BIOLOGICAL ACTIVITY OF 1β-HYDROXY-VITAMIN D_3

Implications for the steric structure of the active form of vitamin D

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1. Introduction

A number of compounds are known to have similar biological activity as the active form of vitamin D_3, the hormonal metabolite 1α,25-dihydroxy-vitamin D_3 (ref [1], 1α25-$(OH)_2D_3$). Among these active materials are 5,6 *trans*vitamin D_3 (B), dihydrotachysterol$_3$(C), 3-desoxy-1α-hydroxy-vitamin D_3 (D) their 25-hydroxy analogues as well as the clinically useful 1α-hydroxy-vitamin D_3 (A, 1α-HOD_3). All these compounds possess the vitamin D_3 skeleton substituted at position 1 with a hydroxy group having the same configuration as 1α-hydroxy-vitamin D_3 (these compounds are drawn up in the respective formulae as 1β-hydroxy derivatives which is their actual configuration, since they all possess the 6,7-*s trans* conformation). However, nothing, has been reported on the activity of vitamin D_3 analogues having the hydroxy group at C-1 in the enantiomeric configuration. Thus in order to establish whether it is essential for the hydroxy function in vitamin D analogues to possess the α-configuration to elicit biological responses, it was necessary to synthesize the 1β-hydroxy-vitamin D_3 (1β-HOD_3) and to determine its activity.

2. Experimental

2.1. *Biological assays*

The biological response of the animals to vitamin D and its derivatives was assessed by daily dosing of the steroids over seven days at which time the amounts of the vitamin and the analogues required to maintain an equivalent response was assessed [2]. This type of bioassay overcomes the problem of different time courses of response which arises in comparing the activity of the vitamin D_3 metabolites.

The intestinal response of the chicks to these steroids was assessed by measuring the changes they effected in intestinal calcium-binding protein (CaBP) levels [3] or by measuring the proportion of an oral dose of ^{45}Ca absorbed [4]. CaBP was determined by the Laurell method of immunoelectrophoresis using pure chick intestinal CaBP as standard. The degree of endochondrial calcification in rat tibia achieved by the steroids was determined by the radiographic method [5]. In a third type of assay the effect of these steroids on calcium mobilisation from bone of rats and chicks was assessed by recording the changes in plasma calcium levels effected by the steroids in animals on a low calcium diet.

2.2. *Chemical synthesis*

2.2.1. 1-keto-previtamin D_3 (G)

(i) A solution of 100 mg 1α-hydroxy-vitamin D_3 (A) in 10 ml dry ethyl ether was treated at room temperature with 350 mg freshly prepared manganese dioxide for 6 h. The reaction mixture was filtered through a celite column and the filtrate was evaporated to dryness at room temperature. Chromatography on silica gel using an ethyl acetate: chloroform mixture (3:7) resulted in 35 mg 1-keto-previtamin D_3 (G). NMR spectrum:

δ ppm 0.74 (3H, *s*, 18-H), 1.69 (3H, *s*, 19-H), 5.99

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and 6.13 (2H, AB *q*, *J* 11 Hz-, 6-H and 7-H), 5.51 (1H, *m*, 9-H) and 4.02 (1H, heptet, *J* 8.6 and 4.3 Hz, 3-H); ultraviolet λ_{max} 287, 236 nm (ϵ 10 000, 9500).

(ii) A solution of 100 mg of 1α-hydroxy-previtamin D_3 (F) in 10 ml ether was treated with 650 mg freshly prepared manganese dioxide for 6 h. Isolation as above resulted in 85 mg 1-keto-previtamin D_3 (G).

2.2.2. 1β-Hydroxy-previtamin D_3 (H)

A solution of 50 mg 1-keto-previtamin D_3 (G) in 20 ml methanol was treated with 100 mg sodium borohydride at 0°C for 30 min, extracted with ether and washed with brine. The ethereal extract was dried over magnesium sulphate and evaporated at 0°C to dryness. The residue was chromatographed on silica gel. Elution with a mixture of ethyl acetate:chloroform (3:7) gave 35 mg of 1β-hydroxy-previtamin D_3 (H). NMR spectrum:

δ ppm 0.70 (3H, *s*, 18-H), 1.70 (3H, *s*, 19-H), 5.56 (1H, *s*, 9-H), 5.78 and 5.94 (2H, AB *q*, *J* 11.5 Hz, 6-H and 7-H), 3.93 (1H, broad *s*, $w_{\frac{1}{2}}$ 11 Hz, 3-H) and 4.22 (1H, *m*, broad *s*, $w_{\frac{1}{2}}$ 10.5 Hz 1-H); ultraviolet, λ_{max} 259 nm (ϵ 10 000).

2.2.3. 1β-Hydroxy-vitamin D_3 (E)

A solution of 30 mg 1β-hydroxy-previtamin D_3 (H) in 10 ml iso-octane was heated under a nitrogen atmosphere at 60°C for 4 h. The solvent was evaporated to dryness and the residue was chromatographed on silica gel. Elution with ethyl either gave a yield of 25 mg 1β-hydroxy-vitamin D_3 (E).

2.3. *Other chemicals and methods*

1α-(OH)D_3 was synthesised as described previously [6] and vitamin D_3 was a crystalline product obtained from Sigma.

Plasma calcium levels were determined by atomic absorption spectroscopy and protein was determined spectrophotometrically.

Animals, Rhode Island Red X Light Sussex chicks (1 day old) were raised on a vitamin D-deficient diet and used after 4 weeks when they were deficient [7]. Piebald weanling rats were raised on a low phosphate vitamin D-deficient diet for two weeks by which time they were rachitic. In the appropriate experiments groups of animals were placed on a low calcium vitamin D-deficient diet. The steroids were dissolved in propylene glycol and administered orally at appropriate levels.

3. Results and discussion

In none of the vitamin D-sensitive biological responses used in this study could an effect with 1β-(OH)D_3 be obtained. As much as 10 μg of this steroid did not produce any detectable amount of CaBP in chick intestine. Since 0.5 μg of vitamin D_3 gives a readily measurable amount of this protein (table 1) the potency of this compound in stimu-

Table 1
Relative effectiveness of vitamin D_3, 1α- and 1β-hydroxy-vitamin D_3 in chick intestinal CaBP synthesis and rat endochondral calcification

Compound	Dose level (μg)	CaBP levels (μg/mg protein)	Dose level (ng)	Calcification score
Vitamin D_3	0.5	9.2	50	3.4
	1.0	24.6	100	4.3
	2.0	34.0	200	6.9
1α(OH)D_3				
	2.0	35.9	50	2.6
	4.0	42.5	100	5.3
1β-(OH)D_3	10.0	ND	200	0

Vitamin D-deficient birds and rats were dosed daily so as to receive the amounts indicated over 7 days. 24 h later the birds were killed and intestinal cytosol prepared for estimation of CaBP (3). ND, no observable precipition line whatsoever. The rats were X-rayed after 7 days and 14 days and the degree of healing assessed. The scores are those obtained after 14 days

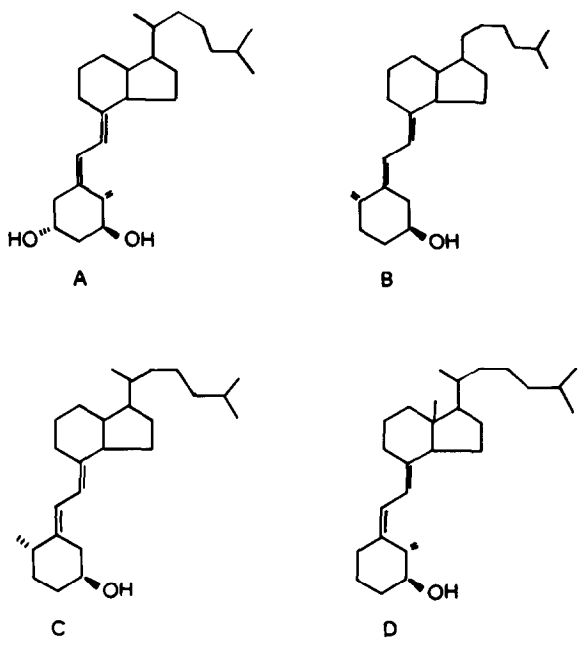

Fig.1. Structures of 1α-HOD$_3$ (A); 5,6-*trans*vitamin D$_3$ (B); dihydrotachysterol$_3$ (C); 3-desoxy-1α-hydroxyvitamin D$_3$ (D).

lating CaBP synthesis must be assessed as less than 5% of vitamin D$_3$. Experience with other compounds with very low vitamin D activity suggests that the activity of 1β-(OH)D$_3$ is less than 1% of the activity of the parent vitamin. Similar findings were obtained in the other bioassay systems so that for example 0.25 μg of the 1β-(OH)D$_3$ failed to produce any effect on calcification in the rat or on elevation of plasma calcium in rat or chick. In both of these latter systems a good response was obtained to increasing amounts of both vitamins D$_3$ and 1α-(OH)D$_3$.

In the course of this work the 1-keto-vitamin D$_3$ compound was prepared, but it was found to be unstable and to undergo rapid re-arrangement to 1-keto-previtamin D$_3$. The biological activity of the 1-keto-vitamin D$_3$ compound was also low in that 0.5 μg was unable to increase the proportion of an oral dose of ^{45}Ca absorbed whereas 125 ng of vitamin D$_3$ gave a measurable response.

Since the discovery of 1,25-(OH)$_2$D$_3$ as the active form of vitamin D$_3$ there have been several studies in the different systems responsive to it of the features of the molecule necessary for biological activity (see ref. [1] for recent review). There are several convincing pieces of

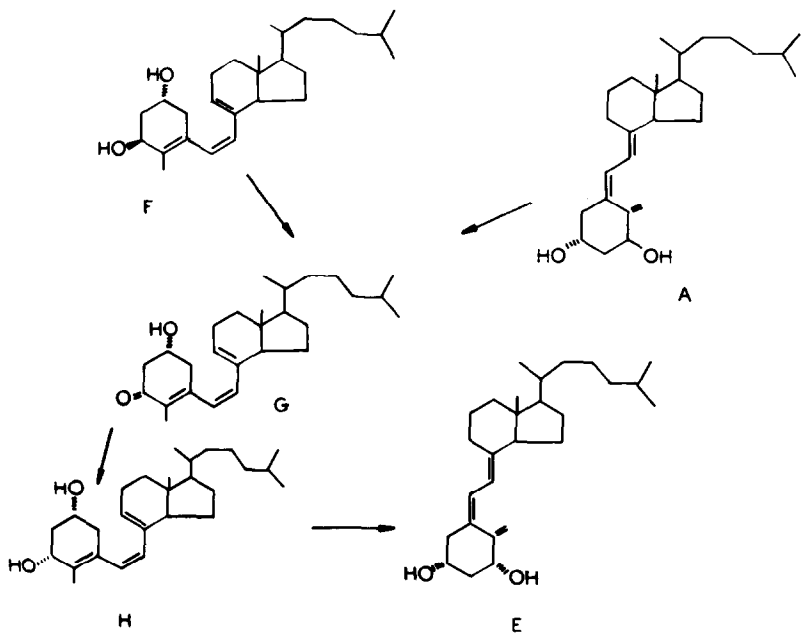

Fig.2. Routes for synthesis of 1β-HOD$_3$. 1β-hydroxyvitamin D$_3$ (E); 1α-hydroxy-previtamin D$_3$ (F); 1-keto-previtamin D$_3$ (G); 1β-hydroxy-previtamin D$_3$ (H).

information that the natural form of the hormone has the hydroxyl group at C-1 in the α-position. Thus the insertion of this hydroxyl group by the 1-hydroxylase to form $1,25\text{-}(OH)_2D_3$ involves loss of ^3H specifically located in the 1α-position of the substrate [7]. Furthermore chemical syntheses have been described which yield specifically $1\alpha 25\text{-}(OH)_2D_3$ and the biological potency of this material corresponds completely with the biologically produced substance [8]. The synthesis of $1\beta\text{-}(HO)D_3$ described here and its very low biological potency confirms that the natural $1,25\text{-}(OH)_2D_3$ has an almost absolute requirement for the 1-hydroxyl group to be in the α-position (i.e., above the plane of the A ring of the vitamin D molecule).

References

[1] Schnoes, H. K. and DeLuca, H. F. (1976) Ann. Revs. Biochem. 46, 631–666.
[2] Holick, M. F., Kasten-Schraufrogel, P., Tavela, T. and DeLuca, H. F. (1975) Arch. Biochem. Biophys. 166, 63–66.
[3] Bar, A. and Wasserman, R. H. (1974) J. Nutr. 104, 1202–1207.
[4] Emtage, J. S., Lawson, D. E. M. and Kodicek, E. (1974) Biochem. J. 144, 339–346.
[5] Kodicek, E. and Lawson, D. E. M. (1967) in: The Vitamins (Gyorgy, P. and Pearson, W. N. eds) pp. 211–244, Acad. Press, New York.
[6] Freeman, D., Archer, A. and Mazur, Y. (1975) Tetrahedron Lett. 43, 261–265.
[7] Lawson, D. E. M., Wilson, P. W. and Kodicek, E. (1969) Biochem. J. 115, 269–277.
[8] Semmler, E. J., Holick, M. F., Schnoes, H. K. and DeLuca, H. F. (1972) Tetrahedron Lett. 40, 4147–4151.