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Synthesis and Biological Activity of (22*E*,24*R*)- and (22*E*,24*S*)-1 α ,24-Dihydroxy-22-dehydrovitamin D₃

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Chemical synthesis of (22*E*,24*R*)- and (22*E*,24*S*)-1,24-dihydroxy- Δ^{22} -vitamin D₃ has been achieved starting with the commercially available dinorcholenic acid acetate. Synthesis involved introduction of the 1-hydroxy group by a reduction of the 1,2-epoxide generated by epoxidation of the 1,4,6-trien-3-one. The side chain on the steroid was then constructed by means of a Wittig reaction followed by introduction of the Δ^7 bond by standard methods and its protection with 1-phenyl-1,2,4-triazoline-3,5-dione. Subsequent reduction of the hydroxy groups in the steroid side chain followed by reduction of the Diels–Alder addition products yielded the both 24-isomers. The 5,7-dienes were irradiated and the corresponding vitamin D compounds isolated. Nuclear magnetic resonance was used to identify individual isomers. The (22*E*,24*S*)-1,24-dihydroxyvitamin D₃ compound bound equally well to the chick intestinal cytosol receptor as 1,25-dihydroxyvitamin D₃, while the 24*R*-isomer was approximately ten times less active. *In vivo*, both isomers were less active than 1,25-dihydroxyvitamin D₃; however, the 24*S*-isomer was considerably more active than the 24*R*-isomer approaching the activity of 1,25-dihydroxyvitamin D₃.

Keywords—1,24-dihydroxy-22-dehydrovitamin D₃; 1,24-dihydroxyvitamin D₃; vitamin D analog; intestinal calcium; calcium mobilization; vitamin D receptor binding activity

Since the discovery of 1 α ,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) as the hormonal metabolite of vitamin D₃,¹⁾ many analogs have been synthesized with the aim of preparing a more active or longer-lasting compound.²⁾ The 1 α -hydroxyl group is essential to elicit vitamin D activity, while the 25-hydroxyl group can be replaced with the 24*R*-hydroxyl group. (24*R*)-1,24-(OH)₂D₃ binds equally well as 1,25-(OH)₂D₃ to the intestinal cytosol receptor,³⁾ but is slightly less biologically active *in vivo*,^{4,5)} while 1,25-(OH)₂D₃ and 1,25-(OH)₂D₂ are equal in receptor binding and biological activity.^{6,7)} It is of interest, therefore, to determine the effect of a C-22 double bond on the biological activity of 1,25-(OH)₂D₃. Thus, we synthesized (22*E*,24*R*)- (12) and (22*E*,24*S*)-1,24-dihydroxy-22-dehydrovitamin D₃ (1,24-(OH)₂- Δ^{22} -D₃) (13) and determined their biological activities.

The key intermediate, 1 α -hydroxydinorcholan-22-al diacetate (5) was prepared by Barton's procedure.⁸⁾ Dinorcholenic acid acetate (1) was reduced with lithium aluminum hydride and subsequently oxidized with dichlorodicyanobenzoquinone to afford the 1,4,6-trien-3-one 2 in 47% yield. The 22-tetrahydropyranyl (THP) ether of 2 was treated with alkaline hydrogen peroxide to give the 1 α ,2 α -epoxide 3 in 41% yield. Reduction of 3 with lithium and ammonium chloride in liquid ammonia–tetrahydrofuran at –78 °C, followed by acetylation and removal of the THP group provided dinorchol-5-ene-1 α ,3 β ,22-triol 1,3-di-acetate (4) in 42% yield.

Oxidation of the 22-alcohol 4 with pyridinium chlorochromate in the presence of sodium

acetate gave the 22-aldehyde **5**. This was coupled with isobutyrylmethylenetriphenylphosphorane⁹⁾ in dimethylsulfoxide at 95 °C to afford the enone **6** in 66% yield from the 22-alcohol **4**. Allylicbromination at C-7 of **6** with *N*-bromosuccinimide, followed by treatment with tetra-*n*-butyl ammonium bromide and then with tetra-*n*-butyl ammonium fluoride¹⁰⁾ gave a mixture of the 4,6-diene and 5,7-diene, from which the 5,7-diene adduct **7** was obtained in 37% yield by treatment with 1-phenyl-1,2,4-triazoline-3,5-dione and chromatographic purification.

Reduction of the enone **7** with sodium borohydride afforded a stereoisomeric mixture of 24-alcohols **8a** and **9a**. This mixture was separated by preparative thin layer chromatography (TLC) (benzene–ethyl acetate, 3 : 1, developed seven times) to give the less polar 24-isomer **8a** (29%, *R_f*=0.53) and the more polar 24-isomer **9a** (43%, *R_f*=0.50). To determine the configuration at the C-24 position, these 24-alcohols were converted into the corresponding (+)-methoxytrifluoromethylphenylacetic acid (MTPA) ester **8b** and **9b**. The proton nuclear magnetic resonance (¹H-NMR) spectra of **8b** and **9b** were compared with those of the (+)-MTPA esters **14b** and **15b**, which were derived from the known (24*S*)-24-alcohol **14a** and its (24*R*)-isomer **15a**,¹¹⁾ respectively. The ¹H-NMR data of the methyl groups of **8b**, **9b**, **14b** and **15b** are shown in Table I. The C-26 and C-27 methyl groups in the less polar isomer **8b** appeared as two doublets. These peaks corresponded closely with those of the (24*S*)-isomer **14b**. On the other hand, the C-26 and C-27 methyl groups in the more polar isomer **9b** appeared as an overlapped doublet. These peaks corresponded closely with those of the (24*R*)-isomer **15b**. Thus, the C-24 configuration of the less polar alcohol **8a** was determined as *S* and that of the more polar alcohol **9a** as *R*.

Treatment of **9a** and **8a** with lithium aluminum hydride gave the (24*R*)-5,7-diene **10** and the (24*S*)-5,7 diene **11**, respectively, in 36% yield. The 5,7-dienes **10** and **11** were irradiated with a medium-pressure mercury lamp in benzene–ethanol for 2.5 min, then refluxed for 1 h to afford (22*E*,24*R*)-1 α ,24-(OH)₂- Δ^{22} -D₃ (**12**) and (22*E*,24*S*)-1 α ,24-(OH)₂- Δ^{22} -D₃ (**13**). The ¹H-NMR data of C-22 and C-23 protons of the (24*R*)-vitamin D₃ analog **12** and those of the (24*S*)-isomer **13** were in good agreement with those of the known (24*R*)-allylic alcohol **15a** and its (24*S*)-isomer **14a**, respectively.

Figure 1 demonstrates the ability of the two synthetic 1,24-(OH)₂D₃ isomers to displace radiolabeled 1,25-(OH)₂D₃ from the chick intestinal receptor. The results demonstrate that the 24*S*-isomer is equally potent as unlabeled 1,25-(OH)₂D₃ in displacing radiolabeled 1,25-(OH)₂D₃ from the receptor. The 24*R*-isomer proved to be approximately one-tenth as active as either 1,25-(OH)₂D₃ or the *S*-isomer. In the stimulation of intestinal calcium transport of rats on a low calcium vitamin D-deficient diet, it is apparent that neither isomer equalled 1,25-(OH)₂D₃ in this capacity (Table II). This contrasts with the results obtained with the chick intestinal receptor in which *S*-isomer equalled 1,25-(OH)₂D₃ in its ability to displace radiolabeled 1,25-(OH)₂D₃ from the receptor. Both isomers were less active than 1,25-

TABLE I. ¹H-NMR (100 MHz) Spectral Data of Methyl Groups in **8b**, **9b**, **14b**, and **15b**

Compound	Chemical shift ^{a)}			
	18-H ₃	19-H ₃	21-H ₃	26-H ₃ and 27-H ₃
8b	0.85	1.08	1.04 (<i>J</i> =7)	0.88 (<i>J</i> =7), 0.92 (<i>J</i> =7)
9b	0.83	1.08	1.04 (<i>J</i> =7)	0.88 (<i>J</i> =7)
14b	0.72	1.04	1.02 (<i>J</i> =7)	0.89 (<i>J</i> =7), 0.93 (<i>J</i> =7)
15b	0.76	1.05	1.04 (<i>J</i> =7)	0.88 (<i>J</i> =7)

a) Shifts are given in ppm and *J* values in Hz.

TABLE II. Increase of Intestinal Calcium Transport and Serum Calcium Concentration in Response to Either (22*E*, 24*R*)-1,24-(OH)₂-Δ²²-D₃, (22*E*, 24*S*)-1,24-(OH)₂-Δ²²-D₃ or 1,25-(OH)₂D₃

Compound given	Intestinal calcium transport (Ca serosal/Ca mucosal)	Serum calcium (mg/100 ml)
None	2.5 ± 0.3 ^{a)}	3.0 ± 0.1 ^{e)}
1,25-(OH) ₂ D ₃	6.4 ± 1.1 ^{b)}	3.8 ± 0.1 ^{f)}
(22 <i>E</i> , 24 <i>R</i>)-1,24-(OH) ₂ -Δ ²² -D ₃	3.4 ± 0.6 ^{c)}	3.4 ± 0.1
(22 <i>E</i> , 24 <i>S</i>)-1,24-(OH) ₂ -Δ ²² -D ₃	3.9 ± 0.4 ^{d)}	3.6 ± 0.1

Weanling male rats were fed a low-calcium, vitamin D-deficient diet for 3 weeks. They were then given 32.5 pmol/d of test compound dissolved in a 0.1 ml mixture of 95% ethanol-propylene glycol (5:95) subcutaneously daily for 7 d. Rats in the control group received the vehicle. Each group consisted of 7 rats.

Standard deviation of the mean.

Significantly different: a) from b) and d) $p < 0.001$, a) from c) $p < 0.005$, b) from c) and d) $p < 0.001$, e) from f) $p < 0.005$.

TABLE III. Increase of Serum Inorganic Phosphorus Concentration and Bone Ash in Response to Either (22*E*, 24*R*)-1,24-(OH)₂-Δ²²-D₃, (22*E*, 24*S*)-1,24-(OH)₂-Δ²²-D₃ or 1,25-(OH)₂D₃

Compound given	Serum inorganic phosphorus (mg/100 ml)	Bone ash (mg)
None	2.4 ± 0.1 ^{a)}	35.0 ± 4.6 ^{e)}
1,25-(OH) ₂ D ₃	3.3 ± 0.4 ^{b)}	53.2 ± 6.9 ^{f)}
(22 <i>E</i> , 24 <i>R</i>)-1,24-(OH) ₂ -Δ ²² -D ₃	2.7 ± 0.4 ^{c)}	35.0 ± 6.7
(22 <i>E</i> , 24 <i>S</i>)-1,24-(OH) ₂ -Δ ²² -D ₃	2.9 ± 0.4 ^{d)}	46.5 ± 4.2 ^{g)}

Weanling male rats were fed a low-phosphorus, vitamin D-deficient diet for 3 weeks. They were then given 32.5 pmol/d of test compound dissolved in a 0.1 ml mixture of 95% ethanol-propylene glycol (5:95) subcutaneously daily for 7 d. Rats in the control group were given the vehicle. Each group consisted of 6–7 rats.

Standard deviation of the mean.

Significantly different: a) from b) $p < 0.001$, c) $p < 0.025$, d) $p < 0.005$, e) from f) and g) $p < 0.001$, f) from g) $p < 0.05$.

whereas the 24*S*-compound was less active than 1,25-(OH)₂D₃ but was clearly effective in this capacity. The rise in serum inorganic phosphorus concentration in animals on low phosphorus diet is a critical response for mineralization of bone. It is evident that all three forms of vitamin D stimulated serum inorganic phosphorus levels; however, neither isomer was equal to 1,25-(OH)₂D₃.

Results presented here illustrate that the biological activity of both *S*- and *R*-isomers is similar to the biological activity observed in similar isomers of the vitamin D series⁵⁾ and both the intestinal receptor and the target organs of vitamin D action are quite tolerant of the introduction of a double bond in the 22-position.

Experimental

Melting points were determined on a hot stage microscope and are uncorrected. Ultraviolet (UV) spectra were obtained in ethanol solution with a Shimadzu UV-200 double-beam spectrometer. ¹H-NMR spectra were run on a Hitachi R-24A spectrometer, a JEOL PS-100 spectrometer or a JEOL FX-400 spectrometer. All NMR spectra were taken in CDCl₃ solution with tetramethylsilane as an internal reference. Mass spectra were obtained with a Shimadzu LKB-9000S spectrometer at 70 eV. Column chromatography was done on silica gel (Merck, 70–230 mesh). Preparative thin layer chromatography was carried out on precoated plates of silica gel (Merck, Silica gel 60 F₂₅₄). The usual work-up refers to dilution with water, extraction with an organic solvent, washing to neutrality, drying

over magnesium sulfate, filtration, and removal of the solvent under reduced pressure. The following abbreviations are used; THF, tetrahydrofuran; THP, tetrahydropyranyl; DHP, dihydropyran; ether, diethyl ether; MTPA, α -methoxy- α -trifluoromethylphenylacetyl; Ph, phenyl; s, singlet; m, multiplet; d, doublet; dd, double doublet; brs, broad singlet.

22-Hydroxy-23,24-dinorchola-1,4,6-trien-3-one (2)—To a solution of 3 β -acetoxydinorcholenic acid (**1**) (7.0 g, 18.04 mmol) in THF (20 ml) lithium aluminium hydride (3.0 g, 78.95 mmol) was added. This mixture was stirred at 60 °C for 14 h. To this reaction mixture water and ethyl acetate were carefully added. Filtration and removal of the solvent gave the residue (5.2 g). This in dioxane (140 ml) was treated with dichlorodicyanobenzoquinone (11.7 g, 51.54 mmol) under reflux for 14 h. After cooling to room temperature the reaction mixture was filtered and the filtrate was evaporated to leave the residue, which was applied to a column of alumina (200 g). Elution with dichloromethane provided the trienone **2** (2.8 g, 47%): mp 156–157 °C (ether), UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 299 (13000), 252 (9200), 224 (12000), $^1\text{H-NMR}$ (CDCl_3) δ : 0.80 (3H, s, 18- H_3), 1.04 (3H, d, $J=6$ Hz, 21- H_3), 1.21 (3H, s, 19- H_3), 3.10–3.80 (3H, m, 22- H_2 and OH), 5.90–6.40 (4H, m, 2-H, 4-H, 6-H, and 7-H), 7.05 (1H, d, $J=10$ Hz, 1-H), MS m/z : 326 (M^+), 311, 308, 293, 267, 112.

1 α ,2 α -Epoxy-22-tetrahydropyranyloxy-23,24-dinorchola-4,6-dien-3-one (3)—The alcohol **2** (2.7 g, 8.28 mmol) in dichloromethane (50 ml) was treated with dihydropyran (1.5 ml, 16.42 mmol) and *p*-toluenesulfonic acid (50 mg) at room temperature for 1 h. The usual work-up (ethyl acetate for extraction) gave a crude product. This was dissolved in MeOH (70 ml), 30% H_2O_2 (4.8 ml) and 10% NaOH–MeOH (0.74 ml) was added, and this mixture was stirred at room temperature for 14 h. The usual work-up (ethyl acetate for extraction) gave a crude product, which was applied to a column of silica gel (50 g). Elution with benzene–ethyl acetate (100:1) provided the epoxide **3** (1.45 g, 41%): mp 113–115 °C (hexane), UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (ϵ): 290 (22000), $^1\text{H-NMR}$ (CDCl_3) δ : 0.80 (3H, s, 18- H_3), 1.07 (3H, d, $J=6$ Hz, 21- H_3), 1.18 (3H, s, 19- H_3), 3.38 (1H, dd, $J=4$ and 1.5 Hz, 1-H), 3.55 (1H, d, $J=4$ Hz, 2-H), 3.30–4.10 (4H, m, 22- H_2 and THP), 4.50 (1H, m, THP), 5.58 (1H, d, $J=1.5$ Hz, 4-H), 6.02 (2H, s, 6-H and 7-H), MS m/z : 342 ($\text{M}^+ - \text{DHP}$), 324 ($\text{M}^+ - \text{THPOH}$), 309, 283, 85.

23,24-Dinorchol-5-ene-1 α ,3 β ,22-triol 1,3-Diacetate (4)—Lithium (3.25 g) was added in small portions to liquid ammonia (130 ml) at –78 °C under an argon atmosphere during 30 min. The mixture was stirred for 1 h at –78 °C, then the epoxide **3** (1.33 g, 3.12 mmol) in dry THF (100 ml) was added dropwise at –78 °C during 30 min and the whole was stirred for 1 h at –78 °C. To this reaction mixture anhydrous NH_4Cl (40 g) was added in small portions at –78 °C during 1 h. After 1.5 h, the cooling bath was removed and most of the ammonia was removed by bubbling argon through the solution. The usual work-up (ether for extraction) gave a crude product (1.23 g). This was treated with acetic anhydride (3 ml) and pyridine (4 ml) at room temperature for 14 h. The usual work-up (ethyl acetate for extraction) gave a crude product (1.3 g). This in methanol (4 ml) and THF (5 ml) was treated with 2 drops of 2 M HCl at room temperature for 2 h. The usual work-up (ether for extraction) gave a crude product (1.1 g), which was applied to a column of silica gel (40 g). Elution with benzene–ethyl acetate (10:1) provided the 1,3-diacetate **4** (575 mg, 42%): oil, $^1\text{H-NMR}$ (CDCl_3) δ : 0.68 (3H, s, 18- H_3), 1.07 (3H, s, 19- H_3), 1.99 (3H, s, acetyl), 2.02 (3H, s, acetyl), 3.02–3.72 (2H, m, 22- H_2), 4.79 (1H, m, 3-H), 4.98 (1H, m, 1-H), 5.46 (1H, m, 6-H), MS m/z : 372 ($\text{M}^+ - \text{CH}_3\text{COOH}$), 313, 312, 297, 279, 253.

1 α ,3 β -Diacetoxy-23,24-dinorcholan-22-al (5)—The 22-alcohol **4** (550 mg, 1.27 mmol) in dichloromethane (20 ml) was treated with pyridinium chlorochromate (836 mg, 3.85 mmol) and sodium acetate (100 mg) at room temperature for 1 h. To this reaction mixture ether (100 ml) was added and the mixture was filtered through a short Florisil column. The filtrate was concentrated to leave the residue, which was applied to a column of silica gel (20 g). Elution with benzene–ethyl acetate (20:1) provided the 22-aldehyde **5** (448 mg, 82%): oil, $^1\text{H-NMR}$ (CDCl_3) δ : 0.70 (3H, s, 18- H_3), 1.07 (3H, s, 19- H_3), 1.09 (3H, d, $J=7$ Hz, 21- H_3), 1.99 (3H, s, acetyl), 2.02 (3H, s, acetyl), 4.79 (1H, m, 3-H), 4.98 (1H, m, 1-H), 5.45 (1H, m, 6-H), 9.45 (1H, d, $J=4$ Hz, 22-H), MS m/z : 310 ($\text{M}^+ - 2 \times \text{CH}_3\text{COOH}$), 295, 253.

(22E)-1 α ,3 β -Diacetoxycholesta-5,22-dien-24-one (6)—To a solution of the 22-aldehyde **5** (420 mg, 0.977 mmol) in dimethyl sulfoxide (30 ml) isobutylmethylphenylphosphorane (2.03 g, 5.87 mmol) was added. This mixture was stirred at 95 °C for 72 h. The usual work-up (ether for extraction) gave a crude product, which was applied to a column of silica gel (10 g). Elution with benzene–ethyl acetate (10:1) provided the enone **6** (392 mg, 81%): oil, $^1\text{H-NMR}$ (CDCl_3) δ : 0.71 (3H, s, 18- H_3), 1.08 (3H, s, 19- H_3), 1.09 (9H, d, $J=7$ Hz, 21- H_3 , 26- H_3 , and 27- H_3), 1.99 (3H, s, acetyl), 2.02 (3H, s, acetyl), 4.79 (1H, m, 3-H), 4.98 (1H, m, 1-H), 5.45 (1H, m, 6-H), 5.96 (1H, d, $J=16$ Hz, 23-H), 6.65 (1H, dd, $J=16$ and 8 Hz, 22-H), MS m/z : 438 ($\text{M}^+ - \text{CH}_3\text{COOH}$), 378 ($\text{M}^+ - 2 \times \text{CH}_3\text{COOH}$), 363, 335, 307, 253, 43.

(22E)-1 α ,3 β -Diacetoxy-5 α -8 α -(3,5-dioxo-4-phenyl-1,2,4-triazolidino)cholesta-6,22-dien-24-one (7)—To a solution of the enone **6** (385 mg, 0.773 mmol) in carbon tetrachloride (20 ml), *N*-bromosuccinimide (193 mg, 1.4 eq) was added and this mixture was refluxed for 25 min under an argon atmosphere. After cooling to 0 °C, the resulting precipitate was filtered off. The filtrate was concentrated below 40 °C to leave the residue. This in THF (15 ml) was treated with a catalytic amount of tetra-*n*-butylammonium bromide at room temperature for 50 min. Then, to this reaction mixture a solution of tetra-*n*-butylammonium fluoride in THF (3.5 ml, 3.5 mmol) was added and the whole was stirred at room temperature for 30 min. The usual work-up (ethyl acetate for extraction) gave a crude 5,7-diene

(380 mg). This in chloroform (15 ml) was treated with a solution of 1-phenyl-1,2,4-triazoline-3,5-dione (95 mg, 0.54 mmol) in chloroform (10 ml) at room temperature for 1 h. Removal of the solvent under reduced pressure gave the residue, which was applied to a column of silica gel (10 g). Elution with benzene–ethyl acetate (5 : 1) provided the triazoline adduct **7** (191 mg, 37%): oil, $^1\text{H-NMR}$ (CDCl_3) δ : 0.83 (3H, s, 18- H_3), 1.01 (3H, s, 19- H_3), 1.08 (9H, d, J = 7 Hz, 21- H_3 , 26- H_3 , and 27- H_3), 1.97 (3H, s, acetyl), 1.98 (3H, s, acetyl), 5.03 (1H, m, 1-H), 5.84 (1H, m, 3-H), 5.96 (1H, d, J = 16 Hz, 23-H), 6.28 (1H, d, J = 8.5 Hz, 6-H or 7-H), 6.41 (1H, d, J = 8.5 Hz, 6-H or 7-H), 6.65 (1H, dd, J = 16 and 8 Hz, 22-H), 7.20–7.60 (5H, m, –Ph), MS m/z : 436 ($\text{M}^+ - \text{PhC}_2\text{N}_3\text{O}_2 - \text{CH}_3\text{COOH}$), 376 (436 – CH_3COOH), 333, 305, 251, 43.

(22E,24R)- and (22E,24S)-1 α ,3 β -Diacetoxy-5 α ,8 α -(3,5-dioxo-4-phenyl-1,2,4-triazolidino)-cholesta-6,22-dien-24-ol (9a and 8a)—The enone **7** (150 mg, 0.224 mmol) in THF (6 ml) and methanol (6 ml) was treated with sodium borohydride (17 mg, 0.448 mmol) at room temperature for 10 min. The usual work-up (ether for extraction) gave a crude product (150 mg), which was subjected to preparative TLC (benzene–ethyl acetate, 3 : 1, developed seven times). The band of R_f value 0.53 was scraped off and eluted with ethyl acetate. Removal of the solvent under reduced pressure gave the less polar (24S)-24-alcohol **8a** (43.2 mg, 28.7%): mp 142–144 °C (ether–hexane), MS m/z : 438 ($\text{M}^+ - \text{PhC}_2\text{N}_3\text{O}_2 - \text{CH}_3\text{COOH}$), 420, 378 (438 – CH_3COOH), (363, 360, 345, 335, 318, 109, 43). The band of R_f value 0.50 was scraped off and eluted with ethyl acetate to give the more polar (24R)-24-alcohol **9a** (64.8 mg, 43.1%): mp 140–142 °C (ether–hexane). MS of **9a** was identical with that of **8a**.

(22E,24S)-1 α ,3 β -Diacetoxy-5 α ,8 α -(3,5-dioxo-4-phenyl-1,2,4-triazolidino)-cholesta-6,22-dien-24-ol (+)-MTPA Ester (8b)—The 24-alcohol **8a** (8.3 mg, 0.0123 mmol) in pyridine (1 ml) was treated with 3 drops of (+)-MTPA–Cl at room temperature for 1 h. The usual work-up (ethyl acetate) provided the MTPA ester **8b** (10.4 mg, 95%): $^1\text{H-NMR}$ (CDCl_3 , 100 MHz) δ : 0.85 (3H, s, 18- H_3), 0.88 (3H, d, J = 7 Hz, 26- H_3), 0.92 (3H, d, J = 7 Hz, 27- H_3), 1.04 (3H, d, J = 7 Hz, 21- H_3), 1.08 (3H, s, 19- H_3), 2.03 (3H, s, acetyl), 2.06 (3H, s, acetyl), 3.27 (1H, m), 3.54 (3H, s, – OCH_3), 6.28 (1H, d, J = 8 Hz, 6-H or 7-H), 6.41 (1H, d, J = 8 Hz, 6-H or 7-H), 7.24–7.56 (5H, m, –Ph).

(22E,24R)-1 α ,3 β -Diacetoxy-5 α ,8 α -(3,5-dioxo-4-phenyl-1,2,4-triazolidino)cholesta-6,22-dien-24-ol 24-(+)-MTPA Ester (9b)—The 24-alcohol **9a** (7.9 mg, 0.0117 mmol) was converted, as described for **8b**, into the MTPA ester **9b** (9.3 mg, 89%): $^1\text{H-NMR}$ (CDCl_3 , 100 MHz) δ : 0.83 (3H, s, 18- H_3), 0.88 (6H, d, J = 7 Hz, 26- H_3 and 27- H_3), 1.04 (3H, d, J = 7 Hz, 21- H_3), 1.08 (3H, s, 19- H_3), 2.03 (3H, s, acetyl), 2.05 (3H, s, acetyl), 3.27 (1H, m), 3.54 (3H, s, – OCH_3), 6.28 (1H, d, J = 8 Hz, 6-H or 7-H), 6.41 (1H, d, J = 8 Hz, 6-H or 7-H), 7.24–7.56 (5H, m, –Ph).

(22E,24S)-6 β -Methoxy-3 α ,5-cyclo-5 α -cholest-22-en-24-ol 24-(+)-MTPA Ester (14b)—The known (24S)-24-alcohol **14a** (10.1 mg, 0.0244 mmol) was converted, as described for **8b**, into the (24S)-MTPA ester **14b** (8.2 mg, 54%): $^1\text{H-NMR}$ (CDCl_3 , 100 MHz) δ : 0.72 (3H, s, 18- H_3), 0.89 (3H, d, J = 7 Hz, 26- H_3), 0.93 (3H, d, J = 7 Hz, 27- H_3), 1.02 (3H, d, J = 7 Hz, 21- H_3), 1.04 (3H, s, 19- H_3), 2.75 (1H, m, 6-H), 3.33 (3H, s, – OCH_3), 3.54 (3H, s, – OCH_3).

(22E,24R)-6 β -Methoxy-3 α ,5-cyclo-5 α -cholest-22-en-24-ol 24-(+)-MTPA Ester (15b)—The known (24R)-24-alcohol **15a** (11.0 mg, 0.0266 mmol) was converted, as described for **8b**, into the (24R)-MTPA ester **15b** (9.4 mg, 56%): $^1\text{H-NMR}$ (CDCl_3 , 100 MHz) δ : 0.76 (3H, s, 18- H_3), 0.88 (6H, d, J = 7 Hz, 26- H_3 and 27- H_3), 1.04 (3H, d, J = 7 Hz, 21- H_3), 1.05 (3H, s, 19- H_3), 2.77 (1H, m, 6-H), 3.36 (3H, s, – OCH_3), 3.57 (3H, s, – OCH_3).

(22E,24R)-Cholesta-5,7,22-triene-1 α ,3 β ,24-triol (10)—The triazoline adduct **9a** (15.0 mg, 0.0223 mmol) in THF (5 ml) was treated with lithium aluminium hydride (5 mg, 0.132 mmol) under reflux for 2 h. To this reaction mixture water was added and the mixture was filtered. The filtrate was concentrated under reduced pressure to leave the residue, which was subjected to preparative TLC (benzene–ethyl acetate, 1 : 1, developed three times). The band of R_f value 0.35 was scraped off and eluted with ethyl acetate. Removal of the solvent provided the 5,7-diene **10** (3.3 mg, 36%), UV $\lambda_{\text{max}}^{\text{EtOH}}$: 294, 282, 272. MS m/z : 414 (M^+), 396, 381, 378, 363, 353, 335, 317, 287, 269, 251, 127, 109.

(22E,24S)-Cholesta-5,7,22-triene-1 α ,3 β ,24-triol (11)—The triazoline adduct **8a** (16.5 mg, 0.0245 mmol) was converted, as described for **10**, to the 5,7-diene **11** (3.6 mg, 36%). The UV and MS of **11** were identical with those of **10**.

(22E,24R)-1 α ,24-Dihydroxy-22-dehydrovitamin D₃ (12)—A solution of the (24R)-5,7-diene **10** (3.3 mg, 7.97 μmol) in benzene (90 ml) and ethanol (40 ml) was irradiated with a medium-pressure mercury lamp through a Vycor filter for 2.5 min with ice-cooling under an argon atmosphere. Then, the reaction mixture was refluxed for 1 h under an argon atmosphere. Removal of the solvent under reduced pressure gave a crude product, which was subjected to preparative TLC (benzene–ethyl acetate, 1 : 1, developed three times). The band of R_f value 0.40 was scraped off and eluted with ethyl acetate. Removal of the solvent under reduced pressure provided the vitamin D₃ analogue **12** (0.59 mg, 18%). This was further purified by high-performance liquid chromatography on a Zorbax SIL normal phase column (4.6 mm i.d. $\times$ 15 cm) at a flow rate of 2 ml/min with 2% methanol in dichloromethane as an eluent. The retention time of **12** was 5.2 min. UV $\lambda_{\text{max}}^{\text{EtOH}}$ 265 nm, $\lambda_{\text{min}}^{\text{EtOH}}$ 228 nm, MS m/z : 414 (M^+), 396, 378, 363, 360, 345, 335, 317, 287, 269, 251, 249, 152, 135, 134, 109. $^1\text{H-NMR}$ (CDCl_3 , 400.5 MHz) δ : 0.57 (3H, s, 18- H_3), 0.87 (3H, d, J = 6.7 Hz, 26- H_3), 0.92 (3H, d, J = 6.7 Hz, 27- H_3), 1.04 (3H, d, J = 6.6 Hz, 21- H_3), 2.32 (1H, dd, J = 13.7 and 6.6 Hz), 2.60 (1H, dd, J = 13.4 and 3.4 Hz), 2.83 (1H, dd, J = 12.6 and 4.0 Hz), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.00 (1H, br s, $W_{1/2}$ = 4.3 Hz, 19-H), 5.33 (1H, br s, $W_{1/2}$ = 4.3 Hz, 19-H), 5.39 (1H, dd, J = 15.2 and 7.1 Hz, 22-H), 5.51 (1H, dd, J = 15.2 and 8.3 Hz, 23-H), 6.01 (1H, d, J = 11.4 Hz, 7-H), 6.38 (1H, d, J = 11.4 Hz, 6-H).

(22E,24S)-1 α ,24-Dihydroxy-22-dehydrovitamin D₃ (13)—The (24S)-5,7-diene **11** (3.5 mg, 8.45 μmol) was trans-

formed, as described for **12**, into the vitamin D₃ form **13** (0.56 mg, 16%). The retention time of **13** under the above-described high performance liquid chromatography (HPLC) conditions was 4.7 min. The UV and MS of **13** were identical with those of **12**. ¹H-NMR (CDCl₃, 400.5 MHz) δ : 0.57 (3H, s, 18-H₃), 0.87 (3H, d, J =6.7 Hz, 26-H₃), 0.92 (3H, d, J =6.7 Hz, 27-H₃), 1.05 (3H, d, J =6.6 Hz, 21-H₃), 2.32 (1H, dd, J =13.7 and 6.6 Hz), 2.60 (1H, dd, J =13.4 and 3.4 Hz), 2.83 (1H, dd, J =12.6 and 4.0 Hz), 4.23 (1H, m, 3-H), 4.43 (1H, m, 1-H), 5.00 (1H, br s, $W_{1/2}$ =4.3 Hz, 19-H), 5.33 (1H, br s, $W_{1/2}$ =4.3 Hz, 19-H), 5.37 (1H, dd, J =15.4 and 7.5 Hz, 22-H), 5.46 (1H, dd, J =15.4 and 8.3 Hz, 23-H), 6.01 (1H, d, J =11.4 Hz, 7-H), 6.38 (1H, d, J =11.4 Hz, 6-H).

Measurement of Biological Activity—Rats: Weanling male rats were purchased from Holzman Co. (Madison, WI), and fed a low-calcium, vitamin D-deficient⁽¹²⁾ diet or a low-phosphorus, vitamin D-deficient⁽¹³⁾ diet for 3 weeks.

Measurement of Intestinal Calcium Transport: Calcium transport activity was measured in everted duodenal sacs as described by Martin and DeLuca.⁽¹⁴⁾

Determination of Serum Calcium and Inorganic Phosphorus: Serum was obtained by centrifugation of clotted blood. Calcium was determined in the presence of 0.1% lanthanum chloride by means of a Perkin-Elmer atomic absorption spectrometer, model 403, while inorganic phosphorus was determined by the colorimetric method of Chen *et al.*⁽¹⁵⁾

Measurement of Bone Ash: After the connective tissue was removed, femurs were extracted successively with ethanol and diethyl ether for 24 h each using a Soxhlet extractor. Fat-free bones were dried in an oven at 100 °C for 24 h and ashed in a muffle furnace at 650 °C for 24 h.

Displacement of 1,25-(OH)₂-[26,27-³H]-D₃ from Chick Intestinal Cytosol Protein for 1,25(OH)₂D₃ by (22E,24R)- or (22E,24S)-1,24-(OH)₂- Δ^{22} -D₃: Graded amounts of either 1,25-(OH)₂D₃, (22E,24R)- or (22E,24S)-1,24-(OH)₂- Δ^{22} -D₃ were dissolved in 0.05 ml of 95% ethanol. Triplicate determination of displacement of 1,25-(OH)₂-[26,27-³H]-D₃ from chick intestinal cytosol binding protein by unlabeled compound was carried out as described by Shepard *et al.*⁽¹⁶⁾

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