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Synthesis of Active Forms of Vitamin D. X.[1] Synthesis of 1α-Hydroxyvitamin D_3

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1α-Hydroxyvitamin D_3 (**20**) was prepared from cholesterol (**1**) in *ca.* 1% overall yield. Hydroboration of the tetrahydropyranyl ether **2** followed by chromic acid oxidation and $NaBH_4$ reduction gave the 6β-ol **5**. Brief treatment of its acetate **6** with acid followed by Jones oxidation gave the 6β-acetoxy-3-one (**7**) in 55% yield from **1**. Introduction of C-1 double bond (64%) was effected by bromination of **7**, followed by dehydrobromination with $CaCO_3$, yielding the 3-oxo-1-ene **9**. Oxidation of **9** with alkaline H_2O_2 afforded the 1α,2α-epoxide **10** (80%) and this was converted by successive 6 steps sequences to 1α-hydroxycholesterol **15** in 43% yield. The 5,7-diene **19** was obtained from the acetate **16** in 40% yield by allylic bromination with N-bromosuccinimide, dehydrobromination with trimethyl phosphite and saponification. Ultraviolet irradiation of **19** in benzene solution followed by thermal isomerization, produced 1α-hydroxyvitamin D_3 (**20**) (20%).

It has been well documented[3] that vitamin D_3, before eliciting its biological activity, must be hydroxylated in the liver on C-25 and then in the kidney on C-1 or C-24. Among those metabolites of vitamin D_3, 1α,25-dihydroxyvitamin D_3 is the most active one and recognized as hormonal active form of vitamin D_3 both in intestine and bone. Since a synthetic analog, 1α-hydroxyvitamin D_3 has been shown[4] to exert a comparable biological activity to 1α,25-dihydroxyvitamin D_3, several research groups[5] have searched for effective synthetic route of this vitamin D analog. This report is a full detail of our synthesis of 1α-hydroxyvitamin D_3, a part of which has already described in a preliminary form.[6]

A key synthetic intermediate is 1α-hydroxycholesterol (**15**) and at the time we had started experiments, there was a report of synthesis of **15** by Pelc and Kodicek.[7] However, when tracing their procedures, it was soon revealed that their results can not be reproduced. Therefore, we have undertaken an independent preparation of **15**, starting from cholesterol (**1**).

Hydroboration of the tetrahydropyranyl (THP) ether **2** with 1M solution of borane-tetrahydrofuran complex, followed by treatment with alkaline hydrogen peroxide afforded 5α-cholestan-3β,6α-diol 3-THP ether (**3**) in 77% yield. Oxidation of **3** by a modified Collins reagent[8] gave the 6-ketone **4** (97%), which was then reduced with sodium borohydride to

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5) *a*) D.H.R. Barton, R.H. Hesse, M.M. Pechet, and E. Rizzardo, *J. Am. Chem. Soc.*, **95**, 2748 (1973); *b*) A. Fürst, L. Labler, W. Meier, and K-H, Pfoertner, *Helv. Chim. Acta*, **56**, 1708 (1973); *c*) R.G. Harrison, B. Lythogoe, and P.W. Wright, *Tetrahedron Letters*, **1973** 3649, *J. Chem. Soc. Perkin I*, 2654 (1974); *d*) C. Kaneko, S. Yamada, A. Sugimoto, Y. Eguchi, M. Ishikawa, T. Suda, M. Suzuki, S. Kakuta, and S. Sasaki, *Steroids*, **23**, 75 (1974); *e*) D. Freeman, A. Acher, and Y. Mazur, *Tetrahedron Letters*, **1975** 261.

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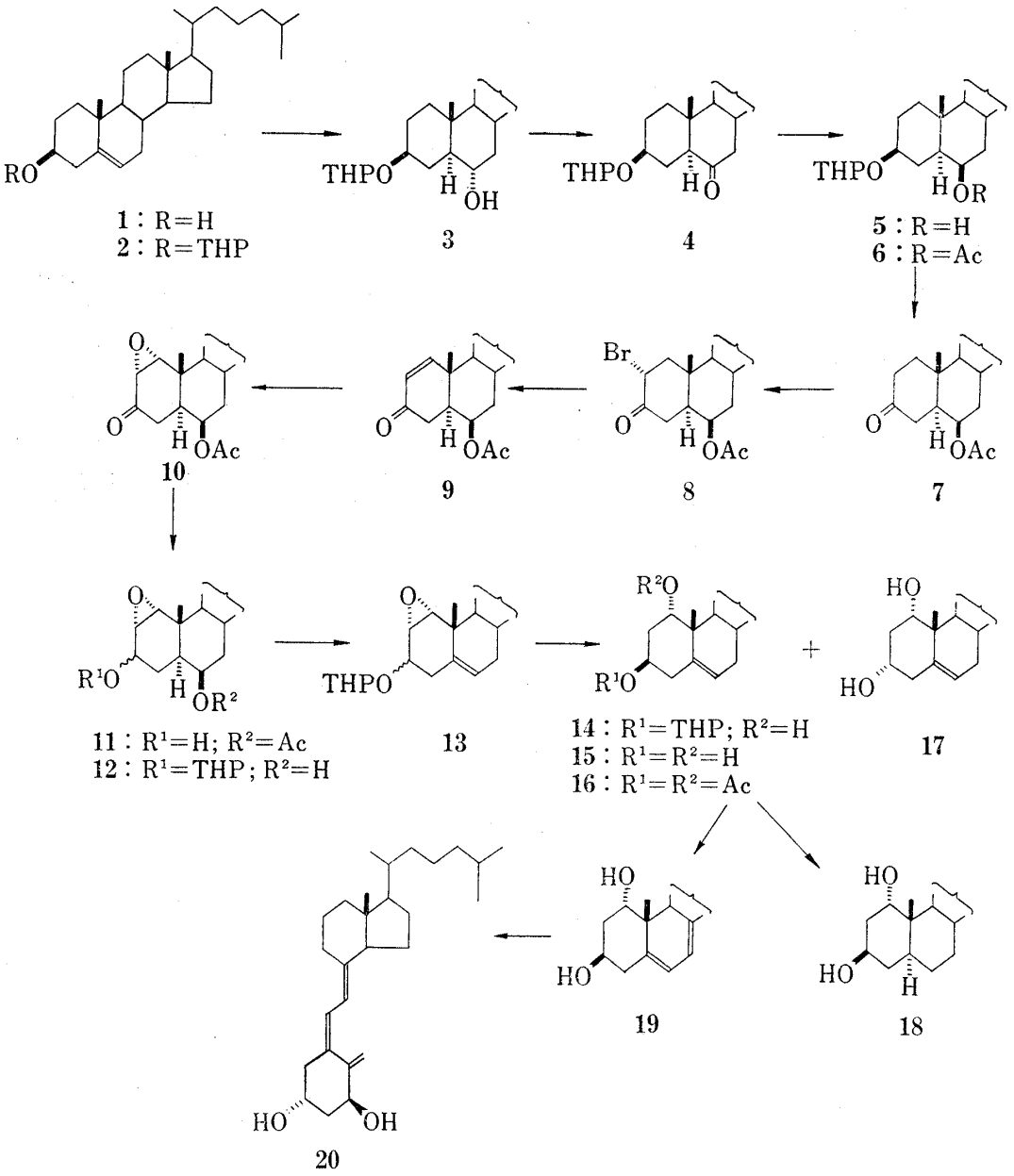
yield the 6β-ol **5** (97%). Both of **3** and **5** showed two distinct spots on thin-layer chromatography but this was ascribed to diastereoisomers due to C-2 of THP group. Stereochemistry of the epimeric alcohols **3** and **5** were deduced from the analogous data reported so far[9] and confirmed by NMR analysis: 19-Me signal of the 6β-ol **5** appeared at 1.01 ppm, significantly lower ($\Delta\delta \simeq 0.2$ ppm) field than that of the 6α-ol **3**.

Acetylation of **5** gave the corresponding acetate **6**, which was treated with acidic methanol and then Jone's reagent to afford the known[8] 3-ketone **7** (65% from **5**). Practically, those 8 steps processes from cholesterol (**1**) can be carried out without purification of intermediates, and the 3-ketone **7** was obtained in 55% over all yield after chromatography of crude product followed by crystallization.

By a slightly modified method of Pelc and Kodicek,[8] the 3-ketone **7** was converted to the 2α-bromide **8** (89%) and then the enone **9** (72%). Epoxidation of **9** with alkaline hydrogen



9) *a*) S. Wolfe, N. Nussim, Y. Mazur, and F. Sondheimer, *J. Org. Chem.*, **24**, 1034 (1959); *b*) D.M.S. Wheeler and M.M. Wheeler in "Organic Reaction in Steroid Chemistry," Vol. 1, J. Fried and J.A. Edwards, Ed., Reinhold, New York, 1972, p. 61.

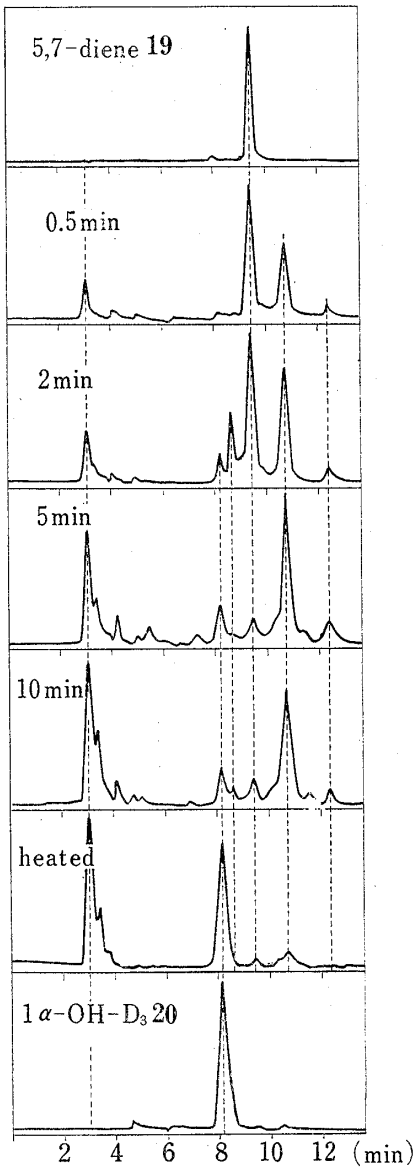


Fig. 1. High Pressure Liquid Chromatography of the UV Irradiation Product of 5,7-Diene **19** (10 mg in 140 ml of Benzene) by a Shimadzu-Dupont 830 Liquid Chromatograph

column, Zorbax SIL (25 cm × 2.1mm); mobile phase 2.5% methanol in methylene dichloride; pressure, 60 kg/cm²; flow rate, 0.26 ml/min.

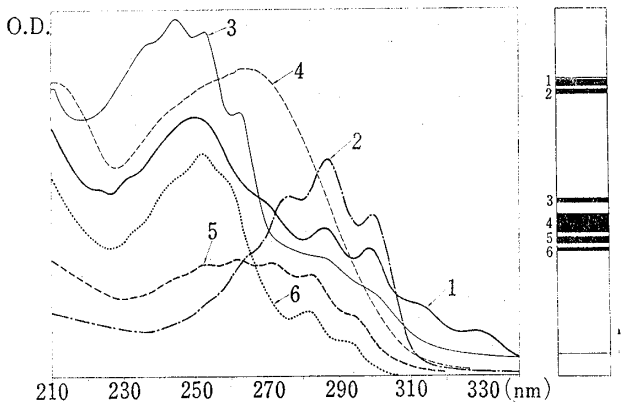


Fig. 2. Ultraviolet Spectra (Left) and Thin-Layer Chromatography (Right) of Irradiation and then Thermally Isomerized Product of 5,7-Diene **19**

The sample was chromatographed on a thin-layer plate (Merck precoated Kiesel gel 60 F_{254}, 0.25 mm thickness) for 4 times with benzene-ethyl acetate (5: 3). Each UV absorbing bands (detected by a Mineralight) was eluted with ethyl acetate and their UV spectra were recorded in ethanol solution.

peroxide gave the 1α,2α-epoxide **10** (80%). Sodium borohydride reduction of the epoxide **10** afforded the 3-alcohol **11**. Attempted separation of the epimeric alcohols was failed at this stage but the ratio of 3α- to 3β-alcohols was estimated as 1: 4 from the isolated yield of the 3α,1α-diol **17** and the 3β,1α-diol **15** (*vide infra*). A slightly higher stereoselectivity (α: β=1: 6) has recently observed[10] with sodium borohydride reduction of a 1α-hydroxy-3-ketone system prepared by a prior epoxide cleavage with aluminium amalgam.

The epoxide **11** was treated with dihydropyran in the presence of *p*-toluenesulfonic acid followed by saponification, to afford the 3-THP-6β-ol **12**. Dehydration of **12** with phosphorus oxychloride regenerated C-5 double bond, producing **13**. Introduction of 1α-hydroxy function was effected by lithium aluminum hydride reduction of **13**. THP group of the product was cleaved under acidic treatment, giving after chromatography the desired 1α-hydroxycholesterol (**15**), together with its 3α-epimer **17**. The conversion of the epoxide **10** to the diols **15** and **17** was carried out without isolation or purification of the intermediates and the overall yields were 43% and 10%, respectively. The nuclear magnetic resonance (NMR) spectrum was steadily consistent with the structure of **15** and hydrogenation of **15** gave the known cholestane-1α,3β-diol (**18**),[11,12] confirming the structure.

10) T.A. Narwid, J.F. Blount, J.A. Iacobelli, and M.R. Uskokovic, *Helv. Chim. Acta*, **57**, 781 (1974).
11) P. Striebel and C. Tamm, *Helv. Chim. Acta*, **37**, 1094 (1954).
12) Further confirmation of structure **15** came from direct comparison (mp, mixed mp and NMR) with a sample alternatively prepared by Dr. Kaneko, *et al.* (Tokyo Medical and Dental University: C. Kaneko, S. Yamada, A. Sugimoto, M. Ishikawa, S. Sasaki, and T. Suda, *Tetrahedron Letters*, **1973**, 2339.

The overall yield of **15** from cholesterol (**1**) was *ca.* 12%. The above synthetic method of 1α-hydroxycholesterol (**15**) is essentially analogous to others,[4,5b] and has been applied[10,13] to synthesis of 1α,25-dihydroxycholesterol which is a key synthetic intermediate of 1α,25-dihydroxyvitamin D_3.

Transformation of 1α-hydroxycholesterol (**15**) into 1α-hydroxyvitamin D_3 (**20**) was carried out according to the established method of vitamin D synthesis. The acetate **16** was brominated with N-bromosuccinimide to give the 7-bromocompound, which was then treated with trimethylphosphite in refluxing xylene.[14] The crude product containing the expected 5,7-diene, was purified after saponification, by silica gel column chromatography to remove the 4,6-diene isomer. The pure 5,7-diene **19** was thus obtained in 40% yield from **15**.

Ultraviolet (UV) irradiation of the 5,7-diene **19** was performed with a medium pressure mercury lamp in benzene solution. The time course of the photochemical reaction was followed by means of high pressure liquid chromatography. As shown in Fig. 1, most of starting compound disappeared after 5 min irradiation by a small scale experiment. However, reaction rate was dependent also on substrate concentration, requiring a longer reaction time for the preparative purposes. The crude irradiation product was directly refluxed in benzene to effect thermal isomerization of precalciferol into calciferol. Ultraviolet spectra of the reaction products fractionated by thin-layer chromatography were shown in Fig. 2.

Band 4 on the thin-layer chromatography showed the expected UV absorption of vitamin D structure and 1α-hydroxyvitamin D_3 (**20**) was isolated from this fraction. The vitamin **20** was alternatively purified by silica gel column chromatography to obtain 20% yield from **19**. The physical data of **20** prepared in the above manner were in good agreement with those of published ones.[5]

Experimental

Melting points were determined on a hot stage microscope and are uncorrected. IR spectra were taken for Nujol solution with 215 Hitachi Grating Infrared Spectrophotometer and optical rotations were determined in chloroform solution. UV spectra were recorded on a Hitachi ESP-3T apparatus with ethanol as solvent. Proton NMR spectra were run on a Varian T 60 or JEOL, JNM-PS-100 spectrometer with deuteriochloroform as solvent and with tetramethylsilane as internal reference. Mass spectra were determined in LKB-9000S. Column chromatography was effected with Wako silica gel C-200. Thin-layer chromatography was carried out on Merck precoated Kieselgel 60, F_{254}.

The following abbreviations were used: THF=tetrahydrofuran; THP=tetrahydropyranyl; s=singlet; d=doublet; m=multiplet; b=broad.

3β-Tetrahydropyranyloxycholest-5-ene (**2**)——To a mixture of cholesterol (98 g, 0.25 mole), *p*-toluenesulfonic acid monohydrate (1.25 g) and CH_2Cl_2 (700 ml), was added a solution of dihydropyran (52.5 g, 0.625 mole) in CH_2Cl_2 (40 ml) under ice-cooling over a period of 10 min. The mixture was stirred at 5° for 20 min. and then at 15° for 1 hr. Saturated solution of $NaHCO_3$ (100 ml) was added and the whole mixture was vigorously shaken. The aqueous layer was extracted with CH_2Cl_2 (250 ml). The combined CH_2Cl_2 extract was dried over K_2CO_3 and the solvent was evaporated off to give the THP ether **2** (109—110 g), mp 152—155° (*n*-hexane).

3β-Tetrahydropyranyloxy-5α-cholestan-6α-ol (**3**)——To a solution of the THP ether **2** (35.4 g, 75 mmole) in THF (300 ml) was added 1M BH_3-THF complex (30 ml) for 4 times on every 30 min period under argon at room temperature. The mixture was stirred at room temperature for 3.5 hr and then added under ice cooling to a mixture of 3N NaOH (50 ml), water (50 ml) and 30% H_2O_2 (40 ml). After stirring for 35 min, K_2CO_3 (22.5 g) was added to the reaction mixture. The aqueous layer was extracted with THF (150 ml). The combined THF layer was dried over $MgSO_4$ and the solvent was evaporated off. The residue was crystallized from *n*-hexane (75 ml) to give the 6α-ol **3** (29 g), mp 149—152°, ν_{max} 3550 cm^{-1} (OH). *Anal.* Calcd. for $C_{32}H_{56}O_3$: C, 78.63; H, 11.55. Found: C, 78.42; H, 11.56%.

13) *a*) E.J. Semmler, M.F. Holick, H.K. Schnoes, and H.F. DeLuca, *Tetrahedron Letters*, **1972**, 4147; *b*) J. Rubio-Lightbourn, M. Morisaki, and N. Ikekawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 1854 (1973). Another synthesis of 1α,25-dihydroxycholesterol: M. Morisaki, J. Rubio-Lightbourn, N. Ikekawa, and T. Takeshita, *ibid.*, **21**, 2568 (1973); D.H.R. Barton, R.H. Hesse, M.M. Pechet, and E. Rizzardo, *J. Chem. Soc, Chem. Comm.*, 203 (1974).

14) F. Hunziker and F.X. Mullner. *Helv. Chim. Acta*, **41**, 70 (1958).

3β-Tetrahydropyranyloxy-5α-cholestan-6-one (4)——To a stirred mixture of pyridine(50 ml) and CH_2Cl_2 (500 ml) was added under ice-cooling, CrO_3 (30 g, 0.3 mole) portionwise. To the resulting red-brown suspension was added a solution of the 6α-ol **3** (24.5 g, 0.05 mole) in CH_2Cl_2 (100 ml) under stirring. Stirring was continued for 50 min. By decantation, the tarry precipitate was removed. Most of CH_2Cl_2 was evaporated under vacuum and the residue was redissolved in benzene. The organic layer was washed with 5% NaOH and water, and dried over $MgSO_4$. Solvent evaporation and crystallization of the residue from methanol gave the 6-ketone **4** (23 g), mp 140—142°, ν_{max}, 1690 cm^{-1} (CO). *Anal.* Calcd. for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 78.90; H, 11.18%.

3β-Tetrahydropyranyloxy-5α-cholestan-6β-ol (5)——To a stirred mixture of the 6-one **4** (76.3 g, 0.157 mole), THF (320 ml), methanol (320 ml) and NaOH (10 mg) was added $NaBH_4$ (19 g) during 15 min. After further stirring for 30 min water (160 ml) was added and stirred for 10 min. Most of the solvent was evaporated off under vacuum and the residue was extracted with benzene (320 ml×2). The organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. Crystallization from *n*-hexane gave the 6β-ol **5** (72 g), mp 156—159°, ν_{max}, 3500 cm^{-1} (OH). *Anal.* Calcd. for $C_{32}H_{56}O_3$: C, 78.63; H, 11.55. Found: C, 78.49; H, 11.45%.

3β-Tetrahydropyranyloxy-6β-acetoxy-5α-cholestane (6)——A mixture of the 6β-ol **5** (73.5 g, 0.15 mole), benzene (300 ml), pyridine (150 ml) and acetic anhydride (150 ml) was heated at 95° for 4 hr, and after cooling to room temperature, poured in ice-water (300 ml). The aqueous layer was extracted with benzene (150 ml). The combined organic fraction was washed with ice-water (200 ml), 1N HCl (200 ml×2) and sat. $NaHCO_3$ (200 ml). Drying over $MgSO_4$ and evaporation of the solvent gave an amorphous solid (80.4 g), which was crystallized from methanol to give the acetate **6**, mp 97—102°, ν_{max}, 1735, 1240, and 1030 cm^{-1}. *Anal.* Calcd. for $C_{34}H_{58}O_4$: C, 76.93; H, 11.01. Found: 76.86; H, 11.08%.

6β-Acetoxy-5α-cholestan-3-one (7)——a) To a suspension of the THP ether **6** (24 g, 0.045 mole) in methanol (450 ml), was added 2N HCl (36 ml) and the mixture was stirred at room temperature for 2 hr, resulting a clear solution. Methanol was evaporated off under vacuum below 50°, and the residue was after addition of water (180 ml), extracted with benzene, washed with water, sat. $NaHCO_3$ and then water, and dried over $MgSO_4$. Evaporation of benzene gave the 3β-ol (21.3 g) as an amorphous solid. This was dissolved in acetone (90 ml) and to this solution, Jones reagent (prepared from conc. H_2SO_4 (12 ml), water (50 ml) and CrO_3 (14 g)) (18 ml) was dropwise added below 20°. Stirring was continued for 30 min and then the precipitate was removed by decantation. Acetone was evaporated off and water was added to the residue. Extraction with isopropyl ether, washing with water and sat. $NaHCO_3$, drying over $MgSO_4$ and the solvent evaporation gave a syrup (20 g). Crystallization from methanol gave the 3-ketone **7** (13.5 g, 67%), mp 99—102° (ref.,[8] 94—97°). ν_{max} 1735, 1710, 1240 and 1040 cm^{-1}.

b) To a solution the THP ether **6** (5.3 g, 0.01 mole) in acetone (30 ml), Jones reagent (14 ml) was dropwise added below 30° under stirring. Stirring was continued for 3 hr at room temperature and the mixture was decanted to remove the precipitate. Acetone was evaporated off and the concentrate was added to water. This was extracted with isopropylether, washed with water, sat. $NaHCO_3$ and water and dried over $MgSO_4$. Evaporation of the solvent gave an amorphous solid, which was crystallized from methanol to give the 3-ketone **7** (3.1 g, 70%), mp 99—102°.

2α-Bromo-6β-acetoxy-5α-cholestan-3-one (8)——a) A solution of bromine (3.4 g, 0.021 mole) in acetic acid (20 ml) was dropwise added to a mixture of the 3-ketone **7** (8.9 g, 0.02 mole), acetic acid (120 ml) and 30% HBr-acetic acid (0.3 ml) at room temperature during 10 min. After stirring 15 min, methanol (15 ml) was added and the mixture was kept in a refrigerator overnight. The resulting precipitate was taken by filtration and washed with methanol (40 ml) to give a colorless crystal (9.0 g). Crystallization from a mixture of CH_2Cl_2 (90 ml) and methanol (90 ml) afforded the 2α-bromide **8** (7.1 g, 68%), mp 197—200° (ref,[8] 196—198°), ν_{max}, 1720 cm^{-1}, δ 0.72 (3H, s, 18-Me), 2.08 (3H, s, Ac), 4.75 (1H, dd, J=14 and 4Hz, 2β-H) and 4.95 ppm (1H, broad s, 6α-H). Mass Spectrum *m/e*: 522 (M^+) and 524 (M+2) (1 : 1). The mother liquor obtained from recrystallization mentioned above was evaporated to give a syrup, which was again crystallized from methanol to give 2α,4α-dibromo-6β-acetoxy-5α-cholestan-3-one (0.7 g), mp 200—204° (decomp.), ν_{max}, 1740 cm^{-1}, δ 0.72 (3H, s, 18-Me), 1.32 (3H, s, 19-Me), 2.08 (3H, s, Ac), 2.65 (1H, dd, J=14 and 6 Hz, 5α-H), 4.9 (2H, m, 2-and 4-H_2) and 5.4 ppm (1H, broad s, 6α–H). Mass Spectrum *m/e*: 540 (M-60), 542 and 544 (1 : 2 : 1). *Anal.* Calcd. for $C_{29}H_{46}O_3Br_2$: C, 57.80; H, 7.71. Found: C, 58.01; H, 7.75.

b) A solution of bromine (prepared from Br_2 (8 g) and acetic acid (40 ml)) was added to a solution of the 3-ketone **7** (8.9 g, o.02 mole) in isopropylether (40 ml) until pale yellow color persist. The mixture was stirred overnight and the precipitate was taken by filtration to afford the 2α-bromide **8** (9.3 g, 89%), mp (decomp.), 200—205°.

6β-Acetoxy-5α-cholest-1-en-3-one (9)——A mixture of the 2α-bromide **8** (10.5 g, 0.02 mole), dimethylformamide (80 ml) and $CaCO_3$ (10 g, 0.1 mole) was stirred with heating with an oil-bath (135—140°) for 3 hr. The precipitate was filtered off and washed with benzene. The combined filtrate was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was crystallized from methanol to give the enone **9** (6.4 g, 72%), mp 103—105° (ref,[8] 95—98°), ν_{max}, 1730, 1670, 1240 and 1035 cm^{-1}, δ 0.74 (3H, s, 18-Me),

1.20 (3H, s, 19-Me), 2.10 (3H, s, Ac), 5.93 (1H, d, $J=10$ Hz, 2-H) and 7.24 ppm (1H, d, $J=10$ Hz, 1-H). Mass Spectrum *m/e*: 442 (M^+), and 382 (M-AcOH).

1,2α-Epoxy-6β-acetoxy-5α-cholestan-3-one (10)——To a stirred mixture of the enone **9** (8.8 g, 0.02 mole), CH_2Cl_2 (40 ml) and methanol (80 ml), 30% H_2O_2 (20 ml, 0.175 mole) was added in one portion under ice-cooling. After addition of 5% NaOH (20 ml, 0.026 mole), the mixture was stirred for 1 hr, and then diluted with water (180 ml). Extraction with CH_2Cl_2, washing with water, drying over K_2CO_3 and evaporation of the solvent gave a white solid, which was crystallized from methanol to give the epoxide **10**, (7.5 g, 82%), mp 141—142°, ν_{max} 1730, 1710, 1235 and 1035 cm^{-1}, δ 0.75 (3H, s, 18-Me), 1.04 (3H, s, 19-Me), 2.08 (3H, s, Ac), 2.24 (2H, broad s, 4-H_2), 3.32 and 3.52 ppm (2H, a pair of d, $J=4$ Hz, 1- and 2-H), Mass Spectrum *m/e*: 398 (M-AcOH).

1,2α-Epoxy-3ξ-tetrahydropyranyloxycholest-5-ene (13)——To a mixture of the epoxyketone **10** (4.6 g, 0.01 mole), methanol (50 ml), ether (50 ml) and NaOH (10 mg) was portionwise added $NaBH_4$ (2.0 g) during 5 min. After stirring at room temperature for 40 min the mixture was concentrated by evaporation of the solvent under reduced pressure. The residue was diluted with water, extracted with isopropylether, washed with sat. $NaHCO_3$ and dried over $MgSO_4$. Evaporation of the solvent gave a white powder (4.6 g), mp 64—69° (from methanol). The crude 3ξ-alcohol **11** (2.3 g) was stirred with a mixture of CH_2Cl_2 (35 ml), *p*-toluenesulfonic acid (10 mg), and dihydropyran (1.3 g) for 40 min. The reaction mixture was diluted with sat. $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. The obtained THP ether (3.1 g) was refluxed in 3% methanolic KOH (35 ml) for 2 hr. The mixture was concentrated and diluted with isopropylether (50 ml) and water (25 ml). The aqueous layer was extracted with isopropylether. The combined organic fraction was washed with water, dried over $MgSO_4$ and evaporated to dryness, giving the 6β-ol **12** (2.7 g). To a solution of the 6β-ol **12** (6.5 g, 0.012 mole) in pyridine (30 ml) was added $POCl_3$ (3.3 ml, 0.036 mole) below 10°. After stirring for 2 hr at room temperature, the mixture was poured on ice-water, and acidified with conc. HCl (10 ml) and extracted with isopropylether. The organic layer was washed with sat. $NaHCO_3$ and water, and dried over $MgSO_4$. Evaporation of the solvent gave a colorless solid **13** (5.3 g).

3β-Tetrahydropyranyloxycholest-5-en-1α-ol (14)——The above obtained crude product (5.3 g) was crystallized from ethanol to give a colorless crystal (4.1 g). This (3.6 g) was dissolved in THF (25 ml) and added to a suspension of $LiAlH_4$ (0.8 g) in THF (50 ml) during 10 min. Stirring was continued for 3.5 hr, and an excess of the reagent was decomposed by a careful addition of water (10 ml). THF was evaporated below 50° under vacuum and the residue was extracted with ether. The ethereal extract was dried over K_2CO_3 and evaporated to give an amorphous solid (3.4 g), which was crystallized from methanol to give the 1α-ol **14** (2.3 g), mp 140—143°. *Anal.* Calcd. for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 79.12; H, 11.18%.

Cholest-5-ene-1α,3β-diol (15)——A mixture of the THP ether **14** (2.5 g), methanol (10 ml), THF (20 ml) and 2N HCl (5 ml) was stirred at room temperature for 2 hr. Most of solvent was evaporated off below 50° under vacuum, and to the residue was added sat. $NaHCO_3$ (50 ml) and water (50 ml). The mixture was extracted with isopropylether and dried over $MgSO_4$. Evaporation of the solvent gave a white solid (1.9 g), which was crystallized from acetone to yield 1α-hydroxycholesterol (**15**), (1.2 g, 60%), mp 153—157°, (from *n*-hexane/acetone), $[\alpha]_D$ −39° ($CHCl_3$), δ 0.68 (3H, s, 18-Me), 1.02 (3H, s, 19-Me), 3.82 (1H, broad s, 1β-H), 3.9 (1H, m, 3α-H) and 5.55 ppm (1H, m, 6-H). M^+ 402. 346 (Calcd. for $C_{27}H_{46}O_2$: 402.349).

Cholest-5-ene-1α,3β-diol Diacetate (16)——A mixture of 1α-hydroxycholesterol (**15**) (1.25 g), acetic anhydride (3 ml) and pyridine (10 ml) was kept at 95° for 4 hr under nitrogen. The solvent were evaporated off and the concentrate was extracted with ether after dilution with water. The organic layer was washed with 1N HCl and then water, dried over Na_2SO_4 and evaporated to dryness. The residue was filtered through silica gel column using benzene as eluant, to afford the diacetate **16** (1.45 g, 95%), mp 101—103° (methanol), $[\alpha]_D^{20}$ −17.0° (*c*, 1.05), δ 0.68 (3H, s, 18-Me), 1.07 (3H, s, 19-Me), 1.99 and 2.01 (6H, a pair of s, acetyl), 4.80 (1H, m, 3α-H), 5.02 (1H, bs, 1β-H) and 5.50 ppm (1H, m, 6-H). *Anal.* Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.71; H, 10.55.

Cholest-5-ene-1α,3α-diol (17)——Crude product **13** (240 mg) was refluxed with $LiAlH_4$ (120 mg) in THF (10 ml) for 1 hr. The usual work-up using ether for extraction gave 3ξ-THPO-1α-ol (239 mg), which was then stirred in a mixture of conc. HCl (0.1 ml) and methanol (20 ml) for 30 min at room temperature. The usual work-up using ether for extraction gave diol mixtures (119 mg), which was applied on silica gel column. Elution with benzene–ether (50: 1) afforded the 1α,3α-diol **17** (20 mg), mp 201—206° (acetone), $[\alpha]_D^{CHCl_3}$ −30°, δ 0.68 (3H, s, 18-Me), 0.98 (3H, s, 19-Me), 3.78 (1H, m, 1β-H), 4.1 (1H, m, 3β-H) and 5.6 ppm (1H, m, 6-H). Further elution with benzene–ether (5: 1) gave 1α-hydroxycholesterol (**15**) (86 mg).

5α-Cholestane-1α,3β-diol (18)——A mixture of 1α-hydroxycholesterol (**15**) (15 mg), PtO_2 (3 mg), cyclohexane (1.0 ml) and acetic acid (0.5 ml) was vigorously shaken under hydrogen at atmospheric pressure for 2 hr. The catalyst was removed by filtration and the filtrate was diluted with ethyl acetate, washed with sat. $NaHCO_3$ and water, and dried over Na_2SO_4. Evaporation of solvent gave a colorless amorphous solid (14 mg). Crystallization from ethanol gave **18**, mp 151—153° (ref,[12] 155°), δ 0.64 (3H, s, 18-Me), 3.8 (1H, bs.

1β-H) and 4.0 ppm (1H, m, 3α-H). Direct comparison (mp. mixed mp, TLC and GLC) with an authentic sample prepared by the method of Striebel and Tamm,[11] confirmed the structure.

Cholesta-5,7-diene-1α,3β-diol (19)——To a refluxing solution of diacetate **16** (1.28 g) in *n*-hexane (14 ml), N-bromosuccinmide (500 mg) was added in one portion. The mixture was refluxed for further 15 min. After cooling to 10°, the resulting precipitate was filtered off. The filtrate was evaporated *in vacuo* below 40° to dryness. The yellow residue was dissolved in xylene (8 ml) and dropped into a refluxing (160—165°) solution of trimethylphosphite (1.0 ml) in xylene (7 ml) during 15 min. The mixture was refluxed for further 1 hr and then evaporated to dryness. The residue was redissolved in ether and washed with 1N HCl, 6% $NaHCO_3$ and water. Drying over Na_2SO_4 and solvent evaporation gave a pale yellow oil. This was heated at 60° for 1 hr in a mixture of 2N KOH-methanol (30 ml), methanol (25 ml) and benzene (25 ml) under nitrogen. The reaction mixture was poured in water and extracted with ethyl acetate. The organic layer was washed with 1N HCl, 6% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated to dryness, affording a pale yellow solid (1.10 g). A part (1.0 g) of this crude product was chromatographed on silica gel (150 g) column. Elution with benzene–ethyl acetate (1 : 1) gave the 5,7-diene **19** (0.38 g, 40% from **16**), mp 157—160° (ref,[5d] 155—158°; ref,[5b] 172—173°), λ_{max}, 272, 282 (ε, 12100) and 294 nm, δ 0.63 (3H, s, 18-Me), 3.7 (1H, bs, 1β-H), 2.1 (1H, m, 3α-H) and 5.6 ppm (2H, AB type q, $J=6$ Hz, 6,7-H_2), Mass Spectrum *m/e*: 400 (M^+). *Anal.* Calcd. for $C_{27}H_{44}O_2$C, 80.94; H, 11.07. Found: C, 80.44; H, 11.31.

1α-Hydroxyvitamin D_3 (20)——The 5,7-diene **19** (50 mg) was irradiated in benzene solution (150 ml) with a medium pressure mercury lamp (Hanovia 654A 36, 200W) through a Vycor filter for 15 min under argon with ice-cooling. The reaction mixture was then directly refluxed for 1.5 hr. The solvent was evaporated under vacuum and the residue was chromatographed on silica gel (30 g) column. Elution with benzene–ethyl acetate (5 : 1) gave 1α-hydroxyvitamin D_3 **20** (10 mg, 20%), mp 135—136.5° (pentane), λ_{max} 264 nm (ε, 18000), λ_{min}, 228 nm (ε, 9000), δ 0.55 (3H, s, 18-Me), 0.87 (6H, d, $J=7$ Hz, 26,27-Me_2), 4.25—4.45 (2H, m, 1,3-H_2), 5.02 and 5.33 (2H, a pair of broad s, 19-H_2) and 6.18 ppm (2H, AB type q, $J=11$ Hz, 6,7-H_2), M^+ 400.336 ($C_{27}H_{44}O_2$ requires 400.334).