Studies on Organic Fluorine Compounds. Part 37.[1] Studies on Steroids. Part 78.[2] Synthesis of 24,24-Difluoro- and 24ξ-Fluoro-25-hydroxyvitamin D_3

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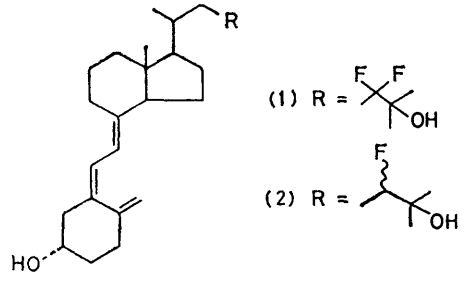
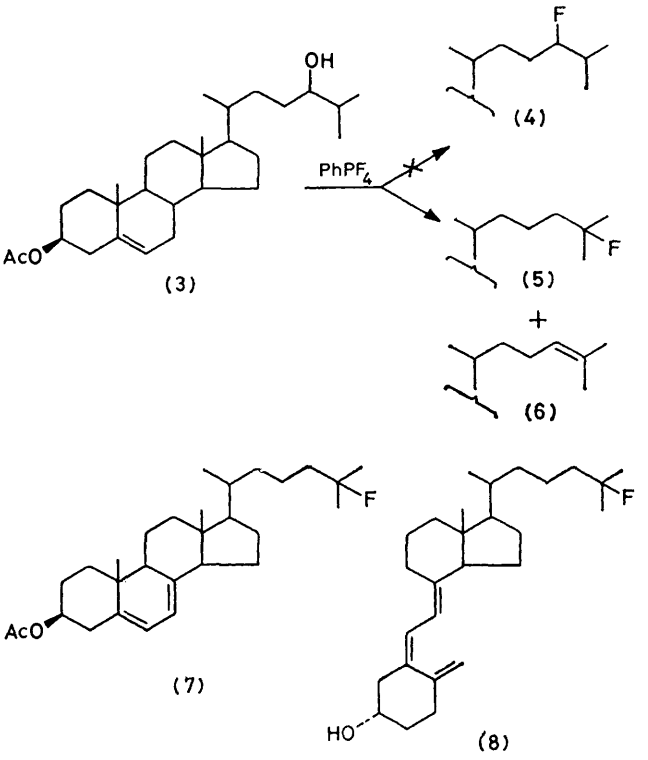
To clarify the physiological significance of C-24 hydroxylation in vitamin D_3 metabolism, vitamin D_3 compounds blocked at C-24 by fluorine substituents, namely 24,24-difluoro-25-hydroxyvitamin D_3 (1) and 24ξ-fluoro-25-hydroxyvitamin D_3 (2) have been synthesized from 3β-hydroxychol-5-en-24-oic acid (13).

It has been demonstrated that vitamin D_3 has to be hydroxylated at various positions before eliciting biological activity.[3] Hydroxylation at the C-1 and C-25 positions produces 1,25-dihydroxy-D_3, which is now regarded as a hormone that plays a central role in the metabolism of calcium and phosphorus. However, the functional role of C-24 hydroxylation, to yield 24(*R*), 25-dihydroxy-D_3 and 1,24(R),25-trihydroxy-D_3, has not been fully clarified.[4,5] Blocking of 24-hydroxylation by substitution with one or two fluorine atoms at C-24 may clarify this problem.[6] In this paper we describe the synthesis of 24,24-difluoro-25-hydroxyvitamin D_3 (1) and 24ξ-fluoro-25-hydroxyvitamin D_3 (2).[7]



24ξ-Fluoro-25-hydroxyvitamin D_3.—Our initial attempts to convert the 24-hydroxycholesteryl acetate (3) into the 24-fluorocholesteryl acetate (4) failed but, instead resulted in formation of the rearranged 25-fluoro-derivative (5). For example, reaction of the acetate (3) with $PhPF_4$[8] in carbon tetrachloride afforded the 25-fluoro-compound (5) and desmosteryl acetate (6) in 30 and 33% yields, respectively. The structure of compound (5) was confirmed by comparison of its n.m.r. spectrum with those of 25-fluoro-7,8-dehydrocholesteryl acetate (7) [9] and 25-fluorovitamin D_3 (8).[10]

It is well known that substitution of a halogen atom (Cl, Br, or I) or a sulphonyloxy-group at a carbon atom adjacent to an electron-withdrawing group (such as a carbonyl, ester, or nitrile group) by a fluoride anion usually affords the corresponding fluoro-compound in good yield, accompanied, in some cases, by the formation of an olefinic compound.[8] When such a reaction is applied to a steroid, it is expected that the olefinic compound, if formed, will be difficult to separate from



the desired fluorinated compound. Thus, we investigated a model reaction for the preparation of the α-fluoro-ester (11) by the use of various leaving groups, fluorinating reagents, solvents, and reaction temperatures (see the Table). With a tosyloxy-group as the leaving group [compound (9)], the combined use of KF, 18-crown-6, and dimethylformamide (DMF) gave satisfactory results. With a bromine atom as the leaving group [compound (10)], the combined use of silver fluoride and acetonitrile proved satisfactory.

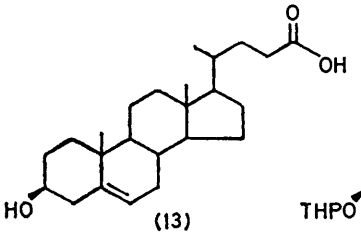
$$\underset{\substack{(9)\ X = OTs\\(10)\ X = Br}}{n\text{-}C_6H_{13}\cdot CHX\cdot CO_2Et} \longrightarrow \underset{(11)}{n\text{-}C_6H_{13}\cdot CHF\cdot CO_2Et} + \underset{(12)}{n\text{-}C_5H_{11}\cdot CH = CHCO_2Et}$$

Based on the above results, we attempted the synthesis of 24-fluoro-25-hydroxyvitamin D_3 (2) using the cholenic acid (13) as a starting material. For the synthesis of

Synthesis of ethyl α-fluoro-octanoate (11)

Compound	Reagent	Solvent	T/°C	t/h	Product yield/% (11)	(12)
(9)	KF, 18-crown-6	DMF	60—70	30	76	5
(10)	AgF	MeCN	reflux	25	89	trace
(10)	KF, 18-crown-6	DMF	120	8	41	42
(10)	$Bu^n_4N^+F^-$	DMF	100	4	43	39

methyl 3β-tetrahydropyranyloxychol-5-ene-24-carboxylate (18),[11] compound (13) was successively treated with dihydropyran–TsOH-*p*, $LiAlH_4$, and TsCl-*p*–pyridine (Ts = tosyl) to afford the tosylate (15) which, in turn, was treated with KCN and 18-crown-6 in DMF to give the cyanide (16) in 48% yield. Hydrolysis of compound (16) with 10% KOH in aqueous ethanol at 140 °C for 18 h gave the carboxylic acid (17) in 63% yield which, on treatment with diazomethane, afforded the methyl ester (18). Generation of the lithium enolate by treatment of the ester (18) with lithium dicyclohexylamide, followed by reaction with iodine in tetrahydrofuran (THF) at −78 °C for 5 min afforded the iodide (19) along with a small amount of the starting ester (18).[12] Since separation of the iodide (19) from the starting ester (16) was difficult, the iodide was converted into the more polar 24-hydroxy compound (20). Thus, the reaction mixture of the iodination reaction was treated with silver trifluoroacetate in the presence of silver oxide in acetonitrile and subsequent alkaline hydrolysis gave the 24-hydroxy-acid, which was treated with diazomethane to afford the methyl 24-hydroxy-ester (20) in 63% overall yield from compound (18). Reaction of the tosylate (21), derived from compound (18), with KF and 18-crown-6 in DMF followed by deprotection afforded the fluoride (22) in 73% yield. Reaction of compound (22) with an excess of the Grignard reagent MeMgI in diethyl ether followed by acetylation gave 24-fluoro-25-hydroxycholesteryl 3β-monoacetate (23) in 80% yield. Following the standard synthesis of vitamin D_3 from cholesterol, the fluorocholesteryl acetate (23) was converted into the desired fluorovitamin D_3 (2) *via* the corresponding 5,7-diene (24). Allylic bromination of compound (23) by *N*-bromosuccinimide (NBS) in CCl_4 and then dehydrobromination with *s*-collidine in refluxing xylene afforded a mixture of the 4,6- and 5,7-dienes from which the desired 5,7-diene (24) was isolated in 18% yield by preparative thin layer chromatography. The 24-fluoro-5,7-diene (24) was saponified with 5%-KOH in methanol and THF at room temperature for 16 h and the resultant alcohol was irradiated in a mixture of ethanol and benzene for 2.5 min at 0 °C. After refluxing for 1 h, the



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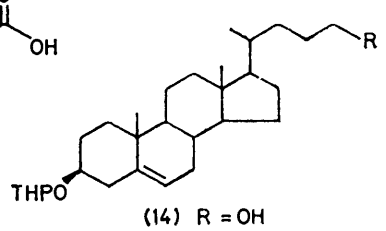
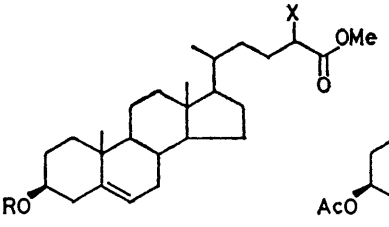
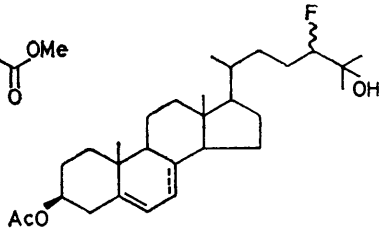


(14) R = OH
(15) R = OTs
(16) R = CN
(17) R = CO_2H
(18) R = CO_2Me



(19) R = THP, X = I
(20) R = THP, X = OH
(21) R = THP, X = OTs
(22) R = H, X = F

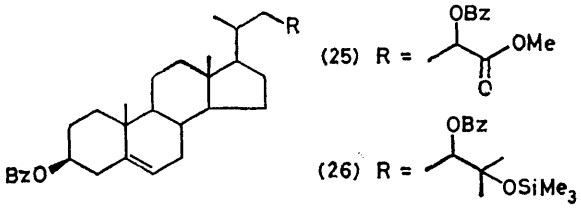


(23) 5-ene
(24) 5,7-diene

THP = tetrahydropyranyl

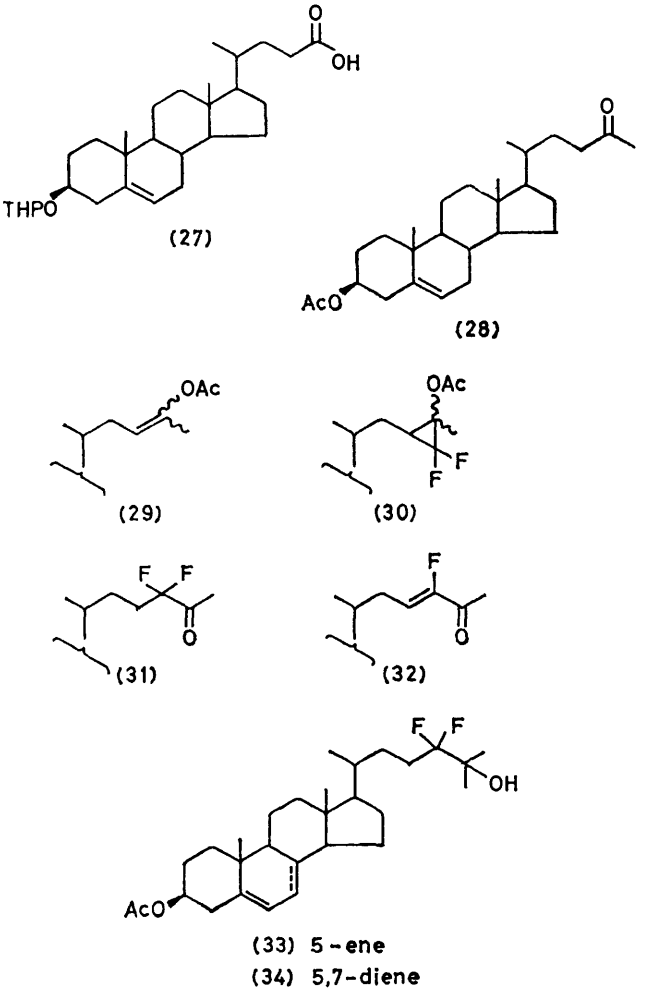
products were fractionated by thin layer chromatography (t.l.c.) and high-pressure liquid chromatography (h.p.l.c.) (Zorbax SIL) to afford the corresponding vitamin D_3 (2) in 16% yield from compound (24). The fluorovitamin D_3 (2) showed the expected u.v. absorption ($\lambda_{max.}$ 263 nm and $\lambda_{min.}$ 228 nm) and mass spectrum.

That the 24-hydroxy-ester (20) is a 1 : 1 epimeric mixture at C-24 was clarified as follows. The 3,24-dibenzoyl ester (25), derived from compound (20), showed twin peaks on h.p.l.c. and, also, compound (20) gave a 1 : 1 mixture of the (24*R*)- and (24*S*)-3,24-dibenzoylcholest-5-en-25-ol trimethylsilyl ethers (26), which were co-chromatographed on h.p.l.c. with authentic samples.[13] From these results the fluorovitamin D_3 (2), prepared above, may be a 1 : 1 epimeric mixture at C-24.



24,24-*Difluoro*-25-*hydroxyvitamin* d_3 (1).—The key steps for the synthesis of 24,24-difluoro-25-hydroxyvitamin D_3 (1) involve difluorocyclopropanation of the enol acetate (29) and subsequent ring opening to give the homologous difluoro-ketone (31) by alkaline hydrolysis of the cyclopropane (30), which has been recently demonstrated by us and others.[14]

Treatment of 3β-tetrahydropyranyloxychol-5-en-24-oic acid (27) with an excess of MeLi followed by deprotection and acetylation gave the methyl ketone (28) in 67% overall yield from compound (13). Enol acetylation of the ketone (28) was effected by refluxing for 7 h in acetic anhydride in the presence of TsOH-*p* to give the diacetate (29) in 72% yield. Reaction of the enol acetate (29) with difluorocarbene, generated by thermolysis of sodium chlorodifluoroacetate in boiling diglyme,[15] afforded the desired cyclopropane (30) in 34% yield along with the starting material (29) in 53% yield. To avoid further cyclopropanation at the 5,6-double bond, the amount of the sodium salt present was controlled (*ca.* 10 mol equiv.). Treatment of compound (30) with LiOH in THF–methanol–water at 20 °C for 2 h followed by reacetylation gave the difluoro-ketone (31) and a stereoisomeric mixture of the α-fluoro-enone (32) in 9.3% and 61% yield, respectively. Reaction of the difluoroketone (31) with an excess of the Grignard reagent MeMgI in diethyl ether and subsequent acetylation furnished the 25-methanol (33) in 85% yield. Conversion of compound (33) into the corresponding vitamin D_3 was achieved by standard synthesis of the vitamin D_3 derivative. Allylic bromination of compound (33) by NBS in CCl_4 and then dehydrobromination with *s*-collidine in refluxing xylene afforded a mixture of the 4,6- and 5,7-dienes. The desired 5,7-diene (34) was isolated in 28% yield by treatment with TsOH-*p* in acetone at 20 °C for 15 h (to transform the 4,6-diene into a less polar material) and then preparative t.l.c. The 24,24-difluoro-5,7-diene (34) was saponified with 5% KOH in methanol at 20 °C for 15 h and then irradiated in a mixture of ethanol and benzene for 2.5 min at 0 °C. After refluxing for 1 h, the products were fractionated by t.l.c. and h.p.l.c. (Zorbax ZIL) to give the 24,24-difluoro-25-hydroxyvitamin D_3 (1), which showed the expected u.v. absorption and mass spectrum.



THP = tetrahydropyranyl

The biological activity of the fluorinated vitamin D_3 analogues (1) and (2) are currently under investigation and some results have been reported.[16]

EXPERIMENTAL

Melting points were determined on a hot-stage microscope and are uncorrected. N.m.r. spectra were recorded on a JEOL JNH-PS-100 or a Varian T-60 spectrometer and chemical shifts are reported in p.p.m. on the δ scale relative to tetramethylsilane. I.r. spectra were recorded using a JEOL A-1 spectrophotometer. Mass spectra were obtained with a Shimadzu LKB-9000S (ionization voltage, 70 eV).

High-pressure liquid chromatography (h.p.l.c.) was performed with a Shimadzu-Du Pont 830 with Zorbax-SIL columns (25 cm × 2.1 mm i.d.). Thin layer chromatography (t.l.c.) was carried out on Merck silica-gel F254 (0.25 mm thick, No. 5 715).

Reaction of 24-*Hydroxycholesterol* 3β-*Acetate* (3) *with* $PhPF_4$.—A mixture of compound (3) (200 mg) and $PhPF_4$ (697 mg) in carbon tetrachloride (10 ml) was stirred for 2 h at 0 °C and then for 1 h at room temperature. The reaction mixture was poured into 5% $NaHCO_3$ solution and extracted with diethyl ether. The organic layer was washed with brine, dried ($MgSO_4$) and chromatographed on silica gel. The fraction which was eluted with benzene and n-hexane (1 : 1 v/v) was recrystallized from methanol and acetone (1 : 1 v/v) to give the 25-fluoride (5) (35 mg). The mother liquor was submitted to preparative t.l.c. [benzene and n-hexane (2 : 1 v/v) as eluant] to give desmosteryl acetate (6) [65 mg (33%)] R_F 0.65—0.52 [17] and 25-*fluorocholesterol* 3β-*acetate* (5) (25 mg; 30%), m.p. 121—122 °C; R_F 0.5—0.4; ν(KBr) 1 740 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.32 (1 H, m, 6-H), 4.58 (1 H, m, 3-H), 1.96 (3 H, s, Ac), and 1.34 (6 H, d, J_{HF} 21 Hz, 26- and 27-H); δ_F ($CDCl_3$ with benzotrifluoride) +72 p.p.m.; *m/e* 386 (M^+ − AcOH).

3β-*Tetrahydropyranyloxychol*-5-*en*-24-*ol* (14).—A mixture of 3β-hydroxychol-5-en-24-oic acid (13) (4.58 g) and dihydropyran (1.84 g) in dichloromethane (30 ml) was stirred for 4 h at 0 °C in the presence of TsOH-*p*. The reaction mixture was then treated with 5% $NaHCO_3$ solution and extracted with dichloromethane. The organic layer was washed with water, dried ($MgSO_4$), and then concentrated under reduced pressure. The residue was dissolved in tetrahydrofuran (THF) (20 ml), and this solution was added to a suspension of $LiAlH_4$ (950 mg) in THF (50 ml). After the reaction mixture had been stirred for 4 h at 60 °C, ethyl acetate was added to destroy the excess of $LiAlH_4$, and then the mixture was treated with ^{2}HCl and extracted with diethyl ether. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure to give a residue which was chromatographed on silica gel. The fraction which was eluted with benzene–diethyl ether (10 : 1 v/v) gave *compound* (14) (4.24 g; 78%) (after recrystallization from methanol and acetone), m.p. 130 °C δ_H ($CDCl_3$ with Me_4Si) 5.36 (1 H, m, 6-H), 4.74 (1 H, m), and 3.94 (1 H, m, 3-H); *m/e* 360 (M^+ − 84) and 342.

3β-*Tetrahydropyranyloxychol*-5-*en*-24-*yl Cyanide* (16).—A mixture of compound (14) (4.24 g), pyridine (1.97 g), and toluene-*p*-sulphonyl chloride (2.38 g) in dichloromethane (20 ml) was stirred for 24 h at room temperature. The reaction mixture was treated with $NaHCO_3$ solution, extracted with diethyl ether, and the organic layer was successively washed with ^{2}HCl and water, dried ($MgSO_4$), and the solvent removed under reduced pressure. The residue was treated with potassium cyanide (568 mg) and 18-crown-6 (2.4 g) in dimethylformamide (DMF) (40 ml) at 60—80 °C for 3 h. The reaction mixture was concentrated under reduced pressure, and water was added to the residue, which was then extracted with diethyl ether. The extract was dried ($MgSO_4$), and then chromatographed on silica gel to give a fraction [eluted with benzene and diethyl ether (30 : 1 v/v)] which gave *compound* (16) (2.07 g; 48%) (after recrystallization from methanol and acetone), m.p. 142—143 °C; ν(KBr) 2 200 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.30 (1 H, m, 6-H) and 4.66 (1 H, m); *m/e* 369 (M^+ −84) and 351 (Found: C, 79.65; H, 10.4; N, 3.2. Calc. for $C_{30}H_{47}NO_2$: C, 79.42; H, 10.44; N, 3.09%).

Methyl 3β-*Tetrahydropyranyloxychol*-5-*ene*-24-*carboxylate* (18).[11]—The cyanide (16) was treated with 10% KOH aqueous ethanolic solution (6 ml of water and 24 ml of ethanol) at 140 °C for 48 h. The reaction mixture was acidified with ^{2}HCl at 0 °C, and extracted with diethyl ether. The organic layer was washed with water, dried ($MgSO_4$), and then chromatographed on silica gel. The fraction which was eluted with benzene and diethyl ether (10 : 1 v/v) gave the acid (17) (1.36 g; 63%) (after recrystallization from methanol and acetone), m.p. 171—172 °C; ν(KBr) 3 000—2 500 and 1 710 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 10.0 (1 H, br, CO_2H), 5.34 (1 H, m, 6-H), 4.72 (1 H, m), and 3.84 (1 H, m, 3-H); *m/e* 388 (M^+ −84) and 370. Treatment of the acid (17) with diazomethane in dichloromethane afforded compound (18) (1.10 g; 79%) (after recrystallization from methanol and acetone), m.p. 159—160 °C; ν(KBr) 1 740 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.34 (1 H, m, 6-H), 4.72 (1 H, m), 3.85 (1 H, m, 3-H), 3.64 (3 H, s, COMe) (Found: C, 76.5; H, 10.3. Calc. for $C_{31}H_{50}O_4$: C, 76.18; H, 10.72%).

Methyl 24-*Hydroxy*-3β-*tetrahydropyranyloxychol*-5-*ene*-24-*carboxylate* (20).—To a solution of dicyclohexylamine (550 mg) in THF (10 ml) under an argon atmosphere was added n-butyl-lithium (2.8 mmol) at −78 °C. The solution was stirred for 10 min at −78 °C and compound (18) (1.215 g; 2.5 mmol), dissolved in THF (15 ml) was then added as drops. The reaction mixture was stirred for a further 10 min at −78 °C and then for 5 min at room temperature. The resultant lithium enolate solution was added in one portion to a THF solution (7 ml) of iodine (711 mg) at −78 °C; the mixture was then stirred for 5 min at −78 °C. After this time it was treated with 0.5N-HCl (20 ml) and extracted with diethyl ether (100 ml × 2). The organic layer was successively washed with $Na_2S_2O_3$ solution and brine and then dried ($MgSO_4$). After removal of the solvent under reduced pressure, the residue was treated with silver trifluoroacetate (633 mg) and silver oxide (928 mg) in acetonitrile (10 ml) and diethyl ether (10 ml) at room temperature for 20 h. A 5% $NaHCO_3$ solution was added to the reaction mixture and the resulting precipitate was filtered off through Čelite, and washed with diethyl ether. The filtrate was extracted with diethyl ether and the extract was washed with water, dried ($MgSO_4$), and then the solvent was removed under reduced pressure. The residue was dissolved in THF (20 ml) and methanol (130 ml) which contained KOH (1 g) and this reaction mixture was stirred for 16 h at room temperature; it was then concentrated under reduced pressure. The residue was acidified with 0.5N-HCl and extracted with diethyl ether. The organic layer was washed with water, dried ($MgSO_4$), and concentrated under reduced pressure. The residue was treated with an excess of diazomethane in dichloromethane at 0 °C and chromatographed on silica gel. The fraction which was eluted with benzene and diethyl ether (5 : 1 v/v) gave *compound* (20) [785 mg, 63% from compound (18)], m.p. 114—117 °C; ν(KBr) 3 300 and 1 745 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.34 (1 H, m, 6-H), 4.70 (1 H, m), 4.16 (1 H, m, 24-H), 3.76 (3 H, s, CO_2Me), and 2.60—2.88 br (1 H, 24-OH); *m/e* 418 (M^+ −84) and 400.

Methyl 3β-*Tetrahydropyranyloxy*-24-*tosyloxychol*-5-*ene*-24-*carboxylate* (21).—A mixture of compound (20) (169 mg), TsCl-*p* (77 mg), and pyridine (64 mg) in dichloromethane was stirred for 4 h at room temperature, and then treated with ^{2}HCl, and extracted with diethyl ether. The organic layer was successively washed with 5% $NaHCO_3$ solution and brine, dried ($MgSO_4$), and the solvent removed under

reduced pressure. The residue was chromatographed on silica gel and the fraction which was eluted with n-hexane and diethyl ether (4 : 1 v/v) gave compound (21) (142 mg, 68%) as a syrup; ν(CH_2Cl_2) 1 770, 1 360, 1 200, 1 180, and 810 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 7.78 (2 H, d), 7.48 (2 H, d), 5.32 (1 H, m, 6-H), 4.76 (1 H, m), 4.72 (1 H, m, 24-H), 3.63 (3 H, s, CO_2Me), and 2.42 (3 H, s, Ar-*Me*); *m/e* 572 (M^+ — 84).

Methyl 24-*Fluoro*-3β-*hydroxychol*-5-*ene*-24-*carboxylate* (22).—A mixture of compound (21) (522 mg), 18-crown-6 (2.64 g), and KF (580 mg) in DMF (40 ml) under an argon atmosphere was stirred for 15 h at 70 °C. Removal of the solvent under reduced pressure gave the residue which was dissolved in diethyl ether and washed with water. The organic layer was dried ($MgSO_4$) and chromatographed on silica gel. The fraction which was eluted with n-hexane and diethyl ether (20 : 1 v/v) gave the pyrano-24-fluoride (293 mg). On deprotection in the usual manner, this afforded *compound* (22) (245 mg, 73%) (after recrystallization from methanol and acetone), m.p. 104—105 °C; δ_H ($CDCl_3$ with Me_4Si) 5.32 (1 H, m, 6-H), 4.86 (1 H, dm, J_{HF} 48 Hz, 24-H), and 3.74 (3 H, s, CO_2Me); *m/e* 420.3030 (Calc. for $C_{26}H_{41}O_3F$: 420.3039).

3β-*Acetoxy*-24-*fluorocholest*-5-*en*-25-*ol* (23).—To a solution of compound (20) (237 mg) in diethyl ether (20 ml) under an argon atmosphere was added a 1M-ethereal solution of MeMgI (3 ml). The mixture was stirred for 20 min at room temperature and the residue was treated with ^{2}HCl, and extracted with diethyl ether. The organic layer was washed with brine, dried ($MgSO_4$), and then chromatographed on silica gel. The fraction which was eluted with n-hexane and diethyl ether (1 : 1 v/v) was treated with pyridine (2 ml) and acetic anhydride (1 ml) in dichloromethane for 12 h at room temperature to afford *compound* (23) (207 mg, 80%) (after recrystallization from methanol and acetone), m.p. 153—154 °C; ν(KBr) 3 400, 1 735, 1 380, 1 240, and 1 030 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 4.14 (1 H, dm, J_{HF} 48 Hz, 24-H), 2.02 (3 H, s, Ac), 1.20 (6 H, s, 26- and 27-H), 1.00 (3 H, s, 19-H), 0.96 (3 H, d, *J* 6 Hz, 21-H), and 0.68 (3 H, s, 18-H); *m/e* 402 (M^+ —60) (Found: C, 75.3; H, 10.4; F, 3.9. Calc. for $C_{29}H_{45}FO_3$: C, 75.3; H, 10.25; F, 4.1%).

3β-*Acetoxy*-24ξ-*fluorocholest*-5,7-*dien*-25-*ol* (24).—A mixture of compound (23) (21 mg) and *N*-bromosuccinimide (NBS) (11 mg) in carbon tetrachloride (2 ml) was refluxed under an argon atmosphere for 45 min. After cooling to room temperature the precipitated imide was filtered off, and the filtrate was concentrated under reduced pressure. To the residue, dissolved in boiling xylene (2 ml), was added a mixture of *s*-collidine (0.5 ml) and xylene (1.5 ml) and the refluxing was continued for a further 10 min. The reaction mixture was cooled to room temperature, treated with dilute HCl, and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and then concentrated under reduced pressure to give a residue which was submitted to p.t.l.c. (benzene–ethyl acetate, 20 : 1 v/v × 3) to give compound (24) (3.8 mg, 18%); $\lambda_{max.}$ (EtOH) 262, 272, and 293 nm; *m/e* 460 (M^+), 445, 401, 400, 385, 313, and 253.

24-*Fluoro*-25-*hydroxyvitamin* D_3 (2).—After treatment of the 5,7-diene acetate (24) (3.4 mg) in THF (2 ml) with 5% KOH in methanol at room temperature for 16 h, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was concentrated under reduced pressure and the residue was purified by p.t.l.c. (benzene–ethyl acetate, 10 : 1 v/v, × 4) to give the 3β-hydroxy-compound (2.5 mg); $\lambda_{max.}$ (EtOH) 263, 272, 283, and 293 nm. A solution of this 3β-hydroxy-compound (2.5 mg) in a mixture of benzene (90 ml) and ethanol (40 ml) was irradiated by a medium-pressure mercury lamp (Hanovia 654A 36, 200W) for 2.5 min and then refluxed for 1 h. After removal of the solvent under reduced pressure, the residue was purified by p.t.l.c. (benzene–ethyl acetate, 5 : 1 v/v, × 3) to give compound (2) (0.52 mg) which was further purified by h.p.l.c. (CH_2Cl_2 as eluant); $\lambda_{min.}$ (EtOH) 228 nm; $\lambda_{max.}$ (EtOH) 263 nm; *m/e* 418 (M^+), 403, 400, 385, 359, 271, 253, 136, and 118.

Methyl 3β,24-*Dibenzoylchol*-5-*ene*-24-*carboxylate* (25).—After treatment of compound (20) (409 mg) with a 1 : 1 mixture of CH_2Cl_2 and methanol (20 ml) in the presence of a catalytic amount of TsOH-*p* at room temperature for 3 h, the reaction mixture was purified by column chromatography (SiO_2). The fraction which was eluted with a mixture of benzene and diethyl ether (3 : 1 v/v) afforded *methyl* 3β,-24-*dihydroxychol*-5-*ene*-24-*carboxylate* (after recrystallization from methanol), m.p. 143—146 °C; δ_H ($CDCl_3$ with Me_4Si) 5.36 (1 H, m, 6-H), 4.18 (1 H, m, 24-H), 3.77 (3 H, s, CO_2Me), and 3.52 (1 H, m, 3-H) (Found: C, 74.55; H, 10.3. Calc. for $C_{26}H_{42}O_4$: C, 74.6; H, 10.11%). Treatment of the dihydroxy ester (60 mg) with benzoyl chloride (100 mg) in pyridine (0.5 ml) and CH_2Cl_2 (2 ml) at room temperature for 1 d followed by the usual extractive work-up and purification by column chromatography (SiO_2, benzene–diethyl ether, 30 : 1 v/v, as eluant) gave the dibenzoate (25) (82 mg, 91%), m.p. 120—121 °C (methanol and acetone); ν(KBr) 1 760 and 1 720 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.40 (1 H, m, 6-H), 5.18 (1 H, m, 24-H), 4.84 (1 H, m, 3-H), 3.74 (3 H, s, CO_2Me), 1.06 (s, 19-H), 0.98 (d, *J* 6 Hz, 21-H), and 0.70 (s, 18-H); *m/e* 595 (M^+ — OMe), 504 (M^+ — BzOH), 489, 399, and 255 (Found: C, 76.4; H, 7.9. Calc. for $C_{40}H_{50}O_6$: C, 76.64; H, 8.04). Compound (25) showed two peaks at R_t 22.8 and 23.8 min, respectively, on h.p.l.c. (hexane–CH_2CCl_2, 10 : 1 v/v, as eluant, 90 kg cm^{-2}).

3β,24-*Dibenzoyloxy*-25-*trimethylsilyloxycholest*-5-*ene* (26).—Reaction of the 3β,24-dihydroxy-ester (192 mg), described above with methyl-lithium (2.75 mmol) in THF (10 ml) at −75 °C (solid CO_2–acetone bath) for 15 min under an argon atmosphere followed by purification by silica-gel column chromatography (benzene–diethyl ether, 5 : 1 v/v, as eluant) gave the crude 24,25-dihydroxycholesterol. After treatment of the triol (60 mg) with pyridine (79 mg) and benzoyl chloride (80 mg) in CH_2Cl_2 (5 ml) at room temperature for 12 h the reaction mixture was purified by silica-gel column chromatography (benzene–diethyl ether, 10 : 1 v/v, as eluant) to give 3β,24-dibenzoyloxycholest-5-en-25-ol (34 mg), m.p. 143—145 °C; δ_H ($CDCl_3$ with Me_4Si) 8.04 (4 H, m), 7.44 (6 H, m), 5.40 (1 H, m, 6-H), 5.00 (1 H, m, 24-H), 4.88 (1 H, m, 3-H), 1.28 (s, 26- and 27-H), 1.07 (s, 19-H), 0.95 (d, *J* 6 Hz, 21-H), and 0.88 (s, 18-H); *m/e* 504 (M^+ — BzOH), 486, 382, and 364. The 3β,24-dibenzoyloxy-25-hydroxy-compound (1 mg) and *N*-trimethylsilylimidazole (50 μl) were warmed at 70 °C for 10 min. The reaction mixture was diluted with water and extracted with hexane. H.p.l.c. of the organic layer (n-hexane–CH_2Cl_2, 5 : 1 v/v, as eluant, 90 kg cm^{-2}) gave two peaks at R_t 6.2 and 8.5 min, respectively. The former peak was identified as the 24*R*-isomer and the latter as the 24*S*-isomer of compound (26) by comparison with authentic samples under the same conditions.

3β-*Tetrahydropyranyloxychol*-5-*en*-24-*oic acid* (27).—A mixture of the 3β-hydroxycholenic acid (13) (5.00 g) and di-

hydropyran (6 ml) in dichloromethane (30 ml) was stirred for 6 h at 0 °C in the presence of a catalytic amount of TsOH-*p*. To the reaction mixture was added 1N-NaOH (5 ml) and the solvent was evaporated off under reduced pressure. The resulting solid was dissolved in EtOH (250 ml) and 2N-NaOH (60 ml) and the mixture stirred for 20 h at room temperature. The solvent was then evaporated off under reduced pressure and the resultant residue was suspended in water (300 ml), acidified with 2N-HCl at 0 °C, and extracted twice with dichloromethane. The organic layer was then washed with water and dried ($MgSO_4$). After removal of the solvent under reduced pressure, recrystallization of the residue from acetone afforded compound (27) (3.538 g), m.p. 156.5—158 °C; a further 1.357 g of (27) was obtained, from the mother liquor.

3β-*Acetoxy*-26,27-*dinorcholest*-5-*en*-24-*one* (28).—To a THF solution (100 ml) of compound (27) (4.895 g, 10.7 mmol) under an argon atmosphere was added methyl-lithium (5% diethyl ether solution, 22 mmol) at −78 °C and the reaction mixture was stirred at 0 °C for 4 h. The reaction mixture was poured into water (200 ml), acidified with 1N-HCl, and extracted with diethyl ether. The organic layer was washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to give a residue which was chromatographed on silica gel. The fraction which was eluted with benzene and diethyl ether (50 : 1 v/v) afforded the crude 3β-tetrahydropyranyloxy-26,27-dinorcholest-5-en-24-one (4.690 g, 94%). A solution of this methyl ketone (4.610 g) in dichloromethane (25 ml) and methanol (20 ml), which contained a catalytic amount of TsOH-*p*, was stirred at room temperature for 24 h. To the reaction mixture was added 1N-NaOH (3 ml) and the solvent was removed under reduced pressure to give a residue which was dissolved in dichloromethane, washed with water, dried ($MgSO_4$) and concentrated to dryness. The residue was dissolved in dichloromethane (30 ml), pyridine (5 ml), and acetic anhydride (5 ml) and the reaction mixture was stirred for 24 h at room temperature. Removal of the solvent under reduced pressure and recrystallization of the residue from methanol and acetone (1 : 2 v/v) gave compound (28) (2.523 g). Further crystallization of the mother liquor gave 1.154 g of (28) to give a total yield of *compound* (28) from (13) of 67%, m.p. 148—151 °C; ν(CH_2Cl_2) 1 730 and 1 720 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.36 (1 H, m, 6-H), 4.60 (1 H, m, 3-H), 2.12 (3 H, s, 25-H), and 2.02 (3 H, s, 3-OAc); *m/e* 354 (M^+ − AcOH), 339, 296, 255, 215, and 213 (Found: C, 78.1; H, 10.35. Calc. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21%).

3β,24-*Diacetoxy*-26,27-*dinorcholest*-5,23-*diene* (29).—Compound (28) (2.73 g, 6.58 mmol) was refluxed for 7 h in acetic anhydride (50 ml) which contained TsOH-*p* (220 mg). After *ca.* 20 ml of acetic anhydride and acetic acid had been distilled off, the reaction mixture was poured into 0.5% NaOH and extracted with mixture of diethyl ether and n-hexane (2 : 1 v/v). The organic layer was washed with water, dried ($MgSO_4$), and evaporated under reduced pressure to give a residue which was chromatographed on silica gel. The fraction which was eluted with benzene and diethyl ether (100 : 1 v/v) afforded *compound* (29) (2.162 g, 72%) (which was recrystallized from methanol), m.p. 109—110 °C; ν(CH_2Cl_2) 1 750 and 1 735 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.40 (1 H, m, 6-H), 5.02 (1 H, m, 23-H), 4.60 (1 H, m, 3-H), 2.14 (3 H, s, 24-OAc), 2.03 (3 H, s, 3-OAc), and 1.90 (3 H, 25-H); *m/e* 396 (M^+ − AcOH), 354, 283, and 253 (Found: C, 76.15; H, 9.7. Calc. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71%).

3β,24-*Diacetoxy*-23,24-(*difluoromethano*)-26,27-*dinorcholest*-5-*ene* (30).—To a hot diglyme solution (15 ml, bath temperature 170—175 °C) of the enol acetate (29) (2.162 g, 4.74 mmol) was added a diglyme solution (35 ml) of sodium chlorodifluoroacetate (10.84 g) in drops (*ca.* 15 min) and then the reaction mixture was refluxed for 20 min. The reaction mixture was cooled to room temperature, poured into water (200 ml) and diethyl ether (100 ml), and the precipitate was filtered off through Celite (No 545) and washed with diethyl ether. After addition of n-hexane (300 ml) to the filtrate, the organic layer was separated off, washed with water (2 × 200 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed on silica gel. The fraction which was eluted with benzene gave the *difluorocyclopropane* (30) (819 mg, 34%) (after the recrystallization from methanol), m.p. 112—115 °C; ν(CH_2Cl_2) 1 760 and 1 730 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.38 (1 H, m, 6-H), 4.60 (1 H, m, 3-H), 2.05 (3 H, s, 24-OAc), and 2.02 (3 H, s, 3-OAc); *m/e* 446 (M^+ − AcOH), 431, 404, and 384 (Found: C, 71.25; H, 8.85; F, 7.5. Calc. for $C_{30}H_{44}F_2O_4$: C, 71.11; H, 8.75; F, 7.50%). The next fraction was eluted with benzene and diethyl ether (25 : 1 v/v) and gave starting enol acetate (29) (1.15 g, 53%).

Base Treatment of the Difluorocyclopropane (30).—A solution of compound (30) (394 mg, 0.78 mmol) in THF (15 ml), water (10 ml), and methanol (4 ml) which contained lithium hydroxide hydrate (140 mg) was stirred for 2.5 h at room temperature. The reaction mixture was diluted with water and extracted with diethyl ether. The organic layer was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was dissolved in dichloromethane (10 ml), pyridine (2.5 ml), and acetic anhydride (2 ml) and stirred for 24 h at room temperature. After removal of the solvent under reduced pressure, the residue was chromatographed on silica gel, and the fraction which was eluted with benzene afforded 3β-*acetoxy*-24,24-*difluoro*-27-*norcholest*-5-*en*-25-*one* (31) (43 mg, 9.3%) (recrystallized from n-hexane), m.p. 135—136.5 °C; ν(KBr) 1 750 and 1 730 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.32 (1 H, m, 6-H), 4.50 (1-H, m, 3-H), 2.26 (3 H, t, J_{HF} 1 Hz, 26-H), and 1.96 (3 H, s, 3-OAc); *m/e* 404 (M^+ − AcOH), 389, 296, 283, and 255. The next fraction to be eluted with benzene afforded (23E)-3β-*acetoxy*-24-*fluoro*-27-*norcholesta*-5,23-dien-25-*one* *E*-(32) (34 mg, 7.7%) (recrystallized from methanol), m.p. 142—143 °C; ν(KBr) 1 730, 1 705, and 1 640 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.62 (1 H, dt, J_{HF} 22, J_{HH} 8 Hz, 23-H), 5.30 (1 H, m, 6-H), 4.52 (1 H, m, 3-H), 2.23 (3 H, d, *J* 5 Hz, 26-H), and 1.96 (3 H, s, 3-OAc); *m/e* 384 (M^+ − AcOH), 369, 283, 255, and 213 (Found: C, 75.35; H, 9.5. Calc. for $C_{28}H_{41}FO_3$: C, 75.63; H, 9.30; F, 4.27%). The last fraction was eluted with benzene and diethyl ether (30 : 1 v/v) and gave (23Z)-3β-*acetoxy*-24-*fluoro*-27-*norcholesta*-5,23-*dien*-25-*one* *Z*-(32) (234 mg, 53%) (recrystallized from methanol), m.p. 169—170 °C; ν(KBr) 1 730, 1 695, and 1 650 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 6.03 (1 H, dt, J_{HF} 34, J_{HH} 8 Hz, 23-H), 5.35 (1 H, m, 6-H), 4.52 (1 H, m, 3-H), 2.27 (3 H, d, *J* 3 Hz, 26-H), and 2.00 (3 H, s, 3-OAc); *m/e* 384 (M^+ − AcOH), 369, 283, and 213 (Found: C, 75.5; H, 9.35; F, 3.95. Calc. for $C_{28}H_{41}FO_3$: C, 75.63; H, 9.30; F, 4.27%).

3β-*Acetoxy*-24,24-*difluorocholest*-5-*en*-25-*ol* (33).—To a solution of compound (31) (70 mg, 0.15 mmol) in diethyl ether (10 ml) under an argon atmosphere was added an ethereal solution of MeMgI (2 ml), prepared from MeI (10 mmol) and Mg (10 mmol) in diethyl ether (12 ml). The reaction mixture was stirred for 15 min at 0 °C after which it was

quenched with dilute HCl, extracted with diethyl ether. The ethereal extract was washed twice with brine, and then dried ($MgSO_4$). Removal of the solvent under reduced pressure gave a residue which was dissolved in dichloromethane (5 ml), pyridine (2 ml), and acetic anhydride (2 ml) and then stirred for 20 h at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel to give a fraction, which was eluted with benzene and diethyl ether (20 : 1 v/v) to afford *compound* (33) (62 mg) (recrystallized from cyclohexane and n-hexane), m.p. 163—164.5 °C; ν(KBr) 3 400 and 1 730 cm^{-1}; δ_H ($CDCl_3$ with Me_4Si) 5.38 (1 H, m, 6-H), 4.60 (1 H, m, 3-H), 2.00 (3 H, s, 3-OAc), 1.28 (6 H, s, 26- and 27-H); *m/e* 420 (M^+ − AcOH), 405, 362, 312, 299, 255, and 213, (Found: C, 72.5; H, 9.7; F, 7.75. Calc. for $C_{29}H_{46}F_2O_3$: C, 72.46; H, 9.65; F, 7.90%).

3β-*Acetoxy*-24,24-*difluorocholest*-5,7-*dien*-25-*ol* (34).—A mixture of compound (33) (30 mg) and NBS (16 mg) in carbon tetrachloride (2 ml) was refluxed under an argon atmosphere for 25 min, and then cooled to room temperature to precipitate the imide. This was filtered off and the filtrate was concentrated under reduced pressure. To the residue, dissolved in boiling xylene (2 ml), was added a mixture of *s*-collidine (0.5 ml) and xylene (1.5 ml); the refluxing was then continued for a further 15 min. The reaction mixture was treated with dilute HCl and extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and then concentrated under reduced pressure to give a residue which was treated with a catalytic amount of TsOH-*p* in acetone (5 ml) for 15 h at room temperature under an argon atmosphere. The reaction mixture was diluted with water, extracted with ethyl acetate, and dried ($MgSO_4$). The extract was thrice purified by p.t.l.c. (benzene–ethyl acetate, 15 : 1 v/v), to give the 5,7-*diene* 3β-*monoacetate* (34) (8.4 mg, 28%); $\lambda_{max.}$ (EtOH) 263, 272, 282, and 292 nm; *m/e* 419 (M^+ − CMe_2OH), 418 (M^+ − 60), 403, and 313.

24,24-*Difluoro*-25-*hydroxyvitamin* D_3 (1).—In a similar manner for the synthesis of the vitamin (2) from compound (24), compound (34) (3 mg) gave the vitamin (1) (0.76 mg); $\lambda_{min.}$ (EtOH) 22 nm; $\lambda_{max.}$ (EtOH) 264 nm; *m/e* 436 (M^+), 421, 418, 403, 377, 271, 253, 136, and 118.

[1/823 *Received*, 22*nd May*, 1981]

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