SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3-DEOXY-1α-HYDROXYVITAMIN D_3 +

by

H. Y. Lam, B. L. Onisko, H. K. Schnoes and H. F. DeLuca

Department of Biochemistry

College of Agricultural and Life Sciences

University of Wisconsin-Madison

Madison, Wisconsin 53706

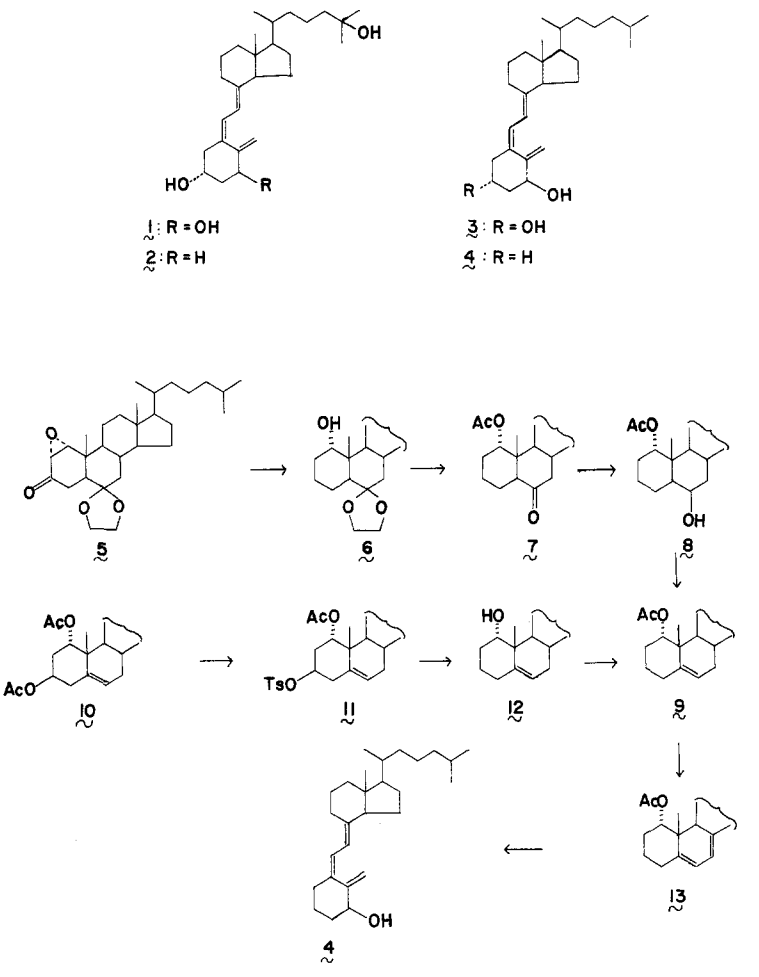
Received June 11,1974

A vitamin D_3 analog, 3-deoxy-1α-hydroxyvitamin D_3 has been synthesized. The compound is active in promoting intestinal calcium transport and bone mineral mobilization.

Conversion of vitamin D_3 to its biologically active metabolite 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$, 1)[1] involves two sequential hydroxylation reactions (1-3). The first, occurring primarily in the liver, leads to 25-hydroxyvitamin D_3 (25-OH-D_3) (2), which in turn serves as substrate for hydroxylation at C-1 by a kidney mitochondrial enzyme. This two-step transformation naturally raises the question of the exact functional significance of the various hydroxy groups in 1,25-$(OH)_2D_3$. Conceivably, the hydroxy groups at C-3 and/or C-25, though essential for the intermediary metabolism of the vitamin, might play no important functional role in determining the expression of vitamin D activity. As part of a general exploration of the structure/activity relationships of the vitamin D system we have prepared, therefore, some simple analogs of 1 which might contribute information bearing on this question. We have already shown (4) that 1α-hydroxyvitamin D_3 (1α-OH-D_3) (3), the 25-deoxy analog of 1 possesses very potent vitamin D activity (intestinal calcium transport, bone calcium mobilization, and antirachitic activity) although its action may well depend on prior hydroxylation at C-25.

+This work was supported by PHS grants No. AM-14881 and No. AM-15512 and the Wisconsin Alumni Research Foundation.

[1]Abbreviations: 1,25-dihydroxyvitamin D_3, 1,25-$(OH)_2D_3$; 25-hydroxyvitamin D_3, 25-OH-D_3; 1α-hydroxyvitamin D_3, 1α-OH-D_3.



SCHEME I

The same compound has since then been prepared by a number of other groups (5-7). Results presented in this communication establish that the 3,25-dideoxy analog of 1 (3-deoxy-1α-hydroxyvitamin D_3, 4) still exhibits vitamin D activity as measured by the intestinal calcium transport and bone calcium mobilization assays

In considering a synthesis of 4 we attempted to make use of intermediates available from an earlier preparation of 1α-OH-D_3 (3) (4). The approaches outlined in Scheme I were, therefore, developed. Epoxyketone 5 (an intermediate of our synthesis of 1α-OH-D_3 (4) and available by procedures described earlier (8)) upon treatment with hydrazine hydrate and subsequent catalytic (Pd/C, H_2)

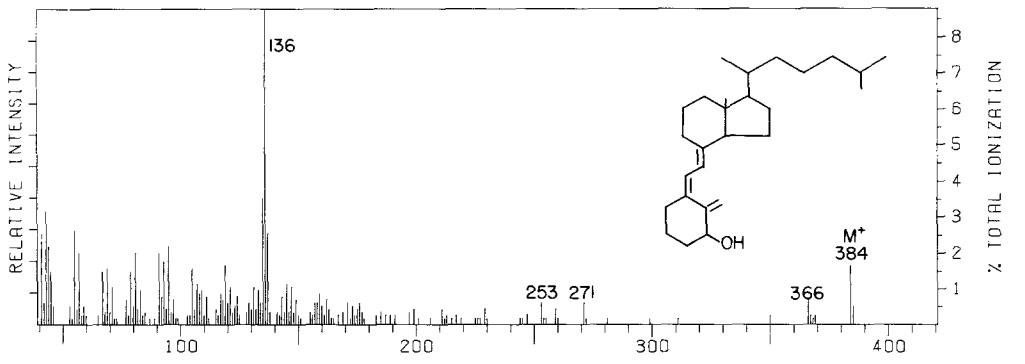


Figure 1. Mass spectrum of synthetic 3-deoxy-1α-hydroxyvitamin D_3.

reduction of the intermediate Δ^2-olefin afforded 1α-hydroxy-derivative 6 (mp 96-98°). Removal of the ketal and acetylation gave 7 (mp 104-105°), which after reduction ($NaBH_4$) to the 6β-alcohol 8 (mp 127-128°) and dehydration ($POCl_3$) yielded the desired Δ^5-cholestene intermediate 9. Another route to 9 involves partial deacetylation of 1α-hydroxy cholesteryl diacetate (10, another intermediate of our previous synthesis of 1α-OH-D_3 (4)), to 1α-acetoxycholesterol, which upon tosylation to 11 and hydride reduction gave 12. Acetylation of 12 then led to 9. Conversion of 9 to the vitamin analog involved the usual allylic bromination and dehydrobromination sequence to 13 (λ_{max} 295, 283, 273 nm), which upon irradiation in ether (9) furnished the previtamin D derivative. The latter was equilibrated to the corresponding vitamin D_3 system by warming in ethanol under nitrogen and subsequent hydrolysis (KOH/MeOH) then yielded 3-deoxy-1α-hydroxyvitamin D_3 (4). Compound 11 exhibited the expected ultraviolet spectrum, λ_{max} 264.5 nm, λ_{min} 229 nm and gave the mass spectrum shown in Figure 1.

Vitamin analog 4 is active in stimulating both intestinal calcium transport and bone mineral mobilization (Table 1). Stimulation of calcium transport is essentially the same as that given by an equivalent dose of 1α-OH-D_3 (3), but the 3-deoxy analog appears to be less active on bone (4). Differential responses of the intestinal and bone systems to several vitamin D metabolites have been observed before (e.g. 12, 13) and in general the bone calcium mobiliza-

Table 1. Intestinal Calcium Transport and Bone Calcium Mobilization Activity of Analog 4 and 1α-OH-D_3 (3).

Dose	Time after dosing (hours)	Ca serosal/Ca mucosal	Serum Ca (mg%)
Control	12.5	1.9 ± 0.2	4.5 ± 0.2
0.25 μg of 4	12.5	3.4 ± 0.4	5.8 ± 0.2
Control	14	1.5 ± 0.2	4.3 ± 0.1
0.25 μg of 1α-OH-D_3	14	2.9 ± 0.2	6.7 ± 0.1

Rats were fed a vitamin D-deficient, low calcium diet for 3-1/2 weeks (10). Groups of six rats then received a dose of 0.25 μg of analog 4 (intrajugularly in 0.05 ml ethanol as solvent), 0.25 μg of 1α-OH-D_3 (3) or 0.05 ml of ethanol alone (controls). The rats were killed at the indicated time after dosing and calcium transport (Ca serosal/Ca mucosal) and bone mineral mobilization (serum calcium, mg%) activity was measured following published procedures (11). Data are expressed as mean ± standard error.

tion system appears more sensitive to structural departures from the 1,25-dihydroxy-substitution pattern than the intestinal calcium transport mechanism. Basic differences in structural requirements of the respective active sites or more trivial effects (e.g. transport to the site of action) seem equally plausible explanations for this phenomenon at present. We consider it unlikely that analog 4 undergoes hydroxylation at C-3 (i.e. to 1α-OH-D_3, 3) in vivo and the very similar activity pattern observed for 1α-OH-D_3-3-methyl ether (14) tends to support this assumption. Our activity data would, therefore, indicate that a C-3 hydroxy group (i.e. the hydroxyl originally present in the steroid precursor of the D-vitamins) plays no crucial functional role in the expression of vitamin D activity. The

question regarding the significance of a C-25-hydroxy group remains open, since analog 4, like the structurally similar dihydrotachysterols (15) may well undergo hydroxylation at that position. Experiments currently in progress in our laboratories may help resolve that ambiguity.

References

1. Holick, M. F., Schnoes, H. K., DeLuca, H. F., Suda, T., and Cousins, R. J. (1971) Biochemistry 10, 2799-2804.
2. Lawson, D. E. M., Fraser, D. R., Kodicek, E., Morris, H. R., and Williams, D. H. (1971) Nature 230, 228-230.
3. Omdahl, J. L., and DeLuca, H. F. (1973) Physiol. Rev. 53, 327-372.
4. Holick, M. F., Semmler, E. J., Schnoes, H. K., and DeLuca, H. F. (1973) Science 180, 190-191.
5. Barton, D. H. R., Hesse, R. H., Pechet, M. M., and Rizzardo, E. (1973) J. Am. Chem. Soc. 95, 2748-2749.
6. Kaneko, C., Yamada, S., Sugimoto, A., Eguchi, Y., Ishikawa, M., Suda, T., Suzuki, M., Kakuta, S., and Sasaki, S. (1974) Steroids 23, 75-92.
7. Harrison, R. G., Lythgoe, B., and Wright, P. W. (1973) Tetrahedron Letters 37, 3649-3652.
8. Semmler, E. J., Holick, M. F., Schnoes, H. K., and DeLuca, H. F. (1972) Tetrahedron Letters 40, 4147-4150.
9. Blunt, J. W., and DeLuca, H. F. (1969) Biochemistry 8, 671-675.
10. Suda, T., DeLuca, H. F., and Tanaka, Y. (1970) J. Nutr. 100, 1049-1052.
11. Martin, D. L., and DeLuca, H. F. (1969) Am. J. Physiol. 216, 1351-1359.
12. Lam, H. Y., Schnoes, H. K., DeLuca, H. F., and Chen, T. C. (1973) Biochemistry 12, 4851-4855.
13. Holick, M. F., Kleiner-Bossaller, A., Schnoes, H. K., Kasten, P. M., Boyle, I. T., and DeLuca, H. F. (1973) J. Biol. Chem. 248, 6691-6696.
14. Chen, A. F., Schnoes, H. K., and DeLuca, H. F., unpublished work.
15. Hallick, R. B., and DeLuca, H. F. (1971) J. Biol. Chem. 246, 5733-5738.