THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF 22-FLUOROVITAMIN D_3: A NEW VITAMIN D ANALOG

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ABSTRACT

We synthesized 22-fluorovitamin D_3 from (22S) cholest-5-ene-3β,22-diol-3β-acetate 2. Compound 2 was treated with diethylaminosulfur trifluoride to give 22-fluorocholest-5-en-3β-acetate 3 and (E) 22-dehydrocholest-5-en-3β-acetate. Compound 3 was treated with N-bromosuccinimide to give a mixture of the respective 5,7- and 4,6-dienes. The 5,7-diene of 3 was separated from the 4,6-diene using the dienophile 4-phenyl-1,2,4-triazoline-3,5-dione. 22-Fluoro-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolino)-cholest-6-en-3β-acetate 4 was purified by flash chromatography and treated with lithium aluminum hydride to generate 22-fluorocholesta-5,7-dien-3β-ol 5. Photolysis of the diene 5, followed by thermal equilibration, resulted in the synthesis of 22-fluorovitamin D_3 7.

The vitamin 7 increased active intestinal calcium transport only at a dose of 50,000 pmol/rat, whereas vitamin D_3 increased intestinal calcium transport at a dose of between 50 and 500 pmol/rat. 22-Fluorovitamin D_3 7 did not mobilize bone and soft tissue calcium at a dose as high as 50,000 pmol/rat, whereas vitamin D_3 was successful in doing so at a dose of 500 pmol/rat. When tested in the duodenal organ culture system, 22-fluorovitamin D_3 7 had approximately 1/25th the potency of vitamin D_3. It did not antagonize the activity of 1,25-dihydroxyvitamin D_3. 22-Fluorovitamin D_3 7 bound to the rat plasma vitamin D binding protein less avidly than vitamin D_3. 22-Fluorovitamin D_3 was bound very poorly to the chick intestinal cytosol receptor for 1,25-dihydroxyvitamin D_3. We conclude that the introduction of fluorine at the C-22 position results in a vitamin D sterol with decreased biologic activity when compared to vitamin D_3. The presence of a fluorine group at C-22 position inhibits the binding of the vitamin to rat vitamin D binding protein when compared to the binding of its hydrogen analog, vitamin D_3.

INTRODUCTION

Several fluorinated vitamin D analogs have been synthesized in order to test the influence of the fluorine atom on the biologic activity of the vitamin D_3 molecule (1-23). 26,27-Hexafluoro-1,25-dihydroxyvitamin D_3, 24,24-difluoro-1,25-dihydroxyvitamin D_3, and 26,27-hexafluoro-25-hydroxyvitamin D_3 are much more active than their respective hydrogen analogs, 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3 (1,5,8,11,13). 3β-Fluorovitamin D_3, 23,23-difluoro-25-hydroxyvitamin D_3, 23,23-difluoro-1,25-dihydroxyvitamin D_3, 25-fluorovitamin D_3, 25-fluoro-1α-hydroxyvitamin D_3, 1α,25-difluorovitamin D_3, and 1-fluorovitamin D_3 are less active than their non-fluorinated counterparts (2,3,6,10,14, 21 - 23). 6-Fluorovitamin D_3 may be an inert vitamin D antagonist (18,20). To further assess the influence of fluorine substitutions on the biologic activity of the vitamin D_3 molecule, we synthesized 22-fluorovitamin D_3 and tested its biologic activity *in vivo* and *in vitro*. We also assessed its ability to bind to rat plasma vitamin D binding protein and to the intestinal cytosol receptor for 1,25-dihydroxyvitamin D_3.

METHODS

General. Infrared spectra were recorded on a Nicolet 5M-X Fourier transform infrared spectrometer (Nicolet Analytical

Instruments, Schaumburg, IL). Ultraviolet spectra (UV) were taken in ethanol with a Beckman Model 35 recording spectrophotometer (Beckman Instruments, Palo Alto, CA). Nuclear magnetic resonance spectra (NMR) were obtained in deuterated chloroform containing 0.1% trimethylsilane on an IBM NR-80 Fourier transform nuclear magnetic resonance spectrometer (IBM Instruments, Danbury, CT). Mass spectra were obtained on a Kratos MS-50/DS-55 mass spectrometer-computer system (Kratos Instruments, UK). Melting points were obtained on a Haake melting point apparatus (Haake Buchler Instruments, Inc., Saddle Brook, NJ). High performance liquid chromatography was performed on a Waters liquid chromatograph equipped with two Model 6000A pumps, a Model 660 gradient programmer from Waters Associates (Milford, MA), and a Kratos Model 783 ultraviolet detector (Kratos Instruments, Ramsey, NJ). Flash chromatography (24) was performed using silica gel (Merck, grade 60, 230-400 mesh). [^{45}Ca]Calcium chloride was obtained from New England Nuclear (Boston, MA). [26,27-^{3}H]25-Hydroxyvitamin D_3 (23 Ci/mmol) and [26,27-^{3}H]1,25-dihydroxyvitamin D_3 (176 Ci/mmol) were obtained from Amersham Corporation (Arlington Heights, IL). 25-Hydroxyvitamin D_3, 1α-hydroxyvitamin D_3, and 1α,25-dihydroxyvitamin D_3 were supplied by Dr. Milan Uskokovic, Hoffman La-Roche (Nutley, NJ). All solvents were distilled prior to use.

Animals. Male, albino, weanling rats (50-60 g) were obtained from the Holtzman Company (Madison, WI). They were maintained in individual overhanging wire cages and were fed a 0.02% calcium, 0.3% phosphorus, vitamin D deficient diet *ad libitum* for a period of 3 to 4 weeks, after which they were used for the experiments described below (25,26).

Biological measurements. Intestinal calcium transport was measured by the method of Martin and DeLuca (27). Serum calcium was measured in the presence of lanthanum with an atomic absorption spectrometer. The duodenal organ culture assay was performed as described before (28,29). Protein-sterol binding assays were performed as described earlier (30,31).

Protocols. In order to determine the optimal time for testing the biologic activity of 22-fluorovitamin D_3 **7**, 50,000 pmol of

the vitamin 7 were administered to 4 groups of rats. Biological activity was tested 4, 12, 24, and 48 h following the administration of the new vitamin analog. Vitamin D_3 or ethanol dosed rats were used as controls. The respective vitamins dissolved in 50 µL of ethanol were given intrajugularly to each rat. A dose response experiment was performed in order to compare the biologic activities of vitamin D_3 and 22-fluorovitamin D_3. Groups of rats received 50, 500, 5,000, or 50,000 pmol of either vitamin D_3 or 22-fluorovitamin D_3 7 dissolved in 50 µL of ethanol 48 h before the experiment; 48 h later they were killed; the duodenum from each rat was removed for measurement of intestinal calcium transport as described. Serum was collected in order to measure total serum calcium. The group of control rats received ethanol alone.

Synthesis of 22-fluorovitamin D_3 7 (Figure 1).

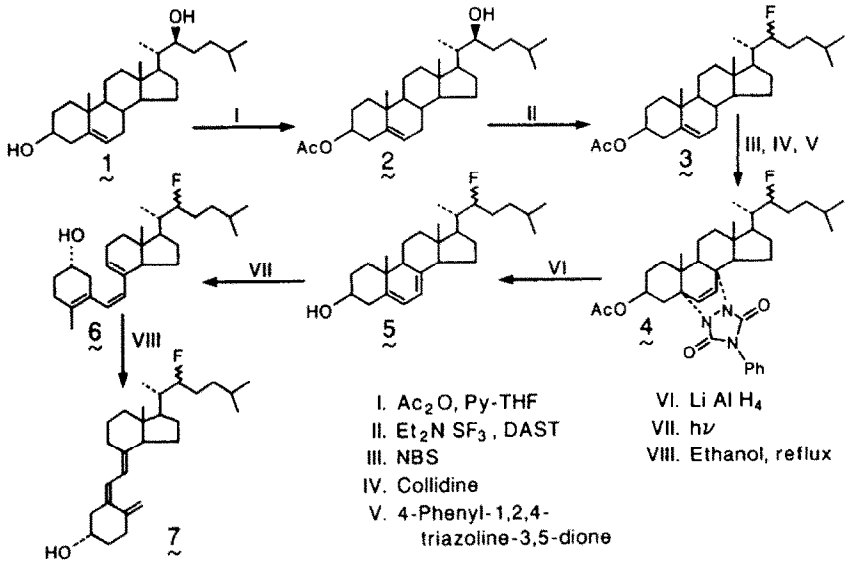

Figure 1. Synthesis of 22-fluorovitamin D_3.

(22S)-Cholest-5-ene-3β,22-diol 3β-acetate 2. (22S)-Cholest-5-ene-3β,22-diol, 1 (1.4517 g, 3.605 mmol) was dissolved in tetrahydrofuran (40 mL). Pyridine (1.115 g, 14.095 mmol) and acetic anhydride (4.187 g, 41.02 mmol) were added to the solution at room temperature. The reaction was stirred for 29 h,

poured into cold water and extracted with ethyl acetate. The organic layer was washed with 5% HCl, dried (Na_2SO_4), and purified by flash chromatography on silica gel (ethyl acetate: hexane::1:4; followed by ethyl acetate) to yield 2 (0.3785 g, 41.3%) m.p. 161.2-161.5°C (from acetone) (lit (32) 162 - 165°C); IR ($CHCl_3$) 3462.5, 1724.5, 1712 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (3H, s, $OCOCH_3$), 3.64 (1H, m, 22-H), 4.58 (1H, m, 3α-H), 5.38 (1H, m, 6-H); MS m/z (assignment, relative intensity) 384 (M^+ - CH_3COOH, 100), 366 (M^+ - CH_3COOH - H_2O, 5.52), 351 (366 - CH_3, 7.18), 255 (M^+ - CH_3COOH - side chain, 7.95).

22-Fluorocholest-5-en-3β-acetate 3 and (E) 22-dehydrocholest-5-en-3β-acetate. Diethylaminosulfur trifluoride (DAST, 1.32 g, 8.189 mmol) in methylene chloride (15 mL) was cooled to -78°C and a solution of 2 (1.0 g, 2.249 mmol) in methylene chloride (15 mL) was added dropwise over a period of 20 min. After 5 min, the cooling bath was removed and reaction was quenched with a 5% sodium bicarbonate solution. In a separate reaction 2 (2.0 g, 4.98 mmol) was reacted with DAST (2.64 g, 10.378 mmol). The combined reaction mixtures were extracted with chloroform, dried (Na_2SO_4), and evaporated. Thin-layer chromatography (TLC, ethyl acetate:hexane::2:100) of reaction showed two close spots. Both components of the mixture were separated by repetitive flash chromatography on silica gel using ethyl acetate:hexane::2:100 v/v. The faster moving component was identified as (E)22-dehydrocholest - 5 -en-3β-acetate (1.2234 g, 42.5%) m.p. 120.3-121.2°C (from methanol) (lit (33) 123-124°C), IR ($CHCl_3$) 1728.3 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.03 (3H, s, $OCOCH_3$), 4.64 (1H, m, 3α-H), 5.18 (1H, m, 22-H), 5.39 (1H, m, 6-H); MS m/z (assignment, relative intensity) 366 (M^+ - CH_3COOH, 100), 351 (M^+ - CH_3COOH - Me, 5.68), 281 (366 - C_6H_{13}, 3.0), 255 (366 - side chain, 5.32).

The slower moving component was identified as 22-fluorocholest-5-en-3β-acetate 3 (1.3058 g, 43.3%), m.p. 123-123.3°C (from methanol); IR ($CHCl_3$) 1726.4 cm^{-1}, ^1H NMR ($CDCl_3$) δ 2.03 (3H, s, $OCOCH_3$), 4.45 (1H, dm, J = 48.2 Hz, 22-H), 4.64 (1H, m, 3α-H), 5.39 (1H, m, 6-H); MS m/z (assignment, relative intensity) 386 (M^+ - CH_3COOH, 6.11), 366 (M^+ - CH_3COOH - HF, 100), 351 (366 - Me, 14.07), 281 (366 - C_6H_{13}), 255 (386 - side chain). High resolution MS calcd. for $C_{27}H_{43}F$ (M^+ - ACOH) 386.338; found 386.3258.

22-Fluoro-5α,8α-(3,5-dioxo-4-phenyl-1,2,4-triazolino)-cholest-6-en-3β-acetate 4. N-Bromosuccinimide (0.2746 g, 1.5445 mmol) and benzoyl peroxide (0.016 g, 0.0661 mmol) were added to a refluxing solution of 3 (0.60 g, 1.3432 mmol) in carbon tetrachloride (120 mL). The reaction was continued for 20 min, cooled to 0°C, and filtered under nitrogen. The solvent was removed under reduced pressure and the crude bromide was dissolved in xylene (90 mL) and collidine (21.0 mL). The mixture was refluxed for 90 min. Ethyl acetate was added to reaction and the organic layer was washed successively with 5% HCl, 5% $NaHCO_3$, and water. The organic layer was dried (Na_2SO_4) and the solvent was removed under vacuum. The residue was passed through a short silica gel flash chromatography column (ethyl acetate:hexane::1:10) to give a mixture of 4,6- and 5,7-dienes. The diene mixture was dissolved in methylene chloride (50 mL) and a solution of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD, 0.18 M) was added dropwise at room temperature until the red color of PTAD persisted. The reaction was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and the crude product was first purified by flash chromatography on silica gel (ethyl acetate:hexane::3:7), followed by HPLC (Varian Micropak Si-10, 50 cm x 8 mm, 8 mL/min, isopropanol:hexane::1.5:100) to give 4 (0.1812 g, 21.8%) m.p. 119.4-120.2°C (from aq. methanol); UV (ethanol) λ_{max} 257, 213 nm; IR ($CHCl_3$) 1751.5, 1732.2, 1699.4 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.06 (3H, s, $OCOCH_3$), 4.46 (1H, dm, J = 47.12 Hz, 22-H), 5.44 (1H, m, 3α-H), 6.22, 6.44 (2H, ABq, J = 7.85 Hz, H-6, H-7), 7.42 (5H, m, aromatic-H); MS m/z (assignment, relative intensity) 384 (M^+ - dienophile, PTAD - CH_3COOH, 100), 364 (384 - HF, 22.35), 253 (384 - side chain, 14.11), 177 (64.7); high resolution MS calcd. for $C_{27}H_{41}F$ 384.3182; found 384.3143.

22-Fluorocholesta-5,7-dien-3β-ol 5. A solution of 4 (0.147 g, 0.2372 mmol) in THF (25 mL) and lithium aluminum hydride (0.296 g, 7.7997 mmol) were refluxed for 3 h. The reaction was quenched by addition of wet ether followed by ethyl acetate and water. Aluminum salts were filtered off and filtrate was extracted with ethyl acetate. Organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. Crude product was purified by flash chromatography on silica gel (ethyl acetate:hexane::3:7) to yield 5 (0.0653 g, 68.38%) m.p. 137.6-138.8°C; UV (ethanol) λ_{max} 293, 282, 271, 262 nm; IR ($CHCl_3$) 3429.7 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.61 (1H, m, 3α-H), 4.47 (1H, dm, J = 47.11 Hz, 22-H), 5.42, 5.58 (2H, m, H-6, H-7); MS m/z (assignment, relative intensity) 402 (M^+, 21.13), 382 (M^+ - HF, 26.28), 369 (M^+ - H_2O - CH_3, 30.20), 349 (M^+ - H_2O - CH_3 - HF,

34.01), 253 (M^+ - side chain - H_2O, 28.11). High resolution MS calcd. for $C_{27}H_{43}FO$ 402.3287; found 402.3216.

22-Fluorovitamin D_3, 7. Diene 5 (0.04 g, 0.0993 mmol) in ether (140 mL) was deoxygenated at ~0°C by bubbling nitrogen through solution. The cold solution was irradiated at 253.7 nm for 2000 sec and at 350 nm for another 2000 sec. Photolyzed mixture was evaporated under reduced pressure and residue was separated by HPLC (Varian Micropak Si-10, 50 cm x 8 mm, 8 mL/min; isopropanol:hexane::1:100) to provide 22-fluoroprevitamin D_3, 6 (λ_{max} 256 nm, λ_{min} 234 nm). Previtamin 6 in ethanol (35 mL) was refluxed under nitrogen for 65 min. Ethanol was removed under reduced pressure and the thermolysis mixture was separated by HPLC (same conditions as above) to give 7 ((10.9646 mg, 27.41%, yield based on UV measurement and assuming ϵ to be 18,200) UV (ethanol) λ_{max} 264 nm, λ_{min} 227 nm); ^1H NMR ($CDCl_3$) δ 3.82 (1H, m, 3α-H), 4.44 (1H, dm, J = 48.2 Hz, 22-H), 4.82 (1H, d, J = 2.24 Hz, 19-H), 5.05 (1H, d, J = 2.24 Hz, 19-H), 6.01, 6.25 (2H, ABq, J = 11.22 Hz, H-6, H-7); MS m/z (assignment, relative intensity) 402 (M^+, 80.51), 384 (M^+ - H_2O, 8.05), 382 (M^+ - HF, 8.88), 369 (25.11), 136 (25.51), 118 (53.82); high resolution MS calcd. for $C_{27}H_{43}FO$ 402.3287; found 402.3381.

RESULTS AND DISCUSSION

Synthesis of 22-fluorovitamin D_3 7 was achieved by selectively protecting the 3β-alcohol in (22S) cholest-5-ene-3β,22-diol 1, as an acetate (41%). Compound 1 was synthesized using 3β-tetrahydropyranyloxy-23,24-dinor-chol-5-en-22-al and isoamyl magnesium bromide. Details of the synthesis are reported elsewhere.[1] Fluorination of (22S) cholest-5-ene-3β,22-diol 3β-acetate 2 with diethylaminosulfur trifluoride resulted not only in the desired 22-fluorocholest-5-en-3β-acetate 3,

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[1]Gill, HS, Londowski, JM, Corradino, R, and Kumar R. Synthesis, biologic activity, and protein binding characteristics of a new vitamin D analog, 22-hydroxyvitamin D_3. Submitted for publication.

but also in a dehydration product (E) 22-dehydrocholest-5-en-3β-acetate in a 1:1 ratio (86%). No rearrangement product such as 21-fluorocholest-5-en-3β-acetate was observed during fluorination. The 5,7-diene was introduced by allylic bromination of 3 with N-bromosuccinimide followed by dehydrobromination of the bromide with collidine. The mixture of 4,6- and 5,7-dienes was titrated with 4-phenyl-1,2,4-triazoline-3,5-dione to obtain 4 (22%). The protecting group was removed using lithium aluminum hydride to regenerate the 5,7-diene 5 (68%). Photolysis of 5 in ether and thermal isomerization of previtamin 6 in ethanol under nitrogen atmosphere completed synthesis of 22-fluorovitamin D_3 7 (27%). The stereochemistry at C-22 could not be assigned precisely as R or S. Based on the mechanism of DAST reactions it is likely that both R and S diasteromers are present (34).

Table 1 shows the results of a time course experiment in which intestinal calcium transport was tested 4, 12, 24, or 48 h after the administration of 50,000 pmol of vitamin D_3 or 22-fluorovitamin D_3 7 intrajugularly to vitamin D deficient rats. Control rats received ethanol alone. Vitamin D_3 increased intestinal calcium transport within 12 h of the administration of the vitamin, and this response was sustained for 48 h. 22-Fluorovitamin D_3 7, however, increased intestinal calcium transport only 48 h after the administration of the

Table 1. Serosal/Mucosal Ratio after Administration of Sterols or Vehicle. Mean ± SEM (n = Number of Animals).

Time	4 h	12 h	24 h	48 h
Control	2.66±0.23 (7)	2.00±0.26 (7)	2.01±0.20 (6)	1.94±0.25 (7)
Vitamin D_3	2.92±0.30 (6)	4.79±0.37* (7)	4.51±0.61* (7)	5.92±0.57* (7)
22-Fluoro-vitamin D_3	1.98±0.15 (7)	2.17±0.23 (7)	2.75±0.39 (7)	3.74±0.40* (6)

*Statistically different from control. Intestinal calcium transport measured by the everted gut sac method at various time points after the administration of 50,000 pmol of vitamin D_3 or 22-fluorovitamin D_3 intrajugularly to vitamin D deficient hypocalcemic rats.

dose. Vitamin D_3 increased serum calcium within 12 h of the administration of the vitamin, and 22-fluorovitamin D_3 7 showed a slight increase in serum calcium at 24 h, but not at 48 h (Table 2).

Table 2. Serum Calcium (mg/dL) after Administration of Sterols or Vehicle. Mean ± SEM (n = Number).

Time	4 h	12 h	24 h	48 h
Control	4.3±0.1 (7)	4.6±0.1 (7)	4.4±0.1 (6)	4.5±0.1 (7)
Vitamin D_3	4.4±0.1 (6)	6.1±0.2* (7)	6.3±0.1* (7)	6.7±0.2* (7)
22-Fluoro-vitamin D_3	4.6±0.2 (7)	4.6±0.1 (7)	4.9±0.2* (7)	4.4±0.1 (6)

*Statistically different from control. Serum calcium concentrations at various time points after the administration of 50,000 pmol of vitamin D_3 or 22-fluorovitamin D_3 to vitamin D deficient hypocalcemic rats.

Based on the intestinal calcium transport responses, the biologic activity of different amounts of the new vitamin analog, 22-fluorovitamin D_3 7, and its non-fluorinated counterpart, vitamin D_3, were tested 48 h after the administration of the appropriate doses. Vitamin D_3 increased intestinal calcium transport at a dose of between 50 and 500 pmol/rat (Table 3); serum calcium was elevated at a dose of 500 pmol/rat. 22-Fluorovitamin D_3 7, however, increased intestinal calcium transport at a dose of 50,000 pmol/rat (Table 3); serum

Table 3. Serosal/Mucosal Ratio and Serum Calcium Response Following Administration of Vitamin D_3 or 22-Fluorovitamin D_3. Mean ± SEM (n = Number).

Compound	Dose (pmol/rat)	n	S/M Ratio	n	Serum Ca (mg/dL)
Vitamin D_3	50	5	3.59 ± 0.25	5	4.9 ± 0.2
	500	7	7.16 ± 0.50*	7	5.0 ± 0.1*
	5,000	8	5.20 ± 1.00*	8	5.7 ± 0.1*
	50,000	6	7.64 ± 0.76*	6	7.0 ± 0.2*
22-Fluoro-	50	10	2.22 ± 0.15	10	4.3 ± 0.1
vitamin D_3	500	9	2.28 ± 0.15	10	4.3 ± 0.1
	5,000	7	2.26 ± 0.24	9	4.2 ± 0.1
	50,000	8	3.45 ± 0.20*	8	4.6 ± 0.1

Serum calcium concentrations or intestinal calcium transport measured by the everted gut sac method following the administration of different doses of either vitamin D_3 or 22-fluorovitamin D_3 to vitamin D deficient rats maintained on a low calcium diet. Control animals received ethanol alone.
Control values for intestinal calcium transport responses were (n = 17); 2.23 ± 0.09.
Control values for serum calcium were (n = 17) 4.5 ± 0.1 mg/dL.
*Statistically different from control using Dunnett's test for multiple comparisons.

calcium did not change. In the organ culture duodenum approximately 2.5×10^{-6} M 22-fluorovitamin D_3 elicited a response comparable to 1×10^{-10} M 1,25-dihydroxyvitamin D_3 (Table 4). 22-Fluorovitamin D_3 appeared to have no antagonistic activity to 1,25-dihydroxyvitamin D_3 and, in fact, appeared to be additive to 1,25-dihydroxyvitamin D_3.

Table 4. Calcium Binding Protein Induction by 22-Fluorovitamin D_3 in Duodenal Organ Culture.

1,25-Dihydroxy-vitamin D_3 (nm)	22-Fluoro-vitamin D_3 (M)	Calcium Binding Protein (µg/100 mg duodenum)
0	0	0
0	2.5×10^{-6}	12.0 ± 0.9
0.1	0	12.3 ± 0.5
0.1	2.5×10^{-6}	19.2 ± 1.6
1	0	27.4 ± 2.9
1	2.5×10^{-6}	38.8 ± 2.2

Calcium binding protein induction by 22-fluorovitamin D_3 or 1,25-dihydroxyvitamin D_3 in the cultured chick embryonic duodenum. Comparing calcium binding protein content induced by 22-fluorovitamin D_3 (2.5×10^{-6} M) and 1,25-dihydroxyvitamin D_3 (0.1 nM) indicates that 22-fluorovitamin D_3 is about 1/25,000th as potent as 1,25-dihydroxyvitamin D_3. This is 1/25th as potent as vitamin D_3 itself in this system. 22-Fluorovitamin D_3 had no antagonist activity; in fact, its activity appears to be additive to that of 1,25-dihydroxyvitamin D_3. On the basis of this assay, 22-fluorovitamin D_3 must be considered a very weak vitamin D agonist in terms of calcium binding protein induction.

The binding of vitamin analog 7 to plasma vitamin D binding protein from the rat was examined. These results are shown in Figure 2. A B-50 value (the B-50 value is the amount

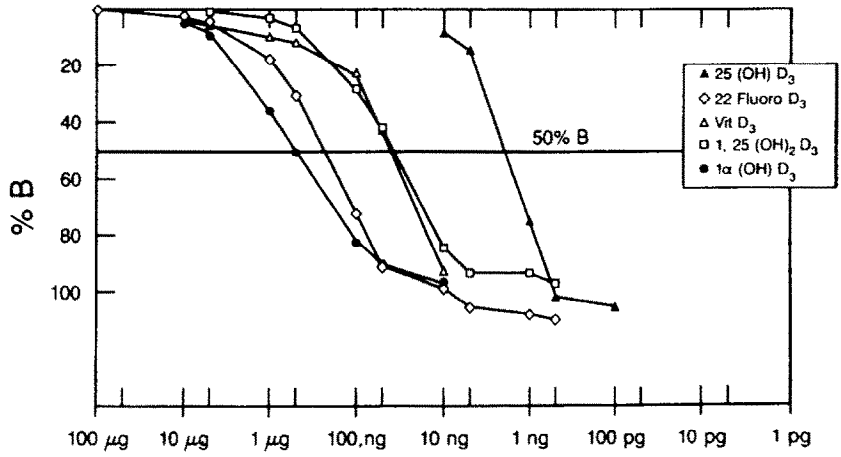

Figure 2. Relative potencies of various vitamin D sterols to displace [^{3}H]25-hydroxyvitamin D_3 from rat plasma vitamin D binding protein.

of sterol needed to displace 50% of the [26,27-^{3}H]25-hydroxyvitamin D_3 from rat vitamin D binding protein) of approximately 5.5×10^{-7} M was obtained for the 22-fluorovitamin D_3 7. This was approximately 100-fold greater than the amount of 25-hydroxyvitamin D_3 (B-50 = 4.62×10^{-9} M) needed to elicit a comparable response. 22-Fluorovitamin D_3 7 was less effective in displacing radiolabeled 25-hydroxyvitamin D_3 from vitamin D binding protein than vitamin D_3 itself (B-50 = 1.01×10^{-7} M). The B-50 value for 1,25-dihydroxyvitamin D_3 and 1α-hydroxyvitamin D_3 were 8.62×10^{-8} and 1.10×10^{-6} M. 22-Fluorovitamin D_3

was extremely ineffective in displacing radiolabeled 1,25-dihydroxyvitamin D_3 from the intestinal cytosol receptor (Figure 3).

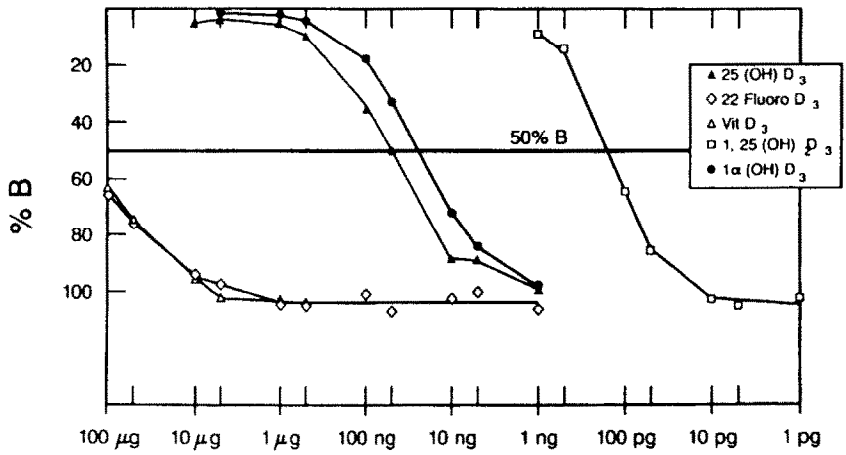

Figure 3. Relative potencies of various vitamin D sterols to displace [^{3}H]1,25-dihydroxyvitamin D_3 from the chick intestinal cytosol receptor.

Based on the biologic information obtained above, we conclude that the introduction of a fluorine at C-22 results in an analog of vitamin D_3 that has weak biologic activity compared to the vitamin itself. The analog 7 induces calcium binding protein in the organ culture duodenum when added in large amounts. It binds very poorly to the chick intestinal cytosol receptor for 1,25-dihydroxyvitamin D_3. It binds to vitamin D binding protein less effectively than vitamin D_3 or 25-hydroxyvitamin D_3.

The effects of fluorine substitution on various parts of the vitamin D molecule give results that are quite variable: in some instances, resulting in compounds that have an increased biologic activity relative to the parent compound, and in other instances, resulting in analogs that have biologic activity that is considerably less than that of the parent analog (1-23). Our results are comparable to those obtained by Nakada et al (21) and Taguchi et al (22) in the case of 23,23-difluoro-25-hydroxyvitamin D_3, in which they observed that the introduction of fluorines at C-23 decreased the bioactivity and the binding of 23,23-difluoro-25-hydroxyvitamin D_3 to the plasma binding protein for 25-hydroxyvitamin D_3. The explanation for the findings observed by us and Nakada et al and Taguchi et al is not clear, but the observations would suggest that even minimal changes in the side chain of the vitamin D_3 molecule influence binding to plasma vitamin D binding protein. In conclusion, substitution of -H at C-22 with -F results in a compound with weak agonist activity and poor binding to vitamin D binding protein.

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