Communications to the Editor

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26,27-DIETHYL-1α,25-DIHYDROXYVITAMIN D_3 AND 24,24-DIFLUORO-24-HOMO-1α,25-DIHYDROXYVITAMIN D_3: HIGHLY POTENT INDUCER FOR DIFFERENTIATION OF HUMAN LEUKEMIA CELLS HL-60

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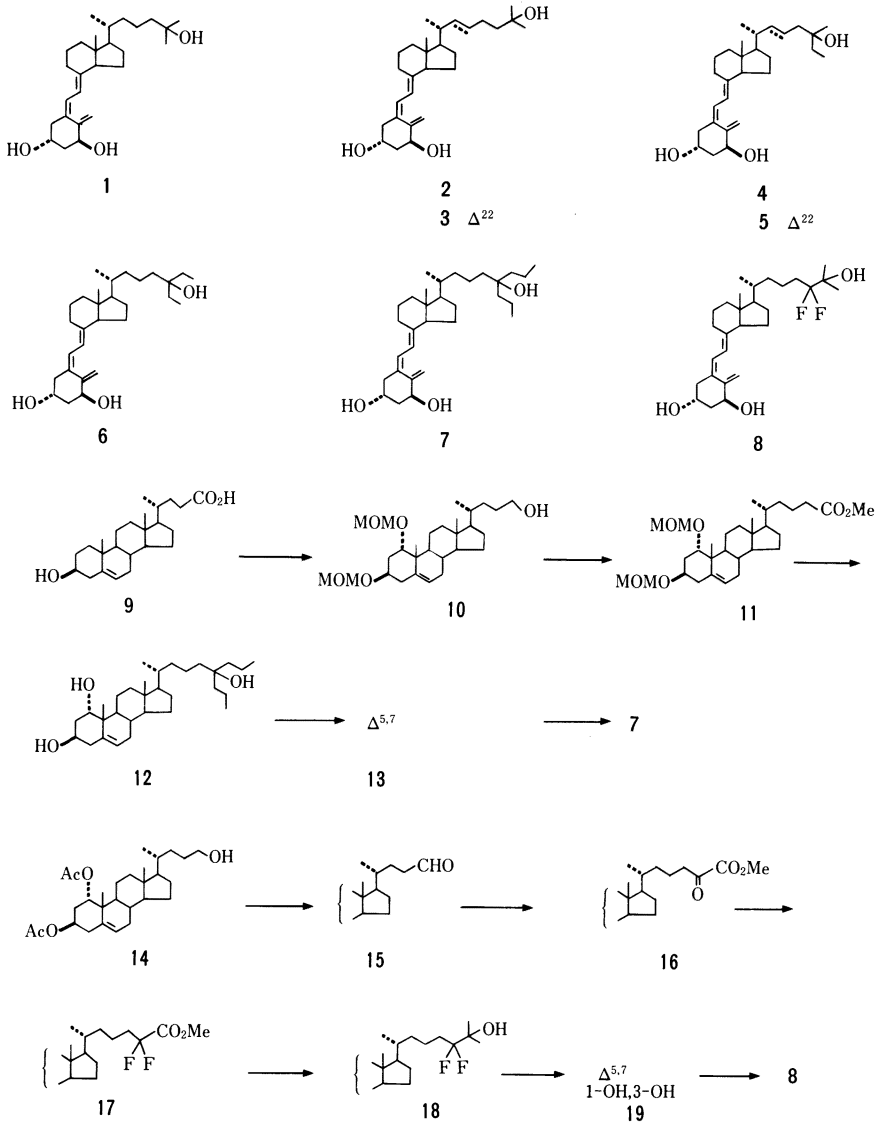
26,27-Diethyl-1α,25-dihydroxyvitamin D_3 (7) and 24,24-difluoro-24-homo-1α,25-dihydroxyvitamin D_3 (8) were synthesized. They had almost no vitamin D activity but were more active than 1α,25-dihydroxyvitamin D_3 (1) in tests for induction of cell differentiation.

KEYWORDS——vitamin D_3 analog; 1α,25-dihydroxyvitamin D_3; 26,27-diethyl-1α,25-dihydroxyvitamin D_3; 24,24-difluoro-24-homo-1α,25-dihydroxyvitamin D_3; cell differentiation; HL-60 cell

Since the discovery of 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) (1), the hormonal form of vitamin D_3, many analogs have been synthesized in order to obtain higher activity and to separate various biological activities.[2)] The target organs for 1,25-$(OH)_2D_3$ (1) have been understood as intestine, bone and kidney. Additional target organs, though previously unrecognized, have been recently identified including brain, stomach, skin, pituitary, parathyroid, pancreas, etc.[3)] A specific receptor for 1,25-$(OH)_2D_3$ (1) was also demonstrated in a number of tumor cells. 1,25-$(OH)_2D_3$ (1) also strongly induces cell differentiation in myeloid leukemia cells.[4)] By testing many analogs of 1,25-$(OH)_2D_3$ (1) for activities, parallel activities were observed usually for the binding affinity to the receptor and for the induction of differentiation.[4)] It seems likely that 1,25-$(OH)_2D_3$ (1) would induce severe hypercalcemia at the concentration required to induce myeloid differentiation *in vivo*. Therefore, separation of these activities is now greatly desired in the search for useful therapeutic agents.

Several compounds have a strong effect on cell differentiation, but weak or almost no calcium regulating effect. They are 1α,25,26-trihydroxy-Δ^{22}-vitamin D_3,[5)] 19-nor-10-oxo-25-hydroxyvitamin D_3,[6)] 26,26,26,27,27,27-hexafluoro-1α-hydroxyvitamin D_3,[7)] and 20-oxa and 22-oxa vitamin D_3 analogs.[8)]

During the course of our structure determination of a uniquely rearranged metabolite of 24-epi-1α,25-dihydroxyvitamin D_2, 24-homo- (2) and 26-homo-1α,25-



dihydroxyvitamin D_3 (4) and their Δ^{22} analogs (3 and 5) were synthesized.[9] These analogs were about ten-fold more potent than 1,25-$(OH)_2D_3$ (1) in inducing differentiation of HL-60 cells in vitro, but in the mobilization of bone calcium, the 24-homo analogs were significantly less active than 1,25-$(OH)_2D_3$ (1), whereas the 26-homo analogs were more active.[10]

In order to obtain further information on the structural requirement for cell differentiation and therapeutic possibility, 26,27-diethyl-1α,25-dihydroxyvitamin D_3 (7) and 24,24-difluoro-24-homo-1α,25-dihydroxyvitamin D_3 (8) were synthesized. Here we describe the synthesis of the compounds (7) and (8) and the results of their preliminary biological tests.

The diethyl analog (7) was synthesized from cholenic acid (9). Cholenic acid (9) was converted to the 24-ol (10) by the same reaction sequence as in our previous reports.[9,11] The 24-alcohol (10) was converted to the 25-methyl ester (11) *via* 24-cyanide (TsCl in pyridine, NaCN in DMSO at 70-80°C, KOH in aqueous EtOH at reflux, excess CH_2N_2) [oil, ν_{max} (neat) 1730 cm^{-1} (carbonyl)] in 54% yield. Grignard reaction of (11) with *n*-propylmagnesium bromide and subsequent acid hydrolysis gave the triol (12) [mp 76-79°C, MS *m/z*, 474 (M^+)] in 66% yield. After acetylation of (12), it was transformed into vitamin D_3 form by the standard method as follows. Bromination at 7-position with *N*-bromosuccinimide in refluxing CCl_4 and dehydrobromination with tetra-*n*-butylammonium fluoride gave a mixture of 4,6-diene and 5,7-diene. Saponification with 5% KOH-MeOH and subsequent preparative TLC afforded the pure 5,7-diene (13) λ_{max}(EtOH); 294, 282, and 271 nm, in 19% yield. Irradiation of the 5,7-diene (13) with a medium pressure mercury lamp through a Vycor filter in benzene-ethanol (2:1) at 0°C for 4 min and thermal isomerization of previtamin under reflux condition in the same solvent, after purification by preparative TLC and HPLC, gave the D_3 analog (7) in 16% yield.

The difluoro analog (8) was synthesized from the known alcohol (14).[12] The alcohol (14) was oxidized to the 24-aldehyde (15) by Swern oxidation. Wittig reaction of (14) with α-tetrahydropyranyloxyphosphonoacetate[13] and subsequent acid hydrolysis gave the keto-ester (16) [oil, $\delta(CDCl_3)$ 2.74 (2H, t, *J*=6.6Hz, 24-H_2)] in 61% yield. Introduction of fluorine atoms into (16) was performed by treatment with DAST[14] in CH_2Cl_2 at r.t. to give the difluoro-ester (17) [oil, ν_{max} ($CHCl_3$) 1210 (F-C-F), 1720 (carbonyl) cm^{-1})] in 69% yield. Grignard reaction of (17) with methylmagnesium bromide followed by acetylation gave the 24-F_2-24-homo-25-ol (18) [oil, $\delta(CDCl_3)$ 0.65 (3H, s, 18-H_3), 1.05 (3H, s, 19-H_3), 1.30 (6H, s, 26- and 27-H_3), 2.02 and 2.05 (6H, each s, acetyl), 4.91 (1H, m, 3-H), 5.08 (1H, m, 1-H), 5.54 (1H, m, 6-H)] in 60% yield. Transformation of (18) into vitamin D_3 form was accomplished by the standard method described above.

The synthetic analogs (7) and (8) had the following spectral data: (7); UV (EtOH) λ_{max}: 265 nm, λ_{min}: 228 nm, MS *m/z*, 472 (M^+), 454, 436, 410, 393, 269, 251, 152, 134. (8); UV (EtOH) λ_{max}: 265 nm, λ_{min}: 228 nm, MS *m/z*, 466 (M^+), 448, 430, 415, 407, 287, 269, 251, 134, 43.

26,27-Dimethyl-1α,25-dihydroxyvitamin D_3 (6) is slightly less active than 1,25-$(OH)_2D_3$ (1) in increasing serum calcium concentration.[11] The effects of this compound on the cell growth and differentiation of human promyecytic leukemia cells (HL-60 cells) *in vitro*[15] was about 2.5 times stronger than 1,25-$(OH)_2D_3$ (1); IC_{50} (concentrations required to inhibit cell growth by 50%) values: 10 nM for (6), and 25 nM for (1).[16]

Surprisingly, both the 26,27-diethyl analog (7) and the 24,24-F_2-homo analog (8) had almost no vitamin D activity, but induced differentiation of HL-60 cells into monocyte-macrophage. Furthermore, these compounds were at least 10 fold more active than 1,25-$(OH)_2D_3$ (1) for cell growth inhibition of HL-60 cells. The IC_{50} values for (7) and (8) were 2 nM and 2-3 nM, respectively, but 25 nM for (1).

It can be concluded that for the vitamin D activity, (intestinal calcium absorption, bone calcium mobilization), the distance between the two hydroxyl groups at the 1- and 25-positions may be critical. Furthermore, the alkyl

substituents at the 25-position should be methyl or ethyl groups. Thus, if the 25-position is substituted by fluorine atoms, the vitamin D activity is extinguished as shown in the 24,24-F_2-24-homo analog (8) and 25-fluoro-1-hydroxyvitamin D_3.[17] On the other hand, the lengthening the side chain by one carbon significantly increases the activity against HL-60 cells. The lengthening at the 26 and 27-positions also causes potent activity for leukemia cells.

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