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SYNTHESIS AND DIFFERENTIATION-INDUCING ACTIVITY OF 1α,24-DIHYDROXY-22-OXA-VITAMIN D_3 ANALOGUES[1]

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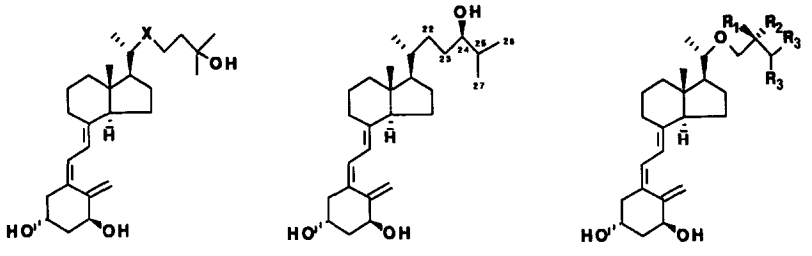
Abstract: The synthesis of four vitamin D_3 analogues, 1α,24(S)- and 1α,24(R)-dihydroxy-22-oxa-vitamin D_3, (5) and (6), and their 26,27-dimethylated homologues, (7) and (8), and the preliminary evaluation of their differentiation-inducing activity *in vitro* are described.

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 of human myeloid leukemia cells (HL-60) from the regulatory effect on calcium and phosphorous metabolism.[2] During our study of side chain modification of 1, we have initially obtained 1α,25-dihydroxy-22-oxa-vitamin D_3 (2) (OCT),[3,4] which shows high activity in inhibition of cellular proliferation and stimulation of cell differentiation with remarkably low calcemic activity.[5] OCT is being clinically investigated for suppression of secondary hyperparathyroidism.[6]

On the other hand, the recent discovery that 1α,24(R)-dihydroxy-vitamin D_3 (3)[7] and MC-903 (4),[8] both hydroxylated at the C-24 position, exhibit essentially equal cell differentiation activity with reduced hypercalcemic action in comparison to 1α,25-$(OH)_2$-D_3 (1), stimulated our interest in 22-oxygenated analogues possessing 24-hydroxy group instead of 25-hydroxy moiety. Accordingly, in this paper we wish to describe the synthesis of 1α,24(S)- and 1α,24(R)-dihydroxy-22-oxa-vitamin D_3, (5) and (6), and their 26,27-dimethylated homologues, (7) and (8), in which enhanced differentiation-inducing effects may be expected as observed previously in the comparison between OCT (2) and 26,27-dimethylated OCT.[4]



(1) X = CH_2; 1α,25-$(OH)_2$-D_3 (3) 1α,24(R)-$(OH)_2$-D_3 (5) R_1 = OH, R_2 = H, R_3 = Me
(2) X = O; OCT (4) 22E-ene-26,27-cyclo; MC-903 (6) R_1 = H, R_2 = OH, R_3 = Me
(7) R_1 = OH, R_2 = H, R_3 = Et
(8) R_1 = H, R_2 = OH, R_3 = Et

Chart 1

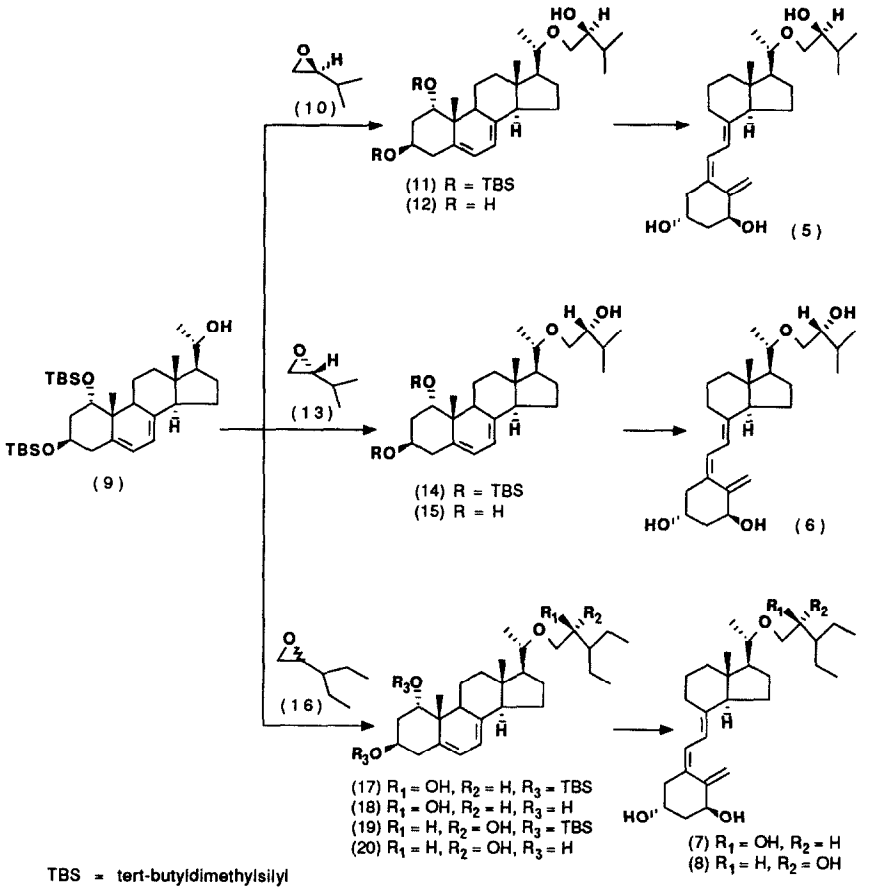


Chart 2

First, the 20(S)-alcohol (9)[3] was alkylated with the (S)-epoxide (10), prepared from D-valine by 3-step sequence[9], in the presence of dibenzo-18-crown-6 and ^{t}BuOK at 100°C to give the 24(S)-alcohol (11) in 34% yield accompanied by the recovery of 9 in 23% yield. Desilylation of 11 with $^{n}Bu_4NF$ provided the triol (12), in 76% yield, which was then converted to 1α,24(S)-dihydroxy-22-oxa-vitamin D_3 (5)[10] by irradiation in ethanol at 0°C using a high pressure mercury lamp through Vycor filter, followed by thermal isomerization under reflux in ethanol in 13% yield.

Next, (R)-epoxide (13), prepared from L-valine, was reacted with the 20(S)-alcohol (9) to afford the 24(R)-alcohol (14) (48% yield based upon the recovery of 9) which was then desilylated (57%), irradiated and thermally isomerized to 1α,24(R)-dihydroxy-22-oxa-vitamin D_3 (6)[11] (14%).

Finally, the reaction between the 20(S)-alcohol (9) and the racemic epoxide (16)[12] provided a mixture of two products, which were separated by silica gel preparative TLC to give the more polar isomer (17) and the less polar isomer (19) in 28% and 20% yields, respectively. By analogy with the behavior on TLC and ^{1}H-NMR spectra of above-mentioned 11 and 14, the more polar isomer (17) was tentatively assigned to the 24(S)-alcohol and the less polar isomer (19) to the 24(R)-alcohol. Both 17 and 19 were transformed to 1α,24(S)-dihydroxy-26,27-dimethyl-22-oxa-vitamin D_3 (7)[13] and 1α,24(R)-dihydroxy-26,27-dimethyl-22-oxa-vitamin D_3 (8)[14], by desilylation, irradiation and thermal isomerization in comparable yields with 5 and 6.

Fig. 1 shows the preliminary results of the differentiation-inducing activity of HL-60 into macrophages *in vitro* estimated by superoxide anion production.[15] Among the four analogues synthesized, 24(S)-isomers, 5 and 7, showed the comparable activities with OCT (2), whereas 24(R)-isomers, 6 and 8, were less active than 2. In the case of MC-903 (4) and its 24(S)-epimer, the similar relationships between the configuration at the C-24 center and the differentiation-inducing activity were also reported.

As reported previously, 26,27-dimethyl OCT was approximately threefold as potent as OCT (2) at the ED_{50} of the differentiation-inducing activity,[4] while clear influence caused by C-26/C-27 homologation was not observed in 1α,24-dihydroxy-22-oxa-vitamin D_3 analogues. Further pharmacological studies, including the binding affinity to the chick embryonic intestinal 1α,25-$(OH)_2$-D_3 receptor, are now under investigation and will be reported elsewhere.

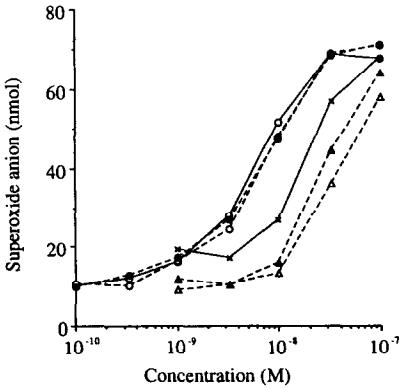


Fig. 1 Comparative Effect of Vitamin D_3 Analogues on the Induction of Superoxide Anion Production of HL-60.

—○—, OCT (2), —×—; 1α,25-$(OH)_2$-D_3 (1), --○--, 5, --◇--, 6, --●--, 7, --▲--, 8.

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References and notes

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10) 5; ^{1}H-NMR δ: 0.53 (3H, s), 0.90 (3H, d, *J*=6.8Hz), 0.97 (3H, d, *J*=6.6Hz), 1.17 (3H, d, *J*=6.2Hz), 3.22-3.36 (2H, m), 3.36-3.49 (2H, m), 4.16-4.28 (1H, br), 4.37-4.48 (1H, br), 5.00 (1H, s), 5.34 (1H, s), 6.02 (1H, d, *J*=11.4Hz), 6.37 (1H, d, *J*=11.4Hz). MS *m/z*: 418 (M^+), 87 (100%). HR-MS Calcd for $C_{26}H_{42}O_4$: 418.3083. Foud: 418.3078. UV λ_{max} nm: 263, λ_{min} nm: 227. $[\alpha]_D$ 56.00 (c=0.1, EtOH).

11) 6; ^{1}H-NMR δ: 0.53 (3H, s), 0.90 (3H, d, *J*=6.8Hz), 0.97 (3H, d, *J*=6.8Hz), 1.18 (3H, d, *J*= 6.0Hz), 3.08 (1H, t, *J*=8.8Hz), 3.20-3.32 (1H, m), 3.36-3.48 (1H, m), 3.67 (1H, d,d, *J*=3.0, 9.1Hz), 4.17-4.28 (1H, br), 4.37-4.48 (1H, br), 4.99 (1H, s), 5.33 (1H, s), 6.02 (1H, d, *J*=11.4Hz), 6.37 (1H, d, *J*=11.4Hz). MS *m/z*: 418 (M^+), 87 (100%). HR-MS Calcd for $C_{26}H_{42}O_4$: 418.3083. Foud: 418.3080. UV λ_{max} nm: 263, λ_{min} nm: 227. $[\alpha]_D$ 46.00 (c=0.1, EtOH).

12) The racemic epoxide (16) was prepared as follows: 2-ethylbutylaldehyde (20.84g), trimethylsulfonium methyl sulfate (45.05g) and 50% aq NaOH (105ml) in CH_2Cl_2 (210ml) was stirred at 40°C for 6 days. The mixture was then diluted with CH_2Cl_2 and H_2O. The separated organic layer was washed with saturated NaCl, dried over $MgSO_4$ and evaporated below 25°C. The crude product was purified by distillation to give 16, 13.36g (56%); bp_{760} 102-117°C. cf. Mosset, P.; Gree, R. *Syn. Commun.* **1985**, *15*, 749.

13) 7; ^{1}H-NMR δ: 0.53 (3H, s), 0.89 (6H, t, *J*=6.8Hz), 1.17 (3H, d, *J*= 6.2Hz), 3.21-3.36 (2H, m), 3.46 (1H, t, *J*=8.6Hz), 3.60-3.72 (1H, br), 4.15-4.27 (1H, br), 4.36-4.46 (1H, br), 4.99 (1H, s), 5.33 (1H, s), 6.03 (1H, d, *J*=11.6Hz), 6.37 (1H, d, *J*=11.6Hz). MS *m/z*: 446 (M^+), 97 (100%). HR-MS Calcd for $C_{28}H_{46}O_4$: 446.3396. Found: 446.3394. UV λ_{max} nm: 263, λ_{min} nm: 227. $[\alpha]_D$ 38.99 (c=0.159, EtOH).

14) 8; ^{1}H-NMR δ: 0.54 (3H, s), 0.89 (6H, t, *J*=6.8Hz), 1.19 (3H, d, *J*= 6.2Hz), 3.16 (1H, t, *J*=9.6Hz), 3.30 (1H, brt, *J*=6.4Hz), 3.61-3.76 (2H,m), 4.16-4.28 (1H, br), 4.37-4.48 (1H, br), 4.99 (1H, s), 5.33 (1H, s), 6.02 (1H, d, *J*=11.4Hz), 6.37 (1H, d, *J*=11.4Hz). MS *m/z*: 446 (M^+), 97 (100%). HR-MS Calcd for $C_{28}H_{46}O_4$: 446.3396. Found: 446.3394. UV λ_{max} nm: 263, λ_{min} nm: 227. $[\alpha]_D$ 43.47 (c=0.115, EtOH).

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