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STUDIES ON VITAMIN D (CALCIFEROL) AND ITS ANALOGS. IX. 1α-HYDROXY-3-EPIVITAMIN D_3: ITS SYNTHESIS AND CONFORMATIONAL ANALYSIS.[1,2]

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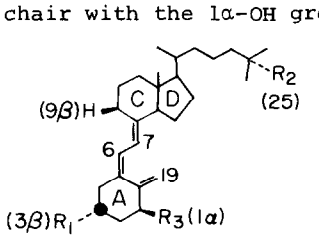
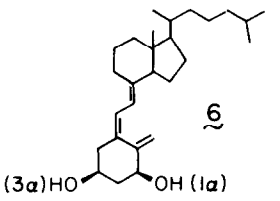
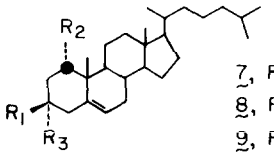
Among steroid hormones, 1α,25-dihydroxyvitamin D_3 (1), a metabolite and the presumed active form of vitamin D_3 (2), is structurally unique.[3] Unlike the classical steroid hormones such as estrogen, aldosterone, testosterone, etc., the seco-steroid 1 lacks the B-ring. Recent ^{1}H NMR studies from this laboratory have shown that the A-ring of 1,[4ab] as well as that of other related seco B steroids,[4] is partitioned between two rapidly equilibrating chair-like conformers. For 1 and for 1α-hydroxyvitamin D_3 (3), a highly potent synthetic analog,[3] the A-ring consists of a ~55/45 mixture of chair-like conformers favoring the chair with the 1α-OH group equatorially oriented.[4a-c] A step in the bio-

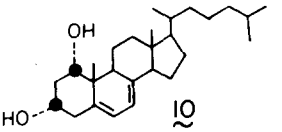


1, $R_1=R_2=R_3=OH$
2, $R_1=OH$; $R_2=R_3=H$
3, $R_1=R_3=OH$; $R_2=H$
4, $R_1=H$; $R_2=R_3=OH$
5, $R_1=R_2=H$; $R_3=OH$





7, $R_1=R_2=OH$; $R_3=H$
8, $R_2=R_3=OH$; $R_1=H$
9, $R_2=R_3=OAc$; $R_1=H$



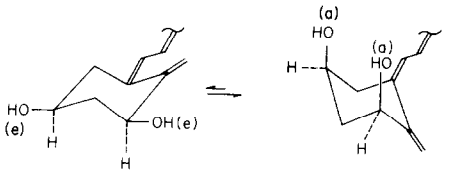
logical action of hormonal steroids is their binding to macromolecules (proteins).[5] In view of the stereostructural specificities toward substrates of enzymes, receptors and other binding proteins, it seemed reasonable to assume that only one of the two chair conformations represents the optimally active molecular topology. At the steroid hormone receptor level for intestinal calcium transport, we have theorized that it is the equatorial 1α-OH conformer that is optimally active.[6] One way to test this hypothesis is through the synthesis and study of analogs appropriately substituted in the A-ring such that this ring is biased in one chair conformer or the other thus orienting the 1α-OH group predominantly axial or predominantly equatorial. A seemingly appropriate position for placing new conformationally biasing substituents is the 3-position since we have already shown that the 3β-OH of 1 or 3 can be removed to produce

the analogs 3-deoxy-1α,25-dihydroxyvitamin D_3 (4)[1] or 3-deoxy-1α-hydroxyvitamin D_3 (5),[7] respectively, which still retain high biological potency in both *in vivo* and *in vitro* assays. It is the purpose of this paper to describe the synthesis and conformational analysis of the C-3 epimer of 3, namely 1α-hydroxy-3-epivitamin D_3 (6).[8] This analog was also reported recently by another laboratory;[4e] we are thus prompted to describe our independent results concerning this substance.

Treatment of 1α-hydroxycholesterol (7, 7.5 mmole), prepared according to Barton's method,[9] with formic acid, triphenylphosphine, and diethyl azodicarboxylate (15 mmole each) in dry tetrahydrofuran (100 ml) for 14 hours (r.t.)[10] and then direct saponfication (20 ml, 20% NaOH/methanol, reflux, 2-3 hours) afforded after work up, chromatography (Woelm alumina III/ether-petroleum ether), and crystallization (methanol), a 76% yield of 1α,3α-dihydroxycholest-5-ene [8: mp 205-208°; NMR ($CDCl_3$, TMS) τ 4.46 (H_6, br, W~12 Hz), 5.92 and 6.25 ($H_{1\beta}$ and $H_{3\beta}$ or vice versa, br, W~8 Hz and 11 Hz respectively), 9.05 ($C_{19}CH_3$, s) and 9.32 ($C_{18}CH_3$, s);[11] mass spectrum (80eV) m/e (rel int) 402 (11, M), 384 (base, M-H_2O), 366 (9, M-2H_2O)]. The diol 8 (5.9 mmole) was acetylated (18 ml 1:1 acetic anhydride/pyridine, 1.8 g 4-dimethylaminopyridine for 12 hrs., r.t.), worked up, chromatographed (as above), and then crystallized (methanol) to give a 94% yield of the diacetate [9: mp 123-125°; NMR ($CDCl_3$, TMS) τ 4.54 (H_6, br, W~11 Hz), 5.00 and 5.10 ($H_{1\beta}$ and $H_{3\beta}$ or vice versa, pseudo t, J ~ 3.0 and 2.5 Hz respectively), 7.97 and 8.02 ($2CH_3O$-, s), 8.94 ($C_{19}CH_3$, s), and 9.30 ($C_{18}CH_3$, s)]. The Δ^5-diacetate 9 was brominated (1,3-dibromo-5,5-dimethylhydantoin) and dehydrobrominated (s-collidine) under conditions exactly as described previously[1] and then the crude diene was directly saponified (5% KOH/methanol, 12 hrs., r. t.). After work up, chromatography (dry column, 10% silver nitrate silica gel, ether), and crystallization (methanol), a 15% yield of 1α,3α-dihydroxycholesta-5,7-diene was obtained [10: mp 182-3°; NMR ($CDCl_3$, TMS) τ 4.10 and 4.57 ($H_{6,7}$, ABq, J_{AB} ~ 6.0 Hz; B exhibited extensive fine structure), 5.76 and 6.33 ($H_{1\beta}$ and $H_{3\beta}$ or vice versa, br, W~10 Hz each), 9.08 ($C_{19}CH_3$, s), and 9.36 ($C_{18}CH_3$, s); uv in ether (ε), λ_{max} 294 (6900), 283 (11,800), 272 (11,200), 263sh (8000), 254sh (4900) nm; mass spectrum (80eV) m/e (rel int) 400 (41, M), 382 (M-H_2O, 52), 364 (M-2H_2O, 52), 341 (84), 251 (76), 197 (base)]. Photolysis of 10 (10-20 mg/100 ml ether; 9.0 minutes) was carried out exactly as previously described[12] and then the pooled concentrates from a number of runs were chromatographed (dry silica gel column/ether) to afford pre-vitamin (λ_{max} 260, λ_{min} 230 nm). After heating the latter (isooctane, 80°, N_2, 3 hrs.), identical chromatographic purification as for the photolysate afforded a ~5% yield of 1α-hydroxy-3-epivitamin D_3 (6), which was obtained as an amorphous foam. The analog 6 proved homogeneous on several thin layer chromatography systems (silica gel G or 10% silver nitrate impregnated silica gel G) and it exhibited the following: NMR (Varian HR300, *ca.* .05 M, $CDCl_3$, TMS), τ 3.58 and 4.02 ($H_{6,7}$, ABq,

J_{AB} ~ 11.4 Hz), 4.73 (H_{19Z}, m), 5.03 (H_{19E}, m), 5.72 ($H_{1\beta}$, m), 5.97 ($H_{3\beta}$, m), 7.18 ($H_{9\beta}$, d, J~13 Hz), 7.46 ($H_{4\beta}$, d, J~13.0 Hz), 7.59 ($H_{4\alpha}$, dd, J~13.0, 5.5 Hz), 9.08 ($C_{21}CH_3$, d, J~6.2 Hz), 9.13 ($C_{26,27}{}^{2}CH_3$, d, J~6.6 Hz), 9.46 ($C_{18}CH_3$, s);[13] uv (ethanol) λ_{max} 263 nm, λ_{min} 227 nm; mass spectrum (80eV) m/e (rel int) 400 (M, 12), 382 (M-H_2O, 22), 364 (M-$2H_2O$, 14), 277 (25), 152 (38), 149 (base), 135 (30), 134 (30), 133 (27).

Not unexpectedly the nmr spectrum of 6 is very similar to that of 3.[13] The magnitude of the splitting observed for 6 ($J_{4\alpha,3\beta}$ ~ 5.5 Hz) and its correlation with the Karplus equation[4] implies that the A-ring exists predominantly (~70/30) in the conformation with the hydroxyl groups diaxial as follows:



This presumably results from an attractive intramolecular hydrogen bonding effect dominating over an unfavorable steric effect.[14] Finally we comment on the efficacy of the inversion reaction 7 → 8 (>76%), which we find surprising in view of the highly complex reaction observed for cholesterol. The 3-epimer of cholesterol was obtained in only <40% yield along with double bond participation and elimination products under exactly identical conditions or with other carboxylic acids (a result which was also recently reported by others[10b]). It is also noteworthy that the 3-monotosylate of 7 reacts cleanly at C-3 with $LiAlH_4$[7b] and with $(CH_3)_2CuLi$[15] whereas the corresponding reactions of cholesteryl tosylate are complicated by double bond participation.[16] We assert that in the reactions of 7 or its 3-monotosylate,[7b] the entering nucleophile associates with the 1α-substituent and is directed to C-3 from the α-side of the steroid.

Footnotes and References:

1. For part VIII of this series, see W. H. Okamura, M. N. Mitra, D. A. Procsal, and A. W. Norman, Biochem. Biophys. Res. Commun., 65, 24 (1975).

2. This study was supported by USPHS Grant AM-16595 and by a grant from the Intramural Research Fund of the University of California, Riverside.

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6. W. H. Okamura, A. W. Norman and R. M. Wing, Proc. Nat. Acad. Sci. U.S.A., 71, 4194 (1974).

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8. The 25-hydroxylated form of 6 has been reported in the literature [E. J. Semmler, M. F. Holick, H. K. Schnoes and H. F. DeLuca, Tetrahedron Lett., 4147 (1972)], but it was not completely characterized.

9. D. H. R. Barton, R. H. Hesse, M. M. Pechet and E. Rizzardo, J. Amer. Chem. Soc., 95, 2748 (1973); experimental details are given in reference 7b.

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11. The chemical shifts for the resonances due to the C_{19} and C_{18} angular methyl groups for a number of 1α and 3α-hydroxylated Δ^5, Δ^6, or $\Delta^{5,7}$ unsaturated cholestanes have been measured. The observed values (calculated values shown in parenthesis based on the parameters given in N. S. Bhacca and D. H. Williams, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, Calif., 1964, pp. 14-24) expressed in hertz downfield from TMS are as follows: 1α-hydroxycholest-5-ene[C_{19} 60.5 (61.5); C_{18} 41.0 (42.0)], 1α-hydroxy-5α-cholest-6-ene [C_{19} 46.0 (46.0); C_{18} 41.0 (42.5)], 3α-hydroxycholest-5-ene [C_{19} 60.0 (60.5); C_{18} 41.0 (41.5)], 3α-hydroxy-5α-cholest-6-ene [C_{19} 44.0 (45.0); C_{18} 41.0 (42.0)], 1α-hydroxycholesterol [C_{19} 61.5 (62.0); C_{18} 41.5 (42.0)], 1α-hydroxy-3-epicholesterol (8) [C_{19} 57.5 (61.5); C_{18} 40.5 (42.5)], 1α,3β-dihydroxy-5α-cholest-6-ene [C_{19} 48.5 (48.0); C_{18} 41.0 (43.0)]; 1α,3α-dihydroxy-5α-cholest-6-ene [C_{19} 43.5 (46.0); C_{18} 41.5 (43.0)]; 1α,3α-dihydroxycholesta-5,7-diene (10) [C_{19} 55.0 (56.0); C_{18} 37.5 (38.5)].

12. S. M. Sine, T. E. Conklin and W. H. Okamura, J. Org. Chem., 39, 3797 (1974).

13. The NMR parameters (300 MHz, $CDCl_3$, TMS) for the closely related 3: τ 3.66 and 4.02 ($H_{6,7}$, ABq, J_{AB} ~ 11.1 Hz), 4.71 (H_{19Z}, m), 5.03 (H_{19E}, m) 5.60 ($H_{1\beta}$, m), 5.81 ($H_{3\alpha}$, m), 7.20 ($H_{9\beta}$, d, J ~ 12.5 Hz), 7.42 ($H_{4\alpha}$, d, J ~ 13.0 Hz), 7.59 ($H_{4\beta}$, dd, J ~ 13.0, 6.5 Hz), 9.07 ($C_{21}CH_3$, d, J ~ 6.1 Hz), 9.12 ($C_{26,27}{}^2CH_3$, d, J ~ 6.6 Hz), 9.46 ($C_{18}CH_3$, s).

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15. W. H. Okamura, S. C. Carey and M. N. Mitra, to be submitted for publication.

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