Synthesis of 1β-Hydroxyvitamin D_3 and 1β,25-Dihydroxyvitamin D_3

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Summary 1β-Hydroxyvitamin D_3 and 1β,25-dihydroxyvitamin D_3 have been prepared from the corresponding 1α-epimers by a convenient three-step process involving the 1-oxoprevitamin forms as the key intermediates.

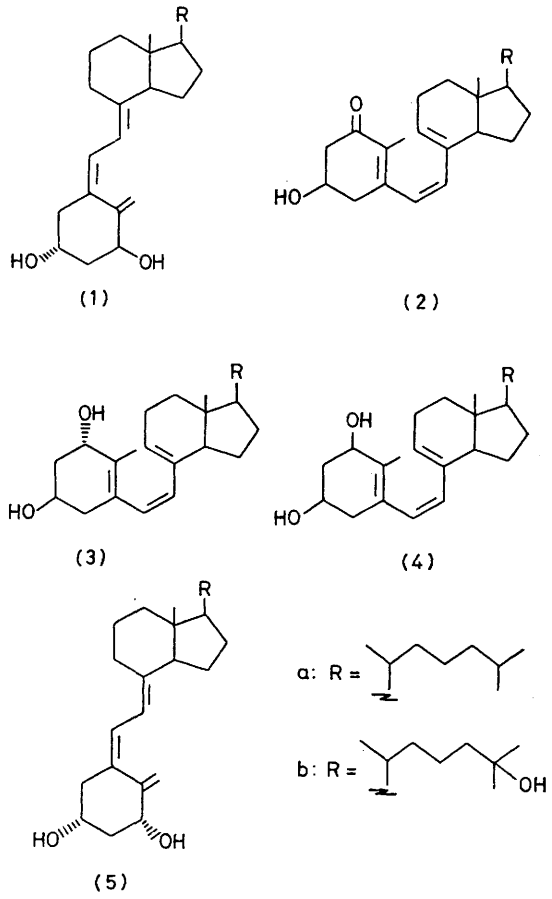
As the tissue-active hormonal metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 (**1b**) elicits full biological response in all systems regulated by the vitamin.[1] A key step in this activity is believed to be the binding of the molecule to tissue-specific, intracellular protein receptors.[1,2]

The crucial importance of the C(1) hydroxy function for vitamin–receptor binding is well established through analogue studies,[3] but little is known about the relationship between molecular configuration, in particular hydroxy-group stereochemistry, and binding affinity. In order to investigate this question we undertook the preparation of selected 1β-hydroxy analogues and report a convenient three-step procedure for the synthesis of these vitamin derivatives from the available 1α-epimers in about 45% overall yield.

Oxidation (activated MnO_2,[4] CH_2Cl_2, 1·5 h) of (**1a**) gave 1-oxoprevitamin D_3 (**2a**) [*m/e* 398 (M^+, 60%), 380 (100), and 365 (10); λ_{max} (Et_2O) 288 (ϵ 7100) and 237 nm (6800); ^{1}H n.m.r. (270 MHz) δ 0·72 (3H, s, 13-Me), 1·80 (3H, s, 10-Me), 4·16 (1H, m, 3-H), 5·47 (1H, narrow m, 9-H), and 6·14 and 6·03 (2H, ABq, *J* 11·9 Hz, 6- and 7-H)] as the only product in 65% yield (or 85% based on recovered starting material). The presence of the linearly conjugated trienone chromophore in (**2a**) probably accounts for the exclusive formation of the previtamin ketone.

This intermediate, a potentially highly useful synthetic precursor to other ring A-modified vitamins (including radiolabelled derivatives), was then reduced (inverse addition $LiAlH_4$, Et_2O, −25 °C) to give, in quantitative yield, a 1:4 mixture of 1α-hydroxyprevitamin D_3 (**3a**) and its 1β epimer (**4a**) which were separated by preparative t.l.c. The salient difference in the n.m.r. spectra of (**3a**) and (**4a**) is the multiplet absorption for the C(1) and C(3) protons. For (**3a**), the peak widths at half height for the signals at δ 4·15 and 4·03 are 10·0 and 23·7 Hz, respectively. This indicates that the proton at δ 4·15 is equatorially oriented while that at δ 4·03 is axial.[5] In the spectrum of (**4a**), the peak widths at half height are 10·7 Hz for the signal at δ 4·2 and 10·5 Hz for the signal at δ 4·03 p.p.m. indicating an equatorial conformation for both protons and, consequently, an axial orientation for the hydroxy-groups at C(1) and C(3). This diaxial arrangement of the hydroxy-functions is due to the 1,3-transannular hydrogen bonding.

Thermal isomerization of (**3a**) and (**4a**), (EtOH, 80 °C, 2·0 h) followed by purification by high-pressure liquid chromatography (h.p.l.c.) produced the respective vitamin analogues (**1a**), which by chromatographic and spectral comparisons proved identical with the original starting material, and (**5a**): *m/e* 400 (M^+, 18%), 382 (35), 364 (25), 152 (100), and 134 (90); λ_{max} (EtOH) 263 nm (ϵ 17,000); ^{1}H n.m.r. (270 MHz) δ 0·55 (3H, s, 13-Me), 0·87 (6H, d, 25-Me_2), 4·11 (1H, narrow m, 3-H), 4·37 (1H, narrow m, 1-H), 5·01 [1H, d, *J* 1·9 Hz, 19(*Z*)-H], 5·29 [1H, d, *J* 1·9 Hz, 19(*E*)-H], and 6·46 and 6·06 (2H, ABq, *J* 11·7 Hz, 6- and 7-H). Epimers (**1a**) and (**5a**) are distinguishable by their chromatographic properties and several spectral characteristics, notably the relative intensities of the diagnostic ions at *m/e* 134 and 152 [(**1a**): 100 and 30 respectively], the u.v. absorption maximum [(**1a**): λ_{max} 265 nm (ϵ 18,200)], and the n.m.r. absorptions of the C(19) methylene protons {(**1a**): δ 5·10 [1H, dd, *J* 1·8 Hz, 19(*Z*)-H] and 5·33 [1H, *J* 1·8 Hz, 19(*E*)-H]}. The splitting of the C(19) protons in (**1a**) is a product of terminal methylenic coupling (*J ca.* 1·8 Hz) and allylic coupling (*J ca.* 1·8 Hz) with C(1)-H whose axial character is due to the favoured equatorial orientation of the C(1) hydroxy-group, and gives rise therefore to a doublet of doublets. In (**5a**), where the C(1) and C(3) hydroxy-groups are hydrogen-bonded in a 1,3-diaxial fashion, allylic coupling is absent because of the orthogonality between the C(1)-H σ-bond and the π-orbitals of the C(10)–C(19) double bond.[6] Therefore, the 19(*E*) and 19(*Z*) proton signals are a simple doublet (*J* 1·9 Hz) reflecting only terminal methylenic coupling.



By an entirely analogous sequence 1β,25-dihydroxyvitamin D_3 (**5b**) was obtained from (**1b**). Initial oxidation resulted in an 80% yield of (**2b**). Reduction of the 1-oxo intermediate gave a 1:3 mixture of (**3b**) and (**4b**) which were separated by h.p.l.c. Thermal isomerization of the previtamins produced (**1b**), identical in all respects to the natural metabolite starting material, and the 1β-epimer (**5b**).†

Preliminary *in vitro* testing in the intestinal cytosol binding assay[7] shows that 1β,25-dihydroxyvitamin D_3 (**5b**)

† All intermediates gave the appropriate spectral data. Compound (**5b**) gave: *m/e* 416 (M^+, 10%), 152 (100), 134 (95), and 59 (85); λ_{max} 263 nm; ^{1}H n.m.r. δ 0·55 and 1·22 (s, 13-Me, 25-Me_2), 4·11 (m, 3-H), 4·37 (m, 1-H), 5·01 [d, *J* 1·9 Hz, 19(*Z*)-H], 5·29 [d, *J* 1·9 Hz, 19(*E*)-H], and 6·43 and 6·06 (ABq, *J* 11·8 Hz, 6- and 7-H).

is $3{\cdot}0 \times 10^3$ times less potent than its 1α-epimer (**1b**), and 1β-hydroxyvitamin D_3 (**5a**) is $1{\cdot}65 \times 10^5$ times less potent in displacing bound 1α,25-dihydroxyvitamin D_3.

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