Synthetic Studies of Vitamin D Analogues. XIV.[1] Synthesis and Calcium Regulating Activity of Vitamin D_3 Analogues Bearing a Hydroxyalkoxy Group at the 2β-Position[2]

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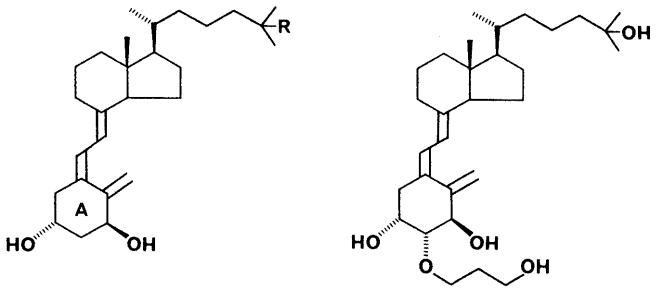
Four vitamin D_3 analogues (7a, 7b, 7c and 7d) bearing a hydroxyalkoxy group at the 2β-position were synthesized from the α-epoxide (5). The C-3 analogue (7b) showed the highest potency for elevating plasma calcium levels in rats. Furthermore, the 25-hydroxylated C-3 analogue (ED-71) (3), prepared from the 25-hydroxylated α-epoxide (9), significantly increased plasma calcium to levels much higher than those in rats administered 1α,25-$(OH)_2$-D_3 (1).

Keywords vitamin D_3 analogue; 1α,25-dihydroxyvitamin D_3; ED-71; plasma calcium level; 3-hydroxypropoxy substituent; 2β-position

Since 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] (**1**) has been shown to induce differentiation in myeloid leukemia cells in addition to its regulatory effect on calcium and phosphorus metabolism, efforts have been concentrated on the synthesis of vitamin D_3 analogues to obtain more potent analogues or to separate these vitamin D_3 activities.[3] Through modifications of the side chain of **1**, we have already obtained analogues exhibiting potent cellular proliferation and differentiation activities with low calcemic action.[4–7]



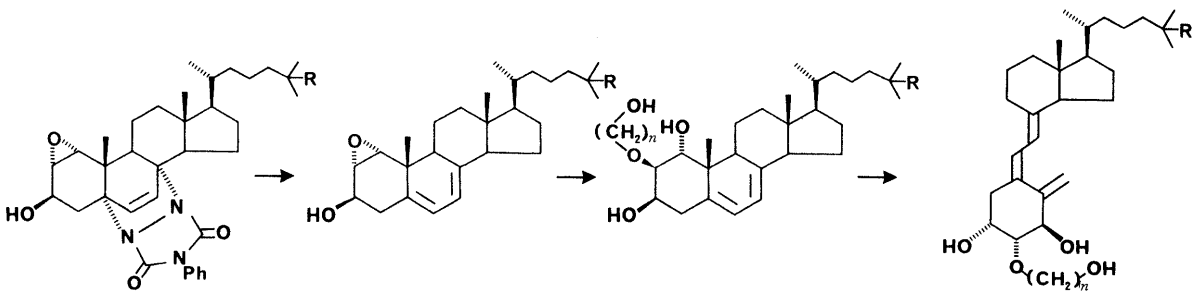
1 : R=OH; 1α,25-$(OH)_2$-D_3
2 : R=H; 1α-OH-D_3

3 : ED-71

Chart 1

On the other hand, the synthesis of certain analogues modified in the A-ring of 1α,25-$(OH)_2$-D_3 (**1**), including fluorinated compounds, has been described in the literature,[8] but biologically promising properties have not been reported to our knowledge. In our work on introducing a substituent into the A-ring of **1**, we have obtained a more calcemic analogue than **1**. In this paper, we will describe the synthesis of a new analogue bearing a 3-hydroxypropoxy substituent at the 2β-position (ED-71) (**3**) and related compounds, and their calcium regulating effects.

First, we focused our attention on the α-epoxide (**5**), readily prepared as described before[1] from the 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) adduct (**4**)[9] which is a key intermediate in an industrial-scale synthesis of 1α-hydroxyvitamin D_3 (1α-OH-D_3) (**2**). Treatment of **5** with various glycols ($n=2$—5) in the presence of potassium *tert*-butoxide (KO^{t}Bu) resulted in stereo and regioselective introduction of hydroxyalkoxy groups into the 2β-position to give pro-vitamin D_3 analogues (**6a**, **6b**, **6c** and **6d**). The stereo and regioselectivities in the nucleophilic opening reaction of the α-epoxy ring of **5** were in accordance with our previous findings.[10] The four pro-vitamin D_3 analogues (**6a**, **6b**, **6c** and **6d**) were then converted to vitamin D_3 analogues (**7a**, **7b**, **7c** and **7d**) by irradiation at 0 °C using a high pressure mercury lamp (400 W, Vycor filter), followed



4 : R=H
8 : R=OH

5 : R=H
9 : R=OH

6a : n=2, R=H
6b : n=3, R=H
6c : n=4, R=H
6d : n=5, R=H
6e : n=3, R=OH

7a : n=2, R=H; C-2
7b : n=3, R=H; C-3
7c : n=4, R=H; C-4
7d : n=5, R=H; C-5
3 : n=3, R=OH; ED-71

Chart 2

by thermal isomerization in boiling tetrahydrofuran (THF). The four vitamin D_3 analogues synthesized and 1α-OH-D_3 (**2**) were examined for biological effects on calcium mobilization in rats. Male Sprague-Dawley (SD) rats were fed a vitamin D-deficient diet containing 0.003% calcium (low Ca/D-deficient diet) for 3 weeks and then vitamin D_3 analogues were orally given every day for 5 d (6.25 μg/kg × 5) under low Ca/D-deficient diet conditions. Plasma calcium levels were measured at 24 h after the last administration. As shown in Fig. 1, all analogues elevated plasma calcium levels and the C-3 analogue (**7b**) showed the strongest activity among them, although 1α-OH-D_3 (**2**) induced only a slight increase of plasma calcium levels under these conditions. The calcium-elevating potencies of these analogues were in the following order: C-3 (**7b**) C-2 (**7a**) C-4 (**7c**) 1α-OH-D_3 (**2**) C-5 (**7d**). These results indicate that the hydroxyalkoxy substituents at the 2β-position enhanced the calcium-regulatory activity of vitamin D.

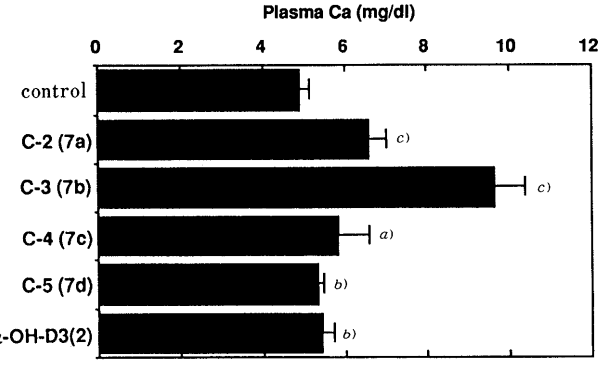


Fig. 1. Comparison of Plasma Calcium Levels in Rats Fed with a Low Ca/D-Deficient Diet after Administration of Vitamin D_3 Analogues: C-2 (**7a**), C-3 (**7b**), C-4 (**7c**), C-5 (**7d**) and 1α-OH-D_3 (**2**) (6.25 μg/kg × 5)

a) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

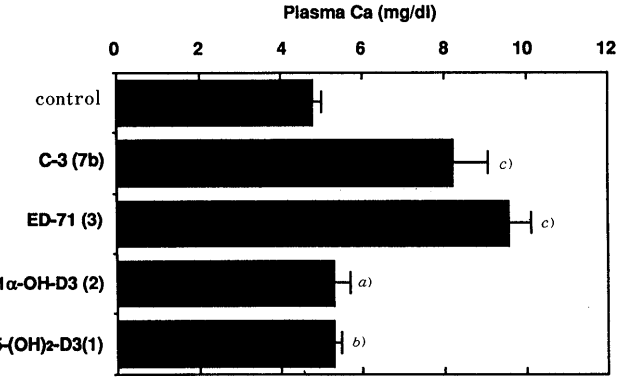


Fig. 2. Comparison of Plasma Calcium Levels in Rats Fed with a Low Ca/D-deficient Diet after Administration of Vitamin D_3 Analogues: C-2 (**7b**), ED-71 (**3**), 1α-OH-D_3 (**2**) and 1α,25-$(OH)_2$-D_3 (**1**) (6.25 μg/kg × 5)

a) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

Next, we turned our attention to the C-3 analogue possessing the 25-hydroxy substituent. The 25-hydroxylated PTAD adduct (**8**)[11] was converted to the 25-hydroxylated α-epoxide (**9**) by retro-cycloaddition reaction in 1,3-dimethyl-2-imidazolidinone (DMI) at 140 °C in 62% yield. Thus, **9** was cleaved by propane-1,3-diol in the presence of KO^{t}Bu to introduce the 3-hydroxypropoxy group at the 2β-position, giving rise to pro-vitamin D_3 (**6e**) in 36% yield. Finally, **6e** was converted to 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) (**3**) by irradiation and thermal isomerization in 23% yield.

Figure 2 compares the plasma calcium levels in rats on a low Ca/D-deficient diet after administration of ED-71 (**3**), C-3 analogue (**7b**), 1α-OH-D_3 (**2**) and 1α,25-$(OH)_2$-D_3 (**1**) (6.25 μg/kg × 5). ED-71 (**3**) significantly increased plasma calcium levels, which reached an almost normal range. It has been confirmed that the half-life of ED-71 (**3**) in the blood stream in rats is much longer than that of 1α,25-$(OH)_2$-D_3 (**1**), due to stronger affinity for vitamin D binding protein in plasma (twice the affinity of 1α,25-$(OH)_2$-D_3).[12] Therefore, the higher potency of ED-71 (**3**) in elevating plasma calcium levels may be explained by its long duration. Further biological properties of ED-71 (**3**), including the effect on bone formation in osteoporotic rats, are under investigation and the structure–activity relationships are being evaluated through synthesis of other 25-hydroxylated analogues having various substituents at the 2β-position; details 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 (NMR) spectra were recorded on a JEOL FX-200 spectrometer with tetramethylsilane as an internal standard. Abbreviations used are s (singlet), d (doublet) and m (multiplet). Ultraviolet (UV) spectra were obtained with a Shimadzu UV-240 in EtOH. Mass (MS) spectra were carried out on a Shimadzu GCMS-QP 1000. High-resolution mass spectra (HRMS) were recorded with VG Auto Spec Q. All reactions were carried out under an atmosphere of dry argon or nitrogen. Preparative thin layer chromatography (TLC) was performed on 20 × 20 cm plates coated with 0.5 mm thickness of Merck Kieselgel 60 containing PF_{254} indicator.

1α,2α-Epoxy-3β,25-dihydroxycholesta-5,7-diene (9) A mixture of **8** (49 mg, 0.08 mmol) in DMI (5 ml) was stirred at 140 °C for 5 h, then poured into H_2O and extracted with AcOEt. The extract was washed with saturated NaCl and dried over $MgSO_4$. Removal of the solvent *in vacuo* left a solid, which was purified by preparative TLC developed twice with AcOEt–*n*-hexane (11 : 9) to give **9** (21 mg, 62%) as a colorless powder, mp

TABLE I. Spectral Data for Pro-vitamin D_3 Analogues (**6**)

Compd.	mp (°C)	^{1}H-NMR ($CDCl_3$) δ ppm	MS *m/z*	UV λ_{max}	Formula	Analysis (%) Calcd C	Calcd H	Found C	Found H
6a	119—125	0.62 (3H, s), 0.87 (6H, d, J=6.6 Hz), 0.94 (3H, d, J=6.4 Hz), 5.32—5.43 (1H, m), 5.65—5.74 (1H, m)	460 (M^+), 442, 424, 38 (100%)	293.5, 281.5, 271, 262 (sh)	$C_{29}H_{48}O_4 \cdot 3/4H_2O$	73.46	10.52	73.31	10.39
6b	114—116	0.62 (3H, s), 0.87 (6H, d, J=6.3 Hz), 0.94 (3H, d, J=6.8 Hz), 5.32—5.39 (1H, m), 5.63—5.73 (1H, m)	474 (M^+), 456, 438, 380 (100%)	293, 282, 271, 262 (sh)	$C_{30}H_{50}O_4 \cdot H_2O$	73.13	10.64	73.29	10.30
6c	99—101	0.63 (3H, s), 0.87 (6H, d, J=6.6 Hz), 0.94 (3H, d, J=6.3 Hz), 5.30—5.40 (1H, m), 5.64—5.73 (1H, m)	488 (M^+), 470, 452, 380, 326 (100%)	293, 281.5, 271, 262 (sh)	$C_{31}H_{52}O_4 \cdot 1/2H_2O$	74.81	10.73	74.63	10.50
6d	141—143	0.62 (3H, s), 0.87 (6H, d, J=6.6 Hz), 0.94 (3H, d, J=6.1 Hz), 5.31—5.39 (1H, m), 5.63—5.72 (1H, m)	502 (M^+), 484, 466, 380 (100%)	293, 281, 271, 262 (sh)	$C_{32}H_{54}O_4$	76.44	10.83	76.46	10.65

TABLE II. Spectral Data for Vitamin D_3 Analogues (7)

Compd.	^{1}H-NMR ($CDCl_3$) δ ppm	MS *m/z*	UV λ_{max}	Formula	HRMS	
					Calcd	Found
7a	0.55 (3H, s), 0.86 (6H, d, J=6.6 Hz), 0.92 (3H, d, J=5.8 Hz), 3.62—3.92 (5H, br), 4.18—4.39 (2H, m), 5.09 (1H, s), 5.49 (1H, s), 6.04 (1H, d, J=10.5 Hz), 6.37 (1H, d, J=10.5 Hz)	460 (M^+), 442, 398, 380, 150 (100%)	262.5	$C_{29}H_{48}O_4$	460.3553	460.3560
7b	0.54 (3H, s), 0.86 (6H, d, J=6.6 Hz), 0.91 (3H, d, J=6.1 Hz), 3.58—4.00 (5H, br), 4.10—4.36 (2H, m), 5.08 (1H, s), 5.49 (1H, s), 6.04 (1H, d, J=10.5 Hz), 6.36 (1H, d, J=10.5 Hz)	474 (M^+), 456, 398, 380, 150 (100%)	263	$C_{30}H_{50}O_4$	474.3709	474.3693
7c	0.54 (3H, s), 0.86 (6H, d, J=6.6 Hz), 0.91 (3H, d, J=6.1 Hz), 3.36—3.88 (5H, br), 4.12—4.36 (2H, m), 5.08 (1H, s), 5.50 (1H, m), 6.04 (1H, d, J=11.4 Hz), 6.36 (1H, d, J=11.4 Hz)	488 (M^+), 470, 452, 398, 380, 150 (100%)	263.5	$C_{31}H_{52}O_4$	488.3866	488.3866
7d	0.55 (3H, s), 0.86 (6H, d, J=6.6 Hz), 0.91 (3H, d, J=5.8 Hz), 3.34—3.82 (5H, br), 4.10—4.36 (2H, m), 5.08 (1H, s), 5.50 (1H, s), 6.04 (1H, d, J=10.8 Hz), 6.36 (1H, d, J=10.8 Hz)	502 (M^+), 484, 466, 380 (100%)	263.5	$C_{32}H_{54}O_4$	502.4022	502.4010

173—175 °C. IR (Nujol): 3330, 3250, 3070, 1310, 1270, 1080, 1070, 950, 850 cm^{-1}. NMR δ: 0.64 (3H, s), 0.97 (3H, d, J=6.3 Hz), 1.05 (3H, s), 1.22 (6H, s), 3.04 (1H, d, J=3.4 Hz), 3.33 (1H, d, J=3.4 Hz), 3.90 (1H, dd, J=10.7, 6.1 Hz), 5.36—5.42 (1H, m), 5.70—5.72 (1H, m). UV λ_{max} nm: 290, 278, 268. MS *m/z*: 414 (M^+), 396, 59 (100%). *Anal.* Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.19; H, 10.02.

1α,3β,25-Trihydroxy-2β-(3-hydroxypropoxy)cholesta-5,7-diene (6e) A mixture of **9** (50 mg, 0.12 mmol), propane-1,3-diol (0.5 ml) and KO^{t}Bu (45 mg, 0.4 mmol) was stirred at 110 °C for 14 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 an oily residue, which was purified by preparative TLC developed with CH_2Cl_2–EtOH (20 : 3) to give **6e** (21 mg, 36%) as a colorless powder, mp 118—120 °C. IR (Nujol): 3350, 1130, 1090, 1050, 1030 cm^{-1}. NMR δ: 0.62 (3H, s), 0.96 (3H, d, J=6.3 Hz), 1.06 (3H, s), 1.22 (6H, s), 5.32—5.42 (1H, m), 5.64—5.73 (1H, m). UV λ_{max} nm: 293, 281.5, 271, 262 (shoulder). MS *m/z*: 490 (M^+), 472, 454, 396, 131 (100%). *Anal.* Calcd for $C_{30}H_{50}O_5$ 3/4H_2O: C, 71.46; H, 10.30. Found: C, 71.44; H, 9.87. Other pro-vitamin D_3 analogues (**6a**, **6b**, **6c** and **6d**) were similarly obtained in 33%, 29%, 55% and 40% yields, respectively. Spectroscopic data are given in Table I.

1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (3) A solution of **6e** (43.3 mg, 0.09 mmol) in THF (310 ml) was irradiated using a 400 W high pressure mercury lamp with a Vycor filter at 0 °C for 2.25 min. The mixture was then refluxed for 2 h and concentrated *in vacuo* to leave a pale yellow oil, which was purified by preparative TLC developed twice with CH_2Cl_2–EtOH (20 : 3) to give **3** (10.0 mg, 23%) as a colorless foam. IR (Nujol): 3360, 1100, 1060, 910 cm^{-1}. NMR δ: 0.55 (3H, s), 0.91 (3H, d, J=6.1 Hz), 1.21 (6H, s), 3.60—4.02 (5H, br), 4.12—4.36 (2H, m), 5.08 (1H, s), 5.49 (1H, s), 6.04 (1H, d, J=10.5 Hz), 6.36 (1H, d, J=10.5 Hz). UV λ_{max} nm: 263. MS *m/z*: 490 (M^+), 472, 454, 396, 59 (100%). HRMS Calcd for $C_{30}H_{50}O_5$: 490.3658. Found: 490.3678. Other vitamin D_3 analogues (**7a**, **7b**, **7c** and **7d**) were similarly obtained in 23%, 14%, 11% and 18% yields, respectively. Spectroscopic data are given in Table II.

Plasma Calcium Level Rats (SD, male, 6 weeks of age), fed low Ca/D-deficient diet for 3 weeks, were orally given various vitamin D_3 analogues (6.25 μg/kg) dissolved in medium chain triglycerides (MCT) every day for 5 d under low Ca/D-deficient diet conditions. Their plasma calcium levels at 24 h after the last administration 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 difference between the control (MCT) and the experimental groups was analyzed by the use of Student's *t*-test.

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