

SYNTHESES OF FOUR ISOMERS OF 25-HYDROXYVITAMIN D₃-26,23-LACTONE¹

Tadashi Eguchi, Suguru Takatsuto, Yutaka Hirano, Masaji Ishiguro²
and Nobuo Ikekawa^{*}

Department of Chemistry, Tokyo Institute of Technology,
Ohokayama, Meguro-ku, Tokyo 152, Japan

Abstract A major metabolite of vitamin D₃ was recently isolated and identified as 25-hydroxyvitamin D₃-26,23-lactone. To determine the configurations at C-23 and C-25 positions, the four stereoisomers were synthesized by stereoselective lactonization method. The two 23R-isomers were obtained by iodolactonization of 22,23-trans-26-acid, and the two 23S-isomers were synthesized by selenolactonization of 22,23-cis-26-acid. The four synthetic isomers and the naturally produced lactone were co-chromatographed on a high performance liquid chromatographic system capable of separating the four isomers. The natural lactone comigrated with synthetic (23S,25R)-isomer determining its structure as (23S,25R)-25-hydroxyvitamin D₃-26,23-lactone.

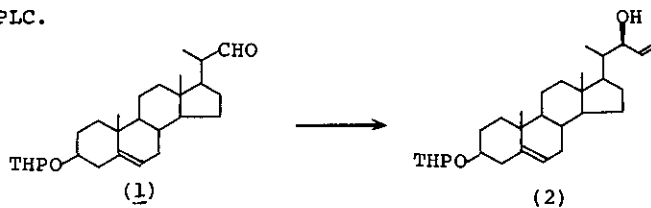
Recently two major metabolites of vitamin D₃ were isolated and identified the structures as 25-hydroxyvitamin D₃-26,23-lactone (25-OH-D₃-26,23-lactone)³ and 23,25-dihydroxyvitamin D₃ (23,25-(OH)₂D₃)⁴ by Wisconsin group. Both metabolites show no significant biological activity and thus their biological functions are unknown. The configuration at C-23 position of 23,25-(OH)₂D₃ was determined as S by us.⁵ To determine the configurations at C-23 and C-25 positions of the natural lactone, four possible stereoisomers should be synthesized. Syntheses of (23R)-lactones were carried out by iodolactonization of 22,23-trans-26-acid, which was reported in a preliminary form.⁶ On the other hand, syntheses of (23S)-lactones were achieved by selenolactonization of 22,23-cis-26-acid. The four synthetic isomers and the naturally produced 25-OH-D₃-26,23-lactone were co-chromatographed on a high performance liquid chromatography showing the natural lactone comigrated with

synthetic (23S,25R)-lactone.⁷ This paper describes the syntheses of four isomers in detail.

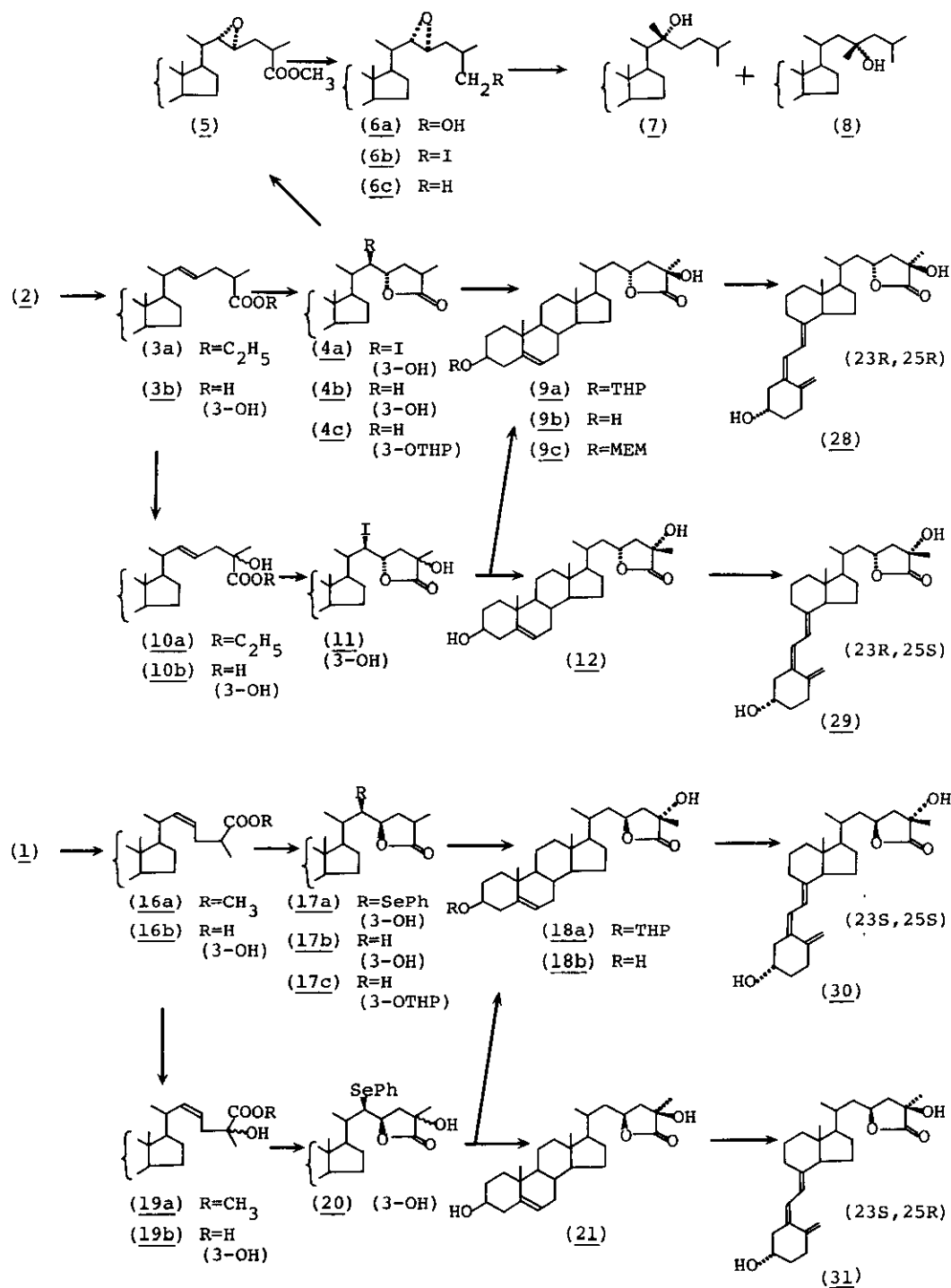
Synthesis of (23R,25R)-isomer

The 22-aldehyde 3-THP ether(1) derived from commercial 22,23-bisnorcholenic acid was the starting material. When the aldehyde was coupled with vinylmagnesium bromide, a mixture of the allylic alcohol was obtained in a 6 : 1 ratio. The less polar major alcohol(2) possesses the 22R-configuration according to the precedents for this mode of the reaction. The Claisen reaction of (2) with ethyl orthopropionate and propionic acid as a catalyst gave 22,23-trans 26-ethyl ester(3a). After hydrolysis of the ester, iodolactonization of the acid(3b) afforded regio- and stereoselectively a single product. The configuration of the lactone (4b) at C-23 position was determined as R by transformation of this compound into 22- and 23-hydroxycholesterols as follows. The lactone(4a) was converted to 22,23-epoxy-26-methyl ester(5) with sodium carbonate and methanol. Reduction of the ester with LiAlH_4 in THF at room temperature gave the 22,23-epoxy-26-ol(6a). Tosylation of the alcohol followed by treatment with sodium iodide in acetone gave the iodide(6b). Removal of iodine of (6b) with tributyltinhydride gave the 22,23-epoxide(6c). Reduction of the epoxide with LiAlH_4 and subsequent removal of the 3-THP group afforded a 1 : 1 mixture of 22-(7) and 23-hydroxycholesterol(8). These hydroxycholesterols were identical with the authentic samples of (22R)-22-hydroxycholesterol(7)⁸ and (23S)-23-hydroxycholesterol(8)⁹, respectively, with respect to the retention times on GLC and HPLC.

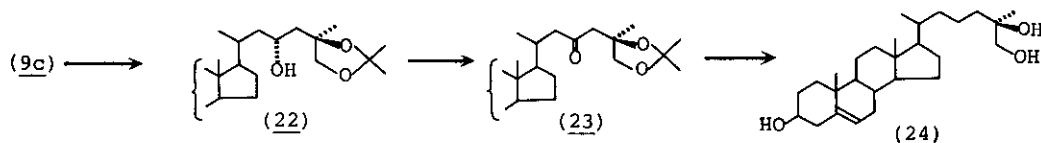
After removal of iodine of the iodolactone(4a) with tributyltinhydride, introduction of a hydroxy group at C-25



position of the lactone(4c) was carried out by oxidation with $\text{MoOPH}(\text{MoO}_5 \cdot \text{Py} \cdot \text{HMPA})$ of the enolate generated by lithium diisopropylamide in THF to give the 25-hydroxylactone(9a). The configuration of C-25 was clarified as R by transformation into 25,26-dihydroxycholesterol(24).¹⁰ The lactone(4b) was converted to the 3-methoxyethoxymethyl ether, and then oxidized to the 25-ol(9c) by MoOPH . The 25-ol was reduced with LiAlH_4 in THF followed by acetonide formation to give the 23,25,26-triol 25,26-acetonide(22). The 23-hydroxy group was oxidized by pyridinium chlorochromate to afford the 23-oxo compound(23), subsequently the tosylhydrazone of (23) was reduced with NaBH_4 to give the 25,26-acetonide. Removal of the protecting



group gave (25R)-25,26-dihydroxycholesterol (24) which was identical with the authentic (25R)-sample prepared by the known procedure.¹⁰ Thus, it is evident that the oxidation of the lactone with MoOPH was performed by the less hindered side attack.

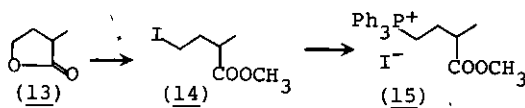


Synthesis of (23R,25S)-isomer

When the ester (3a) was oxidized by MoOPH and then iodolactonization of the hydrolysis product (10b) afforded a non-separable isomeric mixture (11) at C-25 position. After removal of iodine with $n\text{-Bu}_3\text{SnH}$, the mixture of 25-hydroxy-lactone could be separated by chromatography to give the more polar isomer (12), and the less polar isomer (9b) which was identical with the (23R,25R)-25-hydroxy-lactone (9b) obtained previously. The reaction of (10b) into (11) may proceed via the same lactonization mechanism as in the case of (3b) to (4a), thus, the 25-hydroxy-lactone (12) should have (23R,25S)-configuration. The chemical shift of $\text{C}_{23}\text{-H}$ also supported the assignment of the configuration of (12); (23R,25R)-isomer (9b), 4.72 and (23R,25S)-isomer (12), 4.42 ppm.

Synthesis of (23S,25S)-isomer

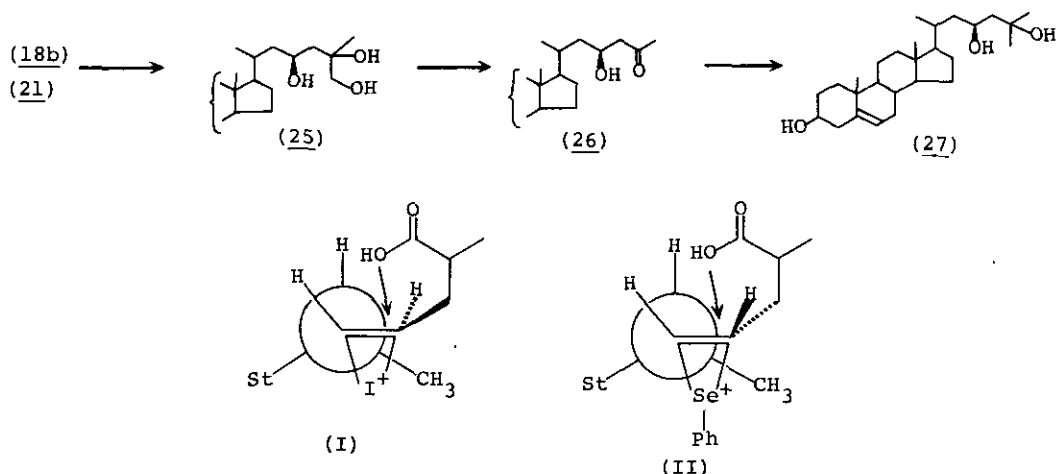
The syntheses of the (23S)-25-OH-26,23-lactones (18b and 21) were accomplished by cyclization of the 22,23-*cis*-26-acid (16b), which was prepared by the following method. α -Methylbutyrolactone (13) was treated with hydroiodic acid to give the iodo-acid, which was converted into the methyl ester (14). The iodide was treated with triphenylphosphine in refluxing benzene for one day to afford the phosphonium salt (15). The reaction of the 22-aldehyde (1) with the ylide solution of (15) in hexa-methylphosphoric triamide (HMPA) gave only the 22,23-*cis*-26-ester (16a).



Iodolactonization of the hydrolysis product (16b) was failed, but lactonization with phenylselenenyl chloride¹¹ gave the lactone (17a) regio- and stereoselectively in high yield. The reduction with $n\text{-Bu}_3\text{SnH}$ and azobisisobutyronitrile resulted in the removal of the phenylseleno group giving rise to the lactone (17b), which was assumed to be an isomeric congener of the (23R)-lactone (4b). This assumption was confirmed by conversion of the 25-hydroxylactone (18b) obtained by MoOPH

hydroxylation of the 3-THP ether(17c) into (23S)-23,25-dihydroxycholesterol(27).⁵ Thus, (18b) was reduced with LiAlH_4 to the 23,25,26-trihydroxycholesterol(25). After NaIO_4 oxidation of (25), Grignard reaction with methylmagnesium iodide provided (23S)-23,25-dihydroxycholesterol(27). From the evidence of less hindered attack by MoOPH , (18a) thus obtained should be (23S,25S)-isomer.

It is interesting to note that the reaction intermediate of the lactonization can be figured out as formula I for trans and formula II for cis double bond indicating to afford (23R)- and (23S)-lactone, respectively.



Synthesis of (23S,25R)-isomer

Another (23S)-lactone was obtained by the following procedure. Selenolactonization of the 22,23-cis-26-acid(19b) prepared by direct hydroxylation of the 22,23-cis-26-ester(16a), afforded isomeric mixture of the lactone. After removal of the phenylseleno group, the mixture(1 : 1) was separated by preparative TLC to give the more polar 25-hydroxy-lactone(21), and the less polar isomer(18b), which was identical with the (23S,25S)-lactone. Consequently, the fourth isomer (21) should have 23S,25R-configuration. The 23S-configuration was also confirmed by the transformation into 23,25-dihydroxycholesterol by the same method described above. The chemical shifts of C₂₃-H of (18b) and (21) exhibited parallel with those of (23R)-isomers; (23S,25S)-isomer(18b), 4.72 and (23S,25R)-isomer(21), 4.43 ppm. The characteristic circular dichroism spectra of those isomers are shown in Figure 1.

The 25-hydroxy-lactones(9b, 12, 18b and 21) synthesized were converted into the corresponding vitamin D compounds through the 5,7-diene by the same method reported previously.¹³ Four isomers of 25-OH-26,23-lactone(28, 29, 30 and 31) obtained as the pure form can be resolved on the HPLC system, and 25-OH-[3 α -³H]D₃

26,23-lactone produced in vitro exactly co-migrated with one of the isomer, (23S,25R)-25-OH-D₃-26,23-lactone(31) as reported elsewhere.⁷

Morris¹⁴ and Yamada¹⁵ reported that the natural lactone has (23R,25S)-configuration, only assuming by the paper describing 25,26-dihydroxyvitamin D₃ may be a precursor of the lactone. But it is incorrect. Recently, Pramanik¹⁶ reported that 25,26-(OH)₂D₃ may not be the precursor of the lactone. During the course of this synthetic work, we obtained clear evidence that (23S)-23,25-(OH)₂D₃ is a far better precursor to the lactone than 25,26-(OH)₂D₃.¹⁷ Thus the configuration as well as the biosynthesis of 25-hydroxyvitamin D₃-26,23-lactone were now elucidated.

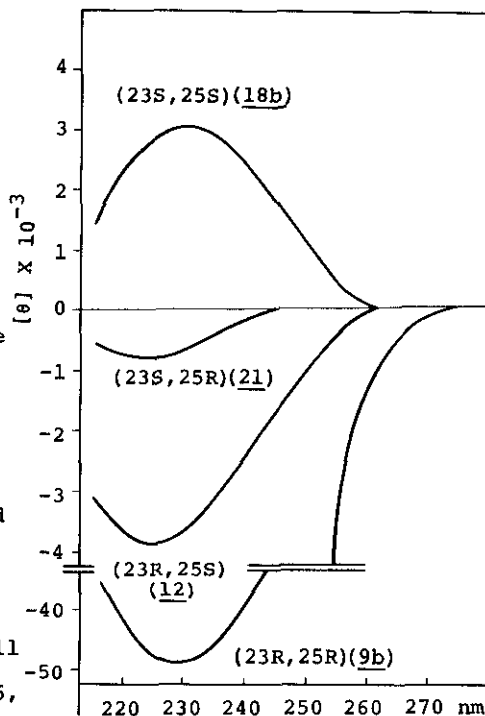


Fig. 1. CD Spectra of four isomers of 25-hydroxycholesterol-26,23-lactone

Acknowledgements

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EXPERIMENTAL

Melting points were determined on a hot stage microscope and uncorrected. The UV spectra were taken on a Shimadzu UV-200 instrument. The NMR spectra were run on a JEOL JNM-PS-100 with CDCl₃ as solvent and with tetramethylsilane as internal standard, unless otherwise stated. Mass spectra were taken on a Shimadzu LKB-9000 mass spectrometer. CD spectra were obtained on a JASCO J-20 spectrometer. Column chromatography was effected with silica gel (Merck, silica gel 60). "The usual work-up" refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying, filtration, and evaporation of the solvent under vacuum. THP refers to tetrahydropyranyl ether, THF to tetrahydrofuran, AcOEt to ethyl acetate, p-TsOH to p-toluenesulfonic acid.

(22R)-25,26,27-Trisnorcholest-5,23-diene-38-tetrahydropyranyloxy-22-ol (2)---

To magnesium (0.072 mol) in dry THF (10 ml) was added 50% vinyl bromide-THF solution (0.68 ml) over 30 min at room temperature under argon. After stirring for 1 hr, a solution of the 22-aldehyde(1) (5.9 g, 14.2 mmol) in dry THF (10 ml) was added at one portion and the mixture was stirred at room temperature for 1 hr. The reaction was quenched by addition of sat. NH_4Cl . The usual work-up, chromatography on silica gel (benzene-AcOEt = 50 : 1) and recrystallization from acetone-methanol gave the 23-olefin (2) (4.4 g) in 70% yield, mp 155-156°C (from acetone-methanol). NMR (CDCl_3) δ : 0.70 (3H, s, 18- H_3), 1.01 (3H, s, 19- H_3), 5.20-6.00 (4H, m, 6-H, 23-H, 24- H_2). MS (m/z): 340 (M^+ -THPOH), 301, 283, 215. Anal. calcd for $\text{C}_{29}\text{H}_{46}\text{O}_3$: C, 78.68; H, 10.21. Found: C, 78.62; H, 10.38.

(22R)-Cholest-5,22-diene-38-tetrahydropyranyloxy-26-oic acid ethyl ester (3a)

--- A solution of the 23-olefin (2) (4.38 g, 9.9 mmol), orthopropionic acid triethyl ester (5.6 ml) and propionic acid (5 drops) in xylene (10 ml) was stirred at reflux for 5 hr under argon. Methanol was added and the mixture was evaporated to dryness under azeotropic condition. Chromatography on silica gel (benzene-AcOEt = 100 : 1) gave the ester (3a) (5.01 g) in 96% yield, mp 104-107.5°C (from acetone-methanol). IR (CHCl_3): 1720 cm^{-1} . NMR (CDCl_3) δ : 0.66 (3H, s, 18- H_3), 0.98 (3H, s, 19- H_3), 1.11 (3H, d, $J=6.6$ Hz, 27- H_3), 1.23 (3H, t, $J=6.9$ Hz, $-\text{CO}_2\text{CH}_2\text{CH}_3$), 4.11 (2H, q, $J=6.9$ Hz, $-\text{CO}_2\text{CH}_2\text{CH}_3$), 5.35 (3H, m, 6-H, 22-H, 23-H). MS (m/z): 424 (M^+ -THPOH), 300, 271, 255. Anal. calcd for $\text{C}_{34}\text{H}_{54}\text{O}_4$: C, 77.52; H, 10.33. Found: C, 77.77; H, 10.37.

Cholest-5-ene-38-hydroxy-22-iodo-26,23-lactone (4a) ---

A mixture of the acid (3b), (2.0 g, 4.83 mmol) and I_2 (3.68 g, 0.014 mol) in dist CH_3CN (30 ml) was stirred at 0°C for 3 hr. 1N- $\text{Na}_2\text{S}_2\text{O}_3$ soln was added and the mixture was extracted with AcOEt. The usual work-up gave the crude product. Chromatography on silica gel (CHCl_3) and recrystallization afforded the pure iodolactone (4a) (1.5 g) in 57% yield, mp 220-224°C (from CHCl_3 -methanol). IR (CHCl_3): 1768 cm^{-1} . NMR (CDCl_3) δ : 0.67 (3H, s, 18- H_3), 3.50 (1H, m, 3-H), 4.10 (1H, dd, $J=5.1, 5.7$ Hz, 22-H), 4.65 (1H, m, 3-H), 5.30 (1H, m, 6-H). MS (m/z): 540 (M^+), 522, 455, 429, 413, 395, 271, 255. High resolution mass spectrum: calcd for $\text{C}_{27}\text{H}_{41}\text{O}_4\text{I}$, 540.2100. Found 540.2102.

Cholest-5-ene-38-hydroxy-26,23-lactone (4b) ---

To a solution of the iodolactone (4a) (346 mg, 0.64 mmol) in dry THF (5 ml) was added n- Bu_3SnH (0.85 ml),

and the mixture was stirred at room temperature under argon atmosphere for 2 hr. After evaporation of the solvent, chromatography on silica gel (benzene : AcOEt = 5 : 1) gave the lactone (4b) (225 mg, 85%), mp 223-224°C (from acetone). IR (CHCl₃): 1760 cm⁻¹. NMR (CDCl₃) δ: 0.70 (3H, s, 18-H₃), 1.00 (3H, s, 19-H₃), 1.27 (3H, d, J=7.5 Hz, 27-H₃), 3.50 (1H, m, 3-H), 4.55 (1H, m, 23-H), 5.35 (1H, m, 6-H). MS (m/z) : 414 (M⁺), 396, 381, 355, 329, 304, 275, 271, 255. High resolution mass spectrum : calcd for C₂₇H₄₂O₃, 414.3134. Found 414.3134.

(23R)-Cholest-5-ene-3β-tetrahydropyranyloxy-26,23-lactone (4c) --- A solution of lactone (4b) (45 mg, 0.11 mmol), dihydropyran (0.2 ml) and a catalytic amount of p-TsOH in dry CH₂Cl₂ was stirred at room temperature for 1 hr. The usual work-up and chromatography on silica gel (benzene : AcOEt = 100 : 1) gave the lactone (4c) in 92% yield, mp 175-177°C (from methanol). IR (CHCl₃): 1760 cm⁻¹. NMR (CDCl₃) δ: 0.70 (3H, s, 18-H₃), 1.01 (3H, s, 19-H₃), 1.29 (3H, d, J=6.6 Hz, 27-H₃), 3.20-4.10 (3H, m, 3-H, 5'-H), 4.40-5.00 (2H, m, 23-H, 1'-H). High resolution mass spectrum: calcd for C₂₇H₄₀O₂ (M⁺-THPOH), 396.3028. Found 396.3030.

Cholest-5-en-22,23-epoxy-3β-tetrahydropyranyloxy-26-oic acid methyl ester (5)
A solution of the iodo-lactone (4a) (222 mg, 0.41 mmol), dihydropyran (0.1 ml) in CH₂Cl₂ (5 ml) containing a catalytic amount of p-TsOH was stirred at room temperature for 30 min. The usual work-up and chromatography on silica gel (benzene : AcOEt = 100 : 1) gave the 3-THP ether (237 mg). A solution of this THP ether (237 mg) and Na₂CO₃ (82 mg) in methanol (6 ml) was stirred at room temperature for 15 hr. The usual work-up and chromatography on silica gel (benzene : AcOEt = 100 : 1) gave the epoxide (5) (187 mg) in 94% yield.

Cholest-5-ene-3β-tetrahydropyranyloxy-22,23-epoxide (6c) --- A mixture of the epoxide (5) (145 mg) and LiAlH₄ (42 mg) in THF was stirred at room temperature for 1 hr. The usual work-up gave a crude 26-alcohol (6a) (133 mg). A mixture of this crude 26-alcohol (6a) (133 mg), and tosyl chloride (50 mg) in pyridine (1 ml) was allowed to stand overnight at 0°C. The usual work-up gave the crude 26-tosylate (140 mg). Then, a mixture of this 26-tosylate (140 mg) and NaI (68 mg) in acetone was stirred at reflux for 1 day. The usual work-up gave the crude 26-iodide (6b) (106 mg). To a solution of this 26-iodide (6b) (106 mg) in THF was added n-Bu₃SnH (0.25 ml) and the mixture was stirred at room temperature for 3 hr under argon. After removal of the solvent, chromatography of the residue on silica gel (benzene) gave the 22,23-epoxide (6c) (66 mg) in 50% yield from (5).

Cholest-5-ene-3β,22-diol (7) and cholest-5-ene-3β,23-diol (8) --- A mixture of

the epoxide (6c) (66 mg) and LiAlH_4 (50 mg) in THF (2 ml) was stirred at reflux for 3 hr. After cooling, the mixture was acidified with 2N-HCl (5 ml) and allowed to stand at room temperature for 1 hr. The usual work-up gave a mixture (1 : 1) of 22-alcohol (7) and 23-alcohol (8). These hydroxycholesterols were identical with the authentic samples of (22R)-22-hydroxycholesterol (7) and (23S)-23-hydroxycholesterol (8), respectively, with respect to the retention times on GLC and HPLC. The retention times on GLC using glass capillary column, 30 m x 0.25 mm i. d.; (22S)-22-OH (7) bis-trimethylsilyl ether (TMSi), 37.11 min; (22R)-22-OH bis-TMSi, 38.11 min; (23R)-and (23S)-23-OH (8) bis-TMSi, 41.22 min, OV-101, 290°. The retention times on HPLC using Zorbax SIL, 15 cm x 4.6 mm i. d.; hexane- CH_2Cl_2 (6 : 1), 20 kg/cm²; (22R)-22-OH (7) bis-(+)- α -methoxy- α -trifluoromethylphenylacetate (MTPA), 7.4 min; (22S)-OH bis-MTPA, 9.0 min; (23R)-23-OH bis-MTPA, 9.4 min; (23S)-23-OH (8) bis-MTPA, 7.8 min.

(23R,25R)-Cholest-5-ene-3 β -tetrahydropyranyloxy-26,23-lactone (9a) --- To a solution of diisopropylamine (0.12 ml) and dry THF (1 ml) was added a solution of n-BuLi in hexane (0.53 ml, 1.6 m M/ml) at -78°C and the mixture was stirred at -78°C for 1 hr under argon. A solution of the lactone (4c) (210 mg, 0.422 mmol) in dry THF (3 ml) was then added dropwise and the mixture was stirred at -78°C for 1 hr. Then, MoOPH (610 mg) was added at once. The mixture was allowed to stand at -78°C overnight and quenched with Na_2SO_3 . The usual work-up and chromatography on silica gel (benzene : AcOEt = 10 : 1) gave the hydroxy-lactone (9a) (175 mg) in 81% yield, mp 193-194°C (acetone-methanol). IR (CHCl_3): 1765 cm^{-1} . NMR (CDCl_3) δ : 0.69 (3H, s, 18- H_3), 0.99 (3H, s, 19- H_3), 1.47 (3H, s, 27- H_3), 3.30-4.05 (3H, m, 3-H, 5'- H_2), 4.70 (2H, m, 23-H, 1'-H), 5.32 (1H, m, 6-H). MS (m/z): 412 (M^+ -THPOH), 397, 368, 324, 291, 255. High resolution mass spectrum: calcd for $\text{C}_{24}\text{H}_{40}\text{O}_3$ (M^+ -THPOH), 412.2977. Found 412.2978.

(23R,25R)-Cholest-5-ene-3 β ,25-dihydroxy-26,23-lactone (9b) --- A solution of the THP ether (9a) (150 mg, 0.29 mmol) and 1 drop of 2N-HCl in THF (2 ml) and methanol (2 ml) was stirred for 2 hr at room temperature. Usual work-up gave the hydroxy lactone (9b) (114 mg) in 91% yield, mp 243-247°C (from CHCl_3 -methanol). IR (CHCl_3): 1765 cm^{-1} . NMR (CDCl_3) δ : 0.71 (3H, s, 18- H_3), 1.02 (3H, s, 19- H_3), 3.50 (1H, m, 3-H), 4.72 (1H, m, 23-H), 5.32 (1H, m, 6-H). MS (m/z): 430 (M^+), 4.2, 397, 340, 319, 255, CD ($c=0.0696$, dioxane): $\theta = -4.95 \times 10^{-4}$ (230 nm). High resolution mass spectrum: calcd for $\text{C}_{27}\text{H}_{42}\text{O}_4$, 430.3083. Found 430.3081.

(22E)-Cholest-5,22-diene-3 β -hydroxy-26-oic acid (3b) --- A solution of the ester (3a) (1.16 g, 2.21 mmol) and 1 drop of 2N-HCl in dioxane (10 ml) and MeOH (10 ml) was stirred for 1 hr, and 5% KOH-MeOH (10 ml) was added to the reaction mixture. The mixture was refluxed for 15 hr, acidified with 2N-HCl and extracted with AcOEt. The usual work-up afforded the acid (3b) (850 mg) in 93% yield, mp 175-178°C (from CHCl₃-methanol). IR (KBr): 1710 cm⁻¹. NMR (CDCl₃ + CD₃OD) δ : 0.68 (3H, s, 18-H₃), 0.98 (3H, s, 19-H₃), 3.50 (1H, m, 3-H), 5.30 (3H, m, 6-H, 22-H, 23-H). MS (m/z): 414 (M⁺), 396, 381, 300. Anal. calcd for C₂₇H₄₂O₃: C, 78.21, H, 10.21. Found: C, 77.96, H, 10.17.

(22E)-Cholest-5,22-diene-25-hydroxy-3 β -tetrahydropyranyloxy-26-oic acid methyl ester (10a) --- To a solution of diisopropylamine (0.63 ml) and THF (4 ml) was added n-BuLi in hexane (2.9 ml, 1.6 mM/ml) at -78°C under argon atmosphere. After stirring for 2 hr, the ester (3a) (1.18 g, 2.24 mmol) in THF (4 ml) was added dropwise and stirring was continued for 2 hr at -78°C. Then, MoOPH (4.86 g) was added at once and the mixture was stirred at -78°C for 18 hr. Sat. Na₂SO₃ soln was added and the mixture was extracted with AcOEt. The usual work-up gave the crude product. Chromatography on silica gel (benzene-AcOEt, 100 : 1) afforded the hydroxy ester (10a) (760 mg) in 63% yield, mp 184-187°C (from acetone-methanol). IR (CHCl₃): 1720, 3450 cm⁻¹. NMR (CDCl₃) δ : 0.67 (3H, s, 18-H₃), 1.01 (3H, s, 19-H₃), 1.27 (3H, s, 27-H₃), 5.30 (3H, m, 22-H, 23-H, 6-H). MS (m/z): 440 (M⁺-THPOH), 367, 271, 255. High resolution mass spectrum: calcd for C₂₉H₄₄O₃, 440.3291 (M⁺-THPOH). Found: 440.3293.

(22E)-Cholest-5,22-diene-3 β ,25-dihydroxy-26-oic acid (10b) --- Compound (10a) was treated in the same manner as (3a) to (3b) to give the hydroxy-acid (10b) in 90% yield, mp 196-199°C (from CHCl₃-methanol). IR (KBr): 1715 cm⁻¹, 3450 cm⁻¹. NMR (CDCl₃ + CD₃OD) δ : 0.68 (3H, s, 18-H₃), 1.01 (3H, s, 19-H₃), 1.28 (3H, s, 27-H₃), 5.30 (3H, m, 22-H, 23-H, 6-H). MS (m/z): 430 (M⁺), 412, 398, 323, 300. High resolution mass spectrum: calcd for C₂₇H₄₂O₄, 430.3083. Found: 430.3086.

Cholest-5-ene-3 β ,25-dihydroxy-22-iodo-26,23-lactone (11) --- Compound (10b) was treated in the same manner as (3b) to (4a) to give the iodo-lactone (11) in 77% yield, mp 203-204°C (from CHCl₃). IR (CHCl₃): 1760 cm⁻¹. NMR (CDCl₃ + CD₃OD) δ : 0.77 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 1.47 (3H, s, 27-H₃), 5.32 (1H, m, 6-H). MS (m/z): 556 (M⁺), 538, 471, 445, 429, 411, 271, 255. High resolution mass spectrum: calcd for C₂₇H₄₀O₄I, 556.2050. Found: 556.2051.

(23R,25S)-Cholest-5-ene-3 β -hydroxy-26,23-lactone (12) --- The iodo-lactone

(11) was treated in the same manner as (4a) to (4b) to give a mixture (1 : 1) of the hydroxy-lactones (9b) and (12), which were separated and purified by flash chromatography on silica gel (Kiesel gel-60, 230-400 mesh, hexane : AcOEt = 2 : 1) to give the less polar isomer (9b) and the desired hydroxy-lactone (12), mp 252-254°C (from acetone-hexane). IR (CHCl₃): 1765 cm⁻¹. NMR (CDCl₃) δ: 0.72 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 1.49 (3H, s, 27-H₃), 3.50 (1H, m, 3-H), 4.42 (1H, m, 23-H), 5.32 (1H, m, 6-H). MS (m/z): 430 (M⁺), 412, 397, 345, 319, 255. CD (c=0.0698, dioxane): θ = -3.70 × 10³ (225 nm). High resolution mass spectrum: calcd for C₂₇H₄₂O₄, 430.3083. Found: 430.3085.

[3-(Methoxycarbonyl)butyl] triphenylphosphonium iodide (15) --- To a refluxing solution of conc. HI (17 ml) was added α-methylbutyrolactone (13) (10 ml) and reflux was continued for 20 min under argon atmosphere. After cooling, the mixture was extracted with ether and the usual work-up gave the iodo-acid, which was treated with slightly excess of CH₂N₂ in ether. Removal of solvent gave the iodo-ester (14) (20 g). A solution of the iodo-ester (14) (20 g) and Ph₃P (32.5 g) in dry benzene (20 ml) was refluxed for 1 day under argon. The precipitate was collected, washed with several portions of benzene and dried *in vacuo* to give the phosphonium salt (15) (27.8 g) in 50% yield from (13).

(22Z)-Cholest-5,22-diene-3β-tetrahydropyranyloxy-26-oic acid methyl ester (16a) --- Sodium hydride (50%) (1.15 g) was washed with several portions of hexane to remove the mineral oil. To this NaH was added dimethyl sulfoxide (20 ml) and the mixture was heated at 75-85°C for 45 min under argon. After cooling to room temperature, 9.9 ml of this solution was added to a solution of phosphonium salt (15) (6 g) in dimethyl sulfoxide and the mixture was stirred at room temperature under argon. After 10 min, the aldehyde (1) (2 g, 4.83 mmol) in hexamethylphosphoric triamide was added to this deep red solution at one portion and stirring was continued for 18 hr. The work-up as usual and chromatography on silica gel (benzene) gave the pure *cis*-ester (16a) (2.24 g) in 90% yield, as an oil. IR (CHCl₃): 1720 cm⁻¹. NMR (CDCl₃) δ: 0.72 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 1.14 (3H, d, J=6.6 Hz, 27-H₃), 3.67 (3H, s, CO₂Me), 5.16 (2H, m, 22-H, 23-H), 5.32 (1H, m, 6-H). MS (m/z): 428 (M⁺-THPOH), 410, 395, 300, 271, 253. High resolution mass spectrum: calcd for C₂₈H₄₂O₂ (M⁺-THPOH), 410.3185. Found: 410.3189.

(22Z)-Cholest-5,22-diene-3β-hydroxy-26-oic acid (16b) --- Compound (16a) was treated in the same manner as (3a) to (3b) to give (16b) in 97% yield, mp 163-164°C (from acetone-hexane). IR (KBr): 1710 cm⁻¹. NMR (CDCl₃) δ: 0.72 (3H, s,

18-H₃), 1.02 (3H, s, 19-H₃), 3.50 (1H, m, 3-H), 5.22 (3H, m, 22-H, 23-H, 6-H). MS (m/z): 414 (M⁺), 396, 381, 300. High resolution mass spectrum: calcd for C₂₇H₄₂O₃, 414.3134. Found: 414.3136.

Cholest-5-ene-3 β -hydroxy-22-phenylseleno-26,23-lactone (17a) --- To a suspension of acid (16b) (200 mg, 0.49 mmol) in dry CH₂Cl₂ (dist from CaH₂) under argon atmosphere at -78°C was added solid PhSeCl (105 mg) and the mixture was stirred at that temperature for 20 hr under argon atmosphere. After evaporation of the solvent, chromatography of the residue on silica gel (benzene : AcOEt = 25 : 1) gave the pure selenolactone (17a) (210 mg) in 76% yield, mp 214.5-217°C (from acetone-hexane). IR (CDCl₃): 1765 cm⁻¹, NMR (CDCl₃) δ : 0.72 (3H, s, 18-H₃), 1.03 (3H, s, 19-H₃), 4.60 (1H, m, 23-H), 5.34 (1H, m, 6-H), 7.20-7.80 (5H, m, PhSe). MS (m/z): 570 (M⁺), 413, 395, 271, 255. High resolution mass spectrum: calcd for C₃₃H₄₆O₃-⁸⁰Se, 570.2612. Found: 570.2612.

Cholest-5-ene-3 β -hydroxy-26,23-lactone (17b) --- n-Bu₃SnH (0.2 ml) and 0.02 M toluene solution of azobisisobutyronitrile (0.4 ml) was added to a solution of the selenolactone (17a) (200 mg, 0.35 mmol) in toluene (10 ml), and the mixture was refluxed for 3.5 hr under argon. After evaporation of the solvent, chromatography of the residue on silica gel (benzene : AcOEt = 10 : 1) gave the lactone (17b) (106.5 mg) in 73% yield, mp 170-172°C (from acetone). IR (CHCl₃): 1763 cm⁻¹. NMR (CDCl₃) δ : 0.72 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 3.50 (1H, m, 3-H), 4.44 (1H, m, 23-H), 5.32 (1H, m, 6-H). MS (m/z): 414 (M⁺), 396, 381, 329, 304. High resolution mass spectrum: calcd for C₂₇H₄₂O₃, 414.3134. Found: 414.3134.

(23S)-Cholest-5-ene-3 β -tetrahydropyranyloxy-26,23-lactone (17c) --- Compound (17c) was treated in the same manner as (4b) to (4c) to give the lactone (17c) in 97.5% yield, mp 189.5-191.5°C (from acetone-hexane). IR (CHCl₃): 1762 cm⁻¹. NMR (CDCl₃) δ : 0.71 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 1.27 (3H, d, J=6.0 Hz, 27-H₃), 3.30-4.20 (3H, m, 3-H, 5'-H₂), 4.42 (1H, m, 23-H), 4.70 (1H, m, 1'-H). High resolution mass spectrum: calcd for C₂₇H₄₀O₂ (M⁺-THPOH), 396.3028. Found: 396.3025.

(23S,25S)-Cholest-5-ene-3 β -tetrahydropyranyloxy-25-hydroxy-26,23-lactone (18a) Compound (17c) was treated in the same manner as (4c) to (5a) to give the hydroxy-lactone (18a) in 60% yield, mp 176-178°C (from acetone-hexane). IR (CHCl₃): 1765 cm⁻¹. NMR (CDCl₃) δ : 0.68 (3H, s, 27-H₃), 3.30-4.20 (3H, m, 3-H, 5'-H₂), 4.70 (2H, m, 23-H, 1'-H), 5.32 (1H, m, 6-H). MS (m/z): 412 (M⁺-THPOH), 397, 368, 324, 291, 255. High resolution mass spectrum: calcd for C₂₇H₄₀O₃ (M⁺-THPOH), 412.2977. Found: 412.2978.

(23S,25S)-Cholest-5-ene-3 β -hydroxy-26,23-lactone (18b) --- Compound (18a) was treated in the same manner as (9a) to (9b) to give the hydroxy-lactone(18b) in 95% yield, mp 247.5-249.5°C (from acetone-hexane). IR (CHCl₃): 1763 cm⁻¹. NMR (CHCl₃) δ : 0.70 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 1.52 (3H, s, 27-H₃), 3.50 (1H, m, 3-H), 4.72 (1H, m, 23-H), 5.34 (1H, m, 6-H). MS (m/z): 430 (M⁺), 412, 397, 340, 319, 255. CD (c=0.104 dioxane): θ = +3.04 \times 10³ (230 nm). High resolution mass spectrum: calcd for C₂₇H₄₂O₄, 430.3083. Found: 430.3081.

(22Z)-Cholest-5,22-diene-25-hydroxy-3 β -tetrahydropyranyloxy-26-oic acid methyl ester (19a) --- Compound (16a) was treated in the same manner as (3a) to (10a) to give the hydroxy-ester (19a) in 70% yield, mp 163-165°C (from acetone-hexane). IR (CHCl₃): 3550, 1725 cm⁻¹. NMR (CDCl₃) δ : 0.72 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 1.23 (3H, s, 27-H₃), 3.75 (3H, s, CO₂Me), 5.30 (3H, m, 22-H, 23-H, 6-H). MS (m/z): 426 (M⁺-THPOH), 365, 271, 255. High resolution mass spectrum: calcd for C₂₈H₄₂O₃, (M⁺-THPOH) 426.3134. Found: 426.3137.

(22Z)-Cholest-5,22-diene-3 β ,25-dihydroxy-26-oic acid (19b) --- Compound (19a) was treated in the same manner as (3a) to (3b) to give the hydroxy-acid (19b) in 98% yield, mp 208-210.5°C (from AcOEt). IR (KBr): 1720 cm⁻¹. NMR (CDCl₃+CD₃OD) δ : 0.75 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 1.41 (3H, s, 27-H₃), 5.30 (1H, m, 6-H). MS (m/z): 430 (M⁺), 412, 398, 323, 300. High resolution mass spectrum: calcd for C₂₇H₄₂O₄, 430.3083. Found: 430.3081.

Cholest-5-ene-3 β ,25-dihydroxy-22-phenylseleno-26,23-lactone (20) --- Compound (19b) was treated in the same manner as (16b) to (17a) to give the selenolactone (20) in 70% yield, mp 203-206°C (from acetone-hexane). IR (CHCl₃): 1765 cm⁻¹. NMR (CDCl₃) δ : 0.72 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 4.67 (1H, m, 23-H), 5.32 (1H, m, 6-H), 7.00-7.80 (5H, m, PhSe). MS (m/z): 586 (M⁺), 429, 411, 325, 271, 255. High resolution mass spectrum: calcd for C₃₃H₄₆O₄⁸⁰Se, 586.2561 Found: 586.2561.

(23S,25R)-Cholest-5-ene-3 β ,25-dihydroxy-26,23-lactone (21) --- Compound (20) was treated in the same manner as (17b) to give a mixture (1 : 1) of the hydroxy-lactones, (18b) and (21), which were separated and purified by preparative TLC (benzene- AcOEt 5 : 1, developed three times) gave the less polar isomer (18b) and the desired hydroxy-lactone (21), mp 248-249°C (from acetone-hexane). IR (CHCl₃): 1763 cm⁻¹. NMR (CDCl₃) δ : 0.71 (3H, s, 18-H₃), 1.02 (3H, s, 19-H₃), 1.51 (3H, s, 27-H₃), 3.50 (1H, m, 3-H), 4.43 (1H, m, 23-H), 5.35 (1H, m, 6-H). MS (m/z): 430 (M⁺), 412, 397, 340, 319, 255. CD (c = 0.066 dioxane): θ = -8.08 \times 10² (225 nm). High resolution mass spectrum: calcd for C₂₇H₄₂O₄, 430.3083. Found: 430.3084.

Cholest-5-ene-3 β ,23,25-triol (27) --- A mixture of the lactone (18b) (20 mg) and LiAlH_4 (10 mg) in THF was stirred at room temperature for 2 hr. The usual work-up gave the crude tetrol (25) (20 mg). A solution of the tetrol (25) (20 mg) and NaIO_4 (12.6 mg) in THF (1 ml) and water (0.3 ml) was stirred at room temperature for 14 hr. The usual work-up gave the crude ketone (26) (18 mg).

Methyl iodide (15 μl) was added to a mixture of Mg (5 mg) in THF (0.5 ml) under argon. After stirring for 1 hr, the crude ketone (26) in THF (1 ml) was added, and the mixture was stirred for 1 hr at room temperature. The usual work up gave the triol (27), which was identical with the authentic sample of (23S)-23,25-dihydroxycholesterol(27) with respect to the spectral data and the retention time of HPLC(benzoate). The lactone (21) gave the same 23,25-dihydroxycholesterol by the similar procedure.

Cholest-5-en-3 β ,23,25,26-tetrol 3-methoxyethoxymethyl ether 25,26-acetonide (22) --- To a solution of the hydroxy-lactone (9c) (234mg) in THF (8ml) was added LiAlH_4 (100mg) at room temperature. After stirring for 30 min, the usual work-up gave the 23,25,26-triol (166mg), which was treated with acetone (10 ml) in the presence of a catalytic amount of p-TsOH at room temperature for 2 hr. The usual work-up and chromatography on silica gel (benzene : AcOEt = 50 : 1) gave the acetonide (22) (110mg) in 68% yield from (9c), mp 96-98°C (from methanol).

Cholest-5-en-3 β ,25,26-triol-23-on 3-methoxyethoxymethyl ether 25,26-acetonide (23) ---A mixture of the alcohol (22) (100mg) and pyridinium chlorochromate (100mg) in CH_2Cl_2 (10 ml) was stirred at room temperature overnight. The usual work-up and chromatography on silica gel (benzene : AcOEt = 50 : 1) gave the ketone (23) (87mg) in 87% yield.

Cholest-5-en-3 β ,25,26-triol (24) ---A mixture of the ketone (23) (32 mg) and tosylhydrazine (50 mg) in methanol (3 ml) was refluxed under argon for 12 hr. To this reaction mixture, NaBH_4 (30 mg) was added and refluxing was continued for 1 hr. The usual work-up gave the crude acetonide, which was treated with a catalytic amount of p-TsOH in methanol (2 ml) at room temperature for 1 hr to give the crude 25,26-diol. Treatment with zinc bromide(10 mg) in CH_2Cl_2 (2ml) at room temperature for 3 hr gave the triol (24), which was identical with the authentic sample of (25R)-25,26-dihydroxycholesterol(24) with respect to the spectral data and the retention time of HPLC(MTPA ester).

Cholest-5-ene-3 β ,25-dihydroxy-26,23-lactone 3-methoxyethoxymethyl ether (9c)
---A mixture of the alcohol (4b) (194mg), methoxyethoxymethyl chloride (0.11 ml)

and diisopropylethylamine (0.17 ml) in CH_2Cl_2 was stirred at room temperature for 3.5 hr. The usual work-up gave 3-methoxyethoxymethyl ether (230 mg), which was treated in the same manner as (4c) to (9a) to give the hydroxylactone (9c) (136 mg) in 56% yield from (4b), mp 175-176°C (from acetone-hexane).

Four isomers of 25-hydroxyvitamin D₃-26,23-lactone (28) (29) (30) (31) ---

Compound (9b) (30 mg) was treated with Ac_2O (0.3 ml) and pyridine (1 ml) at room temperature overnight to give the 3,25-diacetate (31 mg). N-Bromosuccinimide (14.4 mg) was added to a refluxing solution of the diacetate (31 mg) in 2 ml of CCl_4 and reflux was continued under argon. After 20 min, the mixture was cooled with ice-water and the resulting precipitate was filtered off. The filtrate was evaporated to dryness below 40°C. The residue in xylene (1 ml) was added dropwise to a refluxing solution of xylene (1.5 ml) and 2,4,6-collidine (0.5 ml) and refluxing was continued further 20 min under argon. The usual work-up gave a crude $\Delta^{5,7}$ -diene diacetate. This crude diene in acetone (10 ml) was stirred with a catalytic amount of p-TsOH for 18 hr in a dark under argon. A solution of the product in THF (5 ml) and 5% KOH-methanol was stirred for 1.5 hr under argon in a dark. The mixture was acidified with 2N-HCl (2 ml) and extracted with AcOEt. The usual work-up and preparative TLC (benzene-AcOEt, 2 : 1, developed three times) gave the pure $\Delta^{5,7}$ -diene (3.7 mg) in 15% yield. UV(EtOH): λ_{max} 294, 282, 272 nm.

A solution of the $\Delta^{5,7}$ -diene (3.7 mg) in benzene (80 ml) and ethanol (40 ml) was irradiated with a medium pressure mercury lamp through a Vycor filter for 2.5 min at 0°C under argon, and then refluxed under argon for 1 hr. Removal of the solvent and preparative TLC (benzene-AcOEt, 2 : 1, developed twice) gave the vitamin D derivative (28) (700 μg) in 19% yield from $\Delta^{5,7}$ -diene. Other isomers (29), (30) and (31) were obtained from (12), (18b) and (21), respectively, by the same method described above. Further purification of four isomers was carried out by high pressure liquid chromatography (Zorbax SIL, 4.6 mm x 15 cm, 4% 2-propanol in hexane, 2 ml/min). The retention times were: (23R,25R) (28), 8.9 min; (23R,25S) (29), 15.7 min; (23S,25S) (30), 8.3 min; (23S,25R) (31), 14.9 min. Four isomers show the same UV and mass spectra. UV(EtOH): λ_{max} 265, λ_{min} 228 nm. MS(m/z): 428 (M^+), 410, 395, 369, 271, 253, 211, 199, 197, 183, 171, 159, 158, 143, 136, 118. NMR (CDCl_3) δ : (23R,25R)-lactone (28), 0.58 (3H, s, 18- H_3), 1.05 (3H, d, J = 5.8 Hz, 21- H_3), 3.99 (1H, m, 3-H), 4.72 (1H, m, 23-H), 4.86 and 5.10 (2H, a pair of broad s, 19- H_2), 6.07 (1H, d, J = 12.0 Hz, 7-H), 6.28 (1H, d, J = 12.0 Hz, 6-H); (23R,25S)-lactone (29), 0.56 (3H, s, 18- H_3), 1.03 (3H, d, J = 5.5 Hz, 21- H_3), 3.96 (1H, m, 3-H),

4.46 (1H, m, 23-H), 4.82 and 5.05 (2H, a pair of broad s, 19-H₂), 6.03 (1H, d, J=12.0 Hz, 7-H), 6.28 (1H, d, J=12.0 Hz, 6-H); (23S, 25S)-lactone (30), 0.56 (3H, s, 18-H₃), 1.03 (3H, d, J=5.0 Hz, 21-H₃), 3.98 (1H, m, 3-H), 4.72 (1H, m, 23-H), 4.84 and 5.10 (2H, a pair of broad s, 19-H₂), 6.03 (1H, d, J=12.0 Hz, 7-H), 6.28 (1H, d, J=12.0 Hz, 6-H); (23S, 25R)-lactone (31), 0.56 (3H, s, 18-H₃), 1.03 (3H, d, J=5.3 Hz, 21-H₃), 3.96 (1H, m, 3-H), 4.43 (1H, m, 23-H), 4.84 and 5.06 (2H, a pair of broad s, 19-H₂), 6.03 (1H, d, J=12.0 Hz, 6-H), 6.28 (1H, d, J=12.0 Hz, 6-H).

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