Communications to the Editor

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SYNTHESIS OF 1α,25-DIHYDROXY-26,27-DIMETHYLVITAMIN D_3, A HIGHLY ACTIVE ANALOGUE OF 1α,25-DIHYDROXYVITAMIN D_3

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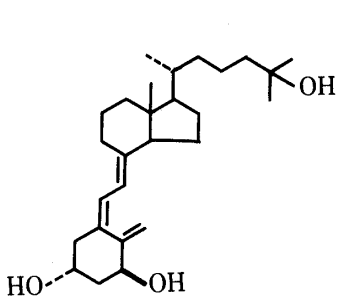
1α,25-Dihydroxy-26,27-dimethylvitamin D_3 (5) was synthesized from 1α,2α-epoxy-24-tetrahydropyranyloxy-22,23-dinorchola-4,6-dien-3-one (7) and was found to be more active than 1α,25-dihydroxyvitamin D_3 (1).

KEYWORDS —— 1α,25-dihydroxyvitamin D_3; 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3; 1α,25-dihydroxy-26,27-dimethylvitamin D_3; vitamin D activity; vitamin D_3 analogue

1α,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (1) is the hormone that mediates calcium and phosphorus metabolism.[1] Since its discovery, much effort has been made in synthesizing many analogues of vitamin D_3 and its metabolites to examine structure-activity relationships and search for agents with augmented or differential activity.[2] We have reported the synthesis and biological activity of 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (2)[3] and 26,26,26,27,27,27-hexafluoro-1α,25-dihydroxyvitamin D_3 ($26,27\text{-}F_6\text{-}1,25(OH)_2D_3$) (3),[4] which were blocked by fluorine for the 24- and 26-hydroxylation, deactivation steps of $1,25(OH)_2D_3$ (1). They were found to be 5-10 times more active than the natural $1,25(OH)_2D_3$ (1), probably because of their slow metabolism. The fluoro vitamins have paved the way for preparing an analogue more active than $1,25(OH)_2D_3$ (1). Based on the same idea of blocking the hydroxylation of $1,25(OH)_2D_3$ (1), we have prepared 1α,25-dihydroxy-24,24-dimethyl-22E-dehydrovitamin D_3 (4)[5] and 1α,25-dihydroxy-26,27-dimethylvitamin D_3 ($1,25(OH)_2\text{-}26,27(Me)_2D_3$) (5). In a biological test, the 24,24-dimethyl compound (4) proved to be slightly less active than $1,25(OH)_2D_3$ (1).[5] However, the 26,27-dimethyl analogue (5) was found to be much more highly active. In this communication, we describe the synthesis of $1,25(OH)_2\text{-}26,27(Me)_2D_3$ (5) and its preliminary biological results.

Our starting material for the target compound (5) is 1α,2α-epoxy-24-tetrahydropyranyloxy-22,23-dinorchola-4,6-dien-3-one (7), which was synthesized from dinorcholenic acid (6) according to our previous paper.[6] Reduction of (7) with lithium and ammonium chloride in liquid ammonia-tetrahydrofuran at -78°C and subsequent treatment with chloromethyl methyl ether and cyclohexyldiethylamine provided the dimethoxymethyl ether (8) in 38% yield. Removal of the tetrahydropyranyl group of (8) with 2 M HCl, followed by Swern oxidation[7] gave the 22-aldehyde (9) [mp 71-72°C, δ($CDCl_3$) 9.61 (1H, d, J = 3 Hz, 22-H)] in 81% yield. The 22-aldehyde (9) was converted into the 23-aldehyde (10) in 74% yield by the following successive reaction: Wittig reaction with methylenetriphenylphosphorane prepared from methyltriphenylphosphonium iodide and n-butyl lithium, selective hydroboration with 9-borabicyclo[3,3,1]nonane, oxidation with 3 M NaOH and 30% H_2O_2, and Swern oxidation.[7] The Wittig reaction of the 23-aldehyde (10) [oil, δ($CDCl_3$) 9.82 (1H, dd, J = 3 and 2 Hz, 23-H)] with (ethoxycarbonylmethylene)triphenylphosphorane, followed by selective hydrogenation with 10% Pd-C in ethyl acetate under normal pressure of hydrogen for 2 h provided the ester (11), oil, in 74% yield. Treatment of (11) with ethylmagnesium bromide and the subsequent removal of the methoxymethyl group with 6 M HCl in aqueous tetrahydrofuran gave 1α,25-dihydroxy-26,27-dimethyl-



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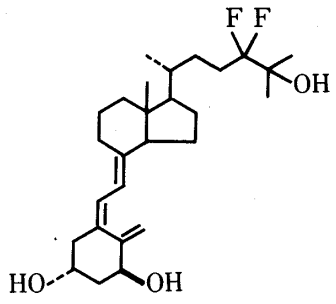

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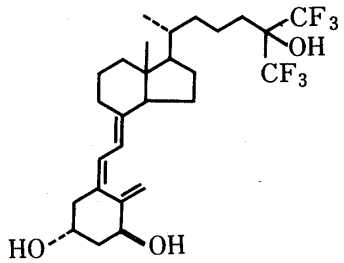

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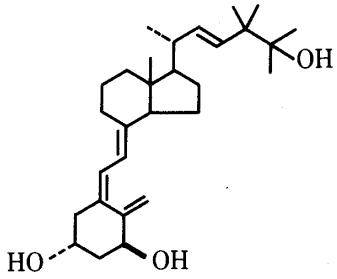

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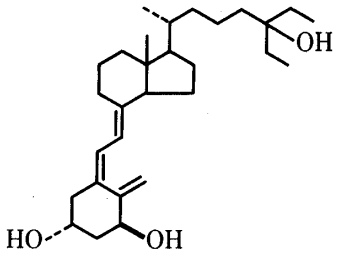

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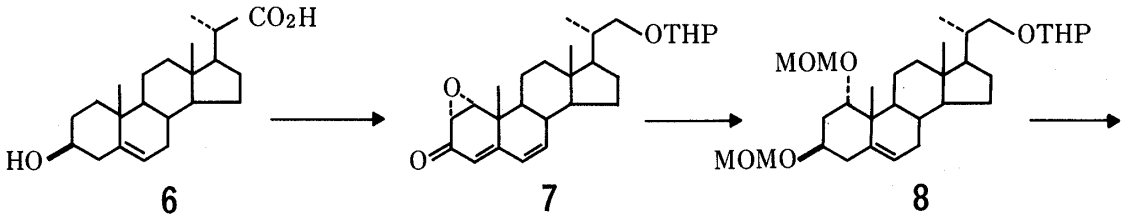

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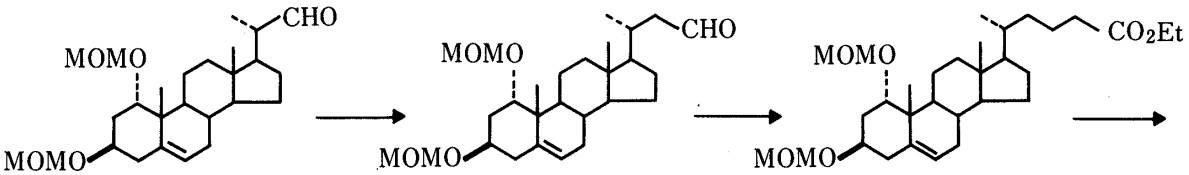

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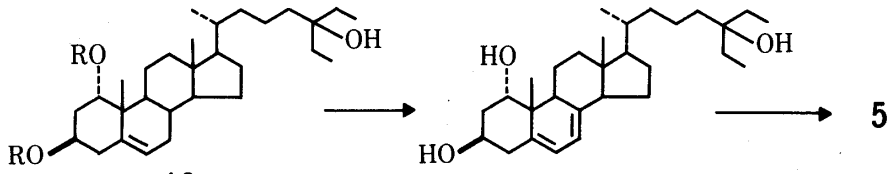

12 R=H
13 R=Ac

14

cholesterol (12) [mp 115-116°C, $\delta(CDCl_3)$ 0.70 (3H, s, 18-H_3), 0.85 (6H, t, J = 7 Hz, $CH_2\underline{CH_3}$), 0.90 (3H, d, J = 6 Hz, 21-H_3), 1.03 (3H, s, 19-H_3), 3.80 (1H, m, 1-H), 3.95 (1H, m, 3-H), 5.56 (1H, m, 6-H)] in 80% yield.

The diacetate (13) was transformed into the vitamin D analogue (5) by the standard method as follows. Bromination at C-7 of (13) with N-bromosuccinimide in refluxing carbon tetrachloride, followed by dehydrobromination with tetra-n-butylammonium fluoride gave a mixture of 4,6- and 5,7-dienes. Saponification of the mixture with 5% KOH/MeOH and the subsequent preparative thin layer chromatography (benzene-ethyl acetate, 2 : 1, developed six times, Rf 0.27) provided the pure 5,7-diene (14), λ_{max}^{EtOH}: 294, 282, and 271 nm, in 26% yield. This was irradiated with a medium-pressure mercury lamp through a Vycor filter in a mixed solvent (benzene-ethanol, 2 : 1) at 0°C for 5 min and the resulting mixture containing previtamin D was refluxed for 1 h. The target vitamin D_3 analogue (5) was isolated in 23% yield by preparative TLC (benzene-ethyl acetate, 2 : 1, developed four times, Rf 0.21). The product (5) had the following spectral data: UV (λ_{max}^{EtOH}: 265 nm, λ_{min}^{EtOH}: 228 nm), MS [FD-MS m/z 444 (M^+), EI-MS m/z 426 (M^+ - 18), 408 (M^+ - 36, base peak), 390 (M^+ - 54), 269 (287 (side chain cleavege) - 18), 251 (287 - 36), 152 (C_7-C_8 cleavage), 134 (152 - 18), 116 (152 - 36)].

According to a preliminary biological test (Table I), increases of serum calcium and inorganic phosphorus concentrations in response to 650 pmol/rat of 1,25(OH)$_2$-26,27(Me)$_2$D$_3$ (5) were much greater that those in response to the same dosage of 1,25(OH)$_2$D$_3$ (1). Even at a dosage of 100 pmol/rat of (5), the responses induced by (5) were greater than those by 650 pmol/rat of (1), indicating that the new vitamin D_3 analogue (5) is several times more active than the natural 1,25(OH)$_2$D$_3$ (1). At a dosage of 100 pmol/rat, the biopotency of 1,25(OH)$_2$-26,27(Me)$_2$D$_3$ (5) in these two bioassays was found to be as highly active as that of 26,27-F$_6$-1,25(OH)$_2$D$_3$ (3), which is the most active vitamin D_3 analogue heretofore prepared.[4b] Furthermore, the 26,27-dimethyl vitamin (5) decreased the activity of serum alkaline phosphatase in rachitic rat more effective than 1,25(OH)$_2$-D$_3$ (1) or 26,27-F$_6$-1,25(OH)$_2$D$_3$ (3), as shown in Table I. Although the exact reason for the surprisingly high activity of 1,25(OH)$_2$-26,27(Me)$_2$D$_3$ (5) is not clear, it may be that, as in the case of 26,27-F$_6$-1,25(OH)$_2$D$_3$ (3), obstruction or blocking of the side chain hydroxylation by the intro-

Table I, Increases in Serum Calcium and Inorganic Phosphorus Concentrations and Decrease in Alkaline Phosphatase Activity in Response to 1,25(OH)$_2$D$_3$, 1,25(OH)$_2$-26,27(Me)$_2$D$_3$, and 26,27-F$_6$-1,25(OH)$_2$D$_3$

Compound given	Amount of compound	Serum Ca (mg/100ml)	Serum inorganic phosphatase (mg/100ml)	Alkaline phosphatase activity (IU/l)
Ethanol	——	9.24 ± 0.12	8.05 ± 0.34	59.5 ± 2.8
1,25(OH)$_2$D$_3$ (1)	650 pmol/rat	9.90 ± 0.11[b]	9.10 ± 0.30[a]	46.9 ± 3.6[a]
1,25(OH)$_2$-26,27(Me)$_2$D$_3$ (5)	650 pmol/rat	11.46 ± 0.20[b]	10.38 ± 0.20[b]	32.7 ± 3.5[b]
	100 pmol/rat	10.49 ± 0.22[b]	9.63 ± 0.24[b]	45.2 ± 3.0[b]
26,27-F$_6$-1,25(OH)$_2$D$_3$ (3)	100 pmol/rat	10.13 ± 0.22[b]	9.65 ± 0.19[b]	52.9 ± 4.4

Significance of difference a) p 0.05, b) p 0.01

The bioassays were carried out according to the published methods.[3,4,8]

duced methyl groups is one plausible explanation. The more detailed biological activity of 1,25-$(OH)_2$-26,27$(Me)_2D_3$ (5) is now being investigated and the results will be reported elsewhere.

In summary, we have succeeded in preparing 1,25$(OH)_2$-26,27$(Me)_2D_3$ (5), which is the first example of a highly active, non-fluorinated vitamin D_3 analogue.[9)]

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REFERENCES AND NOTES

1) H. F. DeLuca and H. K. Schnoes, Ann. Rev. Biochem., 45, 631 (1976), and 52, 411 (1983).
2) N. Ikekawa and T. Takeshita, Yuki Gosei Kagaku Kyokai Shi, 37, 755, 809 (1979).
3) a) Y. Tanaka, H. F. DeLuca, H. K. Schnoes, N. Ikekawa, and Y. Kobayashi, Arch. Biochem. Biophys., 199, 473 (1980); b) S. Okamoto, Y. Tanaka, H. F. DeLuca, Y. Kobayashi, and N. Ikekawa, Am. J. Physiol., 244, E 159 (1983). See also the references; B. D. Kabakoff, N. C. Kendrick, D. Faber, H. F. DeLuca, S. Yamada, and H. Takayama, Arch. Biochem. Biophys., 215, 582 (1982).
4) a) Y. Kobayashi, T. Taguchi, S. Mitsuhashi, T. Eguchi, E. Ohshima, and N. Ikekawa, Chem. Phram. Bull., 30, 4297 (1982); b) Y. Tanaka, H. F. DeLuca, Y. Kobayashi, and N. Ikekawa, Arch. Biochem. Biophys., 229, 348 (1984); c) P. H. Stern, T. Mavreas, Y. Tanaka, H. F. DeLuca, N. Ikekawa, and Y. Kobayashi, J. Pharmacol. Exp. Ther., 229, 9 (1984).
5) H. Sai, S. Takatsuto, and N. Ikekawa, unpublished results.
6) H. Sai, S. Takatsuto, N. Ikekawa, Y. Tanaka, C. Smith, and H. F. DeLuca, Chem. Pharm. Bull., 32, 3866 (1984).
7) A. J. Mancuso, S. L. Huang, and D. Swern, J. Org. Chem., 43, 2480 (1978).
8) A. W. Norman, A. K. Mircheff, T. H. Adams, and A. Spielvogel, Biochim. Biophys. Acta, 215, 348 (1970).
9) Synthesis of some fluoro analogues of 1,25$(OH)_2$-26,27$(Me)_2D_3$ (5) is now in progress in our laboratory.

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