THE JOURNAL OF Organic Chemistry

VOLUME 50, NUMBER 12

JUNE 14, 1985

Synthesis of 6-Fluorovitamin D_3

William G. Dauben,* Boris Kohler,[1] and Alex Roesle

Department of Chemistry, University of California, Berkeley, California 94720

Received August 28, 1984

6-Fluorovitamin D_3 has been synthesized starting with 6-fluoro-7-dehydrocholesteryl acetate, prepared from 6-fluorocholesteryl acetate. The irradiation preparation of the previtamin and the thermal conversion to the vitamin require special conditions. The effect of the fluorine substituent on the chemical synthesis and on the biological activity of the vitamin are discussed.

Vitamin D_3 is an important biological regulator of calcium and phosphorus metabolism.[2] It is now established that the parent vitamin D_3 is sequentially metabolized in various tissues to the steroid hormone 1,25-$(OH)_2$-D_3 (calcitriol) which exerts the highest biological activity of all vitamin D_3 metabolites. This hormonal derivative stimulates the intestinal absorption of calcium (ICA) and phosphorus,[3] and the mobilization of bone calcium (BCM)[2] through a target organ receptor mediated mechanism.[4] All the metabolites so far isolated are polar derivatives, bearing extra hydroxyl, carboxyl, or lactone groups at C-1 or near the extremity of the side chain. In addition to the synthesis of the metabolites, a great amount of synthetic effort has been directed toward the preparation of new derivatives bearing polar groups on the periphery of the molecule. For example, fluorine-substituted vitamin D_3 metabolites have been synthesized in order to evaluate metabolic events obligatory to the vitamin activity.[5] It has been found that 24,24-difluoro-1α,25-dihydroxyvitamin D_3 shows 5–10 times more in vivo activity than the parent 1,25-$(OH)_2$-D_3 steroid hormone.[6]

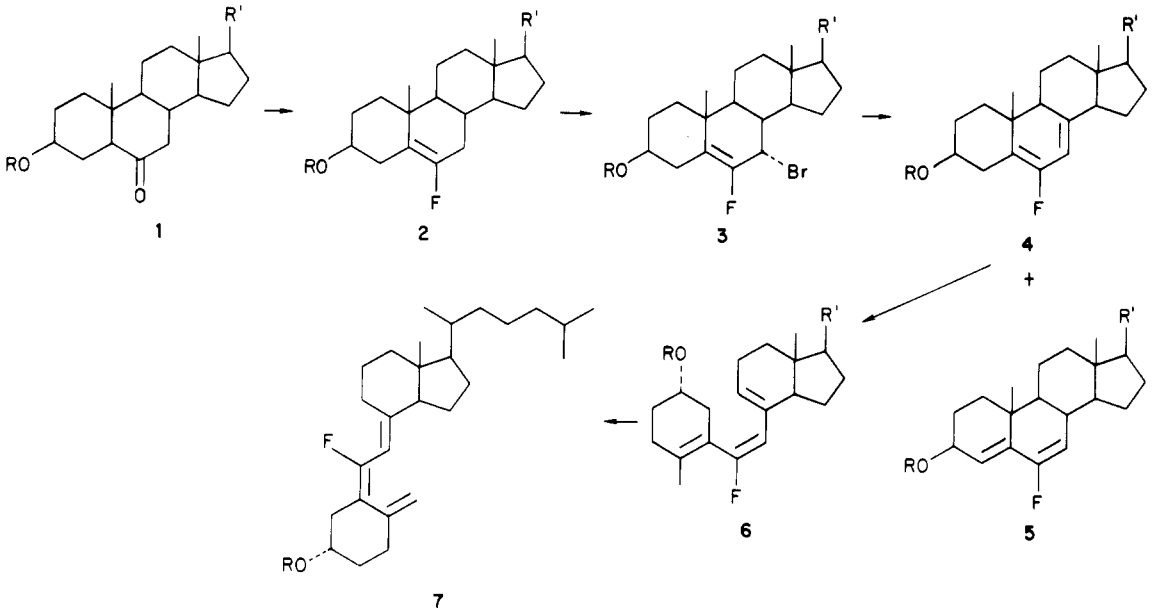
By contrast, little attention has been directed to the role of the triene portion of the vitamin D_3 molecule in relation to the biological activities. Is the role of this portion of the molecule limited solely to geometric requirements or does the π-system interact with the bioreceptors? With these concerns in mind, coupled with our continuing interest in the role of dipolar excited states in polyene photochemistry,[7] the synthesis of 6-fluorovitamin D_3 (6-F-D_3) was undertaken.

6-Fluorocholesteryl acetate (**2a**) was first prepared by Boswell[8] by allowing 6-ketocholestanyl acetate (**1a**) to react with (diethylamino)sulfur trifluoride (DAST). Although a slightly higher yield of **2a** can be obtained by using DAST, the use of piperidinosulfur trifluoride[9] simplifies the synthetic operation and gives **2a** in 55% yield. The desired 7-allylic bromination of the fluoro ene was readily achieved by using *N*-bromosuccinimide (peroxide catalyst plus light) and the crystalline 7α-bromo derivative **3a** was obtained in a 44% yield. The dehydrobromination of **3a** to give in good yield the desired 6-fluoro-7-dehydrocholesteryl acetate (**4a**) proved to be a difficult reaction, clearly showing that the fluorine substituent had affected the process. Many common amine bases employed in the synthesis of the nonfluorinated provitamin gave complex mixtures of numerous products and with *s*-collidine, in particular, there was no indication (by UV spectroscopy) of any of the desired 5,7-diene derivative. The use of trimethyl phosphite in refluxing xylene gave a clean reaction product (84% yield); however, the product was a 2:1 mixture of the 4,6-diene **5a** and the 5,7-diene **4a** (analysis by HPLC). Compared with the corresponding elimination reaction using the nonfluorinated derivative, the trimethyl phosphite elimination with **3a** was veryl slow, 5 days compared to 4 h.

During this latter study, the use of tetra-*n*-butylammonium fluoride at 25 ° was shown to be the most effective agent to produce the provitamin in good yield.[10]

(1) Recipient of Swiss National Foundation Grant No. 82-611-0-78.
(2) Norman, A. W. "Vitamin D: The Calcium Homeostatic Steroid Hormone; "Academic Press: New York, 1979; pp 1–490.
(3) Adams, T. H.; Norman, A. W. *J. Biol. Chem.* **1970**, *245*, 4421.
(4) Norman, A. W.; Roth, J.; Orci, L. *Endocr. Rev.* **1982**, *3*, 331.
(5) For a general survey, see: (a) Ikekawa, N. *J. Steroid Biochem.* **1983**, *19*, 907. (b) Kobayashi, Y.; Taguchi, T. In *Biomed. Aspects Fluorine Chem.* **1982**, 33; *Chem. Abstr.* **1983**, *99*, 158707.
(6) (a) Corrandino, R. A.; DeLuca, H. F.; Tanaka, Y.; Ikekawa, N.; Kobayashi, Y. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 1800. (b) Okamoto, S.; Tanaka, Y.; DeLuca, H. F.; Kobayashi, Y.; Ikekawa, N. *Am. J. Physiol.* **1983**, *244*, E159.
(7) For a general review of the photochemistry in the vitamin system, see: Dauben, W. G.; McInnis, E. L.; Michno, D. M. "Rearrangements in Ground and Excited States"; deMayo, P., Ed.; Academic Press: New York, 1980; Vol. 3.
(8) Boswell, G. A., Jr. U.S. Patent 4 212 815; July 15, 1980; *Chem. Abstr.* **1980**, *93*, 239789.
(9) Markovskij, L. N.; Pashinnik, V. E.; Kirsanov, A. V. *Synthesis* **1973**, 787.

0022-3263/85/1950-2007$01.50/0



a, R=Ac; R'=isooctyl
b, R=H; R'=isooctyl

Further studies also showed that 1,3-dibromo-5,5-dimethylhydantoin was a more effective halogenating agent than *N*-bromosuccinimide. From the preparative standpoint, the finding that standard silica gel column chromatography readily separated the two isomeric diene alcohols **4b** and **5b** was of key importance. Starting with 6-fluorocholesteryl acetate (**2a**) and not purifying any intermediate materials until after the removal of the acetate group by LAH reduction and the desired diene **4b** in an overall yield of 48% and the isomeric 4,6-diene **5b** in 11% yield. The ultraviolet spectra of **4b** and **5b** showed less pronounced structure, and the molecular extinction coefficients are about 30% less than their nonfluorinated counterparts.

The 6-fluoro-7-dehydrocholesterol (**4b**, 6-F-Pro-D_3) was irradiated in a degassed ethereal solution at ~0 °C in a Rayonet reactor using 300-nm light. The reaction was followed by analytical HPLC and stopped when a maximum buildup of the previtamin was observed. The irradiation reaction mixture, using HPLC analysis, showed a 90:10 ratio of 6-fluoroprevitamin D_3 (**6b**) to starting 6-fluoroprovitamin D_3 (**4a**). The two products were readily separated by flash chromatography[11] in an isolated yield of 43% of 6-F-Pre-D_3 and 12% of the 6-F-Pro-D_3, indicating that some decomposition of the previtamin had occurred during the workup and chromatography. Besides these two products, analytical HPLC showed the presence of two very minor ultraviolet-active products which were not isolated. This is an interesting result since in the nonfluorinated series, using 300-nm light, the quasi-photostationary state has the composition of 5% Pro-D_3, 68% Pre-D_3, 19% tachysterol$_3$, and 8% lumisterol$_3$.[12] The apparent inefficient formation of the C-6:C-7 trans isomer 6-fluorotachysterol$_3$ indicates that the fluoro substituent has an effect on the photochemical cis–trans olefin isomerization.

The [1,7]-sigmatropic hydrogen rearrangement to convert the previtamin to the vitamin is normally readily achieved at 80 °C over a period of no longer than 18 h. At this temperature, the 6-F-Pre-D_3 was stable for a period of 24 h. It was found that when an *n*-isooctane solution of **6b** was heated in a sealed tube at 120 °C for 4–5 h, the hydrogen rearrangement did occur and yielded a mixture, as analyzed by HPLC, of 6-F-Pre-D_3 to 6-F-D_3 in a ratio of 15:85. The vitamin **7b** was isolated by preparative TLC as a colorless oil in a yield of 30%. TLC of the crude reaction product showed the presence of some polar material which did not move from the origin upon development of the plate. The isolated 6-F-D_3 had a UV max at 268 nm (ϵ 10300) and was shown to be a single compound by analytical HPLC.

The 6-F-D_3 is very sensitive to air. The decomposition was detected by the broadening of the UV absorption peak, accompanied by a hypsochromic shaft and diminution of the molar extinction coefficient, when the material was allowed to stand in nondegassed cyclohexane solution. The mass spectra of several decomposition products each showed a peak at M + 16 or M + 17, indicating the uptake of oxygen. The 6-F-D_3 can be repurified from the polar products by chromatography with the recovery of 60–70% of the vitamin. Interestingly, the mass spectrum of 6-F-D_3 does not show the characteristic fragmentation of other vitamin D derivatives with the cleavage of the C-7:C-8 double bond.[13]

A complete inhibition of the [1,7]-sigmatropic hydrogen rearrangement has been reported for 19,19-difluoroprevitamin D_3.[14] That this retardation of the reaction may be due to changes in the electron distribution in the triene system is the finding of the nonreversible formation of 19-acetoxyvitamin D_3 from the previtamin.[14] The retardation of the rate of rearrangement in the 6-F-D_3 formation supports this suggestion, but the finding that the equilibrium composition is similar to other vitamin D_3 derivatives suggests that the problem is more complex than previously thought.

In the course of our synthetic studies, the presence of the fluorine substituent created many problems. One of

(10) Rappoldt, M. P.; Hoogendoorn, J.; Pauli, L. F. *Proc. Workshop Vitam. D, 5th* **1982**, 133 (Vitam. D: Chem., Biochem. Clin. Endocrinol. Calcium Metab.); *Chem. Abstr.* **1982**, *97*, 216563.
(11) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(12) Dauben, W. G.; Phillips, R. B. *J. Am. Chem. Soc.* **1982**, *104*, 355.
(13) Okamura, W. H.; Hammond, M. L.; Jacobs, H.J.C.; van Thuijl, J. *Tetrahedron Lett.* **1976**, 4807.
(14) Sialom, B.; Mazur, Y. *J. Org. Chem.* **1980**, *45*, 2201.

the more interesting was the finding that 6-fluorocholesteryl acetate was highly deactivated toward allylic oxidation. For example, 7-ketocholesteryl acetate is formed in high yield by Collins oxidation of cholesteryl acetate,[15] but with the 6-fluoro derivative, even under forcing conditions and long reaction times, the reaction was incomplete and afforded complex mixtures of products, the best isolated yield being <10%. Using a 20-fold excess of the more reactive 3,5-dimethylpyrazole–chromium trioxide reagent,[16] which has been shown to be a superior reagent in the allylic oxidations of cholesteryl benzoate,[17] and extending the reaction time to 48 h (vs. 30 min with the nonfluorinated derivative) gave an 84% yield of 6-fluoro-7-ketocholesteryl acetate.

Preliminary studies[18] of the in vivo biological activity of 6-F-D_3 (7) assessed with vitamin D deficient ducks revealed no agonist intestinal (ICA) or bone (BCM) activity. However, when 6-F-D_3 is administered with the steroid hormone, 1,25-$(OH)_2$-D_3, it serves as an ICA and BCM antagonist. This is the first vitamin D analogue found to be an in vivo antagonist which also shows competitive in vitro binding to the 1,25-dihydroxyvitamin D_3 receptor.

Experimental Section

General Methods. Solvents were dried and/or distilled under a nitrogen atmosphere prior to use when necessary. All reactions, unless specifically mentioned, were conducted under an atmosphere of dry nitrogen.

Spinning disk thin layer chromatography was performed on a Harrison Research Chromatotron Model 7824, with plate thickness and solvent system as noted. HPLC was conducted with a Waters 600A chromatograph connected to a Waters radical compression Model RCM-1000 filled with a 5-μm or 10-μm silica gel column and a Waters R-401 differential refractometer or a Waters R-400 UV detector.

^{1}H NMR spectra were taken on a UCB-250-FT spectrometer. Mass spectral data were collected on an AEI-12 or Consolidated 12-110B mass spectrometer by the Mass Spectrometry Laboratory, College of Chemistry, Berkeley, and elemental analyses were performed by the Microanalytical Laboratory, College of Chemistry, Berkeley.

6-Fluorocholesteryl Acetate (2a).[8] To a magnetically stirred solution of 5.10 g (11.4 mmol) of 6-ketocholesteryl acetate[19] in 50 mL of dry glyme in a Teflon-brand bottle was added, at room temperature, 0.25 mL of 20% fuming sulfuric acid. The solution, under a nitrogen atmosphere, was stirred for 10 min and 10 mL of piperidinosulfur trifluoride[9] was added. The bottle was placed in a preheated oil bath at 50–60 °C, and the solution was stirred at this temperature for 5 days. The solution was cooled to 0 °C and a 5% aqueous sodium bicarbonate solution was carefully added (caution: foaming). The crystals were collected, washed with water, and dried under reduced pressure. The product was recrystallized from methanol to afford 2.81 g (55%) of **2a**: mp 110.0–111.0 °C (lit.[8] mp 110–112 °C, no depression in melting point when mixed with authentic product[20]); $[\alpha]^{25}_D$ −32° ($CHCl_3$, c 0.08); IR (Nujol) 1730, 1465, 1365, 1235, 1040, 980, 905, 875 cm^{-1}; ^{1}H NMR ($CHCl_3$) δ 4.57 (m, 1), 3.03 (dd, 1, J = 7.5 and 13.0 Hz), 2.03 (s, 3), 1.58 (br d, 1, J = 13 Hz), 1.02 (s, 3), 0.98 (d, 3, J = 6.6 Hz), 0.87 (d, 6, J = 6.9 Hz), 0.68 (s, 3); ^{13}C NMR ($CDCl_3$) δ 170.19 (s), 153.51 (d, $J_{^{13}C,F}$ = 253.3 Hz), 115.35 (d, J = 7.5 Hz), 72.69 (s), 36.62 (d, J = 33.7 Hz); mass spectrum (70 eV), m/e (relative intensity) 446 (parent 1), 386 (84), 371 (9), 163 (11), 161 (13), 125 (11), 123 (16), 111 (14), 97 (5), 95 (28), 83 (24), 81 (31), 71 (34), 69 (37), 57 (60), 55 49) 43 (100). Anal. Calcd for $C_{29}H_{47}FO_2$: C, 77.97; H, 10.61. Found: C, 77.81; H, 10.53.

6-Fluoro-7α-bromocholesteryl Acetate (3a). A mixture of 893 mg (1.99 mmol) of 6-fluorocholesteryl acetate (**2a**), 428 mg (2.43 mmol) of N-bromosuccinimide, and 1.0 mg of dibenzoyl peroxide in 15 mL of dry carbon tetrachloride was refluxed, with stirring, for 5 min with a 500-W visible spotlight as the heating source. The mixture was filtered by suction, the solvent was removed on a rotary evaporator, and the oily residue was recrystallized from acetone to yield 460 mg (44%) of **3a** (prisms): mp 116–117 °C; $[\alpha]^{25}_D$ −82° ($CHCl_3$, c 0.15); UV (cyclohexane) λ_{max} 226 nm (ε 1000); IR (neat, thin film) 1740, 1735, 1370, 1240, 1130, 1040, 1015, 985, 910, 740, 680 cm^{-1}; ^{1}H NMR ($CHCl_3$) δ 4.69 (m, 1), 4.55 (br d, 1, J = 8.3 Hz), 3.06 (dd, 1, J = 4.8 and 13.7 Hz), 2.07 (s, 3), 1.95 (br d, 1, J = 13.7 Hz), 1.06 (s, 3), 0.94 (d, 3, J = 5.5 Hz), 0.87 (d, 6, J = 5.9 Hz), 0.71 (s, 3); ^{13}C NMR ($CDCl_3$) δ 170.00 (s), 152.25 (d, $J_{^{13}C,F}$ = 251.4 Hz), 147.13 (d, J = 7.3 Hz), 71.85 (s), 53.62 (d, J = 24.9 Hz); mass spectrum (70 eV), m/e (relative intensity) 446 (3), 402 (10.7), 386 (100), 371 (11), 247 (28), 177 (11), 175 (10), 163 (23), 161 (23), 149 (31), 135 (19), 109 (20), 97 (20), 95 (27), 83 (26), 81 (36), 71 (30), 69 (32), 57 (58), 55 (42). Anal. Calcd for $C_{29}H_{46}BrFO_2$: C, 66.27; H, 8.82; Br, 15.20. Found: C, 66.48; H, 8.67; Br, 15.12.

6-Fluoro-7-dehydrocholesteryl Acetate (4a) and 6-Fluoro-4,6-cholestadien-3β-yl Acetate (5a). To a refluxing solution (160 °C) of 364 mg (0.69 mmol) of 6-fluoro-7α-bromocholesteryl acetate (**3a**) in 10 mL of dry p-xylene contained in a 25-mL, round-bottomed flask equipped with a magnetic stirrer, reflux condenser, and a dry nitrogen inlet was added, slowly, 258 mg (2.08 mmol) of trimethyl phosphite. The solution was heated under reduced pressure, and the oily residue was recrystallized from acetone to give 258 mg (84%) of white crystals of a 2:1 mixture of **4a** and **5a**. A 14-mg sample was easily separated (base-line resolution) by HPLC (solvent ether/mixed hexanes, 1.5:98.5) to give 9.2 mg of pure **5a** and 4.15 mg of **4a**.

The properties of 6-fluoro-7-dehydrocholesteryl acetate (**4a**) are as follows: mp 101–103 °C; $[\alpha]^{25}_D$ −77° ($CHCl_3$, c 0.20); UV (cyclohexane) λ_{max} 274 (ε 6200), 268, 282, 295 nm; IR (CCl_4) 1738, 1680, 1542, 1470, 1365, 1242, 1132, 1042, 1015, 982, 930, 901, 670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.42 (br d, 1, J = 8.8 Hz), 4.70 (m, 1), 3.00 (dd, 1, J = 4.7 and 14.8 Hz), 2.12 (s, 3), 0.99 (s, 3), 0.95 (d, 3, J = 6.5 Hz), 0.88 (d, 3, J = 6.8 Hz), 0.62 (s, 3); mass spectrum (70 eV), m/e (relative intensity) 444 (parent, 2), 384 (100), 369 (11), 271 (5), 229 (6), 215 (7), 201 (7), 176 (20), 163 (12), 161 (56), 159 (85), 137 (15), 123 (7), 95 (10), 81 (11), 57 (16), 55 (17). Anal. Calcd for $C_{29}H_{45}FO_2$: C, 78.33; H, 10.20. Found: C, 78.15; H, 10.14.

The properties of 6-fluoro-4,6-cholestadien-3β-yl acetate (**5a**) are as follows: mp 75–78 °C; $[\alpha]^{25}_D$ −106° ($CHCl_3$, c 0.46); UV (cyclohexane) λ_{max} 233 nm (ε 15 500), 240 nm (ε 15 900); IR (CCl_4) 1738, 1665, 1630, 1540, 1465, 1370, 1238, 1042, 1011, 968, 930, 869, 678 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.73 (br, s, 1), 5.41 (br t, 1, J = 7.9 Hz), 5.26 (br d, 1, J = 16.1 Hz), 2.11 (s, 3), 1.04 (s, 3), 0.91 (d, 3, J = 6.4 Hz), 0.87 (d, 6, J = 6.6 Hz), 0.73 (s, 3); mass spectrum (70 eV), m/e (relative intensity) 444 (parent, 10), 402 (79), 247 (50), 175 (16), 163 (24), 161 (30), 135 (31), 109 (30), 107 (26), 97 (23), 95 (45), 93 (26), 83 (37), 81 (72), 71 (52), 69 (64), 57 (92), 55 (66), 43 (100). Anal. Calcd for $C_{29}H_{45}FO_2$: C, 78.33; H, 10.20. Found: C, 78.08; H, 10.14.

6-Fluoro-7-dehydrocholesterol (4b). A solution of 1.133 g (2.5 mmol) of 6-fluorocholesteryl acetate, 408 mg (1.42 mmol) of 1,3-dibromo-5,5-dimethylhydrantoin, 40 mg (0.24 mmol) of azobis(isobutyronitrile) in 60 mL of spectral-grade n-hexane was heated under reflux (under nitrogen) for 15 min. The mixture was cooled in an ice-bath and filtered, and the solvent was removed on a rotary evaporator. The crude reaction product was dissolved in 30 mL of dry THF and 10 mg (0.03 mmol) of tetrabutylammonium bromide was added, and the solution was stirred for 1 h. To this solution was added 9 mL (9 mmol) of a 1 M solution of tetrabutylammonium fluoride in THF, and the stirring was continued for 2 h in the dark. The reaction solution was dilued with water, the mixture was extracted 3 times with ether, the combined ethereal extract were dried, and the solvent was removed on a rotary evaporator.

The crude dehydrobromination product was dissolved in 20 mL of dry ether and added, slowly at 0 °C, to a suspension of 114

(15) Dauben, W. G.; Lorber, M.; Fullerton, D. S. *J. Org. Chem.* **1969**, *34*, 3587.
(16) Corey, E. J.; Fleet, G. W. J. *Tetrahedron Lett.* **1973**, 4499.
(17) Salmond, W. G.; Barta, M. A.; Havens, J. L. *J. Org. Chem.* **1978**, *43*, 2057.
(18) Wilhelm, F.; Dauben, W. G.; Kohler, B.; Roesle, A.; Norman, A. W. *Archives Biochem. Biophys.* **1984**, *233*, 127.
(19) (a) Dodson, R. M.; Riegel, B. *J. Org. Chem.* **1948**, *13*, 424. (b) Shoppe, C. W. *J. Chem. Soc.* **1948**, 1033.
(20) An authentic sample of **2a** was kindly supplied by G. A. Boswell.

mg (3 mmol) of lithium aluminum hydride and 20 mL of ether, and the mixture was stirred at this temperature for 30 min. At the end of this time, TLC analysis indicated the presence of some unreacted starting material, and an additional 30 mg (0.7 mmol) of lithium aluminum hydride was added. The mixture was stirred for an additional 30 min followed by the addition of 5% aqueous hydrochloric acid until all solid material had dissolved. The material was extracted with ether, the solvent was removed on a rotary evaporator, the the residue was adsorbed on a small amount of silica gel. The adsorbed material was added to 60 g of 70–230-mesh silica gel and the chromatography was conducted with ethyl acetate/mixed hexanes (1:3). The first fractions contained 110 mg (11%) of 6-fluoro-4,6-cholestadien-3-ol (**5b**) and the later fraction yielded 478 mg (48%) of 6-fluoro-7-dehydrocholesterol (**4b**).

The properties of 6-fluoro-7-dehydrocholesterol are as follows: mp 141–143 °C dec (recrystallized from methanol/ether); $[\alpha]^{25}_D$ −94.5° (cyclohexane); UV (cyclohexane) λ_{max} 273 nm (ϵ 8700); IR (thin film) 3540–3080, 2940, 1670, 1460, 1378, 1125, 1070, 835, 795, 740 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.41 (d, 1, J = 8.7 Hz), 3.64 (m, 1), 3.00 (br d, 1, J = 4.5 Hz), 0.98 (s, 3), 0.94 (d, 3, J = 6.2 Hz), 0.87 (d, 6, J = 6.8 Hz), 0.61 (s, 3); mass spectrum (70 eV), m/e (relative intensity) 403 (25), 402 (parent, 61), 370 (58), 369 (82), 339 (27), 229 (20), 189 (21), 175 (31), 163 (54), 161 (53), 131 (37), 122 (93), 111 (23), 109 (27), 97 (37), 95 (38), 85 (25), 83 (45), 81 (39), 71 (53), 69 (63), 67 (21), 57 (83), 55 (76), 43 (100). Anal. Calcd for $C_{27}H_{43}FO$: C, 80.36; H, 10.73. Found: C, 80.60; H, 10.70.

This material was identical with the product formed by saponification of the acetate **4a**.

The properties of 6-fluoro-4,6-cholestadien-3β-ol are as follows: mp 138–139 °C (recrystallized from methanol/ether); $[\alpha]^{25}_D$ −55° (cyclohexane); UV (cyclohexane) λ_{max} 243 nm (ϵ 6100); IR (thin film) 3680–3520, 2920, 2880, 2860, 1710, 1650, 1620, 1450, 1440, 1360, 1050, 830, 790, 750 cm^{-1}; ^{1}H NMR (benzene-d_6) δ 6.18 (s, 1), 5.47 (d, 1, J = 15.9 Hz), 4.3 (m, 1), 3.54 (m, 2), 3.38 (d, 1, J = 13.2 Hz); mass spectrum (70 eV), m/e (relative intensity) 402 (parent, 33), 389 (34), 371 (35), 149 (20), 125 (24), 123 (25), 111 (35), 109 (33), 97 (52), 95 (50), 93 (21), 85 (40), 83 (56), 81 (51), 71 (64), 70 (20), 69 (66), 67 (30), 57 (85), 56 (23), 55 (73), 45 (25), 43 (100).

6-Fluoroprevitamin D_3 (6b). A solution of 69 mg (0.17 mmol) of 6-fluoro-7-dehydrocholesterol (**4b**) in 50 mL of anhydrous ether was placed in a Vycor tube, degassed by a nitrogen purge for 10 min, and the tube was closed with a stopcock and cooled to 0 °C. The tube was placed in a quartz Dewar flask containing ice–water and the solution was irradiated for 45 min in a Rayonet reactor containing 300-nm-type bulbs. The solvent was removed on a rotary evaporator and the residue was separated by flash chromatography[11] using ethyl acetate in mixed hexanes (1:3). The first fractions contained 30 mg (43%) of 6-fluoroprevitamin D_3 and the later fractions yielded 8 mg (12%) of 6-fluoro-7-dehydrocholesterol.

The properties of the oily previtamin are as follows: $[\alpha]^{25}_D$ +3° ($CHCl_3$); UV (ether) λ_{max} 252 nm (ϵ 6000); ^{1}H NMR ($CDCl_3$) δ 5.58 (d, 1, J = 21.7 Hz), 5.44 (d, 1, J = 3.8 Hz), 3.98 (br 1), 2.49 (br d, 1, J = 15.9 Hz), 1.70 (br s, 3), 0.91 (d, 3, J = 6.3 Hz), 0.88 (d, 6, J = 6.3 Hz), 0.68 (s, 3); mass spectrum, exact mass calcd for $C_{27}H_{45}FO$ m/e 402.32976, found 402.32890.

6-Fluorovitamin D_3 (7b). In a glass tube was dissolved 30 mg (0.07 mmol) of 6-fluoroprevitamin D_3 in 9 mL of dry isooctane. The solution was degassed by an argon purge for 5 min, and the tube was sealed with a rubber septum, fastened with a copper wire, and put in an oil-bath at 120–130 °C for 4.5 h. The solution was concentrated under reduced pressure and the product was chromatographed on a preparative silica gel TLC plate with ethyl acetate in mixed hexanes (1:3) in a chromatography tank, filled with argon, to yield 9 mg (30%) of 6-fluorovitamin D_3 as a colorless oil.

The properties of 6-fluorovitamin D_3 are as follows: UV (hexane) λ_{max} 268 nm (ϵ 10 300); c, NMR ($CDCl_3$) δ 6.00 (br d, 1, J = 6.0 Hz), 5.10 (s, 1), 4.93 (s, 1), 4.02 (m, 1), 0.95 (d, 3, J = 6.4 Hz), 0.87 (d, 6, J = 6.4 Hz), 0.66 (s, 3); mass spectrum (70 eV), m/e (relative intensity) 402 (parent, 50), 369 (67), 315 (26), 314 (100), 299 (21), 281 (23), 271 (25), 299 (33), 163 (30), 161 (32), 147 (23), 145 (30), 133 (30), 109 (29), 107 (32), 105 (30), 95 (40), 81 (41).

6-Fluoro-7-oxocholesteryl Acetate. To a suspension of 60 g (0.6 mol) of chromium trioxide, dried several days in a vacuum desiccator over phosphorus pentoxide, in 600 mL of dry, cold (−20 °C) methylene chloride in a 1-L, three-necked, round-bottomed flask equipped with a sealed mechanical stirrer and an oxygen inelt was added, in one portion, 57.6 g (0.6 mol) of recrystallized 3,5-dimethylpyrazole.[16,17] The mixture was stirred for 15 min to give a dark red solution of the oxidant (the presence of moisture will cause the solution to be yellow and not active). To this solution was added 13.2 g (29.5 mmol) of 6-fluorocholesteryl acetate, and the mixture was stirred for 48 h in the dark at −20 °C (cold room) under a slow stream of oxygen. The reaction did not proceed under a nitrogen atmosphere. The mixture was poured into 250 mL of 5 N sodium hydroxide solution and stirred at 0 °C for 2 h. At this temperature, the green thick aqueous phase containing most of the chromium salts separated. The organic phase was separated, washed with 10% aqueous hydrochloric acid, water, and aqueous saturated sodium chloride solution, dried, and concentrated on a rotary evaporator. The residue was purified by chromatography through a short column containing 50 g of silica gel using 5% ethyl acetate/hexane as the eluant. The crude product was recrystallized from ether to yield 13.6 g (84%) of 6-fluoro-7-oxocholesteryl acetate with the following properties: mp 190–192 °C; $[\alpha]^{25}_D$ −34° ($CHCl_3$, c 0.092); UV (cyclohexane) λ_{max} 239 nm (ϵ 6800); IR (thin film) 1730, 1690, 1470, 1376, 1270, 1209, 1130, 1050, 910, 805 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.65 (m, 1), 3.18 (dd, 1, J = 4.6 and 14.3 Hz), 1.07 (s, 3), 0.93 (d, 3, J = 6.3 Hz), 0.86 (d, 6, J = 6.6 Hz), 0.70 (s, 3); mass spectrum (70 eV), m/e (relative intensity) 460 (parent, detected by saturation), 400 (53), 205 (25), 192 (100), 179 (19), 177 (23), 109 (20), 95 (18), 93 (18), 92 (15), 81 (27), 69 (26), 57 (34), 55 (36), 43 (97). Anal. Calcd for $C_{29}H_{45}FO_3$: C, 75.61; H, 9.85. Found: C, 75.90; H, 10.13.

Acknowledgment. This study was supported by Grant No. 00709, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases. We express our appreciation to Dr. G. A. Boswell of the Du Pont Company for his aid, advice, and encouragement. One of us (W.G.D.) thanks the Alexander von Humboldt Foundation for an award and the Max-Planck-Institut für Medizinische Forschung, Heidelberg, for their kind hospitality.

Registry No. **1a**, 18642-27-8; **2a**, 75676-29-8; **3a**, 96109-98-7; **4a**, 96109-99-8; **4b**, 96110-00-8; **5a**, 96110-01-9; **5b**, 96110-02-0; **6b**, 96128-98-2; **7b**, 91625-75-1; 6-fluoro-7-oxocholesteryl acetate, 96110-03-1.