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85. Vitamin D3 Metabolites I. Synthesis of 25-Hydroxycholesterol

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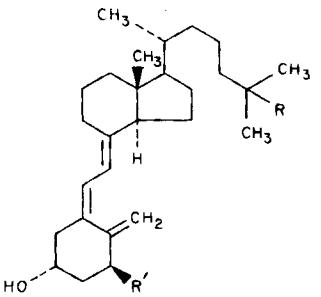
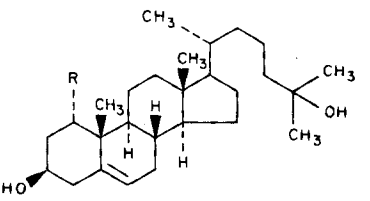
(3. I. 74)

Zusammenfassung. 25-Hydroxycholesterin (**4**) ist in 30proz. Ausbeute in sieben Schritten aus Stigmasterin (**6**) hergestellt worden. Der wichtigste Schritt ist die Umsetzung des Tosylats **11** mit dem Acetylenderivat **13** zu **14** unter Bildung des vollständigen Cholesteringerüsts.

Significant breakthroughs have been made with respect to the structures and the modes of action of the biologically active forms of vitamin D_3 (**1**) [1]. 25-Hydroxycholecalciferol (**2**) [2] and 1α,25-dihydroxycholecalciferol (**3**) [3], the major metabolites of cholecalciferol (**1**), are more potent than **1** in all three criteria of physiological activity, namely, increased calcium transport, bone mineral mobilization, and calcification [1]. Since hydroxylation occurs first in the liver (25-OH) and then in the kidney (1α-OH) before the onset of activity [1], cholecalciferol (**1**) should be viewed



1 R = R′ = H
2 R = OH, R′ = H
3 R = R′ = OH

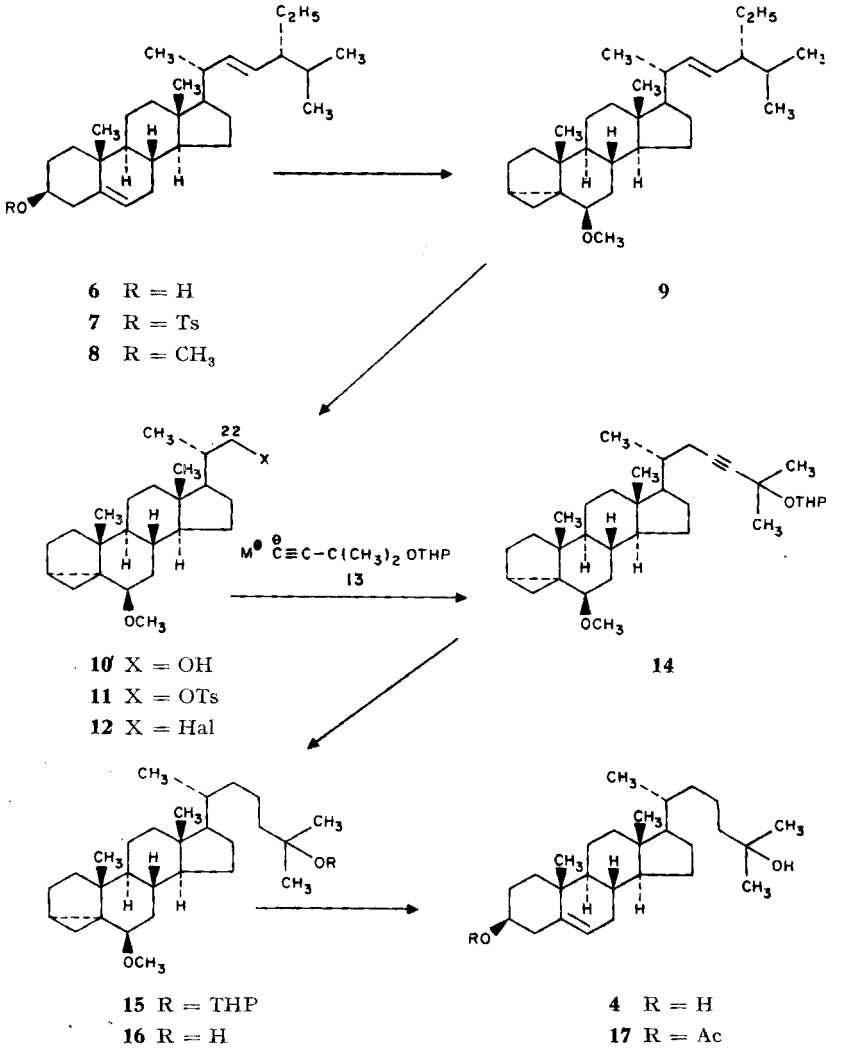


4 R = H
5 R = OH

as the storage form of the vitamin possessing no activity of its own. Thus, the active forms of vitamin D_3, **2** and **3**, are important targets for synthesis [4–6]. In this and the following papers [7] [8] are described several practical syntheses of 25-hydroxycholesterol (**4**) and 1α,25-dihydroxycholesterol (**5**), key intermediates in the preparation of the metabolites **2** and **3**.

25-Hydroxycholesterol (**4**), a minor autoxidation product of cholesterol, has been employed in the syntheses of 25-hydroxycholecalciferol (**2**) [4] and 1α,25-dihydroxycholecalciferol (**3**) [6]. However, in all published accounts to date, 25-hydroxycholesterol (**4**) and similar starting materials were prepared from minor oxidation by-products of 5,6-dibromocholesteryl acetate which are no longer commercially

Scheme



6 R = H
7 R = Ts
8 R = CH_3

9

10 X = OH
11 X = OTs
12 X = Hal

14

15 R = THP
16 R = H

4 R = H
17 R = Ac

available in large quantity. In this report we describe a seven-step synthesis of 25-hydroxycholesterol (**4**) from the readily available plant sterol, stigmasterol (**6**), which is isolated commercially from soybeans [9].

From degradation studies on stigmasterol it is known that the 22,23-double bond is relatively hindered. Thus, the 5,6-double bond must be protected before cleavage of the 22,23-double bond can be carried out efficiently [10]. With this in mind, it seemed that *i*-stigmasteryl methyl ether (**9**) [11] was a logical protected intermediate (*Scheme*).

Stigmasteryl tosylate (**7**) [12] was readily prepared and treated with methanol and pyridine to yield the *i*-methyl ether **9**, in 75% overall yield after separation of the minor product stigmasteryl methyl ether (**8**) by crystallization. The *i*-methyl ether **9** was ozonized at −78° and the ozonide was decomposed under a variety of reducing conditions. Optimum yields (65%) of alcohol **10** were obtained by ozonization in dry methylene chloride containing 1% pyridine, followed by addition of sodium bis-(2-methoxyethoxy)-aluminium hydride in benzene. The amorphous alcohol was fully characterized as the crystalline O-acetyl derivative.

Alcohol **10** was converted into the crystalline tosylate **11** by treatment with *p*-toluenesulfonyl chloride in pyridine at 0° for 3 hours (92% yield). Reaction for a longer time resulted in the production of a considerable amount of the corresponding chloro derivative. This side-reaction illustrates the ease with which certain nucleophiles effect displacement at the primary C(22) center. For example, tosylate **11** was readily converted into iodide **12** (X = I) on treatment with sodium iodide in acetone under mild conditions.

From published analogies [13], we believed that metallated derivatives of 3-methyl-1-butyn-3-yl tetrahydropyranyl ether (**13**) could be C-alkylated by tosylate **11** or iodide **12** (X = I). However, this goal was not immediately realized. When tosylate **11** was treated with the chloromagnesium derivative **13** (M = MgCl) in a variety of solvents, no reaction occurred. Addition of hexamethylphosphoramide as a co-solvent resulted in the formation of the C(22) chloride derivative **12** (X = Cl). Under the same conditions the bromomagnesium acetylide **13** (M = MgBr) yielded the bromo derivative **12** (X = Br) as the major product, but formed none of the desired alkylation product **14**. Substance **14** was finally prepared in 55–65% yield by treatment of either tosylate **11** or iodide **12** (X = I) with **13** (M = Li) in hexamethylphosphoramide-hexane. The corresponding chloro derivative, however, was again formed as a major by-product. Thus, in all conditions used, halide introduction at C(22) constituted a serious side reaction. Clearly, competing halide ions had to be removed before the metallated acetylides **13** could effectively react with tosylate **11**. Therefore, the lithium acetylide **13** (M = Li) was formed in dioxane solution [14] using commercial *n*-butyllithium and 3-methyl-1-butyn-3-yl tetrahydropyranyl ether. The lithium chloride present in commercial *n*-butyllithium was precipitated as the insoluble lithium chloride-dioxane complex and chloride formation was no longer a problem. Thus, when a one-fold excess of this lithium acetylide **13** (M = Li) was heated at reflux with tosylate **11**, reproducible 90% yields of the desired alkylation product **14** were achieved. In this manner the entire carbon framework for 25-hydroxycholesterol was assembled in one step.

The acetylenic bond of **14** was hydrogenated to the saturated ether **15** in quantitative yield using either platinum oxide or 10% Pd/C catalysts in dioxane solution, and no hydrogenolysis of the C(25) oxygen function was observed [15]. Hydrogenation was also carried out successfully in either buffered methanol or ethanol. In the absence of sodium hydrogen carbonate buffer, traces of acidic impurities catalyzed the retro-*i*-rearrangement to give unwanted 3β-methyl and ethyl ethers.

The retro-*i*-rearrangement of the cyclopropyl group of ether **15** was accomplished in various ways. Compound **15** was directly converted into 25-hydroxycholesterol (**4**) in 77% yield by treatment with acidic aqueous dioxane at 80° [16], and into acetate **17** (89%) on exposure to acetic acid at 70°. However, for large scale work it was more advantageous to convert the oily tetrahydropyranyl ether **15** into the highly crystalline alcohol **16** [7] by brief treatment with acidic methanol at 0°, and then rearrange this substance to **4** with acidic aqueous dioxane (75% overall yield). Similarly, alcohol **16** was converted into 25-hydroxycholesteryl 3-acetate (**17**) in 94% yield on heating at 70° with glacial acetic acid. 25-Hydroxycholesterol prepared in the above manner was identical spectrally with authentic material [17], and was readily converted into the 3-acetate derivative **17** [17] on exposure to acetic anhydride in pyridine.

Thus, either 25-hydroxycholesterol (**4**) or its 3-acetate **17** could be prepared efficiently from stigmasterol (**6**) in over 30% yield. The ready availability of these substances makes possible the preparation of the vitamin D_3 metabolites **2** and **3** in quantity.

Experimental Section

Melting points were determined on a *Rinco* Model M-50 melting point apparatus and are uncorrected. IR. spectra were obtained using a *Beckman* IR-9 spectrophotometer. *Raman* data were taken with a *Spex* 1401 double spectrometer using a *Spectra Physics* laser (4880 Å line) for excitation. A *Cary* 14 recording spectrophotometer was used for UV. absorption spectra. A *Perkin Elmer* Model 141 polarimeter was employed for optical rotation measurements. NMR. spectra were determined with *Varian* T-60 and HA-100 spectrometers using tetramethylsilane as the internal reference. Mass spectra were recorded on a *CEC* 21-110B mass spectrometer at 70 eV using a direct insertion probe. Thin layer chromatography (TLC.) was carried out using *Merck* F-254 silica gel plates. The usual work-up of organic solutions means successive washing with 10% sulfuric acid and saturated aqueous sodium hydrogen carbonate, drying over anhydrous magnesium sulfate, and evaporating to dryness.

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

Stigmasteryl Tosylate (**7**) (method of *Steele & Mosettig* [12]). To a solution of 200.0 g (0.485 mol) of stigmasterol (**6**) and 1600 ml of dry pyridine was added 185.0 g (0.970 mol) of *p*-toluenesulfonyl chloride. After stirring at 25° for 16 h the solution was slowly poured into 10% potassium hydrogen carbonate solution. The precipitate was collected by filtration, washed with water and dried *in vacuo* overnight to yield 272.0 g (99%) of stigmasteryl tosylate (**7**), m.p. 141–145°. An analytical sample, prepared by two recrystallizations from acetone, exhibited: m.p. 148–149° (lit. [12] m.p. 147–148°); $[\alpha]_D^{25} = -50.0°$ ($c = 1.04$, $CHCl_3$) (lit. [12] $[\alpha]_D^{25} = -49°$) and gave correct analytical figures for C, H, and S.

i-*Stigmasteryl Methyl Ether* (**9**) *and Stigmasteryl Methyl Ether* (**8**). A solution of 160.0 g (0.282 mol) of stigmasteryl tosylate (**7**) in 1600 ml of methanol and 67 g (0.486 mol) of pyridine was stirred at 75° for 3 h, cooled, concentrated under reduced pressure, poured into water, and extracted with ethyl acetate. The ethyl acetate solution yielded 130.0 g of colorless semisolid by the usual work-up.

Crude stigmasteryl methyl ether (**8**, 28.5 g) was isolated by crystallization of the mixture from acetone-hexane. An analytical sample was obtained by two additional recrystallizations from acetone: white solid, m.p. 120–121°; $[\alpha]_D^{25} = -55.2°$ ($c = 1.16$, $CHCl_3$). – IR. ($CHCl_3$) 1120, 1100 and 975 cm^{-1}. – NMR. ($CDCl_3$) δ 5.31 (*m*, *H*-6); 5.06 (*m*, —C*H*=C*H*—); 3.29 (*s*, OCH_3); 3.01 (*m*, —C*H*—OCH_3); 0.96 (*s*, H_3C—C(19)) and 0.66 (*s*, H_3C—C(18)). – Molecular ion *m/e* 426.

$C_{30}H_{50}O$ (426.73) Calc. C 84.43 H 11.81% Found C 84.74 H 11.91%

The mother liquors contained 90.0 g (75%) of practically pure *i*-stigmasteryl methyl ether (**9**). A small sample of **9** was recrystallized from acetone at 0° to yield colorless cubes: m.p. 52–53° (lit. [11] m.p. 54–55°); $[\alpha]_D^{25} = +34.0°$ ($c = 1.02$, $CHCl_3$) (lit. [11] $[\alpha]_D^{24} = +34.7°$). – IR. ($CHCl_3$) 1090, 1070, and 970 cm^{-1}. – NMR. ($CDCl_3$) δ 5.09 (*m*, —C*H*=C*H*); 3.30 (*s*, OCH_3); 2.75 (*t*, $J = 2.5$ Hz, C*H*—OCH_3); 1.02 (*s*, H_3C—C(19)) and 0.73 (*s*, H_3C—C(18)). – Molecular ion *m/e* 426; correct analytical figures for C and H.

(20S)-20-Hydroxymethyl-6β-methoxy-3α,5-cyclo-5α-pregnane (**10**). A solution of 20.0 g (0.047 mol) of *i*-stigmasteryl methyl ether (**9**) in 400 ml of methylene chloride and 4 ml of pyridine was cooled to –78° and treated with ozonized oxygen (0.056 mol O_3, 20% excess). The reaction vessel was flushed with nitrogen and 27.20 g (0.094 mol) of a 70% benzene solution of sodium bis-(2-methoxyethoxy)-aluminium hydride (Red-Al®) was added. After stirring at –78° for 1 h the mixture was allowed to warm to 0° over a 1 h period and 2 N sulfuric acid was added to decompose an excess hydride. The mixture was poured into water and the product was extracted with methylene chloride. The residue (18.5 g) obtained by the usual work-up of the organic layers was chromatographed on a column of Florisil pre-treated with 1% pyridine in benzene. Starting material (4.11 g) was eluted with benzene. The 5% ether-benzene fractions afforded 10.50 g (65%) of alcohol **10**: glass; $[\alpha]_D^{25} = +47.8°$ ($c = 0.96$, $CHCl_3$). – IR. ($CHCl_3$) 3640 (OH), 1100, 1080 and 1020 cm^{-1}. – NMR. ($CDCl_3$) δ 3.50 (*m*, $CHCH_2O$); 3.32 (*s*, OCH_3); 2.75 (*t*, $J = 2.5$ Hz, C*H*OCH_3); 1.01 (*d*, $J = 7$ Hz, CH_3); 1.00 (*s*, H_3C—C(19)); 0.73 (*s*, H_3C—C(18)); molecular ion *m/e* 346.

O-Acetyl Derivative of **10**: m.p. 124–125°; $[\alpha]_D^{25} = +47.9°$ ($c = 1.19$, $CHCl_3$); IR. ($CHCl_3$) 1735 (C=O), 1260, 1100 and 1080 cm^{-1}; NMR. ($CDCl_3$) δ 3.90 (*m*, CH_2O); 3.35 (*s*, OCH_3); 2.80 (*t*, $J =$ 2.5 Hz, C*H*OCH_3); 2.05 (*s*, $COCH_3$); 1.02 (*s*, H_3C—C(19)); 1.00 (*d*, $J = 7$ Hz, CH_3); 0.74 (*s*, H_3C—C(18)). – Molecular ion *m/e* 388.

$C_{25}H_{40}O_3$ (388.60) Calc. C 77.27 H 10.38% Found C 77.44 H 10.23%

(20S)-6β-Methoxy-20(p-toluenesulfonoxymethyl)-3α,5-cyclo-5α-pregnane (**11**). To a solution of 9.05 g (0.026 mol) of alcohol **10** in 11 ml of pyridine was added dropwise 6.20 g (0.033 mol) of *p*-toluenesulfonyl chloride in 9 ml of pyridine at 0°. After stirring at 0° for 3 h several chips of ice were added, and the mixture was stirred for 5 min to decompose the excess *p*-toluenesulfonyl chloride, then poured into water, and extracted with methylene chloride. The methylene chloride solution yielded by the usual work-up 13.0 g of white solid. Recrystallization from ethyl acetate afforded 12.0 g (92%) of tosylate **11**, m.p. 142–144°. An analytical sample was obtained by an additional crystallization: m.p. 144–145°; $[\alpha]_D^{25} = +30.8°$ ($c = 1.00$, $CHCl_3$). – IR. ($CHCl_3$) 1360, 1190, 1180, 1100, and 950 cm^{-1}. – NMR. ($CDCl_3$) δ 7.50 (A_2B_2, $J(AB) = 8$ Hz, $\Delta\nu = 46$ Hz, aromatic); 3.85 (*m*, $CHCH_2O$); 3.25 (*s*, OCH_3); 2.70 (*t*, $J = 2.5$ Hz, C*H*OCH_3); 2.38 (*s*, CH_3); 0.95 (*s*, H_3C-C(19)); 0.93 (*d*, $J = 7$ Hz, CH_3); 0.62 (*s*, H_3C-C(18)). – Molecular ion *m/e* 500.

$C_{30}H_{44}O_4S$ (500.73) Calc. C 71.95 H 8.86 S 6.40% Found C 71.74 H 8.60 S 6.66%

If the reaction mixture was stirred for 16 h at room temperature, the yield of tosylate **11** dropped to 55–60%. Purification by column chromatography afforded 30–35% of *(20S)-20-chloromethyl-6β-methoxy-3α,5-cyclo-5α-pregnane*: oil; $[\alpha]_D^{25} = +54.7°$ ($c = 1.03$, $CHCl_3$). – IR. ($CHCl_3$) 1100, 1080 and 1020 cm^{-1}. – NMR. ($CDCl_3$) δ 3.48 (*m*, CH_2Cl); 3.29 (*s*, OCH_3); 2.75 (*t*, $J = 2.5$ Hz, C*H*OCH_3); 1.07 (*d*, $J = 7$ Hz, CH_3); 1.01 (*s*, H_3C—C(19)); 0.72 (*s*, H_3C—C(18)). – Molecular ions *m/e* 366 (M^+, ^{37}Cl), 364 (M^+, ^{35}Cl).

(20S)-20-Iodomethyl-6β-methoxy-3α,5-cyclo-5α-pregnane (**12**, $X = I$). A mixture of 1.00 g (0.0020 mol) of tosylate **11**, 3.00 g (0.020 mol) of sodium iodide and 40 ml of dry acetone was heated at reflux for 16 h then cooled, poured into 10% aqueous sodium hydrogen sulfite solution and extracted with ethyl acetate. The organic extract was washed with saturated brine and dried over anhydrous magnesium sulfate to yield 0.880 g of pale yellow solid. The solid was

recrystallized twice from pentane at 0° to yield 0.83 g (92%) of iodide **12** (X = I): m.p. 103–104°; $[\alpha]_D^{25} = +56.7°$ ($c = 1.09$, $CHCl_3$). – IR. ($CHCl_3$) 1095, 1080 and 1020 cm^{-1}. – NMR. ($CDCl_3$) δ 3.30 (*s*, OCH_3); 3.22 (*m*, CH_2I); 2.76 (*t*, $J = 2.5$ Hz, $CHOCH_3$); 1.01 (*d*, $J = 7$ Hz, CH_3); 1.01 (*s*, CH_3–C(19)); 0.75 (*s*, CH_3–C(18)). – Molecular ion *m/e* 456.

$C_{23}H_{37}IO$ (456.45) Calc. C 60.52 H 8.17 I 27.80% Found C 60.63 H 8.15 I 28.04%

3-Methyl-1-butyn-3-yl Tetrahydropyranyl Ether (method of *Barton* [15]). A mixture of 84.1 g (1.00 mol) of 3-methyl-1-butyn-3-ol and 168.2 g (2.00 mol) of 3,4-dihydro-2*H*-pyran was cooled to 0° and 0.05 g of *p*-toluenesulfonic acid monohydrate was added. After stirring for 1 h at 0° and 16 h at 25° the excess dihydropyran was removed under reduced pressure. The residue was poured into sodium hydrogen carbonate solution and extracted with benzene. The benzene solution was washed with water, dried over anhydrous magnesium sulfate, and distilled to yield 119.5 g (71%) of 3-methyl-1-butyn-3-yl tetrahydropyranyl ether: b.p. 30–33°/0.5 Torr (lit. [15] b.p. 57°/3.5 Torr). – IR. ($CHCl_3$) 3310 (C≡C–H), 1125, 1070, and 1030 cm^{-1}. – *Raman* (neat) 2115 (C≡C) cm^{-1}. – NMR. ($CDCl_3$) δ 5.06 (*m*, –O–C*H*–O); 2.44 (*s*, C≡C*H*); 1.51 (*s*, CH_3); 1.48 (*s*, CH_3). – Molecular ion *m/e* 168. – Correct analytical figures for C and H.

6β-Methoxy-25-(2-tetrahydropyranyloxy)-3α,5-cyclo-5α-cholest-23-yne (**14**). To a solution of 0.84 g (0.0050 mol) of 3-methyl-1-butyn-3-yl tetrahydropyranyl ether in 25 ml of dioxane (distilled from sodium) at 5° was added slowly 3.33 ml of 1.5 M *n*-butyllithium in hexane (*Foote Mineral Company*) and the mixture was stirred for 2 h at *ca.* 5° and 2 h at 25°. To this solution was added 1.25 g (0.0025 mol) of tosylate **11** and the mixture was heated at reflux for 72 h. The cooled solution was poured into water and the product was extracted with ethyl acetate. The organic layers were washed with water and saturated brine, dried over anhydrous magnesium sulfate, and evaporated. The residue was chromatographed on a silica gel column. Methylene chloride eluted 1.14 g (92%) of **14**: oil; $[\alpha]_D^{25} = +43.9°$ ($c = 1.09$, $CHCl_3$). – IR. ($CHCl_3$) 1075 and 1030 cm^{-1}. – *Raman* (neat) 2240 (C≡C) cm^{-1}. – NMR. ($CDCl_3$) δ 5.06 (*m*, O–C*H*–O); 3.28 (*s*, OCH_3); 2.76 (*t*, $J = 2.5$ Hz, CH–OCH_3); 1.48 (*s*, CH_3); 1.44 (*s*, CH_3); 1.03 (*d*, $J = 7$ Hz, CH_3); 1.00 (*s*, H_3C–C(19)); 0.76 (*s*, H_3C–C(18)). – Molecular ion *m/e* 496.

$C_{33}H_{52}O_3$ (496.78) Calc. C 79.79 H 10.55% Found C 79.89 H 10.19%

6β-Methoxy-25-(2-tetrahydropyranyloxy)-3α,5-cyclo-5α-cholestane (**15**). A mixture of 0.25 g (0.00050 mol) of **14**, 2 ml of distilled dioxane, 0.1 g of sodium hydrogen carbonate and 0.025 g of 10% palladium-on-carbon was stirred under one atm of hydrogen until gas uptake ceased (24 h). The mixture was diluted with ethyl acetate and filtered through Celite to remove the catalyst. Removal of solvent under reduced pressure yielded 0.25 g (100%) of **15**. An analytical sample was prepared by preparative tlc. (5:1 benzene-ether): oil; $[\alpha]_D^{25} = +40.2°$ ($c = 1.04$, $CHCl_3$). – IR. ($CHCl_3$) 1080 and 1030 cm^{-1}. – NMR. ($CDCl_3$) δ 4.67 (*m*, O–C*H*–O); 3.28 (*s*, OCH_3); 2.74 (*t*, $J = 2.5$ Hz, $CHOCH_3$); 1.17 (*s*, CH_3); 1.16 (*s*, CH_3); 1.00 (*s*, H_3C–C(19)); 0.90 (*d*, $J = 7$ Hz, CH_3); 0.69 (*s*, H_3C–C(18)). – Molecular ion *m/e* 500.

$C_{33}H_{56}O_3$ (500.81) Calc. C 79.15 H 11.27% Found C 79.03 H 11.06%

25-Hydroxy-6β-methoxy-3α,5-cyclo-5α-cholestane (**16**). – *Method A, from the Tetrahydropyranyl Ether* **15**: A solution of 2.50 g (0.0050 mol) of **15** and 60 ml of methanol was cooled to 0° and 0.05 g of *p*-toluenesulfonic acid monohydrate was added. During stirring of the initially homogeneous solution at 0° for 2 h, alcohol **16** crystallized as it formed. After addition of potassium carbonate (0.5 g) the mixture was stirred for 15 min at 0°, then concentrated under reduced pressure, diluted with water, and the product was isolated with ethyl acetate. The organic phase was washed with water and saturated brine, dried over anhydrous magnesium sulfate, and evaporated to dryness. The 2.20 g of solid was recrystallized from hexane to yield 1.70 g (82%) of alcohol **16**, m.p. 153–154°. An additional recrystallization from hexane yielded an analytical sample in thick colorless prisms: m.p. 154–155°; $[\alpha]_D^{25} = +48.2°$ ($c = 0.99$, $CHCl_3$). – IR. ($CHCl_3$) 3620 (OH), 1095 and 1080 cm^{-1}. – NMR. ($CDCl_3$) δ 3.28 (*s*, OCH_3); 2.73 (*t*, $J = 2.5$ Hz, CH–OCH_3); 1.18 (*s*, H_3C–C(26) and H_3C–C(27)); 1.00 (*s*, H_3C–C(19)); 0.90 (*d*, $J = 7$ Hz, CH_3); 0.69 (*s*, H_3C–C(18)). – Molecular ion *m/e* 416.

$C_{28}H_{48}O_2$ (416.69) Calc. C 80.71 H 11.61% Found C 80.78 H 11.91%

Method B, from the Tosylate **11** *without Isolation of Intermediates*: To a solution of 12.60 g (0.075 mol) of 3-methyl-1-butyn-3-yl tetrahydropyranyl ether in 250 ml of dioxane at 5° was

added dropwise 50.0 ml of 1.5 M *n*-butyllithium in hexane. The mixture was stirred for 2 h at 5° and 2 h at room temperature. Then 15.0 g (0.030 mol) of **11** was added and the mixture was heated at reflux for 72 h. Work-up yielded 22.8 g of an oil containing **14**. The oil was dissolved in 150 ml of dioxane and 1.0 g of solid sodium hydrogen carbonate and 0.2 g of 10% palladium-on-carbon were added. The mixture was stirred under one atm of hydrogen until gas uptake ceased (24 h). The solids were removed by filtration and the filtrate was concentrated *in vacuo* to yield 22.0 g of an oil containing **15**. This oil was taken up in 600 ml of methanol at 0°. *p*-Toluenesulfonic acid monohydrate (0.10 g) was added and the mixture was stirred at 0° for 2 h. Potassium carbonate (5.0 g) was added and the mixture was stirred at 0° for 15 min, and was evaporated under reduced pressure. The residue was diluted with water and the product was isolated with ethyl acetate. The 15.0 g of solid was treated with charcoal and recrystallized from hexane to yield 9.50 g (76% overall yield from **11**) of alcohol **16**, m.p. 153–154°.

25-Hydroxycholesterol (**4**). – *Method A, from the Tetrahydropyranyl Ether* **15**: A solution of 5.0 g (0.010 mol) of **15**, 50 ml of dioxane, 50 ml of water, and 0.25 g of *p*-toluenesulfonic acid monohydrate was stirred at 80° for 4 h and cooled [16]. The thick white precipitate was collected by filtration, taken up in methylene chloride, and washed with sodium hydrogen carbonate solution. The solution was dried over anhydrous magnesium sulfate to yield 3.80 g of white amorphous powder which on recrystallization from methanol afforded 3.1 g (77%) of 25-hydroxycholesterol (**4**), m.p. 175–177°. An additional recrystallization from methanol yielded an analytical sample in colorless needles: m.p. 178–180° (lit. [17] m.p. 177–179°); $[\alpha]_D^{25} = -39.0°$ ($c = 1.05$, $CHCl_3$) (lit. [17] $[\alpha]_D^{25} = -38.6°$). – IR. ($CHCl_3$) 3620 (OH), 1050, 1020, 960, 930, and 910 cm^{-1}. – NMR. ($CDCl_3$) δ 5.33 (*m*, *H*-6); 3.48 (*m*, –C*H*–O); 1.19 (*s*, H_3C–C(26) and H_3C–C(27)); 1.00 (*s*, H_3C–C(19)); 0.92 (*d*, $J = 7$ Hz, CH_3); 0.67 (*s*, H_3C–C(18)). – Molecular ion *m/e* 402. – Correct analytical figures figures for C and H.

Method B, from the Alcohol **16**: A mixture of 0.208 g (0.00050 mol) of **16**, 2 ml of water, 6 ml of dioxane, and 0.010 g of *p*-toluenesulfonic acid monohydrate was stirred at 80° for 6 h and cooled. The solid collected by filtration was dissolved in methylene chloride. This solution was washed with aqueous sodium hydrogen carbonate solution and dried over anhydrous magnesium sulfate. Recrystallization from methanol afforded 0.185 g (92%) of 25-hydroxycholesterol (**4**), m.p. 175–177°; $[\alpha]_D^{25} = -38.0°$ ($c = 1.04$, $CHCl_3$).

Method C, from 25-Hydroxycholesteryl 3-acetate (**17**): To a solution of 2.00 g (0.0045 mol) of **17** in 35 ml of methanol was added 0.40 g (0.010 mol) of sodium hydroxide in 5 ml of methanol. After stirring at 50° for 3 h the solution was cooled and evaporated *in vacuo*. The residue was taken up in ethyl acetate and this solution was washed with water and dried over anhydrous magnesium sulfate. Removal of solvent yielded 2.0 g of white solid which was recrystallized from methanol to yield 1.60 g (88%) of 25-hydrocholesterol (**4**), m.p. 175–177°; $[\alpha]_D^{25} = -38.4°$ ($c = 0.99$, $CHCl_3$).

25-Hydroxycholesteryl 3-Acetate (**17**). – *Method A, from the Tetrahydropyranyl Ether* **15**: A mixture of 0.080 g (0.00016 mol) of **15**, and 3 ml of glacial acetic acid was stirred at 70° for 6 h, poured into water and the product isolated with ethyl acetate. The organic solution was washed with saturated aqueous sodium hydrogen carbonate solution and saturated brine, dried over anhydrous magnesium sulfate, and evaporated to dryness. The 0.080 g of solid was recrystallized twice from acetone to yield 0.060 g (89%) of 25-hydroxycholesteryl 3-acetate (**17**), m.p. 137–138°.

An additional recrystallization yielded an analytical sample in colorless prisms: m.p. 139–140°p (lit. [17] m.p. 138.5–140°); $[\alpha]_D^{25} = -41.4°$ ($c = 1.05$, $CHCl_3$) (lit. [17] $[\alpha]_D^{25} = -42.1°$). – IR. ($CHCl_3$) 3620 (OH), 1725 (C=O), 1265, and 1035 cm^{-1}. – NMR. ($CDCl_3$) δ 5.36 (*m*, *H*-6); 4.55 (*m*, –C*H*–O); 2.01 (*s*, $COCH_3$); 1.20 (*s*, H_3C–C(26) and H_3C–C(27)); 1.00 (*s*, H_3C–C(19)); 0.92 (*d*, $J = 7$ Hz, CH_3); 0.67 (*s*, H_3C–C(18)). – MS.: *m/e* 384 ($M^+ - CH_3CO_2H$). – Correct analytical figures for C and H.

Method B, from Alcohol **16**: A solution of 10.0 g (0.024 mol) of **16** in 100 ml of glacial acetic acid was stirred at 70° for 24 h. The cooled solution was concentrated under reduced pressure and the residue was poured onto crushed ice. The solution was neutralized with ice cold 2 N sodium hydroxide solution and the product was isolated with 1:1 methylene chloride-ethyl acetate. The organic layer was washed with water and saturated brine, dried over anhydrous sodium sulfate and evaporated to dryness. The residue (11.0 g) was recrystallized from acetone to yield 10.1 g (94%) of 25-hydroxycholesteryl 3-acetate (**17**), m.p. 138–139°; $[\alpha]_D^{25} = -41.4°$ ($c = 1.05$, $CHCl_3$).

Method C, from 25-Hydroxycholesterol (**4**): To a solution of 0.201 g (0.00050 mol) of **4** in 4 ml of pyridine was added dropwise 1.00 g (0.0095 mol) of 97% acetic anhydride. After standing for 16 h at 25° the mixture was briefly stirred with crushed ice and the product was isolated with ethyl acetate. By the usual work-up the combined organic layers yielded 0.251 g of white solid. Two recrystallizations from acetone afforded 0.184 g (83%) of 25-hydroxycholesteryl 3-acetate (**17**), m.p. 139–140°; $[\alpha]_D^{25} = -42.0°$ ($c = 1.0$, $CHCl_3$).

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86. Vitamin D_3 Metabolites II[1]). Further Syntheses of 25-Hydroxycholesterol

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Zusammenfassung. Zwei weitere Synthesen von 25-Hydroxycholesterin (**10**) ausgehend von Pregnenolon (**1**) bzw. O-acetylpregnenolon (**13**) werden beschrieben. Dabei wird jedesmal auch das noch unbekannte 20(*S*)-25-Hydroxycholesterin (**11**) erhalten. Fernerhin wird ein Zusammenhang zwischen der chemischen Verschiebung der NMR.-Signale der C(21)-Methylgruppe und der Stereochemie am C(20) festgestellt.

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