Synthetic Studies of Vitamin D Analogues. X.[1] Synthesis and Biological Activities of 1α,25-Dihydroxy-21-norvitamin D_3[2]

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1α,25-Dihydroxy-21-norvitamin D_3 (3) was synthesized from 1α-hydroxydehydroepiandrosterone (4). Certain biological properties of 3 were examined in comparison with those of 1α,25-dihydroxyvitamin D_3 (1) and 1α,25-dihydroxy-21-nor-20-oxavitamin D_3 (2) to evaluate the effect of the 21-methyl substituent on biological activities. The differentiation-inducing activity of 3 towards human myeloid leukemia cells was approximately one-fifth of that of 1, while in the binding affinity with chick intestinal cytosolic receptor, 3 was about one-tenth of that of 1. The rather weak effect of 3 on serum calcium levels in normal mice at a dosage of 500 μg/kg (intravenous administration) indicates that the essential importance of the 21-methyl moiety may lie in its effect on the regulation of calcium metabolism.

Keywords 1α,25-dihydroxy-21-norvitamin D_3; 1α,25-dihydroxyvitamin D_3; 1α,25-dihydroxy-21-nor-20-oxavitamin D_3; 1α-hydroxydehydroepiandrosterone; differentiation-inducing activity; calcium metabolism

In recent years, considerable attention has been focused on the synthesis of analogues of 1α,25-dihydroxyvitamin D_3 (**1**) [1α,25-$(OH)_2$-D_3], aiming to separate the differentiation-inducing activity towards human myeloid leukemia cells (HL-60) from the regulatory effect on calcium metabolism.[3] Previously, we synthesized 1α,25-dihydroxy-21-nor-20-oxavitamin D_3 (**2**)[4] and showed that **2** had a high differentiation-inducing activity without the hypercalcemic action.[5] Because **2** lacked the 21-methyl substituent, we were interested in the role of the 21-methyl group in the biological activities of 1α,25-$(OH)_2$-D_3 (**1**). The present report deals with the synthesis of a new analogue with a vacant 21-position, 1α,25-dihydroxy-21-norvitamin D_3 (**3**), and the comparison of its biological activities with those of **1** and **2**.

Synthesis 1α-Hydroxydehydroepiandrosterone (**4**), prepared from dehydroepiandrosterone by microbiological 1α-hydroxylation,[6] was converted to the diene diacetate (**5**) in 98% yield by means of the Wittig reaction with methylidene triphenylphosphorane followed by acetylation.[7,8] The ene reaction of **5** with methyl propiolate in the presence of diethylaluminum chloride gave the triene (**6**) in 66% yield.[7,8] Subsequent catalytic hydrogenation of the C-16 and C-22 double bonds in **6** proceeded selectively from the less congested α-face to afford the ester (**7**),[7,8] which was transformed into the alcohol (**8**) by the following three-step procedure without any purification of intermediates; 1) hydrolysis of the acetoxy and carbomethoxy groups with boiling methanolic potassium hydroxide; 2) protection of the resulting hydroxy groups as the methoxymethyl ether; 3) reduction of the carboxy group in the side chain with lithium aluminum hydride. The hydroxy moiety in **8** was then converted to the bromide (**9**) by means of tosylation with *p*-toluenesulfonyl chloride in pyridine and subsequent bromination with lithium bromide in tetrahydrofuran (THF) at 50 °C in 47% overall yield from the triene (**6**). The bromide (**9**) was treated with lithio-2-methyl-1,3-dithiane in THF to give the dithiane (**10**), in 85% yield, and this was hydrolyzed to the keto-diol (**11**) in 83% yield upon treatment with methyl iodide in boiling aqueous acetone.[9] Addition of excess methylmagnesium bromide to **11** in THF completed the side chain formation, giving rise to the triol (**12**) in 60% yield.[10]

Secondary hydroxy group in **12** were acetylated and the resulting diacetate (**13**) was transformed into the 4-phenyl-1,2,4,-triazoline-3,5,-dione (PTAD) adduct (**14**) in 27% overall yield by the usual three-step process; 1) bromination at the 7-position with *N*-bromosuccinimide (NBS) in boiling *n*-hexane; 2) dehydrobromination by γ-collidine in boiling xylene; 3) addition of PTAD in methylene dichloride.[11] This bromination-dehydrobromination treatment gave a mixture of several components including the 5,7-diene, the 4,6-diene, the unreacted 5-ene, *etc.*, and separation–purification of the 5,7-diene seemed to be difficult. Thus, the more polar PTAD adduct with the 5,7-diene made it easy to isolate the 5,7-diene component from the less polar concomitant which does not react with PTAD. Heating the PTAD adduct (**14**) under reflux with lithium aluminum hydride in THF[11] afforded the pure 5,7-diene (**15**)[12] in 34% yield. Subsequent irradiation of **15** in ethanol at 0 °C

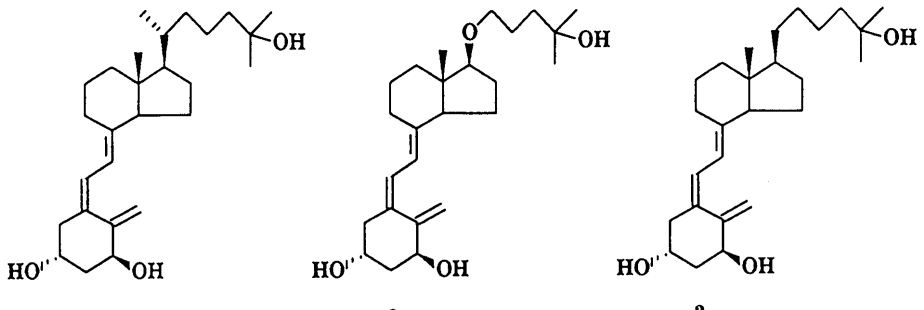

Chart 1

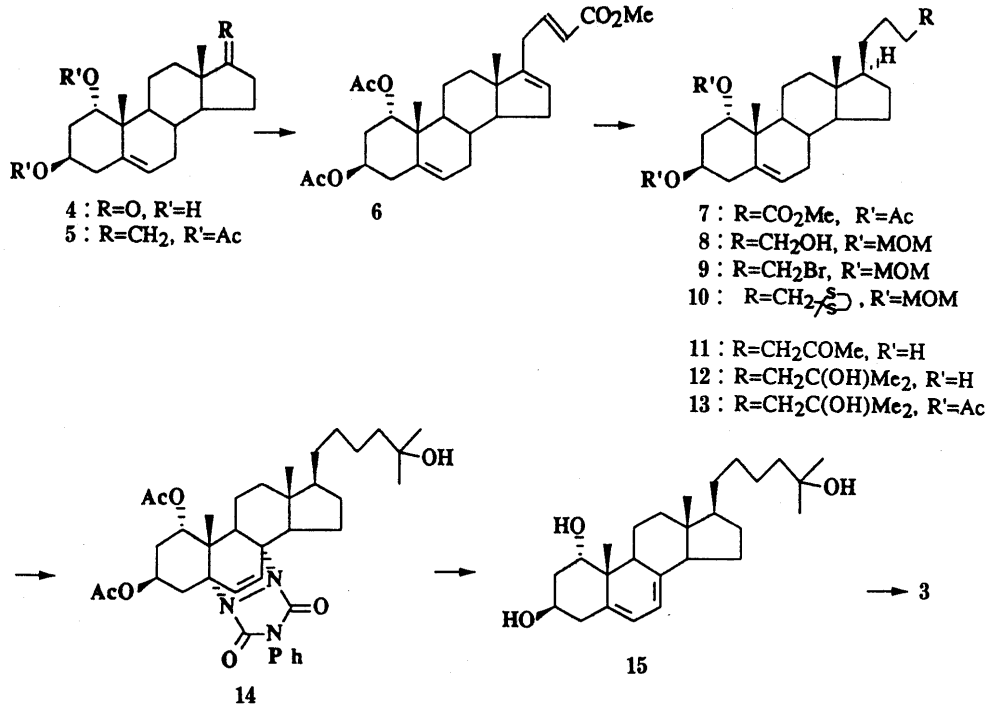

Chart 2

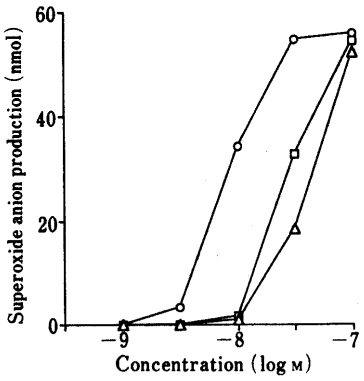

Fig. 1. Comparative Effect of Vitamin D_3 Analogues on the Induction of Superoxide Anion Production of HL-60

1, O—O; 2, △—△; 3, □—□.

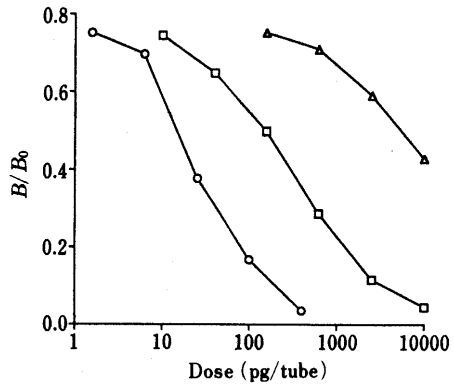

Fig. 2. Competitive Displacement Curves of [^{3}H]1α,25-$(OH)_2$-D_3 from Chick Embryonic Intestinal 1α,25-$(OH)_2$-D_3 Receptor with Vitamin D_3 Analogues

1, O—O; 2, △—△; 3, □—□.

using a high-pressure mercury lamp, followed by thermal isomerization in refluxing ethanol gave 1α,25-dihydroxy-21-norvitamin D_3 (**3**) in 18% yield.

TABLE I. Effect of Vitamin D_3 Analogues on Serum Calcium Levels

Compound	Dose (μg/kg)	Serum calcium (mg/dl)
Control	Vehicle	8.70+0.16
1	2	10.36+0.16[a]
	10	11.04+0.12[a]
2	100	8.80+0.10
	500	8.96+0.13
3	10	8.63+0.13
	100	8.71+0.15
	500	9.68+0.15[a]

Data show the means±S.D. of 6 mice. Significantly different from the control (*a*) $p<0.001$).

Biological Results Figure 1 shows the differentiation-inducing activity of HL-60 into macrophages *in vitro* estimated in terms of superoxide anion generation.[13] 1α,25-Dihydroxy-21-norvitamin D_3 (**3**) and 1α,25-dihydroxy-21-nor-20-oxavitamin D_3 (**2**), had ED_{50} values of 2.9×10^{-8} M and 4.0×10^{-8} M, respectively, compared to 8.3×10^{-9} M for 1α,25-$(OH)_2$-D_3 (**1**). In the binding affinity with chick intestinal cytosolic receptor,[14] **3** and **2** showed one-tenth and one-thousandth of the affinity of **1**, respectively (Fig. 2). The most noteworthy result was the quite low calcemic activity of **3** in normal mice[15] at a dosage of 500 μg/kg (intravenous administration) (Table I).

The replacement of the carbon atom by an oxygen atom at the 20-position made a considerable difference to the binding affinity with chick intestinal cytosolic receptor, but not the differentiation-inducing activity, which was largely retained in the absence of the 21-methyl moiety. On the other hand, the 21-methyl group was apparently important for the regulatory effect on calcium metabolism. Only a moderate increase in serum calcium levels of mice was induced by **3** in comparison with **1**, in spite of a 50-times-higher dose of **3**, while **2** had expectable effects on

serum calcium levels, taking its low binding affinity into consideration. As the receptor-mediated hypercalcemic action of vitamin D is well known, the quite low calcemic activity of **3** suggests that the 21-methyl substituent plays a sterically important role in the realization of the effect on calcium metabolism after binding of vitamin D to its receptor. Further biological properties and physicochemical features under investigation shoud help explain the structural role of the 21-methyl substituent in detail, and will be reported elsewhere.

Experimental

General Methods All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were obtained using a Hitachi 260-30 spectrometer. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL FX-200 spectrometer in $CDCl_3$ with tetramethylsilane as an internal standard. Abbreviations used are s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Mass spectra (MS) were measured on a Shimadzu GCMS-QP 1000 with the ionizing volatage at 20 eV. Ultraviolet (UV) spectra were recorded with a Shimadzu UV-240 in EtOH. All reactions were carried out under an atmosphere of dry argon or nitrogen unless otherwise noted. Flash column chromatography was carried out with Merck Kieselgel 60, 230—400 mesh, and preparative thin layer chromatography (TLC) was performed on 20 × 20 cm plates coated with 0.5 mm of Merck Kieselgel 60 containing PF_{254} indicator.

1α,3β-Diacetoxy-21-norpregna-5,17(20)-diene (5) To a stirred suspension of *t*-BuOK (11.22 g, 90 mmol) in THF (220 ml) was added portionwise methyltriphenylphosphonium bromide (32.15 g, 90 mmol) at room temperature. The mixture was stirred at 50 °C for 3 h. A suspension of **4** (9.07 g, 30 mmol) in THF (100 ml) was then added at room temperature and stirring was continued at room temperature for 15 h. Pyridine (100 ml), Ac_2O (50 ml) and 4-dimethylaminopyridine (1.5 g) were added and the resulting mixture was stirred at room temperature for 24 h, then poured into H_2O, and extracted with Et_2O. The extract was washed with 10% HCl, H_2O, saturated $NaHCO_3$ and saturated NaCl, and dried over $MgSO_4$. Removal of the solvent *in vacuo* left a pale yellow gum, which was purified by flash column chromatography with CH_2Cl_2–*n*-hexane (3 : 2) as the eluant to give **5** (11.30 g, 98%) as colorless needles, mp 108.5—110 °C (MeOH). IR (KBr): 1735, 1655, 1235 cm^{-1}. NMR δ: 0.81 (3H, s), 1.13 (3H, s), 2.05 (6H, s), 4.51 (1H, br d, J = 2.5 Hz), 4.55 (1H, br d, J = 2.5 Hz), 4.68—5.06 (2H, br), 5.45 (1H, br d, J = 6.0 Hz). MS *m/z*: 386 (M^+), 266 (100%). *Anal.* Calcd for $C_{24}H_{34}O_4$: C, 74.57; H, 8.87. Found: C, 74.96; H, 8.91.

Methyl (22*E*)-1α,3β-Diacetoxy-21-norchola-5,16,22-trien-24-oate (6) To a stirred solution of **5** (3.40 g, 8.8 mmol) and methyl propiolate (1.11 g, 13.2 mmol) in CH_2Cl_2 (50 ml) was added dropwise diethylaluminum chloride (15% solution in *n*-hexane, 57 ml, 70 mmol) at room temperature. The resulting mixture was stirred at room temperature for 24 h and quenched by careful addition of saturated $NaHCO_3$. After removal of the solvent *in vacuo*, the residue was taken up with toluene and H_2O. The organic layer was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude **6**, which was purified by flash column chromatography with toluene–AcOEt (20 : 1) as the eluant to give **6** (2.73 g, 66%) as a pale yellow oil. IR (neat): 1730, 1660, 1235 cm^{-1}. NMR δ: 0.79 (3H, s), 1.12 (3H, s), 2.00 (3H, s), 2.03 (3H, s), 3.70 (3H, s), 4.68—5.10 (2H, br), 5.25—5.40 (1H, br), 5.50 (1H, br d, J = 6.0 Hz), 5.80 (1H, d, J = 15.0 Hz), 6.95 (1H, d t, J = 15.0, 8.0 Hz). MS *m/z*: 350 (M^+ − AcOH × 2), 118 (100%).

Methyl 1α,3β-Diacetoxy-21-norchol-5-en-24-oate (7) A solution of **6** (9.95 g, 21.1 mmol) in AcOEt (100 ml) was stirred with 5% Pt/C (1.25 g) at room temperature under an atmosphere of hydrogen for 1.5 h. After removal of the catalyst by filtration, the solvent was evaporated *in vacuo* to give crystalline **7** (9.70 g, crude 97%), which was used without further purification. Recrystallization from MeOH gave analytically pure **7** as colorless needles, mp 114—115 °C. IR (KBr): 1730, 1240 cm^{-1}. NMR δ: 0.60 (3H, s), 1.10 (3H, s), 2.02 (3H, s), 2.05 (3H, s), 3.64 (3H, s), 4.63—5.13 (2H, br), 5.50 (1H, br d, J = 6.0 Hz). MS *m/z*: 354 (M^+ − AcOH × 2), 118 (100%). *Anal.* Calcd for $C_{28}H_{42}O_6$: C, 70.85; H, 8.92. Found: C, 71.11; H, 8.94.

1α,3β-Bis(methoxymethoxy)-21-norchol-5-en-24-ol (8) A solution of **7** (crude 2.64 g) and KOH (85%, 2.70 g) in MeOH (80 ml) was refluxed for 6 h and left at room temperature for 14 h. After removal of the solvent *in vacuo*, the residue was taken up with H_2O, made acidic (pH 2—3) by addition of 10% HCl, and extracted with $CHCl_3$–MeOH (3 : 1). The extract was washed with saturated NaCl, dried over $MgSO_4$, and concentrated *in vacuo* to leave a colorless powder (2.01 g), which was used without further purification. To a stirred mixture of the above-mentioned powder (2.01 g), iso-Pr_2NEt (5.57 ml), THF (20 ml) and dimethylformamide (DMF) (10 ml) was added chloromethyl methyl ether (2.03 ml) at 0 °C. The resulting mixture was then stirred at 45 °C for 14 h, poured into H_2O (100 ml), and extracted with AcOEt. The extract was washed with saturated NaCl, dried over $MgSO_4$, and concentrated *in vacuo* to give a pale yellow oil (2.71 g), which was used without further purification. To a stirred solution of the above-mentioned oil (2.71 g) in THF (40 ml) was added portionwise $LiAlH_4$ (0.99 g) at 0 °C. The mixture was then stirred at room temperature for 2 h and quenched by addition of 10% NaOH at 0 °C. The mixture was diluted with AcOEt and H_2O. The separated organic layer was washed with saturated NaCl, dried over $MgSO_4$ and concentrated *in vacuo* to give gummy **8** (2.40 g), which was used without further purification. Preparative TLC with CH_2Cl_2–AcOEt (9 : 1) afforded analytically pure **8**. IR (neat): 3450, 1050 cm^{-1}. NMR δ: 0.59 (3H, s), 1.02 (3H, s), 3.31 (3H, s), 3.35 (3H, s), 3.60 (2H, br t, J = 6.0 Hz), 5.48 (1H, br d, J = 6.0 Hz).

1α,3β-Bis(methoxymethoxy)-21-norchol-5-en-24-yl Bromide (9) To a stirred solution of **8** (crude 2.40 g) in pyridine (40 ml) was added *p*-TsCl (1.90 g) at 0 °C. The mixture was then left at room temperature for 14 h, poured into H_2O, and extracted with AcOEt. The extract was washed with 6 N HCl, H_2O, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated *in vacuo* to give a pale yellow oil (2.08 g), which was used without further purification. A solution of the abovementined oil (2.08 g) and LiBr (0.90 g) in THF (30 ml) was stirred at 50 °C for 20 h. The mixture was then diluted with AcOEt, washed with H_2O and saturated NaCl, and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude **9**, which was purified by flash column chromatography with *n*-hexane–AcOEt (85 : 15) as the eluant to give **9** (1.39 g, 47% overall yield from **6**) as a colrless gum. IR (neat): 1140, 1100, 1035 cm^{-1}. NMR δ: 0.60 (3H, s), 1.04 (3H, s), 3.32 (3H, s), 3.36 (3H, s), 5.52 (1H, br d, J = 6.0 Hz). MS *m/z*: 453, 451 (M^+ − OMOM), 390 (100%). *Anal.* Calcd for $C_{27}H_{45}BrO_4$: C, 63.15; H, 8,83. Found: C, 63.15; H, 8.80.

1α,3β-Bis(methoxymethoxy)-21,27-bisnor-25,25-trimethylenedithiocholest-5-ene (10) To a stirred solution of 2-methyl-1,3-dithiane (3.50 g, 26 mmol) in THF (35 ml) was added dropwise *n*-BuLi (1.28 M solution in *n*-hexane, 20 ml, 26 mmol) at −78 °C. The mixture was gradually warmed to 4 °C and stirring was continued at 4 °C for 2 h. The mixture was then re-cooled to −78 °C. A solution of **9** (1.34 g, 2.6 mmol) in THF (10 ml) was added dropwise, and stirring was continued at 4 °C for 1 h. After quenching of the reaction by addition of saturated NH_4Cl at 4 °C, the mixture was extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude **10**, which was purified by flash column chromatography with *n*-hexane–AcOEt (85 : 15) as the eluant to give **10** (1.26 g, 85%) as a colorless gum. IR (neat): 1145, 1100, 1035 cm^{-1}. ^{1}H-NMR δ: 0.60 (3H, s), 1.03 (3H, s), 1.61 (3H, s), 3.32 (3H, s), 3.36 (3H, s), 5.50 (1H, br d, J = 6.0 Hz). MS *m/z*: 566 (M^+), 133 (100%). *Anal.* Calcd for $C_{32}H_{54}O_4S_2$: C, 67.80; H, 9.60. Found: C, 67.89; H, 9.68.

1α,3β-Dihydroxy-21,27-bisnorcholest-5-en-25-one (11) A solution of **10** (1.26 g, 2.2 mmol), MeI (9.44 g, 66 mmol) and H_2O (0.8 ml, 44 mmol) in acetone (160 ml) was refluxed mildly for 20 h. After removal of the solvent *in vacuo*, the residue was taken up with CH_2Cl_2 and H_2O. The organic layer was washed with 3% $Na_2S_2O_3$ and saturated NaCl, and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude **11**, which was purified by flash column chromatography with AcOEt–*n*-hexane (3 : 1) as the eluant to give **11** (711 mg, 83%) as colorless plates, mp 101—102 °C (acetone). IR (Nujol): 3450, 1710 cm^{-1}. ^{1}H-NMR δ: 0.59 (3H, s), 1.02 (3H, s), 2.14 (3H, s), 3.70—3.95 (2H, br), 5.54 (1H, br d, J = 6.0 Hz). MS *m/z*: 388 (M^+), 370 (100%). *Anal.* Calcd for $C_{25}H_{40}O_3$: C, 77.27; H, 10.38. Found: C, 77.17; H, 10.54.

1α,3β,25-Trihydroxy-21-norcholest-5-ene (12) To a stirred solution of **11** (160 mg, 0.41 mmol) in THF (10 ml) was added dropwise MeMgBr (1 M solution in THF, 4.1 ml, 4.1 mmol) at −10—−5 °C. The mixture was warmed to room temperature and stirring was continued at room temperature for 30 min. After quenching of the reaction by addition of saturated NH_4Cl at room temperature, the mixture was extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude **12**, which was purified by flash column chromatography with CH_2Cl_2–EtOH (10 : 1) as the eluant

to give **12** (99 mg, 60%) as colorless needles, mp 143—145 °C (acetone). IR (Nujol): 3330, 1460, 1370, 1050 cm^{-1}. ^{1}H-NMR δ: 0.59 (3H, s), 1.02 (3H, s), 1.19 (6H, s), 5.52 (1H, br d, J=6.0 Hz). MS m/z: 404 (M^+), 386 (100%). *Anal.* Calcd for $C_{26}H_{44}O_3$: C, 77.17; H, 10.96. Found: C, 76.89; H, 11.06.

1α,3β-Diacetoxy-25-hydroxy-21-norcholest-5-ene (13) A mixture of **12** (88 mg, 0.22 mmol), 4-dimethylaminopyridine (10 mg), pyridine (10 ml) and Ac_2O (5 ml) was stirred at room temperature for 49 h. The mixture was then poured into H_2O and extracted with AcOEt. The extract was washed with 10% HCl, H_2O, saturated $NaHCO_3$ and saturated NaCl, and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude **13**, which was purified by preparative TLC with CH_2Cl_2–acetone (98 : 2) to give **13** (91 mg, 86%) as a colorless gum. IR (neat): 3460, 1765, 1735, 1230 cm^{-1}. ^{1}H-NMR δ: 0.60 (3H, s), 1.10 (3H, s), 1.43 (3H, s), 1.46 (3H, s), 2.03 (3H, s), 2.06 (3H, s), 4.80—5.13 (2H, br), 5.52 (1H, br d, J=6.0 Hz). MS m/z: 368 ($M^+ - AcOH \times 2$), 118 (100%).

PTAD Adduct of 1α,3β-Diacetoxy-25-hydroxy-21-norcholesta-5,7-diene (14) A mixture of **13** (90 mg, 0.18 mmol), NBS (43 mg, 0.24 mmol) and $NaHCO_3$ (62 mg, 0.74 mmol) in *n*-hexane (10 ml) was refluxed for 1.5 h. The mixture was then diluted with AcOEt, washed with H_2O, 3% $Na_2S_2O_3$, saturated $NaHCO_3$ and saturated NaCl, and dried over $MgSO_4$. Removal of the solvent *in vacuo* left a pale yellow foam, which was used without purification. A mixture of the above-mentioned foam and γ-collidine (0.3 ml) in xylene (10 ml) was refluxed for 1 h. The mixture was diluted with toluene, washed with 10% HCl, H_2O, saturated $NaHCO_3$ and saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left a pale brown foam, which was used without further purification. To a stirred solution of the above-mentioned foam in CH_2Cl_2 (7 ml) was added PTAD (39 mg, 0.22 mmol) in CH_2Cl_2 (3 ml) at room temperature. The mixture was then stirred at room temperature for 30 min. Removal of the solvent *in vacuo* gave crude **14**, which was purified by flash column chromatography with CH_2Cl_2–acetone (50 : 1) to afford **14** (33 mg, 27% overall yield) as a colorless powder. IR (neat): 3450, 1750—1690 (br), 1400, 1230, 1150 cm^{-1}. ^{1}H-NMR δ: 0.75 (3H, s), 1.10 (3H, s), 1.47 (6H, s), 2.03 (3H, s), 2.05 (3H, s), 5.02—5.20 (2H, br), 6.32 (1H, d, J=6.0 Hz), 6.49 (1H, d, J=6.0 Hz), 721—7.59 (5H, br). MS m/z: 486 ($M^+ - PTAD$), 348 (100%).

1α,3β,25-Trihydroxy-21-norcholesta-5,7-diene (15) To a stirred mixture of $LiAlH_4$ (77 mg, 2.0 mmol) in THF (10 ml) was added dropwise **14** (89 mg, 0.13 mmol) in THF (5 ml) at 0 °C. The mixture was then refluxed for 1.5 h, quenched by careful addition of 10% NaOH at 0 °C, and extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left crude **15**, which was purified by flash column chromatography with CH_2Cl_2–EtOH (10 : 1) as the eluant to give **15** (23.5 mg, 34%) as a colorless powder. ^{1}H-NMR δ: 0.60 (3H, s), 1.02 (3H, s), 1.20 (6H, s), 5.38—5.44 (1H, br), 5.72 (1H, d, J=5.7 Hz). MS m/z: 402 (M^+), 197 (100%). UV λ_{max} nm: 293, 282, 271.

1α,25-Dihydroxy-21-norvitamin D_3 (3) A solution of **15** (22.4 mg, 0.06 mmol) in EtOH (400 ml) was irradiated using a 400 W high-pressure mercury lamp with a Vycor filter at 0 °C for 5 min. The mixture was then refluxed mildly for 1.5 h and concentrated *in vacuo* to leave a pale yellow oil, which was purified by preparative TLC developed twice with CH_2Cl_2–EtOH (50 : 7) to give recovered **15** (11.0 mg, 49%) and **3** (2.1 mg, 18% based upon the recovery). IR (neat): 3380, 1470, 1450, 1375, 1060 cm^{-1}. ^{1}H-NMR δ: 0.44 (3H, s), 1.21 (6H, s), 1.25 (3H, br s), 4.14—4.28 (1H, br), 4.34—4.47 (1H, br), 5.00 (1H, s), 5.33 (1H, s), 6.01 (1H, d, J=11.0 Hz), 6.38 (1H, d, J=11.0 Hz). MS m/z: 402 (M^+), 134 (100%). UV λ_{max} nm: 262. λ_{min} nm: 227.

Differentiation-Inducing Activity HL-60 was cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum and 20 μg/ml gentamycin at 37 °C in a humidified atmosphere of 5% CO_2 in air. Induction of differentiation was estimated in terms of the ability of the cells to generate superoxide anion. Vitamin D-induced cells were obtained by seeding HL-60 at 1×10^5/ml in growth medium and culturing for 4 d in the presence of various concentrations of vitamin D_3 analogues. The cells were washed free of the test compounds and suspended in 1.5 ml of reaction mixture containing 80 μM ferricytochrome c (Sigma Chemical Co., St. Louis, MO.) and 500 ng/ml phorbol myristate acetate (Sigma) in 0.1% gelatin Hanks' balanced salt solution without phenol red. The mixture was incubated at 37 °C for 60 min and centrifuged for 10 min at $400 \times g$ at 4 °C. The optical density of the supernatants was determined with a Hitachi U-3200 dual-wavelength (550 *versus* 540 nm) spectrophotometer. The amount of superoxide anion generated was calculated by assuming a molar extinction coefficient of 19.1×10^3/cm.

Binding Affinity with Chick Intestinal Cytosolic Receptor Chick embryonic intestinal 1α,25-$(OH)_2$-D_3 receptor (Yamasa Shoyu Co., Tokyo, Japan) was incubated at 4 °C for 3 h with 10000 dpm of [^{3}H]1α,25-$(OH)_2$-D_3 and various concentrations of vitamin D_3 analogues. Bound and free forms of [^{3}H]1α,25-$(OH)_2$-D_3 were separated by addition of dextran–charcoal and centrifugation. The radioactivity of the receptor-bound [^{3}H]1α,25-$(OH)_2$-D_3 was measured with an Aloka LSC-900.

Determination of Serum Calcium Mice (ddy, male, 7 weeks of age) were intravenously given various doses of vitamin D_3 analogues dissolved in EtOH–saline (1 : 99). Their plasma calcium levels at 20 h after a single intravenous injection were determined by using a calcium assay kit (Wako Pure Chemical Industries, Osaka, Japan). The results are expressed as the mean ±S.D. The statistical significance of the differences between the control and the experimental groups was analyzed by the use of Student's *t*-test.

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References and Notes

1) Part IX: N. Kubodera, K. Miyamoto, M. Akiyama, M. Matsumoto, and T. Mori, *Chem. Pharm. Bull.*, **39**, 3221 (1991).
2) This work was presented at the 110th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1990.
3) Ikekawa and Y. Fujimoto, *Yuki Gosei Kagaku Kyokai Shi*, **46**, 455 (1988).
4) N. Kubodera, K. Miyamoto, K. Ochi, and I. Matsunaga, *Chem. Pharm. Bull.*, **34**, 2286 (1986).
5) J. Abe, M. Morikawa, K. Miyamoto, S. Kaiho, M. Fukushima, C. Miyaura, E. Abe, T. Suda, and Y. Nishii, *FEBS Lett.*, **226**, 58 (1987).
6) R. M. Dodson, A. H. Goldkamp, and R. D. Muir, *J. Am. Chem. Soc.*, **82**, 4026 (1960).
7) A. D. Batcho, D. E. Berger, and M. R. Uskokovic, *J. Am. Chem. Soc.*, **103**, 1293 (1981).
8) A. D. Batcho, D. E. Berger, S. G. Davoust, P. M. Wovkulich, and M. R. Uskokovic, *Helv. Chim. Acta*, **64**, 1682 (1981).
9) M. Fetizon and M. Jurion, *J. Chem. Soc., Chem. Commun.*, **1972**, 382.
10) K. Miyamoto, N. Kubodera, E. Murayama, K. Ochi, T. Mori, and I. Matsunaga, *Synth. Commun.*, **16**, 513 (1986).
11) D. H. R. Barton, T. Shioiri, and D. A. Widdowson, *J. Chem. Soc. (C)*, **1971**, 1968.
12) An alternative synthesis of the triacetate of **15** has been achieved by M. C. Pumar *et al.*; M. C. Pumar, A. Mourino, and L. Castedo, *An. Quim. C-Org. Bioquim.*, **84**, 105 (1988).
13) R. B. Jr. Johnston. C. A. Godzik, and Z. A. Cohn, *J. Exp. Med.*, **148**, 115 (1978).
14) S. Ishizaki, K. Bannai, T. Naruchi, and Y. Hashimoto, *Steroids*, **37**, 33 (1981).
15) A. J. Brown, C. R. Ritter, J. L. Finch, J. Morrissey, K. J. Martin, E. Murayama, Y. Nishii, and E. Slatopolsky, *J. Clin. Invest.*, **84**, 728 (1989).