

## SYNTHESIS AND BIOLOGICAL ACTIVITY OF 3 $\beta$ -FLUOROVITAMIN D<sub>3</sub>: COMPARISON OF THE BIOLOGICAL ACTIVITY OF 3 $\beta$ -FLUOROVITAMIN D<sub>3</sub> AND 3-DEOXYVITAMIN D<sub>3</sub>

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**Summary**—The alteration in the biologic activity of the vitamin D<sub>3</sub> molecule resulting from the replacement of a hydrogen atom with a fluorine atom is a subject of fundamental interest. To investigate this problem we synthesized 3 $\beta$ -fluorovitamin D<sub>3</sub> **6** and its hydrogen analog, 3-deoxyvitamin D<sub>3</sub> **7**, and tested the biologic activity of each by *in vitro* and *in vivo* methods.

Contrary to previous reports which showed that 3 $\beta$ -fluorovitamin D<sub>3</sub> was as active as vitamin D<sub>3</sub> *in vivo*, we found that the fluoro-analog was less active than vitamin D<sub>3</sub>. With regard to stimulation of intestinal calcium transport and bone calcium mobilization in the D-deficient hypocalcemic rat, 3 $\beta$ -fluorovitamin D<sub>3</sub> showed significantly greater biologic activity than its hydrogen analog, 3-deoxyvitamin D<sub>3</sub>. In the organ-cultured, embryonic chick duodenum, 3 $\beta$ -fluorovitamin D<sub>3</sub> was approx 1/1000th as active as the native hormone, 1,25-dihydroxyvitamin D<sub>3</sub>, while 3-deoxyvitamin D<sub>3</sub> was inactive even at  $\mu$ M concentrations, in the induction of the vitamin D-dependent, calcium-binding protein. With regard to *in vitro* activity in displacing radiolabeled 25-hydroxyvitamin D<sub>3</sub> from vitamin D binding protein and radiolabelled 1,25-dihydroxyvitamin D<sub>3</sub> from a chick intestinal cytosol receptor, 3 $\beta$ -fluorovitamin D<sub>3</sub> and 3 $\beta$ -deoxyvitamin D<sub>3</sub> both showed very poor binding efficiencies when compared with vitamin D<sub>3</sub>. Our results show that the substitution of a fluorine atom for a hydrogen atom at the C-3 position of the vitamin D<sub>3</sub> molecule results in a fluorovitamin **6** with significantly more biological activity than its hydrogen analog, 3-deoxyvitamin D<sub>3</sub> **7**.

### INTRODUCTION

The study of fluorinated analogs of vitamin D sterols has received attention recently because of the ability of these analogs to block hydroxylation at C-24 of the side-chain of the sterol and because of their ability to either enhance or decrease the biological activity of the molecule in various *in vitro* and *in vivo* systems [1-7]. The synthesis and biological activity of 3 $\beta$ -fluorovitamin D<sub>3</sub> and the synthesis of 3 $\beta$ -fluorovitamin D<sub>2</sub> and 3 $\beta$ -fluorovitamin D<sub>2</sub> has been previously reported [8-11]. However, in the previously cited work, the newly synthesized fluorosterol was tested at a high dose only and, therefore, no true assessment of biological function could be made. As a result of these biological studies it was concluded that 3 $\beta$ -fluorovitamin D<sub>3</sub> was as active as vitamin D<sub>3</sub> *in vivo* [10]. Protein binding studies with vitamin D binding protein and chick intestinal cytosol receptor were not performed. Studies of the induction of the vitamin D-dependent

calcium binding protein in chick duodenal organ culture were also not performed. We now report a modified route for the synthesis of 3 $\beta$ -fluorovitamin D<sub>3</sub>,<sup>1</sup> and report that, contrary to a previous study, 3 $\beta$ -fluorovitamin D<sub>3</sub> is much less active than vitamin D<sub>3</sub> *in vivo*, but equipotent *in vitro*. However, it is considerably more active than 3-deoxyvitamin D<sub>3</sub>,<sup>2</sup> both *in vivo* and *in vitro*; in fact, 3-deoxyvitamin D<sub>3</sub> is inactive in the *in vitro* induction of the vitamin D-dependent, calcium-binding protein in the organ-cultured duodenum.

### EXPERIMENTAL

#### General

Ultraviolet spectra (u.v.) were taken in methanol with a Beckman Model 35 recording spectrophotometer (Beckman Instruments, Palo Alto, CA). Mass spectra were obtained on a Kratos MS50/DS-55 mass spectrometer-computer system (Kratos Instruments, U.K.). High performance liquid chromatography (HPLC) was performed on a Waters liquid chromatograph featuring two Model M-6000A pumps; a Model 450 u.v. detector, a Model 660 gradient programmer (all from Waters Associates, Milford, MA) and a Model 3380A Hewlett Packard integrator (Hewlett Packard, Avondale, PA). The standard HPLC conditions under which each synthetic compound was purified for final spectroscopic

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<sup>1</sup>Abbreviation: 3 $\beta$ -Fluoro-vitamin D<sub>3</sub>, 5(Z),7(E),3 $\beta$ -fluoro-9,10-seco-5,7,10(19)-cholestadiene.

<sup>2</sup>Abbreviation: 3-Deoxyvitamin D<sub>3</sub>, 5(Z),7(E),3-deoxy-9,10-seco-5,7,10(19)-cholestadiene.

characterization were: Varian Micropak MCH-10, 50 cm  $\times$  8 mm reverse phase column, methanol eluant, 5 ml/min, u.v. detector 265 nm. Nuclear magnetic resonance spectra (NMR) were obtained in deuterated chloroform with 0.1% tetramethylsilane on an IBM NR-80 Fourier transform nuclear magnetic resonance spectrometer (IBM Instruments, Danbury, CT).  $^{45}\text{CaCl}_2$  was obtained from New England Nuclear (New England Nuclear, Boston MA). Biological activity of the various compounds was tested *in vivo* as described previously [12–14]. Binding assays using vitamin D binding protein and chick intestinal cytosol receptor were performed as described before [15]. The induction of the vitamin D-dependent calcium binding protein was studied in organ-cultured duodena as described in detail elsewhere [16, 17].

### Synthesis

**I. Diels Alder reaction of cholesta-5,7-diene-3 $\beta$ -ol 1 with 4-phenyl-1,2,4-triazoline-3,5-dione.** The 4-phenyl-1,2,4-triazoline-3,5-dione reagent was prepared by the method outlined by Cookson *et al.* [18] and purified by sublimation. The triazoline reagent (0.90 g, 5.2 mmol) was dissolved in 25 ml dry acetone and added dropwise to **1** (2.0 g, 5.2 mmol) in 25 ml dry  $\text{CHCl}_3$  until the first sign of a faint pink color persisted. The solvent was removed under reduced pressure resulting in a colorless foam which was recrystallized from acetone to give 5 $\alpha$ ,8 $\alpha$ -(4-phenyl-1,2-urazolo)cholesta-6-ene **2** (2.7 g, 4.6 mmol, 90% yield). u.v.  $\lambda_{\text{max}} = 253$ ,  $\lambda_{\text{min}} = 240$  nm. NMR  $\delta$  3.16 (d of d,  $J = 12.0$  Hz,  $J = 5.3$  Hz, 2H, 4 $\alpha$ -H, 4 $\beta$ -H), 4.43 (m, 1H, 3 $\alpha$ -H), 6.23, 6.42 (ABq, 2H,  $J = 10.4$  Hz, 6, 7-H), 7.41 (m, 5H, Ar). MS:  $m/z$  (assignment, relative intensity) 559 ( $\text{M}^+$ , 0 (not seen)), 384 ( $\text{M}^+$  - Diels Alder adduct,  $\text{C}_{27}\text{H}_{44}\text{O}$ , calculated 384.3381, found 384.3369, 42), 382 ( $\text{M}^+$  - Diels Alder adduct + 2H, 22), 366 (384 -  $\text{H}_2\text{O}$ , 11), 364 (382 -  $\text{H}_2\text{O}$ , 43), 351 (366 -  $\text{CH}_3$ , 28), 349 (364 -  $\text{CH}_3$ , 12), 253 (366 - side chain, 12), 251 (364 - side chain, 21), 177 (adduct + 2H, 100).

### II. Fluorination of 5 $\alpha$ ,8 $\alpha$ -(4-phenyl-1,2-urazolo)cholesta-6-ene **2** with diethylaminosulfur trifluoride (DAST)

Into 25 ml of dry  $\text{CH}_2\text{Cl}_2$  was dissolved the DAST reagent (Aldrich, 1.6 g, 10.0 mmol). The solution was cooled under nitrogen to  $-70^\circ\text{C}$  in a dry ice-acetone bath. Over a 5 min period, **2** (1.2 g, 2.1 mmol) dissolved in 25 ml of dry acetone was added. The solution was stirred for 15 min at dry ice-acetone temperatures. The cooling bath was removed to allow the solution to warm to room temperature for 35 min. The reaction mixture was quenched with 10 ml of 5%  $\text{Na}_2\text{CO}_3$  (5 mmol). An additional 100 ml of  $\text{CH}_2\text{Cl}_2$  was added. The organic layer was separated and then washed with a saturated NaCl (aqueous) solution and a water wash. The organic layer was dried over

$\text{Na}_2\text{SO}_4$  before removal of solvent under reduced pressure to give a yellow oil. The oil was applied to a 35  $\times$  1 cm silica gel 60 column and eluted with 400 ml hexane, 400 ml 2.5% ethyl acetate-97.5% hexane, followed by 400 ml 5% ethyl acetate-95% hexane to give 3 $\beta$ -fluoro-5 $\alpha$ ,8 $\alpha$ -(4-phenyl-1,2-urazolo)cholesta-6-ene **3** (500 mg, 0.88 mmol, 35% yield). The HPLC retention time of **3** was 7.3 min under standard conditions (reverse phase). u.v.  $\lambda_{\text{max}} = 253$  nm,  $\lambda_{\text{min}} = 240$  nm. NMR  $\delta$  3.40 (d of m,  $J = 14.7$  Hz, 2H, 4 $\alpha$  - H, 4 $\beta$  - H), 5.33 (d of m,  $J = 47.2$  Hz, 1H, 3 $\alpha$  - H), 6.25, 6.48 (ABq, 2H,  $J = 13$  Hz, 6, 7-H), 7.41 (m, 5H, Ar). MS: 561 ( $\text{M}^+$ , 0), 386 ( $\text{M}^+$  - adduct,  $\text{C}_{27}\text{H}_{43}\text{F}$ , calculated 386.3354, found 386.3350, 68), 366 (386 - HF, 31), 351 (366 -  $\text{CH}_3$ , 42), 253 (366-side chain, 54), 199 (253 -  $\text{C}_4\text{H}_6$ , 28), 177 (adduct + 2H, 20), 163 (A ring + B ring, 77), 143 (163 - HF, 33).

### III. Reaction of 3 $\beta$ -fluoro-5 $\alpha$ ,8 $\alpha$ -(4-phenyl-1,2-urazolo)cholesta-6-ene **4** with lithium aluminum hydride in THF

Into 50 ml of freshly distilled THF was added **3** (400 mg, 0.71 mmol) along with 400 mg of lithium aluminum hydride. After the mixture was refluxed under argon for 2 h, 10 ml ethyl acetate was added. The mixture was filtered, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated to a slightly yellow oil. The oil was applied to a 25  $\times$  1 cm silica gel 60 column and eluted with 10% ethyl acetate-90% hexane eluant. The first fraction to elute was 3 $\beta$ -fluoro-cholesta-5,7-diene **4** (230 mg, 0.60 mmol, 84% yield). HPLC retention time (standard conditions) was 27 min. u.v.  $\lambda_{\text{max}} = 292$ , 282, 272, 263 nm. NMR  $\delta$  2.55 (m, 2H, 4 $\alpha$ -H, 4 $\beta$ -H), 4.53 (d of m,  $J = 52$  Hz, 1H, 3 $\alpha$ -H), 5.39, 5.61 (ABq,  $J = 6.7$  Hz, 2H, 6, 7-H). MS: 386 ( $\text{M}^+$   $\text{C}_{27}\text{H}_{43}\text{F}$  calculated 386.3354, found 386.3323, 81), 366 ( $\text{M}^+$  - HF, 49), 351 (366 -  $\text{CH}_3$ , 44), 253 (366 - side chain, 100), 199 (253 -  $\text{C}_4\text{H}_6$ , 42), 163 (A ring + B ring, 88), 143 (163 - HF, 55).

### IV. Photolysis of 3 $\beta$ -fluoro-cholesta-5,7-diene **4**

Into 100 ml of absolute ethanol (deoxygenated by bubbling nitrogen) was dissolved **4** (10 mg, 0.025 mmol). The solution was transferred to a quartz photolysis tube cooled with circulating ice water. The solution was irradiated for 10 min with a Hanovia lamp fitted with a Vycor filter ( $\lambda > 280$  nm transmitted). The solvent was removed by rotovap stripping under mechanical vacuum pump pressure maintaining the water bath temperature around  $10^\circ\text{C}$ . The residue was dissolved in methanol- $\text{CHCl}_3$  (2 ml) and injected onto the HPLC under standard conditions to collect 3 $\beta$ -fluoro-9,10-seco-cholesta-5(10), 6-cis, 8-triene **5** (3.5 mg, 0.0091 mmol, 35% yield). Final purification by HPLC under standard conditions showed a retention time of 18.0 min. The yield was determined from the u.v. assuming  $\epsilon = 8860$ . u.v.  $\lambda_{\text{max}} = 265$  nm,  $\lambda_{\text{min}} = 228$  nm. NMR:  $\delta$  1.52 (s, 3H, 19- $\text{CH}_3$ ), 4.80 (d of m,  $J = 48$  Hz, 1H,

$3\alpha$ -H), 5.60, 5.81 (ABq,  $J = 19.9$  Hz, 2H, 6, 7-H). MS: ( $M^+ C_{27}H_{43}F$  calculated 386.3354, found 386.3303, 26), 366 ( $M^+ - HF$ , 14), 273, 386 ( $M^+ - \text{side chain}$ , 20), 253 (366 - side chain, 19), 163 (A ring +  $C_4H_5$ , 36), 143 (163 - HF, 22), 138 (A ring +  $C_2H_2$ , 100), 118 (138 - HF, 10).

V. Thermal *H*-shift of  $3\beta$ -fluoro-9,10-seco-cholesta-5-(10),6-cis, 8-triene **5**

Into 50 ml of methanol (deoxygenated) was dissolved **5** (2.0 mg, 0.0052 mmol). The solution was heated under a nitrogen atmosphere for 3 days at 37°C. The solvent was removed by rotovap stripping under mechanical vacuum pump pressure maintaining the water bath temperature around 10°C. The residue was dissolved in ~2 ml of  $CHCl_3/CH_3OH$  and injected onto the HPLC under standard conditions to collect **5(Z)**, **7(E)**,  $3\beta$ -fluoro-9,10-seco-5,7,10(19)-cholestadiene **6** (1.5 mg, 0.0039 mmol, 75% yield). The retention time was 16.5 min. Yield was determined from u.v. assuming  $\epsilon = 16,880$ . u.v.  $\lambda_{max} = 265$  nm,  $\lambda_{min} = 228$ . NMR:  $\delta$  0.54 (s, 3H, 18- $CH_3$ ), 2.57 (d of d,  $J = 20$  Hz,  $J = 5.3$  Hz, 2H, 4 $\alpha$ -H, 4 $\beta$ -H), 4.75 (d of m,  $J = 48$  Hz, 1H, 3 $\alpha$ -H), 4.87 (d,  $J = 2.7$  Hz, 1H, 19(E)), 5.08 (d(br),  $J = 1.0$  Hz, 1H, 19(Z)), 6.05, 6.25 (ABq,  $J = 16.2$  Hz, 2H, 6, 7-H). MS: 386 ( $M^+ C_{27}H_{43}F$ , calculated 386.3354, found 386.3304), 366 ( $M^+ - HF$ , 26), 351 (366 -  $CH_3$ ), 273 ( $M^+ - \text{side chain}$ , 9), 253 (366 - side chain, 41), 199 (253 -  $C_4H_6$ , 15), 163 (A ring +  $C_4H_5$ , 17), 143 (163 - HF, 26), 138 (A ring +  $C_2H_2$ , 75), 118 (138 - HF, 13).

Synthesis of **5(Z)**, **7(E)**, 3-deoxy-9,10-seco-5,7,10(19)-cholestadiene **7**

The synthesis of **7** was carried out as outlined by Edelstein *et al.* [19]. u.v.  $\lambda_{max} = 265$ ,  $\lambda_{min} = 228$  nm.

NMR  $\delta$  0.55 (s, 3H, 18- $CH_3$ ), 4.77 (d,  $J = 2.7$  Hz, 1H, 19(E)), 4.98 (m, 1H, 19(Z)), 6.04, 6.15 (ABq,  $J = 19.2$  Hz, 2H, 6, 7-H). MS: 368 ( $M^+$ ,  $C_{27}H_{44}$ , calculated 368.3432, found 368.3440, 50), 366 ( $M^+ - 2H$ , 13), 353 ( $M^+ - CH_3$ , 10), 351 (366 - 2H, 5), 255 ( $M^+ - \text{side chain}$ , 27), 253 (366 - side chain, 9), 201 (255 -  $C_4H_6$ , 9), 145 (A ring +  $C_4H_3$ , 23), 120 (A ring +  $C_2H_2$ , 100).

## RESULTS

We report an efficient synthesis of  $3\beta$ -fluorovitamin D<sub>3</sub> (Fig. 1). Cycloaddition of 4-phenyl-1,2,4-triazoline-3,5-dione to **1** gives adduct **2** in 90% yield. Reacting **2** with diethylaminosulfur trifluoride followed by  $Na_2CO_3$  quench and  $NaCl_{(aq)}$  wash gives the  $3\beta$ -fluoro analog **3** in 35% yield. Removal of the protection group from **3** by lithium aluminum hydride in THF gives the  $3\beta$ -fluoroprovitamin **4** in 70% yield. Photolysis of **4** with  $\lambda > 280$  nm gives  $3\beta$ -fluoroprevitamin **5** in 35% yield. Thermal isomerization of **5** in methanol at 37°C affords  $3\beta$ -fluoro-vitamin D<sub>3</sub> **6** in 75% yield.

As shown in Fig. 2,  $3\beta$ -fluorovitamin D<sub>3</sub> causes an increase in intestinal calcium transport at a dose of 500 pmol/rat. This response increases in a dose dependent manner such that at 500,000 pmol/rat the serosal/mucosal intestinal calcium transport *in vitro* is  $5.89 \pm 0.72$ , a value higher than that of the 3-deoxyvitamin D<sub>3</sub> analog but considerably less than that of vitamin D<sub>3</sub>.  $3\beta$ -Fluorovitamin D<sub>3</sub> (Fig. 3) mobilizes bone calcium at a dose of 5,000 pmol/rat whereas the 3-deoxy compound is inert in this respect even at a dose as high as 500,000 pmol/rat.  $3\beta$ -Fluorovitamin D<sub>3</sub> is, however, much less active with respect to bone calcium mobilization than vita-

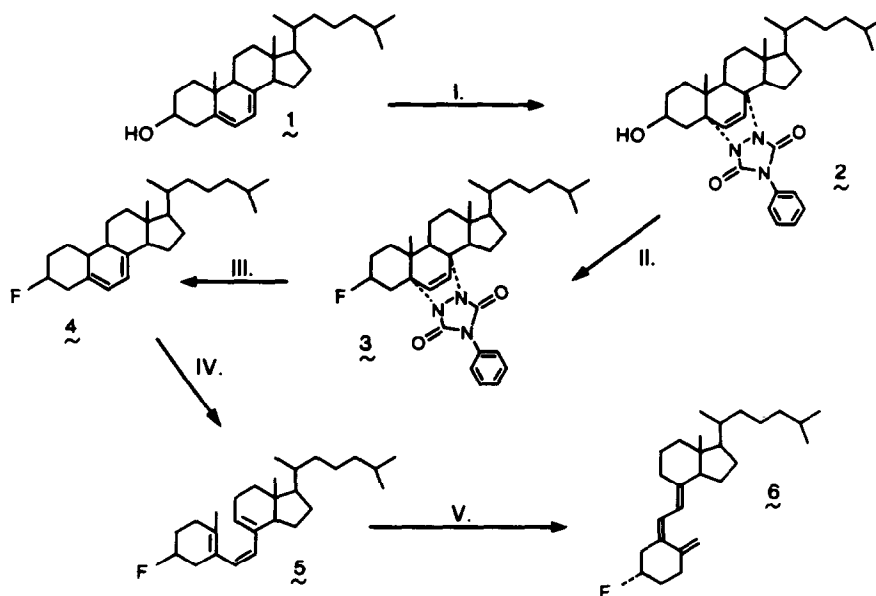


Fig. 1. Synthesis of  $3\beta$ -fluorovitamin D<sub>3</sub> from 7-dehydrocholesterol.

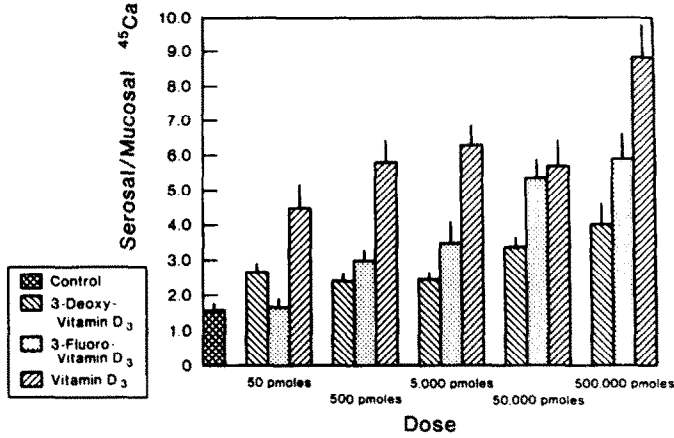


Fig. 2. Intestinal calcium transport in everted duodenal sacs 24 h after the administration of varying doses of vitamin D<sub>3</sub>, 3β-fluorovitamin D<sub>3</sub> or 3-deoxyvitamin D<sub>3</sub> to hypocalcemic vitamin D deficient rats. Data are expressed as mean ± SEM. Vitamin D<sub>3</sub> vs control: 50 through 500,000 pmol *P* < 0.001. 3β-Fluorovitamin D<sub>3</sub> vs control: 50 pmol *P* = NS; 500 and 5000 pmol *P* < 0.003, 50,000 and 500,000 pmol *P* < 0.001. 3-Deoxyvitamin D<sub>3</sub> vs control: 50 through 5000 pmol *P* < 0.03, 50,000 and 500,000 pmol *P* < 0.001.

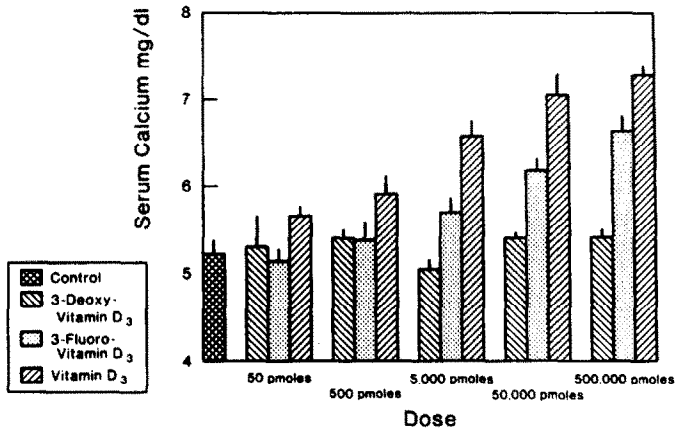


Fig. 3. Bone calcium mobilization response 24 h following the administration of varying doses of vitamin D<sub>3</sub>, 3β-fluorovitamin D<sub>3</sub> or 3-deoxyvitamin D<sub>3</sub> to hypocalcemic vitamin D deficient rats. Data are expressed as mean ± SEM. Vitamin D<sub>3</sub> vs control 50 pmol *P* = NS, 500 pmol *P* < 0.015, 5000 through 500,000 pmol *P* < 0.001. 3β-Fluorovitamin D<sub>3</sub> vs control 50 through 5000 pmol *P* = NS, 50,000 and 500,000 pmol *P* < 0.01. 3-Deoxyvitamin D<sub>3</sub> vs control all doses *P* = NS.

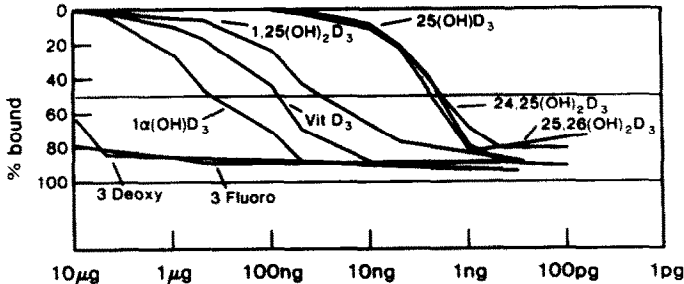


Fig. 4. Ability of various vitamin D analogs to displace radiolabelled 25-hydroxyvitamin D<sub>3</sub> from vitamin D binding protein. B50 values are: 25-hydroxyvitamin D<sub>3</sub> = 4.71 × 10<sup>-9</sup> M; 24(R),25-dihydroxyvitamin D<sub>3</sub> = 4.37 × 10<sup>-9</sup> M; 25(S),26-dihydroxyvitamin D<sub>3</sub> = 5.54 × 10<sup>-9</sup> M; 1,25-dihydroxyvitamin D<sub>3</sub> = 7.33 × 10<sup>-8</sup> M; Vitamin D<sub>3</sub> = 2.16 × 10<sup>-7</sup> M; 1α-hydroxyvitamin D<sub>3</sub> = 1.04 × 10<sup>-6</sup> M; 3-deoxyvitamin D<sub>3</sub> >> 2.5 × 10<sup>-5</sup> M; 3β-fluorovitamin D<sub>3</sub> >> 2.5 × 10<sup>-5</sup> M.

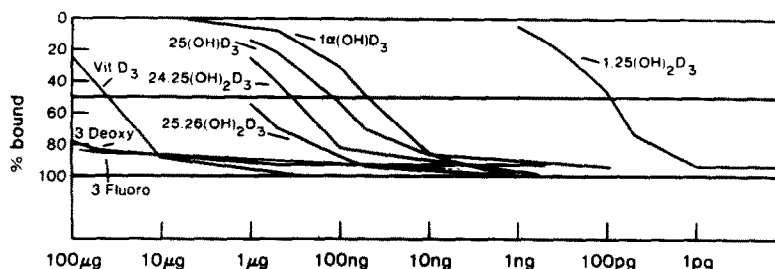


Fig. 5., Ability of various vitamin D analogs to displace radiolabelled 1,25-dihydroxyvitamin D<sub>3</sub> from chick intestinal cytosol receptor. B50 values are: 1,25-dihydroxyvitamin D<sub>3</sub> =  $2.01 \times 10^{-10}$  M; 1 $\alpha$ -hydroxyvitamin D<sub>3</sub> =  $1.1 \times 10^{-7}$  M; 25-hydroxyvitamin D<sub>3</sub> =  $2.86 \times 10^{-7}$  M; 24(R),25-dihydroxyvitamin D<sub>3</sub> =  $7.9 \times 10^{-7}$  M; 25(S),26-dihydroxyvitamin D<sub>3</sub> >  $2.29 \times 10^{-6}$  M; vitamin D<sub>3</sub> =  $1.1 \times 10^{-4}$  M; 3-deoxyvitamin D<sub>3</sub> >  $2.6 \times 10^{-4}$  M; 3 $\beta$ -fluorovitamin D<sub>3</sub> >  $2.5 \times 10^{-4}$  M.

min D<sub>3</sub> which elicits a response as low as 50 pmol/rat. Both 3 $\beta$ -fluorovitamin D<sub>3</sub> and 3-deoxyvitamin D<sub>3</sub> are bound very poorly to vitamin D binding protein and the intestinal cytosol receptor (Figs 4 and 5). As shown in Table 1, 3 $\beta$ -fluorovitamin D<sub>3</sub> induces the synthesis of calcium binding protein in the organ-cultured chick duodenum. It is about 1000 times less potent than 1,25-dihydroxyvitamin D<sub>3</sub> in this system, or about equipotent to vitamin D<sub>3</sub> [16]. The 3-deoxyvitamin D<sub>3</sub> analog is inactive in this system, even at  $\mu$ M concentrations. Neither compound is an antagonist of 1,25(OH)<sub>2</sub>D<sub>3</sub> action. However, 3 $\beta$ -fluorovitamin D<sub>3</sub> produces additive effects on calcium-binding protein synthesis.

#### DISCUSSION

Yakhimovich and Klimashevskii reported successful preparation of 3 $\beta$ -fluorovitamin D<sub>3</sub> by fluorination of cholesterol followed by formation of the 5,7-diene provitamin and photolysis [9]. In another approach, Yakhimovich *et al.*, synthesized the 3 $\beta$ -fluoro-analogs of vitamin D<sub>2</sub> and D<sub>3</sub> by fluorination of the 4-phenyl-1,2,4-triazoline-3,5-dione

cycloadduct of the respective provitamins followed by removal of protecting group and photolysis [11]. In this paper, we report an efficient synthesis of 3 $\beta$ -fluorovitamin D<sub>3</sub> 6 by application of the latter approach of Yakhimovich *et al.* with some modifications. The reaction of 2 with diethylaminosulfur trifluoride followed by quenching with 5% Na<sub>2</sub>CO<sub>3</sub> and washing with saturated NaCl<sub>(aq)</sub> afforded 3 $\beta$ -fluoro-analog 3. Stereochemistry of fluoro-analog 3 was assigned as "β" because: (1) it is established that DAST and sulfur fluorides in general usually replace hydroxyl groups with retention of stereochemistry [19, 20]. (2) The <sup>1</sup>H NMR spectrum of the fluoro analog reported by Yakhimovich to be the 3 $\beta$ -epimer was essentially identical to the <sup>1</sup>H NMR spectrum of 3 [11]. It is reasonable to argue that the steric inhibition created by the presence of the cycloadduct protecting group prevents back side (S<sub>N</sub>2) attack at carbon 3. The epimeric purity was established by (1) the absence of a 3 $\beta$ -H resonance signal from the hypothetical 3 $\alpha$ -fluoro epimer in the <sup>1</sup>H NMR (2) recycling of 4 in the HPLC under standard conditions several times with no evidence of an epimeric mixture.

Table 1. CaBP induction by 3-deoxy D<sub>3</sub> and 3-fluoro D<sub>3</sub> in duodenal organ culture<sup>a</sup>

1,25(OH) <sub>2</sub> D <sub>3</sub> (nM)	3-deoxy D <sub>3</sub> (M)	3-fluoro D <sub>3</sub> (M)	CaBP ( $\mu$ g/100 mg duodenum)
0	0	0	0
0	10 <sup>-8</sup>	0	0
0	10 <sup>-7</sup>	0	0
0	10 <sup>-6</sup>	0	0
1	0	0	10.4 ± 0.9
1	10 <sup>-8</sup>	0	9.1 ± 2.3
1	10 <sup>-7</sup>	0	12.2 ± 2.3
1	10 <sup>-6</sup>	0	13.0 ± 2.7
0	0	10 <sup>-8</sup>	1.1 ± 0.4
0	0	10 <sup>-7</sup>	4.0 ± 1.2
0	0	10 <sup>-6</sup>	12.3 ± 1.2
1	0	10 <sup>-8</sup>	11.1 ± 1.5
1	0	10 <sup>-7</sup>	15.2 ± 1.5
1	0	10 <sup>-6</sup>	20.4 ± 1.0

<sup>a</sup>Values:  $x \pm$  SE; 8 duodena/group.

Comparing CaBP induced by 10<sup>-6</sup> M 3-fluoro D<sub>3</sub> (12.3 ± 1.2) with CaBP induced by 10<sup>-9</sup> 1,25(OH)<sub>2</sub>D<sub>3</sub> (10.4 ± 0.9) suggests that 3-fluoro D<sub>3</sub> is approx 1/1000th as potent as 1,25(OH)<sub>2</sub>D<sub>3</sub> or about equipotent with vitamin D<sub>3</sub> itself in this system. On the other hand, 3-deoxy-D<sub>3</sub> was inactive. Neither analog had any antagonist activity, but the action of 3-fluoro D<sub>3</sub> was additive with that of 1,25(OH)<sub>2</sub>D<sub>3</sub>.

The photolysis of provitamin 4 with a Hanovia lamp fitted with a Vycor filter ( $\lambda > 280$  nm transmitted) was carried out in absolute ethanol cooled by circulating ice water. Photolysis at 254 nm resulted in a complex mixture while photolysis at 350 nm showed no reaction. The progress of the photolysis was checked at regular time intervals by injecting aliquots of the photolysate directly onto the HPLC under standard conditions. The conversion of provitamin 4 (retention time 27 min) to the previtamin 5 (retention time 18 min) and the side product (retention time 21 min) was monitored as a function of reaction time so as to maximize the yield of 5 and minimize the accumulation of side product. The previtamin 5 was isolated by preparative HPLC and then heated to 37°C in deoxygenated methanol for 3 days. The thermal rearrangement of previtamin 5 (retention time 18 min) to vitamin 6 (retention time 16 min) was also followed by HPLC. The conversion of 5 to 6 seemed to reach equilibrium under these temperature conditions with the ratio of vitamin 6 to previtamin 5 approx 95:5 as shown by the HPLC u.v. detector integration. After the final purification of vitamin 6 was carried out by HPLC, the unreacted previtamin 5 was then again subjected to 37°C in methanol in order to maximize the conversion to vitamin 6. Fluorinated analogs 5 and 6 showed acute sensitivity to thermal decomposition especially during the removal of solvents. It was necessary to strip away the solvent (methanol) in the rotovap under mechanical vacuum pump pressure maintaining the water bath temperature around 5–10°C in order to minimize decomposition upon isolation.

Contrary to previous reports, we observed that the 3 $\beta$ -fluorovitamin D<sub>3</sub> 6 was less active than vitamin D<sub>3</sub> *in vivo*. On the other hand, fluorovitamin 6 was much more active than the hydrogen analog, 3-deoxyvitamin D<sub>3</sub> 7, in both *in vivo* systems as well as in the organ-cultured embryonic chick duodenum. The enhanced biological activity of the fluorovitamin could be related to unique chemical and physical properties of the fluorine atom or alternatively different rates of metabolism of the fluoro compound when compared with its hydrogen analog.

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#### REFERENCES

- Corradino R. A., Ikekawa N. and DeLuca H. F.: Induction of calcium-binding protein in organ-cultured chick intestine by fluoro analogs of vitamin D<sub>3</sub>. *Archs biochem. Biophys.* **208** (1981) 273–277.
- Ikekawa N.: Synthesis and biological activity of vitamin D<sub>3</sub> analogues. *J. steroid Biochem.* **19** (1983) 907–911.
- Napoli J. L., Fivizzani M. A., Schnoes H. K. and DeLuca H. F.: 1-Fluorovitamin D<sub>3</sub>, a vitamin D<sub>3</sub> analogue more active on bone-calcium mobilization than on intestinal-calcium transport. *Biochemistry* **18** (1979) 1641–1646.
- Onisko B. L., Schnoes H. K., DeLuca H. F. and Glover R. S.: Metabolism and biological activity of 25-fluorocholecalciferol, 24-dehydrocholecalciferol and 25-dehydrocholecalciferol in the rat. *Biochem. J.* **182** (1979) 1–9.
- Paaren H. E., Fivizzani M. A., Schnoes H. K. and DeLuca H. F.: 1 $\alpha$ ,25-Difluorovitamin D<sub>3</sub>: An inert vitamin D analog. *Archs biochem. Biophys.* **209** (1981) 579–583.
- Tanaka Y., DeLuca H. F., Kobayashi Y., Taguchi T., Ikekawa N. and Morisaki M.: Biological activity of 24,25-difluoro-hydroxyvitamin D<sub>3</sub>. *J. biol. Chem.* **254** (1979) 7163–7167.
- Tanaka Y., DeLuca H. F., Kobayashi Y. and Ikekawa N.: 26,26,26,27,27,27-Hexafluoro-1,25-dihydroxyvitamin D<sub>3</sub>: A highly potent, long-lasting analog of 1,25-dihydroxyvitamin D<sub>3</sub>. *Archs biochem. Biophys.* **229** (1984) 348–354.
- Klimashevskii V. M., Yakhimovich R. I. and Vendt V. P.: 3 $\alpha$ 1'fa-ftor-9,10-sekokholes-tatrienu-5,7,10(19)-ftoro analoha vitaminu D<sub>3</sub>. *Ukr. Biokhim. Zh.* **46** (1974) 627–630.
- Yakhimovich R. I. and Klimashevskii V. M.: Synthesis of 3 $\beta$ -fluoro-cholesta-5,7-diene, provitamin D. *Ukr. Biokhim. Zh.* **46** (1974) 124–127.
- Yakhimovich R. I., Klimashevskii V. M. and Segal G. M.: Synthesis and biological activity of 3 $\alpha$ -fluoro-9,10-secocholesta-5,7,10(19)-triene—a fluoro derivative of vitamin D<sub>3</sub>. *Khim. Farm. Zh.* **10** (1976) 58–64.
- Yakhimovich R. I., Fursaeva N. F. and Pashinnik V. E.: Synthesis of 3 $\beta$ -fluorovitamins D<sub>2</sub> and D<sub>3</sub>. *Khim. Prir. Soedin.* **4** (1980) 580–581.
- Nagubandi S., Kumar R., Londowski J. M., Corradino R. A. and Tietz P. S.: The role of vitamin D glucosiduronate in calcium homeostasis. *J. clin. Invest.* **66** (1980) 1274–1280.
- Martin D. L. and DeLuca H. F.: Influence of sodium on calcium transport by the rat small intestine. *Am. J. Physiol.* **216** (1969) 1351–1359.
- Suda T., DeLuca H. F. and Tanaka Y.: Biologic activity of 25-hydroxycholecalciferol in rats. *J. Nutr.* **100** (1970) 1049–1052.
- Kumar R., Cohen W. R., Silva P. and Epstein F. H.: Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. *J. clin. Invest.* **63** (1979) 342–344.
- Corradino R. A.: Calcium-binding protein of intestine: Induction by biologically significant cholecalciferol-like steroids *in vitro*. *J. steroid Biochem.* **9** (1978) 1183–1187.
- Corradino R. A.: Induction of calcium-binding protein in embryonic chick duodenum *in vitro*: Direct assessment of biopotency of vitamin D-steroids. In *Vitamin D, Basic and Clinical Aspects* (Edited by R. Kumar). Martinus Nijhoff, Boston (1984) pp. 325–341.
- Cookson R. C., Gupte S. S., Stevens I. D. R. and Watts C. T.: 4-Phenyl-1,2,4-triazoline-3,5-dione. *Organic Synthesis* **51** (1971) 121–127.
- Edelstein S., Sheves M., Mazur Y., Bar A. and Hurwitz S.: Synthesis and biological action of 3-deoxy-vitamin D<sub>3</sub> and 3-deoxy-25-hydroxyvitamin D<sub>3</sub>. *FEBS Lett.* **97** (1979) 241–244.
- Sheppard W. A. and Sharts C. M.: *Organic Fluorine Chemistry*. W. A. Benjamin, New York (1969) p. 602.