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Syntheses of 24,25-Dihydroxyvitamin D_2, 24,25-Dihydroxy-22-dehydrovitamin D_3, 25-Hydroxy-24-oxo-22-dehydrovitamin D_3 and 22,24,25-Trihydroxyvitamin D_3[1)]

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Four vitamin D_2 and D_3 metabolites, 24,25-dihydroxyvitamin D_2 (**8**), 24,25-dihydroxy-22-dehydrovitamin D_3 (**10**), 25-hydroxy-24-oxo-22-dehydrovitamin D_3 (**12**) and 22,24,25-trihydroxyvitamin D_3 (**14**), were synthesized from ergosterol (**1**) *via* the hydroxyketone (**4**) as a common key intermediate.

Keywords——24,25-dihydroxyvitamin D_2; 24,25-dihydroxy-22-dehydrovitamin D_3; 25-hydroxy-24-oxo-22-dehydrovitamin D_3; 22,24,25-trihydroxyvitamin D_3; vitamin D; ergosterol; high-performance liquid chromatography

There are two of vitamin D,[2)] namely D_2 and D_3, which differ in the side chain structure but have practically the same biological activity in mammals, including human beings. These two vitamins are known to be similarly metabolized to 25-hydroxyvitamin D (25-OH-D) in the liver and subsequently to 1α,25-dihydroxyvitamin D [1α,25-$(OH)_2$-D] or 24*R*,25-dihydroxyvitamin D [24*R*,25-$(OH)_2$-D] in the kidney when plasma calcium concentrations are lower or higher than normal.[3)] Many studies have been done on the further metabolism of 25-OH-D_3 and 1α,25-$(OH)_2$-D_3. The 26,23-lactone,[4)] 23-hydroxy,[5)] 23,24-dihydroxy,[6)] 24,26-dihydroxy,[6)] 23-oxo,[6)] 24-oxo[6,7)] and 23-hydroxy-24-oxo[8-10)] derivatives of 25-OH-D_3 and 26,23-lactone,[11)] 23-hydroxy[12)] and 24-oxo[13,14)] derivatives of 1α,25-$(OH)_2$-D have been isolated and identified as *in vivo* or *in vitro* metabolites. The mechanisms of metabolism have been well discussed[15,16)] and some of the isolated metabolites were chemically synthesized.[17-21)] However, few reports have appeared on the metabolism of vitamin D_2, and the metabolic fate of the double bond at the 22-position and the methyl group at the 24*S*-position in the side chain of vitamin D_2 remains to be clarified. We now report the syntheses of four potential metabolites, 24,25-$(OH)_2$-D_2 (**8**), 24,25-dihydroxy-22-dehydrovitamin D_3 [Δ^{22}-24,25-$(OH)_2$-D_3] (**10**), 25-hydroxy-24-oxo-22-dehydrovitamin D_3 [Δ^{22}-24-oxo-25-OH-D_3] (**12**) and 22,24,25-trihydroxyvitamin D_3 [22,24,25-$(OH)_3$-D_3] (**14**).

First, we investigated the synthesis of 24,25-$(OH)_2$-D_2 (**8**). Though the synthesis of **8** from stigmasterol was reported by Jones *et al.*,[22,23)] their synthetic route is rather complicated. Therefore, we have developed an improved synthesis of **8** by modification of their procedure. As shown in Chart 1, ergosterol (**1**) was converted into the known 20-aldehyde (**3**) *via* a route involving protection of the 5,7-diene group and ozonolysis according to Barton *et al.*[24)] Aldol condensation of **3** with 3-methyl-3-(tetrahydropyran-2-yloxy)butan-2-one proceeded very smoothly to afford the hydroxyketone (**4a** and **4b**) as a diastereomeric mixture; **4a**

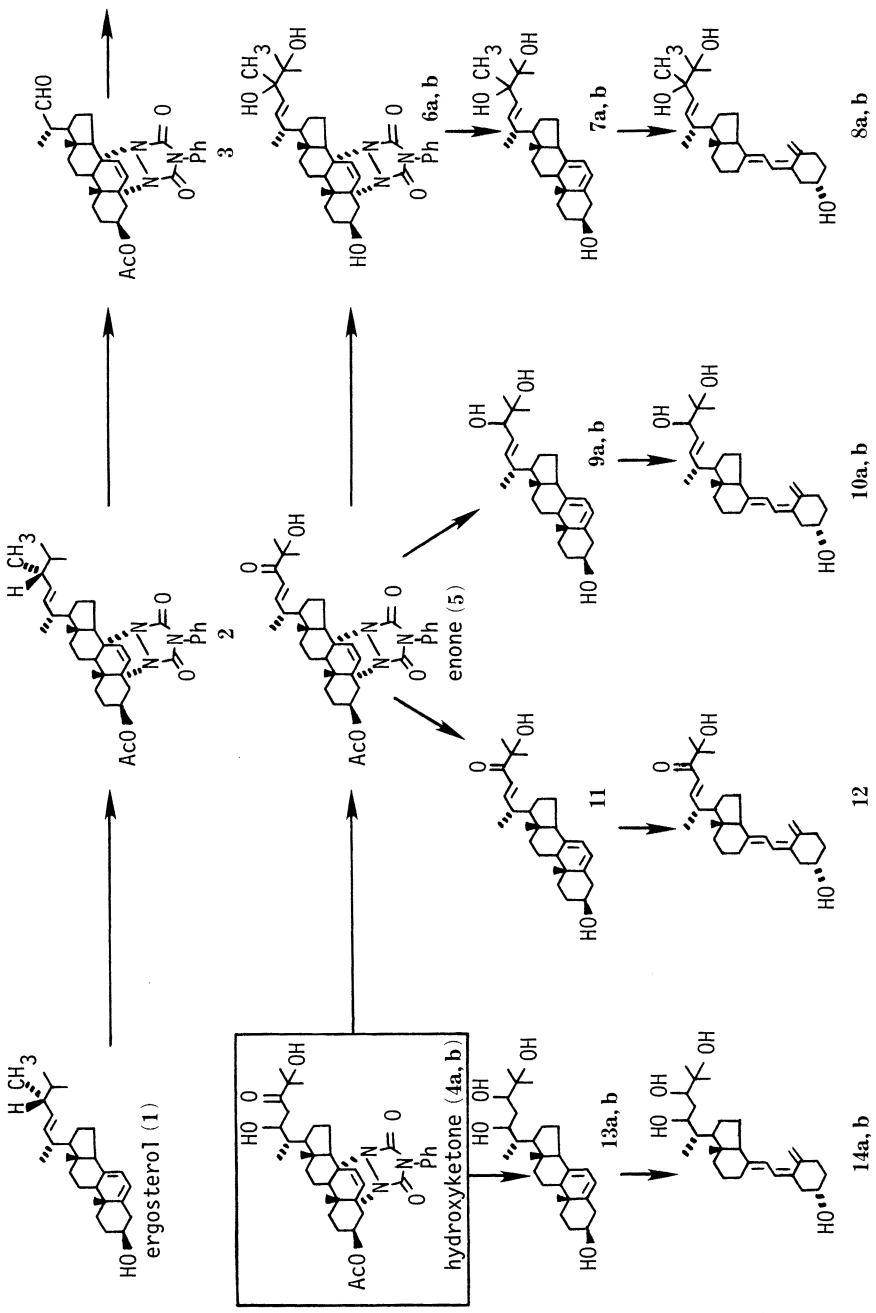

Chart 1. Synthetic Scheme

and **4b** were separated, both as colorless crystals, by preparative thin layer chromatography (TLC) using silica gel. The yields of the less polar (**4a**: $Rf=0.33$) and more polar (**4b**: $Rf=0.19$) 22-isomers were 18 and 21%, respectively, though their stereochemistry at the 22-position has not been clarified. Dehydration of a mixture of **4a** and **4b** with *p*-toluenesulfonic acid afforded the known enone (**5**) in 90% yield, which exhibited the proton nuclear magnetic resonance (^{1}H-NMR) signals of two newly formed olefinic protons at 6.36 (d, $J=15$ Hz, 23-H) and 7.03 (dd, $J=15$, 8 Hz, 22-H). The conversion of **3** to **5** was carried out according to Eyley and Williams,[25] who obtained **5** directly without isolation of **4**. Methylation of **5** with methyllithium afforded the methylated 24,25-glycols (**6**) in 60% yield, and these were refluxed with lithium aluminum hydride ($LiAlH_4$) in tetrahydrofuran (THF) to afford the desired 24,25-dihydroxyprovitamin D_2 [24,25-$(OH)_2$-pro-D_2, **7a** and **7b**] as a mixture of diastereomers in 70% yield.

The two diastereomers were completely separated by high-performance liquid chromatography (HPLC) on a Zorbax SIL column with 2.5% isopropanol in *n*-hexane as the mobile phase, as shown in Fig. 1, to afford the respective diastereomers in almost equal amounts; the less polar (peak 1) was confirmed to be (24*S*)-24,25-$(OH)_2$-pro-D_2 (**7a**) and the more polar compound (peak 2) to be the (24*R*)-isomer (**7b**) by converting them into the corresponding 24,25-$(OH)_2$-D_2 (**8a** and **8b**) by ultraviolet (UV) irradiation followed by thermal isomerization. Figure 2 shows the HPLC profiles of **7a** and **7b** before and after photochemical and thermal isomerization as representatives of the provitamin forms (**7**, **9**, **11** and **13**).

When **7a** and **7b** were irradiated by a monochromatic UV ray at 295 nm obtained from a spectroirradiator, the peaks decreased while those of the respective previtamin forms (**15a** and **15b**) with retention times of 46.3 and 49.4 min increased. Upon thermal isomerization by refluxing the irradiated ethanolic solutions for 2 h, the previtamin peaks were converted to those of the vitamin D forms (**8a** and **8b**) with retention times of 32.0 and 34.0 min, respectively. These products were purified by HPLC. The eluates corresponding to the respective peaks were carefully collected and the solvent was evaporated off under reduced pressure to give the respective vitamins (**8a** and **8b**) in pure form.

On co-chromatography (HPLC) with authentic **8a** and **8b**, kindly donated by Dr. Y. Mazur and Dr. G. Jones, the purified vitamins showed retention times of 32.0 and 34.0 min, and were confirmed to be (24*S*)-24,25-$(OH)_2$-D_2 (**8a**) and (24*R*)-24,25-$(OH)_2$-D_2 (**8b**), respectively.

Secondly, we synthesized Δ^{22}-24,25-$(OH)_2$-D_3 (**10a** and **10b**). Δ^{22}-24,25-$(OH)_2$-pro-D_3 (**9a** and **9b**) was produced from the enone (**5**) by a double reduction procedure. Reduction of **5**

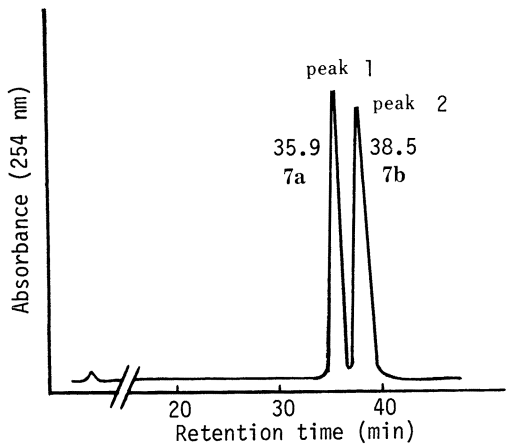

Fig. 1. HPLC Separation of the Diastereomeric Mixture of 24,25-$(OH)_2$-D_2 (**7a** and **7b**)

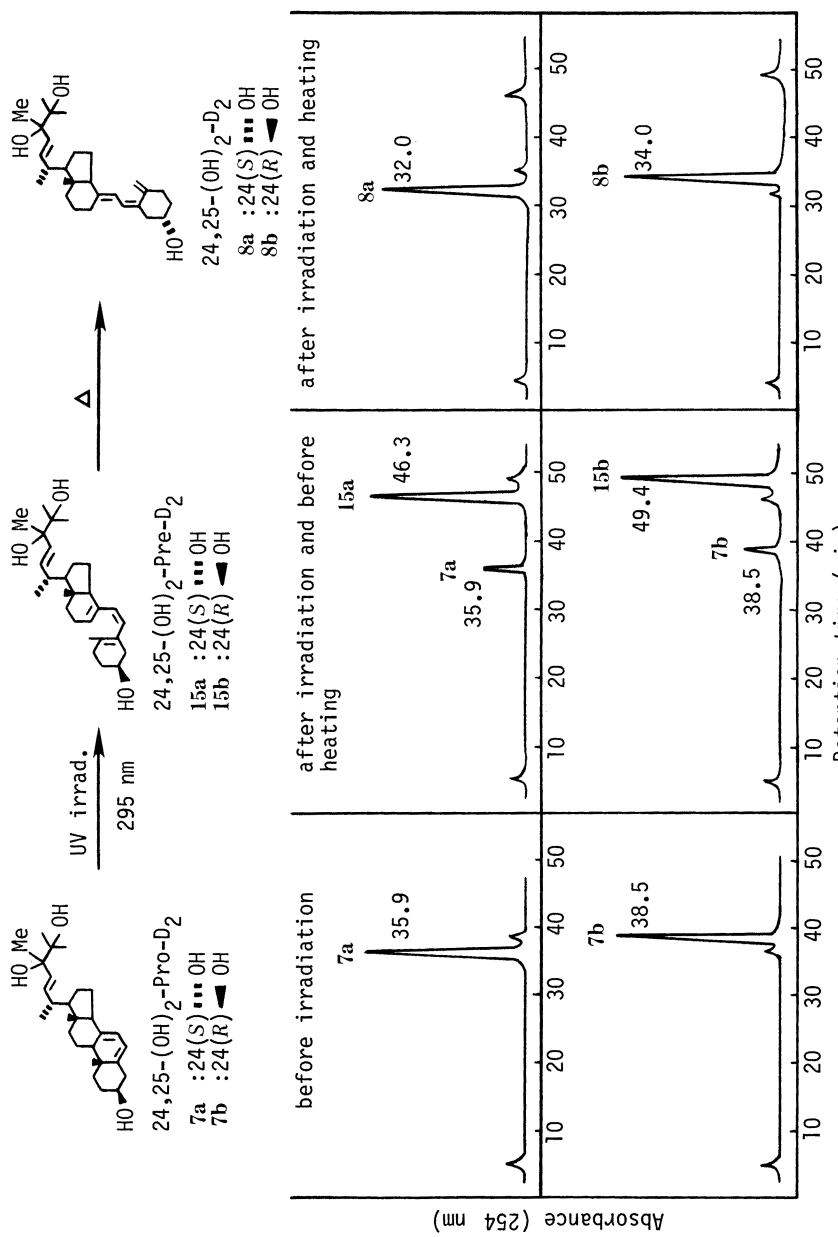

Fig. 2. HPLC Profiles of **7a** and **7b** before and after Photochemical and Thermal Isomerization

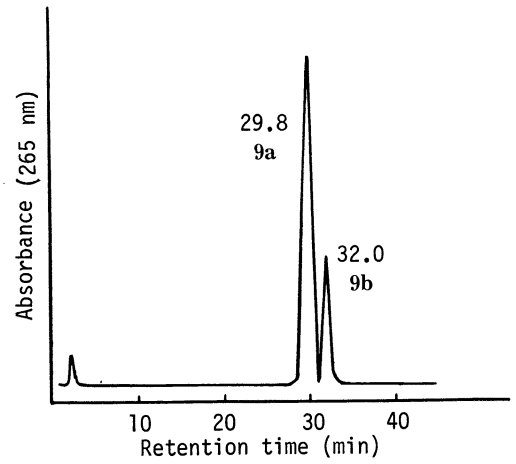

Fig. 3. HPLC Separation of the Diastereomeric Mixture of the 24-ol Provitamins (**9a** and **9b**)

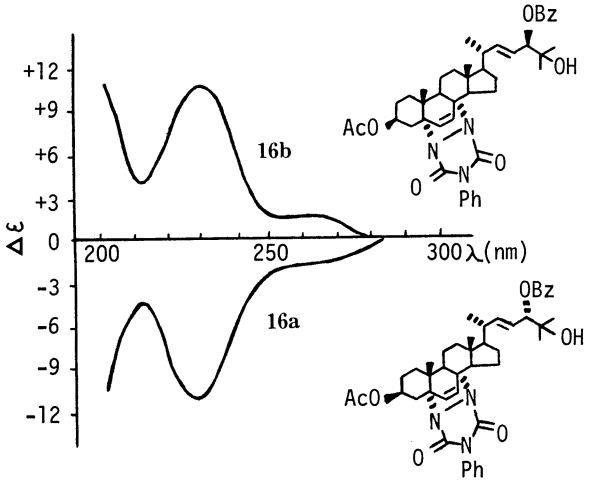

Fig. 4. CD Spectra of the 24-Benzoates (**16a** and **16b**)

with $LiAlH_4$ in THF afforded the two provitamins (**9a** and **9b**) in the ratio of 3:1. On the other hand, chemoselective reduction of the carbonyl group at the 24-position in **5** with lithium tri-*sec*-butylborohydride (L-selectride) in THF at −78 °C followed by deprotection of the triazoline ring with $LiAlH_4$ gave **9a** and **9b** in the ratio of 6:1; these products were separated by HPLC on a Zorbax SIL column with 5.5% isopropanol in *n*-hexane as a mobile phase, as shown in Fig. 3. The absolute configurations at the 24-position of **9a** and **9b** were assigned as 24*S* and 24*R*, respectively, on the basis of the following considerations.

Since the 24*S*-isomer of 24,25-$(OH)_2$-pro-D_2 (**7a**) was eluted faster than the 24*R*-isomer (**7b**) on HPLC, as shown in Fig. 1, we considered that the faster and later peaks observed in Fig. 3 might also be due to the 24*S*- and 24*R*-isomers of the provitamin forms (**9a** and **9b**), respectively. The faster elution of the 24*S*-isomers (**7a** and **9a**) than the respective 24*R*-isomers (**7b** and **9b**) is in good agreement with the results on the analogous epimers of 24-hydroxyderivatives of the vitamin D_3 series reported by Sai *et al.*[26] The provitamin D forms (**9a** and **9b**) were converted into the respective benzoates (**16a** and **16b**), and their circular dichroism (CD) spectra were measured. As shown in Fig. 4, a strongly negative Cotton curve in **16a** and a positive one in **16b** were observed. Therefore, the prediction of the "exciton chirality method" developed by Gonnella *et al.*[27] is that **9a** and **9b** have 24*S* and 24*R*

configurations, respectively.

As mentioned above, the reduction of **5** with the two hydride reagents resulted in the preferential formation of the 24*S*-ol (**9a**) rather than the 24*R*-ol (**9b**). Sai *et al.*[26] have also reported that the reduction of the Δ^{22}-24-one system in the vitamin D_3 series with sodium borohydride gave the (24*S*)-24-alcohol as the major product. The preferential formation of **9a** over **9b** can be explained as follows. The most stable conformation for the enone (**5**) may be that shown in Chart 2, in which the 20-hydrogen is *syn* to the *s-cis* enone system involving hydrogen bonding with the 25-hydroxyl group. Hydride ion would attack the carbonyl group preferentially from the *re*-face due to the presence of the more bulky steroidal skeleton than the methyl group in **5**. When we used L-selectride as a bulky hydride agent, stereoselectivity in the reduction of the enone system increased, as described above. Corey *et al.*[28] reported the same effect on the chirality at the α-position in hydride reduction of the α,β-unsaturated enone system.

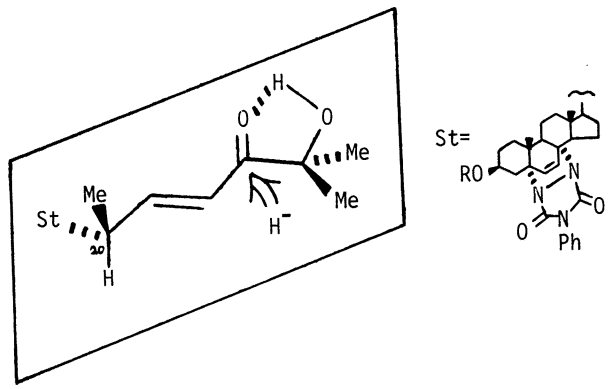

Chart 2. Presumed Mechanism of the Reduction of **5**

As in the syntheses of **8a** and **8b**, the provitamins (**9a** and **9b**) were converted into the respective vitamin forms (**10a** and **10b**) upon UV irradiation followed by thermal isomerization.

Finally, we also synthesized Δ^{22}-25-OH-24-oxo-D_3 (**12**) and 22,24,25-$(OH)_3$-D_3 (**14a** and **14b**). The enone (**5**) was heated at 120 °C in ethylene glycol in the presence of anhydrous K_2CO_3 to afford Δ^{22}-25-OH-24-oxo-pro-D_3 (**11**) as a result of retro-1,4-cycloaddition. On the other hand, reduction of each of the two separated hydroxyketones (**4a** and **4b**) with $LiAlH_4$ in THF afforded the respective 22,24,25-$(OH)_3$-pro-D_3 (**13a** and **13b**). The compounds were characterized spectrally except for their absolute configurations at the 22- and 24-positions. The provitamin D forms (**11**, **13a** and **13b**) thus obtained were similarly converted into the respective vitamin D forms (**12**, **14a** and **14b**) upon UV irradiation followed by thermal isomerization.

Experimental

All melting points were measured on a micro hot-stage apparatus (Yanagimoto) and are uncorrected. ^{1}H-NMR spectra were obtained on a Varian XL-200 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a JEOL LMS-01SG or a Hitachi M-80 spectrometer. UV spectra were obtained on a Hitachi 323 spectrophotometer in ethanol and infrared (IR) spectra were recorded on a Hitachi IR-215 spectrometer in $CHCl_3$. CD spectra were obtained on a JASCO J500C spectropolarimeter in ethanol. Preparative TLC was carried out on precoated plates of silica gel (Kieselgel $60F_{254}$, 2 or 0.5 mm thickness, Merck). HPLC was performed on a Shimadzu LC-3A or LC-4A high-performance liquid chromatograph equipped with a Shimadzu SPD 2AS detector (set at 265 nm, 0.005 absorbance unit full scale) or a Shimadzu UVD-2 (set at 254 nm, 0.001 absorbance

unit full scale) and a Zorbax SIL column (4.6 i.d. × 250 mm or 6.2 i.d. × 250 mm, DuPont) with 2.5 or 5.5% isopropanol in *n*-hexane as a mobile phase. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, THF = tetrahydrofuran, DHP = dihydropyran, $LiAlH_4$ = lithium aluminum hydride.

3β-Acetoxy-22,25-dihydroxy-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidino)cholesta-6-en-24-one (4a and 4b)——A solution of *n*-butyllithium (0.3 ml of 15% solution in *n*-hexane) was added to a cooled (−78 °C) solution of diisopropylamine (75 mg, 0.7 mmol) in dry THF (3 ml) with stirring. A solution of 3-methyl-3-(tetrahydropyran-2-yloxy)butan-2-one[25] (68 mg, 0.67 mmol) in dry THF (2 ml) was then added dropwise at −78 °C over 15 min. The mixture was stirred at the same temperature for 1 h and then a solution of the 20-aldehyde (**3**)[24] (200 mg, 0.36 mmol) in dry THF (4 ml) was added. After being stirred at −78 °C for 2 h, the reaction mixture was brought back to room temperature. Diethyl ether (Et_2O) and water were added and the whole was vigorously shaken, then allowed to stand at room temperature. The separated organic layer was washed with brine, dried (Na_2SO_4) and evaporated. The resulting residue was dissolved in THF (10 ml) containing 1.5 N HCl (2 ml) and allowed to stand at room temperature for 3 h. Et_2O was added, and the solution was washed with 5% Na_2CO_3 solution and brine, dried (Na_2SO_4), and evaporated to give crude **4**, which was subjected to preparative TLC (developing solvent: Et_2O–acetone, 97 : 3). The band of *Rf* 0.32 was scraped off and extracted with $CHCl_3$–MeOH. Removal of the solvent under reduced pressure gave the less polar hydroxyketone (**4a**) as colorless crystals (42 mg, 18%): mp 143—145.5 °C (Et_2O–*n*-hexane). High-resolution MS *m/z*: Calcd for $C_{27}H_{40}O_3$ ($M^+ - PhC_2N_3O_2 - CH_3COOH$): 412.2974. Found: 412.2970. ^{1}H-NMR δ: 0.85 (3H, s, 13-Me), 1.00 (3H, s, 10-Me), 1.00 (3H, d, *J* = 6 Hz, 20-Me), 1.38 (6H, s, 25-Me_2), 2.03 (3H, s, 3-OAc), 4.22 (1H, m, 22-H), 5.48 (1H, m, 3α-H), 6.30, 6.46 (each 1H, d, *J* = 8 Hz, 6- and 7-H), 7.44 (5H, m, Ar).

The band of *Rf* 0.19 on preparative TLC was scraped off and extracted with $CHCl_3$–MeOH to give the more polar hydroxyketone (**4b**) (49 mg, 21%): mp 153—154 °C (Et_2O–*n*-hexane) as colorless crystals. High-resolution MS *m/z*: Calcd for $C_{27}H_{40}O_3$ ($M^+ - PhC_2N_3O_2 - CH_3COOH$): 412.2975. Found: 412.2974. ^{1}H-NMR δ: 0.82 (3H, s, 13-Me), 0.99 (3H, d, *J* = 6 Hz, 20-Me), 1.00 (3H, s, 10-Me), 1.38 (6H, s, 25-Me_2), 2.03 (3H, s, 3-OAc), 2.50 (1H, dd, *J* = 17, 2.5 Hz, 23-H), 2.92 (1H, dd, *J* = 17, 9 Hz, 23-H), 4.27 (1H, br d, *J* = 9.5 Hz, 22-H), 5.48 (1H, m, 3-H), 6.28, 6.46 (each 1H, d, *J* = 8 Hz, 6- and 7-H), 7.42 (5H, m, Ar).

(22*E*)-3β-Acetoxy-25-hydroxy-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidino)cholesta-6,22-dien-24-one (5)——A mixture of the hydroxyketones (**4a** and **4b**) (100 mg, 0.15 mmol) was dissolved in benzene and a small amount of *p*-toluenesulfonic acid was added. This mixture was stirred at room temperature for 2 h, and solid K_2CO_3 was added for neutralization. The mixture was extracted with benzene and the extract was dried (Na_2SO_4). Removal of the solvent gave the enone (**5**) as a colorless glass (90 mg, 90%). High-resolution MS *m/z*: Calcd for $C_{27}H_{38}O_2$ ($M^+ - PhC_2N_3O_2 - CH_3COOH$): 394.2869. Found: 394.2860. IR ν_{max} cm^{-1}: 3400, 1750, 1730, 1700. ^{1}H-NMR δ: 0.83 (3H, s, 13-Me), 0.98 (3H, s, 10-Me), 1.12 (3H, d, *J* = 6 Hz, 20-Me), 1.38 (6H, s, 25-Me_2), 2.02 (3H, s, 3-OAc), 3.24 (1H, m, 9-H), 5.45 (1H, m, 3α-H), 6.25, 6.42 (each 1H, d, *J* = 8 Hz, 6- and 7-H), 6.36 (1H, d, *J* = 15 Hz, 23-H), 7.03 (1H, dd, *J* = 15, 8 Hz, 22-H), 7.42 (5H, m, Ar).

(22*E*)-3β,24,25-Trihydroxy-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidino)ergosta-6,22-diene (6)——A solution of methyllithium (0.6 mmol/ml) in Et_2O (2 ml) was added with stirring to a solution of the enone (**5**) (100 mg, 0.16 mmol) in THF (5 ml) at 0 °C under argon. The solution was brought back to room temperature, stirred for 0.5 h and then treated with 5% HCl solution. The reaction mixture was extracted with ethyl acetate, and the extract was dried (Na_2SO_4) and evaporated. The crude product was purified by preparative TLC (developing solvent: CH_2Cl_2–MeOH = 9 : 1) to give the 24,25-glycol (**6**) (57 mg, 60%) as a colorless glass. High-resolution MS *m/z*: Calcd for $C_{28}H_{44}O_3$ ($M^+ - PhC_2N_3O_2$): 428.3287. Found: 428.3229. IR ν_{max} cm^{-1}: 3500, 1730, 1700. ^{1}H-NMR δ: 0.84 (3H, s, 13-Me), 0.99 (3H, s, 10-Me), 1.07 (3H, d, *J* = 6 Hz, 20-Me), 1.20 (6H, s, 25-Me_2), 1.28 (3H, s, 24-Me), 4.48 (1H, m, 3α-H), 5.60 (2H, m, 22- and 23-H), 6.26, 6.42 (each 1H, d, *J* = 8 Hz, 6- and 7-H), 7.46 (5H, m, Ar).

(22*E*)-3β,24,25-Trihydroxyergosta-5,7,22-triene (7a and 7b)——The 24,25-glycol (**6**) (20 mg, 0.03 mmol) in dry THF (5 ml) was treated with $LiAlH_4$ (21 mg, 0.56 mmol) under reflux for 3 h. After cooling, the excess reagent was destroyed by adding a few drops of water. The mixture was extracted with ethyl acetate, dried (Na_2SO_4) and evaporated. The crude product was purified by HPLC on a Zorbax SIL column (4.6 i.d. × 250 mm) with 2.5% isopropanol in *n*-hexane as a mobile phase at a flow rate of 2.0 ml/min to give (24*S*)-24,25-$(OH)_2$-pro-D_2 (**7a**) (4.2 mg, 33%) as a colorless glass and (24*R*)-24,25-$(OH)_2$-pro-D_2 (**7b**) (4.3 mg, 34%) as a colorless glass.

7a: High-resolution MS *m/z*: Calcd for $C_{28}H_{44}O_3$: 428.3289. Found: 428.3271. UV λ_{max}^{EtOH} nm: 264, 272, 281, 292. IR ν_{max} cm^{-1}: 3500, 1600. ^{1}H-NMR δ: 0.64 (3H, s, 13-Me), 0.96 (3H, s, 10-Me), 1.07 (3H, d, *J* = 6 Hz, 20-Me), 3.66 (1H, m, 3α-H), 5.42 (1H, m, 6- or 7-H), 5.60 (3H, m, 22,23-H and 6- or 7-H).

7b: High-resolution MS *m/z*: Calcd for $C_{28}H_{44}O_3$: 428.3289. Found: 428.3273, UV λ_{max}^{EtOH} nm: 264, 272, 281, 292. IR ν_{max} cm^{-1}: 3500, 1600. ^{1}H-NMR δ: 0.64 (3H, s, 13-Me), 0.96 (3H, s, 10-Me), 1.07 (3H, d, *J* = 6 Hz, 20-Me), 3.67 (1H, m, 3α-H), 5.42 (1H, m, 6- or 7-H), 5.60 (3H, m, 22,23-H and 6- or 7-H).

(24*S*)-24,25-Dihydroxyvitamin D_2 (8a)——A solution (4 ml) of (24*S*)-24,25-$(OH)_2$-pro-D_2 (**7a**) in ethanol (0.5 mg/ml) was placed in a quartz cell (10 × 10 × 40 mm) in a spectroirradiator and irradiated with monochromatic light at 295 nm. The irradiated solution was refluxed for 2 h and the solvent was evaporated off. The crude product was purified by HPLC under the same conditions as above to give (24*S*)-24,25-$(OH)_2$-D_2 (**8a**) (0.2 mg, 10%) as a

colorless glass: High-resolution MS *m/z*: Calcd for $C_{28}H_{44}O_3$: 428.3288. Found: 428.3307. UV λ_{max}^{EtOH} nm: 265; λ_{min}^{EtOH} nm: 228. ^{1}H-NMR δ: 0.50 (3H, s, 13-Me), 0.98 (3H, d, J=6 Hz, 20-Me), 1.14, 1.16, 1.20 (each 3H, s, 24-Me and 25-Me$_2$), 3.88 (1H, m, 3α-H), 4.76, 4.99 (each 1H, br s, 19-H$_2$), 5.50 (2H, m, 22- and 23-H), 5.97, 6.20 (each 1H, d, J= 11 Hz, 6- and 7-H).

(24*R*)-24,25-Dihydroxyvitamin D$_2$ (8b)——As described for the conversion of **7a** to **8a**, (24*R*)-24,25-(OH)$_2$-pro-D$_2$ (**7b**) (2.0 mg, 0.005 mmol) was converted into (24*R*)-24,25-(OH)$_2$-D$_2$ (**8b**) (0.2 mg, 10%) as a colorless glass: High-resolution MS *m/z*: Calcd for $C_{28}H_{44}O_3$: 428.3287. Found: 428.3271. UV λ_{max}^{EtOH} nm: 265; λ_{min}^{EtOH} nm: 228. ^{1}H-NMR δ: 4.84, 5.08 (each 1H, br s, 19-H$_2$), 5.64 (2H, m, 22- and 23-H).

(22*E*)-3β,24,25-Trihydroxycholesta-5,7,22-trienes (9a and 9b) Reduction with LiAlH$_4$——The enone (**5**) (60 mg, 0.1 mmol) in dry THF was treated with $LiAlH_4$ (70 mg, 1.8 mmol) under reflux for 3 h. After cooling, the excess reagent was destroyed by adding a few drops of water. The mixture was extracted with ethyl acetate, and the extract was dried (Na_2SO_4) and evaporated. The crude product was purified by HPLC on a Zorbax SIL column (4.6 i.d. × 250 mm) with 5.5% isopropanol in *n*-hexane as a mobile phase at a flow rate of 1.0 ml/min to give (24*S*)-Δ^{22}-24,25-(OH)$_2$-pro-D$_3$ (**9a**) (18.5 mg, 45%) and (24*R*)-Δ^{22}-24,25-(OH)$_2$-pro-D$_3$ (**9b**) (7 mg, 17%) as colorless crystals.

9a: mp 203—204 °C (MeOH–*n*-hexane). High-resolution MS *m/z*: Calcd for $C_{27}H_{42}O_3$: 414.3131. Found: 414.3112. UV λ_{max}^{EtOH} nm: 292, 280, 272, 262. ^{1}H-NMR δ: 0.64 (3H, s, 13-Me), 0.94 (3H, s, 10-Me), 1.06 (3H, d, J= 6 Hz, 20-Me), 1.13 and 1.18 (each 3H, s, 25-Me$_2$), 3.62 (1H, m, 3α-H), 3.82 (1H, d, J=7 Hz, 24-H), 5.42 (2H, m, 22- or 23- and 6- or 7-H), 5.62 (2H, m, 22- or 23- and 6- or 7-H).

9b: mp 206—207 °C (MeOH–*n*-hexane). High-resolution MS *m/z*: Calcd for $C_{27}H_{42}O_3$: 414.3132. Found: 414.3111. UV λ_{max}^{EtOH} nm: 292, 280, 272, 262. ^{1}H-NMR δ: 0.64 (3H, s, 13-Me), 0.95 (3H, s, 10-Me), 1.08 (3H, d, J= 6 Hz, 20-Me), 1.16 and 1.20 (each 3H, s, 25-Me$_2$), 3.64 (1H, m, 3α-H), 3.86 (1H, d, J=7 Hz, 24-H), 5.42 (2H, m, 22- or 23- and 6- or 7-H), 5.60 (2H, m, 22- or 23- and 6- or 7-H).

(22*E*)-3β,24,25-Trihydroxycholesta-5,7,22-trienes (9a and 9b). Reduction with L-Selectride and LiAlH$_4$——The enone (**5**) (15 mg, 0.024 mmol) was dissolved in dry THF (2 ml) and 0.03 ml (0.03 mmol) of 1.0 M L-selectride solution in THF was slowly added at −78 °C. The reaction mixture was kept at −78 °C for 2 h with stirring, then brought back to room temperature, and the hydrolyzed with 0.01 ml of 3 N NaOH solution. The organoborane was then decomposed with 0.01 ml of 30% H_2O_2. The reaction mixture was extracted with Et_2O, washed with water, dried (Na_2SO_4) and evaporated to give 3β,24,25-trihydroxy-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidino)cholesta-6,22-diene (**17**) (10 mg, 71%) as a colorless glass. ^{1}H-NMR δ: 0.83 (3H, s, 13-Me), 0.98 (3H, s, 10-Me), 1.06 (3H, d, J= 6 Hz, 20-Me), 1.13 and 1.20 (each 3H, s, 25-Me$_2$), 3.82 (6/7H, d, J=7 Hz, 24-H), 3.86 (1/7H, d, J=7 Hz, 24-H), 4.46 (1H, m, 3-H), 5.46 (1H, dd, J=15, 7 Hz, 23-H), 5.65 (1H, dd, J=15, 8 Hz, 22-H), 6.28 and 6.44 (each 1H, d, J=8 Hz, 6- and 7-H), 7.40 (5H, m, Ar).

This triol (**17**) (5 mg, 0.008 mmol) in dry THF (5 ml) was treated with $LiAlH_4$ (9.3 mg, 0.24 mmol) under reflux for 3 h. The crude product was purified by HPLC using a Zorbax SIL column (4.6 i.d. × 250 mm) with 5.5% isopropanol in *n*-hexane as a mobile phase at a flow rate of 1.0 ml/min to give **9a** (1.9 mg, 57%) and **9b** (0.3 mg, 9%).

3β-Acetoxy-24-benzoyloxy-25-hydroxy-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolidino)cholesta-6,22-dienes (16a and 16b)——Methanol (1 ml) and $NaBH_4$ (1.7 mg, 0.045 mmol) were added to a solution of the enone (**5**) (15 mg, 0.024 mmol) in dry THF (1 ml). The mixture was kept at room temperature for 2 h with stirring, then extracted with ethyl acetate. The extract was evaporated to give a residue, which was dissolved in $CHCl_3$ (2 ml). Benzoyl chloride (0.03 ml) was added, and the resulting reaction mixture was stirred in the presence of *N*,*N*-diisopropyl ethylamine at room temperature for 3 h. The usual work-up ($CHCl_3$ extraction) and purification by HPLC on a Zorbax SIL column (4.6 i.d. × 250 mm) with 8% isopropanol in *n*-hexane as a mobile phase at a flow rate of 0.4 ml/min gave the 24*S*-benzoate (**16a**) (4 mg, 24%) as a colorless glass and the 24*R*-benzoate (**16b**) (3.8 mg, 22%) as a colorless glass.

16a: ^{1}H-NMR δ: 2.04 (3H, s, 3-OAc), 5.32 (1H, d, J=8 Hz, 24-H), 5.50 (2H, m, 22- or 23-H and 3α-H), 5.76 (1H, m, 22- or 23-H), 6.26 and 6.42 (2H, d, J=8 Hz, 6- and 7-H), 7.48 (8H, m, Ar), 8.10 (2H, d, J=8 Hz, Ar).

16b: ^{1}H-NMR δ: 0.82 (3H, s, 13-Me), 1.00 (3H, s, 10-Me), 1.06 (3H, d, J=6 Hz, 20-Me), 1.28 (6H, s, 25-Me$_2$), 2.02 (3H, s, 3-OAc), 5.32 (1H, d, J=8 Hz, 24-H), 5.50 (2H, m, 22- or 23-H and 3α-H), 5.76 (1H, m, 22- or 23-H), 6.28 and 6.44 (each 1H, d, J=8 Hz, 6- and 7-H), 7.48 (8H, m, Ar), 8.10 (2H, d, J=8 Hz, Ar).

Upon reduction with $LiAlH_4$, the benzoates (**16a** and **16b**) thus isolated were unambigously converted into the respective alcohols (**9a** and **9b**), which were identical with the respective alcohols prepared directly by reduction of the enone (**5**) with $LiAlH_4$.

(22*E*)-24,25-Dihydroxy-22-dehydrovitamin D$_3$ (10a and 10b)——In the same manner as described for the synthesis of **8**, (24*S*)-Δ^{22}-24,25-(OH)$_2$-pro-D$_3$ (**9a**) and (24*R*)-Δ^{22}-24,25-(OH)$_2$-pro-D$_3$ (**9b**) (each 4 mg, 0.01 mmol) were converted into (24*S*)-Δ^{22}-25-(OH)$_2$-D$_3$ (**10a**) as a colorless glass (0.6 mg, 15%) and (24*R*)-Δ^{22}-(24*R*)-24,25-(OH)$_2$-D$_3$ (**10b**) as a colorless glass (0.6 mg, 15%), respectively.

10a: High-resolution MS *m/z*: Calcd for $C_{27}H_{42}O_3$: 414.3131. Found: 414.3125. UV λ_{max}^{EtOH} nm: 265; λ_{min}^{EtOH} nm: 228. ^{1}H-NMR δ: 0.56 (3H, s, 13-Me), 1.04 (3H, d, J=6 Hz, 20-Me), 1.16 and 1.20 (each 3H, s, 25-Me$_2$), 3.86 (1H, d, J=7 Hz, 24-H), 3.96 (1H, m, 3α-H), 4.84 and 5.07 (each 1H, br s, 19-H$_2$), 5.44 (1H, dd, J=15, 7 Hz, 23-H), 5.64 (1H, dd, J=15, 8 Hz, 22-H), 6.04 and 6.26 (each 1H, d, J=11 Hz, 6- and 7-H).

10b: High-resolution MS *m/z*: Calcd for $C_{27}H_{42}O_3$: 414.3132. Found: 414.3132. UV λ_{max}^{EtOH} nm: 265; λ_{min}^{EtOH} nm:

228. ^{1}H-NMR δ: 0.56 (3H, s, 13-Me), 1.05 (3H, d, J=6 Hz, 20-Me), 1.16 and 1.20 (each 3H, s, 25-Me$_2$), 3.83 (1H, d, J=7 Hz, 24-H), 3.96 (1H, m, 3α-H), 4.83 and 5.06 (each 1H, br s, 19-H$_2$), 5.42 (1H, dd, J=15, 7 Hz, 23-H), 5.60 (1H, dd, J=15, 8 Hz, 22-H), 6.05 and 6.25 (each 1H, d, J=11 Hz, 6- and 7-H).

(22*E*)-3β,25-Dihydroxycholesta-5,7,22-trien-24-one (11)——A mixture of the enone (**5**) (20 mg, 0.03 mmol), anhydrous K_2CO_3 and furan (2.8 g, 0.04 mmol) in ethylene glycol was heated at 120 °C for 5 h under N_2. The mixture was then extracted with ethyl acetate, and the extract was washed with 5% HCl, saturated aqueous Na_2CO_3 and brine, and dried (Na_2SO_4). After evaporation of the solvent, the crude product was purified by HPLC on a Zorbax SIL column (4.6 i.d. × 250 mm) with 2.5% isopropanol in *n*-hexane as a mobile phase at a flow rate of 1.5 ml/min to give the Δ^{22}-25-OH-24-oxo-pro-D$_3$ (**11**) (7 mg, 54%) as a colorless glass. High-resolution MS *m/z*: Calcd for $C_{27}H_{40}O_3$: 412.2974. Found: 412.2969. UV λ_{max}^{EtOH} nm: 292, 282, 272, 262. ^{1}H-NMR δ: 0.51 (3H, s, 13-Me), 0.93 (3H, s, 10-Me), 1.02 (3H, d, J=6 Hz, 20-Me), 1.38 and 1.40 (each 3H, s, 25-Me$_2$), 3.64 (1H, m, 3α-H), 5.40 and 5.58 (each 1H, m, 6- and 7-H), 6.40 (1H, d, J=15 Hz, 23-H), 7.10 (1H, dd, J=15, 9 Hz, 22-H).

(22*E*)-25-Hydroxy-24-oxo-22-dehydrovitamin D$_3$ (12)——In the same manner as described for the synthesis of **8**, Δ^{22}-25-OH-24-oxo-pro-D$_3$ (**11**) (0.5 mg, 0.001 mmol) was converted into Δ^{22}-25-OH-24-oxo-D$_3$ (**12**) (0.015 mg, 3%) as a colorless glass. High-resolution MS *m/z*: Calcd for $C_{27}H_{40}O_3$: 412.2976. Found: 412.2989. UV λ_{max}^{EtOH} nm: 265: λ_{min}^{EtOH} nm: 228.

3β,22,24,25-Tetrahydroxycholesta-5,7-diene (13a and 13b)——A solution of the hydroxyketone (**4a**) (40 mg, 0.06 mmol) in dry THF (10 ml) was treated with $LiAlH_4$ (70 mg, 1.8 mmol) under reflux for 3 h. After cooling, excess reagent was destroyed by adding a few drops of water. The mixture was extracted with ethyl acetate, and the extract was dried (Na_2SO_4) and evaporated. The crude product was purified by HPLC on a Zorbax SIL column (6.2 i.d. × 250 mm) with 15% isopropanol in *n*-hexane as a mobile phase at a flow rate of 1.7 ml/min to give the 22,24,25-(OH)$_3$-pro-D$_3$ (**13a**) (18 mg, 70%) as a colorless glass. High-resolution MS *m/z*: Calcd for $C_{27}H_{44}O_4$: 432.3236. Found: 432.3234. UV λ_{max}^{EtOH} nm: 292, 280, 272, 262. ^{1}H-NMR δ: 0.64 (3H, s, 13-Me), 0.94 (3H, s, 10-Me), 0.98 (3H, d, J=6 Hz, 20-Me), 1.18 and 1.21 (each 3H, s, 25-Me$_2$), 3.60 (2H, m, 3α- and 22-H), 3.94 (1H, m, 24-H), 5.40 and 5.58 (each 1H, m, 6- and 7-H).

In the same manner as described for **13a**, the hydroxyketone (**4b**) (40 mg, 0.06 mmol) was converted into 22,24,25-(OH)$_3$-pro-D$_3$ (**13b**) (18 mg, 70%) as a colorless glass. High-resolution MS *m/z*: Calcd for $C_{27}H_{44}O_4$: 432.3237. Found: 432.3230. UV λ_{max}^{EtOH} nm: 292, 280, 272, 262. ^{1}H-NMR δ: 0.63 (3H, s, 13-Me), 0.94 (3H, s, 10-Me), 0.98 (3H, d, J=6 Hz, 20-Me), 1.16 and 1.20 (each 3H, s, 25-Me$_2$), 3.60 (2H, m, 3α- and 22-H), 3.96 (1H, d-like, J=7 Hz, 24-H) 5.42 and 5.58 (each 1H, m, 6- and 7-H).

22,24,25-Trihydroxyvitamin D$_3$ (14a and 14b)——In the same manner as described for the synthesis of **8**, 22,24,25-(OH)$_3$-pro-D$_3$ (**13a**) (4 mg, 0.01 mmol) was converted into 22,24,25-(OH)$_3$-D$_3$ (**14a**). The crude product was purified by HPLC on a Zorbax SIL column (4.6 i.d. × 250 mm) with 5.5% isopropanol in *n*-hexane as a mobile phase at a flow rate of 1.7 ml/min to give **14a** as a colorless glass. High-resolutions MS *m/z*: Calcd for $C_{27}H_{44}O_4$: 432.3237. Found: 432.3217. UV λ_{max}^{EtOH} nm: 265; λ_{min}^{EtOH} nm: 228. ^{1}H-NMR δ: 0.56 (3H, s, 13-Me), 0.97 (3H, d, J=6 Hz, 20-Me), 1.20 and 1.24 (each 3H, s, 25-Me$_2$), 3.60 (1H, m, 22- or 24-H), 4.00 (2H, m, 22- or 24-H and 3α-H), 4.85 and 5.08 (each 1H, br s, 19-H$_2$), 6.07 and 6.27 (each 1H, d, J=11 Hz, 6- and 7-H).

Similarly, **13b** (4 mg, 0.01 mmol) was converted into **14b** as a colorless glass. High-resolution MS *m/z*: Calcd for $C_{27}H_{44}O_4$: 432.3236. Found: 432.3235. UV λ_{max}^{EtOH} nm: 265; λ_{min}^{EtOH} nm: 228. ^{1}H-NMR δ: 0.54 (3H, s, 13-Me), 0.98 (3H, d, J=6 Hz, 20-Me), 1.17 and 1.21 (each 3H, s, 25-Me$_2$), 3.73 (3H, m, 3α-, 22- and 24-H), 4.84 and 5.06 (each 1H, br s, 19-H$_2$), 6.05 and 6.25 (each 1H, d, J=11 Hz, 6- and 7-H).

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