Inhibitors of sterol synthesis. Chemical synthesis and properties of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one and 25,26,26,26,27,27,27-heptafluorocholesterol and their effects on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured mammalian cells

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Abstract A side-chain fluorinated $\Delta^{8(14)}$-15-ketosterol has been synthesized from 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VII**) as part of a program to prepare new analogs of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**), a potent regulator of cholesterol metabolism. 3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**) was prepared in five steps from **VII** in 38% overall yield. Dehydration of **VII** via the *ortho*-nitrophenylselenide to the 23-ene, followed by addition of $(CF_3)_2CFI$ gave 3β-acetoxy-23R-iodo-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one. Reductive deiodination with tributyltin hydride, followed by hydrolysis of the acetate gave **VIII.** 25,26,26,26,27,27,27-Heptafluorocholest-5-en-3β-ol (**XXI**) was prepared in eight steps in 31% overall yield from 3α,6α-diacetoxy-5β-cholanic acid (**XIII**). Compound **XIII** was reduced with borane–methyl sulfide to the corresponding 24-hydroxysteroid, which was converted to 3α,6α-diacetoxy-25,26,26,26,27,27,27-heptafluoro-5β-cholestane (**XVIII**) by reactions analogous to those developed for the preparation of **VIII** from **VII.** Conversion of **XVIII** via the 3α,6α-diol to the 3α,6α-ditosylate, followed by heating with potassium acetate in dimethylformamide and subsequent hydrolysis gave **XXI.** Full ^1H and ^{13}C NMR assignments are presented for **VIII, XXI,** and intermediates involved in their synthesis. ^{13}C NMR assignments for 3α,6α-dihydroxy-5β-steroids have been corrected, and stereochemical assignments were established for the side-chain methylene protons of **VIII, XXI,** and most synthetic intermediates. Compound **VIII** lowered the levels of HMG-CoA reductase activity in CHO-K1 cells and in HepG2 cells with a potency comparable to that of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**). In contrast, 25,26,26,26,27,27,27-heptafluorocholest-5-en-3β-ol had little or no effect on reductase activity in CHO-K1 cells. These combined results indicate that metabolism of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) to 26- and 25-oxygenated species is not required for the suppressive action of **I** on the levels of HMG-CoA reductase activity in CHO-K1 cells and HepG2 cells.—**Swaminathan, S., W. K. Wilson, F. D. Pinkerton, N. Gerst, M. Ramser, and G. J. Schroepfer, Jr.** Inhibitors of sterol synthesis. Chemical synthesis and properties of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one and 25,26,26,26,27,27,27-heptafluorocholesterol and their effects on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured mammalian cells. *J. Lipid Res.* 1993. **34:** 1805–1823.

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3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) is a potent inhibitor of cholesterol biosynthesis in cultured mammalian cells and lowers the levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity and of two other enzymes involved in the enzymatic formation of mevalonic acid (1–5). **I** has significant hypocholesterolemic action upon oral administration to rats (6–8), mice (6), baboons (9), and rhesus monkeys (10). **I** serves as an alternative substrate for acyl coenzyme A:cholesterol acyltransferase (ACAT) and inhibits the oleoyl-CoA-dependent

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; AIBN, 2,2'-azobisisobutyronitrile; BSTFA, bis(trimethylsilyl)trifluoroacetamide; CHO-K1, Chinese hamster ovary (cells); COSYDEC, ω_1-decoupled ^1H-^1H correlation spectroscopy; DEPT, distortionless enhancement by polarization transfer; GC, gas chromatography; HepG2, human hepatoma (cells); HETCOR, ^1H-^{13}C shift correlated spectroscopy; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPLC, high performance liquid chromatography; IR, infrared; MEM, minimal Eagle's medium; mp, melting point; MS, mass spectrometry or mass spectrum; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; TLC, thin-layer chromatography; TMS, trimethylsilyl; UV, ultraviolet.

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esterification of cholesterol in jejunal microsomes (11). Oral administration of **I** to rats lowers the levels of ACAT activity in jejunal microsomes (12) and inhibits the intestinal absorption of cholesterol (13, 14).

The 15-ketosterol **I** is metabolized to cholesterol in cell-free preparations from rat liver (15, 16) and in intact animals (17–22), and a scheme has been presented to account for the overall metabolism of **I** to cholesterol (16). Cholesterol and cholesteryl esters have been shown to be the major metabolites of **I** found in blood and tissues at 48 h after its intravenous administration to bile duct-cannulated rats (19, 21). However, a quantitatively more important fate of **I** under these conditions is very rapid conversion to polar metabolites which are excreted in bile (19, 21) and of which a significant fraction undergoes enterohepatic circulation (19). Further studies (23–25) have demonstrated that the major metabolites formed upon incubation of **I** with rat liver mitochondria in the presence of NADPH are (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (25R-**II**), (25S)-3β,26-dihydroxy-5α-cholest- 8(14)-en-15-one (25S-**II**), 3β-hydroxy-15-keto-5α-cholest-8(14)-en-26-oic acid (**III**), and 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (**IV**) (**Fig. 1**). Synthetic 25R-**II** and **IV** have been shown to have high potency, equivalent to **I**, in lowering the levels of HMG-CoA reductase activity in cultured mammalian cells (23, 26–28), whereas synthetic **III** had relatively low potency in lowering reductase activity (29). The results of further studies in bile duct-cannulated rats and in HepG2 cells (J. S. Pyrek, S. Numazawa, G. T. Emmons, N. Gerst, F. D. Pinkerton, and G. J. Schroepfer, Jr., unpublished data.) have indicated significant conversion of **I** to polar metabolites; this metabolism appears to be initiated by oxidation at C-26. Two potential in vivo metabolites, (25R)-5α-cholest-8(14)-ene-3β,15β,26-triol (**V**) (30) and 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (**VI**) (31, 32) have been shown to be highly active and moderately active inhibitors, respectively, of HMG-CoA reductase activity in cultured mammalian cells.

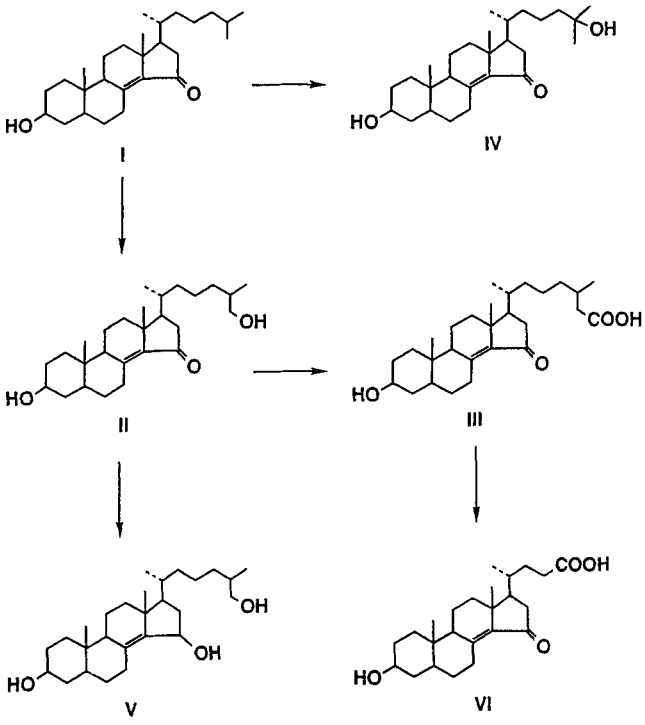

Fig. 1. Conversion of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) to side-chain oxygenated metabolites.

The high activity of these polar metabolites of the 15-ketosterol **I** raises the possibility that its effects in cultured cells and/or in intact animals may be partially or totally due to metabolites of **I.** That **I** itself is capable of lowering HMG-CoA reductase activity is strongly indicated by the high activity of **I** in CHO-K1 cells (2, 3, 5), a cell line in which little or no metabolism of **I** has been detected, apart from the formation of its fatty acid esters (5). To explore this matter further, we sought the chemical synthesis of the 25,26,26,26,27,27,27-heptafluoro analog of **I.** We recently found that oxidation of the 3β-acetate derivative of **I** with a mixture of trifluoroacetic anhydride, hydrogen peroxide, and sulfuric acid gives 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VII**) in remarkably high yield (31, 32). The availability of **VII,** selectively protected at C-3, provided a key intermediate for the chemical synthesis of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**). Described herein are the synthesis and properties of **VIII** and its effects on the levels of HMG-CoA reductase activity in two mammalian cell lines. Also presented are the chemical synthesis of 25,26,26,26,27,27,27-heptafluorocholesterol (**XXI**), its properties, and its effects on the levels of HMG-CoA reductase activity in CHO-K1 cells.

EXPERIMENTAL PROCEDURES AND RESULTS

Materials and methods

Triethylborane, tributyltin hydride, tributylphosphine, and hyodeoxycholic acid were purchased from Aldrich Chemical Company (Milwaukee, WI). 2-Iodoheptafluoropropane was obtained from Strem Chemicals, Inc. (Newburyport, MA). 2,2'-Azobisisobutyronitrile (AIBN) was obtained from Janssen Chimica (San Diego, CA). 2-Nitrophenyl selenocyanate was prepared according to Sharpless and Young (33). 3β-Acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VII**) was prepared as described previously (31, 32). 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) was prepared as described previously (34). Cholesterol was purified by way of its dibromide derivative (35).

Powdered minimal Eagle's medium (MEM), phosphate-buffered saline (PBS; KCl, 2.7 mM; KH_2PO_4, 1.2 mM,

NaCl, 137 mM; and Na_2HPO_4, 8.1 mM), and trypsin were purchased from Gibco Laboratories (Grand Island, NY). Ham's F12 medium (36) was obtained from Irvine Scientific (Irvine, CA). Fetal calf serum was purchased from Whittaker M.A. Bioproducts (Elkhart, IN). Lux tissue culture plasticware was from Miles Scientific (Elkhart, IN). (3RS)-[3-^{14}C]HMG-CoA (56 mCi per mmol) and (3RS)-[2-^{3}H]mevalonolactone (176 mCi per mmol) were purchased from Amersham Corporation (Arlington Heights, IL). Chinese hamster ovary (CHO-K1) cells and human hepatoma (HepG2) cells were obtained from the American Type Culture Collection (Rockville, MD).

Thin-layer chromatography (TLC) was carried out on silica gel G plates (Analtech; Newark, DE) or on aluminum-backed silica gel 60 plates (EM Separations; Gibbstown, NJ) or, in the case of assays of HMG-CoA reductase activity, Whatman LK5D plates (American Scientific Products, Houston, TX). Components of the plates were visualized after spraying with 5% ammonium molybdate(VI) in 10% sulfuric acid followed by heating. Solvent systems were: SS-1, 35% ether in hexane; SS-2, 15% ethyl acetate in hexane; SS-3, 20% ethyl acetate in hexane. High performance liquid chromatography (HPLC) was carried out using a Waters 600 Multisolvent Delivery System and a Shimadzu SPD 6A UV detector with a 5-μm Customsil ODS reversed phase column (250 mm × 4.6 mm) and a flow rate of 1 ml per min. The HPLC column was purchased from Custom LC (Houston, TX). Capillary gas chromatography (GC) was carried out on a Shimadzu GC-9A unit using splitless injection with nitrogen (1.3 kg per cm^2) as the carrier gas. The injector and detector were maintained at 290°C and the column temperature was programmed as follows: 100°C for 3 min; then 100°C to 250°C at 20°C per min; then 250°C for 10 min. The column used was Rt_x 1701 (15 m × 0.25 mm ID; 14% cyanopropylphenyl, 86% methyl polysiloxane; 0.1 μm film thickness; Restek Corporation; Bellefonte, PA). Trimethylsilyl (TMS) ether derivatives of the sterols were prepared using a 1:1 mixture of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine for 1 h under nitrogen at room temperature, followed by evaporation to dryness under nitrogen. GC samples were injected in hexane (1 μl).

Melting points (mp) were measured with a Thomas-Hoover apparatus in sealed, evacuated capillary tubes. Ultraviolet (UV) spectra were recorded on an IBM 9430 spectrophotometer using ethanol as the solvent. Infrared (IR) spectra were obtained on a Mattson Galaxy 6020 Fourier-transform IR spectrometer with KBr pellets. Overlapping carbonyl IR absorbances were distinguished by resolution enhancement (Gaussian apodization of the interferogram). Low resolution mass spectra (MS) were recorded on a Shimadzu QP-1000 quadrupole spectrometer with an electron energy of 70 eV and direct inlet sample introduction. GC–MS analyses were carried out as described previously (24, 25, 37) using a DB-5 capillary column (15 m × 0.25 mm; 5% phenyl, 95% methyl polysiloxane; 0.1 μm film thickness; J & W Scientific, Inc.; Folsom, CA) with direct introduction of the effluent into the ion source of the mass spectrometer (Extrel ELQ-400). Ions A, B, and C have been defined previously (24). High resolution MS were recorded on a Kratos MS-50DA spectrometer at the Midwest Center for Mass Spectrometry (Lincoln, NE).

Nuclear magnetic resonance (NMR) spectra were measured on an IBM AF300 (300.1 MHz for ^{1}H, 75.5 MHz for ^{13}C), a Bruker AMX500 (500.1 MHz for ^{1}H), or a Bruker AC250 (235.4 MHz for ^{19}F) spectrometer in $CDCl_3$ solution and referenced to internal tetramethylsilane (^{1}H), $CDCl_3$ at 77.0 ppm (^{13}C), or $CFCl_3$ (^{19}F). ^{19}F chemical shifts were measured from spectra of 64k points over a 212-ppm spectral width; ^{19}F coupling constants were measured from spectra of individual resonances using 16k points and a spectral width of ~1 ppm. Standard Bruker software was used to acquire DEPT (distortionless enhancement by polarization transfer), COSYDEC (ω_1-decoupled ^{1}H-^{1}H correlation spectroscopy, 0.2-s fixed evolution period τ_e, δ ~0.5–2.5, 256 increments), and HETCOR (^{1}H-^{13}C shift correlated spectroscopy; ~50 increments, δ ~0.6–2.6 in the ^{1}H dimension) spectra. Coupling constants were derived from line spacings of resolution-enhanced spectra and confirmed in some cases by homodecoupling experiments or spin simulation with NMR (Calleo Scientific Software; Ft. Collins, CO). PC Model (Macintosh version 4.4; Serena Software, Bloomington, IN) was used to calculate molecular mechanics structures of sterols and to predict vicinal ^{1}H NMR coupling constants. ^{1}H and ^{13}C NMR assignments were made from a combination of DEPT, HETCOR, and COSYDEC spectra in conjunction with ^{1}H and ^{13}C chemical shift comparisons and substituent increments (29, 32, 37–39). ^{1}H NMR stereochemical assignments were made by chemical shift and coupling constant comparisons (29, 32, 38).

Effects of added sterols on the levels of HMG-CoA reductase activity in cultured mammalian cells

CHO-K1 cells were maintained in a lipid-rich medium (Ham's F12 medium (36) supplemented with 5% fetal calf serum) in a humidified atmosphere of 5% CO_2–95% air at 37°C. Each experiment was initiated by inoculating 3.75 × 10^5 cells into 100-mm dishes containing lipid-rich medium (10 ml), followed by incubation for 48 h. The medium was aspirated and, after rinsing the plates with PBS (10 ml), the cells were incubated for 18 h in lipid-deficient media (10 ml; Ham's F12 medium supplemented with 5% delipidated (40) fetal calf serum) for 18 h. The cells then were incubated for 4 h at 37°C with fresh lipid-deficient media (10 ml) containing the oxygenated sterols. Lipid-deficient media containing the oxygenated sterols

were prepared by the addition of ethanol solutions of the sterols to the lipid-deficient medium, followed by equilibration at room temperature for at least 6 h prior to storage at 4°C. The ethanol concentration in all experimental media was constant. After incubation of the cells in the lipid-deficient media containing the various concentrations of the oxygenated sterols (from 0.0 μM to 2.5 μM) for 4 h at 37°C, the media were removed, the plates were rinsed with ice-cold PBS, and the cells were harvested by scraping into ice-cold PBS (5 ml) containing dithiothreitol (5 mM). Detergent-solubilized cell preparations were obtained for assay of HMG-CoA reductase activity using the method of Brown, Dana, and Goldstein (41). Replicate assays (n = 3) were carried out as described by Pinkerton et al. (3), except that the specific activity of the (3RS)-[3-^{14}C]HMG-CoA was 20,000 dpm per nmol.

HepG2 cells were maintained in a lipid-rich medium (MEM supplemented with 20% fetal calf serum and glutamine (5 mM)) in a humidified atmosphere of 5% CO_2-95% air at 37°C. Each experiment was initiated by inoculating 10^6 cells into 100-mm dishes containing the lipid-rich medium (10 ml), followed by incubation for 72 h. The medium was aspirated and, after rinsing the plates with PBS (10 ml), the cells were incubated with lipid-deficient medium (10 ml; MEM) supplemented with 10% delipidated (40) fetal calf serum and glutamine (5 mM). Lipid-deficient media containing the oxygenated sterols were prepared as described above. After incubation of the cells in the lipid-deficient media containing various concentrations of the oxygenated sterols (from 0.0 μM to 2.5 μM) for 6 h at 37°C, the media were removed, the plates were rinsed with ice-cold PBS, and the cells were harvested and assayed for HMG-CoA reductase activity as described above for the case of the CHO-K1 cells.

Chemical synthesis of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (Fig. 2)

*3β-Acetoxy-5α-chola-8(14),23-dien-15-one (**X**).* To a mixture of 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VII;** 1.217 g; 2.93 mmol) and 2-nitrophenyl selenocyanate (0.86 g; 3.8 mmol) in a dry round-bottom flask was added tetrahydrofuran (15 ml) under nitrogen. Tributylphosphine 0.95 ml; 3.8 mmol) was added dropwise to the reddish solution over ~2 min. After stirring the blackish-yellow mixture at room temperature for 2 h, the tetrahydrofuran was evaporated, and the residue was adsorbed on silica gel (7 g). The resulting solid was passed through a silica gel column (15 cm × 8 mm) using methylene chloride–ethyl acetate–hexane (2:1:7,500 ml) as the eluting solvent. The eluate was evaporated to dryness to give the crude *ortho*-nitrophenyl selenide **IX** (1.8 g); IR, ν_{max} 2940, 2855, 1736, 1701, 1626, 1591, 1514, 1451, 1377, 1358, 1331, 1304, 1256, 1221, 1123, 1098, 1030, and 727 cm^{-1}; ^{13}C NMR,

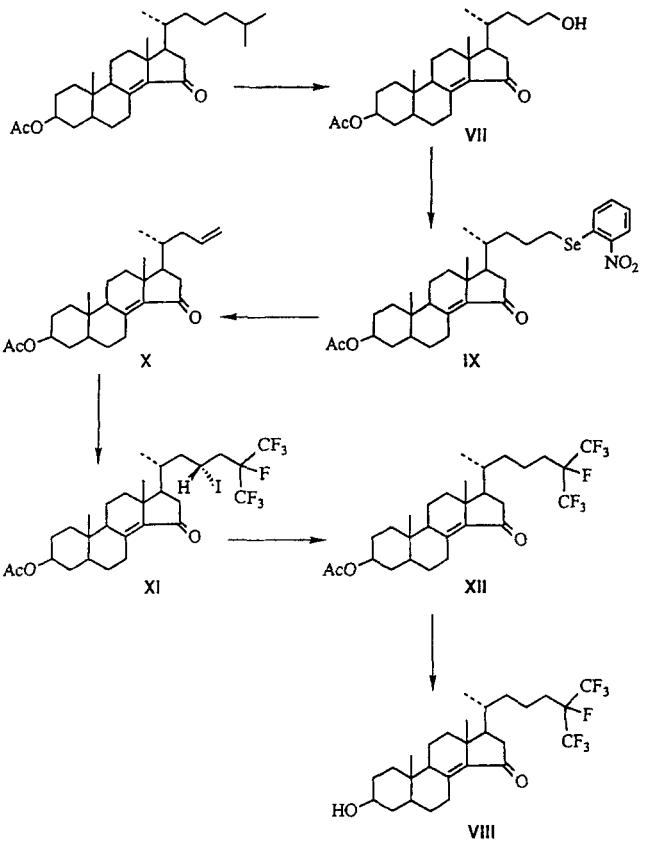

Fig. 2. Chemical synthesis of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**).

Table 1; ^{1}H NMR, **Table 2;** single component on TLC (solvent system, 30% ethyl acetate in hexane; R_f 0.51).

To the selenide (1.8 g) in tetrahydrofuran (20 ml) was added 30% hydrogen peroxide (1.5 ml) dropwise. After stirring at room temperature for 4 h, tetrahydrofuran was evaporated, and the residue was poured into water (100 ml). The resulting mixture was extracted with ethyl acetate (3 × 20 ml) and washed with aqueous $NaHCO_3$ and water. The residue (1.16 g) obtained upon evaporation of the solvent was passed through a silica gel column (16 cm × 8 mm) using 4% ethyl acetate in hexane as the solvent. Evaporation of the solvent and recrystallization from methanol gave **X** (0.85 g; 73% yield); mp, 158.5–159°C; IR, ν_{max} 2974–2824, 1738, 1701, 1624, 1468, 1454, 1441, 1377, 1358, 1256, 1221, 1198, 1175, 1155, 1144, 1125, 1103, 1086, 1030, 991, and 909 cm^{-1}; ^{13}C and ^{1}H NMR and MS, Tables 1, 2, and **Table 3,** respectively; single component (>99%) on TLC in two solvent systems (SS-1, R_f 0.48; SS-2, R_f 0.49).

*3β-Acetoxy-23R-iodo-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**XI**).* 3β-Acetoxy-5α-chola-8(14),23-dien-15-one (**X;** 197 mg; 0.494 mmol) was dissolved in hexane (50 ml) in a round-bottom flask fitted with a septum. 2-Iodoheptafluoropropane (0.14 ml; 0.988 mmol)

TABLE 1. ^{13}C NMR chemical shifts for the F_7-15-ketosterol **VIII**, F_7-cholesterol (**XXI**), and synthetic intermediates[a]

Carbon	IX[b]	X	XI	XII[c]	VIII	XIII	XIV	XVI	XVII	XVIII[d]	XIX	XX[e]	XXI
C-1	36.19	36.21	36.24	36.24	36.48	34.94	34.95	34.99	35.00	35.01	35.59	34.69	37.23
C-2	27.14	27.16	27.17	27.17	31.09	26.34	26.34	26.38	26.41	26.40	30.11	27.23	31.62
C-3	73.12	73.12	73.12	73.14	70.79	73.67	73.65	73.64	73.65	73.64	71.51	79.59	71.76
C-4	33.55	33.57	33.58	33.59	37.71	26.14	26.14	26.19	26.21	26.21	29.29	26.36	42.26
C-5	43.84	43.86	43.87	43.89	44.07	45.26	45.28	45.32	45.33	45.34	48.48	46.18	140.76
C-6	28.96	28.97	28.96	28.99	29.10	70.93	70.93	70.92	70.88	70.92	67.99	81.68	121.62
C-7	27.45	27.43	27.48	27.49	27.57	31.19	31.21	31.25	31.24	31.25	34.78	31.99	31.87
C-8	150.65	150.37	150.78	150.75	151.17	34.52	34.53	34.58	34.56	34.59	34.82	34.71	31.87
C-9	50.63	50.65	50.67	50.68	50.81	39.76	39.78	39.83	39.80	39.84	39.88	39.33	50.07
C-10	38.64	38.62	38.68	38.68	38.74	35.96	35.96	36.00	36.02	36.02	35.93	36.01	36.48
C-11	19.44	19.45	19.45	19.47	19.51	20.60	20.61	20.63	20.64	20.66	20.73	20.40	21.05
C-12	36.83	36.78	36.84	36.88	36.91	39.78	39.80	39.75	39.84	39.84	39.99	39.48	39.73
C-13	42.50	42.46	42.73	42.53	42.51	42.80	42.75	42.79	43.03	42.84	42.83	42.70	42.35
C-14	140.07	140.18	139.93	140.03	139.90	56.05	56.08*	56.09	56.13	56.11	56.21	55.77*	56.69
C-15	207.45	207.66	206.77	207.29	207.34	23.98	24.01	24.07	23.98	24.03	24.19	23.80	24.23
C-16	42.28	42.29	41.78	42.22	42.22	27.99	28.12	28.11	27.85	28.17	28.22	27.98	28.20
C-17	50.58	50.26	50.78	50.62	50.60	55.83	56.05*	55.73	56.01	55.96	56.08	55.67*	55.89
C-18	18.76	18.78	18.92	18.77	18.75	11.94	11.93	11.99	12.07	11.95	11.96	11.81	11.82
C-19	12.77	12.77	12.81	12.79	12.87	23.18	23.19	23.23	23.24	23.24	23.49	22.75	19.37
C-20	34.34	34.50	36.30	34.32	34.28	35.17	35.43	35.67	37.41	35.43	35.53	35.36	35.52
C-21	19.11	19.13	17.95	18.94	18.91	18.16	18.56	18.53	17.29	18.41	18.37	18.25	18.47
C-22	36.19	40.22	46.65[f]	35.61	35.59	30.64	31.73	40.50	47.17[f]	35.92	35.93	35.75	36.01
C-23	24.82	136.07	21.99[f]	17.96[g]	17.93[g]	30.90	29.25	137.20	22.61[f]	18.01[g]	18.11[g]	18.02[g]	18.11[g]
C-24	26.42	116.48	40.69[h]	29.26[i]	29.25[i]	179.58	63.39	115.71	40.81[h]	29.37[i]	29.39[i]	29.23[i]	29.40[i]
C-25			[j]	91.69[k]	91.70[k]				[j]	91.77[k]	91.80[k]	[j]	91.84[k]
C-26			120.31*[l]	121.04[l]	121.04[l]				120.39*[l]	121.07[l]	121.10[l]	[j]	121.13[l]
C-27			120.66*[l]	121.04[l]	121.04[l]				120.67*[l]	121.07[l]	121.10[l]	[j]	121.13[l]
Acetate	21.37	21.35	21.37	21.36		21.24[m]	21.34[m]	21.37[m]	21.36[m]	21.38			
	170.62	170.56	170.61	170.62		170.50[m]	170.47	170.43	170.43	170.45			
Mnemonic	SeAr	Δ^{23}	23-I	F_7-Ac	F_7-OH	COOH	24-OH	Δ^{23}	23-I	diAc	diOH	diTs	F_7-OH

[a]Chemical shifts referenced to the $CDCl_3$ signal at 77.0 ppm. Data obtained at 75 MHz in $CDCl_3$ solution at a concentration of 0.05–0.2 M. Signals marked by an asterisk may be interchanged within a column.
[b]Aromatic signals of selenide **IX:** δ 133.68, 133.51, 128.91, 126.43, 125.27.
[c]Signals corresponding to a minor (~15%) component: δ 51.02 (s), 49.65 (t), 42.50 (s), 42.20 (t), 41.97 (t), 35.83 (d), 35.58 (t), 34.30 (d), 19.65 (q), 18.74 (q), 18.63 (q).
[d]Signals corresponding to a minor (~15%) component: δ 56.41, 50.52, 36.71, 28.27, 18.82, 11.84.
[e]Tosylate signals of **XX:** δ 144.62, 134.37, 129.75, 129.72, 127.49, 127.42, 21.54.
[f]Signals broadened; also, C-22 of **XVII**, J_{CF} ~3 Hz.
[g]d, J_{CF} ~5 Hz.
[h]d, J_{CF} 18.0 ± 0.3 Hz.
[i]d, J_{CF} 21.0 ± 0.5 Hz.
[j]Signals too weak to be measured accurately.
[k]d of septet, J_{CF} 202.1 ± 0.3 Hz and 31.7 ± 0.1 Hz.
[l]q of d, J_{CF} 287 ± 1 Hz and 28 ± 1 Hz.
[m]Slight resolution (~0.02–0.05 ppm) of the acetate signals was observed.

and triethylborane (1 M solution in hexanes; 0.1 ml; 0.0988 mmol) were successively added. After 4 h at room temperature, TLC analysis (three developments with 10% ethyl acetate in hexane) showed only a trace amount of starting material. The mixture was passed through a column of silica gel (6 g) using hexane (50 ml) and 5% ethyl acetate in hexane (200 ml) as the eluting solvents. Evaporation of the solvent under reduced pressure gave **XI** (310 mg; 90% yield); mp, 139–142°C; ^{13}C and ^1H NMR and MS, Tables 1, 2, and **Table 4,** respectively; single component on TLC in one solvent system (SS-1, R_f 0.45) and one major (~95%) component (R_f 0.43) and a minor component (R_f 0.48) in another solvent system (SS-2). The ^1H and ^{13}C spectra showed an additional set of signals (Tables 1 and 2) of ~15% intensity of the major component; this observation appears to be compatible with formation of the 23S-epimer of **XI.**

*3β-Acetoxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**XII**).* To a solution of the iodide **XI** (310 mg; 0.446 mmol) and AIBN (10 mg) in tetrahydrofuran (4 ml) was added tributyltin hydride (0.16 ml; 0.603 mmol) under argon. After 5 h, water (20 ml) was added and the resulting mixture was extracted twice with ether (10 ml portions). The ether solution was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue (300 mg) was subjected to chromatography on a silica gel (6 g) column. Using 4% ethyl acetate in hexane as the eluting solvent, fractions 8 ml in volume were collected. The contents of fractions 20–56 were pooled (227 mg) and recrystallized from methanol to

TABLE 2. ^{1}H NMR chemical shifts for the F_7-15-ketosterol **VIII**, F_7-cholesterol (**XXI**), and synthetic intermediates[a–c]

Atom	IX[d]	X	XI[e]	XII	VIII	XIII	XIV	XVI	XVII[f]	XVIII	XIX	XX[g]	XXI
H-1α	[h]	1.25	1.25	1.25	1.20	1.81	1.82	1.82	1.82	1.82	1.79	1.72	1.08
H-1β	[h]	1.74	1.75	1.74	1.73	1.11	1.11	1.11	1.12	1.11	1.06	0.91†	1.85
H-2α	[h]	1.87	1.87†	1.86	1.85	1.43	1.43	1.43	1.43	1.43	1.35	1.51	1.84
H-2β	[h]	1.46	1.46†	1.46	1.38	1.72	1.72	1.72	1.72	1.72	1.69	1.72†	1.50
H-3	4.73	4.73	4.73	4.73	3.65	4.70	4.71	4.70	4.71	4.71	3.63	4.30	3.52
H-4α	[h]	1.71	1.72	1.71	1.68	1.60	1.60	1.60	1.60	1.59	1.42	1.61	2.30
H-4β	[h]	1.35	1.35	1.35	1.27	1.84	1.83	1.83	1.84	1.84	1.90	1.76	2.23
H-5α	1.47	1.47	1.48†	1.48	1.42	1.76	1.76	1.76	1.77	1.76	1.62	1.48	
H-6α	[h]	1.49	1.49†	1.49†	1.49								5.35
H-6β	[h]	1.34	1.35†	1.34	1.35	5.15	5.15	5.15	5.15	5.15	4.06	4.79	
H-7α	[h]	1.58	1.57†	1.59	1.59	1.20	1.20	1.20	1.20	1.20	1.14	1.23	1.53
H-7β	4.12	4.13	4.12	4.13	4.14	1.69	1.69	1.69	1.69	1.69	1.66	1.64	1.97
H-8β						1.53	1.53	1.53	1.54	1.53	1.46	1.39	1.46
H-9α	1.87	1.88	1.88	1.88	1.86	1.42	1.44†	1.42†	1.43	1.43†	1.38	1.26†	0.93
H-11α	[h]	1.65	1.67	1.66	1.66	1.40	1.42‡	1.42‡	1.41	1.42†	1.42	1.30*	1.49‡
H-11β	[h]	1.55†	1.55†	1.54	1.54	1.20†	1.20‡	1.22	1.22†	1.22†	1.20	1.26‡	1.49‡
H-12α	[h]	1.25	1.27	1.27	1.26	1.17†	1.18†	1.15	1.16	1.16	1.15†	1.11†	1.17
H-12β	2.09	2.09	2.12	2.10	2.10	1.98	2.00	1.99	2.00	1.99	1.98	1.94	2.01
H-14α						1.16	1.15	1.15	1.14	1.16	1.15	1.08	1.00
H-15α						1.56	1.55	1.55	1.56	1.56	1.60	1.47	1.60
H-15β						1.09	1.08	1.09	1.13	1.09	1.08	0.99	1.09
H-16α	2.36	2.40	2.33	2.33	2.33	1.87	1.85	1.87	1.82	1.82	1.83	1.80	1.82
H-16β	2.07	2.07	2.13	2.05	2.05	1.30	1.27	1.29	1.34	1.26	1.25	1.23	1.26
H-17α	1.49	1.47	1.58†	1.47	1.47	1.12	1.13	1.13	1.22	1.11	1.12	1.09	1.10
H-18	0.979	0.985	1.032	0.982	0.983	0.650	0.651	0.656	0.698	0.651	0.648	0.597	0.688
H-19	0.731	0.733	0.739	0.735	0.719	0.975	0.976	0.975	0.983	0.976	0.911	0.802	1.011
H-20	[h]	1.68	1.84	1.62	1.63	1.45	1.41	1.48	1.64	1.42	1.42†	1.39	1.42
H-21	1.026	1.022	0.984	1.029	1.029	0.926	0.930	0.918	0.886	0.928	0.928	0.897	0.941
H-22R[i]	[h]	1.87	1.27‡	1.41	1.41	1.81	1.43†	1.80	1.18	1.40†	1.42†	1.42‡	1.41
H-22S[i]	[h]	2.18	1.88	1.16	1.16	1.35	1.07	2.18	1.94	1.10	1.09	1.07	1.09
H-23R[i]	[h]	5.76	4.33	1.48	1.48	2.26	1.45*	5.77	4.34	1.44	1.44	1.42	1.44
H-23S[i]	[h]			1.64	1.64	2.39	1.64*			1.63	1.64	1.61	1.63
H-24R[i]	2.88*	5.02	2.83	1.99	1.99		~3.60*	4.98	2.82	1.97	1.97	1.96	1.97
H-24S[i]	2.90*	5.02	3.04	2.05	2.06		~3.62*	4.98	3.03	2.05	2.05	2.03	2.05
Ac	2.030	2.026	2.029	2.025		2.017	2.013	2.013	2.018	2.018			
						2.041	2.038	2.036	2.044	2.041			
Mnemonic	SeAr	Δ^{23}	23-I	F_7-Ac	F_7-OH	COOH	24-OH	Δ^{23}	23-I	diAc	diOH	diTs	F_7-OH

[a]Data obtained at 500.1 MHz in $CDCl_3$ solution at a concentration of 0.01–0.1 M. Chemical shifts referenced to $Si(CH_3)_4$ signal.

[b]Chemical shifts are generally accurate to 0.01 ppm except for values marked by † (± 0.02 ppm) or ‡ (± 0.05 ppm).

[c]Selected ^{1}H coupling constants in Hz (average of observed values, deviations generally ≤0.2 Hz): H-1α (**XI, XII, VIII**) td, 13.5, 3.5; (**XIV, XVII, XVIII, XIX**) dt, 14.2, 3.5; H-1β (**X, XI, XII, VIII**) dt, 13.3, 3.5, (**XVI, XVII, XIX**) td, 14.3, 3.5; H-3α (**X, XI, XII, VIII**) tt, 11.3, 4.8, (**XXI**) tdd, 11.1, 5.1, 4.2; H-3β tt, 11.3, 4.7; H-4α (**XII, VIII**) ddt 12.5, 5.0, 2.5, (**XIII, XIV, XVI, XVII, XX**) td, 12.7, 11.6, (**XXI**) ddd, 13.1, 5.1, 2.2; H-4β (**X, XI, XII, VIII**) td, 12.3, 11.2, (**XIX**) dddd, 12.5, 4.6, 3.6, 2.2, (**XXI**) ddq, 13.2, 10.9, 2.6; H-5α (**VIII**) tt, 12.1, 2.9; H-5β, dt, 13.2, 4.4; H-6 (**XXI**) dt, 5.3, 2.0; H-6β (**X, XII**) qd, 13.3, 4.2, (**VIII**) qd, 12.3, 3.9, (**XIII, XIV, XVI, XVII, XVIII, XIX, XX**) dt, 12.3, 4.8; H-7α (**X**) dddd, 14.1, 13.0, 5.8, 1.1, (**XIII, XIV, XVI, XVII, XVIII, XX**) q, 12.2; H-7β (**IX, X, XI, XII, VIII**) ddd, 14.2, 4.2, 2.0, (**XIII, XIV, XVI, XVII, XVIII, XIX, XX**) dt, 12.3, 4.2, (**XXI**) dtd, 17.1, 5.0, 2.7; H-9α (**XII, VIII**) dd, 10.3, 7.2, (**XXI**) ddd, 12.0, 10.8, 5.2; H-11α (**X, XI, VIII**) ddt, 14.0, 7.1, 3.5; H-12α (**XII**), td, 13.4, 3.8, (**XXI**) td, 12.7, 4.7; H-12β (**IX, X, XI, XII, VIII**) dt 12.5, 3.5, (**XIV, XVI, XVIII, XIX**) dt, 11.9, 2.9, (**XXI**) ddd 12.6, ~4, ~3; H-15α (**XXI**) dddd, 11.9, 9.8, 6.9, 3.0; H-16α (**IX, X, XI, XII, VIII**) dd, 18.3, 7.8, (**XVI, XIX, XX, XXI**) dddd, 13.2, 9.6, 9.2, 5.9; H-16β (**IX, X, XI, XII, VIII**) dd, 18.4, 12.4, (**XVI, XIX, XXI**) dddd, 13.3, 11.1, 9.6, 3.1; H-17α (**X, VIII**) ddd, 12.4, 10.0, 7.9, (**XVII, XIX, XX**) q, 9.7; H-21 d, 6.6; H-22R (**XVII**) ddd, 14.9, 12.0, 2.1; H-22S (**XII, VIII**) dddd, 13.2, 11.0, 8.6, 4.7, (**X, XVI**) dddt, 13.9, 6.0, 3.3, 1.7; H-23 (**X, XVI**) dddd‡, ~17, ~10, 8.3, 6.0, (**XI, XVII**) dddd, ~12, ~10, ~3, ~2; H-23R (**XIII**) ddd, 15.7, 10.3, 5.3; H-23S (**XIII**) ddd, 15.9, 9.7, 6.4; H-24R (**XI, XVII**) br ddd, ~16, ~14 (J_{HF}), 9.2, (**XII**) br dddd, 20 (J_{HF}), ~15, ~11, 5; H-24S (**XI, XVII**) dddd, 28.7 (J_{HF}), 16.2, 4.2, ~1. Two couplings observed to be identical may differ by up to ~1 Hz. Coupling patterns showing additional lines from second-order effects at 500 MHz are marked by ‡.

[d]Aromatic signals of selenide **IX:** δ 8.29 (br d, ~8 Hz, 1H), 7.52 (m, 2H), 7.31 (m, 1H).

[e]Signals corresponding to a minor (~15%) component: δ 4.3 (m), 4.13 (ddd, H-7β), 2.8 (m), 2.37 (dd, 18.1, 7.7 Hz, H-16α), 1.09 (d, 6.5 Hz), 0.73 (s).

[f]Signals corresponding to a minor (~15%) component: δ 2.8 (m), 0.97 (d, H-21), 0.66 (s).

[g]Tosylate signals of **XX:** δ 7.79 (d‡, 8.4 Hz), 7.72 (d‡, 8.4 Hz), 7.35 (d‡, 8.4 Hz), 7.33 (d‡, 8.4 Hz), 2.47 (s), 2.46 (s); additional lines in aromatic region indicated $J_{AA'}$ ~2 Hz.

[h]Not assigned.

[i]Pro-R and pro-S assignments for C-22, C-23, and C-24 protons; values marked with an asterisk may be interchanged.

TABLE 3. Mass spectral data for 3β-acetoxy-5α-chola-8(14),23-dien-15-one (**X**), 3β-acetoxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**XII**), and 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**)

	X			XII			VIII		
Suggested Assignment	*m/z* (%)			*m/z* (%)			*m/z* (%)		
M^+	398 (100)[a]	$C_{26}H_{38}O_3$	(+1.0)[b]	568 (100)[a]	$C_{29}H_{39}O_3F_7$	(−2.0)[b]	526 (100)[a]	$C_{27}H_{37}O_2F_7$	(0.0)[b]
M-2H							524 (2)	$C_{27}H_{35}O_2F_7$	(+1.4)
M-CH_3	383 (21)	$C_{26}H_{35}O_3$	(+1.3)	553 (8)	$C_{28}H_{36}O_3F_7$	(+0.8)	511 (10)	$C_{26}H_{34}O_2F_7$	(+0.5)
M-H_2O	380 (11)	$C_{26}H_{36}O_2$	(+0.2)	550 (14)	$C_{29}H_{37}O_2F_7$	(+1.7)	508 (19)	$C_{27}H_{35}OF_7$	(+2.0)
M-CH_3-H_2O	365 (30)	$C_{25}H_{33}O_2$	(−0.3)	535 (3)			493 (51)	$C_{26}H_{32}OF_7$	(+1.1)
M-C_2H_3O	355 (2)	$C_{24}H_{35}O_2$	(−1.0)						
Ion D-H_2O	339 (11)								
M-CH_3COOH	338 (8)	$C_{24}H_{34}O$	(+0.2)	508 (10)	$C_{27}H_{35}OF_7$	(+3.2)			
M-SC	329 (6)	$C_{21}H_{29}O_3$	(−0.4)	329 (14)	$C_{21}H_{29}O_3$	(−1.6)	287 (11)	$C_{19}H_{27}O_2$	(−0.2)
M-CH_3COOH-CH_3	323 (43)	$C_{23}H_{31}O$	(0.0)	493 (60)	$C_{26}H_{32}OF$	(−1.1)			
M-CH_3COOH-H_2O	320 (5)			490 (6)	$C_{27}H_{33}F_7$	(+3.2)			
M-SC-H_2O	311 (4)			311 (55)	$C_{21}H_{27}O_2$	(−0.6)	269 (82)	$C_{19}H_{25}O$	(+0.1)
M-CH_3COOH-CH_3-H_2O	305 (17)	$C_{23}H_{29}O$	(+2.1)	475 (8)	$C_{26}H_{30}F_7$	(−1.3)			
M-SC-28	301 (5)	$C_{19}H_{25}O$	(+0.5)	301 (7)			259 (5)		
M-SC-$2H_2O$							251 (19)	$C_{19}H_{23}$	(−1.7)
M-SC-CH_3COOH	269 (14)			269 (17)	$C_{19}H_{25}O$	(−0.9)			
M-SC-CH_3COOH-H_2O	251 (19)	$C_{19}H_{23}$	(−0.9)	251 (37)	$C_{19}H_{23}$	(−0.9)			
Ion A	243 (5)	$C_{17}H_{25}O$	(+0.2)	413 (15)	$C_{20}H_{24}OF_7$	(−0.5)	413 (11)	$C_{20}H_{24}OF_7$	(−2.1)
Ion B				402 (8)	$C_{19}H_{25}OF_7$	(−1.8)	402 (13)	$C_{19}H_{25}OF_7$	(−1.8)
Ion A-14				399 (12)			399 (11)	$C_{19}H_{22}OF_7$	(−1.6)
Ion B-CH_3				387 (23)	$C_{18}H_{22}OF_7$	(−0.3)	387 (35)	$C_{18}H_{23}OF_7$	(−0.5)
Ion A-28				385 (5)			385 (5)		
M-SC-CH_3COOH-28	241 (5)								
Other	309 (2)			465 (4)			411 (5)		
	281 (6)			411 (8)					
	275 (4)			401 (6)					
	263 (6)			373 (4)					

[a]Ion abundances from Shimadzu instrument (70 eV, direct probe), expressed as percentage of base peak ($m/z > 100$).
[b]Difference (in millimass units) of observed mass from calculated value.

give **XII** (211 mg; 83% yield); mp, 187–188°C; UV, λ_{max} 258 nm (ε 14,200); ^{13}C and ^1H NMR and MS, Tables 1, 2, and 3, respectively; single component on TLC in one solvent system (SS-1, R_f 0.45) and one major (~98%) component (R_f 0.45) and a minor component (R_f 0.48) in another solvent system (SS-2).

*3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**).* A solution of the acetate **XII** (59 mg) in methanol (4 ml) was stirred with potassium carbonate (30 mg) for 5 h at room temperature. Ethyl acetate (10 ml) and water (20 ml) were added and the resulting mixture was extracted twice with ethyl acetate (25 ml portions). The organic extract was washed with water (10 ml), dried over anhydrous sodium sulfate, and evaporated to dryness. The resulting residue (51 mg) was subjected to chromatography on a silica gel column (3.5 cm × 0.8 cm). Using 5% ethyl acetate in hexane (100 ml) and 10% ethyl acetate in hexane (250 ml) as the eluting solvents, fractions 50 ml in volume were collected. The contents of fractions 4–7 were pooled to give, after evaporation of the solvent, 49 mg of material which was then recrystallized from hexane to give **VIII** (38 mg; 69% yield); mp, 177–179°C; UV λ_{max} 259 nm (ε 13,600); IR, ν_{max} 3500, 2934, 2861, 1699, 1624, 1472, 1452, 1381, 1362, 1310, 1219, and 1134 cm^{-1}; ^{13}C and ^1H NMR and MS, Tables 1, 2, and 3, respectively; ^{19}F NMR, δ −76.76 and −76.96 (F-26 and F-27, AB system, $^3J_{FF}$ ~7 Hz, $^4J_{FF}$ ~9 Hz), −184.28 (F-25, dd of septet, $^3J_{HF}$ 20.9 and 20.0 Hz, $^3J_{FF}$ 6.7 Hz). HPLC analyses at 259 nm and 210 nm on a Spherisorb ODS-II column (solvent, 7% methanol in water) showed a single component (>99.8% purity) with a retention time of 6.92 min. GC–MS analysis of the 3β-trimethylsilyl derivative showed a single component; MS, *m/z* 598 (52; M^+), 583 (7; M-CH_3), 580 (2; M-H_2O), 508 (13; M-TMSOH), 493 (30; M-TMSOH-CH_3), 490 (6; M-TMSOH-H_2O), 475 (6), 413 (15; ion A), 402 (15; ion B), 399 (5), 387 (20; ion B-CH_3), 359 (4; M-SC), 341 (20; M-SC-H_2O), 331 (ion C; 4), 291 (4), 284 (10), 269 (20; M-SC-TMSOH), and 251 (100; M-SC-TMSOH-H_2O); TLC, HPLC, and GC, **Table 5.**

Chemical synthesis of 25,26,26,26,27,27,27-heptafluorocholesterol (Fig. 3)

*3α,6α-Diacetoxy-5β-cholan-24-ol (**XIV**).* The starting material, 3α,6α-diacetoxy-5β-cholan-24-oic acid (**XIII**) (42), was prepared by acetylation of hyodeoxycholic acid with acetic anhydride–pyridine and purified by recrystallization from ether–hexane; ^{13}C and ^1H NMR and MS, Tables 1, 2, and **Table 6,** respectively. To a solution of

TABLE 4. Mass spectral data for 3β-acetoxy-23R-iodo-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**XI**) (from *m/z* 250)

Ion (*m/z*)		Suggested Assignment	Elemental Composition	Observed Exact Mass	
694	(100)[a]	M^+	$C_{29}H_{38}O_3F_7I$	694.1741	(−1.3)[b]
679	(6)	$M-CH_3$			
676	(9)	$M-H_2O$			
634	(8)	$M-CH_3COOH$			
619	(53)	$M-CH_3COOH-CH_3$			
601	(5)	$M-CH_3COOH-CH_3-H_2O$			
568	(12)	M−I + H	$C_{29}H_{39}O_3F_7$	568.2763	(−2.4)
567	(5)				
566	(3)	M−HI	$C_{29}H_{37}O_3F_7$	566.2611	(−1.9)
549	(6)				
539	(12)	Ion A			
528	(7)	Ion B			
525	(5)				
513	(20)	Ion $B-CH_3$			
507	(7)				
493	(9)	$M-CH_3COOH-CH_3-I+H$	$C_{26}H_{32}OF_7$	493.2333	(−0.9)
489	(11)				
401	(7)				
399	(5)				
398	[(21)]*	$M-C_3F_7I$	$C_{26}H_{29}O_3$	398.2810	(−1.1)
329	(15)	M−SC	$C_{21}H_{29}O_3$	329.2110	(−0.6)
311	(58)	$M-SC-H_2O$	$C_{21}H_{27}O_2$	311.2000	(−1.2)
301	(9)	$M-SC-C_2H_4$	$C_{19}H_{25}O_3$	301.1792	(−1.2)
269	(23)	$M-SC-CH_3COOH$	$C_{19}H_{25}O$	269.1905	(−0.5)
251	(53)	$M-SC-CH_3COOH-H_2O$	$C_{29}H_{23}$	251.1801	(+0.1)

[a]Ion abundances from Shimadzu instrument (70 eV, direct probe), expressed as percentage of base peak.
[b]Difference (in millimass units) of observed mass from calculated value.
*Ion present in high abundance (21%) in the high resolution MS of **XI**. This ion was of low abundance in low resolution MS.

XIII (2.092 g, 4.4 mmol) in tetrahydrofuran under argon at 0°C was added borane–methyl sulfide (2 M, 2.2 ml, 4.4 mmol). The reaction was stirred for 16 h at room temperature and quenched by addition of ice water (50 ml). The layers were separated, and the aqueous layer was extracted with ethyl acetate (3 × 15 ml). The combined organic layers were washed with water, dried over anhydrous sodium sulfate, and concentrated to a foamy white oil (1.906 g, 94% crude yield). A portion of the oil was purified by column chromatography (12 g silica gel; elution with 8:92 ethyl acetate–hexane, followed by 12:88 ethyl acetate–hexane) to give an analytical sample: mp, 80.5–83.0°C; single component on TLC (R_f 0.74, 1:1 ethyl acetate–hexane); IR, ν_{max} 3500, 2980–2820, 1736, 1726, 1466, 1454, 1377, 1366, 1242, 1028 cm^{-1}; high resolution MS, calcd. for $C_{24}H_{38}O$ ($M-CH_3COOH-CH_3COOH$), 342.2923, found 342.2929; ^{13}C and ^1H NMR and MS, Tables 1, 2, and 6.

TABLE 5. Chromatographic properties of cholesterol, 25,26,26,26,27,27,27-heptafluorocholesterol (**XXI**), 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**), and 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**)

	TLC[a]		HPLC[b]			GC[c]		
			Free Sterols					
Sterol	Free Sterol A	Acetate B	C	D	E	Free Sterol F	Acetate F	TMS Ether F
F_7-15-Ketosterol **VIII**	0.17	0.32	3.70	4.51	5.63	19.04	21.74	17.27
15-Ketosterol **I**	0.20	0.39	5.51	7.45	10.12	25.76	30.22	22.50
F_7-Cholesterol (**XXI**)	0.33	0.54	6.69	10.86	17.55	12.84	13.78	12.24
Cholesterol	0.35	0.55	15.07	26.78	45.70	15.32	17.01	14.34

[a]Thin-layer chromatography (silica gel G): A, hexane–ethyl acetate 3:2; B, hexane–ethyl acetate 4:1.
[b]High performance liquid chromatography (reversed phase): C, methanol; D, methanol–water 98:2; E, methanol–water 95:5.
[c]Gas chromatography (Rt_x 1701 column) under operating conditions (F) presented in Materials and Methods.

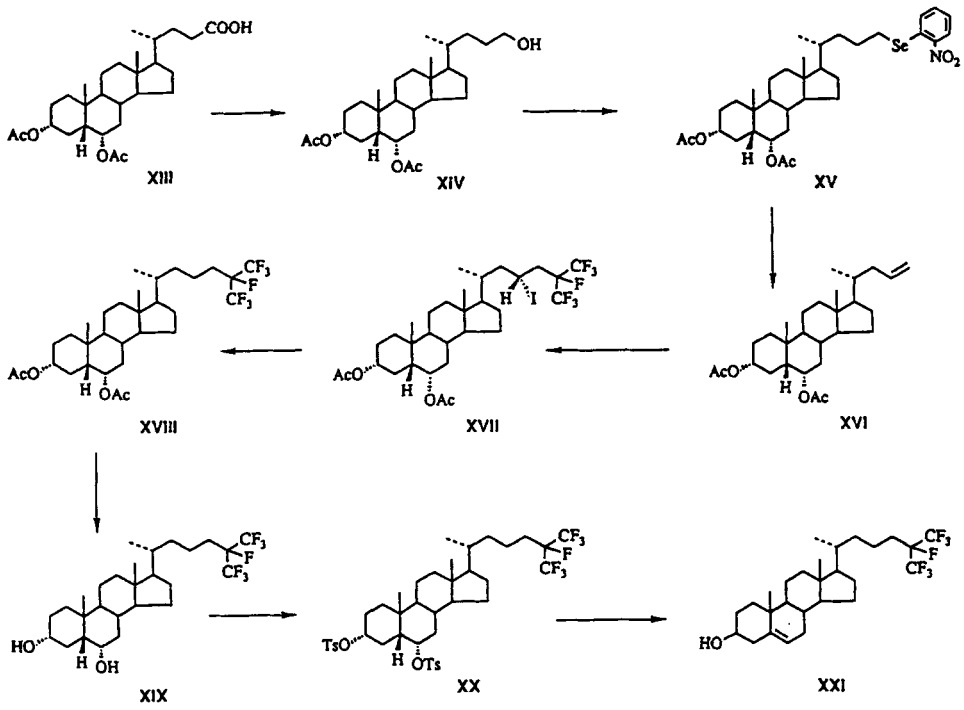

Fig. 3. Chemical synthesis of 25,26,26,26,27,27,27-heptafluorocholesterol.

*3α,6α-Diacetoxy-24-(2-nitrophenylseleno)-5β-cholane (**XV**).*[2] A mixture of diacetate **XIV** (3.236 g, 7.0 mmol) and 2-nitrophenyl selenocyanate (2.06 g, 9.1 mmol) under argon was dissolved in tetrahydrofuran (20 ml), and tributylphosphine (2.26 ml, 9.1 mmol) was added to the resulting solution. The reaction mixture was stirred at room temperature for 2 h, followed by evaporation of the solvent. The residue was adsorbed onto silica gel (9 g) and purified by silica gel chromatography (14 × 2.5 cm column; elution with 8% ethyl acetate in hexane; 100-ml fraction volumes). Evaporation of fractions 13–27 gave **XV** as a foamy yellow solid (4.0 g): single component on TLC in SS-3 (R_f 0.41); IR, ν_{max} 2980–2820, 1736, 1589, 1566, 1516, 1452, 1364, 1331, 1304, 1246, 1028, 731 cm^{-1}; high resolution MS, calcd. for $C_{34}H_{49}NO_6{}^{80}Se$, 647.2725, found 647.2744; MS (asterisk indicates isotope cluster consistent with the presence of selenium atom), *m/z* 647* (7, M^+), 527* (6, $M-2CH_3COOH$), 512* (9, $M-2CH_3COOH-CH_3$), 492* (7), 373 (10), 341 (5), 325 (25), 313 (6), 296* (4), 282* (14), 272* (6), 264* (7), 255 (26), 253* (22), 107 (98), 105 (100); ^{13}C and ^1H NMR, Tables 1 and 2.

*3α,6α-Diacetoxy-5β-chol-23-ene (**XVI**).* To a solution of selenide **XV** (4.0 g, 6.2 mmol) in tetrahydrofuran (30 ml) was added hydrogen peroxide (3.1 ml, 31 mmol) under argon. The reaction mixture was stirred for 4 h at room temperature, stored overnight at 4°C, and poured into water (400 ml). Extraction with ether (3 × 60 ml), followed by washing of the organic layer with 10% aqueous $NaHCO_3$ (2 × 40 ml) and water (40 ml), drying over anhydrous sodium sulfate, and evaporation gave a residue (3.1 g). The crude product was purified by silica gel chromatography (22 × 2 cm column, elution with 2% ethyl acetate in hexane; 75-ml fraction volumes). Evaporation of fractions 7–20 resulted in a pale yellow solid (2.4 g) that was recrystallized from methanol to give colorless needles (2.2 g from two crops, 71% yield from **XIV**): mp 159–161°C; single component on TLC in SS-3 (R_f 0.65); IR, ν_{max} 3075, 2980–2820, 1736, 1726, 1638, 1466, 1449, 1362, 1252, 1242, 1026, 912 cm^{-1}; high resolution MS, calcd. for $C_{26}H_{40}O_2$ ($M-CH_3COOH$) 384.3028, found 384.3022; ^{13}C and ^1H NMR and MS, Tables 1, 2, and 6.

*3α,6α-Diacetoxy-23R-iodo-25,26,26,26,27,27,27-heptafluoro-5β-cholestane (**XVII**).* To a slurry of olefin **XVI** (2.191 g, 4.93 mmol) in hexane (90 ml) was added 2-iodoheptafluoropropane (1.4 ml, 9.86 mmol) and triethylborane (1 M, 2 ml, 1.97 mmol), and the solution was stirred at room temperature in the dark. After 2 h, the disappearance of ion *m/z* 324 ($M-CH_3COOH-CH_3COOH$ of **XVI**) in the mass spectrum indicated that the reaction was complete. The volatile material was evaporated to a residue that was subjected to silica gel chromatography (22 × 1.4 cm column; elution with 4% ethyl acetate in

[2] Selenides **IX** and **XV** and ditosylate **XX** were not isolated as crystalline solids. Although melting points are not reported, these compounds appeared as single components on TLC and showed ≥ ~95% purity by ^1H NMR (300 or 500 MHz).

TABLE 6. Mass spectral data for 3α,6α-dioxygenated steroid intermediates in the chemical synthesis of F_7-cholesterol (**XXI**)[a]

Suggested Assignment[b]	XIII	XIV	XVI	XVII	XVIII	XIX	XX
M-ROH	416 (1)	402 (1)	384* (1)	680 (0.6)	554* (0.3)	512* (100)	
M-ROH-CH_3			369* (1)	665 (0.3)	539* (0.5)	497* (70)	
M-2ROH	356 (100)	342* (100)	324* (100)	620* (100)	494* (100)	494* (77)	494* (100)
M-2ROH-CH_3	341 (20)	327* (14)	309* (11)	605 (13)	479* (12)	479* (53)	479* (27)
M-2ROH-C_4H_6	302 (2)	288* (3)	270 (2)	566 (3)	440* (3)	440* (14)	440* (18)
M-2ROH-C_8H_{12}	248 (7)	234* (7)		512* (8)	386* (6)	386* (7)	386* (12)
M-2ROH-C_9H_{13}	235 (8)	221* (5)	203* (7)	499* (15)	373* (8)	373* (14)	373* (19)
M-2ROH-pSC	283 (1)	283* (2)	283* (57)[c]	283* (9)	283* (1)	283* (4)	283 (2)
M-2ROH-SC	255 (11)	255* (13)	255* (4)	255* (13)	255* (4)	255* (24)	255* (9)
$C_{17}H_{24}$	228 (15)	228* (12)	228* (9)	228* (19)	228* (7)	228* (31)	228* (1)
$C_{16}H_{21}$	213 (29)	213* (30)	213* (31)	213* (46)	213* (20)	213* (76)	213* (11)
C_8H_{11}	107 (14)	107* (15)	107* (20)	107* (28)	107* (9)	107* (79)	107* (54)
C_8H_9	105 (17)	105* (19)	105* (21)	105* (34)	105* (10)	105* (66)	105* (45)
Other ions	315 (2)	384 (1)[d]	343 (6)[c]	565 (4)	439 (4)	453 (16)[c]	331 (6)[c]
	301 (3)	324* (1)[d]	275 (5)[c]	493 (23)[c]		439 (17)[c]	
	248 (7)		267 (4)[c]	451 (12)		400 (14)[c]	
			253 (4)[c]	253 (6)[c]		387 (14)[c]	
			241 (29)[c]			273 (10)[c]	
			215 (20)[c]			231 (53)	
R =	$COCH_3$	$COCH_3$	$COCH_3$	$COCH_3$	$COCH_3$	H	$SO_2C_6H_4CH_3$

[a]Major ions above *m/z* 100 in mass spectra acquired at 70 eV by direct-probe. Relative intensities as % of base peak. Ions also observed in the high resolution mass spectrum and compatible (± 3.0 millimass units) with the suggested assignments are indicated by an asterisk.

[b]M-2ROH-C_4H_6 is compatible with a retro Diels-Alder reaction in ring A, and the subsequent two entries are compatible with loss of ring A and part of ring B; pSC refers to the portion of the sterol side chain containing carbons 22-27.

[c]Compatible with loss of C_3H_5 (allyl radical) from other ions.

[d]Compatible with loss of H_2O from other ions.

[e]High-resolution MS is compatible with the following assignments or formulas: **XVI,** *m/z* 267, $C_{20}H_{27}$; *m/z* 275, $C_{18}H_{27}O_2$; *m/z* 253, $C_{19}H_{25}$; *m/z* 241, $C_{18}H_{25}$; *m/z* 215, $C_{16}H_{23}$; **XVII,** *m/z* 493, M-2CH_3COOH-I; *m/z* 253, $C_{19}H_{25}$; **XIX,** *m/z* 453, M-2H_2O-C_3H_5; *m/z* 439, M-2H_2O-C_4H_7; *m/z* 400, M-2H_2O-C_7H_{10}; *m/z* 387, M-2H_2O-C_8H_{11}; *m/z* 273, M-H_2O-SC; **XX,** *m/z* 331, M-2TsOH-$C_{12}H_{19}$.

hexane; 75-ml fraction volumes). Evaporation of fractions 3–10 gave a white solid (3.085 g, 85% yield): single component on TLC in SS-3 (R_f 0.64); IR, ν_{max} 2980–2820, 1736, 1726, 1466, 1454, 1378, 1366, 1289, 1242, 1159, 1146, 1028 cm^{-1}; high resolution MS, calcd. for $C_{27}H_{36}F_7I$ (M-CH_3COOH-CH_3COOH) 620.1750, found 620.1770; ^{13}C and ^1H NMR and MS, Tables 1, 2, and 6; ^{19}F NMR, δ −77.24 (quintet, ~8.8 Hz, F-26 or F-27), −78.03 (qd, 9.3, 6.7 Hz, F-26 or F-27), −184.68 (m compatible with analysis as ddqq, 28.7, ~15, ~8, 6.7 Hz, F-25). An additional set of signals of ~15% intensity of major component was observed in the ^1H and ^{13}C spectra (Tables 1 and 2) and in the ^{19}F spectrum: δ −77.60 and −77.05 (both rough quintets, ~8 Hz, F-26 and F-27), −187.75 (m, F-25). This observation appears to be compatible with formation of the 23S-epimer of **XVII.**

*3α,6α-Diacetoxy-25,26,26,26,27,27,27-heptafluoro-5β-cholestane (**XVIII**).* To a solution of iodide **XVII** (3.072 g, 4.15 mmol) and AIBN (0.36 g) in tetrahydrofuran (25 ml) was added tributyltin hydride (1.9 ml, 7.06 mmol) under nitrogen, and the reaction was stirred at room temperature. After 4 h, disappearance of the *m/z* 620 ion (M-CH_3COOH-CH_3COOH of **XVII**) indicated completion of the reaction. The solvent was evaporated to a residue that was purified by silica gel chromatography (16 × 1.5 cm column; elution with 3% ethyl acetate in hexane; 22-ml fraction volumes). Evaporation of fractions 2–9 gave a solid (2.7 g) that was recrystallized from methanol to furnish **XVIII** as colorless needles (1.95 g, 76% yield): mp 146–147°C; single component on TLC in SS-3 (R_f 0.65); IR, ν_{max} 2980–2820, 1738, 1726, 1468, 1456, 1366, 1312, 1238, 1157, 1028 cm^{-1}; high resolution MS, calcd. for $C_{29}H_{41}O_2F_7$ (M-CH_3COOH) 554.2995, found 554.2978; ^{13}C and ^1H NMR and MS, Tables 1, 2, and 6.

*25,26,26,26,27,27,27-Heptafluoro-5β-cholestane-3α,6α-diol (**XIX**).* To a solution of diacetate **XVIII** (1.372 g, 2.23 mmol) in tetrahydrofuran (9 ml) was added methanol (9 ml) and potassium acetate (926 mg, 6.7 mmol). The reaction mixture was stirred at room temperature. After 4 h, TLC analysis (30% ethyl acetate in hexane) showed disappearance of starting material and appearance of two lower spots attributed to a monoacetate and the diol. After 24 h, the reaction was poured into water (150 ml), and the aqueous phase was extracted with ether (2 × 40 ml). The combined organic extracts were dried over anhydrous sodium sulfate and evaporated to a residue (1.21 g). Purification by silica gel chromatography (18 × 1.4 cm column; elution with 25% ethyl acetate in hexane (250 ml), 35% ethyl acetate in hexane (250 ml), and 85% ethyl acetate in hexane; 75-ml fraction volumes) gave a white foam (1.152 g) that was recrystallized from hexane to provide **XIX** as colorless needles (1.128 g, 95% yield):

mp 150–151°C; single component on TLC in ethyl acetate (R_f 0.39); IR, ν_{max} 3400, 2980–2820, 1468, 1377, 1346, 1312, 1221, 1159, 1038, 940, 719 cm^{-1}; high resolution MS, calcd. for $C_{27}H_{39}OF_7$ (M–H_2O) 512.2889, found 512.2885; ^{13}C and ^1H NMR and MS, Tables 1, 2, and 6.

*3α,6α-Bis(*para*-toluenesulfonyloxy)-25,26,26,26,27,27,27-heptafluoro-5β-cholestane (**XX**).*[2] Pyridine (9 ml) was added to a mixture of diol **XIX** (1.108 g, 2.09 mmol) and *para*-toluenesulfonyl chloride (1.3 g, 7.00 mmol), and the solution was kept at room temperature for 24 h. The mixture was poured into cold 5% sulfuric acid (200 ml) and extracted with ether (3 × 40 ml). The combined organic extracts were washed with water, dried over anhydrous sodium sulfate, and concentrated to a residue (1.902 g) that was purified by silica gel chromatography (18 × 1.4 cm column; elution with 4% ethyl acetate in hexane; 75-ml fraction volumes). Evaporation of fractions 11–16 gave **XX** as a white brittle foam (1.76 g, 100% crude yield): single component on TLC in SS-3 (R_f 0.53); IR, ν_{max} 2980–2820, 1599, 1468, 1456, 1360, 1223, 1190, 1177, 945, 924, 849, 669 cm^{-1}; high resolution MS, calcd. for $C_{27}H_{37}F_7$ (M–2TsOH), 494.2783, found 494.2761; ^{13}C and ^1H NMR and MS, Tables 1, 2, and 6.

*25,26,26,26,27,27,27-Heptafluorocholest-5-en-3β-ol (**XXI**).* A solution of ditosylate **XX** (1.69 g) and potassium acetate (12.5 g) in N,N-dimethylformamide (40 ml) was heated at 110°C for 5 h. The mixture was cooled, poured into water (160 ml), and extracted with ether (3 × 50 ml). The extract was washed with water (2 × 40 ml), dried over anhydrous sodium sulfate, and concentrated to a residue (1.12 g). The residue was dissolved in methanol–tetrahydrofuran (25 ml, 3:2 mixture), and KOH (1.9 g) and water (0.4 ml) were added. The reaction mixture was kept at room temperature for 3 h and poured into water (200 ml). The resulting precipitate (1.02 g) was collected by filtration and purified by silica gel chromatography (12 × 1 cm column; elution with 8% ethyl acetate in hexane; 22-ml fraction volumes). Evaporation of fractions 8–18 gave a solid that was recrystallized from methanol to give **XXI** as colorless plates (783 mg, 76% yield): mp, 149–150.5°C; single component on TLC in SS-3 (R_f 0.41); IR, ν_{max} 3450, 2980–2820, 1470, 1441, 1379, 1358, 1317, 1279, 1221, 1159, 1136, 1051, 1020, 937, 719 cm^{-1}; high resolution MS, calcd. for $C_{27}H_{39}OF_7$, 512.2889, found 512.2906; MS, *m/z* 512 (52, M^+), 510 (1; M–2H), 497 (10, M–CH_3), 494 (38, M–H_2O), 479 (26, M–CH_3–H_2O), 455 (4), 453 (5), 452 (5), 427 (35), 401 (42), 373 (10), 345 (3), 331 (5), 319 (4), 291 (4), 273 (7, M-SC), 255 (17, M–SC–H_2O), 231 (11), and 213 (33), 107 (100), 105 (87); ^{13}C and ^1H NMR, Tables 1 and 2; ^{19}F NMR, δ −76.75 and −76.96 (F-26 and F-27, AB system shown in **Fig. 4,** $^3J_{FF}$ ~7 Hz, $^4J_{FF}$ ~9 Hz), −184.14 (F-25, dd of septet, $^3J_{HF}$ 21.1 and 19.9 Hz, $^3J_{FF}$ 6.6 Hz); TLC, HPLC, and GC, Table 5.

NMR experiments to determine stereochemical assignments of the C-24 protons of F_7 sterols

The upfield H-24 signal of **VIII** and **XII** and the downfield H-24 signal of **XX** and **XXI** were largely unobstructed by other signals. In each case, the H-24 signals were approximately compatible with an analysis as dddd of septet with coupling constants of 20, ~15, ~11, ~5, and ~1 Hz, as judged by first-order simulation. The 20-Hz couplings were identified as J_{HF} based on coupling patterns of the ^{19}F NMR spectra and on splitting of the H-24 correlations in f_1 of COSYDEC spectra of **XII, XXI,** and other F_7 sterols. The other couplings were attributed respectively to geminal (~15 Hz), vicinal anti (~11 Hz), vicinal gauche (~5 Hz), and four-bond couplings to fluorine at C-26 and C-27 (~1 Hz). Irradiation of the upfield H-23 signal of **XX** or **XXI** led to loss of the ~5-Hz coupling of the downfield H-24 signal, and irradiation of the downfield H-23 signal resulted in loss of the ~11-Hz coupling. Results of other decoupling experiments are given in the discussion. Homodecoupling experiments were carried out by irradiation with a field of ~40 Hz, which led to Bloch-Siegert shifts of 0.01–0.005 ppm for resonances 0.3–0.6 ppm distant from the center of irradiation. Exact analysis of the effects of decoupling on the H-24 signals was hampered by the presence of the numerous small couplings that led to severe line broadening. The slight reduction in the magnitude of coupling constants observed in the decoupled spectra appears to be due to effects analogous to those observed in single-frequency off-resonance decoupled ^{13}C spectra.

COSYDEC experiments provided additional support for the H-24 assignments described in the discussion section. COSYDEC (τ_e 200 ms) correlations between C-23 and C-24 protons were limited to H-23R/H-24S and H-23S/H-24R (i.e., upfield to upfield signals and downfield to downfield signals). This result is compatible with the proposed assignments since optimal cross peak intensity is observed for protons related by couplings of 2.5, 7.5, and 12.5 Hz in COSYDEC experiments with τ_e of 200 ms.

Comparisons of the chromatographic properties of cholesterol, 25,26,26,26,27,27,27-heptafluorocholesterol (XXI), 3β-hydroxy-5α-cholest-8(14)-en-15-one (I), and 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (VIII)

The chromatographic mobilities of cholesterol and its F_7 analog (**XXI**) as well as the 15-ketosterol (**I**) and its F_7 analog (**VIII**) on TLC (silica gel G plates) are shown in Table 5 along with data for the acetate derivatives of the same compounds. The F_7 substitution had little or no effect on the TLC mobilities of cholesterol or cholesteryl acetate. Similar findings have been made in silicic acid-

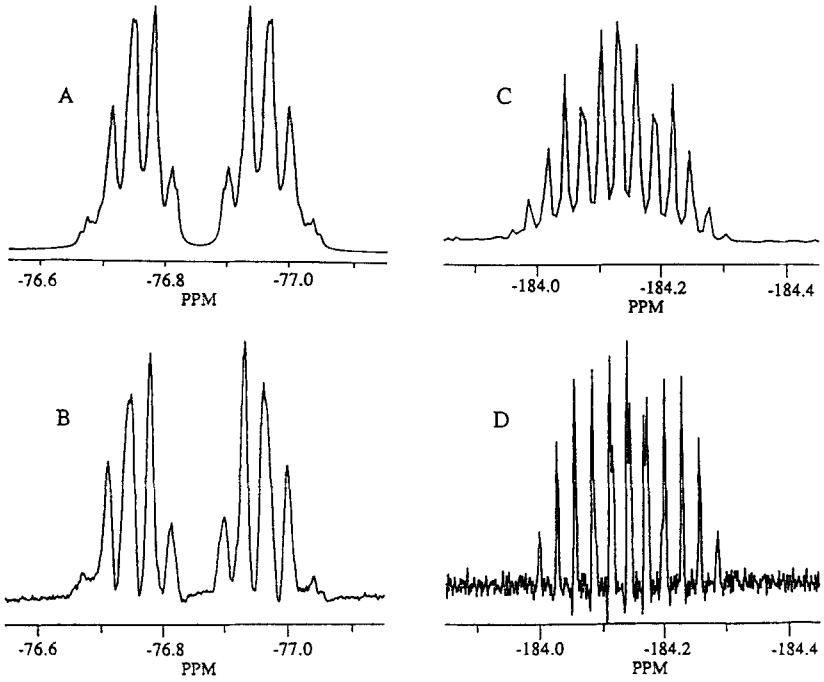

Fig. 4. ^{19}F NMR spectra of the F_7-cholesterol (**XXI**). A: Simulated spectrum of F-26 and F-27. B: Observed spectrum of F-26 and F-27. C: Spectrum of F-25 at digital resolution of 0.8 Hz. D: Spectrum of F-25 at digital resolution of 0.01 Hz with moderate resolution enhancement.

Super Cel column chromatography; cholesterol and its F_7 analog **XXI** showed almost identical behavior with the F_7 sterol being very slightly more polar (N. Gerst and G. J. Schroepfer, Jr., unpublished data). The F_7-15-ketosterol **VIII** was slightly more polar on TLC than the 15-ketosterol **I** and this difference was clearer in the case of the acetate derivatives of the two sterols.

Cholesterol and its F_7 analog **XXI** as well as the 15-ketosterol **I** and its F_7 analog **VIII** were readily separable by either reversed phase HPLC or by capillary GC, as shown in Table 5. Introduction of the F_7 substitution resulted in a considerable shortening of HPLC and GC retention times for both cholesterol and the 15-ketosterol. The acetate ester and trimethylsilyl ether derivatives of the F_7 sterols also showed markedly shorter retention times on GC than those of the corresponding sterols lacking the fluorine substitution. In contrast, the F_7 substitution had much less effect on the mobility of the sterols or their acetates on TLC using silica gel.

Effects of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (VIII) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (I) on the levels of HMG-CoA reductase activity in CHO-K1 cells and in HepG2 cells

The effects of the 15-ketosterol **I** and its F_7 analog **VIII** on HMG-CoA reductase were studied in both CHO-K1 cells and in HepG2 cells. The F_7 analog of the 15-ketosterol was highly active in lowering the elevated levels of HMG-CoA reductase activity induced by transfer of the CHO-K1 cells to lipid-deficient media (**Table 7**). The potency of the F_7 analog **VIII** did not differ significantly from that of the 15-ketosterol **I**. The F_7 analog **VIII** was also highly active in suppressing the elevation of the levels of HMG-CoA reductase activity induced by transfer of HepG2 cells to lipid-deficient media (Table 7). The potency of **VIII** was comparable to that of **I**.

Effects of 25,26,26,26,27,27,27-heptafluorocholesterol (XXI) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (I) on the levels of HMG-CoA reductase activity in CHO-K1 cells

The effects of the 15-ketosterol **I** and of the F_7 analog of cholesterol (**XXI**) were studied in CHO-K1 cells. While the 15-ketosterol **I** was highly active in lowering the elevated levels of HMG-CoA reductase activity induced by transfer of the CHO-K1 cells to lipid-deficient media, the F_7-cholesterol (**XXI**) had little or no effect on reductase activity (**Table 8**).

DISCUSSION

A strategy for the chemical synthesis of the F_7-ketosterol **VIII** should take into account the difficulty of efficiently protecting the $\Delta^{8(14)}$-15-keto function and the desirability of constructing the $\Delta^{8(14)}$-15-keto group prior to introduction of the heptafluoro functionality. These considerations together with retrosynthetic analysis and literature prece-

TABLE 7. Effects of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) and 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**) on the levels of HMG-CoA reductase activity in CHO-K1 cells and in HepG2 cells

	HMG-CoA Reductase Activity			
	CHO-K1 Cells[a]		HepG2 Cells[b]	
Concentration	**I** n = 5	**VIII** n = 4	**I** n = 2	**VIII** n = 2
μM	*% of control activity*			
0.0	100.0	100.0	100.0	100.0
0.1	56.2 ± 6.5	68.3 ± 6.1	58.1 ± 3.0	70.2 ± 9.5
0.25	43.5 ± 2.4	49.1 ± 5.4	42.3 ± 5.4	44.9 ± 15.3
0.5	34.8 ± 2.1	35.6 ± 6.2	37.8 ± 0.4	41.3 ± 5.7
1.0	28.6 ± 2.3	29.0 ± 5.0	33.1 ± 3.2	28.9 ± 5.9
2.5	27.4 ± 3.3	22.2 ± 4.2	26.0 ± 10.0	21.4 ± 1.4

[a]Results are expressed as mean ± SEM; n = number of experiments. In each experiment, triplicate assays of HMG-CoA reductase activity were made. No significant ($P < 0.05$) difference existed between the levels of inhibition by **I** and **VIII** at each of the concentrations studied.

[b]Average of two experiments with each value determined in triplicate. Variation is expressed as average deviation from the mean.

dent (43–45) suggested a strategy of coupling a C_{22}, C_{23}, or C_{24} 15-ketosteroid with a small carbon fragment containing a perfluorinated isopropyl moiety. A direct route to a C_{24}-15-ketosterol was provided by our recent finding that **VII** can be prepared in high yield by reaction of the acetate of **I** with a mixture of trifluoroacetic anhydride, hydrogen peroxide, and sulfuric acid, followed by treatment of the crude product with triethylamine and methanol (32). Perfluorinated iodides are known to undergo facile radical-catalyzed addition to olefins (43, 46), a reaction that, as shown by results presented herein, does not require protection of the $\Delta^{8(14)}$-15-keto functionality. To achieve the desired coupling, the 24-hydroxyl of **VII** had to be dehydrated to the olefin **X.** This was accomplished by treatment of **VII** with 2-nitrophenyl selenocyanate (33) and tributylphosphine (47) to give selenide **IX,** which was oxidized with hydrogen peroxide to furnish the Δ^{23} steroid **X** in 73% yield. Olefin **X** underwent facile alkylation with $(CF_3)_2CFI$ in the presence of triethylborane to give iodide **XI,** a reaction that proceeded with remarkable stereospecificity (see below). Reductive deiodination of **XI** with tributyltin hydride (48) and AIBN gave acetate **XII.** Mild hydrolysis of the acetate with potassium carbonate in methanol provided the free sterol **VIII.** This five-step synthesis furnished **VIII** from monoacetate **VII** in 38% overall yield. Sterols **XII** and **VIII** showed the appropriate absorbance at 258–259 nm (ε 14,200 and 13,600 respectively) consistent with retention of the $\Delta^{8(14)}$-15-ketone functionality (26, 29, 32, 37). Further, the IR, MS, and ^1H and ^{13}C NMR data on **VIII** and on each of the intermediates in its synthesis were fully in accord with the assigned structures. Although numerous side-chain fluorinated derivatives of sterols and D vitamins have been prepared (43, 49), **VIII** appears to represent the first 25,26,26,26,27,27,27-heptafluoro steroid derivative.

TABLE 8. Effects of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) and 25,26,26,26,27,27,27-heptafluorocholesterol (**XXI**) on the levels of HMG-CoA reductase activity in CHO-K1 cells

	HMG-CoA Reductase Activity[a]	
Concentration	**I**	**XXI**
μM	*% of control activity*	
0.0	100.0 ± 1.4[b]	100.0 ± 5.0[c]
0.1	60.7 ± 3.3	92.6 ± 1.8
0.25	38.1 ± 1.4	97.2 ± 0.8
0.5	27.7 ± 0.9	89.1 ± 5.1
1.0	26.2 ± 0.8	82.6 ± 2.5
2.5	27.9 ± 4.1	88.1 ± 0.8

[a]Variation expressed as ± SD of replicate (n = 3) assays of HMG-CoA reductase activity.

[b,c]Mean values for controls were 401 and 367 pmol per min per mg protein, respectively.

A useful starting material for preparing F_7-cholesterol (**XXI**) was hyodeoxycholic acid. Its 3α,6α-dihydroxy functionality, protected as the diacetate, served as a masked 3β-hydroxy-Δ^5 moiety, and the 24-carboxylic acid function provided a handle for constructing the F_7 side chain. This strategy has been used previously to prepare side-chain fluorinated analogs of cholesterol from a C_{23} synthon derived from hyodeoxycholic acid (43). In our case, conversion of the 24-carboxylic acid group to the 24-hydroxyl derivative enabled us to use the synthetic methodology developed for preparing the F_7-15-ketosterol **VIII.** Thus, the 3α,6α-diacetate of hyodeoxycholic acid was reduced with borane–methyl sulfide to the 24-hydroxyl derivative **XIV.** In a sequence of reactions

analogous to the preparation of **XII** from **VII,** the 24-hydroxy steroid **XIV** was converted to 25,26,26,26,27,27,27-heptafluoro-3α,6α-diacetoxy-5β-cholestane (**XVIII**). These reactions proceeded in consistently good yield in both series provided that the reagents were fresh and the steroid starting materials of high purity. Hydrolysis of diacetate **XVIII** and tosylation of the resulting diol afforded the 3α,6α-ditosylate **XX.** Using a procedure originally developed for the synthesis of Δ^5-cholenic acid from hyodeoxycholic acid (50, 51) and subsequently applied to the preparation of side-chain derivatized analogs of cholesterol (42, 43), the 3α,6α-ditosylate **XX** was converted to the desired F_7-cholesterol **XXI** in 76% yield. This series of eight reactions (31% overall yield) represents the first chemical synthesis of 25,26,26,26,27,27,27-heptafluorocholest-5-en-3β-ol.

The F_7-15-ketosterol **VIII,** F_7-cholesterol (**XXI**), and all synthetic intermediates were characterized by TLC, IR, ^1H and ^{13}C NMR, MS, and high resolution MS. The F_7 functionality had notable effects on spectral and chromatographic properties. The IR spectra of F_7 sterols **VIII** and **XXI** showed a series of strong bands attributable to C–F stretching vibrations (52) in the region from 940 to 1300 cm^{-1}. Outside this region, the spectra were similar to those of 15-ketosterol **I** and cholesterol, respectively. The ^{13}C NMR spectrum of the F_7 sterols **VIII, XII, XVIII, XIX,** and **XXI** showed the expected C–F coupling patterns for resonances corresponding to C-26/27 (quartet of doublets), C-25 (doublet of septet), C-24 (doublet), and C-23 (doublet); the magnitudes of the C–F coupling constants (Table 1) were as anticipated.[3] In contrast to most sterols having the usual C_8H_{17} side chain, the C-26 and C-27 resonances of these F_7 sterols appear to be isochronous. The ^{19}F NMR spectra of F_7 sterols **VIII** and **XXI** did show resolved signals for F-26 and F-27, which appeared as a deceptively simple pair of quartets at low digital resolution. Spectra reacquired under conditions of high resolution revealed additional lines of low intensity and shoulders on some strong lines, features that were reproduced in a simulated spectrum (Fig. 4). The F-25 resonance was also interpretable only after the spectrum was reacquired at high resolution (Fig. 4). The coupling constants derived from analysis of these signals provided information about the side-chain conformation of the F_7 sterols (see below).

The F_7 moiety significantly deshielded the C-23 and C-24 protons in the ^1H NMR spectrum of the F_7 sterols. This effect eliminated the virtual coupling that, together with signal overlap, usually makes the coupling pattern of protons in the C_8H_{17} side chain of sterols extremely difficult to interpret. The upfield H-22 signal (δ 1.16) in the 500 MHz spectrum of **XII** was entirely isolated from other signals and could be analyzed as a first-order spin system. Of the observed couplings (13.2, 10.9, 8.6, 4.6 Hz), 13.2 Hz was assigned as the geminal coupling, and one anti (10.9 Hz) and one gauche (4.6 Hz) coupling were attributed to coupling to the C-23 protons. The remaining coupling (8.6 Hz) indicated that this proton is anti to H-20 in the predominant conformation. The upfield H-22 signal therefore corresponds to the pro-S proton. Similar reasoning was used to establish stereochemical assignments for the C-22 protons of other steroids. Examination of coupling constants in Table 2 (together with homodecoupling experiments in the case of **XVII**) showed that the downfield C-22 proton of Δ^{23} steroids **X** and **XVI** and of iodide **XVII** is gauche to H-20 (pro-S in these cases).[4] The upfield H-22 signal of bile acids has recently been assigned as the proton anti to H-20 (pro-S) (53).

Once the stereochemical assignments for the C-22 protons were established, homodecoupling experiments could be used to assign the pro-R and pro-S protons at C-23. Thus, irradiation at δ 1.63 (H-20 and the downfield H-23 proton) caused the H-22 pro-S signal of **XII** to collapse from dddd (13.1, 10.9, 8.6, 4.6 Hz) to dd (12.7, 10.4 Hz). Because other decoupling experiments had established $J_{20\text{-}22S}$ as 8.6 Hz, the downfield H-23 proton must be gauche to H-22S and therefore pro-S ($J_{22S\text{-}23R}$ = 4.6 Hz). In an analogous experiment, collapse of the upfield H-24 signals of **VIII** and **XII** (br dddd, 20, ~15, ~11, ~5 Hz) to br ddd (19, ~15, ~9 Hz) upon irradiation of H-23S indicated that the upfield H-24 proton is gauche to H-23S and therefore pro-R. The foregoing assignments, which are based on the reasonable assumption that the sterol side chain is mainly in a fully extended conformation (54), were supported by the results of additional decoupling experiments. Stereochemical assignments of side-chain protons of other F_7 sterols were made by chemical shift comparisons. These findings appear to represent the first stereochemical assignments of methylene protons in a saturated sterol side chain.

The stereochemical assignments for the C-22 protons of iodide **XVII** also provided a basis for determining the stereochemistry at C-23. Analysis of the coupling patterns

[3]The ^{13}C and ^{19}F NMR spectra of the F_7 sterols were compared with spectra observed for $ICH_2CH_2CF(CF_3)_2$ (obtained from Strem Chemicals): ^{13}C NMR (125 MHz, ^1H decoupled) δ 120.53 (d of q, 285, 28 Hz), 91.61 (d of septet, 204, 32 Hz), 34.26 (d, 20 Hz), −9.97 (s); ^{19}F NMR (235.4 MHz) δ −76.60 (br s), −76.62 (br s), −184.46 (t of septet, 19.1, 6.9 Hz). Coupling constants were not readily obtained from the two downfield ^{19}F signals, but simulation with $^3J_{FF}$ = 6.9 Hz and $^4J_{FF}$ ~9 Hz gave a reasonable approximation to the observed peak shapes.

[4]The C-22 protons of sterols with a C_8H_{17} side chain generally resonate at δ ~1.0 and δ ~1.35. Occasionally, the upfield H-22 signal can be analyzed sufficiently to show that this proton is anti to H-20 (W. K. Wilson and G. J. Schroepfer, Jr., unpublished results). In each case examined among sterols, bile acids, and Δ^{23} sterols, the downfield signal has been assigned to the proton nearest C-16.

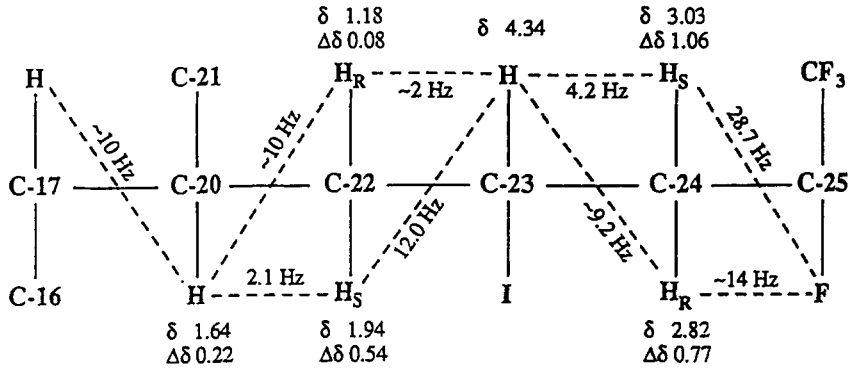

Fig. 5. ^{1}H NMR chemical shifts and coupling constants for iodide **XVII** shown on a projection of the extended conformation. The NMR data was derived from a combination of the ^{1}H spectrum, the COSYDEC spectrum, and homodecoupling experiments. The Δδ values represent the substituent increments for introduction of the 23-iodide and were calculated by subtracting chemical shifts for **XVII** from corresponding chemical shifts of the 23-protio analog **XVIII.** Note that the priority rules for the RS nomenclature reverse the designations for the C-22 and C-24 protons in **XVII** relative to those of **XVIII.**

of the side-chain protons of **XVII** together with decoupling experiments led to the coupling constants given in **Fig. 5.** Because of long-range couplings affecting all the isolated ^{1}H side-chain signals, the values shown are accurate to only ca. ± 0.5 Hz. Fig. 5 also shows the ^{1}H chemical shift increments due to the 23-iodo substituent. ^{1}H substituent increments tabulated for 3α- and 3β-iodo steroids (55) indicate that introduction of iodine leads to downfield shifts of ~0.5–0.8 ppm for vicinal protons gauche to iodine and ~0.2 ppm for anti protons. Thus, the small substituent increment for H-22R (0.08 ppm) indicates that H-22R is anti to iodine. Furthermore, the large $J_{H22S-H23}$ value implies that H-23 is anti to H-22S. These conditions are met only if the C-23 stereochemistry is R and the side chain is predominantly in the extended conformation.[5] The large $J_{H24S-F25}$ value reveals that the terminus of the side chain also adopts a single conformation to avoid steric interactions between iodine and the CF_3 groups. In contrast, sterols with a C_8H_{17} or the $C_8H_{10}F_7$ side chain described here show approximately equal populations of rotamers with C-26 and C-27 anti to C-23, as indicated by the observation that J_{24R-25} and J_{24S-25} are approximately equal (53) and that C-26 and C-27 and their proton or fluorine substituents have similar or identical chemical shifts. (It should be noted that the ^{19}F trifluoromethyl signals are not exactly isochronous even in an achiral model for the F_7 sterols.[3]) The unusually large substituent increment for H-24S of **XVII** (Fig. 5) evidently results from the aforementioned conformational differences between **XVII** and other F_7 sterols.

The similarity of the ^{1}H and ^{13}C NMR chemical shifts in the side chains of iodides **XI** and **XVII** demonstrate that the C-23 configuration of **XI** is also R. Formation of the 23R isomer as the major product is mechanistically reasonable. The free radical addition appears to occur in a stepwise manner (56) in which the final stereochemistry is determined by the conformation of a postulated C_{27} intermediate with a free radical site at C-23. Examination of models suggests that reaction of the radical with $(CF_3)_2CFI$ to form the 23S isomer is partially blocked by the C-20 methyl group. However, some of the 23S isomer appears to be formed, as judged by the NMR signals of a minor component (~15%) present in preparations of **XI** and **XVII.**

In the course of characterizing the intermediates in the preparation of F_7-cholesterol, we have established reliable ^{1}H and ^{13}C NMR assignments for 3α,6α-diacetoxy-5β-steroids. A combination of DEPT and HETCOR data in conjunction with chemical shift comparisons provided facile ^{13}C assignments for quaternary, methine, and methyl carbons as well as for methylene carbons in rings C and D (57). The remaining carbons (C-1, C-2, C-4, C-7) could not be assigned from HETCOR spectra because the chemical shifts of their attached protons were not known. The COSYDEC (58) spectrum of 3α,6α-diacetate **XIII** showed H-6β correlated to signals at δ_H 1.20 and 1.69 in addition to a correlation to the known H-5β signal at δ_H 1.76. The only unassigned ^{13}C resonance in the HETCOR spectrum correlated to protons at these chemical shifts was assigned to C-7 (δ_C 31.10). Correlation of H-3β in the COSYDEC spectrum

[5]Modeling of the 23R-iodide by molecular mechanics indicated that the fully extended side chain conformation is >2 kcal/mol lower in energy than any of the eight conformers with C-24 gauche to C-20 and/or C-25 gauche to C-22. The absence of coupling constants intermediate in magnitude between gauche and anti (e.g., 5–8 Hz for ^{1}H-^{1}H couplings and ~20-Hz for ^{19}F-^{1}H couplings) provided additional evidence that the side chain of iodides **XI** and **XVII** is predominantly in a single conformation. In the case of the 23S-iodide, steric interaction between the iodine and the C-20 methyl raises the energy of the fully extended conformation, and preliminary calculations suggest that the most stable conformer has a bent side chain.

to signals at δ_H 1.41, 1.60, 1.71, and 1.83 established the assignment for C-1 by process of elimination (δ_C 34.86, δ_H 1.11 and 1.81). The 500 MHz ^{1}H spectrum showed a nearly isolated quartet at δ 1.60 with a coupling constant of ~12.2 Hz. Of the C-2 and C-4 protons, only H-4α is expected to have three large couplings, and C-4 was immediately identified from the HETCOR spectrum. Completion of the ^{13}C assignments for **XIII** allowed spectra of the other 3α,6α-diacetates to be assigned by comparisons; the spectra of diol **XIX** and ditosylate **XX** were assigned by similar reasoning. Despite the congestion of the ^{1}H and COSYDEC spectra, numerous coupling patterns and signal correlations could be established to confirm these assignments. Complete ^{1}H NMR assignments have not previously been reported for 5β-steroids functionalized at C-3 and C-6. Our ^{13}C assignments appear to differ from those presented earlier by others for C-2, C-4, and C-7 of 3α,6α-dihydroxy-5β-steroids (59, 60).

The low resolution MS data for the new compounds (Tables 3, 4, and 6) were fully compatible with the assigned structures. In general, the suggested fragment ion assignments for the $\Delta^{8(14)}$-15-ketosteroids (**X, XI, XII, VIII**) are in accord with the observed high-resolution MS data and with assignments made previously for the 15-ketosterol **I** and its side-chain oxygenated derivatives (24–29, 32, 37). In each of the $\Delta^{8(14)}$-15-ketosterols, the molecular ion (M^+) was the base peak. Fragment ions of high abundance (> 40%) corresponding to M–CH_3COOH–CH_3 were observed in the spectra of **X, XI,** and **XII** and for M–H_2O–CH_3 in the MS of the free sterol **VIII.** Noteworthy were the presence of ions of significant abundance corresponding to ion B and ion B–CH_3 (**Fig. 6**) in the MS of **XI, XII,** and **VIII.** These ions are diagnostic for $\Delta^{8(14)}$-15-ketosterols (24–29, 32, 37). Ions of high abundance (>50%) corresponding to M–SC–H_2O were observed in the MS of **XI, XII,** and **VIII.** However, ions of significant abundance corresponding to ion D (loss of carbon atoms C_{22} through C_{27} and its substituents), ion D–H_2O, ion C (M–SC–28), and ion C–H_2O (ions of significant abundance in the MS of the 15-ketosterol **I** and its side-chain oxygenated derivatives (24–29)) were absent in the MS of **XI, XII,** and **VIII.**

The mass spectral fragmentation pattern of F_7-cholesterol (**XXI**) was analogous to that of cholesterol (61). Of the synthetic intermediates in the preparation of **XXI,** the 3α,6α-diol **XIX** showed a strong molecular ion and numerous fragment ions of high abundance. Selenide **XV** gave several unassigned ions that appear to involve fragmentation in $SePhNO_2$ moiety. The mass spectra of the other diacetates and the ditosylate were dominated by ions corresponding to M–2CH_3COOH or M–2TsOH (base peak). No molecular ions were observed, and other ions above *m/z* 250 were generally of rather low intensity ≤20%). Except for the loss of allyl radical (M–C_3H_5) from the Δ^{23} olefin **XVI,** these fragments represented chiefly the loss of methyl, ring A, or ring A together with part of ring B from the base peak fragment ions.

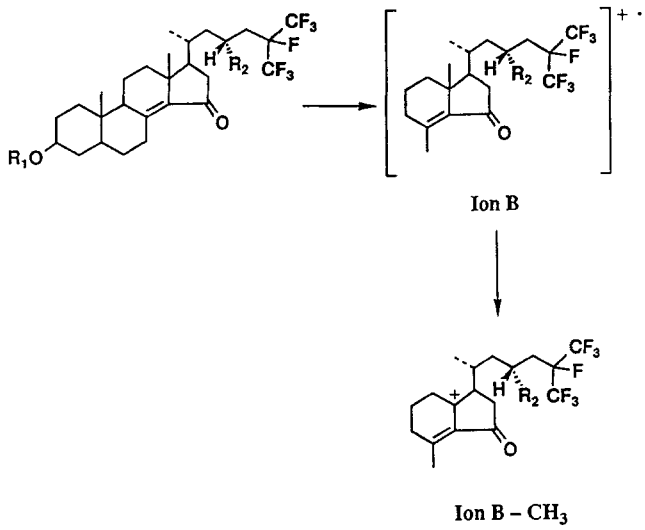

Fig. 6. Suggested mass spectral fragmentation processes for 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**) and its derivatives (R_1 = H or CH_3CO; R_2 = H or I). The designation for ion B corresponds to that used previously (29, 32) for 3β-hydroxy-5α-cholest-8(14)-en-15-one and its side-chain oxygenated derivatives.

The chromatographic properties of the F_7 sterols are worthy of note. F_7-Cholesterol and the F_7-15-ketosterol were very similar to the corresponding compounds lacking the fluorine substitution on TLC on silica gel and on silicic acid-Super Cel column chromatography. However, very striking separations of the F_7-cholesterol (**XXI**) from cholesterol and the F_7-15-ketosterol (**VIII**) from the 15-ketosterol were achieved on reversed phase HPLC and on capillary GC (Table 5).

The 15-ketosterol **I** is one of the most potent oxysterols in lowering HMG-CoA reductase activity in cultured mammalian cells (1, 4). Several metabolites of **I** oxygenated at C-26 or C-25 have also been shown to be highly active in lowering HMG-CoA reductase activity (23, 26–30). This situation raised the possibility that these metabolites of **I** might be responsible for the activity of the 15-ketosterol. However, in CHO-K1 cells, in which **I** shows high potency in lowering the levels of HMG-CoA reductase activity (2, 3, 5), little or no metabolism of **I** to polar metabolites was observed (5). In contrast, major metabolism of **I** to its 26-hydroxy analog has been demonstrated in HepG2 cells (J. S. Pyrek, S. Numazawa, G. T. Emmons, N. Gerst, F. D. Pinkerton, and G. J. Schroepfer, Jr., unpublished data). The results presented herein demonstrate that the lowering of HMG-CoA reductase activity in both CHO-K1 cells and in HepG2 cells by **I** does not require its metabolism to 26-hydroxy or 25-hydroxy analogs. The F_7-15-ketosterol **VIII,** in which oxi-

dation at C-26 or C-25 is precluded by the fluorine substitution, lowered HMG-CoA reductase activity in both CHO-K1 cells and in HepG2 cells, with a potency equivalent to that of the 15-ketosterol **I** (Table 7). This result, coupled with previous findings (5), indicates the inherent activity of the 15-ketosterol in CHO-K1 cells and in HepG2 cells. Nonetheless, in those cells and animals in which the 15-ketosterol undergoes significant metabolism to side-chain hydroxylated sterols, these polar metabolites may be important in the expression of the actions of **I**.

The remote possibility that the F_7 substitution itself results in a major suppressive effect on the levels of HMG-CoA reductase activity was excluded by the demonstration that 25,26,26,26,27,27,27-heptafluorocholesterol has little effect on the levels of HMG-CoA reductase activity in CHO-K1 cells over the range of concentrations studied (0.1 μM to 2.5 μM) (Table 8). For example, the mean levels of HMG-CoA activity (relative to control values) were, at a concentration of 0.25 μM sterol, 62% lower for the F_7-15-ketosterol whereas the F_7-cholesterol showed no effect. At the highest concentration studied (2.5 μM), the reductase levels were 72% lower in the case of the F_7-15-ketosterol and only marginally lower (−12%) with the F_7-cholesterol.

The F_7-cholesterol **XXI** may also be of value in a number of other types of research. The F_7 substitution in the side chain represents a potentially valuable form of labeling that may find use for internal standards in GC–MS studies of enzymatic oxidation and/or autoxidation in the sterol nucleus of cholesterol and other sterols. The F_7 cholesterol also constitutes a potentially valuable starting material for chemical syntheses of other F_7 analogs of oxysterols (such as 7-ketocholesterol, 7α- and 7β-hydroxycholesterol, and the 5,6-epoxides of cholesterol). Such F_7 oxysterol analogs would permit evaluation of blockage of the side chain metabolism of these sterols on the effects of the parent sterols in cultured cells and in animals. For example, the lack of sustained action of 7-ketocholesterol on serum cholesterol levels (62, 63) may be related to its rapid metabolism to polar metabolites (as yet unidentified) which are rapidly excreted in bile (62). The F_7 substitution, by blocking the formation of major polar metabolites, may provide for sufficient levels of the oxysterol in vivo to permit the demonstration of significant hypocholesterolemic action. The F_7-cholesterol may also be of interest with respect to its effects in synthetic and/or natural membrane preparations.

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