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SYNTHESIS AND BIOLOGICAL CHARACTER OF 1β-HYDROXYLATED VITAMIN D_3 ANALOGUES[1]

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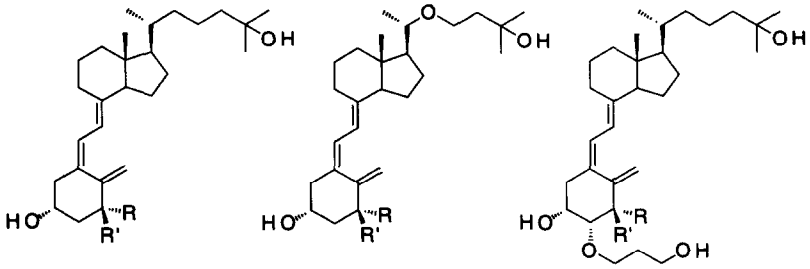
Abstract: The synthesis of 1β,25-dihydroxy-22-oxavitamin D_3 and 1β,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 and the preliminary *in vitro* biological evaluation are described.

Since 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] (1) has been shown to induce differentiation in myeloid leukemia cells in addition to its regulatory effect on calcium and phosphorous metabolism, efforts have been concentrated on the synthesis of vitamin D_3 analogues to separate these vitamin D_3 activities or to obtain more potent analogues[2]. We have already reported that two characteristic analogues of 1α,25$(OH)_2D_3$ (1), namely 1α,25-dihydroxy-22-oxavitamin D_3 (OCT) (2) having potent cellular proliferation and differentiation activities with low calcemic liability[3] and 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71) (3) showing highly calcemic character and strong affinity to bone[4]. OCT is being clinically investigated as a candidate for treatment of secondary hyperparathyroidism[5] and psoriasis[6]. ED-71 is anticipated to be a useful therapeutic agent for osteoporosis[7].



(1); R = H, R' = OH
(4); R = OH, R' = H

(2); R = H, R' = OH
(5); R = OH, R' = H

(3); R = H, R' = OH
(6); R = OH, R' = H

Chart 1

On the other hand, Norman and co-workers recently reported that 1β,25-dihydroxyvitamin D_3 [1β,25(OH)$_2$D$_3$] (4), the diastereomer of 1α,25(OH)$_2$D$_3$ (1) at the C-1 position, is devoid of activity as an agonist for transcaltachia and is a potent stereospecific antagonist of 1α,25(OH)$_2$D$_3$(1) stimulation of the nongenomic transcaltachia response[8]. These finding stimulated our interest in the biological characters of the 1β-hydroxylated diastereomers of OCT (2) and ED-71 (3). Accordingly, in this paper we wish to describe the synthesis of 1β,25-dihydroxy-22-oxavitamin D_3 (1β-OCT) (5) and 1β,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (1β-ED-71) (6). Their affinity to bovine thymus vitamin D receptor (VDR) and rat vitamin D binding protein (DBP) and their antiproliferative activity for human promyeloid leukemia cells (HL-60) are also reported as a preliminary *in vitro* biological evaluation.

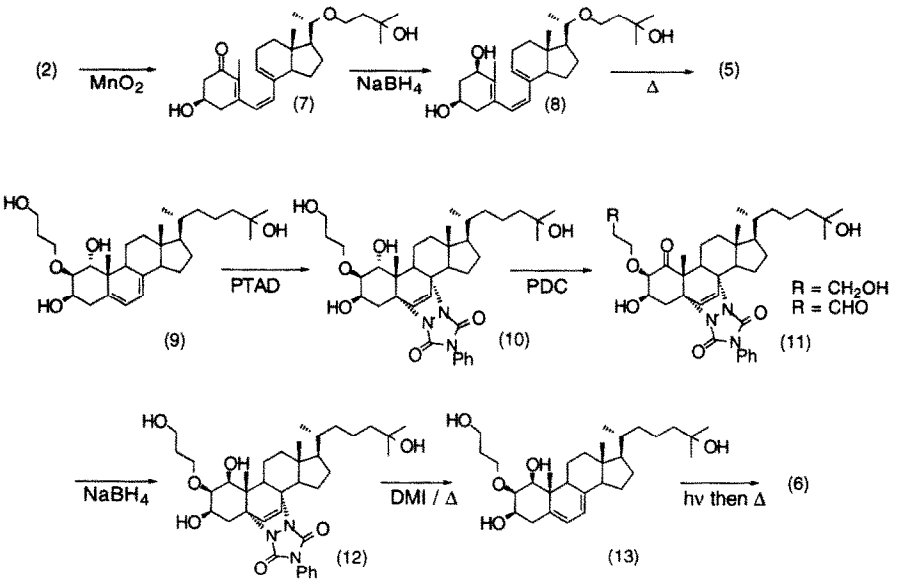


Chart 2

First, we undertook the synthesis of 1β-OCT (5). The allylic alcohol moiety of OCT (2) was oxidized by MnO_2[9] giving the 1-keto compound (7) with concomitant isomerization of the triene system in 54% yield. The stereoselective hydride reduction of the carbonyl group in 7 was achieved by $NaBH_4$ at the less congested α-face to afford the pre-1β-OCT (8)[9], which was then thermally isomerized to 1β-OCT (5)[10] in 34% yield from 7.

Because the MnO_2 oxidation of the hindered alcohol moiety of ED-71 (3) resulted in a complicated reaction mixture, we adopted a different sequence to obtain 1β-ED-71 (6). The 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) adduct (10), prepared from the known 5,7-diene (9) and PTAD, was oxidized by pyridinium dichromate (PDC)[11] to provide the mixture of keto-alcohol and keto-aldehyde (11) in 56% yield. The mixture was then stereoselectively reduced to the alcohol (12) by $NaBH_4$ in 71% yield. Although the reaction condition has not been optimized, PDC-oxidation and $NaBH_4$-reduction procedure is assumed to be applicable as a general method for the inversion of the sterically hindered 1α-hydroxy group in steroidal compounds. The 5,7-diene system in 13 was regenerated in 68% yield by simply heating 12 in 1,3-dimethyl-2-imidazolidinone (DMI) at 140°C[12]. Finally, 13 was converted to 1β-ED-71 (6)[13] by irradiation in EtOH at 0°C using a high pressure mercury lamp through a Vycor filter followed by thermal isomerization under reflux in EtOH in 8% yield.

Compound		Binding affinity for DBP and VDR VDR	DBP	Antiproliferative activity on HL-60 cells
1α,25(OH)$_2$D$_3$	(1)	1	1	1
1β,25(OH)$_2$D$_3$	(4)	0.2	2.9	0.2
OCT	(2)	0.6	0.002	3.1
1β-OCT	(5)	0.007	0.007	0.003
ED-71	(3)	0.7	4.1	
1β-ED-71	(6)	0.003	6.7	

Table 1

Table 1 shows the relative binding potencies of each derivative for DBP[14] and VDR[15] measured by competitive displacement with [^{3}H]-1α,25(OH)$_2$D$_3$. The 1β-hydroxylated diastereomers (4, 5 and 6) exhibit higher binding potencies for DBP than the corresponding 1α-hydroxylated derivatives (1, 2 and 3), though the affinities of 4, 5 and 6 for VDR are weakened when compared to 1, 2 and 3. Relative antiproliferative activities[16] of each derivative for HL-60 are also shown in Table 1 and well correlate with their affinity for VDR. Further *in vivo* biological properties including intestinal calcium transport and bone mobilization under investigation should help explanation of the structure-function relationship of 1β-hydroxylated derivatives and will be reported elsewhere.

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10) 5: NMR ($CDCl_3$) δ: 0.54 (3H, s), 1.19 (3H, d, *J*=5.9Hz), 1.24 (6H, s), 3.21-3.34 (1H, m), 3.43-3.55 (1H, m), 3.79-3.89 (1H, m), 4.05-4.15 (1H, m), 4.31-4.40 (1H, m), 5.00 (1H, s), 5.29 (1H, s), 6.06 (1H, d, *J*=10.9Hz), 6.44 (1H, d, *J*=10.9Hz). MS (*m/z*): 418 (M^+), 69 (100%). HRMS Calcd for $C_{26}H_{42}O_4$ 418.3083. Found 418.3112. UV λ_{max}nm: 263, λ_{min}nm: 221.
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13) 6: NMR ($CDCl_3$) δ: 0.54 (3H, s), 0.94 (3H, d, *J*=6.5Hz), 1.22 (6H, s), 3.58-3.64 (1H, m), 3.84 (2H, t, *J*=5.4Hz), 3.91 (2H, t, *J*=5.4Hz), 4.01-4.10 (1H, m), 4.28-4.33 (1H, brs), 5.08 (1H, s), 5.38 (1H, s), 6.03 (1H, d, *J*=12.0Hz), 6.43 (1H, d, *J*=12.0Hz). MS (*m/z*): 490 (M^+), 60 (100%). HRMS Calcd for $C_{30}H_{50}O_5$ 490.3658. Found 490.3706. UV λ_{max}nm: 264, λ_{min}nm: 227.
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