SYNTHESIS AND BIOLOGICAL ACTION OF 3-DEOXY-VITAMIN D_3 AND 3-DEOXY-25-HYDROXYVITAMIN D_3

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1. Introduction

$1,25(OH)_2D_3$ is considered to be the active metabolite of vitamin D, particularly in the intestine. This compound is produced in vivo from vitamin D by C-25 hydroxylation in the liver, followed by C-1 hydroxylation in the kidney. When $1,25(OH)_2D_3$ interacts with intestinal cells, a specific CaBP is formed.

The characteristic functional groups of $1,25(OH)_2D_3$ are the 1α, 3β and 25-hydroxy groups. Of these, only the 3β-hydroxyl is present in the parent vitamin molecule. Therefore, the 2-step transformation of the active form raises the question of the significance of this hydroxy group for the intermediary metabolism and the subsequent biological activity of the vitamin. Previous studies have shown however, that 3-deoxy analogues of $1,25(OH)_2D_3$ are only slightly less potent biologically than the natural metabolite [1–3].

This paper reports the chemical synthesis of 3-deoxy-D_3 and 3-deoxy-$25(OH)D_3$ and their biological activity in chicks. The latter was evaluated by monitoring growth, plasma calcium, bone ash, duodenal CaBP and the renal $25(OH)D_3$-1-hydroxylase.

Abbreviations: D_3, vitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; $1(OH)D_3$, 1-hydroxyvitamin D_3; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; CaBP, calcium-binding protein; TLC, thin-layer chromatography

2. Experimental

2.1. *Measurements of biological activity*

Day-old White Leghorn males kept in a windowless room were fed for 28 days a semi-purified vitamin D-deficient diet containing 1.2% calcium and 0.7% phosphorus [4]. At the age of 21 days, the chicks were divided into 5 groups. Birds of each group were injected subcutaneously either with daily doses of 0.25 μg D_3, 0.25 or 100 μg 3-deoxy-D_3, or 0.25 or 100 μg 3-deoxy-$25(OH)D_3$. The sterols were dissolved in propylene glycol.

At the age of 28 days, heparinized blood samples were obtained from 6 birds of each group before they were killed by dislocation of the neck. Plasma calcium concentration was determined by an EGTA titration using an automatic titrator (Precision Systems, USA). The duodenal mucosa was homogenized [5] and assayed for CaBP using a radioimmunoassay with a sensitivity of 30 pg [6].

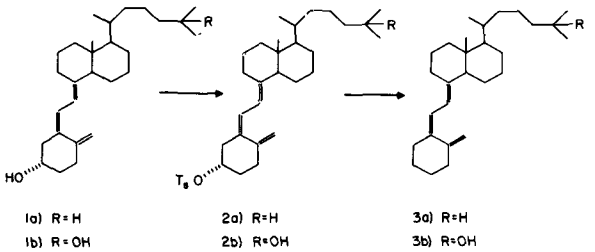
Bones were cleaned of adhering tissues, dried and ashed [7].

2.2. *Synthesis of 3-deoxy-25(OH)D_3 and 3-deoxy-D_3 (scheme 1)*

2.2.1. 3-Tosyl-$25(OH)D_3$ (2b)

150 mg of $25(OH)D_3$ (1b) were dissolved in 4 ml dry pyridine. The solution was treated with 115 mg tosyl chloride and left at room temperature (25°C) overnight. The reaction mixture was then extracted

Scheme 1
Synthetic steps for the preparation of 3-deoxy-D_3 and 3-deoxy-25(OH)D_3



with diethyl ether, washed with cold 5% aq. HCl and with water, and dried over $MgSO_4$. The diethyl ether was evaporated and the residue was chromatographed on a preparative silica-gel TLC plate with diethyl ether. The resulting compound (2b) (100 mg) (ultraviolet λ_{max} 264 nm, 232 nm; ϵ, 17 500, 14 000 l. mol^{-1} . cm^{-1}) showed by ^{1}H NMR spectroscopy signals at δ 0.55, 1.13, 4.66, 4.76, 4.99, 5.87, 6.02, 7.26 and 7.71 ppm, *m/e* (M^+) 554.

2.2.2. 3-Deoxy-D_3 (3a)

D_3-tosylate (2a) [8] 100 mg were dissolved in 6 ml dry tetrahydrofuran and chilled to 0°C. The solution was treated with 0.2 ml 2 M lithium triisobutylborohydride solution and was left for 30 min at 0°C and 6 h at room temperature (25°C). Excess hydride was decomposed with water. The organoborane was oxidized with 5 ml 3 N NaOH and 5 ml 30% H_2O_2. The mixture was then extracted with pentane, washed with water and dried over $MgSO_4$. The dry residue was chromatographed on a preparative silica-gel TCL plate with hexane. The resulting compound, 3-deoxy-D_3 (3a) (20 mg) (λ_{max}, 264 nm; ϵ, 17 500 l. mol^{-1} cm^{-1}), showed by ^{1}H NMR spectroscopy signals at δ 0.55, 4.66, 4.92, 5.92 and 6.02 ppm, *m/e* (M^+) 366.3464 (calc. 366.3453).

2.2.3. 3-Deoxy-25(OH)D_3 (3b)

3-Tosyl-25(OH)D_3 (2b), 100 mg, were treated as above but the resulting reaction mixture was chromatographed on a preparative silica-gel TLC plate with hexane/diethyl ether (2:1, v/v). The resulting compound 3-deoxy-25(OH)D_3 (3b) (15 mg) (λ_{max} 264 nm; ϵ, 17 500 l. mol^{-1} cm^{-1}) showed by ^{1}H NMR spectroscopy signals at δ 0.55, 1.13, 4.66, 4.92, 5.92 and 9.02 ppm, *m/e* (M^+) 384.3375 (calc. 384.3392).

2.3. *Renal 25(OH)D_3-1-hydroxylase*

Three vitamin D-deficient chicks were killed by decapitation, their kidneys were removed, washed with cold saline, pooled and homogenised. The homogenate was assayed for 1-hydroxylase activity by 10 min incubation with [26, 27-^{3}H]25(OH)D_3 or [26, 27-^{3}H]3-deoxy-25(OH)D_3 as substrates [9]. The concentration of either substrate was 15 ng/1.5 ml incubation mixture. The metabolites produced were chromatographed on Sephadex LH-20 columns. The columns (1 × 65 cm) were equilibrated and developed with chloroform : petroleum ether 40°–60° : methanol (75:23:2, v/v/v) [10].

2.4. *Radioactive materials and measurements*

[26, 27-^{3}H]25(OH)D_3 (spec. radioact. 9.0 Ci/mmol) was obtained from the Radiochemical Centre (Amersham, Bucks).

[26, 27-^{3}H]3-deoxy-25(OH)D_3 was prepared in our laboratories from [26, 27-^{3}H]25(OH)D_3 as above.

All radioactivity measurements were carried out in a Packard Tri-Carb automatic liquid-scintillation spectrometer no. 3390. Lipid samples were counted for radioactivity in a solution of 100 mg 1,4-bis-(5-phenyloxazol-2-yl) benzene and 4 mg 2,5-diphenyloxazole/l. toluene. Quenching was corrected for by using the automatic external standardization and correlation curve for ^{3}H.

3. Results

Results shown in table 1 indicate that final body weight and % bone ash were significantly ($P < 0.05$) higher in the D_3 and the high 3-deoxy-25(OH)D_3-treated chicks than in the other 3 groups which did not differ significantly ($P > 0.05$) from each other. Plasma calcium concentration was normal only in the D_3 and the high 3-deoxy-25(OH)D_3-treated birds. Hypocalcemia was observed in all other groups, although in the birds which were treated with high doses of 3-deoxy-D_3 the severity of the hypocalcemia was reduced.

Duodenal CaBP was high in the D_3-treated chicks,

Table 1
Body weight, plasma calcium, bone ash and duodenal CaBP of chicks treated with the various vitamin D derivatives

Vitamin D derivative	Dose (μg/day)	Final body weight (g)	Plasma calcium (mg/dl)	Bone ash (%)	Duodenal CaBP (mg/g)
D_3	0.25	222 ± 4[a,f]	10.6 ± 0.2[a]	37.0 ± 0.7[a]	1405 ± 97[a]
3-Deoxy-D_3	0.25	174 ± 13[b]	6.3 ± 0.1[b]	28.3 ± 0.9[b]	11 ± 2[b]
3-Deoxy-D_3	100	176 ± 9[b]	8.4 ± 0.7[c]	29.2 ± 0.3[b]	133 ± 17[c]
3-Deoxy-25(OH)D_3	0.25	178 ± 15[b]	5.9 ± 0.2[b]	27.6 ± 0.9[b]	45 ± 3[d]
3-Deoxy-25(OH)D_3	100	222 ± 15[a]	10.7 ± 0.4[a]	38.1 ± 0.5[a]	924 ± 170[e]

[a–e] Means designated by different letters differ significantly ($P < 0.01$)
[f] Mean ± SEM, obtained from 6 birds

slightly lower in the high 3-deoxy-25(OH)D_3-treated chicks, and very low in the rest. The levels measured in the low 3-deoxy-D_3-treated chicks were close to the detection limit. Slightly higher levels were measured in the low 3-deoxy-25(OH)D_3-treated chicks, and still higher levels for high 3-deoxy-D_3-treated chicks. Since the most important functional group of 1,25$(OH)_2D_3$ is the hydroxyl at C-1, the observed biological effectiveness of 3-deoxy-25(OH)D_3, which was found to be lower than that of D_3, suggested that 1-hydroxylation of 3-deoxy-D_3 might be impaired. In order to test this possibility, kidney homogenates obtained from vitamin D-deficient chicks were incubated with either [26, 27-^{3}H]25(OH)D_3 or [26, 27-^{3}H]3-deoxy-25(OH)D_3. Results given in fig.1 show that the conversion of [26, 27-^{3}H]3-deoxy-25(OH)D_3 into its corresponding 1-hydroxylated derivative was impaired. The efficiency of the conversions of [26, 27-^{3}H]3-deoxy-25(OH)D_3 and of [26, 27-^{3}H]25(OH)D_3 to their 1-hydroxylated derivatives were estimated at 3.8% and 14.3%, respectively.

4. Discussion

This report details the chemical synthesis of 3-deoxy-D_3 and 3-deoxy-25(OH)D_3, which lack the 3β-hydroxyl of the natural vitamin. The synthesis was achieved in 2 steps from D_3 or from 25(OH)D_3, respectively. Of the several criteria used here to evaluate the biological activity of these analogues of vitamin D, intestinal CaBP appeared to be most sensitive, in agreement with [11,12]. Furthermore,

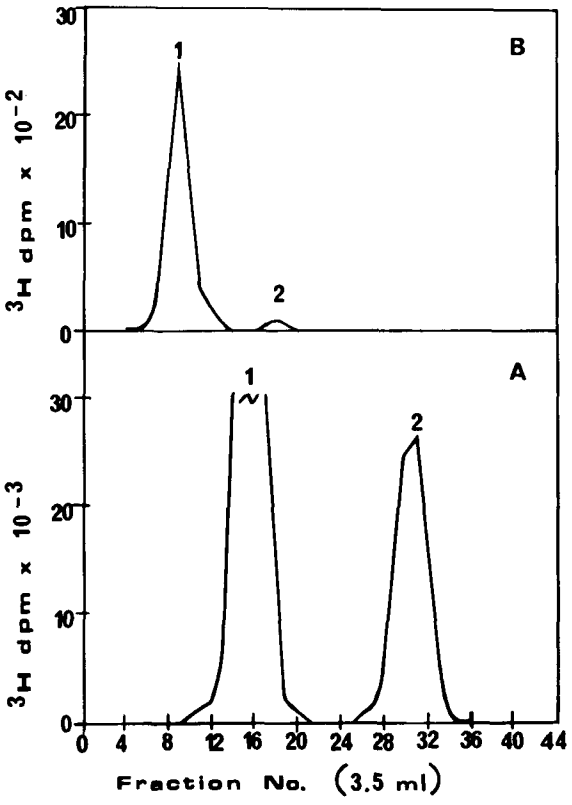


Fig.1. Sephadex LH-20 chromatography of lipid extracts from kidney homogenates which were incubated with [26, 27-^{3}H]25(OH)D_3 (A) and [26, 27-^{3}H]3-deoxy-25(OH)D_3 (B). Curves: (A) peaks 1 and 2 are 25(OH)D_3 and 1,25$(OH)_2D_3$, respectively; (B) peaks 1 and 2 are 3-deoxy-25(OH)D_3 and 3-deoxy-1,25$(OH)_2D_3$, respectively.

the present radioimmunoassay with its high sensitivity, made possible the distinction between vitamin D analogues which possess a low biological activity.

A 0.25 μg D_3/day dose was sufficient to promote normal weight gain, bone ash, plasma calcium and duodenal CaBP. The two 3-deoxy-analogues given at the same doses were found to be inactive, although a slight response in terms of duodenal CaBP was noted with 3-deoxy-25(OH)D_3. With doses as high as 100 μg/day, 3-deoxy-25(OH)D_3 produced a response similar to the lower dose of D_3. The 3-deoxy-D_3, on the other hand, produced only marginal responses. Thus, 3-deoxy-25(OH)D_3 is more potent biologically than 3-deoxy-D_3, and the 3β-hydroxyl is required for the 25-hydroxylation step of D_3.

The low biological activity of 3-deoxy-25(OH)D_3 relative to D_3 itself, suggested that the 3β-hydroxyl was required for the 1-hydroxylation step in the kidney. This has been demonstrated also by the poor in vitro conversion of this analogue to its 1-hydroxy-derivative by kidney homogenates. However, the 3-deoxy-analogues which had already been hydroxylated at C-1, were shown [3] only slightly inferior to 1,25$(OH)_2D_3$. Therefore, the 3β-hydroxy group is more important for the intermediary metabolism of the vitamin than for its action on the target organ.

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