Studies on 1α-Hydroxy Derivatives of Vitamin D₃. I. Syntheses of 1α-Hydroxyvitamin D₃ and 1α,25-Dihydroxyvitamin D₃

TADASHI SATO, HIROSHI YAMAUCHI, YOSHIKANE OGATA, MASAHIKO TSUJI, TOSHINOBU KUNI, KENGO KAGEI, SHOJI TOYOSHIMA, and TADASHI KOBAYASHI

Research and Development Division, Eisai Co., Ltd. and Department of Hygienic Chemistry, Kobe Women’s College of Pharmacy

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1α-Hydroxyvitamin D₃ (XIVα) was synthesized from cholesterol (Ia) in ca. 1.5% overall yield. 1α,25-Epoxycholest-4-en-3-one (Iva) readily obtained from Ia was converted to 1α,25-epoxycholest-5-en-3-one (Va) by the modified deconjugation reaction with t-BuOK in DMSO. Reduction of Va with Ca(BH₄)₂ and then with LiAIH₄ gave 1α-hydroxycholesterol (VIIa) in 15.6% yield from Ia. Allylic bromination and subsequent dehydrobromination of the diacetate of VIIa afforded 1α,3β-diacetoxysterola-5,7-diene (Xa), whose saponification gave the corresponding diol (XIIa). 1α-Hydroxyprovitamin D₃ (XIIa) in ethanol was irradiated with the ultraviolet lights in the range between 275 and 310 nm through a newly found filter solution. The formed 1α-hydroxyprovitamin D₃ (XIIa) was thermally isomerized into 1α-hydroxyvitamin D₃. The yield of XIVα from XIIa was ca. 25%

These procedures were applied to 25-hydroxycholesterol (Ib) and 1α,25-dihydroxyvitamin D₃ (XIVb) was obtained ca. 0.4% overall yield from Ib.

Keywords—1α-hydroxyvitamin D₃; 1α,25-dihydroxyvitamin D₃; 1α,25-epoxycholest-5-en-3-one; 1α,25-epoxycholest-5-en-25-ol-3-one; cholesta-5,7-diene-1α,3β-diol; cholesta-5,7-diene-1α,3β-25-triol; deconjugation

It has been documented that vitamin D₃ is hydroxylated on the 25 position in the liver and subsequently on the 1α-position in the kidney to yield 1α,25-dihydroxyvitamin D₃ (XIVb), the active form of the vitamin showing the biological activity in stimulating intestinal calcium transport and bone mineral mobilization. It has been also documented that 1α-hydroxyvitamin D₃ (XIVa) is metabolized into XIVb in the liver and shows similar biological activity to XIVb. Since XIVα can be easily synthesized than XIVb, the former must

1) Location: a) Kawashima, Hashima, Gifu; b) Koishikawa 4-chome, Bunkyo-ku, Tokyo; c) 19-1, Matsuyamahita-machi 4-chome, Higashinada-ku, Kobe.
be a valuable substitute for the latter. The synthesis of XIVa has been reported by several research groups, but most of them were complicated and gave poor yield except the attractive route reported by Barton et al. We tried to find a synthetic route giving good yield and then established the route as shown in Chart 1. The synthesis of XIVb by using a similar route is also described in this paper.
Kaneko et al. reported the following two routes using the deconjugation procedures for the synthesis of cholesta-5,7-diene-1α,3β-diol (XIIa). Their first method included the conversion of cholesta-1,4-dien-3-one to cholesta-1,5-dien-3-one by the deconjugation procedure, followed by NaBH₄ reduction, hydroboration and alkaline hydrogen peroxide oxidation to yield 1α-hydroxycholesterol (VIIIa). However, since this method gave large quantities of by-products including 2α-hydroxycholesterol, the overall yield of XIIa from cholesta-1,4-dien-3-one was poor (ca. 2.6%). Morisaki et al. reported a modified method on the 1α-hydroxylation using oxymercuration-demercuration reaction instead of the hydroboration-oxidation, but the yield was still unsatisfactory. In the second method of Kaneko et al., cholesta-1,4,6-trien-3-one was converted to cholesta-1,5,7-trien-3-one by the deconjugation procedure, followed by Ca(BH₄)₂ reduction to yield cholesta-1,5,7-trien-3β-ol. The triazolone adduct of cholesta-1,5,7-trien-3β-ol was converted to the 1,2-epoxy compound by m-chloroperbenzoic acid oxidation, followed by the reaction with LiAlH₄ to yield XIIa. However, since the epoxidation of the triazolone adduct gave much more quantities of the 1β,2β-epoxide (yield: 50—55%) than the expected 1α,2α-epoxide (yield: 30—35%), the overall yield from Ia was also poor (ca. 3%).

Although these are interesting and attractive methods using the deconjugation procedures, the weak points in the idea of 1α-hydroxy group as mentioned above caused to give poor yield. In order to find a convenient method giving higher yield than them, we investigated the conversion of 1α,2α-epoxycholesta-4-en-3-one (IVa) to 1α,2α-epoxycholesta-5-en-3-one (Va) by the deconjugation procedure which was reported to fail by Pec and Kodicek. Since it is known that the epoxidation of IIa predominantly gives 1α,2α-epoxycholesta-4,6-dien-3-one (IIIa) (more than 90% yield) and that the reaction of the 1α,2α-epoxy compounds with LiAlH₄ usually gives the corresponding 1α-hydroxyl compounds in good yield, high yield of VIIIa must be obtained if the conversion of IVa to Va proceeds smoothly. After investigating the deconjugation reaction, we found that the conversion went smoothly when the condition on the deconjugation procedure was strictly chosen.

Another characteristic in our route is the modification on the photochemical conversion of XIIa to 1α-previtamin D₃ (XIIIa) by ultraviolet (UV) ray's irradiation through a newly found filter solution. This filter solution which is stable during irradiation can cut off the undesirable UV lights in the ranges below 275 nm and above 310 nm for the photochemical conversion. Finally, we established a convenient synthetic route of XIIa including the two characteristics mentioned above as shown in Chart 1.

Cholesta-1,4,6-trien-3-one (IIa) obtained by oxidation of Ia with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) was oxidized with hydrogen peroxide in 10% methanolic KOH solution according to Glotter et al. to give IIIa in good yield (40.5% yield from Ia). 1α,2α-Epoxycholesta-4-en-3-one (IVa) obtained by the catalytic hydrogenation of IIIa was readily converted in good yield to Va (78% from IVa) by the deconjugation procedure with t-BuOK in dimethyl sulfoxide (DMSO) under argon atmosphere, followed by pouring into ice-cold KH₂PO₄ solution and immediately extracting with ethyl acetate. Since the 5-en-3-one (Va) is sensitive to aqueous alkaline solution and regenerates the 4-en-3-one (IVa),
the protonation should be performed in the weak acidic solution using KH₂PO₄ to give good results. The infrared (IR) spectrum of Va showed the absorption at 1715 cm⁻¹ indicating the presence of isolated ketone and the nuclear magnetic resonance (NMR) as shown in Fig. 1 supported the structure of Va.

1₂,2₂-Epoxycholesterol-5-en-3-one (Va) was reduced with Ca(BH₄)₂ to give the mixture of two isomers, 1₂,2₂-epoxycholesterol-5-en-3β-ol (VIa) and 1₂,2₂-epoxycholesterol-5-en-3α-ol (VIIa). These were purified by silica gel column chromatography and VIa was obtained in ca. 45% yield from IVa. Reaction of VIa with LiAlH₄ afforded VIIIa in high yield of more than 95%. The overall yield of VIIIa from Ia was 15.6%.

1₂,3₂-Diacetoxycholesterol-5-ene (Xa) obtained by acetylation of VIIIa was converted to 1₂,3₂-diacetoxycholesta-5,7-diene (Xia) by allylic bromination at 7-position with 1,3-dibromom-5,5'-dimethylhydantoin (DDH), followed by dehydrobromination with trimethylphosphite (TMP) according to conventional methods. Purification of the crude product by silica gel column chromatography, gave Xia in 51% yield from Xa. Saponification of Xia gave XIIa as colorless needles in 39% yield from Xa.

A solution of XIIa (200 mg) in 400 ml of ethanol was irradiated for 45 min under argon atmosphere with a 200 W high-pressure mercury lamp through the newly found filter solution which contained nickel sulfate, copper sulfate, quinine hydrochloride and sulfuric acid in water. Barton et al.¹⁰,¹¹ mentioned that the UV lights in the ranges below 275 nm and above 310 nm, especially between 310 and 330 nm, gave undesirable by-products on the photochemical reaction of XIIa. They used an organic filter solution consisting of carbon disulfide, toluene and methanol,¹¹ but it was unstable to UV irradiation. On the other hand, our used filter solution was stable during UV irradiation. Effect of wavelength on the photochemical reaction of XIIa will be reported in the forthcoming paper.

The irradiated solution was condensed to about one-fourth and then heated at 75° to reach the equilibrium of XIIIa and XIVa. The thermal isomerized mixture was purified on a silica gel column to afford XIVa which was crystallized from n-hexane to give colorless needles (mp 124—125°). The yield of the crystals from XIIa was ca. 25%. On the other hand, another purification was also performed. The irradiated solution was evaporated and then applied to Sephadex column chromatography. The resulting XIIIa was thermally isomerized into XIVa by refluxing in ethanol solution and the mixture was purified on a silica gel column, followed by crystallization from n-hexane to give the crystalline XIVa. The yield from XIIa in this procedure was also ca. 25%.

The overall yield of XIVa from Ia was 1.5%. Since it was a little less than that of Barton et al.¹⁰ (2.2%) but better than those of other research groups (less than 1%), our route was thought to be in practical use.

The synthesis of XIVb was also investigated by using a similar route to that of XIVa. Several research groups have reported on the synthesis of XIVb,¹² but most of their routes were too complicated to give good yield except the attractive one reported by Barton et al.¹² The route to synthesize XIVb the route used for the synthesis of XIVa was applied to 25-hydroxycholesterol (Ib)¹³ obtained from desmosterol.

1₂,2₂-Epoxycholesterol-4-en-25-ol-3-one (IVb) obtained from Ib by the same route as described for IVa was converted to 1₂,2₂-epoxycholesterol-5-en-25-ol-3-one (Vb) by the deconjugation

reaction with t-BuOK in DMSO. The spectral data of Vb were similar to those of Va. 1z,2z-Epoxycholestan-5-en-25-ol-3-one (Vb) was reduced with Ca(BH₄)₂, followed by purification on a silica gel column to give 1z,2z-epoxycholestan-5-en-3β,25-diol (VIb). Reaction of VIb with LiAlH₄ afforded 1z,25-dihydroxycholesterol (VIIIb).

1z,3β,25-Triacetoxycholestan-5-ene (Xb) obtained by acetylation of VIIIb was converted to 1z,3β,25-triacetoxycholestan-5,7-diene (XIb) by allylic bromination at 7-position, followed by dehydrobromination according to conventional methods. After purification of XIb by silica gel chromatography, it was saponified. Purification of the saponified mixture on a Sephadex column followed by crystallization from methanol gave cholesta-5,7-diene-1z,3β,25-triol (XIIb) as colorless needles in ca. 20% yield from Xb.

The solution of XIIb (200 mg) in 400 ml of ethanol was irradiated in the same manner as described in XIIa. The irradiated mixture was purified by Sephadex column chromatography to give 1z,25-dihydroxyprevitamin D₃ (XIIIb). This was thermally isomerized into XIVb by refluxing in ethanol solution and the mixture was purified on a sephadex LH-20 column to afford XIVb as an oil. After further purification by silica gel column chromatography and crystallization from benzene-ethyl acetate, XIVb was obtained as colorless needles (mp 100°-103°). The overall yield of XIVb from Ib was ca. 0.4%.

**Experimental**

Melting points were determined with a Yanagimoto Micro Melting Point apparatus and uncorrected. Infrared (IR) spectra were obtained either in Nujol or in KBr disks on a Hitachi EPI-G3 infrared spectrometer. UV spectra were obtained on a Shimadzu UV-210 spectrometer. NMR spectra were obtained on a JEOL JNM-FS-100 spectrometer with CDCl₃ as solvent and chemical shifts (δ) were given as ppm relative to tetramethylsilane as an internal standard. The following abbreviations were used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, b=broad. Mass spectra (MS) were recorded on a JEOL JMS-01SG spectrometer. Optical rotations were measured with a Yanagimoto ORD-50D polarimeter.

**1a,2a-Epoxycholestan-4-en-3-one (IVA)—** 1z,2z-Epoxycholestan-4,6-dien-3-one (IIIa, 39.7 g), which was obtained in 40.5% yield by oxidation of cholesterol (Ia) with DDQ and subsequently with hydrogen peroxide in 10% methanolic KOH solution, was catalytically hydrogenated in the presence of 10% Pd-CaCO₃ (2.8 g) in benzene (300 ml) until hydrogen uptake equivalent to 1.08 mol was observed. After filtering off the catalyst, the filtrate was evaporated under reduced pressure. Crystallization of the resulting residue from methanol gave 3.0 g of IVa, mp 127°-130°, UV λmax nm (ε): 246.5 (13900). NMR δ: 3.40 (1H, d, J=4 Hz, 1β-H), 3.60 (1H, d, J=4 Hz, 3β-H), 5.80 (1H, b, 4-H). MS m/e: 298 (M⁺). Anal. Calcd. for C₂₉H₄₂O₂: C, 81.85; H, 10.62. Found: C, 81.06; H, 10.79.

**1a,2a-Epoxycholestan-5-en-3-one (Va)—** A solution of IVa (16 g) in dry tetrahydrofuran (THF, 100 ml) was added to the suspension of t-BuOK (8.8 g) in DMSO (100 ml), dried over molecular sieves 4Å with stirring under argon atmosphere. After stirring for further 25 min, the reaction mixture was poured into 11% KH₂PO₄ solution (300 ml) containing crushed ice (ca. 500 g) and immediately extracted with ethyl acetate. The organic layer was washed with saturated NaCl solution, dried over anhydrous MgSO₄, then evaporated to dryness under reduced pressure. Crystallization of the resulting residue from methanol gave 12.5 g of Va as crystals. Small portion of this crystals was recrystallized from methanol to afford colorless crystals (Va), mp 113°-115°, IR νmax cm⁻¹: 1715 (C=O). NMR δ: 3.02 (2H, s, 4-H₂), 3.28 (1H, d, J=4 Hz, 1β-H), 3.43 (1H, d, J=4 Hz, 2β-H), 5.45 (1H, d, J=6 Hz, 6-H).

**1a,2a-Epoxycholestan-5-en-3β-ol (VIA) and 1a,2a-Epoxycholestan-5-en-3α-ol (VIIa)—** To the solution of powdered CaCl₂ (48 g) in dry THF (850 ml) and ethanol (2.5 l), NaBH₄ (5.6 g) dissolved in dry THF (70 ml) and ethanol (150 ml) was added. After stirring for 3 h at room temperature, the crude residue of Va obtained above (24 g) was added to the resulting Ca(BH₄)₂ solution during 30 min. After continuous stirring for further 1.5 h at room temperature, the solvent was evaporated off under reduced pressure below 40°. A mixture of water (2 l) and acetic acid (40 ml) was added. The mixture was extracted with ethyl acetate, washed with saturated NaCl solution and dried over anhydrous Na₂SO₄. The ethyl acetate solution was evaporated to dryness below 40° under reduced pressure to afford a yellow crystalline residue. This was chromatographed on a silica gel column. Elution with n-hexane-ethyl acetate (9:1 v/v) and crystallization from methanol gave 13.0 g of VIA, mp 110°-112°, NMR δ: 3.09 (1H, d, J=4 Hz, 1β-H), 3.18 (1H, d, J=4 Hz, 2β-H), 3.90 (1H, m, 3α-H), 5.50 (1H, d, J=5 Hz, 6-H). MS m/e: 400 (M⁺). Elution with n-hexane-ethyl acetate (6:1 v/v) gave 1.8 g of VIIa, mp 139°-141° (methanol). NMR δ: 3.20 (1H, d, J=4 Hz, 1β-H), 3.48 (1H, t, J=4 Hz, 2β-H), 4.10 (1H, m, 3α-H), 5.54 (1H, d, J=5 Hz, 6-H). MS m/e: 400 (M⁺).
Cholest-5-ene-1α,3β-diol (VIIIa) — To the suspension of LiAlH₄ (10 g) in THF (420 ml) was added 50 g of Vla in THF (420 ml) during 1 hr at 60°C. The solution was continuously stirred for further 30 min at 60°C and excess LiAlH₄ was destroyed with addition of water. The solvent was evaporated off below 60°C under reduced pressure, and to the resulting residue was added a mixture of water (800 ml) and conc. HCl (80 ml). The mixture was extracted with CHCl₃, washed with saturated NaCl solution and dried over anhydrous Na₂SO₄. After evaporating the solvent under reduced pressure, the resulting residue was crystallized from methanol to give 47.5 g of colorless needles (VIIIa), mp 163–164°C, [α]D²⁰ = -36.0° (c=0.428, CHCl₃).
NMR δ: 3.82 (1H, b, W₁/2=8 Hz, 1β-H), 3.96 (1H, m, W₁/2=24 Hz, 3β-H), 5.58 (1H, d, J=5 Hz, 6-H). MS m/e: 402.3487 (M⁺, C₃₇H₇₄O₃ requires 402.3497), 384 (M⁺–H₂O), 366 (M⁺–2H₂O), 351 (M⁺–2H₂O–CH₃). Anal. Calcd. for C₃₇H₇₄O₃: C, 80.54; H, 11.52. Found: C, 80.51; H, 11.45.

Cholest-5-ene-1α,3α-diol (IXa) — VIIa (201 mg) was reduced with LiAlH₄ (75 mg) in THF (10 ml) and worked up according to the similar method as described above. Crystallization from acetone gave 132 mg of IXa, mp 222–233°C. NMR δ: 3.70 (1H, m, W₁/2=10 Hz, 1β-H), 4.08 (1H, m, W₁/2=10 Hz, 3β-H), 5.52 (1H, d, J=5 Hz, 6-H). MS m/e: 402 (M⁺).

1α,3β-Diacetoxycholest-5-ene (Xa) — VIIa (14 g) was dissolved in a mixture of acetic anhydride (100 ml) and pyridine (60 ml). After heating for 2 hr at 90°C, the solution was poured into ice-cold water and then extracted with ethyl acetate. The organic layer was washed with water, 1 N HCl and saturated NaHCO₃ solution, and dried over anhydrous MgSO₄. Evaporation of the solvent under reduced pressure gave crystalline solid, which was recrystallized from methanol to give 16.1 g of colorless needles (Xa), mp 101–103°C, [α]D²⁰ = -17.1° (c=1.185, CHCl₃).
NMR δ: 2.01 (3H, s, acetyl-CH₃), 2.05 (3H, s, acetyl-CH₃), 4.99 (1H, m, W₁/2=24 Hz, 3α-H), 5.12 (1H, b, W₁/2=8 Hz, 1β-H), 5.50 (1H, m, 6-H). MS m/e: 426 (M⁺–CH₃COOH), 366 (M⁺–2CH₃COOH), 351 (M⁺–2CH₃COOH–CH₃), 247, 211.

1α,3β-Diacetoxycholest-5,7-diene (XIa) — To a refluxing solution of Xa (24.3 g) in n-hexane (800 ml) was added DDO (9.1 g) and the solution was refluxed for 10 min. After it was cooled to room temperature, the resulting precipitate was filtered off. The filtrate was evaporated to dryness below 40°C under reduced pressure. The resulting yellow residue was dissolved in xylene (800 ml) and then dropped in a refluxing solution of 3M (8 ml) and xylene (170 ml) during 30 min. After refluxing for further 2 hr, the solvent was evaporated off under reduced pressure. The residue was dissolved in n-hexane, which was washed with water and evaporated to dryness under reduced pressure. The resulting residue was purified by chromatography on a silica gel column. Elution with n-hexane–ethyl acetate (95: 5 v/v) gave 13.35 g of XIa as crystals, mp 118–119°C. UV λmax nm (ε): 263.0, 271.0, 282.0 (12000), 294.0. Recrystallization from methanol gave colorless needles, mp 119.5–120°C. UV λmax nm (ε): 263.0, 271.0, 282.0 (12000), 294.0. NMR δ: 2.03 (3H, s, acetyl-CH₃), 2.08 (3H, s, acetyl-CH₃), 5.00 (2H, m, 1α-H and 3α-H), 5.38 (1H, m, 6-H or 7-H), 5.68 (1H, m, 6-H or 7-H). MS m/e: 484 (M⁺), 424 (M⁺–CH₃COOHH), 364 (M⁺–2CH₃COOH), 251 (M⁺–side chain–CH₃).

Cholest-5,7-diene-1α,3β-diol (XIIa) — 2.9 g of the crude crystalline powder of XIa was added to 5% KOH in a mixture of water–methanol–benzene (1:14: 5 v/v) and heated at 40°C for 1 hr under argon atmosphere. The saponified mixture was poured into water and then extracted with ethyl acetate. The organic layer was washed with water, dried over anhydrous Na₂SO₄ and then evaporated to dryness under reduced pressure. Crystallization of the resulting residue from methanol gave 1.7 g of XIIa as colorless needles, mp 171–175°C (under argon) (ref., 90) 172–173°C; ref., 94) 155–158°C, ref., 90 157–160°C). UV λmax nm (ε): 271.5, 282.5 (12000), 294.3. NMR δ: 3.71 (1H, b, W₁/2=8 Hz, 1β-H), 4.02 (1H, m, W₁/2=27 Hz, 3α-H), 5.34 (1H, m, 6-H or 7-H), 5.59 (1H, d, J=9 Hz, 6-H or 7-H). MS m/e: 400 (M⁺), 392 (M⁺–H₂O), 364 (M⁺–2H₂O), 344, 312, 296 (M⁺–side chain–H₂O), 251 (M⁺–side chain–2H₂O), 227.

1α-Hydroxyvitamin D₃ (XIIIa) — Method A: XIIa (200 mg) in ethanol (400 ml) was irradiated for 43 min under argon atmosphere with a 200 W high-pressure mercury lamp (Taika Kogyo, HL-B-V) through the filter solution (prepared by dissolving 200 g of NaSO₄·6H₂O, 2 g of CuSO₄·5H₂O, 0.1 g of quinine hydrochloride and 4 ml of conc. H₂SO₄ in water to make 1000 ml) in order to use the UV lights in the range between 275 and 310 nm. In order to keep the irradiating solution below 40°C, the outside of the mercury lamp was cooled by circulating the cooled filter solution and the reaction vessel was also cooled from outside with ice-cold water. The combined irradiated solution (sum of XIIa: 1 g) was condensed to about 500 ml under reduced pressure and heated at 75°C for 2.5 hr under argon atmosphere to isomerize the formed XIIIa into XIVa. After evaporation of the solvent under reduced pressure, the resulting residue was purified by chromatography on a silica gel column. Elution with benzene–ethyl acetate (4: 1 v/v) gave 254 mg of XIVa, mp 124–125°C (n-hexane). UV λmax nm (ε): 265.5 (17100); 258.5 (28800). NMR δ: 3.90–4.64 (2H, m, 1α-H and 3α-H), 5.01 and 5.32 (2H, a pair of m, 19-H), 6.01 (1H, d, J=11 Hz, 7-H), 6.39 (1H, d, J=11 Hz, 6-H). MS m/e: 400.336 (M⁺, C₃₆H₇₄O₃ requires 400.334), 382 (M⁺–H₂O), 364 (M⁺–2H₂O), 287 (M⁺–side chain), 269 (M⁺–side chain–H₂O), 152, 154. IR νmax cm⁻¹: 3413, 3092, 3035, 1645, 1631, 1602, 1048, 956, 900.

Method B: The combined irradiated solution from five runs described in method A was evaporated under reduced pressure and the resulting residue was chromatographed on a Sephadex LH-20 column. Elution with CHCl₃–n-hexane (65: 35 v/v) gave XIIIa as an oil, UV λmax nm: 259. The oil was dissolved in ethanol (100 ml) and heated at 75°C for 2.5 hr under argon atmosphere to isomerize XIIIa into XIVa.
evaporation of the solvent under reduced pressure, the resulting residue was chromatographed on a silica gel column with benzene-ethyl acetate (4:1 v/v). Crystallization from n-hexane gave 254 mg of fine colorless needles (XIVa).

**Stability of Filter Solutions**—(i) Barton's Filter Solution. This solution was prepared by dissolving 24 ml of toluene and 4 ml of carbon disulfide in methanol to make 1000 ml. The filter solution (1.7 l) was UV irradiated with a 100 W high-pressure mercury lamp (Taika Kogyo, HL8-V), and it was circulated with a cooling-circulator during UV irradiation. The optical density of the solution at 295 nm was 1.05 and it was increased to 1.50 after UV irradiation for 30 min. A white precipitate was formed by UV irradiation for more than 17 min.

(ii) Our Filter Solution: The filter solution was prepared by dissolving 200 g of NiSO$_4$·6H$_2$O, 2 g of CuSO$_4$·5H$_2$O, 0.1 g of quinine hydrochloride and 4 ml of conc. H$_2$SO$_4$ in water to make 1000 ml. The solution (2.4 l) was irradiated with a 200 W high-pressure mercury lamp (Taika Kogyo HL8-V) in the same manner as described above. The optical density of the solution was 0.75, which remained unchanged after irradiation for 90 min.

1a, 2a-Epoxycholesten-4-en-25-ol-3-one (IVb) — 5 g of 1a, 2a-epoxycholesta-4,6-dien-25-ol-3-one (IIb) obtained from 25-hydroxycholesterol (1b) in 35% yield according to Barton et al. Tas catalytically hydrogenated in the presence of 10% Pd-CaCO$_3$ (0.5 g) in benzene. After filtering off the catalyst, the filtrate was evaporated under reduced pressure. The resulting residue was chromatographed on a silica gel column. Elution with n-hexane–ethyl acetate (2:1 v/v) and crystallization from n-hexane–ethyl acetate gave 4.0 g of IVb, mp 166—167°C. UV $\lambda_{max}$ nm (ε): 246.5 (12900). NMR δ: 1.20 (6H, s, 26-CH$_3$ and 27-CH$_3$), 1.24 (3H, s, 19-CH$_3$), 3.40 (1H, d, J = 2 and 4 Hz, 2β-H), 3.55 (1H, d, J = 4 Hz, 1β-H), 5.70 (1H, b, $W_1/2$ = 4 Hz, 4-H). MS m/z: 414 (M$^+$), 396 (M$^+$–H$_2$O). Anal. Calcd. for C$_{29}$H$_{43}$O: C, 78.21; H, 10.21. Found: C, 78.32; H, 10.37.

1a, 2a-Epoxycholesten-5-en-25-ol-3-one (Vb) — A solution of IVb (3.0 g) in THF (30 ml) was added to the suspension of t-BuOK (1.65 g) in DMSO (19 ml) with stirring under argon atmosphere. After stirring for 25 min, the reaction mixture was poured into 6.15% K$_2$PO$_4$ solution (100 ml) containing crushed ice and then immediately extracted with ethyl acetate. The usual work-up gave a crude residue (3.6 g). Treatment of the resulting residue with cold methanol afforded pale yellow crystals (Vb), mp 74—81°C. NMR δ: 1.21 (6H, s, 26-CH$_3$ and 27-CH$_3$), 3.08 (2H, s, 4-H$_2$), 3.55 (1H, d, J = 4 Hz, 1β-H), 3.54 (1H, d, J = 4 Hz, 2β-H), 5.56 (1H, m, $W_1/2$ = 8 Hz, 6-H), 5.72 (1H, m, impurity due to C-4 proton of unreacted IVb).

1a, 2a-Epoxycholesten-3,β,25-triol (VIb) — The crude deconjugation product (Vb) (3 g) was reduced with NaBH$_4$ as described for Vla. After extracting with ethyl acetate, the solution was evaporated under reduced pressure to give a yellow oil. This oily residue was purified by silica gel column chromatography. Elution with n-hexane–ethyl acetate (4:1 v/v) and crystallization from ethyl acetate gave 1.3 g of colorless crystals (VIb), mp 156—158°C. NMR δ: 1.20 (6H, s, 26-CH$_3$ and 27-CH$_3$), 3.06 (1H, d, J = 4 Hz, 1β-H), 3.16 (1H, d, J = 4 Hz, 2β-H), 3.36 (1H, q, J = 8 and 10 Hz, 3α-H), 5.50 (1H, m, $W_1/2$ = 4 Hz, 6-H). MS m/z: 416 (M$^+$), 398 (M$^+$–H$_2$O), 380 (M$^+$–2H$_2$O). Anal. Calcd. for C$_{29}$H$_{43}$O$_2$: C, 77.83; H, 10.65. Found: C, 77.54; H, 10.60.

**Cholesterol-5-en-1a,3β,25-triol (VIIIb) —** Vlb (1.0 g) was reduced with LiAlH$_4$ (0.5 g) in THF (35 ml) as described for VIIA. Extraction with methylene chloride and evaporation of the solution under reduced pressure gave a residue which was chromatographed from acetone–benzene to give 810 mg of VIIIb, mp 170.5—172.5°C, [α]$_D$ = −33.3° (c = 0.3, CHCl$_3$). NMR δ: 1.20 (6H, s, 26-CH$_3$ and 27-CH$_3$), 3.85 (1H, m, W$_1/2$ = 8 Hz, 1β-H), 4.00 (1H, m, W$_1/2$ = 24 Hz, 3α-H), 5.60 (1H, m, W$_1/2$ = 10 Hz, 6-H). MS m/z: 418.3446 (M$^+$, C$_{29}$H$_{40}$O$_3$ requires 418.3447), 400 (M$^+$–H$_2$O), 382 (M$^+$–2H$_2$O), 364 (M$^+$–3H$_2$O).

**Cholesterol-5-en-1a,3α,25-triol (IXb) —** To the suspension of LiAlH$_4$ (1 g) in THF (70 ml) was added 2 g of the crude Ca(BH$_4$)$_2$ reduction product (VIIb+Vlb) of VIb in THF (50 ml) during 20 min at room temperature. The solution was heated at 60°C for 90 min and then worked up according to the similar method as described for VIIA to give a crystalline residue. The residue was chromatographed on a silica gel column with n-hexane–ethyl acetate (1:1 v/v) to give 221 mg of colorless prisms (IXb), mp 212.5—214.5°C. NMR δ: 1.22 (6H, s, 26-CH$_3$ and 27-CH$_3$), 3.76 (1H, m, W$_1/2$ = 8 Hz, 1β-H), 4.12 (1H, m, W$_1/2$ = 8 Hz, 3α-H), 5.58 (1H, m, W$_1/2$ = 10 Hz, 6-H). MS m/z: 416 (M$^+$), 400 (M$^+$–H$_2$O), 382 (M$^+$–2H$_2$O), 364 (M$^+$–3H$_2$O). Anal. Calcd. for C$_{29}$H$_{42}$O: C, 77.46; H, 11.08. Found: C, 77.52; H, 11.14.

Further elution with the same solvent mixture gave 0.51 g of VIIb as colorless needles.

1a,3β,25-Triacetoxycholesten-5-ene (Xb) — VIIIb (3.25 g) was dissolved in a mixture of acetic anhydride (45 ml) and pyridine (3 ml). After refluxing for 3 hr on an oil bath, the mixture cooled to room temperature was poured into ice-cold water and then extracted with ethyl acetate. After washing with saturated NaHCO$_3$ solution and water, the organic layer was dried over anhydrous Na$_2$SO$_4$. Evaporation of the solvent under reduced pressure gave an oily residue, which was chromatographed on a silica gel column. Elution with 2% ethyl acetate in benzene gave 3.47 g of Xb as an oil. NMR δ: 1.39 (6H, s, 26-CH$_3$ and 27-CH$_3$), 1.94, 1.99 and 2.02 (9H, a trio of s, acetyl-CH$_3$), 4.90 (1H, m, W$_1/2$ = 24 Hz, 3α-H), 5.06 (1H, m, W$_1/2$ = 8 Hz, 1β-H), 5.54 (1H, m, W$_1/2$ = 8 Hz, 6-H). MS m/z: 484 (M$^+$–CH$_3$COOH), 424 (M$^+$–2CH$_3$COOH), 364 (M$^+$–3CH$_3$COOH).
Cholesta-5,7-diene-1α,3β,25-triol (XII)—To a refluxing solution of Xb (3.7 g) in n-hexane (200 ml) was added DDH (1.2 g) and the solution was refluxed for 30 min. After cooling to room temperature, the resulting precipitate was filtered off. The filtrate was evaporated below 40° under reduced pressure. The resulting oily residue was dissolved in xylene (40 ml) and then dropped in a refluxing mixed solution of TMP (28 ml) and xylene (120 ml) during 30 min. After refluxing for further 150 min, the solution was evaporated under reduced pressure. The resulting residue was dissolved in ethyl acetate, washed with water and evaporated to dryness under reduced pressure. The residue was chromatographed on a silica gel column with n-hexane–ethyl acetate (7:1 v/v) to give ca. 1.6 g of crude solid (XIIb). This solid was added to 5% KOH in a mixture of water–methanol–benzene (1:14:5) and heated at 40° for 1 hr under argon atmosphere. The mixture was worked up by conventional method. The resulting residue was purified by Sephadex LH-20 column chromatography. Elution with CHCl₃–n-hexane–methanol (65:35:2) gave a crystalline residue. Recrystallization from methanol gave 618 mg of XIIb, mp 211—213°. UV λmax (acetone) nm (ε): 264.0, 271.6, 282.4 (11100), 294.2. NMR δ: 1.22 (6H, s, 26-CH₃ and 27-CH₃), 3.77 (1H, t, J = 4 Hz, 1β-H), 4.07 (1H, m, W1/2 = 24 Hz, 3α-H), 5.39 (1H, m, W1/2 = 12 Hz, 6-H or 7-H), 5.72 (1H, d, d, J = 6 and 2 Hz, 6-H or 7-H). MS m/e: 416 (M⁺), 398 (M⁺–H₂O), 383 (M⁺–H₂O–CH₃), 380 (M⁺–2H₂O), 365 (M⁺–2H₂O–CH₃), 362 (M⁺–3H₂O), 287 (M⁺–side chain), 269 (M⁺–side chain–H₂O), 251 (M⁺–side chain–2H₂O), 227.

1α,25-Dihydroxyvitamin D₃ (XIVb)—XIIb (200 mg) in ethanol (400 ml) was irradiated for 45 min under argon atmosphere with a 200 W high-pressure mercury lamp through the filter solution in the same manner as described for XIIa. Evaporation of the solvent under reduced pressure gave an oily residue from the two runs (400 mg) was chromatographed on a Sephadex LH-20 column. Elution with CHCl₃–n-hexane–methanol (62:35:3 v/v) gave 1α,25-dihydroxyvitamin D₃ (XIVb) as an oil. The oil was dissolved in ethanol (50 ml) and heated at 75° for 200 min under argon atmosphere to isomerize XIIb into XIVb. After evaporation of the solvent under reduced pressure, the resulting residue was purified by chromatography on a Sephadex LH-20 column. Elution with CHCl₃–n-hexane–methanol (62:35:3 v/v) gave 146 mg of oil, which was further purified by chromatography on a silica gel. Elution with benzene–ethyl acetate (3:2 v/v) and crystallization from benzene–ethyl ether afforded 76 mg of colorless fine needles (XIVb), mp 100—103°. UV λmax (acetone) nm (ε): 264.5 (16600); 225.0 (9600). NMR δ: 1.22 (6H, s, 26-CH₃ and 27-CH₃), 4.22 (1H, m, W1/2 = 11.4 Hz, 3α-H), 4.42 (1H, t, J = 5.7 Hz, 3β-H), 5.00 and 5.32 (2H, a pair of b.s, 19-H₂), 5.00 and 5.32 (2H, a pair of b.s, 19-H₂), 6.00 (1H, d, J = 11.4 Hz, 7-H) and 6.38 (1H, d, J = 11.4 Hz, 6-H). MS m/e: 416.3272 (M⁺) (Calcd. for C₂₉H₂₂O₄ 416.3291), 398 (M⁺–H₂O), 380 (M⁺–2H₂O), 362 (M⁺–3H₂O), 251 (M⁺–side chain–3H₂O), 152, 134.

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