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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(3. I. 74)

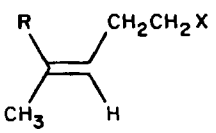
Zusammenfassung. Zwei weitere Synthesen von 25-Hydroxycholesterin (**10**) ausgehend von Pregnenolon (**1**) bzw. O-acetylpregnenolon (**13**) werden beschrieben. Dabei wird jedesmal auch das noch unbekannte 20(*S*)-25-Hydroxycholesterin (**11**) erhalten. Fernerhin wird ein Zusammenhang zwischen der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

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

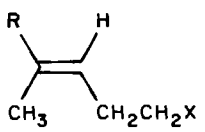
The importance of 25-hydroxycholesterol (**10**) as an intermediate for the synthesis of vitamin D_3 metabolites has been well documented [1]. Historically, 25-hydroxycholesterol was only available *via* 25-nor-24-oxo-cholesterol, a degradation product of cholesterol which is formed in poor yield [2]. Recently *Morisaki et al.* [3] have described a synthesis of 25-hydroxycholesterol beginning with fucosterol, a marine sterol isolated from brown *algae* [4]. The low availability of fucosterol, however, precludes its usefulness as a commercial starting material. We now report two practical syntheses of **10** from pregnenolone and its acetate, respectively.

Synthesis *via* a Protected Δ^5-Double Bond Precursor. – 3β-Hydroxypregn-5-en-20-one (**1**) (*Scheme 1*) being both inexpensive and readily available was our choice of starting material. Since the proposed synthetic scheme involved the hydrogenation of a $\Delta^{20(22)}$-double bond in a later stage of the sequence, the possibility of concomitant reduction of the Δ^5-double bond was circumvented by transformation of **1** into the known *i*-steroid **2** [5]. Elaboration of the C(17) side chain into an eight-carbon unit containing a 25-hydroxyl group was performed in two stages. Treatment of ketone **2** with vinylmagnesium chloride led to an 86% yield of recrystallized allylic alcohol **3**. The stereochemistry of the newly formed asymmetric center at C(20) has been assigned the (*S*)-configuration. The assignment is based on *Cram*'s rule in conjunction with a recent publication by *Gut et al.* [6] dealing with the stereochemistry of *Grignard* additions to 20-keto steroids. The side chain of alcohol **3** was readily extended to a properly functionalized seven-carbon unit upon treatment with two equivalents of diketene [7] in refluxing decalin using *s*-collidine as a catalyst. In this fashion, a 1:2 mixture of the *cis*- and *trans*-keto olefins **4** and **5** was obtained in 60–70% yield.

The stereochemistry of **4** and **5** has been assigned based on their respective NMR. spectra. Recently, the chemical shifts of a number of vinyl methyl groups of some trisubstituted acyclic olefins of type **A** and **B** have been published [8]. These data suggest that when the vinyl methyl group is *cis* to the olefinic proton the chimical shift of the methyl group appears at a lower field than that of the *trans*-isomer. In the *cis*-system **A**, the methyl group generally appears between δ 1.67 and 1.70 ppm



A

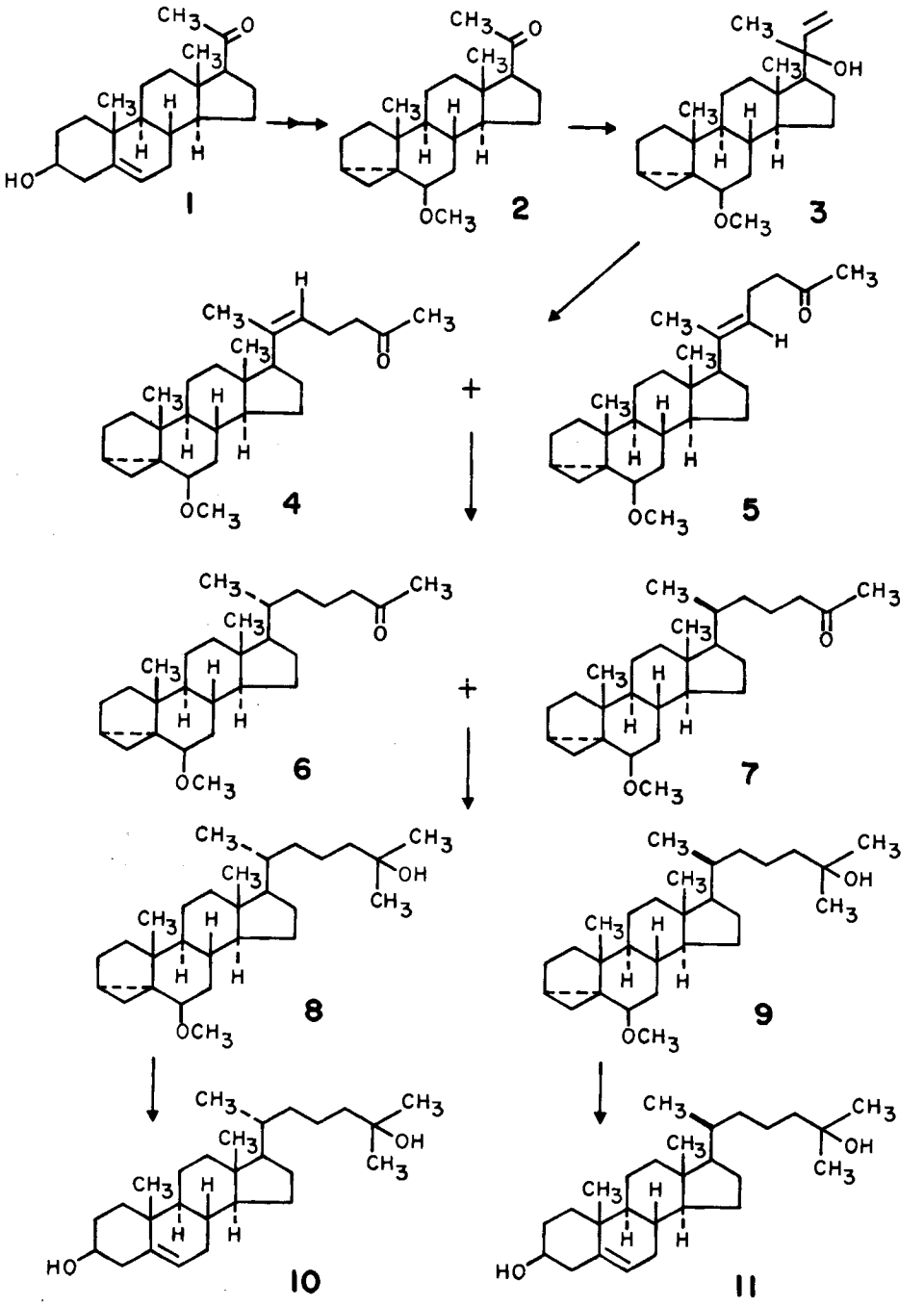


B

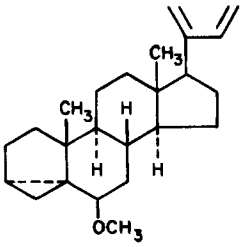
while in the *trans*-isomer **B** the methyl group is found between δ 1.59 and 1.61 ppm. The vinyl methyl of olefin **5** appears at δ 1.61 ppm while that of **4** lies at δ 1.68 ppm.

Alternatively, the *Carroll* rearrangement could be effected in a 60% yield by heating an intimate mixture of the allylic alcohol **3** and a 10% molar excess of ethyl acetoacetate [9] at 180° for 1.2 h. The product ratio was again found to be 1:2 favoring formation of the *trans*-isomer **5**. Although unnecessary, the olefin mixture **4** and **5** could be separated by column or thick layer chromatography.

Scheme 1



Whether or not the *Carroll* rearrangement was performed using diketene or ethyl acetoacetate, a third minor component from the reaction mixture could be isolated. From the NMR. and UV. spectra of this substance, its structure has been deduced to be that of diene **12**.



12

The crude mixture of keto olefins **4** and **5** was reduced over platinum oxide catalyst in 95% ethanol and gave an approximate 1.5:1 mixture of saturated ketones **6** and **7**. The crude mixture of ketones was treated with methylmagnesium iodide and produced a mixture of the saturated alcohols **8** and **9**. The desired 20(*R*)-isomer **8** was separated by crystallization and obtained in a 26% overall yield from the allylic alcohol **3**. Reversal of the hydrogenation-methyl *Grignard* sequence also produces alcohol **8**, but it is technically more desirable to perform the sequence as described above. It should also be mentioned that hydrogenation of either the pure *cis*- or *trans*-keto olefins (**4** or **5**) under the identical conditions as above also led to a near equal mixture of **6** and **7**.

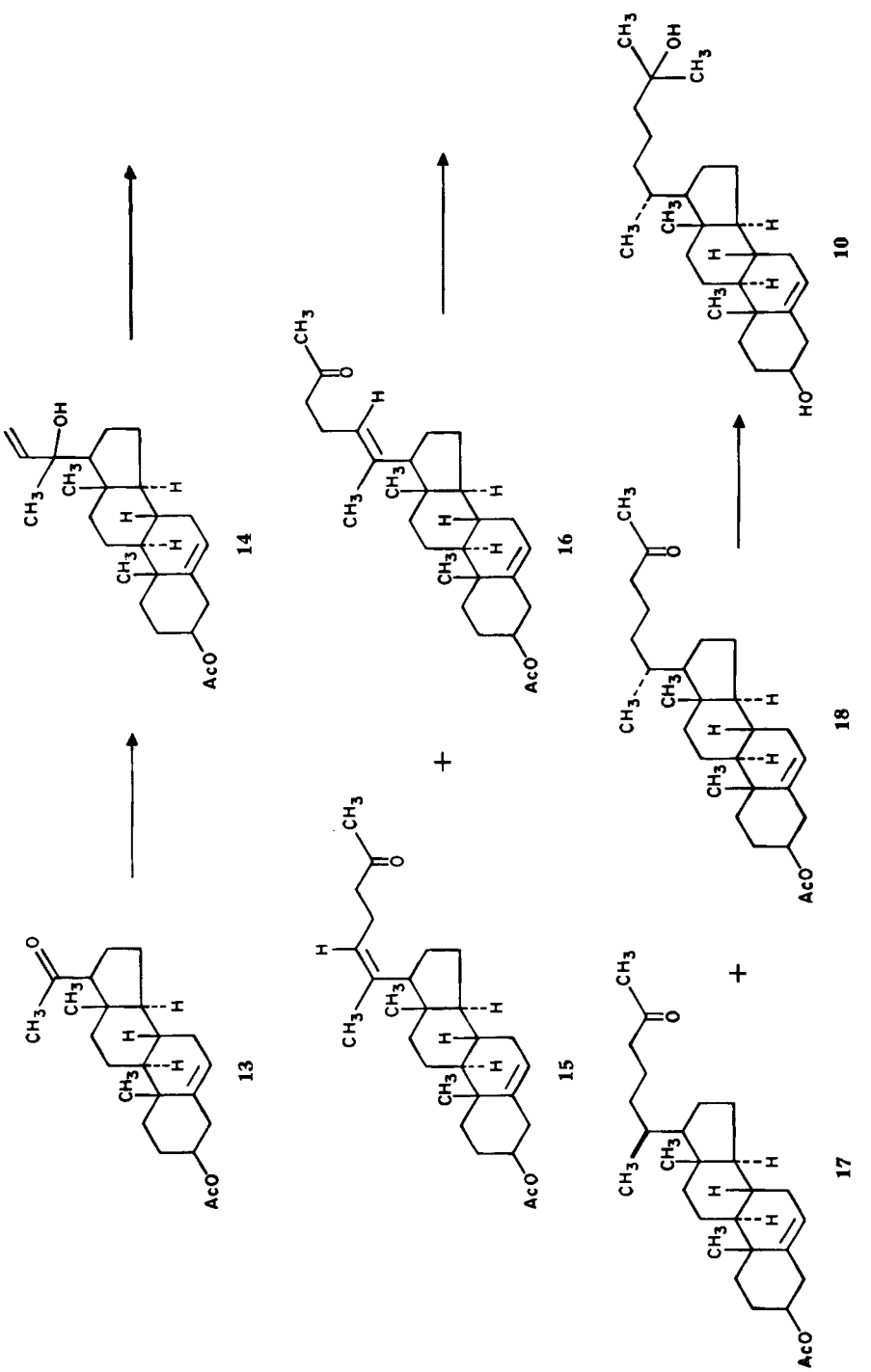
With the side chain now fully elaborated, acid catalyzed solvolysis of the *i*-steroid **8** in aqueous dioxane [10] led to the desired 25-hydroxycholesterol (**10**) in 93% yield. The overall yield from pregnenolone (**1**) was 17%.

The mother liquors from the crystallization of alcohol **8** are rich in alcohol **9**. With significant quantities of the alcohol **9** available having the unnatural configuration at C(20), it was desirable to prepare the previously unknown cholesterol isomer **11**. Thus, when a portion of these mother liquors were solvolysed as above, a 56% yield of 20(*S*)-25-hydroxycholesterol (**11**) was obtained.

Synthesis *via* an Unprotected Δ^5-Double Bond Precursor. – Subsequent to completion of the above described synthesis, we had discovered that protection of the Δ^5-double bond was unnecessary during hydrogenation of the $\Delta^{20,22}$-double bond. This led to a much simplified procedure and a significantly higher overall yield for the preparation of 25-hydroxycholesterol (**10**). Our starting material for the synthesis was O-acetylpregnenolone (**13**) (*Scheme 2*).

Treatment of **13** in dichloromethane at $-78°$ with a two-fold excess of vinylmagnesium chloride afforded the allylic alcohol **14** in *ca.* 90% yield. Extension of the C(20) vinyl side chain was then accomplished as described previously using diketene with *s*-collidine as a catalyst in refluxing decalin. Thus, a 72% yield of a 1:2 mixture of the crystalline *cis*- and *trans*-keto olefins **15** and **16** was obtained. The mixture could be fully separated by chromatography or partially by fractional crystallization. However, this was not necessary. Catalytic hydrogenation of the

Scheme 2



mixture of **15** and **16** over prereduced platinum oxide catalyst led to the known 20(*R*)-ketone **18** (50% yield) [11], which was readily separated from the 20(*S*)-isomer **17** by crystallization from 95% ethanol. A similar selective hydrogenation of a $\Delta^{20,22}$-double bond was recently reported by *Ikan et al.* [12] in their synthesis of β-sitosteryl acetate. Using their conditions (10% Pd/C, ethyl acetate) for the reduction of the Δ^5-double bond, however, we did not observe stereospecific reduction of the $\Delta^{20,22}$-double bond as they have reported. When either the pure *cis*- or *trans*-keto olefin (**15** or **16**), was hydrogenated, a 40–50% yield of ketone **18** was obtained. These results again indicate that both isomeric olefins are hydrogenated with about equal selectivity.

Conversion of **18** to 25-hydroxycholesterol (**10**) was accomplished in 88% yield upon treatment with an excess of methylmagnesium chloride. The overall yield for the seven-step sequence is 25–28%. This synthesis represents therefore the most efficient and preferred route.

Stereochemistry of C(20) in Cholesteryl Compounds. – In a recent publication by *Schneider* [13] on the synthesis of 11-oxygenated cholesterols and derivatives, several criteria were presented which could prove useful in distinguishing between cholesteryl compounds isomeric at C(20). These methods include: (1) specific rotation, (2) infrared spectroscopy, (3) melting point, (4) relative mobility on tlc., and (5) NMR. spectroscopy, and are briefly discussed (except for NMR. data) in relation to a number of examples.

In the above described syntheses of 25-hydroxycholesterol, several pairs of 20(*R*)- and 20(*S*)-intermediates were formed. We have found that the members of these isomeric pairs could be distinguished by the chemical shift of their C(21) methyl groups regardless of whether or not both isomers are available for comparison. The data in the Table show that in all four isomeric pairs the chemical shift of the C(21) methyl group in the (*R*)- or 'natural'-series lies downfield relative to the corresponding (*S*)- or 'unnatural'-series. The 20(*R*)-compounds have an average resonance absorption at δ 0.93 ppm with the individual resonance frequencies falling over a range of δ 0.91 to 0.95 ppm. For the C(21) methyl groups in the (*S*) series, the average resonance absorption was found to be δ 0.84 ppm over a range of 0.83 to δ 0.86 ppm. The average difference, $\Delta(\delta_R^{Av}-\delta_S^{Av})$, between the (*R*)- and (*S*)-isomers of the two series is found to be δ 0.09 ppm. Based on the rather narrow range in which the C(21) methyl groups appear in both series, and the significant difference between their average resonance frequencies, it appears that NMR. spectroscopy would be the most useful diagnostic tool in determining the configuration at C(20) of isomeric cholesterol derivatives.

Chemical Shifts ($CDCl_3$) *and Specific Rotations* ($CHCl_3$) *of Isomeric C(20) Cholesteryl Compounds*

20(*R*)-Compound	C(21) (ppm)	$[\alpha]_D^{25}$	20(*S*)-Compounds	C(21) (ppm)	$[\alpha]_D^{25}$
6	δ 0.94	+47.97	7	δ 0.84	+38.87
8	δ 0.91	+51.58	9	δ 0.83	+35.23
10	δ 0.93	−38.39	11	δ 0.84	−41.50
18	δ 0.95	−45.26	17	δ 0.86	−59.72

The relative magnitude of the specific rotations for each isomeric pair was found to be in agreement with *Schneider*'s observations. In all cases the (*S*)-isomers were found to have a lower or more negative rotation (see Table). However, any prediction of stereochemistry using these criteria alone should be approached with great caution and would also be limited by the fact that one must have either pure samples of both isomers available or know the rotation of the isomer not in hand. In conjunction with NMR. spectroscopy, however, it appears that the relative magnitudes of the specific rotations for a pair of isomers would contribute significantly as supporting evidence in the assignment of the configuration of C(20) cholesteryl isomers.

Experimental Section

Melting points were determined on either a *Thomas Hoover* or a *Reichert* melting point apparatus and are uncorrected. IR. spectra were recorded on either a *Beckman* IR-9 or a *Perkin Elmer* 621. For recording of NMR. and UV. spectra, and performing thin layer chromatography (tlc.) see [1]. Mass spectra were recorded on either a *Jeolco* 01SG or a *CEC* 21-110B spectrometer at 70 eV using a direct insertion probe. Column chromatography was carried out on either *Merck* silica gel 60 or a 3:1 mixture of silica gel 60 and *Merck* PF 254 silica gel. All solvents and reagents were purified and/or dried before using according to generally accepted procedures. Petroleum ether used had b.p. 30–60°.

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

20(S)-6β-Methoxy-20-vinyl-3α,5-cyclo-5α-pregnan-20-ol (**3**). To 224 ml (0.56 mol) of vinylmagnesium chloride solution in 700 ml of dry tetrahydrofuran under a nitrogen atmosphere at room temperature was added dropwise an 800 ml solution of anhydrous tetrahydrofuran containing **2** (100 g, 0,303 mol). The mixture was stirred for 2.74 h, refluxed for 1 h, and then stirred at room temperature for 16 h. The excess *Grignard* reagent was destroyed with saturated sodium sulfate solution. Filtration, evaporation of the solvent *in vacuo*, and recrystallization of the residue from petroleum ether gave 78.7 g of allylic alcohol **3**. Several more crops afforded a total of 93.1 g (86% yield) of **3**. Several recrystallizations from petroleum ether afforded the analytical sample: m.p. 92.5–94°; $[\alpha]_D^{25} = +28.05°$ (c = 1.0376, $CHCl_3$). – IR. ($CHCl_3$): 3600 cm^{-1} (–OH). – NMR. ($CDCl_3$): δ 6.10 (*q*, 1 H, $-CH=CH_2$); 5.15 (*m*, 1 H, $-CH=CHH$); 4.92 (*m*, 1 H, $-CH=CHH$).

$C_{24}H_{38}O_2$ (358.60) Calc. C 80.40 H 10.68% Found C 80.65 H 10.56%

Mixture of 6β-Methoxy-3α,5-cyclo-27-nor-5α-cholest-20(22)-cis-*en-25-one* (**4**) *and 6β-Methoxy-3α,5-cyclo-27-nor-5α-cholest-20(22)*-trans-*en-25-one* (**5**). – *Method A, using Diketene;* To a stirred suspension of allylic alcohol **3** (98.7 g, 0.276 mol) in 300 ml of decalin under a nitrogen atmosphere was added *s*-collidine (9.8 g, 0.081 mol) followed by diketene (46.7 g, 0.552 mol). The mixture was heated to 210° for 50 min, and then the solvent, *s*-collidine, and excess diketene were removed by vacuum distillation (100°/1.0–0.07 Torr). The residue was dissolved in 300 ml of toluene, maleic anhydride (30.0 g, 0.303 mol) was added, and the total heated to 100° for 3 h. The solvent was removed *in vacuo* to give 155.4 g of a dark brown syrup which was dissolved in 3 l of ether and washed with 4 × 250 ml of 1 N sodium hydroxide solution. The base extracts were washed with ether which was combined with the above ether fraction and dried over 500 g anhydrous sodium sulfate. Filtration and evaporation of the solvent *in vacuo* gave 109.7 of a syrup which was dissolved in 500 ml of benzene and filtered through a 2.5 cm × 10 cm bed of silica gel. The silica gel was washed with 2.5 l of benzene and the solvent evaporated *in vacuo* to give 94.95 g of crude viscous orange syrup. Vpc. analysis (5% SE-30 column, 6 ft. × 1/4 in., 67 ml/min flow rate, 280° oven temp.) of the crude product mixture showed the presence of two major components in a ratio of approximately 2:1 consisting of 70 to 75% of the product mixture for a yield of 60%. The analytical samples were obtained by preparative thick layer chromatography on silica gel (11:1 benzene-ether).

trans-Isomer **5**: oil (Rf = 0.47); $[\alpha]_D^{25} = +37.74°$ (c = 1.0709, $CHCl_3$). – IR. ($CHCl_3$): 1715 (C=O), 1660 cm^{-1} (C=C). – NMR. ($CDCl_3$): δ 5.11 (*m*, 1 H, $-CH=C$); 3.29 (*s*, 3 H, $-OCH_3$); 2.75

(*t*, 1 H, –C*H*OCH$_3$); 2.10 (*s*, 3 H, –COC*H*$_3$); 1.61 (*s*, 3 H, C=CC*H*$_3$); 1.00 (*s*, 3 H, C(19)); 0.54 (*s*, 3 H, C(18)). – Molecular ion at *m/e* 398.

$C_{27}H_{42}O_2$ (398.63) Calc. C 81.35 H 10.62% Found C 81.03 H 10.58%

cis Isomer **4**: oil (Rf = 0.57); $[\alpha]_D^{25} = -3.46°$ (*c* = 0.9815, $CHCl_3$). – IR. ($CHCl_3$): 1715 cm^{-1} (C=O). – NMR. ($CDCl_3$): δ 5.20 (*m*, 1 H, –C*H*=C); 3.30 (*s*, 3 H, –OC*H*$_3$); 2.75 (*t*, 1 H, C*H*–OCH$_3$); 2.11 (*s*, 3 H, –COC*H*$_3$); 1.68 (*d*, 3 H, *J* = 8 Hz, –CH=CC*H*$_3$); 1.01 (*s*, 3 H, C(19)); 0.78 (*s*, 3 H, C(18)). – Molecular ion at *m/e* 398.

$C_{27}H_{42}O_2$ (398.63) Calc. C 81.35 H 10.62% Found C 81.59 H 10.55%

The crude mixture was used in the next step.

Method B, using Ethyl Acetoacetate: To the allylic alcohol **3** (0.50 g, 1.44 mmol) under a nitrogen atmosphere was added ethyl acetoacetate (0.205 g, 1.58 mmol) and the reaction vessel heated to 180° for 1.25 h. All volatile materials were removed under high vacuum and the crude tan oily residue was chromatographed on thick layer plates (silica gel, 20:1 benzene/ether, 2 developments). The major product was the *trans*-keto olefin **5**, 0.224 g (40% yield), Rf = 0.47 and the minor product was the *cis*-keto olefin **4**, 0.105 g (19% yield), Rf = 0.57, for a total yield of 59%. – NMR., IR. and Mass- spectra, and tlc. behaviour of each product were identical with those of the corresponding authentic material.

Mixture of 20(R)-6β-Methoxy-3α,5-cyclo-27-nor-5α-cholestan-25-one (**6**) and *20(S)-6β-Methoxy-3α,5-cyclo-27-nor-5α-cholestan-25-one* (**7**). A crude mixture of **4** and **5** (6.62 g) was hydrogenated at one atmosphere in 60 ml of 95% ethanol using platinum oxide catalyst (0.66 g). After 0.5 h, hydrogen uptake had ceased at 676 ml (125% of 545 ml calculated total hydrogen uptake including catalyst). Celite was added, the catalyst filtered, and the solvent evaporated *in vacuo* to give 6.49 g of a straw colored viscous oil. Tlc. (silica gel, 11:1 benzene/ether) of this oil showed two major products along with several minor impurities which were not investigated. The two major products were isolated and characterized by preparative thick layer chromatography and were present in a ratio of approximately 1.5:1.

First major component: 20(*R*)-isomer **6**, oil (Rf = 0.32); $[\alpha]_D^{25} = +47.97°$ (*c* = 1.0715, $CHCl_3$). – IR. ($CHCl_3$): 1715 cm^{-1} (C=O). – NMR. ($CDCl_3$): δ 3.33 (*s*, 3 H, –OC*H*$_3$); 2.78 (*t*, 1 H, *J* = 2.5 Hz, –C*H*–OCH$_3$); 2.15 (*s*, 3 H, C*H*$_3$–CO–); 1.03 (*s*, 3 H, C(19)); 0.94 (*d*, 3 H, *J* = 6.5 Hz, C(21)); 0.70 (*s*, 3 H, C(18)). – Molecular ion *m/e* 400.

$C_{27}H_{44}O_2$ (400.65) Calc. C 80.94 H 11.07% Found C 80.64 H 10.88%

Second major component: 20(*S*)-isomer **7**, oil (Rf = 0.34); $[\alpha]_D^{25} = +38.87°$ (*c* = 1.0650, $CHCl_3$). – IR. (CHCl): 1715 cm^{-1} (C=O). – NMR. ($CDCl_3$): δ 3.83 (*s*, 3 H, –OC*H*$_3$); 2.77 (*t*, 1 H, *J* = 2.5 Hz, –C*H*–OCH$_3$); 2.13 (*s*, 3 H, C*H*$_3$CO); 1.03 (*s*, 3 H, C(19)); 0.84 (*d*, 3 H, *J* = 6.5 Hz, C(21)); 0.71 (*s*, 3 H, C(18)). – Molecular ion *m/e* 400.

$C_{27}H_{44}O_2$ (400.65) Calc. C 80.94 H 11.07% Found C 80.76 H 10.92%

The crude ketone mixture was used in the next step.

20(R)-6β-Methoxy-3α,5-cyclo-5α-cholestan-25-ol (**8**) *and 20(S)-6β-Methoxy-3α,5-cyclo-5α-cholestan-25-ol* (**9**). To a crude mixture of ketones **6** and **7** (92.84 g) dissolved in 600 ml of anhydrous ether at room temperature under a nitrogen atmosphere was added dropwise over a 30 min period, 89.6 ml (0.252 mol) of a 2.8 M etheral solution of methylmagnesium iodide diluted with 200 ml of anhydrous ether. After stirring for 1.5 h at room temperature, tlc. (silica gel, 11:1 benzene/ether) showed absence of starting material. The excess *Grignard* reagent was destroyed by careful dropwise addition of 15 ml of saturated sodium sulfate solution. Then anhydrous sodium sulfate (300 g) was added, the solution stirred for 1 h, and filtered through a pad of Celite. The filter cake was washed with ether (4 × 500 ml) followed by dichloromethane (4 × 250 ml). The solution was concentrated to 1 l, washed with 10% sodium hydrogen sulfite (500 ml), then immediately with 8% sodium hydrogen carbonate solution. The organic fraction was dried over anhydrous sodium sulfate (500 g), filtered and the solvent evaporated *in vacuo* to a semi-solid mass which was crystallized from 400 ml of hexane to give 34.0 g of crude **8**. Recrystallization of the substance from hexane afforded four crops, total weight 27.3 g. The mother liquors from the 34.0 g crop upon concentration and keeping in the cold for 3 days gave an additional 3.1 g for a total weight

of 30.4 g (26.5% overall yield from **3**). Several recrystallizations from hexane afforded the analytical sample: m.p. 155–157°; $[\alpha]_D^{25} = +49.07°$ (c = 0.9945, $CHCl_3$). – IR. ($CHCl_3$): 3615 cm^{-1} (–OH). – NMR. ($CDCl_3$): δ 3.30 (s, 3 H, –CH–OCH_3); 2.75 (t, 1 H, J = 3 Hz, –CH–OCH_3); 1.20 (s, 6 H, C(26) and C(27)); 1.02 (s, 3 H, C(19)); 0.92 (d, 3 H, J = 6 Hz, C(21)); 0.71 (s, 3 H, C(18)). – Molecular ion m/e 416.

$C_{28}H_{48}O_2$ (416.69) Calc. C 80.71 H 11.61% Found C 80.61 H 11.82%

The mother liquors from the initial crop of **8** were rich in the 20(*S*)-isomer **9**. Purification by thick layer chromatography gave the analytical sample: oil (Rf = 0.18, 11:1 benzene/ether); $[\alpha]_D^{25} = +35.23°$ (c = 0.5195, $CHCl_3$). – IR. ($CHCl_3$): 3615 cm^{-1} (–OH). – NMR. ($CDCl_3$): δ 3.29 (s, 3 H, –OCH_3); 2.75 (t, 1 H, J = 3 Hz, –CH–OCH_3); 1.20 (s, 6 H, C(26) and C(27)); 1.01 (s, 3 H, C(19)); 0.83 (d, 3 H, J = 6 Hz, C(21)); 0.71 (s, 3 H, C(18)). – Molecular ion m/e 416.

$C_{28}H_{48}O_2$ (416.69) Calc. C 80.71 H 11.61% Found C 80.42 H 11.40%

25-Hydroxycholesterol (**10**) (method of *McKennis* [10]). To 10 ml of dioxane containing 1.5 ml of water at room temperature under a nitrogen atmosphere was added alcohol **8** (0.50 g, 1.2 mmol) and the mixture heated to 80–85° until it became homogeneous. Then *p*-toluenesulfonic acid (0.06 g) was added and heating was continued for 6 h at 80°. The reaction mixture was cooled, poured into 10 ml of 2N sodium carbonate, and extracted with 3 × 15 ml of dichloromethane. The combined extracts were washed with 10 ml of water, dried over anhydrous sodium sulfate and the solvent evaporated *in vacuo* to give 0.50 g of white crystalline solid. Recrystallization from methanol gave 0.45 g (93% yield) of *25-hydroxycholesterol* (**10**), m.p. 174–176°. The analytical sample was obtained by thick layer chromatography (silica gel, 98:2 chloroform/ether) followed by recrystallization from methanol: m.p. 179–181°; $[\alpha]_D^{25} = -38.39°$ (c = 1.0576, $CHCl_3$). – IR. ($CHCl_3$): 3615 cm^{-1} (–OH). – NMR. ($CDCl_3$): δ 5.34 (m, 1 H, –CH=); 3.47 (m, 1 H, –CH–OH); 1.20 (s, 6 H, –$C(CH_3)_2$); 1.00 (s, 3 H, C(19)); 0.93 (d, 3 H, J = 5.5 Hz, C(21)); 0.67 (s, 3 H, C(18)). – Molecular ion m/e 402.

$C_{27}H_{46}O_2$ (402.66) Calc. C 80.54 H 11.52% Found C 80.38 H 11.46%

20(S)*-25-Hydroxycholesterol* (**11**) (method of *McKennis* [10]). To 100 ml of dioxane diluted with 15 ml of water was added the 20(*S*)-alcohol **9** (4.5 g, 10.0 mmol) followed by *p*-toluenesulfonic acid (0.54 g). The mixture was heated at 80° for 18 h, then cooled, and kept at room temperature for 36 h. The white crystalline precipitate was collected and dried affording 2.14 g of *20*(S)*-25-hydroxycholesterol* (**11**), m.p. 180–185°. The filtrate was poured into 100 ml of water containing 50 ml of 2N sodium carbonate and extracted with 4 × 50 ml of chloroform. The combined extracts were dried over anhydrous sodium sulfate and evaporated *in vacuo*. The oily residue was dissolved in pentane and seeded to give an additional 0.20 g of product, m.p. 175–180° (56% total yield). The analytical sample was recrystallized several times from chloroform: m.p. 189.5–190.5°; $[\alpha]_D^{25} = -41.50°$ (c = 0.9278, $CHCl_3$). – IR. ($CHCl_3$): 3615 cm^{-1} (–OH). – NMR. ($CDCl_3$): δ 5.34 (m, 1 H, –C=CH); 3.45 (m, 1 H, –CHOH); 1.22 (s, 6 H, C(26) and C(27)); 1.02 (s, 3 H, C(19)); 0.84 (d, 3 H, J = 6 Hz, C(21)); 0.68 (s, 3 H, C(18)). – Molecular ion m/e 402.

$C_{27}H_{46}O_2$ (402.66) Calc. C 80.54 H 11.52% Found C 80.81 H 11.67%

20(S)*-3β-Acetoxy-20-hydroxy-vinylpregn-5-en* (**14**). To a vigorously stirred solution of acetate **13** (75 g, 0.21 mol) in 400 ml of dry dichloromethane cooled to –78° under a nitrogen atmosphere was added dropwise over a period of 3.75 h, 460 ml of a solution of vinylmagnesium chloride prepared by diluting 250 ml of 2.04M vinylmagnesium chloride with 250 ml of dry dichloromethane. After tlc. (*Merck* Silica Gel 60) indicated the absence of starting material, the dry ice bath was removed and immediately 90 ml of saturated sodium sulfate solution was added dropwise over a period of 5 min. After the neutralized mixture had been stirred at room temperature for 0.5 h, it was dried over sodium sulfate, filtered through a Celite plug, and the solvent evaporated *in vacuo* to give 82 g of crude allylic alcohol **14** which was used in the next step. The analytical sample was obtained by several recrystallizations from methanol: m.p. 163–164°; $[\alpha]_D^{25} = -69.70°$ (c = 1.1142, $CHCl_3$). – IR. ($CHCl_3$): 3615 (–OH), 1730 cm^{-1} (–OAc). – NMR. ($CDCl_3$): δ 5.98 [d of d, 1 H, J = 11 Hz (*cis*) and 17 Hz (*trans*), $HC(22)$]; 5.38 (m, 1 H, –C=CH–); 5.13 [d of d,

1 H, J = 2 Hz (*gem*) and 17 Hz (*trans*), *H*C(23)]; 4.95 [*d* of *d*, 1 H, J = 2 Hz (*gem*) and 11 Hz (*cis*), *H*C(23)].

$C_{25}H_{38}O_3$ (386.58) Calc. C 77.68 H 9.91% Found C 77.96 H 9.75%

Mixture of Keto Olefins **15** *and* **16**. To crude allylic alcohol **14** (5.0 g, 13 mmol) suspended in 25 ml of decalin under a nitrogen atmosphere was added *s*-collidine (0.5 g) and freshly distilled diketene (2.18 g, 26.0 mmol). The mixture was heated at 80° for 30 min and then at 200° for 70 min. Distillation (25–110°/5–0.1 Torr) of the excess diketene and decalin gave 5.88 g of viscous residue which was chromatographed on 200 g of silica gel (3:1 mixture of *Merck* silica gels 60 and PF 254). After elution with 2500 ml of benzene, elution with 50:1 benzene/ether (1500 ml) afforded 3.96 g (72% yield) of a 2:1 mixture of two isomeric products which could be completely separated by preparative thick layer chromatography (silica gel, elution with 98:2 benzene/ether).

The *major isomer* was **16**. The analytical sample was recrystallized from 95% ethanol, m.p. 120–121°; $[\alpha]_D^{25} = -53.06°$ (c = 1.0215, $CHCl_3$). – IR. ($CHCl_3$): 1725 cm^{-1} (broad, —OC(O)CH_3, —C(O)CH_3) – NMR ($CDCl_3$): δ 5.38 (*m*, 1 H, —C*H*=); 5.13 (*m*, 1 H, *H*C(22)); 4.60 (*m*, 1 H, CH_3CO_2CH—); 2.13 (*s*, 3 H, —COCH_3); 2.02 (*s*, 3 H, CH_3CO_2—); 1.65 (*s*, broad, 3 H, CH_3=C); 1.01 (*s*, 3 H, C(19)); 0.52 (*s*, 3 H, C(18)). – Molecular ion *m/e* 426.

$C_{28}H_{42}O_3$ (426.64) Calc. C 78.83 H 9.92% Found C 78.87 H 10.07%

The *minor isomer* was **15**. The analytical sample was recrystallized from pentane: m p 84–86°; $[\alpha]_D^{25} = -117.15°$ (c = 0 9654, $CHCl_3$). – IR. ($CHCl_3$): 1725 cm^{-1} (broad, —OC(O)CH_3, —C(O)CH_3).– NMR. ($CDCl_3$): δ 5.38 (*m*, 1 H, —C*H*=); 5.22 (*m*, 1 H, *H*C(22)); 4.64 (*m*, 1 H, CH_3CO_2CH—); 2.12 (*s*, 3 H, —COCH_3); 2.02 (*s*, 3 H, CH_3CO_2—); 1.69 (*s*, broad, 3 H, CH_3C=); 1.01 (*s*, 3 H, C(19)); 0.65 (*s*, 3 H, C(18)). – MS.: *m/e* 366 (*M* – HOAc).

$C_{28}H_{42}O_3$ (426.64) Calc. C 78.83 H 9.92% Found C 78.85 H 10.02%

20(R)-*3β-O-Acetyl-27-norcholesteryl-25-one* (**18**) *by Hydrogenation of a Mixture of* **15** *and* **16**. A mixture of the *cis*- and *trans*-keto olefins **15** and **16** (1.86 g, 4.37 mmol) in 80 ml of 95% ethanol was hydrogenated at one atmosphere over prereduced platinum oxide catalyst (0.186 g). Hydrogen uptake ceased at 80 ml (76% of theoretical). After approximately 75% of the hydrogen had been taken up a precipitate began to appear (product) but had no effect on the reaction. A small amount of dichloromethane was added, the catalyst filtered, and the solvent evaporated *in vacuo* to give 1.9 g of crude product which was recrystallized to give 0.85 g of **18**, m.p. 138–140°. A second crop afforded an additional 0.086 g, m.p. 129–132° for a total yield of 0.93 g (50%). The analytical sample was recrystallized from 95% ethanol: m.p. 139–140°; $[\alpha]_D^{25} = -45.26°$ (c = 1.0032, $CHCl_3$). – IR. ($CHCl_3$): 1720 cm^{-1} (broad, —OAc, C=O). – NMR. ($CDCl_3$): δ 5.29 (*m*, 1 H, —C*H*=), 4.64 (*m*, 1 H, AcOC*H*), 2.15 (*s*, 3 H, CH_3CO—), 2.05 (*s*, 3 H, CH_3COO—), 1.03 (*s*, 3 H, C(19)), 0.95 (*d*, 3 H, J = 6 Hz, C(21)), 0.69 (*s*, 3 H, C(18)). MS.: spec. *m/e* 368 (*M* – HOAc).

$C_{28}H_{44}O_3$ (428.66) Calc. C 78.46 H 10.35% Found C 78.56 H 10.64%

25-Hydroxycholesterol (**10**) *from Keto Acetate* **18**. To keto acetate **18** (0.156 g, 0.365 mmol) dissolved in 10 ml of anhydrous ether at room temperature under a nitrogen atmosphere was added dropwise methylmagnesium chloride (0.43 ml of a 3.35 M solution in tetrahydrofuran diluted with 10 ml of ether). The mixture was stirred at room temperature for 1.5 h and the excess *Grignard* reagent destroyed by the dropwise addition of saturated sodium sulfate solution. After addition of anhydrous sodium sulfate and filtration of the salts, evaporation of the solvent gave 0.174 g of crude product. Recrystallization from 95% ethanol afforded 0.129 g (88% yield) of *25-hydroxycholesterol* (**10**), m.p. 177–180°, which was spectroscopically and chromatographically (Rf = 0.28, silica gel, chloroform/ethanol 98:2) identical with authentic material.

Stereochemistry of C(20) Cholesteryl Compounds. For all eight isomers studied in $CDCl_3$ solution (concentration about 0.05 mol%) the C(21) methyl groups appeared in the NMR. spectra (*Varian* HA-100 spectrometer) as doublets having a coupling constant of J = 6 Hz. The resonance frequencies were measured from an internal standard of tetramethylsilane to the center of the doublet.

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

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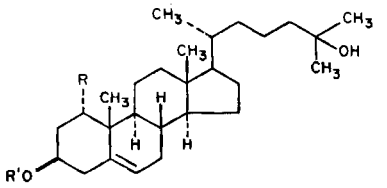
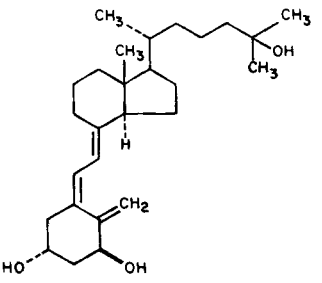
(3. I. 74)

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].