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Synthesis of 24,24-Difluoro-1α,25-dihydroxyvitamin D_3

24,24-Difluoro-1α,25-dihydroxyvitamin D_3 (**1**) has been synthesized from 24,24-difluoro-5β-cholestane-3α,25-diol (**2**) as an antimetabolic analogue of 1α,25-dihydroxyvitamin D_3 to study the role of the 24-hydroxylation in the metabolism of vitamin D_3.

Keywords——antimetabolic analogue of 1α,25-dihydroxyvitamin D_3; 24,24-difluoro-5β-cholestane-3α,25-diol; role of 24-hydroxylation of vitamin D_3 in the metabolism; biologically potent vitamin D analogue; NMR spectra

Vitamin D_3 must be metabolically hydroxylated first in the liver at the 25-position and subsequently in the kidney at the 1α-position to afford 1α,25-dihydroxyvitamin D_3 before it can function.[1] Another important hydroxylation occurs at the 24-position under the conditions whereby the 1α-hydroxylation is suppressed[2] and leads 25-hydroxyvitamin D_3 to 24,25-dihydroxyvitamin D_3[3] and 1α,25-dihydroxyvitamin D_3 to 1α,24,25-trihydroxyvitamin D_3.[4] Although the 24-hydroxylated metabolites have significant activity[5] and 24,25-dihydroxyvitamin D_3 is one of the major metabolites, the role of these metabolites has not been clearly understood.

To study the biological importance of 24-hydroxylation in the function of vitamin D, we undertook the synthesis of the analogues of 25-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3 blocked at the 24-position with fluorine atoms and in the previous paper,[6] reported

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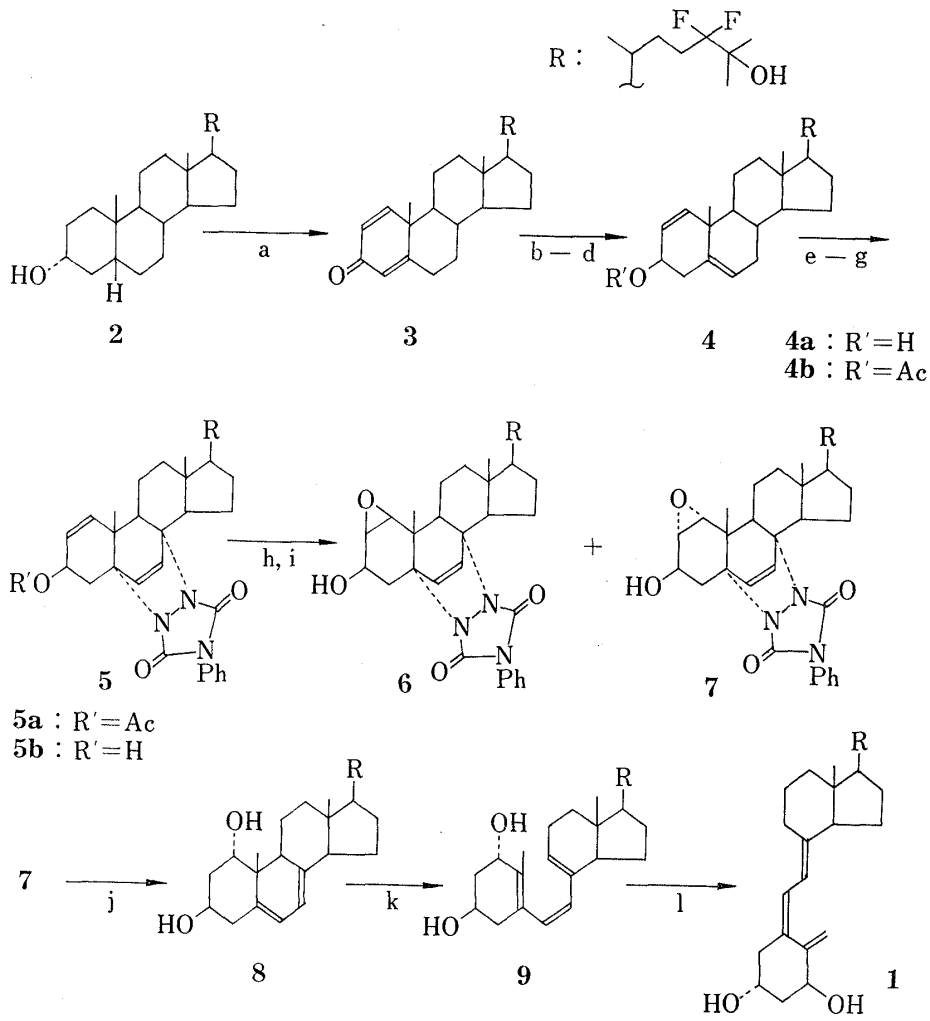
successful synthesis of 24,24-difluoro-25-hydroxyvitamin D_3. We now wish to report the first synthesis of 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (**1**).

The synthesis of the title compound (**1**) was performed as shown in Chart 1 using 24,24-difluoro-5β-cholestane-3α,25-diol (**2**) as starting material, which was prepared from lithocholic acid in 20% overall yield and used in the synthesis of 24,24-difluoro-25-hydroxyvitamin D_3[6] as a key intermediate.



a) DDQ, dioxane, refl. b) *t*-BuOK, DMSO, 15°, then H_2O. c) $Ca(BH_4)_2$, EtOH–MeOH, −10°. d) Ac_2O, py. e) NBS, hexane–benzene, refl. f) Collidine, xylene, refl. g) 4-Phenyl-1,2,4-triazoline-3,5-dione. h) NaOH, EtOH. i) *m*-CPBA, $CHCl_3$. j) LAH, THF, refl. k) ***hν***, ether 1) EtOH, RT.

Chart 1

Oxidation of **2** with DDQ gave the dienone (**3**) (mp 176—177°) which was converted to the 1,5-diene-3β-ol (**4**) [mp 171—172°; *m/e* 436; NMR ($CDCl_3$) δ 0.74 (3H, s), 1.12 (3H, s) 1.34 (6H, s), 4.22 (1H, m), 5.45 (1H, m), 5.59 (1H, d, J=10 Hz), 5.83 (1H, dd, J=10, 2 Hz)] by deconjugation followed by reduction with $Ca(BH_4)_2$.[7] The acetate (**4b**) of **4a** was allowed to allylic bromination and subsequent dehydrobromination to afford a mixture of trienes from which the desired 1,5,7-triene was isolated by Diels-Alder adduct formation with 4-phenyl-1,2,4-triazoline-3,5-dione to give **5a** [mp 203—204°; *m/e* 476; NMR ($CDCl_3$) δ 0.83

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(3H, s), 1.10 (3H, s), 1.27 (6H, s), 2.06 (3H, s), 6.23 (1H, d, J=8 Hz), 6.50 (1H, d, J=8 Hz); IR ($CHCl_3$) 1740, 1690 cm^{-1}] in 36% yield. To introduce hydroxyl function at the 1α-position, the acetate (**5a**) was hydrolysed to afford **5b**, which was oxidized with m-CPBA to give isomeric two epoxides, less polar **6** and more polar **7** in 53% and 41% yields, respectively. The structures of **6** and **7** were determined by comparing their chromatographic and spectroscopic properties [**6**: mp 168—169°; *m/e* 450; NMR ($CDCl_3$) δ 0.78 (3H, s), 0.98 (3H, s), 1.29 (6H, s), 4.99 (1H, m), 6.23 (1H, d, J=8 Hz), 6.40 (1H, d, J=8 Hz), **7**: mp 217—218°; *m/e* 450; NMR ($CDCl_3$) 0.84 (3H, s), 1.05 (3H, s), 1.27 (6H, s), 4.90 (1H, m), 6.13 (1H, d, J=8 Hz), 6.40 (1H, d, J=8 Hz)] with those reported for closely related compounds.[8] Reduction of the α-epoxide (**7**) with LAH effected removal of the protecting group as well as opening of the epoxide ring to give the provitamin D, 24,24-difluoro-1α,25-dihydroxy-7-dehydrocholesterol (**8**) [mp 188—189°; *m/e* 452; UV (EtOH) 272, 282, 294 nm; NMR ($CDCl_3$) δ 0.63 (3H, s), 0.95 (3H, s), 1.31 (6H, s), 3.76 (1H, m, w/2=7 Hz), 4.06 (1H, m, w/2=30 Hz), 5.40 (1H, dd, J=5, 3 Hz), 5.74 (1H, d, J=5 Hz)] in 25% yield. The structure of **8** was based on its spectral properties and the examples similar to this.[7] Irradiation of **8** (high pressure mercury lamp, Vycor filter) followed by chromatographic separation (Sephadex LH-20, hexane: $CHCl_3$ 35: 65) afforded the previtamin D (**9**) (UV 260 nm) in 25% yield. On standing at room temperature for two weeks, the previtamin (**9**) was completely transformed into the desired vitamin D (**1**) in 71% yield. The structure of 24,24-difluoro-1α,25-dihydroxyvitamin D_3 (**1**) thus obtained was confirmed by its mass (*m/e* 452, 251, 134), UV (EtOH, 266 nn), and NMR spectra [$CDCl_3$, δ 0.56 (3H, s, 18-Me), 0.95 (3H, d, J=5 Hz, 21-Me), 1.31 (6H, s, 26,27-Me), 4.20 (1H, m, 1β- or 3α-H), 4.40 (1H, m, 3α- or 1β-H), 5.34 (1H, br s, 19-H), 5.02 (1H, br s, 19-H), 6.05 (1H, d, J=11 Hz, 7-H), 6.38 (1H, d, J=11 Hz, H-6)].

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