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Synthesis and Biological Activity of 1α-Fluoro-25-hydroxyvitamin D_3

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trans Diaxial opening with potassium hydrogen difluoride of the 1β,2β-epoxy-3β-ol derived from cholenic acid gave the 1α-fluoro-2β,3β-diol derivative. Construction of the 25-hydroxy cholesterol side chain and deoxygenation of the 2β-hydroxy group afforded 1α-fluoro-3β,25-dihydroxycholest-5-ene, which was transformed into 1α-fluoro-25-hydroxyvitamin D_3. A single dose of 1.3 μg of 1α-fluoro-25-hydroxyvitamin D_3 produced neither an intestinal calcium transport response nor a bone calcium mobilization response in vitamin D-deficient rats. In the same rats, 50 ng of 25-hydroxyvitamin D_3 produced a marked response. In contrast, 1α-fluoro-25-hydroxyvitamin D_3 was 30 times more active than 25-hydroxyvitamin D_3 in binding to the chick intestinal receptor for 1α,25-dihydroxyvitamin D_3. Addition of 1α-fluoro-25-hydroxyvitamin D_3 to human promylocytic leukemia cells resulted in strong phagocytic activity.

Keywords——vitamin D_3; 1α,25-dihydroxyvitamin D_3; 1α-fluoro-25-hydroxyvitamin D_3; calcium transport; cytosol receptor protein; cell differentiation

Metabolic hydroxylation of vitamin D_3 at the C-1 and C-25 positions produces the hormonally active form of vitamin D_3, 1α,25-dihydroxyvitamin D_3, which mediates the metabolism of calcium and phosphorus. Although the physiological actions of the hormone on the target tissues are well established,[1] those of (24*R*)-24,25-dihydroxyvitamin D_3, a major circulating metabolite of vitamin D_3 remain to be established.[2] Vitamin D_3 analogues which possess a hydroxyl group at the C-24 and/or C-25 position and fluorine at the 1α-position may be a useful research tool for this purpose. Since 1α-hydroxylation, which is an important step for eliciting biological activity, is blocked in these analogues, the biological importance of the 24- and/or 25-hydroxyl groups can be examined independently of that of the 1α-hydroxyl group. In this paper we describe the synthesis of 1α-fluoro-25-hydroxyvitamin D_3 (**19b**) and its biological activities.

Treatment of cholenic acid (**1**) with 2,3-dihydropyran in the presence of *p*-toluenesulphonic acid and the subsequent reduction with lithium aluminium hydride were followed by protection of the resulting 24-ol as the methoxymethyl ether to provide the ether **2** in 80% yield. Hydroboration of **2** with BH_3–tetrahydrofuran complex, followed by alkaline H_2O_2 oxidation, gave, after removal of the tetrahydropyranyl group, the 3,6-diol, which was oxidized with Jones reagent to give the 3,6-diketone **3** in 60% overall yield. This was converted into the 6β-acetoxy-3-oxosteroid **4** in 71% yield by the following successive reactions; reduction with lithium aluminium hydride to the 3β,6β-diol, acetylation, selective saponification of the 3-acetate, and Jones oxidation. After exchange of the protecting group of the side chain to an acetyl group, the 3-ketone **5a** was brominated with pyridinium hydrobromide perbromide[3] and then dehydrobrominated with $CaCO_3$ in refluxing dimethyl-

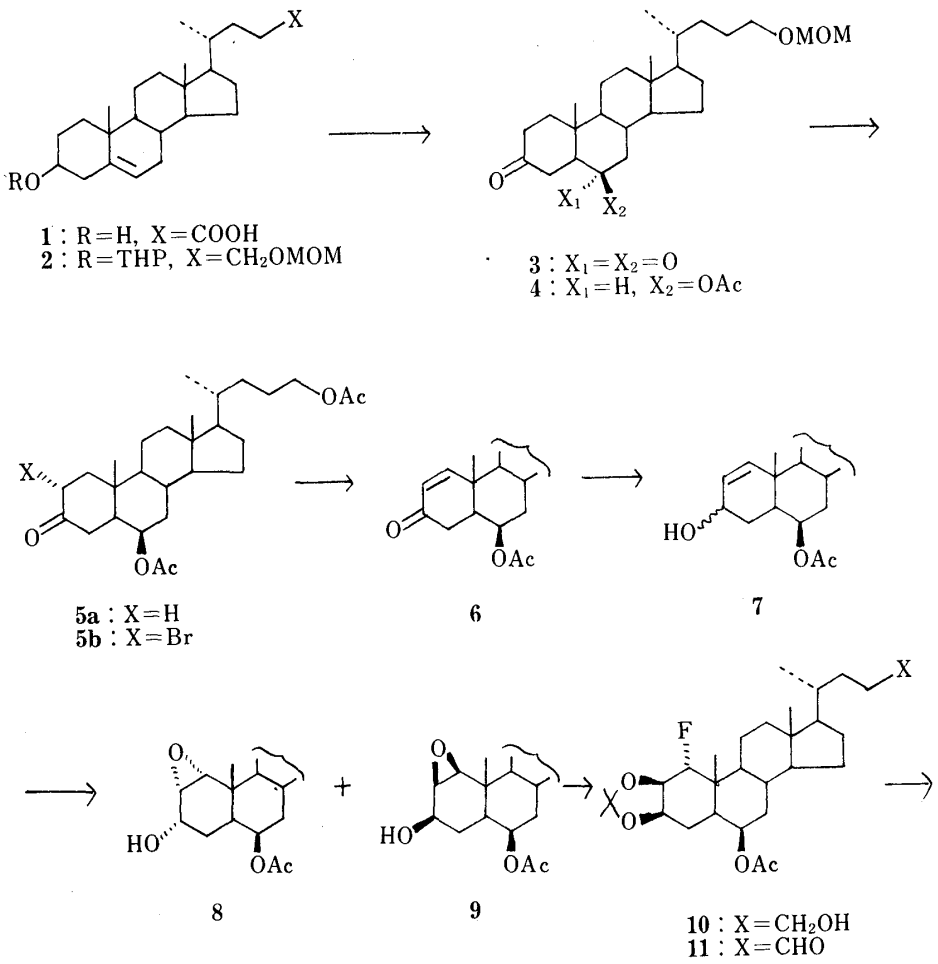

Chart 1

formamide to provide the 1-en-3-one **6** in 68% overall yield from **4**. Reduction of **6** with sodium borohydride in the presence of $CeCl_3 \cdot 7H_2O$[4] gave an epimeric mixture of the allylic alcohol **7**. Epoxidation of **7** with *m*-chloroperbenzoic acid proceeded according to Henbest's rule.[5] Chromatographic separation of the resulting mixture gave the less polar 1α,2α-epoxide **8** and the more polar 1β,2β-epoxide **9** in 43 and 45% yields, respectively, from the enone **6**. The stereochemical assignment of **8** and **9** was based on proton nuclear magnetic resonance (^{1}H-NMR) analysis. The epoxides **8** and **9** showed the characteristic signals due to 1-H and 2-H at δ 3.02 (2H, singlet) and at δ 3.22 (2H, singlet), respectively, which are in accordance with those of the known 6β-acetoxy-1α,2α-epoxy-5α-cholestan-3α-ol and 6β-acetoxy-1β,2β-epoxy-5α-cholestan-3β-ol,[6] respectively.

Introduction of fluorine at the 1α-position of the β-epoxide **9** was achieved by our method as described in the preceding paper.[6] Treatment of **9** with potassium hydrogen difluoride in ethyleneglycol at 140 °C (accompanied by deacetylation at the C-24 position) and the subsequent acetonide formation gave the 1α-fluoroacetonide **10** in 45% yield. Swern oxidation[7] of **10** followed by Wittig reaction with the ylide derived from isopropyltriphenylphosphonium iodide and saponification provided the 24-ene **12** in 61% yield, and **12** was dehydrated with $POCl_3$–pyridine to give the 5,24-diene **13**. Oxymercuration and demercuration of the diene **13** proceeded selectively to afford the 25-hydroxy compound **14** in 81% yield. In its ^{1}H-NMR spectrum, the fluoro compound **14** showed characteristic signals due to 1β-H at δ 4.74 as a double doublet with the coupling constants of 44 and 3 Hz and those due to 19-H at δ 1.16 as a doublet (3 Hz). These data are in good agreement with those of the known 1α-fluoro-2β,3β-isopropylidenedioxycholest-5-ene.[6] Thus, the configuration of fluorine at the

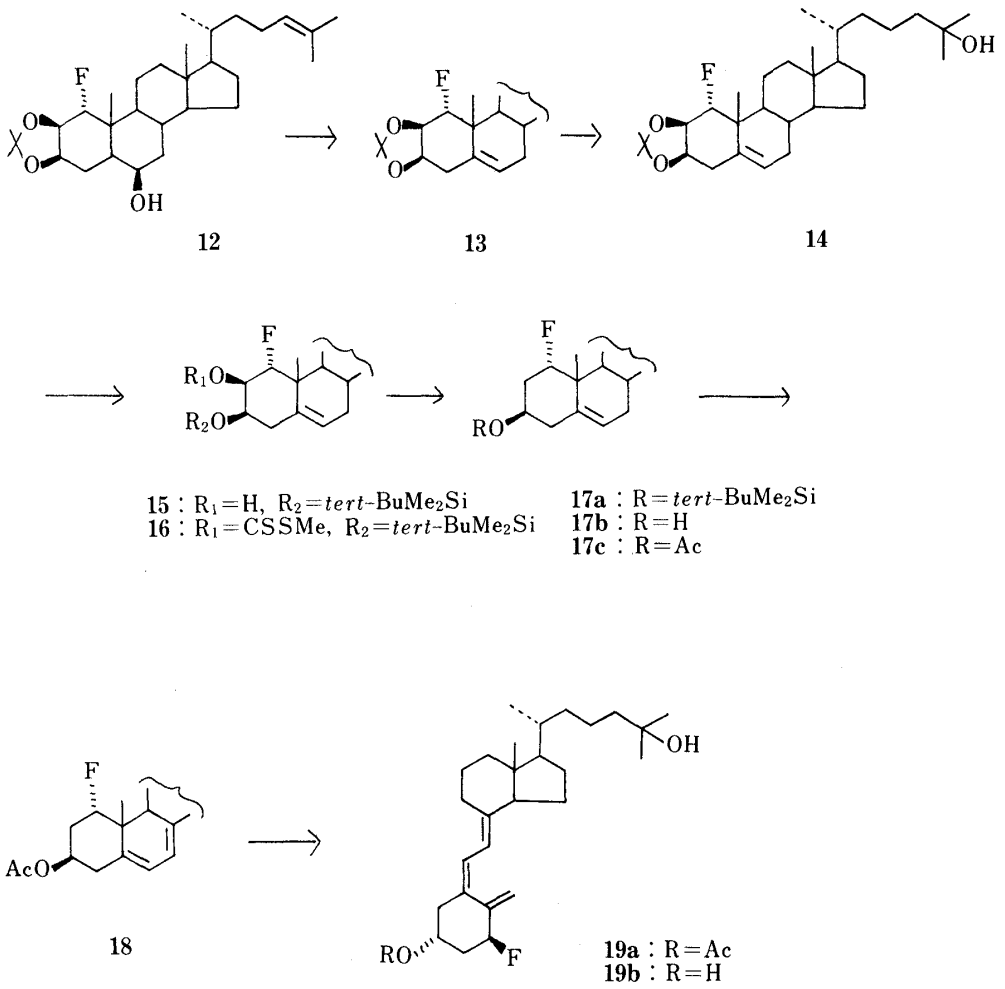

Chart 2

C-1 position was unambiguously confirmed.

Removal of the acetonide protecting group and the subsequent selective silylation with *tert*-butyldimethylsilyl chloride and imidazole in dimethylformamide gave the 3β-monosilyl ether **15** in 96% yield, and this product was converted to the 2β-monoxanthate ester **16**. Reduction of **16** with tributyltinhydride afforded, after removal of the silyl group, 1α-fluoro-3β,25-dihydroxycholest-5-ene (**17b**) in 73% yield. The 1α-fluorosterol **17b** showed the expected downfield shift[6] of 3-hydrogen at δ 3.90 in its ^{1}H-NMR spectrum owing to the 1,3-diaxial interaction between 1α-fluorine and 3α-hydrogen.

Transformation of **17b** into 1α-fluoro-25-hydroxyvitamin D_3 (**19b**) was carried out according to the known procedure.[8] Allylic bromination of the acetate **17c** with *N*-bromosuccinimide was followed by dehydrobromination with tetrabutylammonium fluoride to give a mixture of the 4,6-diene and 5,7-diene, from which the desired 5,7-diene **18** was isolated in 29% yield. The 5,7-diene **18** in benzene–ethanol was irradiated with a medium pressure mercury lamp through a Vycol filter for 3 min and then refluxed for 1 h to provide the vitamin D_3 acetate **19a** in 19% yield. The 5,7-diene **18** was recovered in 44% yield. Saponification and purification by high performance liquid chromatography gave 1α-fluoro-25-hydroxyvitamin D_3 (**19b**) in 32% yield.

Biological Activities

The biological effects of 1α-fluoro-25-hydroxyvitamin D_3 (**19b**) and 25-hydroxyvitamin D_3 on intestinal calcium transport[9] and on bone calcium mobilization measured in terms of

TABLE I. Increase in Intestinal Calcium Transport and Serum Calcium Concentration in Response to 1α-F-25-OH-D_3 and 25-OH-D_3

Compound given	Amount of compound	Intestinal Ca transport (Ca serosal/Ca mucosal)	Serum Ca (mg/100 ml)
EtOH	—	$2.8 \pm 0.4^{*a)}$	$2.8 \pm 0.1^{d)}$
25-OH-D_3	250 ng/rat	$5.5 \pm 0.7^{b)}$	$3.5 \pm 0.1^{e)}$
1αF-25-OH-D_3	1.3 μg/rat	$3.1 \pm 1.0^{c)}$	$2.6 \pm 0.2^{f)}$
Significance of difference		*b*) from *a*) $p<0.001$	*e*) from *d*) $p<0.001$
(Performed by the student's '*t*' test)		*c*) from *a*) N.S.	*f*) from *d*) N.S.

Weanling male rats were obtained from Haltzman Co., Madison, Wis., and were fed a low Ca vitamin D-deficient diet[9] for 3 weeks, and allowed water *ad libitum*. They were then divided into three groups of 6 rats each. Rats in the control group received 0.05 ml of 95% ethanol by intrajugular injection. Rats in the second group received 250 ng/rat of 25-hydroxyvitamin D_3, while rats in the third group received 1.3 μg/rat of 1α-fluoro-25-hydroxyvitamin D_3 in 0.05 ml of 95% ethanol. Twenty-four hours later, intestinal Ca transport and serum Ca concentration (bone Ca mobilization) were measured as described by Martin[10] and Tanaka.[11]

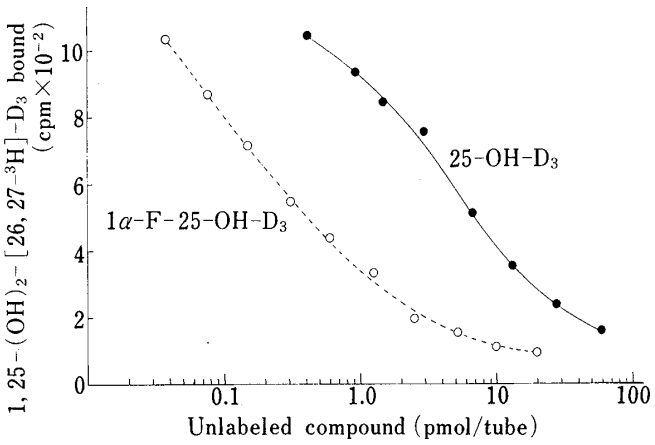

Fig. 1. Binding Activity of 1α-Fluoro-25-hydroxyvitamin D_3 to Chick Intestinal Cytosol Receptor Protein for 1α,25-Dihydroxyvitamin D_3

The binding activity of 1α-fluoro-25-hydroxyvitamin D_3 (**19b**) to chick intestinal cytosol protein for 1α,25-dihydroxyvitamin D_3 was examined by measuring the displacement of 1α,25-dihydroxy-[26,27-^{3}H]-vitamin D_3 from the protein by the fluoro analogue (**19b**). Various concentrations of 1α-fluoro-25-hydroxyvitamin D_3 (**19b**) were dissolved in 50 μl of 95% ethanol and added to the cytosol receptor in the presence of 1α,25-dihydroxy-[26,27-^{3}H]-vitamin D_3 as described by Shepard *et al.*[12] Each point represents the mean value from duplicate determinations.

the serum calcium concentration[10] were compared. The results are shown in Table I. 1α-Fluoro-25-hydroxyvitamin D_3 (**19b**) at a dosage level of 1.3 μg failed to stimulate intestinal calcium transport or bone calcium mobilization.

The binding activity of (**19b**) to chick intestinal cytosol receptor protein for 1α,25-dihydroxyvitamin D_3[11] was compared with that of 25-hydroxyvitamin D_3 (Fig. 1). 1α-Fluoro-25-hydroxyvitamin D_3 exhibited binding activity 30 times that of 25-hydroxyvitamin D_3.

A concentration of 0.26 μg/ml of 1α-fluoro-25-hydroxyvitamin D_3 elicited 40% differentiation in human promyelocytic leukemia cells (HL60) as shown by phagocytic activity.[13] In the same test, a concentration of 0.05 μg/ml of 1α,25-dihydroxyvitamin D_3 or 0.55 μg/ml for (24*R*)-24,25-dihydroxyvitamin D_3 produced the same response.

Experimental

Melting points were determined with a hot-stage microscope and are uncorrected. ^{1}H-NMR spectra were taken with a Hitachi R-24-A or a JEOL PS-100 spectrometer in $CDCl_3$ with Me_4Si as an internal standard. Mass spectra (MS) were determined with a Shimadzu LKB-9000S or a Hitachi M-80 mass spectrometer at 70 eV. Ultraviolet (UV) spectra were obtained in EtOH solution with a Shimadzu UV-200 double beam spectrophotometer. Column chromatography was done on silica gel (E. Merck, 70—230 mesh). Preparative thin layer chromatography (TLC) was carried out on precoated plates of silica gel (E. Merck, 0.25 mm thickness). The usual work-up refers to dilution with water, extraction with the organic solvent indicated in parenthesis, washing of the extract to neutrality, drying over $MgSO_4$, filtration, and removal of the solvent under reduced pressure. The following abbreviations are used: THF, tetrahydrofuran; THP, tetrahydropyranyl; EtOAc, ethyl acetate; C_6H_6, benzene; *p*-TsOH, *p*-toluenesulphonic acid; $LiAlH_4$, lithium aluminium hydride; $NaBH_4$, sodium borohydride; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ether, diethyl ether; CH_2Cl_2, dichloromethane; MeOH, methanol; AcOH acetic acid.

24-Methoxymethoxy-3β-tetrahydropyranyloxychol-5-ene (2)——A mixture of cholenic acid (**1**) (30 g, 95.9 mmol), 2,3-dihydropyran (150 ml), and *p*-TsOH (1.0 g) in C_6H_6 (450 ml) and THF (300 ml) was stirred at room temperature for 1 h. $LiAlH_4$ (15 g) was added to the reaction mixture and the whole was stirred at room temperature for 1 h. Excess reagent was destroyed with water, and the resulting inorganic salts were filtered off and washed with THF and EtOAc. Removal of the solvent gave the crude 3β-THP ether (48 g). This in dioxane (150 ml) was treated with chloromethyl methyl ether (10 ml) and *N,N*-diethylcyclohexylamine (60 ml) at room temperature for 10 h. The usual work-up (EtOAc) and chromatography on silica gel (600 g) with hexane–EtOAc (10 : 1) gave the ether **2** (37.3 g, 80%), mp 101—105 °C (MeOH–H_2O). δ 0.65 (3H, s, 18-H_3), 0.90 (3H, d, J=6 Hz, 21-H_3), 1.00 (3H, s, 19-H_3), 3.30 (3H, s, OCH_3), 3.60 (2H, t, J=6 Hz, 24-H_2), 4.65 (2H, d, J=5 Hz, O–CH_2–O–CH_3), and 5.25 (1H, m, 6-H). High resolution MS, Calcd for $C_{26}H_{42}O_2$ (M^+ −THPOH) *m/z* 386.3187, Found *m/z* 386.3200.

24-Methoxymethoxy-5α-cholane-3,6-dione (3)——The 5-ene **2** (37 g, 75.8 mmol) in THF (100 ml) was treated with 1 M BH_3–THF complex solution (100 ml) at room temperature overnight. After destruction of the excess reagent with water, 3 M NaOH (60 ml) and 30% H_2O_2 (60 ml) were added. The mixture was stirred at 40 °C for 30 min. The usual work-up (ether) gave the 3,6-diol, which was treated with 2 eq of Jones reagent in acetone (300 ml) at −15 °C for 30 min. The usual work-up (EtOAc) and chromatography on silica gel (200 g) with C_6H_6–EtOAc (10 : 1) gave the 3,6-diketone **3** (19.1 g, 60%), mp 118—120 °C (MeOH). δ 0.70 (3H, s, 18-H_3), 0.95 (3H, d, J=6 Hz, 21-H_3), 0.96 (3H, s, 19-H_3), 3.33 (3H, s, OCH_3), 3.46 (2H, t, J=6 Hz, 24-H_2), and 4.59 (2H, s, O–CH_2–O–CH_3). High resolution MS, Calcd for $C_{25}H_{38}O_3$ (M^+ −MeOH) *m/z* 386.2822, Found *m/z* 386.2812.

6β-Acetoxy-24-methoxymethoxy-5α-cholan-3-one (4)——The diketone **3** (8.38 g, 20 mmol) in THF was treated with $LiAlH_4$ (2.0 g) at room temperature for 1 h. Addition of water, filtration, and removal of the solvent gave the 3,6-diol (6.78 g), which was treated with acetic anhydride (60 ml) under reflux for 2 h. The usual work-up (EtOAc) gave the crude 3,6-diacetate. This in THF (50 ml) was treated with 5% KOH–MeOH (10 ml) at room temperature for 1 h. The usual work-up (EtOAc) gave the 3β-ol, which was then treated with 1 eq of Jones reagent in acetone (200 ml) at −15 °C for 15 min. The usual work-up (ether) and chromatography on silica gel (200 g) with C_6H_6–EtOAc (10 : 1) gave the 3-oxosteroid **4** (6.5 g, 71%), mp 93—95 °C (MeOH). δ 0.70 (3H, s, 18-H_3), 0.90 (3H, d, J=6 Hz, 21-H_3), 1.17 (3H, s, 19-H_3), 2.04 (3H, s, acetyl), 3.32 (3H, s, OCH_3), 3.46 (2H, d, J=6 Hz, 24-H_2), 4.60 (2H, s, O–CH_2–O–CH_3), and 4.85 (1H, m, 6α-H). High resolution MS, Calcd for $C_{26}H_{42}O_3$ (M^+ −AcOH) *m/z* 402.3136, Found *m/z* 402.3140.

6β,24-Diacetoxy-5α-cholan-3-one (5a)——The 3-ketone **4** (12.6 g, 27.3 mmol) in aqueous dioxane (200 ml) was treated with *p*-TsOH (20 mg) at 80—100 °C for 5 h. The usual work-up (EtOAc) gave the residue, which was treated with acetic anhydride (8 ml) and pyridine (30 ml) at room temperature for 2 h. The usual work-up (EtOAc) and chromatography on silica gel (50 g) with hexane–EtOAc (8 : 1) gave the acetate **5a** (10.8 g, 86%), mp 131—133 °C (hexane). δ 0.70 (3H, s, 18-H_3), 0.90 (3H, d, J=6 Hz, 21-H_3), 1.16 (3H, s, 19-H_3), 2.01 (6H, s, acetyl), 4.00 (2H, t, J=6 Hz, 24-H_2), and 4.95 (1H, m, 6α-H). High resolution MS, Calcd for $C_{28}H_{44}O_5$ (M^+ −AcOH) *m/z* 400.2979, Found *m/z* 400.2989.

6β,24-Diacetoxy-5α-chol-1-en-3-one (6)——The acetate **5a** (4.36 g, 9.48 mmol) in AcOH (50 ml) was treated with pyridinium bromoperbromide[3] (3.64 g, 11.4 mmol) at 50 °C for 20 min. The usual work-up (ether) gave the bromide **5b** (5.1 g), mp 194—196 °C (MeOH). This in DMF (40 ml) was treated with $CaCO_3$ (4.0 g) under reflux for 2 h. The usual work-up (EtOAc) and chromatography on silica gel (100 g) with hexane–EtOAc (10 : 1) gave the enone **6** (3.19 g, 74% from **5a**), mp 145—147 °C (hexane). λ_{max}: 233 nm (ε 8300), δ 0.75 (3H, s, 18-H_3), 0.94 (3H, d, J=6 Hz, 21-H_3), 1.18 (3H, s, 19-H_3), 2.05 (3H, s, acetyl), 2.09 (3H, s, acetyl), 4.00 (2H, t, J=6 Hz, 24-H_2), 4.96 (1H, m, 6α-H), 5.78 (1H, d, J=10 Hz, 2-H), and 7.02 (1H, d, J=10 Hz, 1-H), High resolution MS, Calcd for $C_{26}H_{38}O_2$ (M^+ −AcOH) *m/z* 398.2822, Found *m/z* 398.2838.

6β,24-Diacetoxy-1α,2α-epoxy-3α-hydroxy-5α-cholane (8)——The enone **6** (1.0 g, 2.18 mmol) and $CeCl_3 \cdot 7H_2O$ (1.0 g, 2.62 mmol) were dissolved in MeOH (40 ml) and THF (40 ml). $NaBH_4$ (130 mg, 3.27 mmol) was added in one portion and stirring was continued for 5 min. The usual work-up (EtOAc) gave a mixture of allylic alcohol **7**. This in CH_2Cl_2 (120 ml) was treated with *m*-chloroperbenzoic acid (750 mg, 4.36 mmol) at room temperature for 18 h. $Ca(OH)_2$ (5.0 g) was added and the whole was stirred at room temperature for 1 h. The precipitated salts were filtered

off and evaporation of the solvent gave the crude epoxides, which were separated by flash chromatography (3.5 cm i.d. × 15 cm, Kieselgel 60, 230—400 mesh, E. Merck). Elution with EtOAc–hexane (2 : 1) gave the less polar α-epoxide **8** (448 mg, 43%), mp 165—167 °C (hexane–EtOAc). δ 0.70 (3H, s, 18-H_3), 0.90 (3H, d, J=6 Hz, 21-H_3), 1.13 (3H, s, 19-H_3), 2.03 (6H, s, two acetyls), 3.02 (2H, s, 1β-H and 2β-H), 3.98 (2H, t, J=6 Hz, 24-H_2), 4.00 (1H, m, 3β-H), and 4.96 (1H, m, 6α-H), High resolution MS, Calcd for $C_{26}H_{40}O_4$ (M^+ −AcOH) m/z 416.2928, Found m/z 416.2905.

6β,24-Diacetoxy-1β,2β-epoxy-3β-hydroxy-5α-cholane (9)——Further elution with the same solvent gave the more polar β-epoxide **9** (450 mg, 45%), mp 156—158 °C (hexane–EtOAc). δ 0.74 (3H, s, 18-H_3), 0.94 (3H, d, J=6 Hz, 21-H_3), 1.12 (3H, s, 19-H_3), 2.06 (6H, s, two acetyls), 3.22 (2H, s, 1α-H and 2α-H), 4.00 (3H, m, 24-H_2 and 3α-H), and 4.92 (1H, m, 6α-H), High resolution MS, Calcd for $C_{26}H_{40}O_4$ (M^+ −AcOH) m/z 416.2928, Found m/z 416.2922.

6β-Acetoxy-1α-fluoro-24-hydroxy-2β,3β-isopropylidenedioxy-5α-cholane (10)——The β-epoxide **9** (528 mg, 1.21 mmol) in ethyleneglycol (7 ml) was treated with potassium hydrogen difluoride (2.5 g) at 140 °C for 7 h. The usual work-up (EtOAc) gave the crude product, which was treated with acetone (100 ml) containing a catalytic amount of p-TsOH at room temperature for 1 h. The usual work-up (EtOAc) and chromatography on silica gel (20 g) with C_6H_6–EtOAc (15 : 1) gave the 1α-fluoroacetonide **10** (264 mg, 45% from **9**) as an amorphous solid. δ 0.70 (3H, s, 18-H_3), 0.90 (3H, d, J=6 Hz, 21-H_3), 1.11 (3H, d, J=3 Hz, 19-H_3), 1.30 and 1.50 (6H, s × 2, acetonide), 2.02 (3H, s, acetyl), 3.58 (2H, t, J=6 Hz, 24-H_2), 4.06—4.25 (2H, m, 2α-H and 3α-H), 4.70 (1H, dd, J=44 and 3 Hz, 1β-H), and 4.94 (1H, m, 6α-H), High resolution MS, Calcd for $C_{27}H_{43}FO_3$ (M^+ −AcOH) m/z 434.3198, Found m/z 434.3212.

6β-Acetoxy-1α-fluoro-2β,3β-isopropylidenedioxy-5α-cholan-24-al (11)——DMSO (294 μl) was added to a solution of CH_2Cl_2 (3 ml) and oxalylchloride (172 μl) at −78 °C under argon and the whole was stirred at −78 °C for 10 min. Then, asolution of the alcohol **10** (377 mg, 0.763 mmol) in CH_2Cl_2 (2 ml) was added dropwise at −78 °C. After 15 min, triethylamine (1.07 ml) was added and stirring was continued for 5 min. The usual work-up (CH_2Cl_2) gave the aldehyde **11** (366 mg) as an oil. δ 0.70 (3H, s, 18-H_3), 1.10 (3H, d, J=3 Hz, 19-H_3), 1.32 and 1.49 (6H, s × 2, acetonide), 2.03 (3H, s, acetyl), 3.92—4.50 (2H, m, 2α-H and 3α-H), 5.02 (1H, m, 6α-H), and 9.82 (1H, t, J=2 Hz, 24-H).

1α-Fluoro-6β-hydroxy-2β,3β-isopropylidenedioxy-5α-cholest-24-ene (12)——A solution of n-BuLi in hexane (1.6 M, 1.05 ml) was added dropwise to a suspension of isopropyltriphenylphosphonium iodide (480 mg) in THF (4 ml) at 0 °C under argon. The mixture was stirred at 0 °C for 15 min. A solution of the aldehyde **11** (369 mg) in THF (2 ml) was added to the resulting ylide solution at 0 °C. The mixture was stirred at room temperature for 1 h. The usual work-up (EtOAc) gave the crude product, which was treated with 5% KOH–MeOH (20 ml) at 60 °C for 3 h. The usual work-up (EtOAc) and chromatography on silica gel (10 g) with C_6H_6–EtOAc (10 : 1) gave the 6β-ol **12** (220 mg, 61% from **10**) as an amorphous solid. δ 0.70 (3H, s, 18-H_3), 0.92 (3H, d, J=6 Hz, 21-H_3), 1.12 (3H, d, J=3 Hz, 19-H_3), 1.36 and 1.54 (6H, s × 2, acetonide), 1.60 and 1.68 (6H, s × 2, 26-H_3 and 27-H_3), 3.85 (1H, m, 6α-H), 4.00—4.40 (2H, m, 2α-H and 3α-H), 4.74 (1H, dd, J=44 and 3 Hz, 1β-H), and 5.02 (1H, m, 24-H).

1α-Fluoro-2β,3β-isopropylidenedioxycholesta-5,24-diene (13)——The 6β-ol **12** (163 mg, 0.342 mmol) in pyridine (4 ml) was treated with $POCl_3$ (0.1 ml) at 0 °C for 3 h. The usual work-up (EtOAc) and chromatography on silica gel (2 g) with C_6H_6 gave the diene **13** (120 mg, 77%), mp 119—120 °C (MeOH–H_2O). δ 0.70 (3H, s, 18-H_3), 0.92 (3H, d, J=6 Hz, 21-H_3), 1.16 (3H, d, J=3 Hz, 19-H_3), 1.36 and 1.54 (6H, s × 2, acetonide), 1.60 and 1.68 (6H, s × 2, 26-H_3 and 27-H_3), 4.00—4.40 (2H, m, 2α-H and 3α-H), 4.74 (1H, dd, J=44 and 3 Hz, 1β-H), 5.02 (1H, m, 24-H), 5.48 (1H, m, 6-H), High resolution MS, Calcd for $C_{30}H_{47}FO_2$ (M^+) m/z 458.3557, Found m/z 458.3540.

1α-Fluoro-25-hydroxy-2β,3β-isopropylidenedioxycholest-5-ene (14)——A mixture of mercury diacetate (145 mg, 0.455 mmol), water (0.8 ml) and THF (0.4 ml) was added dropwise to a solution of the diene **13** (173 mg, 0.379 mmol) in THF (0.8 ml) at 0 °C. The mixture was stirred at room temperature for 30 h and then 3 M NaOH (120 μl) and $NaBH_4$ (240 mg) in 3 M NaOH (1.2 ml) were added. The resulting mercury was filtered off and the filtrate was extracted with EtOAc. The usual work-up and chromatography on silica gel (2 g) with C_6H_6–EtOAc (10 : 1) gave the 25-ol **14** (146 mg, 81%) as an amorphous solid. δ 0.69 (3H, s, 18-H_3), 0.92 (3H, d, J=6 Hz, 21-H_3), 1.16 (3H, d, J=3 Hz, 19-H_3), 1.22 (6H, s, 26-H_3 and 27-H_3), 1.36 and 1.54 (6H, s × 2, acetonide), 4.00—4.42 (2H, m, 2α-H and 3α-H), 4.74 (1H, dd, J=44 and 3 Hz, 1β-H), and 5.50 (1H, m, 6-H), MS m/z 476 (M^+), 461, 459, 443, 418, 400, 380, 345 (base peak), 305, 287, 245, 111.

1α-Fluoro-2β,25-dihydroxy-3β-(*tert*-butyldimethylsilyloxy)cholest-5-ene (15)——The acetonide **14** (146 mg, 0.306 mmol) in THF–MeOH (1 : 1, 10 ml) was treated with a catalytic amount of p-TsOH at room temperature for 16 h. The usual work-up (EtOAc) gave the triol (131 mg), mp 240 °C (dec.) (hexane–EtOAc). A mixture of the triol (131 mg) DMF (8 ml), *tert*-butyldimethylsilyl chloride (100 mg, 0.61 mmol), and imidazole (62 mg, 0.92 mmol) was stirred at room temperature for 4 h. The usual work-up and chromatography on silica gel (10 g) with C_6H_6–EtOAc (15 : 1) gave the 3β-silyl ether **15** (162 mg, 96%) as an amorphous powder.

***O*-[1α-Fluoro-25-hydroxy-3β-(*tert*-butyldimethylsilyloxy)cholest-5-en-2β-yl]-*S*-methyl Dithiocarbonate (16)**——A mixture of the 3β-silyl ether **15** (162 mg, 0.294 mmol), NaH dispersion (60%, 28 mg), imidazole (4 mg), and THF (12 ml) was refluxed for 3 h under argon. Carbon disulphide (1 ml) was added and refluxing was continued for 0.5 h. Then, methyl iodide (1 ml) was added and the mixture was refluxed for 0.5 h. The usual work-up (EtOAc) and preparative TLC (C_6H_6–EtOAc (5 : 1), developed once) gave the xanthate **16** (76 mg, 40%) as an oil. Rf=0.77, δ 0.65 (3H, s, 18-H_3), 0.84 (9H, s, *tert*-butyl), 0.92 (3H, d, J=6 Hz, 21-H_3), 1.02 (3H, d, J=3 Hz, 19-H_3), 1.20 (6H, s, 26-H_3

and 27-H_3), 2.56 (3H, s, SCH_3), 3.98 (1H, m, 3-H), 4.70 (LH, dd, $J=44$ and 3 Hz, 1α-H), 5.50 (1H, m, 6-H), 5.98 (1H, m, 2β-H).

1α-Fluoro-3β,25-dihydroxycholest-5-ene (17b)——The xanthate **16** (76 mg, 0.118 mmol) in xylene (8 ml) was treated with tributyltinhydride (70 mg, 0.241 mmol) under reflux for 2 h. Removal of the solvent and preparative TLC (C_6H_6–EtOAc, 5 : 1, developed once) gave the silyl ether **17a** (60 mg, 95%), $Rf=0.75$. This in THF (10 ml) was treated with tetrabutylammonium fluoride (1 M, 2 ml) at room temperature overnight. The usual work-up (EtOAc) and chromatography on silica gel (5 g) with C_6H_6–EtOAc (10 : 1) gave the diol **17b** (34.4 mg, 77%), mp 177—179 °C (hexane–$CHCl_3$). δ 0.68 (3H, s, 18-H_3), 0.92 (3H, d, $J=6$ Hz, 21-H_3), 0.98 (3H, d, $J=2$ Hz, 19-H_3), 1.22 (6H, s, 26-H_3 and 27-H_3), 3.90 (1H, m, 3α-H), 4.64 (1H, dm, $J=48$ Hz, 1β-H), and 5.50 (1H, m, 6-H), High resolution MS, Calcd for $C_{27}H_{45}O_2F$ (M^+) m/z 420.3401, Found m/z 420.3406.

3β-Acetoxy-1α-fluoro-25-hydroxycholesta-5,7-diene (18)——The diol **17b** (16 mg, 0.038 mmol) was treated with acetic anhydride (1 ml) and pyridine (3 ml) at room temperature for 5 h. The usual work-up (EtOAc) and chromatography on silica gel (2 g) with C_6H_6–EtOAc (10 : 1) gave the acetate **17c** (16.6 mg, 95%). The acetate **17c** (16.6 mg) in CCl_4 (1 ml) was treated with *N*-bromosuccinimide (9 mg) under reflux for 50 min. The reaction mixture was cooled to room temperature and the precipitate was filtered off. The filtrate was concentrated to give the crude bromide, which was dissolved in THF (5 ml) containing a small amount of tetrabutylammonium bromide at room temperature under argon. After 50 min in the dark, tetrabutylammonium fluoride (1 M THF solution, 0.3 ml) was added and the mixture was stirred at room temperature for 30 min in the dark. The usual work-up (EtOAc) gave a mixture of the 4,6-diene and 5,7-diene, which was treated with acetone (10 ml) containing a catalytic amount of *p*-TsOH for 9 h under argon in the dark. The usual work-up (EtOAc) and preparative TLC (C_6H_6–EtOAc, 10 : 1, developed three times) gave the 5,7-diene **18** (4.82 mg, 29%), $Rf=0.25$. λ_{max}: 292, 280, 269, and 261 nm.

1α-Fluoro-25-hydroxyvitamin D_3 3-Acetate (19a)——A solution of the 5,7-diene **18** (4.8 mg) in C_6H_6 (80 ml) and EtOH (40 ml) was irradiated with a medium pressure mercury lamp through a Vycol filter at 0 °C under argon for 3 min. The reaction mixture was then refluxed for 1 h under argon. Removal of the solvent and preparative TLC (C_6H_6–EtOAc (10 : 1), developed three times) gave the vitamin D_3 acetate (**19a**) (0.918 mg, 19%), $Rf=0.60$. λ_{max}: 270 and 243 nm, λ_{min}: 255 and 224 nm, MS m/z 400 ($M^+ - AcOH$) and 380. The UV spectrum was identical with that of the reported 1α-fluorovitamin D_3 3-acetate.[6] The starting 5,7-diene **18** (2.1 mg, 44%, $Rf=0.54$) was recovered.

1α-Fluoro-25-hydroxyvitamin D_3 (19b)——The acetate **19a** (0.918 mg) in THF (2 ml) was treated with 5% KOH–MeOH (2 ml) at room temperature overnight. The usual work-up (EtOAc) gave a crude product, which was purified by high-performance liquid chromatography (Shimadzu LC-3A; column, Zorbax SIL, 0.46 mm i.d. × 15 cm; eluant, CH_2Cl_2; flow rate, 2.0 ml/min; t_R, 5.0 min) to give the vitamin D_3 analogue **19b** (0.272 mg, 32%). λ_{max}: 270 and 243 nm, λ_{min}: 255 and 224 nm, δ (400 MHz), 0.54 (3H, s, 18-H_3), 0.92 (3H, d, $J=6$ Hz, 21-H_3), 1.22 (6H, s, 26-H_3 and 27-H_3), 4.23 (1H, m, 3α-H), 5.14 (1H, ddd, $J=49$, 6, and 2 Hz, 1β-H), 5.11 and 5.39 (2H, br s × 2, 19-H_2), 6.03 and 6.41 (2H, ABq, $J=11$ Hz, 6-H and 7-H), High resolution MS, Calcd for $C_{27}H_{42}O_2$ ($M^+ - HF$) m/z 398.3183, Found m/z 398.3196. The characteristic UV spectrum was identical with that of the reported 1α-fluorovitamin D_3.[6]

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