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Synthesis of 24,24-Difluoro-1 α ,25-dihydroxyvitamin D₃

24,24-Difluoro-1 α ,25-dihydroxyvitamin D₃ (1) has been synthesized from 24,24-difluoro-5 β -cholestane-3 α ,25-diol (2) as an antimetabolic analogue of 1 α ,25-dihydroxyvitamin D₃ to study the role of the 24-hydroxylation in the metabolism of vitamin D₃.

Keywords—antimetabolic analogue of 1 α ,25-dihydroxyvitamin D₃; 24,24-difluoro-5 β -cholestane-3 α ,25-diol; role of 24-hydroxylation of vitamin D₃ in the metabolism; biologically potent vitamin D analogue; NMR spectra

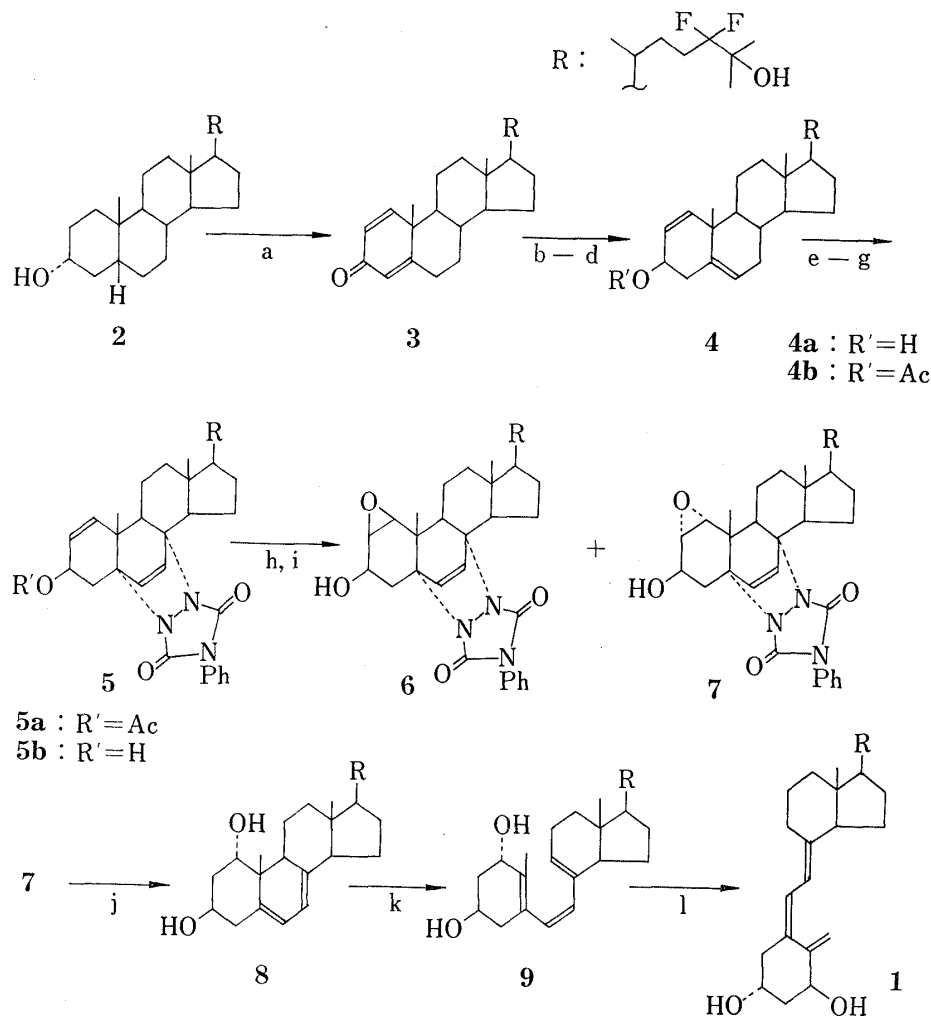
Vitamin D₃ must be metabolically hydroxylated first in the liver at the 25-position and subsequently in the kidney at the 1 α -position to afford 1 α ,25-dihydroxyvitamin D₃ before it can function.¹⁾ Another important hydroxylation occurs at the 24-position under the conditions whereby the 1 α -hydroxylation is suppressed²⁾ and leads 25-hydroxyvitamin D₃ to 24,25-dihydroxyvitamin D₃³⁾ and 1 α ,25-dihydroxyvitamin D₃ to 1 α ,24,25-trihydroxyvitamin D₃.⁴⁾ Although the 24-hydroxylated metabolites have significant activity⁵⁾ and 24,25-dihydroxyvitamin D₃ 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 D₃ and 1 α ,25-dihydroxyvitamin D₃ 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 D₃. We now wish to report the first synthesis of 24,24-difluoro-1 α ,25-dihydroxyvitamin D₃ (**1**).

The synthesis of the title compound (**1**) was performed as shown in Chart 1 using 24,24-difluoro-5 β -cholestane-3 α ,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 D₃⁶⁾ as a key intermediate.



a) DDQ, dioxane, refl. b) *t*-BuOK, DMSO, 15°, then H₂O. c) Ca(BH₄)₂, EtOH-MeOH, -10°. d) Ac₂O, 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, CHCl₃. j) LAH, THF, refl. k) *hv*, ether l) EtOH, RT.

Chart 1

Oxidation of **2** with DDQ gave the dienone (**3**) (mp 176–177°) which was converted to the 1,5-diene-3 β -ol (**4**) [mp 171–172°; *m/e* 436; NMR (CDCl₃) 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(BH₄)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 (CDCl₃) 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- $1\alpha,25$ -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- $1\alpha,25$ -dihydroxyvitamin D₃ (**1**) thus obtained was confirmed by its mass (m/e 452, 251, 134), UV (EtOH, 266 nm), 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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