CHEMICAL SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3-DEOXY-1α-HYDROXYVITAMIN D_3 AN ANALOG OF 1α,25-$(OH)_2$-D_3, THE ACTIVE FORM OF VITAMIN D_3

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Summary

The chemical synthesis of 3-deoxy-1α-hydroxyvitamin D_3 from cholesterol is described. This steroid is a highly important analog of the hormonally active form of vitamin D, 1α,25-dihydroxyvitamin D_3; it is the only analog presently available for structure-function studies which lacks the 3β-hydroxyl but retains the key 1α-hydroxyl of 1α,25-dihydroxyvitamin D_3. The new steroid is highly biologically active; it stimulated intestinal calcium absorption significantly more rapidly than vitamin D_3 and as rapidly as 1α,25-dihydroxyvitamin D_3.

Introduction

It is now well documented that the steroid 1α,25-dihydroxyvitamin D_3[1] is the likely hormonally active form of vitamin D in the intestine in terms of stimulating intestinal calcium transport (1-3). 1α,25-$(OH)_2$-D_3 is some 5-15 X more active than the parent vitamin D_3 and 3-5 X more active than its precursor 25-OH-D_3 (4,5).

One intriguing question relating to the high biological activity of 1α,25-$(OH)_2$-D_3 concerns a detailed examination of the structure-function relationships of this steroid (see Figure 1). What are the relative contributions to biological response of the characteristic functional groups i.e. the 1α, 3β, and 25 hydroxyls, the C-19-methylene, the triene system and side-chain skeleton? It is already apparent that the most important functional group on 1α,25-$(OH)_2$-D_3 is the 1α-hydroxyl since it is well documented that neither 25-OH-D_3 nor vita-

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

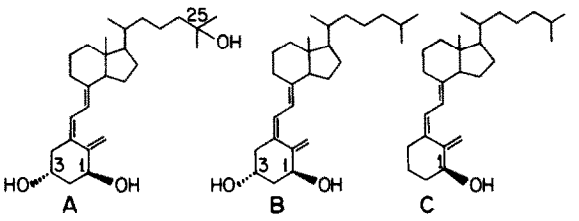

Figure 1. Structure of 1α,25-dihydroxyvitamin D_3 (A), 1α-hydroxyvitamin D_3 (B) and 3-deoxy-1α-hydroxyvitamin D_3 (C).

min D_3 are biologically active in anephric animals (6)[2] or man (10). The synthesis of 1α-OH-D_3 has already been reported (11-13). Furthermore, this compound also is highly biologically active in anephric animals (14) and humans with kidney failure (Coburn et al., submitted for publication). The results of Brumbaugh and Haussler (15) suggest that while 1α-OH-D_3 is functional in a 1α,25-$(OH)_2$-D_3 steroid receptor assay, its activity is markedly increased if it has become 25-hydroxylated.

To date no data are available concerning the importance of the 3β-hydroxyl of 1α,25-$(OH)_2$-D_3 for biological activity. It is the purpose of this communication to report the chemical synthesis and biological effectiveness of 3-deoxy-1α-hydroxy-vitamin D_3. This analog lacks this 3β-OH functional group.

Experimental

The new analog 3-D-1α-OH-D_3 (see Figure 1, C) was synthesized from cholesterol as summarized in Figure 2. Cholesterol was converted in two steps as previously described (16,11) to the epoxydienone E, which was reduced successively with lithium aluminum hydride and then lithium in ammonia to give F and G respectively. The 1α-hydroxycholest-5-ene, G, could also be synthesized from E by first conversion to 1α-hydroxycholesterol, H, by Barton's method (11). The latter, H, was selectively tosylated (p-toluenesulfonyl chloride, pyridine) and then reduced (lithium aluminum hydride) to give I and G, respectively. The structure of G was established from the fact that it exhibited appropriate

[2]The production of 1α,25-$(OH)_2$-D_3 occurs via a two-step hydroxylation process; the 25-OH is introduced in the liver (7) and the 1α-OH in the kidney (8,9).

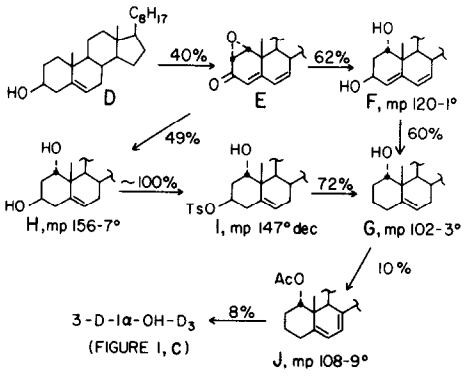

Figure 2. Synthesis of 3-D-1α-OH-D_3 (C) from cholesterol (D).

spectroscopic (NMR, IR, and mass spectrometry) and microanalytical data (Calcd: C, 83.87, H, 11.99. Found: C, 83.71, H, 12.34) and that it was convertible by catalytic hydrogenation to the known 1α-hydroxy-5α-cholestane (17). A detailed description of the experimental procedure will be forthcoming (Mitra et al., in press). The alcohol G was acetylated (acetic anhydride, pyridine), brominated (1,3-dibromo-5,5-dimethylhydantoin), and then dehydrobrominated (trimethylphosphite) to give the provitamin J. The latter was saponified (potassium hydroxide), photochemically irradiated (Hanovia 100 watt mercury arc, quartz, ether) and then heated to give 3-D-1α-OH-D_3. The production of the vitamin from G is well documented for converting other Δ^5-enes to the corresponding vitamin (18). The analog 3-D-1α-OH-D_3 has thus far been obtained only as a liquid. The material used for bioassay described below was homogeneous by TLC, exhibited the characteristic vitamin D ultraviolet spectrum with λ max 263 nm and λ min 227 (see Figure 3), and revealed an appropriate parent ion (m/e 384) in its mass spectrum.

The 3-D-1α-OH-D_3 was assayed along with 1α,25-$(OH)_2$-D_3, 1α-OH-D_3 and vitamin D_3 by the procedure of Hibberd and Norman (19) for ability to stimulate intestinal calcium transport in rachitic (vitamin D-deficient) chicks. These results are presented in Table 1.

Results and Discussion

This is the first reported chemical synthesis of an analog of 1α,25-

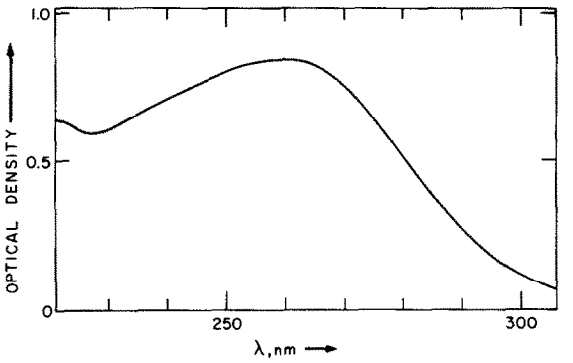

Figure 3. Ultraviolet absorption of 3-deoxy-1α-hydroxyvitamin D_3.

$(OH)_2$-D_3 or vitamin D_3 which lacks the 3β-hydroxyl group. The synthesis of 3-D-1α-OH-D_3 was accomplished in 9-10 steps from cholesterol. This is only 1-2 steps greater than the previously reported chemical synthesis of 1α-OH-D_3 from cholesterol (11,12).

The results presented in Table 1 clearly demonstrate that 3-D-1α-OH-D_3 is highly biologically active in stimulating intestinal calcium transport in vitamin D deficient chicks. At the doses of 3-D-1α-OH-D_3 employed, the maximum transport level was 7 X greater than that of the rachitic, (-D) control birds and was even 1.5 X greater than the response obtained from administering D_3 or 1α,25-$(OH)_2$-D_3.

There is a characteristic time lag of 24-36 hours before the appearance of the biological response when the parent vitamin D is administered (20), which is a reflection of its obligatory metabolism to 1α,25-$(OH)_2$-D_3. Only 9-12 hours are required for the maximal biological response when 1α,25-$(OH)_2$-D_3 (4) or its analog, 1α-OH-D_3, (14) are bioassayed. Thus it is of considerable interest that 3-D-1α-OH-D_3 produced a greater response at 12 hours than at 24 hours in a manner similar to 1α,25-$(OH)_2$-D_3. The simplest interpretation is that the 3β-hydroxyl of 1α,25-$(OH)_2$-D_3 is not obligatorily required for elicitation of a biological response in the intestine. The slightly slower time for maximal response (12 hrs vs 9 hrs) of 3-D-1α-OH-D_3 as compared to 1α,25-$(OH)_2$-D_3 is possibly a reflection of a requirement for 25-hydroxylation

Table 1

Biological Response:
Stimulation of Intestinal Calcium Transport[a]

Compound	Administered dose	Time of assay after dosing	Intestinal Calcium[b] Absorption (plasma $^{45}Ca^{2+}$)	Relative Enhancement over control
	(nmoles)	(hours)	(cpm/0.20 ml±SEM)	
Control	None	-	430 ± 15	1.0
D_3	1.3	10	620 ± 18	1.4
D_3	1.3	24	1360 ± 40*	3.2
D_3	2.6	24	2060 ± 65*	4.8
D_3	26.0	24	1730 ± 72*	4.0
$1\alpha,25\text{-}(OH)_2\text{-}D_3$	0.6	10	1950 ± 68*	4.5
$1\alpha,25\text{-}(OH)_2\text{-}D_3$	0.6	24	780 ± 21	1.8
$1\alpha\text{-}OH\text{-}D_3$	1.6	10	2010 ± 52*	4.7
$1\alpha\text{-}OH\text{-}D_3$	0.8	24	1920 ± 64*	4.5
3-D-1α-OH-D_3	26.0	9	1047 ± 67*	2.4
3-D-1α-OH-D_3	26.0	12	3000 ± 220*	7.0
3-D-1α-OH-D_3	26.0	24	1930 ± 95*	4.5
3-D-1α-OH-D_3	5.2	24	1880 ± 96*	4.4

[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 exactly as described by Hibberd and Norman (19). 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 for groups of 6-8 birds.

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

of these steroids as suggested by Brumbaugh and Haussler (15), and this laboratory (Norman and Procsal, unpublished observations). There are no reports of

enzymes in higher organisms capable of 3β-hydroxylation. The 3β-OH is introduced into the steroid nucleus at the squalene to lanosterol step in the pathway of cholesterol biosynthesis (21).

It remains to the future to precisely define the structure requirements of $1\alpha,25\text{-}(OH)_2\text{-}D_3$ necessary for optimum biological function. In light of the recent report of Okamura et al. (22) an essential concern will be a description of the three-dimensional dynamic topology of active species. However, it is already apparent that $3\text{-}D\text{-}1\alpha\text{-}OH\text{-}D_3$ will be a very important component of this program.

Acknowledgements

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