Synthetic Studies of Vitamin D Analogues. X.¹⁾ Synthesis and Biological Activities of 1 α ,25-Dihydroxy-21-norvitamin D₃²⁾

Noboru KUBODERA,* Katsuhito MIYAMOTO, Masahiko MATSUMOTO, Takehiko KAWANISHI, Hiroyuki OHKAWA, and Takashi MORI

Exploratory Research Laboratories, Chugai Pharmaceutical Co., Ltd., 1-135 Komakado, Gotemba, Shizuoka 412, Japan. Received August 8, 1991

1 α ,25-Dihydroxy-21-norvitamin D₃ (3) was synthesized from 1 α -hydroxydehydroepiandrosterone (4). Certain biological properties of 3 were examined in comparison with those of 1 α ,25-dihydroxyvitamin D₃ (1) and 1 α ,25-dihydroxy-21-nor-20-oxavitamin D₃ (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 μ g/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 1 α ,25-dihydroxy-21-norvitamin D₃; 1 α ,25-dihydroxyvitamin D₃; 1 α ,25-dihydroxy-21-nor-20-oxavitamin D₃; 1 α -hydroxydehydroepiandrosterone; differentiation-inducing activity; calcium metabolism

In recent years, considerable attention has been focused on the synthesis of analogues of 1 α ,25-dihydroxyvitamin D₃ (1) [1 α ,25-(OH)₂-D₃], 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 1 α ,25-dihydroxy-21-nor-20-oxavitamin D₃ (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 1 α ,25-(OH)₂-D₃ (1). The present report deals with the synthesis of a new analogue with a vacant 21-position, 1 α ,25-dihydroxy-21-norvitamin D₃ (3), and the comparison of its biological activities with those of 1 and 2.

Synthesis 1 α -Hydroxydehydroepiandrosterone (4), prepared from dehydroepiandrosterone by microbiological 1 α -hydroxylation,⁶⁾ was converted to the diene diacetate (5) in 98% yield by means of the Wittig reaction with methylenetriphenylphosphorane 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 me-

thoxymethyl 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 THF¹¹⁾ 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 D₃ Analogues on the Induction of Superoxide Anion Production of HL-60

1, $\bigcirc \rightarrow \bigcirc$; 2, $\triangle - \triangle$; 3, $\square - \square$.

Fig. 2. Competitive Displacement Curves of [^3H]1 α ,25-(OH) $_2$ -D $_3$ from Chick Embryonic Intestinal 1 α ,25-(OH) $_2$ -D $_3$ Receptor with Vitamin D $_3$ Analogues

1, ○—○; 2, △—△; 3, □—□.

using a high-pressure mercury lamp, followed by thermal isomerization in refluxing ethanol gave 1 α ,25-dihydroxy-21-norvitamin D₃ (**3**) in 18% yield.

TABLE I. Effect of Vitamin D₃ Analogues on Serum Calcium Levels

Compound	Dose ($\mu\text{g/kg}$)	Serum calcium (mg/dl)
Control	Vehicle	8.70 $\pm$ 0.16
1	2	10.36 $\pm$ 0.16 ^{a)}
	10	11.04 $\pm$ 0.12 ^{a)}
2	100	8.80 $\pm$ 0.10
	500	8.96 $\pm$ 0.13
3	10	8.63 $\pm$ 0.13
	100	8.71 $\pm$ 0.15
	500	9.68 $\pm$ 0.15 ^{a)}

Data show the means $\pm$ 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⁾ 1 α ,25-Dihydroxy-21-norvitamin D₃ (3) and 1 α ,25-dihydroxy-21-nor-20-oxavitamin D₃ (2), had ED₅₀ values of 2.9×10^{-8} M and 4.0×10^{-8} M, respectively, compared to 8.3×10^{-9} M for 1 α ,25-(OH)₂-D₃ (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 mice¹⁵⁾ at a dosage of 500 μ g/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 physico-chemical features under investigation should 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. ^1H -Nuclear magnetic resonance (^1H -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 voltage 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 PF₂₅₄ indicator.

1 α ,3 β -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), Ac₂O (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 H₂O, and extracted with Et₂O. The extract was washed with 10% HCl, H₂O, saturated NaHCO₃ and saturated NaCl, and dried over MgSO₄. Removal of the solvent *in vacuo* left a pale yellow gum, which was purified by flash column chromatography with CH₂Cl₂–*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 C₂₄H₃₄O₄: C, 74.57; H, 8.87. Found: C, 74.96; H, 8.91.

Methyl (22*E*)-1 α ,3 β -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 CH₂Cl₂ (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 NaHCO₃. After removal of the solvent *in vacuo*, the residue was taken up with toluene and H₂O. The organic layer was washed with saturated NaCl and dried over MgSO₄. 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, dt, *J* = 15.0, 8.0 Hz). MS *m/z*: 350 (M⁺ – AcOH $\times$ 2), 118 (100%).

Methyl 1 α ,3 β -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 $\times$ 2), 118 (100%). Anal. Calcd for C₂₈H₄₂O₆: C, 70.85; H, 8.92. Found: C, 71.11; H, 8.94.

1 α ,3 β -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 H₂O, made acidic (pH 2–3) by addition of 10% HCl, and extracted with CHCl₃–MeOH (3:1). The extract was washed with saturated NaCl, dried over MgSO₄, 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-Pr₂NEt (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 H₂O (100 ml), and extracted with AcOEt. The extract was washed with saturated NaCl, dried over MgSO₄, 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 LiAlH₄ (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 H₂O. The separated organic layer was washed with saturated NaCl, dried over MgSO₄, and concentrated *in vacuo* to give gummy **8** (2.40 g), which was used without further purification. Preparative TLC with CH₂Cl₂–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).

1 α ,3 β -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 H₂O, and extracted with AcOEt. The extract was washed with 6*N* HCl, H₂O, saturated NaHCO₃ and saturated NaCl, dried over MgSO₄, and concentrated *in vacuo* to give a pale yellow oil (2.08 g), which was used without further purification. A solution of the above-mentioned 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 H₂O and saturated NaCl, and dried over MgSO₄. 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 colorless 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 C₂₇H₄₄BrO₄: C, 63.15; H, 8.83. Found: C, 63.15; H, 8.80.

1 α ,3 β -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 NH₄Cl at 4 °C, the mixture was extracted with AcOEt. The extract was washed with saturated NaCl and dried over MgSO₄. 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. ^1H -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 C₃₂H₅₄O₄S₂: C, 67.80; H, 9.60. Found: C, 67.89; H, 9.68.

1 α ,3 β -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 H₂O (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 CH₂Cl₂ and H₂O. The organic layer was washed with 3% Na₂S₂O₃ and saturated NaCl, and dried over MgSO₄. 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. ^1H -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 C₂₅H₄₀O₃: C, 77.27; H, 10.38. Found: C, 77.17; H, 10.54.

1 α ,3 β ,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 NH₄Cl at room temperature, the mixture was extracted with AcOEt. The extract was washed with saturated NaCl and dried over MgSO₄. Removal of the solvent *in vacuo* left crude **12**, which was purified by flash column chromatography with CH₂Cl₂–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} . $^1\text{H-NMR}$ 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 $\text{C}_{26}\text{H}_{44}\text{O}_3$: C, 77.17; H, 10.96. Found: C, 76.89; H, 11.06.

1 α ,3 β -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} . $^1\text{H-NMR}$ 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 ($\text{M}^+ - \text{AcOH} \times 2$), 118 (100%).

PTAD Adduct of 1 α ,3 β -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% $\text{Na}_2\text{S}_2\text{O}_3$, 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} . $^1\text{H-NMR}$ 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), 7.21–7.59 (5H, br). MS m/z : 486 ($\text{M}^+ - \text{PTAD}$), 348 (100%).

1 α ,3 β ,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. $^1\text{H-NMR}$ 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.

1 α ,25-Dihydroxy-21-norvitamin D₃ (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} . $^1\text{H-NMR}$ δ : 0.44 (3H, s), 1.21 (6H, s), 1.25 (3H, brs), 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 $\mu\text{g}/\text{ml}$ 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 $1 \times 10^5/\text{ml}$ in growth medium and culturing

for 4 d in the presence of various concentrations of vitamin D₃ 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 $19.1 \times 10^3/\text{cm}$.

Binding Affinity with Chick Intestinal Cytosolic Receptor Chick embryonic intestinal $1\alpha,25\text{-(OH)}_2\text{-D}_3$ receptor (Yamasa Shoyu Co., Tokyo, Japan) was incubated at 4 °C for 3 h with 10000 dpm of [^3H] $1\alpha,25\text{-(OH)}_2\text{-D}_3$ and various concentrations of vitamin D₃ analogues. Bound and free forms of [^3H] $1\alpha,25\text{-(OH)}_2\text{-D}_3$ were separated by addition of dextran-charcoal and centrifugation. The radioactivity of the receptor-bound [^3H] $1\alpha,25\text{-(OH)}_2\text{-D}_3$ 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 D₃ 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 $\pm$ 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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