_{min})^2/(4.2F_{min})^2]$$

Most bond distances and angles of the side chains did not improve and the temperature factor of some atoms became large. This indicates that disorder and/or considerable thermal motion exists in the side chain region. A

difference Fourier of this region contained some fairly weak peaks. From their positions, however, no model for a possible disorder could be deduced. The form factors used were those given by Cromer and Mann.[14] All calculations were performed on a DEC 10 computer system using the X-RAY 72 program system.[15]

DESCRIPTION AND DISCUSSION OF THE STRUCTURE

The atomic parameters are listed in Table 1. A list of final observed and calculated structure factors can be obtained from this Department. The numbering of atoms and the bond distances and angles of the two cholesteryl sulfate molecules A and B are shown in Figs. 1 and 2. These values are as expected considering the high standard deviations. The geometry of the cholesterol skeleton is in agreement with that observed in other cholesterol derivatives.[16–20] The two independent molecules differ in their conformation about the C(20)–C(22) bond (Fig. 3).

Fig. 4 shows the tail to tail packing of cholesteryl sulfate molecules in a double layer ar-

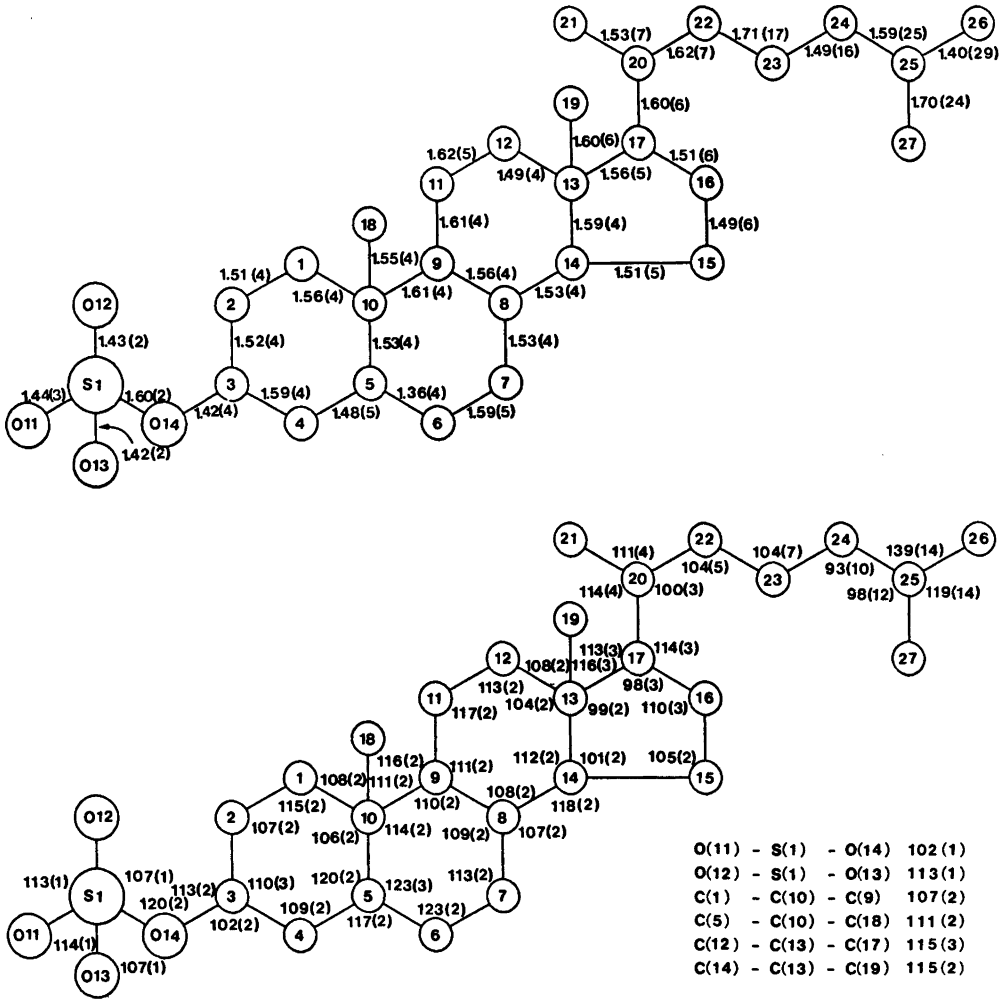

Fig. 1. Atom numbering and bond distances (Å) and angles (°) of molecule A. Estimated standard deviations are given in parentheses.

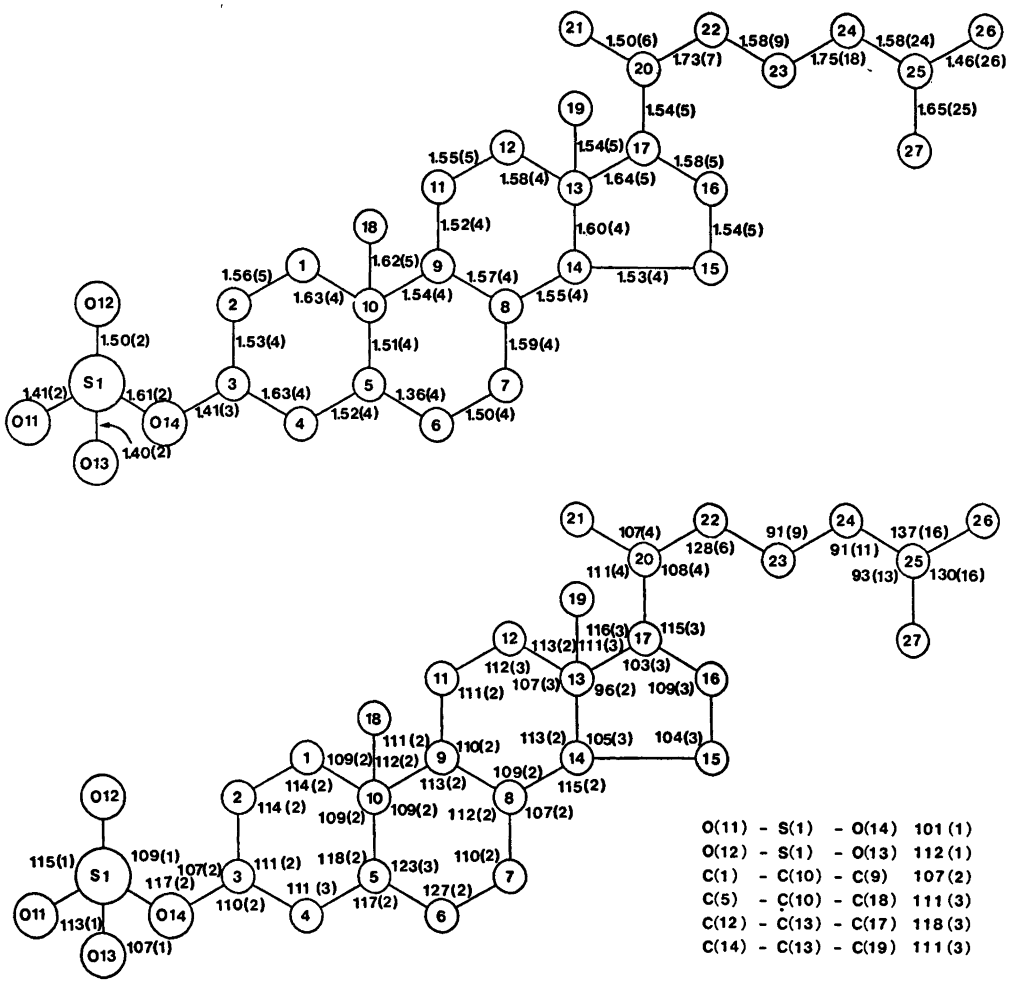

Fig. 2. Atom numbering and bond distances (Å) and angles (°) of molecule B. Estimated standard deviations are given in parentheses.

rangement. The two halves of the double layer are not identical in that each half contains only one type of the two independent molecules. Both molecules are tilted 75° towards the layer boundary.

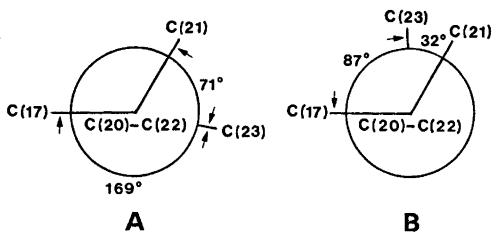

Fig. 3. The molecular conformation about the bond C(20) – C(22) in molecules A and B.

The sulfate groups, the rigid steroid skeleta and the branched hydrocarbon side chains form three regions with different packing character within the double layer.

The sulfur atoms and sodium ions lie close to a plane parallel to the *ac*-face. Each sodium atom is roughly octahedrally surrounded by six oxygens at distances between 2.31 and 2.63 Å (Table 2). Four of these belong to the sulfate groups and lie approximately in one plane while the two other oxygens belong to water

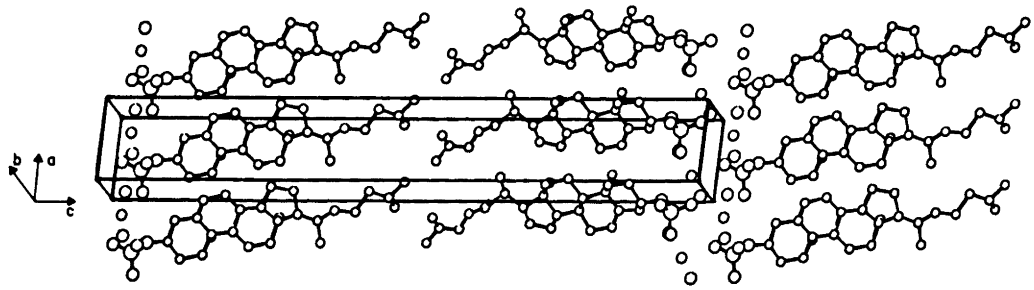

Fig. 4. The molecule arrangement of cholesteryl sulfate.

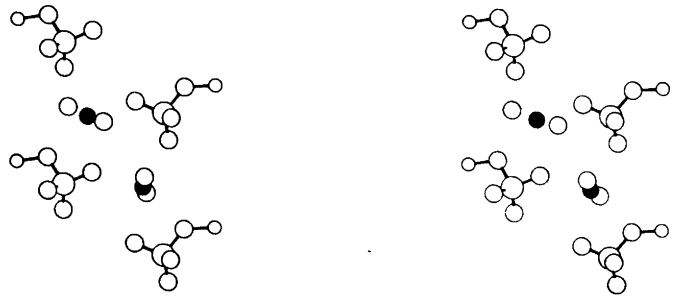

Fig. 5. Stereoscopic view of the polar region.

molecules. The polar region is visualized stereographically in Fig. 5 and torsion angles about the ester bond are shown in Fig. 6. Short intermolecular oxygen-oxygen distances are given in Table 3. All of these involve water molecules and may represent hydrogen bonds.

Table 2. Sodium oxygen distances (Å) less than 3.5 Å and their estimated standard deviations ($\times 10^2$).

Na(1) – O(1)	(0,0,0) [a]	2.40(3)
Na(1) – O(2)	(0,0,0)	2.32(3)
Na(1) – O(A11)	(0,0,0)	2.53(3)
Na(1) – O(A12)	(1,0,0)	2.42(3)
Na(1) – O(A14)	(0,0,0)	2.63(2)
Na(1) – O(B11)	(0,0,−1)	2.34(3)
Na(2) – O(3)	(0,0,0)	2.42(3)
Na(2) – O(4)	(0,0,0)	2.31(3)
Na(2) – O(A11)	(0,0,1)	2.37(2)
Na(2) – O(B11)	(−1,0,0)	2.50(3)
Na(2) – O(B12)	(0,0,0)	2.49(3)
Na(2) – O(B14)	(−1,0,0)	2.59(2)

[a] The figures within parentheses indicate translations in the directions *a*, *b* and *c* of the second atom.

The lateral packing of the steroid skeleta is different from that found in other cholesteryl derivatives. In cholesteryl esters [18,19] the skeleta pack laterally in a double row arrangement in which the projecting methyl groups face each other and can interdigitate due to a displacement of the steroid skeleta in the direction of their maximum extension. In cholesteryl sulfate the skeleta pack in single rows only. In Fig. 7 this lateral arrangement is shown for molecule A (the packing of molecule B is similar). The methyl groups project into the space between two skeleta of the adjacent row. This arrangement also differs from that of the

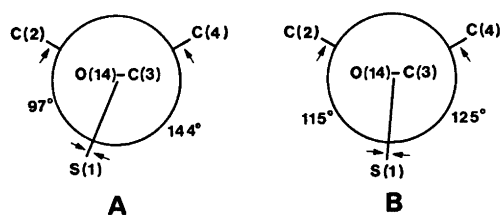

Fig. 6. The molecular conformation about the bond O(14) – C(3) in molecules A and B.

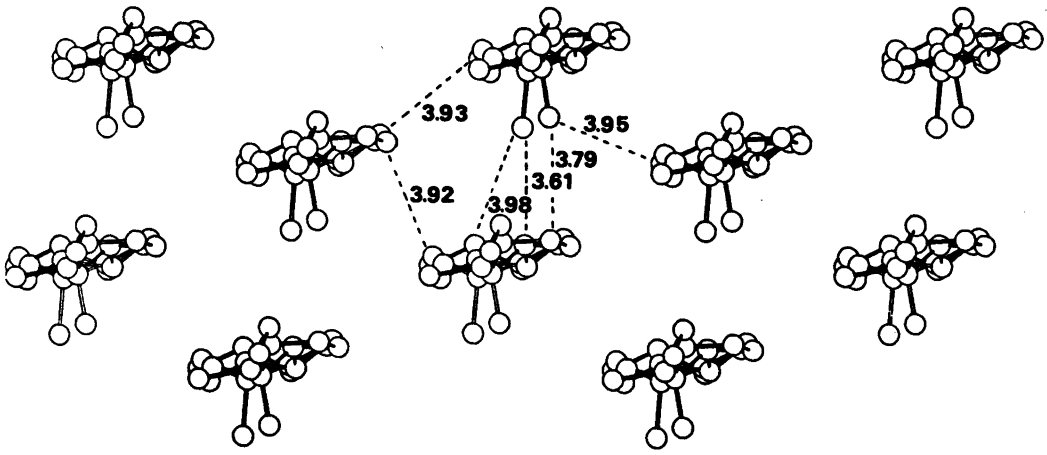

Fig. 7. The lateral arrangement of the steroid skeleta of molecule A.

cholesteryl esters in that adjacent skeleta show only a small mutual displacement in their direction of maximum extension. Both types of lateral packing are about equally effective with a cross sectional area of 37 Å² per molecule.

These packing requirements of the bulky steroid groups leave the side chains with unusually large space. This no doubt explains the observed disorder and/or large thermal vibrations in the chain packing region. Though the skeleta planes of molecule A and B have different orientation in the unit cell their side chains become roughly parallel due to conformational differences about the C(20 – C(22) bond (Fig. 3).

The side chains tilt by an angle of approximately 60° towards the layer boundary. The lateral distance between the chains then corresponds to a cross section area of about 35 Å² per chain which can be compared with a value of 21 Å² for hexagonal α-phase adopted by many long-chain compounds near their melting point. In cholesteryl iodide [16] and in 7-bromo-cholesteryl-bromide [17] the side chains pack more effectively being in contact also with the sterol skeleta.

The arrangement of the steroid skeleta of cholesteryl sulfate appears to be of interest when considering the copacking of cholesterol and long chain lipids in biological membranes. Recent single crystal analyses of a cholesteryl ester,[18] phosphatidylethanolamine [21] and cerebroside [22] have shown that the hydrocarbon chains of these complex lipids arrange according to earlier unknown hybrid packing modes. These chain matrices have a striking resemblance in geometry and cross sectional area with the cholesterol packing pattern in cholesteryl sulfate which in principle allows a random replacement of a cholesterol molecule by two hydrocarbon chains of a complex lipid.[23]

Table 3. Intramolecular oxygen-oxygen distances (Å) less than 3.2 Å and their estimated standard deviations (× 10²).

O(A12)---O(1)	(–1,–1,0) [a]	3.17(4)
O(A13)---O(1)	(–1,0,0)	2.94(4)
O(A13)---O(2)	(0,1,0)	2.96(3)
O(B13)---O(3)	(0,0,0)	2.87(4)
O(B13)---O(4)	(1,1,0)	2.93(3)
O(1)---O(2)	(0,1,0)	3.00(4)
O(1)---O(4)	(1,1,–1)	3.14(4)
O(2)---O(3)	(0,–1,–1)	3.07(3)
O(3)---O(4)	(0,1,0)	2.97(4)

[a] The figures within parentheses indicate translations in the directions *a*, *b* and *c* of the second atom.

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