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87. Vitamin D_3 Metabolites III[1]). Synthesis and *X*-ray Analysis of 1α, 25-Dihydroxycholesterol

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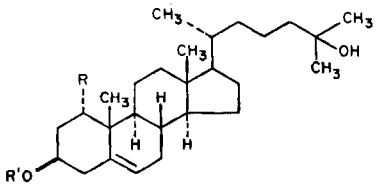
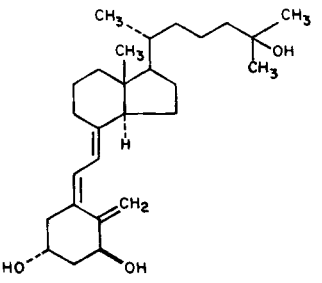
(3. I. 74)

Zusammenfassung. Die Synthese von 1α,25-Dihydroxycholesterin (**1**) aus 3-O-Acetyl-25-hydroxy-cholesterin (**2**) in 18proz. Gesamtausbeute wird beschrieben. Die Struktur von **1** wurde durch *Röntgen*-Strahlendiffraktion bewiesen.

1α,25-Dihydroxycholesterol (**1**) is a key intermediate in the synthesis of 1α,25-dihydroxycholecalciferol (**3**) [2], the most active vitamin D_3 metabolite [3]. We now report a synthesis of **1** from 25-hydroxycholesterol 3-acetate (**2**) [1] [4] which is particularly attractive from a preparative point of view, the overall yield attaining 18%. The structure of synthetic **1** was fully confirmed by *X*-ray analysis.



1 R = OH, R′= H
2 R = H, R′= Ac



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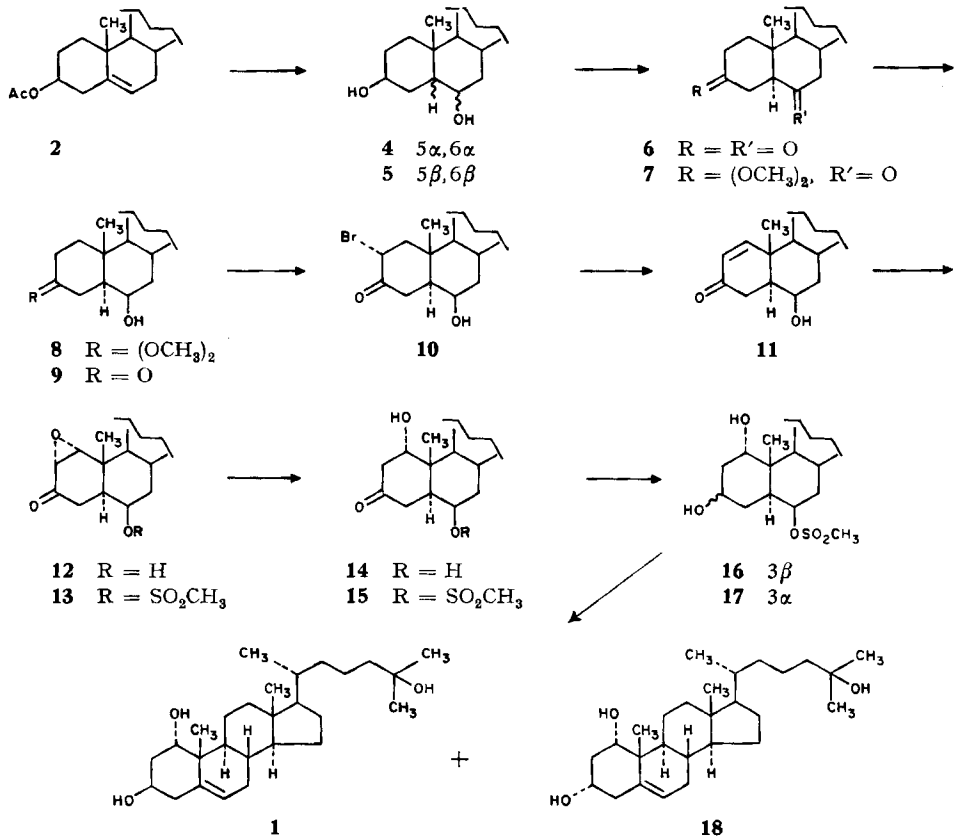
[1]) Part II, see [1].

Hydroboration of 25-hydroxycholesteryl 3-acetate (**2**) [4] with an excess of diborane at 0° followed by addition of alkaline hydrogen peroxide afforded a mixture of alcohols from which the major component was isolated and shown to be triol **4**. None of the minor components were investigated but in analogy with an earlier study of the hydroboration of cholesterol [5] the presence of isomeric triol **5** could be assumed. In subsequent experiments, the crude reaction mixture was oxidized with *Jones* reagent to the crystalline diketone **6** in 74% overall yield. In order to convert the carbonyl at C(6) into a β-hydroxyl group which would later serve as functionality for introduction of the Δ^5-double bond, the carbonyl at C(3) had to be protected. This was accomplished in quantitative yield by heating **6** in dry methanol with a catalytic amount of oxalic acid, thus affording the monoketal **7**. The NMR. spectrum of the crude reaction product showed the presence of only two methoxyl groups, thus indicating complete regioselectivity and stereochemical integrity of the C(5) ring juncture. Heating diketone **6** in dry methanol at 55° with an equal weight of selenium dioxide [6] also produced ketal **7** in quantitative yield. The former procedure, however, was more practical.

Reduction of ketone **7** with sodium borohydride was stereoselective and afforded the dihydroxy ketal **8** in 76% yield. Hydrolysis of **8** in acetonitrile/tetrahydrofuran



with dilute hydrochloric acid proceeded smoothly and led to the ketone diol **9** in 98% yield. Preferably, conversion of diketone **6** into **9** was carried out in a single reaction vessel to give an 88% overall yield without isolation or purification of intermediates **7** and **8**.

Bromination of ketone **9** in dioxane produced the 2α-bromoketone **10** which was dehydrohalogenated to the Δ^1-enone **11**. Epoxyketone **12** was then prepared by treatment of **11** with hydrogen peroxide under basic conditions. The overall yield for the three-step sequence is 71%.

Lithium aluminium hydride reduction of epoxy ketones of type **12** has been shown [7] to be stereochemically non-selective with respect to the carbonyl at C(3). Similarly, sodium borohydride produced a mixture of C(3) epimers containing a significant amount of the undesired 3α-hydroxy isomer [8]. These results suggested the use of a reducing agent which would cleave the epoxide function to give the β-hydroxyketone **14**. Borohydride reduction of **14** was expected to lead more selectively to the desired 1α,3β-arrangement of the ring A hydroxyl groups [2]. Aluminium amalgam was selected as the reagent of choice since it has recently been shown to effect the reductive cleavage of an α,β-epoxyketone [9]. When epoxide **12** was treated with a large excess of aluminium amalgam it was cleanly converted to the crystalline β-hydroxyketone **14** in high yield.

Our attention was then focused on masking the C(6) hydroxyl group of **12** to alleviate the problem of having to distinguish between the chemically similar 1α- and 6β-hydroxyl groups of **14** after epoxide cleavage. Since a methanesulfonate ester could perform this task and also be readily eliminated to form the Δ^5-double bond, **12** was converted to the mesylate **13** (65% yield). During mesylation, a small amount of the $\Delta^{24,25}$-olefin derived from **13** was also formed. Cleavage of epoxy ketone **13** with aluminium amalgam then led to the 1α-hydroxyketone **15**. Sodium borohydride reduction of this ketone gave a separable mixture of epimeric alcohols **16** and **17**. It was more practical, however, to separate the C(3) epimers after the introduction of the Δ^5-double bond. Treatment of the mixture of **16** and **17** with lithium carbonate in dimethylformamide at 115° for 15 min led after chromatography to the desired 1α,25-dihydroxycholesterol (**1**) in 62% overall yield based on epoxymesylate **13**. The crystalline 3α-epimer **18** was obtained in 11% overall yield from **13**.

X-Ray Analysis of 1α,25-Dihydroxycholesterol (**1**). At the time we had prepared triol **1**, there were no physical data reported for this compound in the literature. Also,

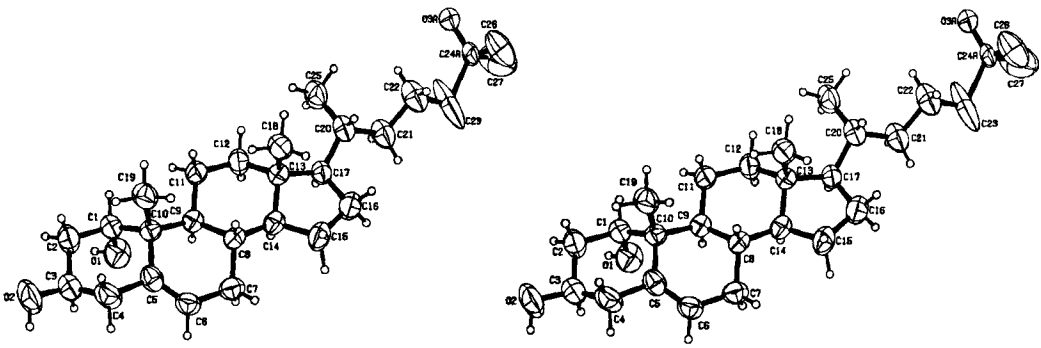


Table I. *Final Atomic Parameters for 1 with Standard Deviations in Parentheses*

Atom	X	Y	Z	B
O(1)	0.4230(3)	0.314	0.3548(6)	*
O(2)	0.4569(4)	0.361(2)	0.6599(8)	*
O(3)A	0.0236(6)	0.089(3)	-0.2567(12)	*
O(3)B	0.0094(9)	0.558(3)	-0.3187(18)	*
C(1)	0.3902(4)	0.232(2)	0.4199(8)	*
C(2)	0.4192(5)	0.210(2)	0.5194(9)	*
C(3)	0.4338(5)	0.389(2)	0.5650(10)	*
C(4)	0.3872(5)	0.509(2)	0.5725(9)	*
C(5)	0.3542(4)	0.522(2)	0.4765(7)	*
C(6)	0.3380(4)	0.677(2)	0.4406(9)	*
C(7)	0.3031(4)	0.700(2)	0.3511(9)	*
C(8)	0.2757(4)	0.527(2)	0.3213(7)	*
C(9)	0.3152(4)	0.374(2)	0.3214(7)	*
C(10)	0.3405(4)	0.342(2)	0.4272(7)	*
C(11)	0.2938(4)	0.200(2)	0.2746(9)	*
C(12)	0.2635(4)	0.228(2)	0.1757(8)	*
C(13)	0.2208(4)	0.369(2)	0.1847(7)	*
C(14)	0.2474(4)	0.537(2)	0.2212(7)	*
C(15)	0.2069(5)	0.681(2)	0.2046(11)	*
C(16)	0.1760(5)	0.622(2)	0.1104(10)	*
C(17)	0.1947(4)	0.433(2)	0.0870(7)	*
C(18)	0.1814(4)	0.303(2)	0.2545(8)	*
C(19)	0.3037(5)	0.239(2)	0.4905(8)	*
C(20)	0.1517(4)	0.317(2)	0.0378(8)	*
C(21)	0.1334(4)	0.399(3)	-0.0629(9)	*
C(22)	0.0821(4)	0.327(3)	-0.1084(9)	*
C(23)	0.0684(5)	0.408(5)	-0.2031(13)	*
C(24)A	0.0242(11)	0.261(4)	-0.2693(24)	*
C(24)B	0.0204(10)	0.379(9)	-0.2545(22)	*
C(25)	0.1670(5)	0.126(3)	0.0215(10)	*
C(26)	-0.0266(4)	0.333(4)	-0.2037(12)	*
C(27)	0.0163(8)	0.320(4)	-0.3663(16)	*
HO(1)	0.453	0.25	0.353	7.0
H(1)	0.380	0.11	0.393	5.0
HO(2)	0.466	0.47	0.689	9.0
H(2)A	0.451	0.14	0.512	5.0
H(2)B	0.398	0.14	0.566	5.0
H(3)	0.458	0.46	0.522	5.0
H(4)A	0.397	0.63	0.596	5.0
H(4)B	0.365	0.45	0.625	5.0
H(6)	0.350	0.79	0.473	5.0
H(7)A	0.324	0.74	0.297	5.0
H(7)B	0.278	0.79	0.367	5.0
H(8)	0.250	0.50	0.373	5.0
H(9)	0.342	0.42	0.279	5.0
H(11)A	0.271	0.15	0.323	5.0
H(11)B	0.323	0.12	0.266	5.0
H(12)A	0.246	0.11	0.152	5.0
H(12)B	0.286	0.27	0.124	5.0
H(14)	0.274	0.56	0.173	5.0
H(15)A	0.225	0.81	0.196	6.0
H(15)B	0.185	0.70	0.263	6.0
H(16)A	0.183	0.70	0.052	6.0
H(16)B	0.139	0.62	0.118	6.0
H(17)	0.222	0.44	0.038	5.0
H(18)A	0.198	0.27	0.320	5.0
H(18)B	0.164	0.19	0.226	5.0
H(18)C	0.155	0.40	0.264	5.0
H(19)A	0.320	0.22	0.558	5.0
H(19)B	0.295	0.12	0.459	5.0
H(19)C	0.271	0.31	0.498	5.0
H(20)	0.122	0.32	0.082	5.0
H(21)A	0.161	0.37	-0.106	6.0
H(21)B	0.130	0.53	-0.051	6.0
H(22)A	0.055	0.35	-0.061	6.0
H(22)B	0.086	0.19	-0.113	6.0
H(25)A	0.197	0.12	-0.020	6.0
H(25)B	0.138	0.06	-0.016	6.0
H(25)C	0.174	0.07	0.084	6.0

* Anisotropic thermal parameters are given in Table II.

the stereochemistry of the C(1) hydroxyl group of 1,25-dihydroxycholecalciferol had not been firmly established. It was decided therefore to confirm the structure of **1** by *X*-ray analysis. The result of the analysis is shown in the Figure and confirms the $1\alpha,3\beta$-arrangement of the ring A hydroxyl groups. It can also be seen that both C(5) and C(6) are trigonal and that the C(5)–C(6) bond is shortened, the C–C distance being 1.32 Å.

Stereodrawing of 1 (see Fig. on p. 783). Only one of the two conformations adopted by the side chain is shown. The thermal ellipsoids are scaled to 50% probability. The hydrogen atoms are shown as spheres of arbitrary size. The hydrogen atoms on C(23), C(26), C(27) and O(3) are not shown.

Crystals of **1**, obtained from acetone, are monoclinic, space group $C2$. There are four molecules in a unit cell of dimensions $a = 26.26\ (2)$, $b = 7.485\ (5)$, $c = 13.674\ (5)$ Å, $\beta = 93.36\ (5)$ °. The intensity data were measured on a *Hilger-Watts* model Y290 diffractometer. Nickel filtered Cu $K\alpha$ radiation and pulse height discrimination were used. Of the 3032 accessible reflections with $\Theta < 76°$, 2019 had intensities significantly greater than background. The structure was solved by a multiple solution procedure [10]. During the preliminary refinement of the structure, it became apparent that some of the atoms in the side chain were disordered. It appeared that this chain randomly assumes either of two conformations in the crystal. The positions of C(24) and O(3) in the two conformations were well resolved and accordingly four half-atoms, C(24)A, C(24)B, O(3)A and O(3)B, were used to describe the disorder. However, the positions of the two methyl groups C(26) and C(27) are about the same in the two conformers, hence it was not possible to represent them by four half-atoms. The two positions of the methylene carbon C(23) were not clearly resolved and, therefore, only one atom was used for C(23).

Refinement of the structure was carried out by full-matrix least squares. Near the end of the refinement hydrogen atoms were introduced at their calculated positions for all atoms except C(23), C(26), C(27) and O(3). In the final cycles of least squares, the hydrogens were included in the structure factor calculations but their parameters were not varied. Anisotropic thermal parameters were used for all atoms except the hydrogens, which had isotropic temperature factors.

Table II. *Final Anisotropic Thermal Parameters for 1 with Standard Deviations in Parantheses*

	4	3	4	4	4	4
Atom	B11x10	B22x10	B33x10	B12x10	B13x10	B23x10
O(1)	17(1)	31(2)	94(6)	10(5)	9(2)	6(10)
O(2)	37(3)	38(3)	127(8)	26(8)	-44(4)	-72(14)
O(3)A	16(3)	19(4)	73(10)	1(7)	-8(4)	2(15)
O(3)B	27(4)	31(6)	145(19)	1(13)	3(7)	77(27)
C(1)	16(2)	19(2)	68(7)	2(6)	-4(3)	-4(11)
C(2)	20(2)	26(3)	71(7)	15(7)	-9(3)	-20(13)
C(3)	21(2)	24(3)	87(9)	3(7)	-12(3)	-12(14)
C(4)	20(2)	26(3)	77(8)	-3(7)	-6(3)	-48(14)
C(5)	13(2)	24(3)	54(6)	5(5)	0(2)	-17(11)
C(6)	16(2)	19(2)	71(7)	-2(5)	0(3)	-24(11)
C(7)	17(2)	16(2)	77(7)	-2(6)	3(3)	-8(11)
C(8)	14(1)	14(2)	56(5)	4(5)	4(2)	9(9)
C(9)	14(1)	16(2)	50(5)	2(4)	2(2)	4(9)
C(10)	14(1)	15(2)	52(5)	1(4)	1(2)	-5(9)
C(11)	18(2)	16(2)	76(7)	11(5)	-8(3)	-20(11)
C(12)	17(2)	21(2)	68(7)	14(6)	-8(3)	-14(12)
C(13)	13(1)	16(2)	52(5)	1(4)	1(2)	2(9)
C(14)	15(2)	17(2)	54(6)	4(5)	4(3)	17(10)
C(15)	23(2)	21(3)	95(10)	26(7)	-8(4)	-5(13)
C(16)	24(2)	22(3)	80(9)	16(7)	-7(4)	6(13)
C(17)	13(1)	20(2)	50(6)	2(5)	-1(2)	10(9)
C(18)	16(2)	26(3)	58(6)	-10(6)	0(3)	28(11)
C(19)	20(2)	27(3)	53(6)	-18(7)	-3(3)	21(11)
C(20)	14(1)	28(3)	55(6)	1(6)	0(2)	10(11)
C(21)	16(2)	38(4)	66(7)	-5(7)	-3(3)	30(14)
C(22)	17(2)	47(5)	68(7)	-9(9)	-1(3)	41(18)
C(23)	14(2)	136(16)	111(12)	-56(15)	-17(4)	261(39)
C(24)A	21(5)	19(5)	114(22)	5(12)	-36(8)	-16(26)
C(24)B	9(4)	108(19)	49(16)	29(24)	5(6)	138(49)
C(25)	25(3)	29(3)	70(8)	8(8)	-15(4)	-18(14)
C(26)	13(2)	59(7)	128(13)	0(10)	2(4)	-22(28)
C(27)	36(5)	56(8)	145(18)	-44(17)	1(7)	5(33)

The final discrepancy index is $R = 0.117$ for the 2019 observed data. The final atomic coordinates and thermal parameters are given in Tables I and II.

The anisotropic temperature factor has the form

$$\exp[-(h^2B_{11} + k^2B_{22} + l^2B_{33} + 2hkB_{12} + 2hlB_{13} + 2klB_{23})].$$

Experimental Section

For technical data see [1].

We express our gratitude to the staff of the Physical Chemistry Department of *Hoffmann-La Roche Inc.* for their assistance in this work.

25-Hydroxy-5α-cholestan-3,6-dione (**6**). To an ice-cooled solution of 50.00 g (0.113 mol) of acetate **2** in 75 ml of anhydrous tetrahydrofuran was added dropwise 0.465 mol (465 ml) of borane (1 M tetrahydrofuran solution). The mixture was stirred in an ice bath for 1.5 h and at room temperature for 1.5 h, then cooled in an ice bath, and 140 ml of 3 N sodium hydroxide solution was added dropwise followed by the dropwise addition of 140 ml of 30% hydrogen peroxide. Stirring was continued in the ice bath for 0.4 h and then at room temperature for 0.5 h. The total was then poured into 1500 ml of saturated brine and extracted with 500 ml and 6 × 300 ml of chloroform. The combined organic extracts were washed with 1 l of water, dried over anhydrous sodium sulfate, filtered, and the solvent evaporated *in vacuo*, to give 51.0 g of crude product. [A small portion of material was chromatographed and eluted with ethyl acetate/acetone 10:1 to give **4**. The analytical sample was obtained after several recrystallizations from 95% ethanol: m.p. 218–219°; $[\alpha]_D^{25} = +56.12$ ($c = 0.8357$, $CHCl_3$) – IR. (KBr): 3375 cm^{-1} (strong and broad, –OH's) – NMR. ($SO(CD_3)_2$): δ 4.34 (*d*, 1H, $J = 4$ Hz, –*OH*); 4.15 (*d*, 1H, $J = 6$ Hz, –*OH*); 3.95 (*s*, 1H, –*OH*); 1.04 (*s*, 6H, $-COH(CH_3)_2$); 0.88 (*d*, 3H, $J = 5$ Hz, C(21)); 0.72 (*s*, 3H, C(19)); 0.61 (*s*, 3H, C(18)) – MS.: *m/e* 403 (*M* – OH).

$C_{27}H_{48}O_3$ (420.68) Calc. C 77.09 H 11.50% Found C 76.77 H 11.31%

The suspension of the total finely ground crude product in 575 ml of ice cooled acetone was added to 73.5 ml of *Jones* reagent (27.72 g chromium trioxide, 23 ml of conc. sulfuric acid diluted to 100 ml with water). The mixture was stirred 0.5 h at ice bath temperature, 2 h at room temperature, and then refrigerated at 0° for 12 h. The excess *Jones* reagent was destroyed with 2-propanol, the solution decanted away from the precipitate and poured into 800 ml of water. The precipitate was washed with 9 × 250 ml of dichloromethane and the washings were used to extract the aqueous layer. The combined dichloromethane washings were washed with saturated brine, dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to give 52.9 g of crude product. Recrystallization from acetone afforded two crops of **6** (total weight 34.7 g, 72% yield). Several recrystallizations from acetone afforded the analytical sample: m.p. 184–188°: $[\alpha]_D^{25} = +3.62°$ ($c = 1.0279$, $CHCl_3$). – IR.: 3615 cm^{-1} (–OH), 1712 (2 × C=O). – NMR. ($CDCl_3$): δ 1.21 (*s*, 6H, $-COH(CH_3)_2$); 0.96 (*s*, 3H, C(19)); 0.94 (*d*, 3H, $J = 5$ Hz, C(21)); 0.70 (*s*, 3H, C(18)). – MS.: *m/e* 398 (*M* – H_2O).

$C_{27}H_{44}O_3$ (416.62) Calc. C 77.83 H 10.65% Found C 77.86 H 11.00%

25-Hydroxy-3,3-dimethoxy-5α-cholestan-6-one (**7**). To a solution of 1.0 g (2.4 mmol) of diketone **6** in 75 ml of dry methanol was added 30 mg of oxalic acid. The mixture was refluxed for 16 h with the condensate passing over 15 g of Type 3A molecular sieves. The solvent was evaporated *in vacuo*, the residue dissolved in 100 ml of chloroform, the solution was wahed with 50 ml of 2 N sodium carbonate, and then with 50 ml of water. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to give 1.10 g (100% yield) of ketal **7** as a semi-solid which was not further purified: $[\alpha]_D^{25} = -3.85°$ ($c = 1.0382$, $CHCl_3$). – IR. ($CHCl_3$): 3270 cm^{-1} (–OH), 1700 (–C=O). – NMR. ($CDCl_3$): δ 3.24 (*s*, 3H, $-OCH_3$); 3.10 (*s*, 3H, $-OCH_3$); 1.22 [*s*, 6H, $-COH(CH_3)_2$]; 0.93 (*d*, 3H, $J = 5$ Hz, C(21)); 0.75 (*s*, 3H, C(19)); 0.67 (*s*, 3H, C(18)). – MS.: *m/e* 462 (*M*).

$C_{20}H_{50}O_4$ (462.72) Calc. C 75.28 H 10.80% Found C 75.12 H 10.20%

6β,25-Dihydroxy-3,3-dimethoxy-5α-cholestane (**8**). To an ice-cooled solution of 13.8 g (30 mmol) of ketal **7** in 250 ml of methanol was added dropwise a solution of 1.2 g (30 mmol) of sodium borohydride in 10 ml of water (stabilized with several drops of 1 N sodium hydroxide solution). After 1 h, tlc. (silica gel, ethyl acetate) indicated absence of starting material. The mixture was poured

into 1 l of water and extracted with chloroform (emulsions can be broken up by addition of saturated brine). The combined extracts were dried over sodium sulfate, filtered, and evaporated. Crystallization from acetone (50 ml) gave 8.68 g of diol **8**. The mother liquors were chromatographed on 430 g of silica gel and eluted with ethyl acetate/dichloromethane 1:1. An additional 1.88 g of **8** was obtained for a total of 10.56 g (76% yield). The analytical sample was obtained after several recrystallizations from acetone: m.p. 162–165°; $[\alpha]_D^{25} = +13.35°$ ($c = 0.9365$, $CHCl_3$). – IR. ($CHCl_3$): 3620 cm^{-1} (–OH). – NMR. ($CDCl_3$): δ 3.79 (*m*, 1H, –C*H*OH); 3.21 (*s*, 3H, CH_3O–); 3.13 (*s*, 3H, CH_3O–); 1.20 (*s*, 6H, C(26) and C(27)); 1.01 (*s*, 3H, C(19)); 0.92 (*d*, 3H, $J = 6$ Hz, C(21)); 0.68 (*s*, 3H, C(18)). – MS.: *m/e* 464 (*M*).

$C_{29}H_{54}O_2$ (464.73) Calc. C 74.95 H 11.28% Found C 75.19 H 11.23%

6β,25-Dihydroxy-5α-cholestan-3-one (**9**). To 2.39 g (5.1 mmol) of diol **8** dissolved in 125 ml of acetonitrile/tetrahydrofuran 1:1 at room temperature was added 5 ml of 6N hydrochloric acid. After stirring for 0.5 h, tlc. (ethyl acetate/dichloromethane 4:1) indicated absence of starting material. The mixture was poured into 125 ml of water and extracted with chloroform. The combined organic extracts were dried over sodium sulfate and evaporated *in vacuo*. Crystallization of the residue from acetone afforded 1.49 g of ketone **9**. The mother liquors were chromatographed on 70 g of silica gel and eluted with ethyl acetate/dichloromethane 4:1. An additional 0.63 g of **9** was obtained for a total of 2.12 g (98% yield). Several recrystallizations from acetone gave the analytical sample: m.p. 192–194°; $[\alpha]_D^{25} = +16.4°$ ($c = 0.580$, $CHCl_3$). – IR. ($CHCl_3$): 3610 cm^{-1} (–OH), 1705 (–C=O). – NMR. ($CHCl_3$): δ 3.72 (broad, 1H, –C*H*–OH); 1.21 (*s*, 9H, C(19), C(26), C(27)); 0.93 (*d*, 3H, $J = 5$ Hz, C(21)); 0.72 (*s*, 3H, C(18)). – MS.: *m/e* 400 ($M - H_2O$).

$C_{27}H_{46}O_3$ (418.66) Calc. C 77.46 H 11.08% Found C 77.73 H 11.14%

6β,25-Dihydroxy-5α-cholestan-3-one (**9**) *from 25-Hydroxy-5α-cholestan-3,6-dione* (**6**) *without Isolation of Intermediates* **7** *and* **8**. To the diketone **6** (3.0 g, 7.2 mmol) suspended in 125 ml of anhydrous methanol was added 25 mg of oxalic acid. The mixture was refluxed and the condensate passed over molecular sieves. After 3 h, tlc. (dichloromethane/ethyl acetate 9:1) indicated complete reaction. The mixture was cooled to room temperature and solid sodium borohydride was added in 100, 25, and 50 mg batches (total, 4.6 mmol). When tlc. indicated no more starting material, 100 ml of water, 50 ml of acetonitrile, and 6 ml of 6N hydrochloric acid were added. The mixture was stirred for 1 h at room temperature, then poured into water, and extracted with chloroform. The organic phase was dried over sodium sulfate and filtered. Evaporation of the solvent *in vacuo* and crystallization of the residue afforded three crops of **9** for a total of 2.64 g (88% yield). The product was identical to material prepared by the stepwise procedure above.

2α-Bromo-6β,25-dihydroxy-5α-cholestan-3-one (**10**). To a solution of 50.16 g (120 mmol) of ketone **9** in 480 ml of anhydrous dioxane, under a nitrogen atmosphere and cooled in ice water was added rapidly 212 ml of a 0.6M solution of bromine (ca. 127 mmol) in dioxane. Immediately after this addition the pale yellow mixture was poured into 1680 ml of saturated sodium hydrogen carbonate solution. The white precipitate was filtered, dried under high vacuum over phosphorus pentoxide, and crystallized from 400 ml of 95% ethanol (ice bath) to give 46.7 g (two crops) of bromide **10**. The mother liquors (14.6 g) were chromatographed and eluted with ethyl acetate/dichloromethane 1:1 to give an additional 3.5 g of **10** for a total weight of 50.2 g (84% yield). Recrystallization from 95% ethanol gave the analytical sample: m.p. 174–176°; $[\alpha]_D^{25} = +25.62°$ ($c = 1.015$, $CHCl_3$). – IR. ($CHCl_3$): 3610 cm^{-1} (–OH), 1725 (–C=O). – NMR. ($CDCl_3$): δ 4.76 (*d* of *d*, 1H, $J = 13$ Hz, $J = 6$ Hz, –COC*H*Br); 3.77 (*m*, 1H, –C*H*OH); 1.26 (*s*, 3H, C(19)); 1.19 (*s*, 6H, –COH(CH_3)$_2$); 0.92 (*d*, 3H, $J = 6$ Hz, C(21)); 0.71 (*s*, 3H, C(18)). – MS.: *m/e* 478 ($M - H_2O$).

$C_{27}H_{45}BrO_3 \cdot {}^1/_3H_2O$ (503.52) Calc. C 64.41 H 9.14 Br 15.87%
Found ,, 64.40 ,, 9.13 ,, 15.87%

6β,25-Dihydroxy-5α-cholest-1-en-3-one (**11**). A mixture of 7.11 g (14.3 mmol) of bromide **10**, 7.11 g (82 mmol) of lithium bromide, and 7.11 g (96.4 mmol) of lithium carbonate in 70 ml of dry dimethylformamide under a nitrogen atmosphere was heated at 170° for 0.5 h, then cooled at room temperature, poured into 1 l of water, and extracted with ethyl acetate. The organic extracts were washed with water, dried over sodium sulfate, filtered, and evaporated *in vacuo*. The crystalline residue was recrystallized from acetone giving three crops of enone **11**, 5.21 g. The mother liquors were chromatographed on 76 g of silica gel and eluted with ethyl acetate-dichloromethane affording an additional 0.157 g of **11** for a total weight of 5.36 g (90% yield). The analytical

sample was recrystallized from acetone: m.p. 191–194°; $[\alpha]_D^{25} = +28.99°$ ($c = 1.0005$, $CHCl_3$). – IR. ($CHCl_3$): 3620 (–OH), 1670 cm^{-1} (–C(O)–CH=CH–). – NMR. ($CDCl_3$): δ 7.05 (*d*, 1H, $J =$ 10 Hz, –C*H*=); 5.79 (*d*, 1H, $J = 10$ Hz, –C(O)–C*H*=C); 3.88 (*m*, 1H, –C*H*OH); 1.18 (*s*, 9H, C(19), C(26), C(27)); 0.91 (*d*, 3H, $J = 3$ Hz, C(21)); 0.70 (*s*, 3H, C(18)). – UV.: λ_{max}^{EtOH} 230 nm (enone, $\varepsilon = 9{,}000$). – MS.: *m/e* 416 (*M*).

$C_{27}H_{44}O_3$ (416.65) Calc. C 77.84 H 10.64% Found C 77.69 H 10.60%

1α,2α-Epoxy-6β,25-dihydroxy-5α-cholestan-3-one (**12**). To a solution of 5.27 g (12.6 mmol) of enone **11** in 170 ml of methanol, cooled in an ice/salt bath, was added with rapid stirring 12.1 ml of 30% hydrogen peroxide followed immediately by 6.3 ml of 6N sodium hydroxide solution. After 0.5 h, tlc. (ethyl acetate/dichloromethane 4:1) indicated that the reaction was complete. The mixture was poured into 175 ml of water and extracted with chloroform. The combined extracts were dried over sodium sulfate, filtered, and the solvent evaporated *in vacuo*. The solid residue was crystallized from acetone affording 5.07 g of epoxide **12**. The mother liquors were chromatographed on 4.0 g of silica gel and eluted with dichloromethane/ethyl acetate 3:1 affording an additional 0.132 g of **12** for a total of 5.2 g (95% yield). The analytical sample was obtained by several recrystallizations from acetone; m.p. 197–199°; $[\alpha]_D^{25} = +86.52°$ ($c = 0.8830$, $CHCl_3$). – IR. ($CHCl_3$): 3615 (–OH's), 1720 cm^{-1} (–C=O). – NMR. ($CDCl_3$): δ 3.81 (*m*, 1H, –C*H*OH); 3.40 (*d*, 1H, $J = 4$ Hz, –CO–C*H*(O)–C*H*–); 3.20 (*d*, 1H, $J = 4$ Hz, –CO–C*H*(O)–C*H*–); 1.20 (*s*, 6H, C(26) and C(27)); 1.05 (*s*, 3H, C(19)); 0.93 (*d*, 3H, $J = 6$ Hz, C(21)); 0.72 (*s*, 3H, C(18)). – MS.: *m/e* 414 ($M - H_2O$).

$C_{27}H_{44}O_4$ (432.65) Calc. C 74.96 H 10.25% Found C 74.94 H 10.15%

1α,2α-Epoxy-25-hydroxy-6β-methylsulfonyloxy-5α-cholestan-3-one (**13**) *and 1α,2α-Epoxy-6β-methylsulfonyloxy-5α-cholest-24-en-3-one*. To a rapidly stirred, cooled solution (– 6°) of 3.11 g (7.2 mmol) of epoxide **12** in 8 ml of dry pyridine was added 0.876 g (0.6 ml) of methanesulfonyl chloride. After 12 h, tlc. (dichloromethane/ethyl acetate 2:1) indicated the presence of starting material, more methanesulfonyl chloride (0.090 g) was added, the mixture kept at – 6° for an additional 16 h, then poured into 50 ml of water, and extracted with 3 × 40 ml of methylene chloride. The combined organic extracts were washed with 80 ml of 1N hydrochloric acid, followed by 80 ml of water, and dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent *in vacuo*, the residue (4.0 g) was chromatographed on 300 g of silica gel and eluted with dichloromethane/ethyl acetate 2:1.

Eluted first from the column was 0.504 g of *1α,2α-epoxy-6β-methylsulfonyloxy-5α-cholest-24-en-3-one* (14% yield). Several recrystallizations from ethanol gave the analytical sample: m.p. 162–164.5°; $[\alpha]_D^{25} = +52.53°$ ($c = 0.9995$, $CHCl_3$). – IR. (KBr): 1717 cm^{-1} (–C=O), 1353, 1180 (–OSO_2CH_3), 907 (epoxide). – NMR. ($CDCl_3$): δ 5.06 (*m*, 1H, –C*H*=); 4.77 (*m*, 1H, –C*H*–OSO_2CH_3); 3.43 (*d*, 1H, $J = 5$ Hz, –C(O)–C*H*(O)–C); 3.23 (*d*, 1H, $J = 5$ Hz, –C(O)–C(O)–C*H*); 2.96 (*s*, 3H, –OSO_2CH_3); 2.34 (*s*, 2H, –C*H*$_2$–C(O)–); 1.66 (*s*, 3H, =C(*CH*$_3$)–CH$_3$); 1.59 (*s*, 3H, =C(CH$_3$)–*CH*$_3$); 1.00 (*s*, 3H, C(19)); 0.92 (*d*, 3H, $J = 5$ Hz, C(21)); 0.72 (*s*, 3H, C(18)). – MS.: *m/e* 492 (*M*).

$C_{28}H_{44}O_5S$ (492.72) Calc. C 68.26 H 9.00 S 6.51% Found C 68.27 H 8.78 S 6.41%

Continued elution then afforded 2.58 g of mesylate **13**. Recrystallization from ether gave 2.39 g (65%) of product. Several recrystallizations from acetone/petroleum ether gave the analytical sample: m.p. 95–96°; $[\alpha]_D^{25} = +39.44°$ ($c = 1.0430$, $CHCl_3$). – IR. (KBr): 3500 cm^{-1} (–OH), 1720 (–C=O), 1353, 1180 (–OSO_2CH_3), 907 (epoxide). – NMR. ($CDCl_3$): δ 4.76 (*m*, 1H, –C*H*OSO_2CH_3); 2.32 (*s*, 2H, *HC*(4)); 1.28 (*s*, 6H, –COH(C*H*$_3$)$_2$); 0.99 (*s*, 3H, C(19)); 0.91 (*d*, 3H, $J = 5$ Hz, C(21)); 0.71 (*s*, 3H, C(18). – MS.: *m/e* 492 ($M - H_2O$).

$C_{28}H_{46}O_6S$ (510.73) Calc. C 65.84 H 9.08 S 6.28% Found C 65.93 H 9.11 S 6.10%

1α,25-Dihydroxycholesterol (**1**) *and 1α,25-Dihydroxy-epicholesterol* (**18**). To 2.04 g (4 mmol) of *1α,2α-epoxy-25-hydroxy-6β-methylsulfonyloxy-5α-cholestan-3-one* (**13**) dissolved in 25 ml of 95% ethanol containing 1.5 ml of 10% sodium hydrogen carbonate and cooled to – 15° was added an excess of aluminium amalgam (8–10 g). The mixture was stirred at –15° and monitored until tlc. showed the complete disappearance of starting material. The reaction mixture was diluted with

100 ml of chloroform, filtered through a pad of Celite, and the solvent evaporated *in vacuo* to give 2.0 g of crude *1α,25-dihydroxy-6β-methylsulfonyloxy-5α-cholestan-3-one* (**15**) (used in the next step). – NMR. ($CDCl_3$) δ 4.80 (*m*, 1H, –C*H*OSO$_2$CH$_3$); 4.0 (*m*, 1H, –C*H*OH); 3.0 (*s*, 3H, –OSO$_2$C*H*$_3$); 1.22 (*s*, 6H, –COH(C*H*$_3$)$_2$); 1.12 (*s*, 3H, C(19)); 0.93 (*d*, 3H, $J = 6$ Hz, C(21)); 0.73 (*s*, 3H, C(18)).

To the solution of 2 g of crude **15** in 20 ml of 95% ethanol, cooled to – 5°, was added dropwise 0.5 ml of a solution containing 0.042 g (1.1 mmol) of sodium borohydride and 1.20 ml of 1 N sodium hydroxide. After 0.5 h an additional 0.010 g of solid sodium borohydride was added and stirring continued for an additional 0.5 h. The excess borohydride was destroyed with 3 ml of 10% acetic acid, the mixture diluted with 50 ml of water, and extracted with 4 × 50 ml of chloroform. The combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* to give 2.1 g of a mixture of crude **16** and **17** (used in the next step). – NMR. ($CDCl_3$): δ 4.85 (*m*, 1H, –C*H*OSO$_2$CH$_3$); 4.03 (broad *m*, 1H, –C*H*OH); 3.75 (*m*, 1H, –C*H*OH); 3.0 (*s*, 3H, –CHOSO$_2$C*H*$_3$).

To the solution of 2.1 g of crude **16** and **17** in 25 ml of dry dimethylformamide at room temperature under a nitrogen atmosphere was added 2.2 g (0.027 mol) of lithium carbonate. After heating at 115° for 1.33 h, the mixture was cooled, poured into 100 ml of water, and extracted with 4 × 50 ml of ethyl acetate. The combined organic extracts were washed with 5 × 50 ml of water, dried over anhydrous sodium sulfate, filtered, and the solvent evaporated *in vacuo* to give 1.7 g of residue. Crystallization from acetone afforded 0.952 g of material which was chromatographed on 72 g silica gel with 95% chloroform – 5% methanol to give 0.717 g of **1** (tlc. homogeneous).

Column chromatography (ethyl acetate) of the residue (0.838 g) from the mother liquors gave 0.111 g of the less polar *epicholesterol derivative* **18**. Recrystallization from acetone gave the analytical sample: m.p. 197–199°; $[\alpha]_D^{25} = -4.68°$ ($c = 0.8545$, $CHCl_3$). – IR. (KBr): 3350 cm^{-1} (broad, –OH). – NMR. ($CDCl_3$ + MeOD): δ 5.56 (*m*, 1H, –C*H*=); 4.08 (*m*, 1H, –C*H*OH); 3.73 (*m*, 1H, –C*H*OH); 1.19 (*s*, 6H, –COH(C*H*$_3$)$_2$); 0.97 (*s*, 3H, C(19)); 0.92 (*d*, 3H, $J = 5$ Hz, C(21)); 0.68 (*s*, 3H, C(18)). – MS.: *m/e* 418 (*M*), *m/e* 400 (*M* – H_2O), *m/e* 382 (*M* – 2H_2O).

$C_{27}H_{46}O_3$ (418.66) Calc. C 77.46 H 11.08% Found C 77.42 H 11.08%

Further elution with the same solvent system afforded an additional 0.297 g of *1α,25-dihydroxycholesterol* (**1**) (tlc. homogeneous) for a total weight of 1.01 g (62% overall yield from **13**). Several recrystallizations from ethyl acetate gave the analytical sample: m.p. 162–164°; $[\alpha]_D^{25} = -36.9°$ ($c = 0.13$, $CHCl_3$). – IR. (KBr): 3400 cm^{-1} (broad –OH). – NMR. ($CDCl_3$): δ 5.59 (*m*, 1H, –C*H*=); 3.89 (broad *m*, 1H, –C*H*OH); 3.83 (*m*, 1H, –C*H*OH); 1.19 [*s*, 6H, –COH(C*H*$_3$)$_2$]; 1.02 (*s*, 3H, C(19)); 0.92 (*d*, 3H, $J = 5$ Hz, C(21)); 0.67 (*s*, 3H, C(18)). – MS.: *m/e* 418 (*M*), *m/e* 400 (*M* – H_2O).

$C_{27}H_{46}O_3$ (418.66) Calc. C 77.46 H 11.08% Found C 77.26 H 11.27%

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