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SYNTHESIS AND IMMUNOREGULATING ACTIVITY OF VITAMIN D ANALOGUES BEARING PREGNANE SIDE CHAINS#1)

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Abstract: The synthesis of Vitamin D analogues bearing pregnane side chains and their immunoregulating activity in mice are described.

Since 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] (1) was shown to inhibit the proliferation and induce the differentiation of murine leukemia cells into monocyto-macrophages in addition to its regulatory effect on calcium and phosphorous metabolism,[2] many studies have shown that 1 is a potent modulator not only for suppressing proliferation and inducing differentiation of numerous normal and tumor cells, but also for regulating the functions of immunologically competent cells such as T and B lymphocytes.[3] These results prompted chemists to synthesize vitamin D analogues to separate these differentiation-inducing activity and immunoregulating effects from the potent hypercalcemic action.[4] During our investigation of the structure-activity relationship of vitamin D analogues, we took an interest in the immunoregulating potential of hexanorvitamin D analogues, in which an absence of calcemic properties would be expected due to the truncated side chain (C-22 - C-27). It is well-known that the side chain from C-22 to C-27 of 1 is essential for vitamin D activity (bone calcium mobilization (BCM) and intestinal calcium absorption (ICA)).[5] Accordingly, in this paper we wish to describe the synthesis of three hexanorvitamin D analogues bearing the pregnane side chain, cortisone-type (3), aldosterone-type (4), and pregnenolone-type (5) analogues, and their immunoregulating activity.

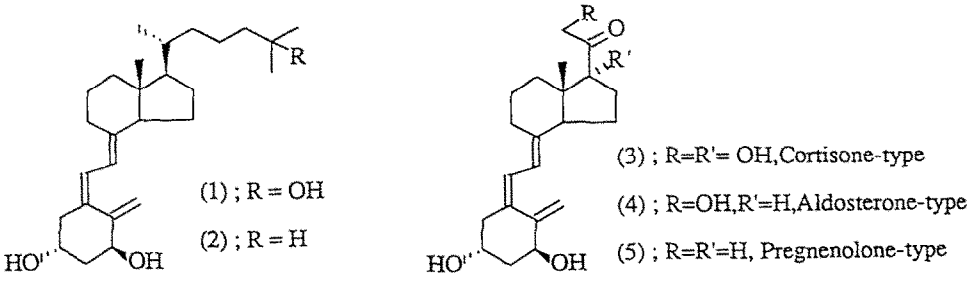

Chart 1

#Dedicated to Dr. Masatomo Hamana, Professor Emeritus, Kyushu University on the occasion of his 75th birthday.

In our synthesis of 3, 4, and 5, the 17-ketosteroid (6) was chosen as the starting material.[6] The construction of cortisone-type and aldosterone-type side chain from 6 was carried out first.[7] The addition of methyl lithiomethoxyacetate to 6 at -60°C for 3 hours gave the adduct (7), in 70% yield. Subsequently, 7 was treated with thionyl chloride in pyridine for 2 hours at -20°C to afford the ester (8), in 71% yield, which was then transformed into the alcohol (9) by DIBAL in 82% yield. Treatment of 9 with MCPBA in CH_2Cl_2 gave cortisone-type side chain of 10 in 40% yield after silylation of the primary hydroxy moiety. Subsequent irradiation of 10 with a 400W mercury lamp through a Vycor filter, followed by thermal isomerization in refluxing hexane and elimination of the silyl groups with trifluoroacetic acid, gave rise to cortisone-type analogue (3).[8]

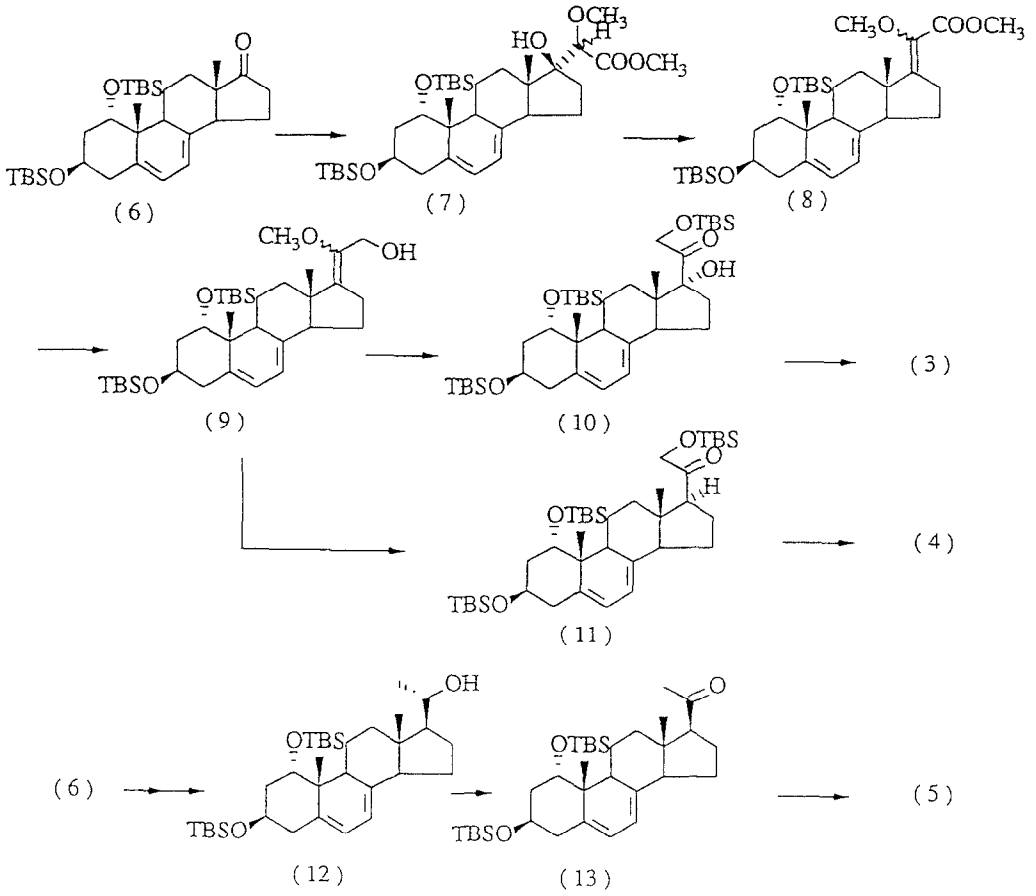

Chart 2

Next, the aldosterone-type side chain of 11 was formed from 9 by hydrolysis with aqueous oxalic acid and silylation in 79% yield. Then 11 was irradiated, thermally isomerized and desilylated to give aldosterone-type analogue (4).[9]

Finally, the ketone (13), obtained from the alcohol (12)[6] by PCC in 70% yield, was converted to pregnenolone-type analogue (5)[10] by the same sequence described above.

Among three hexanorvitamin D analogues synthesized, pregnenolone-type analogue (5) showed the most significant effect on the primary immune response in BALB/C mice immunized with 1 x 10^7 sheep red blood cells (SRBC),[11] while the effect of cortisone-type (3) and aldosterone-type (4) analogues was moderate. Fig. 1 shows the number of antibody-forming cells of mice which orally received either 0.2 or 1.0 μg/kg of 5 or 0.2 μg/kg of 1α-hydroxyvitamin D_3 (1α-OH-D_3) (2). On the other hand the *in vitro* measurement of the binding affinity with chick intestinal cytosolic receptor[12] disclosed that 5 has only 1/10,000 the affinity of 1α,25-$(OH)_2$-D_3 (1), and 3 and 4 have less than 1/100,000. The administration of 5 to rats deficient in vitamin D showed no effect on BMC and ICA at a dose of 125μg/kg (x 5, i.v.). Thus, an interesting biological feature of 5, retaining high immunoregulating activity without hypercalcemic action, is shown. Further biological properties of this analogue are now under investigation.

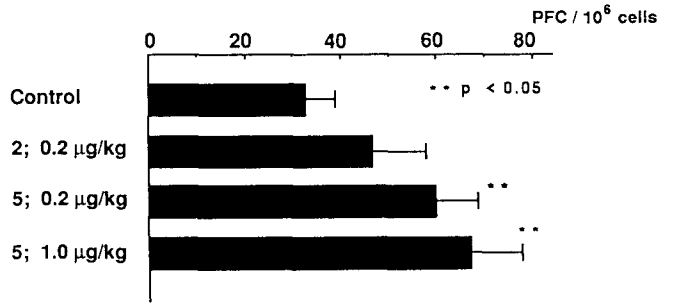

Fig. 1. Effect of pregnenolone-type analogue (5) and 1α-OH-D_3 (2) on the primary anti-SRBC response.

Acknowledgement; We are grateful to Drs. J. Abe, M. Fukushima, Y. Takita and E. Kamijo for biological experiments.

References and notes

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8) 3; ^{1}H-NMR ($CDCl_3$, δ) 0.58 (3H,s), 2.35 (1H,dd,*J*=12.5, 6.3Hz), 2.55-2.96 (4H,m), 3.10 (1H,t,*J*=4.9Hz), 4.21-4.31 (1H,m), 4.35 (1H,dd,*J*=20.0, 4.9Hz), 4.38-4.51 (1H,m), 4.66 (1H, dd,*J*=20.0, 4.9Hz), 5.01 (1H,t,*J*=1.5Hz), 5.35 (1H,t,*J*=1.5Hz), 6.09 (1H,d,*J*=11.4Hz), 6.38 (1H,d,*J*=11.4Hz). UV (EtOH, λmax, nm) 264.
9) 4; ^{1}H-NMR ($CDCl_3$, δ) 0.53 (3H,s), 2.52-2.68 (2H,m), 2.87 (1H,d,*J*=12.0Hz), 3.26 (2H,brs), 4.12-4.34 (3H,m), 4.38-4.49 (1H,m), 4.98 (1H,t,*J*=1.7Hz), 5.33 (1H,t,*J*=1.7Hz), 6.05 (1H,d,*J*=10.8Hz), 6.34 (1H,d,*J*=10.8Hz). UV (EtOH, λmax, nm) 262. $[\alpha]_D^{23}$ 2.81 (*c*=0.07, EtOH). MS *m/z* 346 (M^+), 134 (100%). HRMS Calcd for $C_{21}H_{30}O_4$: 346.2144. Found: 346.2146.
10) 5; ^{1}H-NMR ($CDCl_3$, δ) 0.51 (3H,s), 2.13 (3H,s), 2.23-2.37 (1H,m), 2.52-2.73 (3H,m), 2.84 (1H,brd,*J*=12.8Hz), 4.15-4.28 (1H,br), 4.36-4.47 (1H,br), 4.99 (1H,t,*J*=1.4Hz), 5.33 (1H,t,*J*=1.4Hz), 6.04 (1H,d,*J*=11.4Hz), 6.36 (1H,d,*J*=11.4Hz). UV (EtOH, λmax, nm) 263. $[\alpha]_D^{23}$ 9.20 (*c*=1.00, EtOH). MS *m/z* 330 (M^+), 133 (100%). HRMS Calcd for $C_{21}H_{30}O_3$: 330.2195. Fond: 330.2176.
11) SRBC (1 x 10^7) were intravenously injected into BALB/C mice (7 weeks) to give a suboptimal immune response. Various dose of vitamin D analogues were orally administered twice after immunization (0 and 24 hours). Splenic direct plaque-forming cell (PFC) assay was performed according to the method of Cunningham and Szenberg[13] on day 3 after immunization. Detailed method is shown in the following literature; Abe, J.; Takita, Y.; Nakano, T.; Miyaura, C.; Suda, T.; Nishii, Y. *Endocrinology*, **1989**, 124, 2645.
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