STUDIES ON VITAMIN D AND ITS ANALOGS. VIII. 3-DEOXY-1α,25-DIHYDROXYVITAMIN D_3, A POTENT NEW ANALOG OF 1α,25-$(OH)_2$-D_3.

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Summary

The seven step chemical synthesis of 3-deoxy-1α,25-dihydroxyvitamin D_3 from 1α,25-dihydroxycholesterol is described. This steroid is a potentially most interesting analog of the hormonally active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3 since it lacks only the 3β-hydroxyl group. The newly synthesized steroid stimulated intestinal calcium transport virtually as effectively as 1α,25-dihydroxyvitamin D_3. Further, the new compound is the most effective analog known to date in terms of its ability to compete with 1α,25-dihydroxyvitamin D_3 in an *in vitro* intestinal receptor assay.

It appears well documented that 1α,25-dihydroxyvitamin D_3,[1] the active form of vitamin D_3, is a steroid hormone both from a structural and functional point of view (1,2). This metabolite is the most active form of vitamin D known. One intriguing question concerns the relative contributions of the various functional groups and topological features of this potent metabolite to the elicitation of its biological responses (3). In this regard, our laboratories (4-7), as well as Lam *et al.* (8), recently reported the synthesis of the analog 3-D-1α-OH-D_3, which lacks both the 3β- and 25-OH groups of 1α,25-$(OH)_2$-D_3. In an *in vivo* chick intestinal calcium transport assay this analog was found to be capable of producing a significant biological response (5). However, a larger dose and a longer time were required to produce the maximal response for 3-D-1α-OH-D_3 than were required for 1α,25-$(OH)_2$-D_3 (5,7). It seemed possible

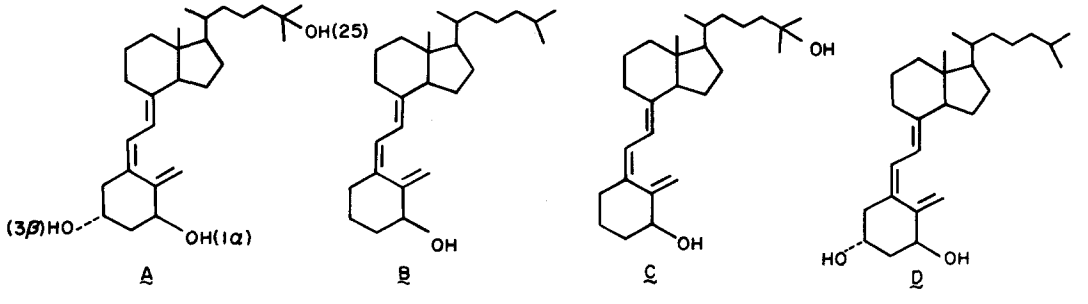
[1]Abbreviations employed are : 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3]; 3-deoxy-1α-hydroxyvitamin D_3 [3-D-1α-OH-D_3], 3-deoxy-1α,25-dihydroxyvitamin D_3, [3-D-1α,25-$(OH)_2$-D_3]; 1α-hydroxyvitamin D_3, [1α-OH-D_3]; 25-hydroxyvitamin D_3 [25-OH-D_3]; deoxyribonucleic acid, DNA.

that this result was due to the requirement of 3-D-1α-OH-D_3 to undergo 25-hydroxylation to give 3-D-1α,25-$(OH)_2$-D_3, before acting physiologically. This appeared likely because two other analogs, 5,6-trans-vitamin D_3 (9) and dihydrotachysterol$_3$ (10) have recently been shown to undergo 25-hydroxylation prior to production of biological responses. It is the purpose of this communication to describe the chemical synthesis of 3-D-1α,25-$(OH)_2$-D_3 and to report on certain aspects of its biological activity.

Experimental

1α,25-Dihydroxycholesteryl p-Toluenesulfonate F. The 1α,25-dihydroxycholesterol E was prepared from 25-hydroxycholesterol as previously described (11). The 25-hydroxycholesterol was a generous gift provided by Dr. Milan Uskoković of Hoffmann-La Roche, Nutley (12). A mixture of E (500 mg, 1.19 mmole), dry pyridine (5 ml), and p-toluenesulfonyl chloride (575 mg, 3 mmole) was maintained below 0° (freezer) for 30 hours. After work-up, crystallization of the residue from acetone-petroleum ether afforded 552 mg (81%, mp 138-9° dec.) of pure F: nmr ($CDCl_3$, TMS), τ2.08 and 2.54 (4HAr ring, ABq, J_{AB} ~8.0 Hz), 4.4 (H-6, br m), 5.1 (H-3α, br, W~20 Hz), 6.1 (H-1β, br, W~8 Hz), 7.52 ($ArCH_3$, s), 8.78 (C-26-27, $2CH_3$, s), 9.01 (C-19, CH_3, s), 9.07 (C-21, CH_3, d, J~5.5 Hz), and 9.33 (C-18, CH_3, s).

1α,25-Dihydroxycholest-5-ene G. A solution of lithium aluminum hydride (1.033 g, 27 mmole) and F (570 mg, 1.00 mmole) in anhydrous ether (150 ml) was refluxed for 20 hours. The cooled mixture was quenched by successive addition of water (1.03 ml), aqueous 15% sodium hydroxide (1.03 ml), and water (3.09 ml). After filtering and stripping, the crude residue was purified by chromatography on silica gel (petroleum ether-benzene) to afford 336 mg of G. Crystallization (acetone-petroleum ether) afforded 282 mg (70%, double mp 127-8° and 135-6°) of pure G: nmr ($CDCl_3$, TMS) τ4.5 (H-6, br m), 6.3 (H-1β, br, W~7 Hz), 8.78 (C-26-27, $2CH_3$, s), 8.98 (C-19, CH_3, s), 9.06 (C-21, CH_3, d, J~5.5 Hz), and 9.32 (C-18, CH_3, s); mass spectrum (80 eV) m/e 402 (parent ion).



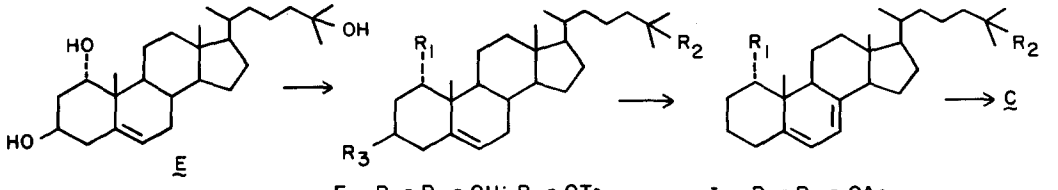
A B C D

Figure 1. Structure of 1α,25-dihydroxyvitamin D_3 (A); 3-deoxy-1α-hydroxyvitamin D_3 (B); 3-deoxy-1α,25-dihydroxyvitamin D_3 (C); 1α-hydroxyvitamin D_3 (D).

1α,25-Diacetoxycholest-5-ene H. A mixture of G (195 mg, 0.484 mmole), dry pyridine (4 ml), and acetic anhydride (4 ml) was heated at 90° for 24 hours. After work-up with water and ether, the crude diacetate was eluted through a short column of silica gel (2% acetone in benzene) to afford H (~100%) sufficiently pure for the preparation of I. Crystallization of H (methanol) afforded material with mp 106-107°: nmr ($CDCl_3$, TMS) τ4.5 (H-6, br m), 4.95 (H-1β, br, W~6 Hz), 7.93 and 8.02 (2 CH_3CO-, s), 8.56 (C-26-27, $2CH_3$, s), 8.93 (C-19, CH_3, s), 9.08 (C-21, CH_3, d, J~5.5 Hz), and 9.32 (C-18, CH_3, s); mass spectrum (80 eV) m/e 486 (parent ion).

1α,25-Dihydroxycholesta-5,7-diene J. The diacetate (222.8 mg, 0.46 mmole) in 1:1 benzene-hexane was brominated with 1,3-dibromo-5,5-dimethylhydantoin (67.5 mg, 0.23 mmole) exactly as previously described (6). The crude bromo compound in xylenes (10 ml) was added dropwise to refluxing s-collidine (14 ml) under nitrogen. The mixture was refluxed for an additional 30 minutes, cooled, and then after the usual work-up, the residue was chromatographed on 10% silver nitrate impregnated silica gel (linear gradient between equal volumes of petroleum ether and 1:1 ether-petroleum ether). Fractions containing I (λ_{max} 280 nm) and a more polar material (λ_{max} 312 nm) were pooled and concentrated. The residue was saponified (5% methanolic KOH, 45 ml, 25°, 12 hours) and then the crude diol J was purified by chromatography on silica gel (linear gradient between equal volumes of petroleum ether and ether). Fractions containing pure (tlc, uv) J (17 mg) were combined and concentrated. Crystallization (CH_3OH-H_2O) afforded material with mp 151-152°: nmr ($CDCl_3$, TMS) τ4.28 and 4.55 (H-6-7, ABq, J_{AB} 6 Hz), 6.31 (H-1β, br, W~6 Hz), 8.76 (C-26-27, 2 CH_3, s), 9.01 (C-21, CH_3, d, J~5.5 Hz) 9.06 (C-19, CH_3, s), and 9.35 (C-18, CH_3, s); mass spectrum (80 eV) m/e 400 (parent ion).

3-Deoxy-1α,25-dihydroxyvitamin D_3 C. The provitamin J (12 mg) in ether (100 ml) with ice cooling under nitrogen was photochemically irradiated (Hanovia 100 watt medium pressure mercury arc, quartz vessel with a NO. 9700 Corex filter sleeve) for 8.0 minutes. Concentration afforded a residue which was chromatographed on silver nitrate impregnated silica gel as described above. The fractions rich in previtamin (λ_{max} 258 nm) were pooled and then concentrated. The residue was heated in isooctane at 75° for 2.25 hours. Chromatography of the residue twice on silver nitrate impregnated silica gel as above afforded the purified C (1.4 mg, 12%). The purified C was homogeneous (free from J and previtamin and exhibited a single spot by tlc), showed λ_{max} 263 nm and λ_{min} 228 nm in the uv, and exhibited a parent ion of m/e 400 (parent ion) in the mass spectrum (80 eV).



F, $R_1 = R_2 = OH$; $R_3 = OTs$

G, $R_1 = R_2 = OH$; $R_3 = H$

H, $R_1 = R_2 = OAc$; $R_3 = H$

I, $R_1 = R_2 = OAc$

J, $R_1 = R_2 = OH$

Figure 2. Structure of 1α,25-dihydroxycholesterol (E); compounds F-J are referred to in the Experimental Section.

Bioassay (in vivo). The 3-D-1α,25-(OH)2-D3 was assayed along with 1α,25-$(OH)_2$-D_3 according to the procedure of Hibberd and Norman (13) for ability to stimulate intestinal calcium transport in vitamin D-deficient (rachitic) chicks. The results are given in Table 1.

Bioassay (in vitro). 3-D-1α,25-$(OH)_2$-D_3, 3-D-1α-OH-D_3 and 1α,25-$(OH)_2$-D_3 were assayed for their ability to interact in a competitive binding assay which is highly specific for 1α,25-$(OH)_2$-D_3 (14). The competitive binding assay is dependent upon the ability to duplicate, in vitro, the conditions which permit the saturable binding of tritiated 1α,25-$(OH)_2$-D_3 to chick intestinal chromatin in vivo (15). It consists of incubating Triton X-100 washed chromatin fractions

Table 1

Stimulation of Intestinal Calcium Transport[a]

Compound	Administered Dose	Time of Assay after Dosing	Intestinal Calcium Absorption (plasma $^{45}Ca^{2+}$)[b]	Relative Enhancement over Control
	(nmoles)	(hours)	(cpm/0.2ml ± SEM)	
Control (-D)	none	--	310 ± 15	1.0
1α,25-$(OH)_2$-D_3	6.5	8	1100 ± 30*	3.5
1α,25-$(OH)_2$-D_3	6.5	12	1200 ± 60*	3.9
1α,25-$(OH)_2$-D_3	6.5	16	1230 ± 40*	4.0
1α,25-$(OH)_2$-D_3	6.5	36	580 ± 15	1.9
1α,25-$(OH)_2$-D_3	0.26	12	1000 ± 40*	3.2
1α,25-$(OH)_2$-D_3	1.30	12	1010 ± 25*	3.2
3D-1α,25-$(OH)_2$-D_3	6.5	8	750 ± 30*	2.4
3D-1α,25-$(OH)_2$-D_3	6.5	12	800 ± 20*	2.4
3D-1α,25-$(OH)_2$-D_3	6.5	16	1060 ± 40*	3.4
3D-1α,25-$(OH)_2$-D_3	6.5	36	400 ± 12	1.3
3D-1α,25-$(OH)_2$-D_3	0.26	16	370 ± 20	1.2

[a]The steroids were administered intraperitoneally in 0.20 ml of 1,2-propanediol: ethanol, 1:1. At the indicated time an assay of intestinal calcium transport was carried out (13). For this assay 4.0 mg of $^{40}Ca^{2+}$ + $^{45}Ca^{2+}$ (2μCi) are placed in a duodenal loop, in vivo. Thirty minutes later the appearance of $^{45}Ca^{2+}$ is measured in the blood. Each number is the average ± SEM of groups of 8-10 birds.

[b]Values indicated by a * are significantly different from the control (-D) at $p < 0.01$.

(750 μg DNA), cytosol fraction (105,000 xg supernatant), 20 pmoles of ^{3}H-1α,25-(OH)$_2$-D$_3$, and the analog of choice at 25° for 45 min. Then the chromatin is reisolated by centrifugation, washed twice with 4 ml volumes of 0.5% Triton X-100 in 0.01 M Tris-HCl and the tritium content of the organic solvent extract of the pellet determined by liquid scintillation counting.

Results and Discussion

This report describes the details of the chemical synthesis of 3-D-1α,25-(OH)$_2$-D$_3$, which lacks only the 3β-OH of the natural metabolite 1α,25-(OH)$_2$-D$_3$. The synthesis was achieved in seven steps from 1α,25-dihydroxycholesterol, but it requires two more steps (81% and 70% yields) to synthesize 3-D-1α,25-(OH)$_2$-D$_3$ than it does 1α,25-(OH)$_2$-D$_3$ from the same intermediate. However, the new analog is an important compound for evaluating more critically our present understanding of structure-function relationships for vitamin D$_3$.

The results given in Table 1 clearly indicate that 3-D-1α,25-(OH)$_2$-D$_3$ is quite biologically active in terms of its ability to stimulate the intestinal absorption of calcium. It appears to be at least as active as 1α,25-(OH)$_2$-D$_3$ when assayed at comparable dose levels. It is known that there is a lag of 24-36 hours before the appearance of the biological response when cholecalciferol (D$_3$) is administered (1) which reflects the necessity for metabolism to 1α,25-(OH)$_2$-D$_3$. While the onset of biological response to 3-D-1α,25-(OH)$_2$-D$_3$ (12-16 hours) is significantly more rapid than that of vitamin D$_3$, it is still slightly slower than that of 1α,25-(OH)$_2$-D$_3$ (8-12 hours).

As shown in Figure 3, 3-D-1α,25-(OH)$_2$-D$_3$ is a most effective competitor for the cytosol-chromatin receptor for 1α,25-(OH)$_2$-D$_3$. The concentrations required for a 50% decrease in maximal radioactivity bound to the chromatin were 1:8:900:900:5000:>10,000 for 1α,25-(OH)$_2$-D$_3$: 3-D-1α,25-(OH)$_2$-D$_3$: 25-OH-D$_3$: 1α-OH-D$_3$: 3-D-1α-OH-D$_3$: D$_3$ respectively. Thus while the absence of the 3β-hydroxyl does reduce the effectiveness of the competitor (1/8 as effective as 1α,25-(OH)$_2$-D$_3$), it is a much smaller effect than that observed for the separate absence of the 1α-hydroxyl or 25-hydroxyl (both 1/900 as effective as 1α,25-(OH)$_2$-D$_3$). Further 3D-1α,25-(OH)$_2$-D$_3$ is 600 x more effective a competitor than 3-D-1α-OH-D$_3$, while 1α,25-(OH)$_2$-D$_3$ is 900 x more effective than 1α-OH-D$_3$. These results emphasize the

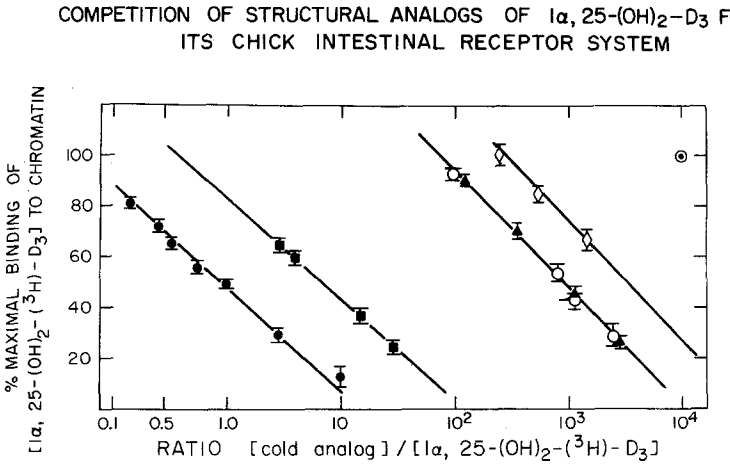


Figure 3. Competition of cholecalciferol analogs with tritiated 1α,25-$(OH)_2$-D_3 for its chick intestinal receptor system, in vitro. Increasing concentrations of nonradioactive analogs were incubated with a reconstituted cytosol-chromatin receptor system with 2.0 x 10^{-8}M 1α,25-$(OH)_2$ [^{3}H]-D_3. The percent of the maximal amount of radioactivity bound to the chromatin is plotted as a function of the relative concentration of analog and 1α,25-$(OH)_2$-[^{3}H]-D_3 present in the assay mixture (●—●) 1α,25-$(OH)_2$-D_3; (◇—◇) 3-D-1α-OH-D_3; (■—■) 3-D-1α,25-$(OH)_2$-D_3; (○—○) 1α-OH-D_3; (▲—▲) 25-(OH)-D_3; (⊙) vitamin D_3.

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importance of the 25-hydroxyl. Clearly both the 1α-OH and 25-OH are essential structural features of vitamin D steroids which may interact in this system. Further studies are currently in progress to evaluate the structure-function relationship of the interaction of 3-D-1α,25-$(OH)_2$-D_3 and related cogeners in other vitamin D target systems.

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