Inhibitors of sterol synthesis. Metabolism-based design and construction of a new analog of 3β-hydroxy-5α-cholest-8(14)-en-15-one and its effects in cultured mammalian cells and in rats

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Abstract 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) is a potent regulator of cholesterol metabolism. In the present study, the 7α-methyl-25,26,26,26,27,27,27-heptafluoro analog (**X**) of **I** has been synthesized with the goal of blocking not only the side chain oxidation of **I** but also its conversion to cholesterol. **X** was prepared in seven steps from the known 7α-methyl analog (**IX**) of **I**. Treatment of the acetate of **IX** with a mixture of trifluoroacetic anhydride, hydrogen peroxide, and sulfuric acid gave 3β-acetoxy-7α-methyl-24-hydroxy-5α-chol-8(14)-en-15-one (**XII**) in remarkably high (68%) yield. Dehydration of **XII** via the *ortho*-nitrophenylselenide to the 23-ene, followed by addition of $(CF_3)_2CFI$ gave (23*R*)-3β-acetoxy-7α-methyl-23-iodo-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**XV**). Reductive deiodination of **XV** with tributyltin hydride, followed by hydrolysis of the acetate gave 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**X**). The F_7-7α-methyl-15-ketosterol **X** lowered the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in CHO-K1 cells with a potency equivalent to that of **I**. **X** showed significant hypocholesterolemic action upon oral administration to rats, with a potency far in excess of the 7α-methyl-15-ketosterol **IX** lacking the F_7 substitution. In marked contrast to **I**, **X** showed little or no suppression of food consumption in rats. Upon oral administration of **X** to rats, low levels of **X** (relative to cholesterol), characterized by chromatographic and gas chromatography–mass spectrometric methodologies, were observed in serum, liver, and small intestine. No material was observed with the expected properties of F_7-7-methylcholesterol (or potential intermediates in its possible formation from **X**). In contrast to **I**, **X** lowered serum cholesterol levels at dosages at which no effect on food consumption was observed.—**Swaminathan, S., A. U. Siddiqui, N. Gerst, F. D. Pinkerton, A. Kisic, L. J. Kim, W. K. Wilson, and G. J. Schroepfer, Jr.** Inhibitors of sterol synthesis. Metabolism-based design and construction of a new analog of 3β-hydroxy-5α-cholest-8(14)-en-15-one and its effects in cultured mammalian cells and in rats. *J. Lipid Res.* 1995. **36:** 767–786.

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

The 15-ketosterol **I** is metabolized to cholesterol in in vitro systems (15, 16) and in intact animals (14, 17–22), and a scheme has been presented to account for the overall metabolism of **I** to cholesterol (16) (**Fig. 1**). 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

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

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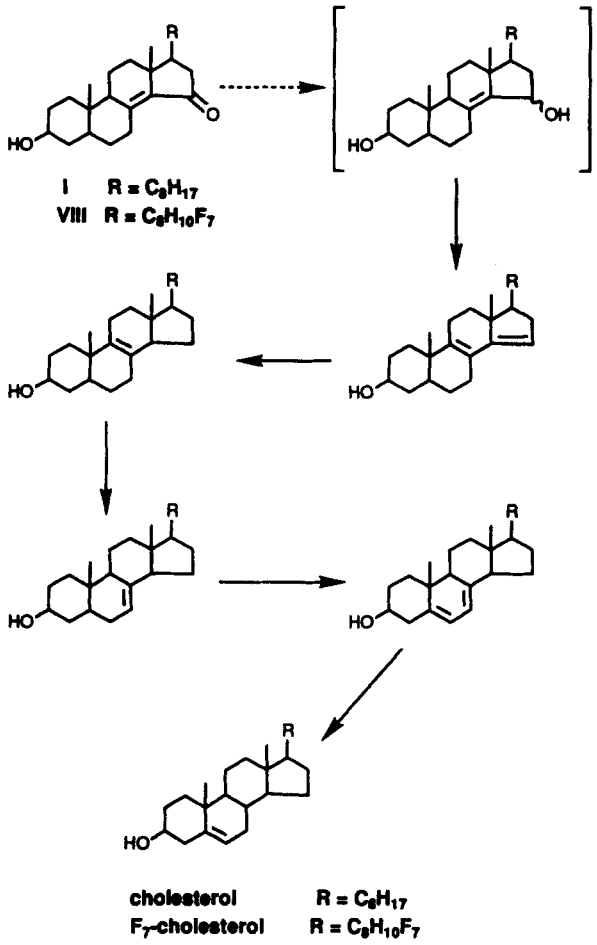

Fig. 1. Scheme for the overall conversion of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) to cholesterol and of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**VIII**) to 25,26,26,26,27,27,27-heptafluorocholesterol (adapted from ref. 16) wherein R = C_8H_{17} or $C_8H_{10}F_7$.

circulation (19). Other 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. 2**). Synthetic 25R-**II** and **IV** were as potent as **I** in lowering the levels of HMG-CoA reductase activity in cultured mammalian cells (23, 26–28), whereas synthetic **III** has 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 metabolism of **I** to polar metabolites by a pathway that 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) have been shown to be highly active and moderately active suppressors, respectively, of HMG-CoA reductase activity in cultured mammalian cells.

The high potency of these polar metabolites of the 15-ketosterol in lowering reductase activity raised the possibility that the effects of **I** in cultured cells and/or in intact animals may be partially or totally due to these metabolites. That **I** itself is capable of lowering HMG-CoA reductase activity is strongly indicated by its effectiveness 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 and also to test the possibility that blocking the side chain oxidation of **I** might provide even more effective hypocholesterolemic action, we prepared the 25,26,26,26,27,27,27-heptafluoro analog of **I** (32). This synthesis was based on our recent finding 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). 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**) (32). Synthetic **VIII**, in which side-chain oxidation at C-26 and C-25 is blocked by the fluorine substitution, was found to be highly active in lowering HMG-CoA reductase activity in CHO-K1 cells and in HepG2 cells, with potencies equivalent to those of the parent 15-ketosterol **I** (32). These results, coupled with the finding that 25,26,26,26,27,27,27-heptafluorocholesterol (containing the same F_7 substitution as in **VIII**) had little or no effect on reductase activity, demonstrated the high inherent activity of the 15-ketosterol and showed that metabolism to 26- or 25-hydroxy analogs was not required for suppression by **I** of HMG-CoA reductase activity in the cultured cells.

Dietary administration of **VIII** to rats indicated several very favorable features of the F_7 substitution (33). First, **VIII** was found to be highly active in lowering serum cholesterol levels, with significant hypocholesterolemic action at dosage levels far below that required for this action with **I**. In addition, and in marked contrast to **I** (6–8, 33), administration of **VIII**, even at high dosage levels, had little or no suppressive effect on food consumption (33). Thus, introduction of the F_7 substitution into **I** resulted in very promising effects upon administration to intact animals. A possibly undesirable feature was the presence of significant quantities of F_7-cholesterol in blood and liver, indicating significant conversion of **VIII** to the F_7 analog of cholesterol.

Stimulated by these findings, we sought the preparation of an analog of the 15-ketosterol in which not only its side chain metabolism was blocked but also its conversion to cholesterol. To this end we prepared the 7α-methyl analog

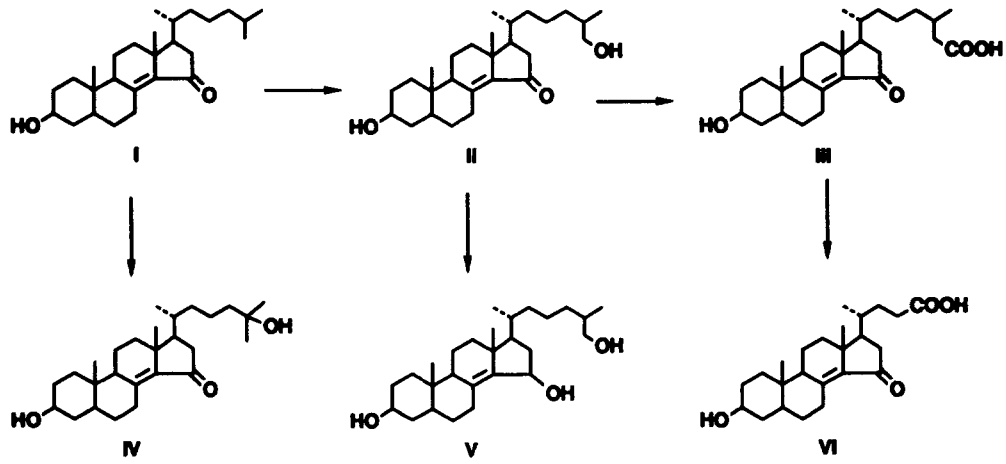

Fig. 2. Conversion of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) to side-chain oxygenated metabolites.

of **I**, 3β-hydroxy-7α-methyl-5α-cholest-8(14)-en-15-one (**IX**) and studied its effects in cultured cells and in intact animals (34). The 7α-methyl 15-ketosterol **IX** showed high potency in lowering HMG-CoA reductase activity in CHO-K1 cells and significant hypocholesterolemic action upon dietary administration to rats (34). These findings, coupled with the observations that administration of **IX** to rats had little or no effect on food consumption and did not lead to the accumulation of 7-methylcholesterol, prompted our construction of 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**X**) (**Fig. 3**) and evaluation of its effects in cultured cells and intact animals.

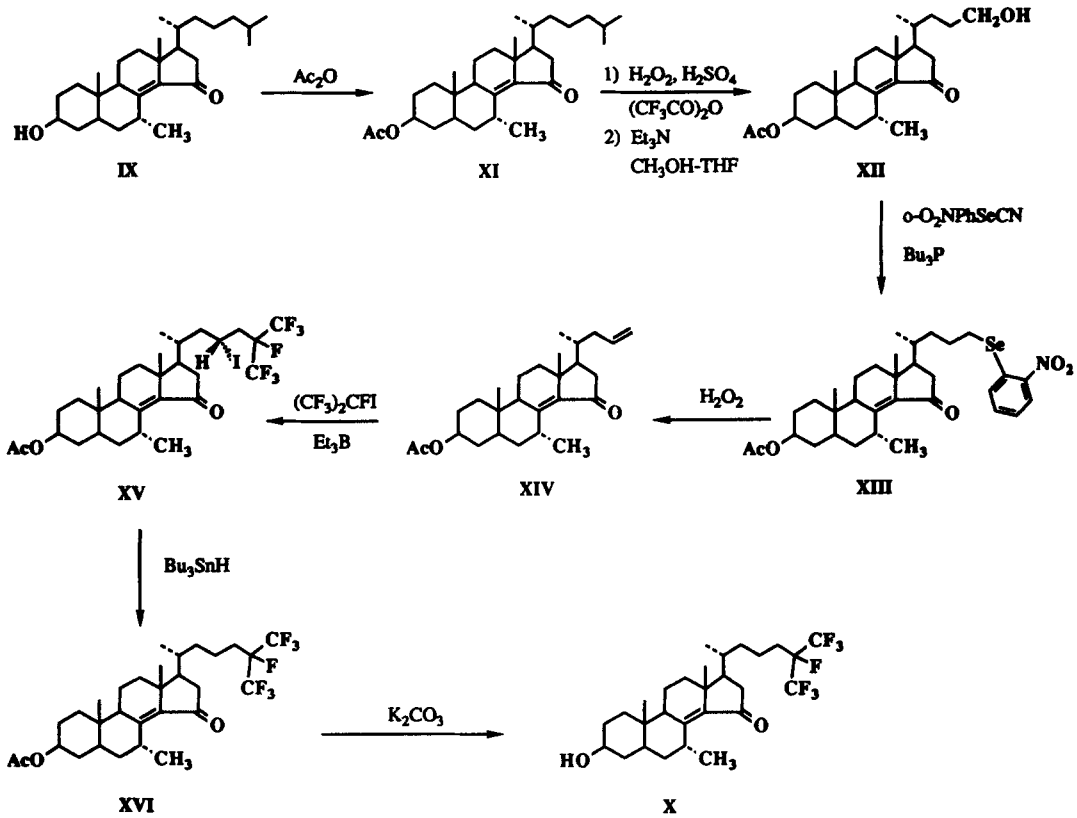

Fig. 3. Chemical synthesis of 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**X**) from 3β-hydroxy-7α-methyl-5α-cholest-8(14)-en-15-one (**IX**).

EXPERIMENTAL PROCEDURES AND RESULTS

Materials and methods

Triethylborane, tributyltin hydride, and tributylphosphine 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). *Ortho*-nitrophenyl selenocyanate was prepared according to Sharpless and Young (35). 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) was prepared as described previously (36). 3β-Hydroxy-7α-methyl-5α-cholest-8(14)-en-15-one (**IX**) was prepared as described previously (34). Cholesterol was purified by way of its dibromide derivative (37). [7(n)-^{3}H]Cholesterol (15 Ci per mmol) and [1,2,6,7(n)-^{3}H]cholesteryl oleate (65.8 Ci per mmol) were obtained from Amersham (Arlington Heights, IL). [2,4-^{3}H]3β-hydroxy-5α-cholest-8(14)-en-15-one (13.5 mCi per mmol) was prepared by a minor modification of a procedure described previously (38).

Melting points, ultraviolet (UV) spectra, and infrared (IR) spectra were measured as described previously (32). 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). Components on the plates were detected after spraying with 5% ammonium molybdate(VI) in 10% sulfuric acid followed by heating. Solvent systems were: SS-1, 15% ethyl acetate in hexane; SS-2, 20% ethyl acetate in hexane; SS-3, 30% ethyl acetate in hexane; SS-4, 40% ethyl acetate in hexane; SS-5, 5% ether in benzene; SS-6, 50% ether in benzene; SS-7, 5% water in methanol; SS-8, 10% water in methanol. Unless otherwise specified, column chromatography was performed on silica gel (70–230 mesh; Aldrich Chemical Co.). Medium pressure liquid chromatography (MPLC) was done on a Lobar column (440 mm × 37 mm i.d., Lichroprep Si 60, 40–63 μm; EM Separations), and fraction volumes were 20 ml. Steroid samples were adsorbed onto silica gel by rotary evaporation from an ethyl acetate solution of the steroid-containing silica gel (~3 g per g of steroid) and eluted from a small column onto the main MPLC column. High performance liquid chromatography (HPLC) was performed isocratically on a Waters HPLC system with a model 481 UV detector and a 5-μm Spherisorb or Customsil C_{18} analytical column (250 mm × 4.6 mm; Custom LC; Houston, TX). Unless stated otherwise, HPLC analyses were done at a flow rate of 1.0 ml/min with UV detection at 259 nm. Solvents for analytical studies were HPLC grade. Colorimetric assay of cholesterol and cholesteryl esters in effluents of silicic acid-Super Cel columns was carried out using the color reagent described by Abell et al. (39).

Capillary gas chromatography (GC) was carried out using splitless injection on a Shimadzu GC-9A unit (1.3 kg per cm^2 nitrogen). The column used was DB-5 (30 m × 0.25 mm; 5% phenyl, 95% methyl polysiloxane; 0.1 μm film thickness; J & W Scientific, Inc.; Folsom, CA). The injector and flame-ionization detector were maintained at 290°C and the column temperature was programmed as follows: 200°C for 3 min; 200°C to 280°C at 20°C per min; and 280°C for 15 min. Trimethylsilyl (TMS) ether derivatives of the sterols were prepared using a 1:1 mixture (200 μl) of bis(trimethylsilyl)trifluoroacetamide (BSTFA) and pyridine for 1 h under nitrogen at room temperature, followed by evaporation at 40°C to a residue that was dissolved in hexane (100 μl) from which aliquots (1 μl) were taken for GC analysis. 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) using a 15 m DB-5 capillary column with direct introduction of the effluent into the ion source of the mass spectrometer (Extrel ELQ-400). 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 in $CDCl_3$ solution on an IBM AF300 (300.1 MHz for ^{1}H, 75.5 MHz for ^{13}C, ~22°C), a Bruker AMX500 (500.1 MHz for ^{1}H, 27°C), or a Bruker AC250 (235.4 MHz for ^{19}F, ~22°C) spectrometer and referenced to internal tetramethylsilane (^{1}H), $CDCl_3$ at 77.0 ppm (^{13}C), or internal $CFCl_3$ (^{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, τ_e 0.2 s) (40), and HETCOR (^{1}H-^{13}C shift-correlated spectroscopy; ~50 increments, δ 0.6–2.6 in the ^{1}H dimension), and nuclear Overhauser effect (NOE) difference spectra (500 MHz, low-power irradiation for 1.0 s, 90° read pulse, 2.7-s acquisition time, 16 scans per cycle, non-degassed sample). Irradiation power in NOE experiments was sufficient to reduce the intensity of the irradiated line by ~80%. Saturation difference spectra were acquired under NOE conditions as done previously (34) and gave isolated resonances with 90° square pulses provided that the irradiated line was > 5 Hz from lines of other resonances. Force field calculations were done with PC Model (Macintosh version 4.4, Serena Software, Bloomington, IN) and used to predict ^{1}H-^{1}H NMR vicinal coupling constants. The purity of sterol samples was determined by HPLC, TLC, and ^{1}H NMR (500 MHz, after sufficient magnification of the ver-

tical scale in the methyl and δ 2–6 spectral regions to detect a 1% impurity).

The effects of **I** and the F_7-7α-methyl-15-ketosterol **X** on HMG-CoA reductase activity were studied in CHO-K1 cells as described previously (32). The effects of **I** and of the 7α-methyl-F_7 analog **X** on the levels of ACAT activity in jejunal microsomes, isolated by a modification of the method of Suckling, Stange, and Dietschy (41), were assayed using minor modifications (26) of the conditions described by Helgerud, Haugen, and Norum (42).

The effects of dietary administration of **X** were studied in male Sprague-Dawley rats, which were purchased from Harlan Sprague-Dawley (Houston, TX) and housed in pairs for 6 days on a light (6:00 AM–6:00 PM)–dark cycle and fed a basal diet (Purina Formulab 5008) and water ad libitum. The animals were then divided into groups of eight animals each, such that the mean values of serum cholesterol and body weight were approximately the same. The animals were then housed individually and, unless indicated otherwise, were provided diet and water ad libitum. The body weight and food consumption of the individual rats were determined daily. Blood for serum sterol concentrations was obtained at ~8:00 AM from the tail vein on days 5 and 9 and "neck blood" was obtained at the time of death on the morning of day 10. The experiments were terminated with ketamine anesthesia (0.2 ml; 100 mg/ml) followed by decapitation. The following organs were removed, cleaned, and weighed: liver, heart, small intestine, kidneys, adrenal glands, testes, and spleen.

Two experiments involving the dietary administration of the F_7-7α-methyl-15-ketosterol **X** were performed. Experiment 1 involved five groups of rats. The first group represented ad libitum control animals with free access to the basal diet (mean body weight on day 0, 164.9 ± 2.9 g). The other four groups received **X** at levels of 0.05%, 0.075%, 0.10%, and 0.125% by weight in diet, respectively (corresponding to 0.93, 1.39, 1.85, and 2.31 μmol per g of diet, respectively). The mean body weights in these four groups on day 0 were 161.9 ± 2.9, 163.8 ± 3.4, 165.8 ± 3.4, and 161.9 ± 4.1 g, respectively. In Experiment 2, **X** was administered at a level of 0.15% by weight in diet (2.78 μmol per g of diet). This experiment involved three groups of rats, an ad libitum control group, a sterol-treated group, and a pair-fed control group that received basal diet but only in the amount consumed by its individual counterpart in the sterol-treated group on the previous day. The mean values of body weight on day 0 in the three groups were 165.9 ± 2.8, 163.5 ± 3.2, and 164.8 ± 3.6 g, respectively.

A third experiment involved the dietary administration of the parent 15-ketosterol **I** at a level of 0.05% in diet (1.25 μmol per g of diet). The mean values of body weight on day 0 in the control and experimental animals were 173.3 ± 2.9 and 174.3 ± 2.4 g, respectively.

Serum cholesterol was measured using a commercial assay kit ("Single Vial"; Boehringer Mannheim Diagnostics; catalog number 236691). Because the levels of **X** in serum constituted a low but significant percentage (~4–6%) of total sterols in serum (vide infra), and as **X** also serves as a substrate for the cholesterol oxidase components of the enzyme kit, the levels of cholesterol in serum during the period of administration of **X** were determined by capillary GC analyses. The same analyses provided quantitative data on the levels of **X** in serum. Routine capillary GC analyses were carried out on a DB-5 column as described above, using stigmasterol as an internal standard. Routine saponification of samples (100 μl) involved mild alkaline hydrolysis (potassium carbonate (200 mg) in methanol (1 ml) for 3 h at 55°C), to avoid base-catalyzed decomposition of the F_7-7α-methyl-15-ketosterol, followed by extraction with hexane (3 × 3 ml). After evaporation to dryness under nitrogen, the samples were silylated as described above and subjected to GC analysis.

Statistical comparisons were made using a paired Student's *t* test. Unless indicated otherwise, variation is expressed as standard error of mean.

3β-Acetoxy-7α-methyl-5α-cholest-8(14)-en-15-one (XI)

A mixture of **IX** (6.25 g), acetic anhydride (10 ml), and pyridine (15 ml) was heated until the sterol dissolved. After standing overnight, the reaction mixture was poured into water (1000 ml), and the resulting precipitate was filtered and washed with 5% HCl (100 ml), 5% sodium bicarbonate (200 ml), and water (1000 ml). Recrystallization from methanol gave **XI** as colorless needles (5.85 g, 81% yield): mp 118.5–119.5°C; TLC, single component in SS-2 (R_f 0.87) and SS-5 (R_f 0.69); HPLC in 9:1 methanol-2-propanol, t_R 7.5 min (99.6% purity); high resolution MS, calcd. for $C_{30}H_{48}O_3$ 456.3603, found 456.3589; UV λ_{max} 260 nm (ε 13400); ^{1}H NMR, ^{1}H-^{1}H NMR coupling constants, ^{13}C NMR, and MS, **Tables 1, 2, 3, and 4.**

3β-Acetoxy-7α-methyl-24-hydroxy-5α-chol-8(14)-en-15-one (XII)

Caution: The following reaction should be conducted in a vented flask behind a safety shield. Although we have conducted without incident over 20 such oxidations of $\Delta^{8(14)}$-15-ketosterols on a scale up to 48 mmol of sterol (estimated 395 mmol of trifluoroperacetic acid at ~0.7 M concentration, prepared using 30% hydrogen peroxide), a small explosion has been reported in an unrelated application of trifluoroperacetic acid as an oxidizing agent at high concentration (5.4 M, prepared using 90% hydrogen peroxide) (43).

To a mechanically stirred mixture of trifluoroacetic anhydride (141 ml) and sulfuric acid (57.8 ml; 96%) maintained at −6°C to −3°C was added a solution of 30% hydrogen peroxide (14 ml) dropwise over a period of 20

TABLE 1. ^{1}H NMR chemical shifts for the F_7-7α-methyl-15-ketosterol **X** and synthetic intermediates[a]

	XI	XII	XIII[b]	XIV	XV	XVI	X
H-1α	1.24	1.25	1.25	1.24	1.25	1.25	1.20
H-1β	1.73	1.73	1.73	1.73	1.73	1.73	1.71
H-2α	1.86	1.86	1.86	1.86	1.86	1.86	1.86
H-2β	1.46	1.46	1.46	1.45	1.46	1.46	1.37
H-3α	4.75	4.75	4.75	4.75	4.75	4.75	3.66
H-4α	1.64	1.63	1.64	1.64	1.64	1.64	1.60†
H-4β	1.30	1.30	1.30	1.30	1.30	1.30	1.23
H-5α	1.70	1.71	1.71	1.71	1.71	1.71	1.65
H-6α	1.19	1.20	1.20	1.20	1.20	1.20	1.20
H-6β	1.55	1.55	1.55	1.55	1.56	1.55	1.57
H-7β	4.38	4.38	4.38	4.38	4.37	4.38	4.38
H-9α	2.13	2.13	2.13	2.13	2.13	2.14	2.12
H-11α	1.64	1.65	1.64	1.64	1.66	1.65	1.65
H-11β	1.52	1.52	1.52	1.52	1.54	1.53	1.54
H-12α	1.22	1.23	1.22	1.22	1.24	1.24	1.23
H-12β	2.09	2.09	2.08	2.08	2.12	2.09	2.09
H-16α	2.35	2.36	2.36	2.39	2.33	2.33	2.33
H-16β	2.06	2.08	2.09	2.08	2.14	2.07	2.07
H-17α	1.42	1.44	1.44	1.44	1.55	1.43	1.43
H-18	0.972	0.980	0.981	0.985	1.033	0.983	0.985
H-19	0.730	0.730	0.730	0.730	0.738	0.733	0.718
H-20	1.57	1.61	1.64	1.68	1.83	1.61	1.61
H-21	0.994	1.022	1.022	1.016	0.982	1.024	1.024
H-22R	1.33	1.47	1.58	1.87	1.30	1.42	1.42
H-22S	1.06	1.15	1.30	2.18	1.89	1.15	1.15
H-23R	1.33	1.48	1.70	5.76	4.34	1.47	1.47
H-23S	1.19	1.64	1.85			1.65	1.65
H-24R	1.10*	3.61*	2.87	5.02	2.83	1.99	1.99
H-24S	1.15*	3.63*	2.91	5.02	3.04	2.05	2.05
H-25	1.52						
H-26	0.865						
H-27	0.868						
7α-Me	1.004	1.002	1.002	0.996	0.996	1.002	1.014
Acetate	2.029	2.028	2.028	2.026	2.029	2.029	
Side chain	C_8H_{17}	24-OH	24-SeAr	Δ^{23}	$C_8H_{10}F_7I$	$C_8H_{10}F_7$	$C_8H_{10}F_7$

[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, are generally accurate to 0.01 ppm except for values marked by † (± 0.02 ppm). Stereochemical assignments marked by * are uncertain.

[b] Aromatic signals of selenide **XIII:** δ 7.31 (ddd, 8.3, 6.3, 2.1 Hz, 1H), 7.51 (m, 2H), 8.29 (dd, 8.2, 1.3 Hz, 1H).

min. Acetate **XI** (7.70 g, 16.9 mmol) was, with continued vigorous stirring, added in one portion and the temperature of the reaction mixture was maintained at about −2°C. Within 1 h the mixture turned to a thick slurry. With continued vigorous stirring, the slurry changed to a clear, light yellow-colored, homogeneous, mobile solution after ~3.5 h. TLC (SS-3) of an ethyl acetate extract of an aliquot of the reaction mixture indicated completion of the reaction as judged by consumption of almost all of the starting material (R_f 0.68) and the presence of a major component (R_f 0.12) and minor components with R_f values of 0.49 (3β,24-diacetate) and 0.63 (3β-acetate-24-trifluoroacetate). The reaction was poured into ice water (1200 ml) and extracted with ethyl acetate (3 × 200 ml). The organic extracts were washed with aqueous sodium sulfite (200 ml), 5% KOH solution (until the washings were pH 9), and 2% HCl solution (until the washings were pH ~5), followed by drying over sodium sulfate and evaporation to a residue (9.9 g). To a solution of the residue in tetrahydrofuran–methanol 1:4 (120 ml) was added triethylamine (2 ml), and the reaction mixture was stirred at room temperature for 2 h, after which time TLC showed complete hydrolysis. The mixture was evaporated to a residue that was purified by column chromatography on silica gel (30 × 3.6 cm column, elution with 15% and 30% ethyl acetate in hexane) to give monoacetate **XII** (~5.1 g, ~68% yield). An analytical sample was prepared by MPLC (50 × 1 cm column; elution with 15% ethyl acetate in hexane). Evaporation of fractions 76–124 gave **XII:** mp 199.5–201.5°C; TLC, single component in SS-4 (R_f 0.34) and SS-6 (R_f 0.31); HPLC in SS-8, t_R 7.7 min (99% purity); IR, ν_{max} 3450, 2980–2820, 1732, 1694, 1613, 1245, 1045, 1032 cm^{-1}; high resolution MS, calcd. for $C_{27}H_{42}O_4$ 430.3083, found 430.3082; ^{1}H NMR, ^{1}H-^{1}H NMR coupling constants, ^{13}C NMR, and MS, Tables 1, 2, 3, and 4.

TABLE 2. ^{1}H-^{1}H NMR coupling constants for F_7-7α-methyl-15-ketosterol **X** and synthetic intermediates[a]

	XI[b]	XII	XIII[b]	XIV	XV	XVI	X
1α-1β	13.1	13.2	13.2	13.3	13.2	13.2	13.1
1α-2α	3.8	3.8†	3.6	3.6		3.7	3.6
1α-2β	14.0†	13.8†	13.9	13.7	13.6†	13.7	13.8
1β-2α	3.3†	3.4†	3.5†	3.6†	3.2†	3.4†	3.3
1β-2β	3.8	3.6†	3.5†	3.6†	3.8	3.7	3.7
2α-2β	12.7	12.8	12.6		12.8†	12.8	12.8
2α-3α	4.9	4.7	4.8	4.8	4.7	4.8	4.7
2β-3α	11.4†	11.7	11.7	11.4†	11.7†	11.5	11.2
3α-4α	4.8†	4.8	4.8	4.8	5.0	4.8	4.7
3α-4β	11.4†	11.2	11.2	11.4†	11.1†	11.3	11.1
4α-4β	12.5†	12.2†	12.2†	12.2†	12.4	12.3†	12.2†
4α-5α	3.1†	3.3	3.3†	3.4	3.1	3.3	3.3†
4β-5α	12.5†	12.8	12.8†	12.9†	12.4†	12.8†	12.6†
5α-6α	3.4	3.3	3.3	3.4	3.4	3.4	3.0†
5α-6β	13.0	12.9	13.2†	13.1	13.0†	12.9	13.1†
6α-6β	13.7	13.7	13.6	13.7	13.7	13.7	13.3
6α-7β	1.5	1.4	1.4	1.5	1.5	1.6	1.5
6β-7β	6.0	6.0	5.9	6.0	6.0	6.0	5.9
7β-CH_3	7.3	7.3	7.2	7†	7.3†	7.3†	7.3
9α-11α	7.0	7.2	7.0	7.2	6.9	7.1	7.1
9α-11β	10.4	10.5	10.4	10.5	10.5	10.4	10.5
11α-11β	13.9	13.8	14.0†	13.9†	13.8	14.0	13.9
11α-12α	3.7†	3.9†	3.5†	3.7	3.5†	3.5†	3.9
11α-12β	3.8†	3.6†	3.4†	3.4	3.6†	3.5†	3.3
11β-12α	14.0	14.1†	14.0†	13.9†	14.1	14.0	14.0
11β-12β	3.5	3.6	3.6	3.6	3.6†	3.5†	3.6
12α-12β	12.6	12.8†	12.6	12.6	12.6	12.6	12.7
16α-16β	18.4	18.3	18.3	18.3	18.2	18.2	18.2
16α-17α	7.8	7.7	7.8	7.8	7.8	7.7	7.8
16β-17α	12.6	12.6	12.5	12.5	12.5	12.5	12.6
17α-20	9.7	9.7	9.6	10.1	9.2†	9.9	9.7
20-21	6.6	6.6	6.6	6.3†	6.8†	6.7	6.7
20-22R		2.9	3.1	8.0†	10.4†	3.0	3.0
20-22S		8.6	8.5	3.4	2.4†	8.7	8.7
22-22		13.2†	13.4	13.9	14.6	13.3	13.4
22R-23R		6.5††	5.2	8.3	2.0†	11.0	5.7††
22R-23S		10.5††	11.0			5.3	11.4†
22S-23R		11.5†	10.9	6.0	12.5†	10.8	11.0
22S-23S		5††	4.6			4.6	4.8
23-23		13††	13.5				12.0††
23R-24R		6.6†	8.8	10†† [c]	9.2†	10.5††	11.7††
23R-24S		6.6†	6.2	17†† [d]	4.0†		5.7††
23S-24R		6.6†	6.4			5.0†	5.0
23S-24S		6.6†	8.9				
24-24		10.3	11.4		15.9†	13.0††	
Side chain	C_8H_{17}	24-OH	24-SeAr	Δ^{23}	$C_8H_{10}F_7I$	$C_8H_{10}F_7$	$C_8H_{10}F_7$

[a] Data obtained at 500.1 MHz in 0.02-0.1 M $CDCl_3$ solution (27°C). Accuracy is ca. ± 0.2 Hz except for couplings marked by † (± 0.5 Hz) or by †† (± 1 Hz). Most compounds showed $J_{2\alpha-4\alpha}$ of ~2.1 Hz.

[b] Additional couplings for **XI:** J_{24R-25} 6.5† Hz, J_{24S-25} 6.5† Hz, J_{25-26} 6.8 Hz, J_{25-27} 6.7 Hz.

[c] *Cis* coupling.

[d] *Trans* coupling.

3β-Acetoxy-7α-methyl-5α-chola-8(14),23-dien-15-one (XIV)

To a mixture of monoacetate **XII** (5.426 g, 12.6 mmol) and *ortho*-nitrophenyl selenocyanate (3.72 g; 16.4 mmol) was added dry tetrahydrofuran (45 ml) under nitrogen. Tributylphosphine (4.0 ml; 16.1 mmol) was added dropwise to the reddish-colored solution over ~2 min, and the blackish-yellow mixture was stirred at room temperature for 2 h. After evaporation of the solvent, the residue was adsorbed onto silica gel (16 g) and chromatographed on a silica gel column (20 × 2.8 cm) using methylene chloride–ethyl acetate–hexane 10:5:85 (500 ml) as the eluting solvent. The eluate was evaporated to dryness to give the crude nitrophenyl selenide (**XIII;** 6.8 g, 88% yield). An analytical sample was obtained by recrystallization from ethyl acetate–hexane: mp, 158.5–159.5°C; TLC, single component in SS-2 (R_f 0.38) and SS-5 (R_f 0.44); HPLC in methanol, t_R 6.0 min (99% purity); IR, ν_{max} 2980–2820, 1728, 1696, 1607, 1510, 1329, 1304, 1242,

TABLE 3. ^{13}C NMR chemical shifts for the F_7-7α-methyl-15-ketosterol **X** and synthetic intermediates[a]

	XI	XII	XIII[b]	XIV	XV	XVI	X
C-1	36.23	36.19	36.21	36.20	36.24	36.24	36.48
C-2	27.24	27.20	27.20	27.21	27.24	27.24	31.16
C-3	73.18	73.17	73.09	73.12	73.13	73.13	70.79
C-4	33.60	33.56	33.56	33.57	33.59	33.60	37.74
C-5	37.84	37.81	37.79	37.81	37.84	37.85	37.97
C-6	34.70	34.66	34.65	34.66	34.66	34.70	34.86
C-7	27.52	27.53	27.51	27.50	27.58	27.58	27.64
C-8	155.97	156.29	156.30	156.13	156.57	156.49	157.00
C-9	45.75	45.73	45.70	45.70	45.78	45.76	45.90
C-10	38.58	38.57	38.56	38.57	38.62	38.62	38.70
C-11	19.26	19.22	19.19	19.22	19.22	19.24	19.30
C-12	36.86	36.82	36.80	36.75	36.83	36.86	36.90
C-13	42.44	42.40	42.39	42.39	42.66	42.39	42.45
C-14	139.90	139.74	139.58	139.68	139.48	139.55	139.43
C-15	207.65	207.48	206.92	207.21	206.38	206.81	206.96
C-16	42.60	42.46	42.43	42.48	41.95	42.44	42.45
C-17	50.51	50.40	50.30	50.00	50.48	50.42	50.39
C-18	19.00	18.99	19.00	19.04	19.16	19.01	19.03
C-19	12.32	12.29	12.29	12.30	12.34	12.31	12.43
C-20	34.42	34.21	34.24	34.44	36.24	34.28	34.29
C-21	19.20	19.13	19.10	19.13	17.92	18.92	18.92
C-22	35.77	31.49	36.17	40.20	46.62	35.57	35.58
C-23	23.57	29.03	24.83	136.11	21.98	18.05[c]	18.04[c]
C-24	39.31	63.07	26.40	116.46	40.74[d]	29.21[e]	29.22[e]
C-25	27.91				[f]	91.69[g]	91.69[g]
C-26	22.49				[f]	121.04[h]	121.04[h]
C-27	22.72				[f]	121.04[h]	121.04[h]
7α-Me	21.46	21.46	21.45	21.44	21.51	21.45	21.50
Acetate	21.35	21.33	21.33	21.33	21.35	21.31	
	170.61	170.66	170.53	170.54	170.61	170.57	
Side chain	C_8H_{17}	24-OH	24-SeAr	Δ^{23}	$C_8H_{10}F_7I$	$C_8H_{10}F_7$	$C_8H_{10}F_7$

[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.
[b]Aromatic signals of selenide **XIII:** δ 125.24, 126.36, 128.92, 133.40, 133.60, 146.78.
[c]d, J_{CF} ~5 Hz.
[d]d, J_{CF} 17.2 Hz.
[e]d, J_{CF} 20.6 Hz.
[f]Signals too weak to be measured accurately.
[g]d of septet, J_{CF} 202.4 ± 0.1 Hz and 31.7 ± 0.1 Hz.
[h]q of d, J_{CF} 287.3 ± 1 Hz and 28 ± 1 Hz.

1036, 729 cm⁻¹; high resolution MS, calcd. for $C_{33}H_{45}NO_5{}^{80}Se$ 615.2463, found 615.2439; UV λ_{max} 229 nm (ε 18500), 257 (29800), 390 (4000); ^1H NMR, ^1H-^1H NMR coupling constants, and ^{13}C NMR, Tables 1, 2, and 3.

To the selenide (6.8 g) in tetrahydrofuran (70 ml) was added 30% hydrogen peroxide (5.5 ml) dropwise. The mixture was stirred at room temperature for 6 h, tetrahydrofuran was evaporated, and the residue was poured into water (600 ml). The resulting mixture was extracted with ethyl acetate (3 × 100 ml) and washed with aqueous $NaHCO_3$ (100 ml). The residue (5 g) obtained upon evaporation of the solvent was passed through silica gel to give a yellow residue (3.9 g) that was further purified by MPLC (50 × 2.5 cm column containing 120 g silica gel, elution with 3% ethyl acetate in hexane). Evaporation of fractions 75–114 and recrystallization from methanol gave olefin **XIV** (2.753 g, 60% yield): mp, 155.5–156.5°C; TLC, SS-1 (R_f 0.71) and SS-6 (R_f 0.59); HPLC in *methanol, t_R 5.2 min (97% purity);* IR, ν_{max} 3073, 2980–2820, 1730, 1696, 1613, 1244, 1115, 1032, 912 cm⁻¹; high resolution MS, calcd. for $C_{27}H_{40}O_3$ 412.2977, found 412.2985; UV λ_{max} 261 nm (ε 13900); ^1H NMR, ^1H-^1H NMR coupling constants, ^{13}C NMR, and MS, Tables 1, 2, 3, and 4.

Compound **XIV** was also conveniently prepared from **XII** without isolation of the selenide intermediate. To a solution of the 24-hydroxy-C_{24} steryl acetate **XII** (10.7 g; 24.8 mmol) and *ortho*-nitrophenyl selenocyanate (7.34 g; 32.3 mmol) in dry tetrahydrofuran (100 ml) was added tributylphosphine (8.2 ml; 32.9 mmol), and the reaction was stirred for 1.5 h at room temperature. After the reaction was complete as judged by TLC, the mixture was cooled to 10°C and hydrogen peroxide (11 ml; ~50% solution) was added. The reaction was stirred at room temperature for 3 h and poured into water (800 ml). The mixture was extracted with ether (3 × 15 ml), and the organic

TABLE 4. Mass spectral data for 3β-hydroxy-7α-methyl-5α-cholest-8(14)-en-15-one (**IX**), 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**X**), and synthetic intermediates[a]

Suggested Assignment[b]	IX[c]	XI	XII	XIII	XIV	XVI	X
M^+	414 (51)	456* (45)	430* (56)	615* (15)	412* (100)	582* (66)	540* (31)
$M-CH_3$	399 (15)	441* (10)	415* (11)	600 (1)	397* (16)	567 (6)	525 (3)
$M-H_2O$	396 (61)	438* (63)	412* (56)	597* (2)	394* (47)	564* (72)	522 (35)
$M-H_2O-CH_3$	381 (32)	423* (9)	397* (16)	582* (4)	379* (95)	549 (14)	507 (16)
$M-ROH-H_2O-CH_3$	363 (5)	363* (9)	337* (10)	564 (1)	319* (50)	489* (24)	489* (7)
$M-CH_3COOH$		396* (2)	370 (1)		352* (10)	522* (3)	
$M-CH_3COOH-CH_3$		381* (17)	355* (18)	540 (4)	337* (30)	507 (21)	
$M-CH_3COOH-H_2O$		378* (4)	352* (7)	537 (2)	334* (13)	504 (15)	
Ion $D-H_2O$	311 (6)	353* (8)	353* (8)	353* (6)	353* (7)		
M-SC	301 (15)	343* (17)	343* (13)	343* (8)	343* (13)	343* (11)	301* (10)
$M-SC-H_2O$	283 (100)	325* (100)	325* (100)	325* (100)	325* (42)	325* (58)	283* (100)
$M-SC-H_2O-14$	269 (6)	311* (14)	311* (6)	311* (7)	311* (11)	311* (5)	269 (5)
$M-SC-CH_3COOH$		283* (32)	283* (13)	283* (9)	283* (19)	283* (14)	
$M-SC-ROH-H_2O$	265 (17)	265* (85)	265* (47)	265* (62)	265* (59)	265* (85)	265* (26)
Ion C	273 (7)	315* (7)	315* (2)	315* (5)	315* (5)	315* (2)	273* (4)
Ion A	[301]	301* (5)	275* (2)		257* (4)	427* (5)	427* (2)
Ion $B-CH_3$	275 (5)	275* (5)	249* (3)	434 (1)	231* (7)	401* (5)	401* (6)
m/z 107	107 (30)	107 (44)	107 (34)	107 (23)	107 (49)	107 (100)	107 (28)
m/z 105	105 (28)	105 (40)	105 (31)	105 (20)	105 (60)	105 (91)	105 (33)
Other ions		287* (6)[d] 273* (11)[f]		409 (7) 395 (7)[e] 339 (6) 335 (5) 272 (8)[e]	295 (5)[e] 293 (5)[e] 289 (6)[e] 269 (7)[e] 255 (9)[e] 251 (8)[e]	441 (5)[e] 425 (5)[e] 413* (9)[d] 399* (19)[f] 371 (5) 255 (6)[e] 251 (5)[e]	413* (5)[d] 399* (10)[f] 329 (3)
R =	H	Ac	Ac	Ac	Ac	Ac	H
Side chain	C_8H_{17}	C_8H_{17}	24-OH	24-SeAr	Δ^{23}	$C_8H_{10}F_7$	$C_8H_{10}F_7$

[a] Major ions above *m/z* 100; 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 marked by an asterisk.

[b] SC, side chain; ion A, M-ring A-H; ion B, M-ring $A-CH_2+2H$; ion C, $M-SC-C_2H_4$; ion D, loss of part of side chain by cleavage of C20-C22 bond. Precise definitions of these ions are given in ref. 24.

[c] Data from ref. 34.

[d] Compatible with assignment as M-ring $A-H-CH_2$.

[e] High resolution MS results were compatible with the following formulas: **XIII**, *m/z* 395, $C_{27}H_{39}O_2$; *m/z* 272, $C_{11}H_{14}NO_2{}^{80}Se$ (side chain ion); **XIV**, *m/z* 295, $C_{21}H_{27}O$; *m/z* 293, $C_{22}H_{29}$; *m/z* 289, $C_{19}H_{29}O_2$; *m/z* 269, $C_{19}H_{25}O$; *m/z* 255, $C_{19}H_{27}$ and $C_{18}H_{23}O$ (two ions); *m/z* 251, $C_{19}H_{23}$; **XVI**, *m/z* 441, $C_{22}H_{28}OF_7$; *m/z* 425, $C_{21}H_{24}OF_7$; *m/z* 255, $C_{18}H_{23}O$; *m/z* 251, $C_{19}H_{23}$.

[f] Compatible with assignment as M-ring $A-H-C_2H_4$.

layer was dried over sodium sulfate and concentrated to a yellow residue (~20 g). The crude product was dissolved in CH_2Cl_2 (20 ml) and subjected to column chromatography on silica gel (80 g). Elution with 3% ethyl acetate in hexane gave a yellow solid (8.7 g) which was precipitated from hot methanol (200 ml) and water (200 ml) to give a pale cream-colored solid, which was again precipitated from methanol-water to give **XIV** (8.05 g; 79% yield), melting at 155-156°C.

(23*R*)-3β-Acetoxy-7α-methyl-23-iodo-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (XV)

To a slurry of olefin **XIV** (2.753 g; 6.67 mmol) in hexane (200 ml) were added successively triethylborane (2.3 ml; 2.3 mmol; 1 M solution in hexane) and 2-iodoheptafluoropropane (2 ml; 14.1 mmol). The reactants dissolved, and the solution was stirred in the dark at room temperature for 6 h. Evaporation of volatile material gave **XV** (4.107 g): single major component on TLC in SS-1 (R_f 0.67) and SS-5 (R_f 0.61); high resolution MS, calcd. for $C_{30}H_{40}O_3F_7I$ 708.1910, found 708.1888; UV λ_{max} 260 nm (ε 15300); ^{19}F NMR (6:1:1 mixture), major component, δ −76.22 (quintet; ~8.8 Hz; F-26 or F-27), −78.06 (qd; 9.3, 6.9 Hz; F-26 or F-27), −184.64 (m compatible with analysis as ddqq; ~28, ~15, ~8, 6.9 Hz; F-25), signals of minor components, δ −76.77, −76.97 and δ −77.28, −77.39 (A_3B_3 portions of two A_3B_3X systems each appearing as a pair of quartets with J ~8 Hz, F-26 and F-27), δ −184.29, −184.51 (multiplets having the general appearance of F-25 resonance of major component, F-25); ^1H NMR, $^1H-^1H$ NMR coupling constants, and ^{13}C NMR of major component, Tables 1, 2, and 3.

3β-Acetoxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (XVI)

Tributyltin hydride (2.04 ml, 7.58 mmol) was added under argon to a solution of the iodide **XV** (3.993 g; 5.64 mmol) and AIBN (0.31 g) in tetrahydrofuran (35 ml). The reaction was stirred at room temperature for 6 h and stored overnight at −15°C. Water was added and the resulting mixture was extracted with ether (2 × 10 ml). The combined ether extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness.

The residue (7.1 g) was dissolved in hexane (80 ml) and subjected to column chromatography (13 × 2.0 cm column). After removal of tributyltin hydride by elution with hexane (600 ml), the sterol was eluted with 5% ethyl acetate in hexane (300 ml). Evaporation gave a residue (3.59 g), of which 3.49 g was adsorbed onto silica gel (14 g) and subjected to MPLC on $AgNO_3$-silica gel (44). The column (100 × 2.5 cm, 120 g of 10% $AgNO_3$-silica gel) was eluted with 2% ethyl acetate in hexane (600 ml), followed by 3% ethyl acetate in hexane. Evaporation of fractions 122–395 gave **XVI** (2.99 g, 91% yield): mp 128–129°C; TLC, single component in SS-2 (R_f 0.58) and SS-5 (R_f 0.75); HPLC in SS-7, t_R 9.0 min, (97% purity); IR, ν_{max} 2980–2820, 1732, 1697, 1613, 1314, 1279, 1242, 1157, 1132, 1032 cm^{-1}; high resolution MS, calcd. for $C_{30}H_{41}O_3F_7$ 582.2944, found 582.2928; UV λ_{max} 260 nm (ϵ 14000); ^1H NMR, ^1H-^1H NMR coupling constants, ^{13}C NMR, and MS, Tables 1, 2, 3, and 4.

3β-Hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (X)

A solution of the acetate **XVI** (2.9 g) in degassed methanol (50 ml) and degassed tetrahydrofuran (25 ml) was stirred with potassium carbonate (1.376 g) for 3 h at room temperature. Ethyl acetate (250 ml) and water (100 ml) were added, and the organic layer was washed with water (3 × 250 ml), dried over anhydrous sodium sulfate, and evaporated to dryness. The resulting residue (2.65 g, ~99% purity by HPLC and TLC) was dissolved in CH_2Cl_2 (10 ml) and subjected to chromatography on a silica gel column (45 g, 70–230 mesh, 11 × 3.5 cm). The column was successively eluted with 5% ethyl acetate in hexane (500 ml), 7% ethyl acetate in hexane (1000 ml), 10% ethyl acetate in hexane (250 ml), and 12% ethyl acetate in hexane. Evaporation of fractions 10–40 gave acetate **XVI** (86 mg), and fractions 128–197 gave the free sterol **X** (2.51 g, 93% yield): mp, 153.5–154.5°C; TLC, single component in SS-4 (R_f 0.37) and SS-6 (R_f 0.44); HPLC in SS-8, t_R 10.1 min (100% purity); IR, ν_{max} 3400, 2980–2820, 1694, 1674, 1595, 1314, 1278, 1219, 1159,

TABLE 5. Mass spectral data of the TMS derivative of authentic 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**X**) and **X** found in serum and in the free and esterified forms in rat liver and small intestine after its administration to rats at a level of 0.15% in diet for 10 days[a]

		Relative Abundance in Mass Spectra of TMS Derivative of **X**					
		Synthetic	Liver		Small Intestine		Serum
Ion (*m/z*)	Suggested Assignment[b]		Free	Ester	Free	Ester	
612	M^+	38	17	43	33	45	65
597	M-CH_3	7	3	6	5	6	9
594	M-H_2O	15	9	13	12	16	25
579	M-CH_3-H_2O	3	2	3	2	3	3
522	M-TMSOH	3	3	3	2	2	4
507	M-TMSOH-CH_3	12	7	10	9	12	15
504	M-TMSOH-H_2O	11	7	9	11	15	14
489	M-TMSOH-CH_3-H_2O	18	12	19	13	18	32
427	Ion A	4	3	4	3	4	5
416	Ion B	4	3	3	4	4	5
413	Ion A-14	6	3	4	4	5	6
401	Ion B-CH_3	6	3	6	4	5	6
399	Ion A-28	11	7	9	8	9	10
373	M-SC	8	4	8	6	7	8
355	M-SC-H_2O	28	20	39	37	38	42
283	M-SC-TMSOH	9	8	9	9	9	10
265	M-SC-TMSOH-H_2O	100	100	100	100	100	100
107		27	23	38	32	27	42
105		25	26	39	29	24	38

[a] Major ions above *m/z* 100; mass spectra acquired at 70 eV by GC-MS. Relative intensities as % of base peak.
[b] SC, side chain; ion A, M-ring A-H; ion B, M-ring A-CH_2 + 2H. Precise definitions of these ions are given in ref. 24.

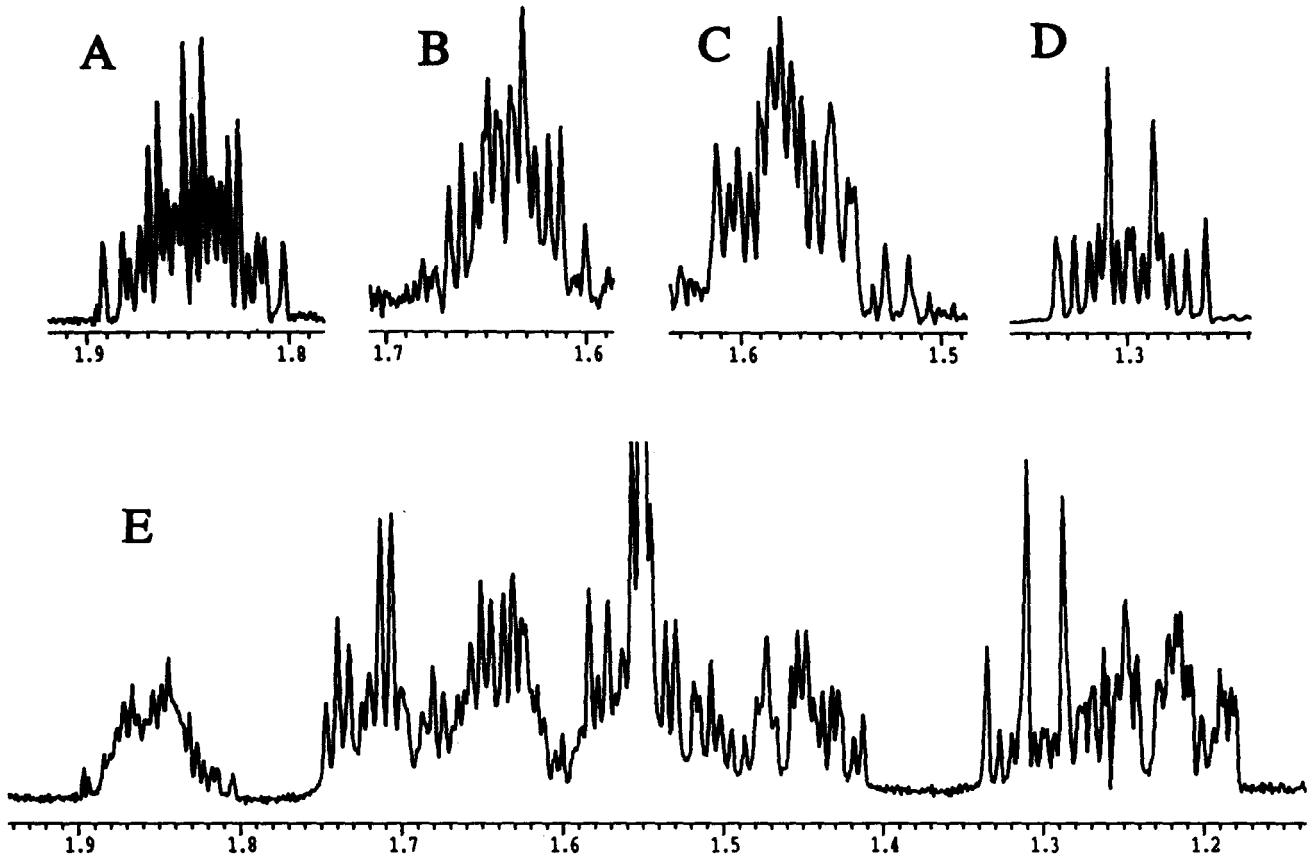

Fig. 4. Isolated ^{1}H NMR multiplets for side-chain protons of selenide **XIII:** panel A, H-23S; panel B, H-20; panel C, H-22R; panel D, H-22S and H-4β. Spectra were obtained by saturation difference spectroscopy except for panel B, which is from an NOE difference spectrum (irradiation of H-18). A portion of the usual ^{1}H NMR spectrum of **XIII** is shown in panel E. All spectra are resolution enhanced.

1130, 1055 cm^{-1}; high resolution MS, calcd. for $C_{28}H_{39}O_2F_7$ 540.2838, found 540.2830; UV λ_{max} 261 nm (ϵ 13800); ^{19}F NMR, δ −76.76 and −76.96 (A_3B_3 portion of A_3B_3X system; $^3J_{FF}$ ~7 H, $^4J_{FF}$ ~9 Hz; F-26 and F-27), −184.30 (dd of septet; $^3J_{HF}$ 21 and 20 Hz, $^3J_{FF}$ ~ 6.7 Hz; F-25); ^{1}H NMR, ^{1}H-^{1}H NMR coupling constants, ^{13}C NMR, and MS, Tables 1, 2, 3, and 4. GC-MS analysis of the 3β-trimethylsilyl derivative showed a single component. The MS data for the TMS ether of **X** are presented in **Table 5.**

Assignment of ^{1}H, ^{13}C, and ^{19}F NMR signals

^{19}F spectra were interpreted as described previously for other F_7 sterols (32). The orientation (45) of signals in HETCOR spectra of the F_7 sterols showed that $J_{C24\text{-}F}$ and $J_{H24\text{-}F}$ (pro-R and pro-S) have the same sign. The ^{1}H and ^{13}C NMR assignments (Tables 1 and 3) were established by standard procedures (46) from a combination of HETCOR, COSYDEC, DEPT, and other 1D spectra in conjunction with chemical shift comparisons (31, 32, 34). Precise ^{1}H NMR chemical shifts were obtained from COSYDEC spectra as described previously (34). ^{1}H-^{1}H coupling constants (Table 2) were calculated by averaging all appropriate line spacings (first-order analysis) of resolution-enhanced multiplets in saturation difference spectra (47, 48), NOE difference spectra, and usual ^{1}H spectra.

^{1}H NMR assignments for the pro-R and pro-S protons of C-22, C-23, and C-24 of the F_7-sterols **X, XV,** and **XVI** were made by chemical shift comparisons with other F_7-sterols, for which assignments had been established previously (32). The C-22 protons of the Δ^{23} steroid **XIV** were also assigned by chemical shift comparison (32).[2] Stereochemical assignments for the other C_{24} steroids (**XII** and **XIII**) were based on application of a Karplus relationship (49) to coupling constants derived from difference spectra. In the case of selenide **XIII,** saturation difference and NOE difference spectra furnished isolated multiplets for H-20, H-22R, H-22S, and H-23S (**Fig. 4**).

[2] Note that the priority rules for RS nomenclature lead to a reversal of the designations for the C-22 protons of the Δ^{23} steroid **XIV** and 23-iodide **XV** relative to those of other steroids described here. Designations for the C-23 protons are similarly reversed for **X, XII, XIII, XIV,** and **XVI** relative to those of **XI** and other sterols with a C_8H_{17} side chain.

First-order analysis of these complex signals (ddqd 9.6, 8.6, 6.5, 3.1 Hz; dddd, 13.4, 11.0, 5.2, 3.0 Hz; dddd, 13.4, 10.9, 8.4, 4.6 Hz; and ddddd, 13.5, 11.1, 8.8, 6.4, 4.7 Hz, respectively) led to coupling assignments compatible only with the stated stereochemical assignments and a predominantly extended conformation for the steroid side-chain. Similar analysis was carried out for the bile alcohol **XII,** although strong coupling effects and signal overlap limited the precision of the resulting coupling constants.

Effects of 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (X) on the levels of HMG-CoA reductase activity in CHO-K1 cells and on the levels of ACAT activity in rat jejunal microsomes

The F_7-7α-methyl analog (**X**) 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 6**). The potency of **X** did not differ significantly from that of the 15-ketosterol **I. X** also showed high potency, similar to that of **I,** in the inhibition of the oleoyl-CoA-dependent esterification of cholesterol by rat jejunal microsomes (Table 6).

TABLE 6. Effects of 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**X**) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) on the levels of HMG-CoA reductase activity in CHO-K1 cells and on the levels of ACAT activity in rat jejunal microsomes

	HMG-CoA Reductase Activity	
Sterol Concentration	**X**[a]	**I**[b]
μM	*% of control*	
0.0	100.0	100.0
0.1	59.2 ± 6.8	62.5 ± 2.8
0.25	46.5 ± 2.1	45.8 ± 2.0
0.50	40.9 ± 1.6	36.6 ± 1.6
1.0	23.2 ± 0.6	28.8 ± 1.4
2.5	21.9 ± 1.3	23.6 ± 1.6
	ACAT Activity[c]	
	X	**I**
	% of control	
0	100.0	100.0
1	72.7 ± 5.5	64.8 ± 2.0
2	60.3 ± 3.5	52.0 ± 2.2
4	40.6 ± 1.7	35.3 ± 1.9
8	25.4 ± 1.7	19.2 ± 0.9
16	10.9 ± 0.4	7.0 ± 0.3

[a]Mean ± SD for replicate (n = 3) assays of HMG-CoA reductase activity.

[b]Mean ± SEM of 40 independent experiments in which triplicate determinations of enzyme activity were made at each concentration.

[c]Mean ± SEM of 3 independent experiments in which quadruplicate determinations of enzyme activity were made at each concentration.

Effects of dietary administration of 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (X) on food consumption, changes in body weight, and on weights of selected organs

In marked contrast to the case of the parent 15-ketosterol **I,** dietary administration of the F_7-7α-methyl-15-ketosterol **X** had little effect on food consumption.[3] Also in marked contrast to the case of the parent 15-ketosterol **I,** dietary administration of **X** had little effect on the growth of the rats.[4] The major effect of **X** was on the weight of small intestine. Administration of **X,** at all doses studied except for 0.05%, was associated with an increased weight of small intestine. This effect was related to dosage (**Table 7**) and showed the largest increase (+41%; $P = 0.0001$) at the highest dose level (0.15%). It is noteworthy that the increases in the weight of small intestine associated with administration of either **IX** or **X** were less than those observed with a comparable dosage of the parent 15-ketosterol **I.**[5]

[3]At dosages of 0.05% and 0.075% in diet, **X** had no effect on food consumption on any of the days studied. At a dosage of 0.10% in diet, slightly lower (−8.2%, −9.2%, and −5.5%; $0.01 < P < 0.05$) mean values of food consumption were observed on days 5, 6, and 7, respectively. At a dosage of 0.125% in diet, slightly lower mean values of food intake were observed on days 4 (−13.7%; $P < 0.01$), 5 (−11.8%; $0.01 < P < 0.05$), 6 (−10.8%; $P < 0.01$), 7 (−15.4%; $0.01 < P < 0.05$), 9 (−7.9%; $P < 0.01$), and 10 (−15.5%; $0.01 < P < 0.05$), respectively. The marginal effects of **X** on food consumption observed at these two dosages were not accentuated upon raising the dose level to 0.15% in diet. In this case, only slight effects were observed on days 5 (−11.8%; $P = 0.016$), 6 (−12.3%; $P = 0.008$), and 9 (−11.5%; $P = 0.034$).

[4]At dosage levels of 0.05% and 0.075% in diet, **X** had no effect on mean values of body weight on any of the 10 days of the experiments. At a dosage of 0.10% in diet, the mean values for body weight of the treated rats were lower ($0.01 < P < 0.05$) than ad libitum control animals on days 3 (−6.4%), 5 (−5.8%), 6 (−6.3%), 7 (−6.1%), 8 (−5.9%), 9 (−5.1%), and 10 (−6.1%). However, at the higher dosage of 0.125% in diet, the mean values for body weight did not differ from those of the control animals except for day 9 (−8.1%; $0.01 < P < 0.05$). Similarly, at an even higher dosage level (0.15%) the mean values for body weights of the treated animals were only slightly lower than those of ad libitum control animals only on days 8 (−7.0%; $P = 0.032$), 9 (−6.7%; $P = 0.032$), and 10 (−7.1%; $P = 0.017$).

[5]Administration of **X** at the various dosages studied had no effect on the weight of kidneys, heart, or testes. **X** had no effect on the mean weights of liver (relative to ad libitum controls) at each of the dosages from 0.05% to 0.125% in diet. At a level of **X** of 0.15% in diet, the mean value for liver weight was higher (+21%, $P = 0.0085$) than that of the ad libitum controls. When liver weights were analyzed with respect to percentage of total body weight, mean values for animals receiving **X** were slightly higher than those of ad libitum controls: 0.05% **X** (+9.3%; $P = 0.026$), 0.075% **X** (+10.3%; $P = 0.002$), 0.10% **X** (11.0%; $P = 0.0003$), 0.125% **X** (+10.3%; $P = 0.032$), and 0.15% **X** (+15.0%; $P = 0.038$). Administration of **X** had no significant effect on mean weights of spleen except at a dose of 0.125% at which the mean value of spleen was lower (−10.3%; $P = 0.008$) than that of the ad libitum control animals. At the same dosage level, no significant difference from control animals was observed when the spleen weight was expressed as a percentage of total body weight. Moreover, at the highest dose studied (0.15%), **X** had no effect on the weight of spleen. **X** had no effect on mean values of the weight of adrenals except at the dosage of 0.125% (+11.1%; $P = 0.043$). However, at the highest dose (0.15%) tested, **X** had no effect on adrenal weight.

TABLE 7. Comparison of the effects of dietary administration of 3β-hydroxy-7α-methyl-5α-cholest-8(14)-en-15-one (IX), 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (X), and 3β-hydroxy-5α-cholest-8(14)-en-15-one (I) on the weight of small intestine

Sterol	Level in Diet		Percentage Difference from ad Libitum Control	
	% by Weight	μmol per g Diet	From Weight in g	From % of Total Body Weight
IX[a]	0.10	2.42	+24.1[c]	+19.1[c]
	0.15	3.62	+24.2[c]	+30.7[c]
	0.20	4.84	+60.7[c]	+80.1[d]
X	0.050	0.93	+3.5[ns]	+8.2[ns]
	0.075	1.39	+11.0[d]	+12.5[c]
	0.100	1.85	+16.8[c]	+23.7[d]
	0.125	2.31	+21.9[c]	+34.4[c]
	0.150	2.78	+31.4[c]	+41.5[d]
I	0.05	1.25	+30.4[d]	+33.2[d]
	0.100[b]	2.50	+84.9[d]	+157.8[d]

[a] Data from ref. 34.
[b] Data from ref. 33.
[c] $0.002 < P < 0.02$.
[d] $P \leq 0.002$.
ns, Not significant ($P > 0.05$).

Effects of dietary administration of 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (X). Studies of the chemical nature of sterols in blood, liver, and small intestine

The liver (11.04 g) of one rat that received the F_7-7α-methyl-15-ketosterol **X** (0.15% in diet) for 10 days was homogenized with a 2:1 mixture (300 ml) of $CHCl_3$ and methanol. The resulting mixture was filtered and, after washing of the filter twice with $CHCl_3$–methanol 2:1 (50-ml portions), the filtrate was mixed with water (100 ml). The separated organic phase was washed with water and evaporated to dryness under reduced pressure. The residue was dissolved in toluene (10 ml) and aliquots (20 μl) were removed for GC analysis. One portion was subjected to mild alkaline hydrolysis using potassium carbonate (1 g) in methanol (3 ml) for 3 h at 55°C, followed by extraction of the sterols with methyl-*tert*-butyl ether (3 × 10 ml). GC analysis of the TMS ethers showed one major component with the retention time of the TMS ether of cholesterol (14.37 min), which was preceded by a less abundant component with the retention time (13.08 min) of the TMS derivative of authentic **X** and followed by two very minor components with retention times of 16.08 min and 18.0 min. The latter two minor components corresponded in retention time and MS to the TMS ethers of the plant sterols campesterol and β-sitosterol present in the basal chow diet.

The remainder of the total lipid extract of liver was applied to a silicic acid–Super Cel 1:1 column (100 cm × 2.2 cm) along with internal standards of [7(n)-^{3}H]cholesterol (10^6 dpm), [1,2,6,7(n)-^{3}H]cholesteryl oleate (10^6 dpm), and [2,4-^{3}H]3β-hydroxy-5α-cholest-8(14)-en-15-one (10^6 dpm). The column was eluted with toluene which, at fraction 50, was changed to toluene–ether 92:8. Fractions 15 ml in volume were collected.

The contents of fractions 31 to 40, corresponding to the location of cholesterol, were subjected to reversed-phase HPLC on a semi-preparative C_{18} column using methanol (3 ml per min) as solvent. One major component with the retention time (22.44 min) of authentic cholesterol was observed. Two minor components with retention times of 18.92 min and 25.28 min from the HPLC column were observed. GC–MS of the TMS ether of the former component showed a single substance with an MS compatible with that of the TMS ether of a Δ^5-3-hydroxysterol with a side chain containing a methyl group and one carbon–carbon double bond. The latter component (t_R 25.28 min) represented a mixture of two substances (incompletely separated from cholesterol on the HPLC column) with the retention times and MS of the TMS derivative of β-sitosterol and campesterol. The three minor sterols were also present in the commercial chow diet used in these experiments.

The contents of fractions 13 to 18, corresponding to the location of cholesteryl esters were saponified with ethanolic KOH as described above, and the resulting free sterols were subjected to reversed-phase HPLC on a semi-preparative C_{18} column using methanol (3 ml per min) as solvent. Two components were noted in the sterol region, a major component with retention time of 22.19 min (corresponding to that of cholesterol) and a minor component at 24.06 min. GC–MS analysis of the TMS derivative of the latter component showed material with the retention time and MS of the TMS ether of campesterol.

The contents of fractions 19 to 30, corresponding to the mobility of fatty acid esters of the 15-ketosterol (and of the F_7-7α-methyl-15-ketosterol) were combined and subjected

to mild alkaline hydrolysis as described above. The resulting free sterols were subjected to reversed-phase HPLC on a semi-preparative C_{18} column (solvent, methanol–water 9:1; 3 ml per min). The material with the retention time (13.56 min) of the F_7-7α-methyl-15-ketosterol was analyzed by GC–MS in the form of its TMS derivative. The resulting chromatogram showed a single component (> 99%) with the same retention time and MS (Table 5) as that of the TMS ether of authentic **X.** Independent GC analysis on a DB-5 column (using an internal standard of the TMS ether of cholesterol) indicated the level of **X** (in the ester form) of ~16.1 μg per g of liver.

The contents of fractions 70 to 80, corresponding to the mobility of 15-ketosterols **I** and **X,** were combined and subjected to reversed-phase HPLC on a semi-preparative C_{18} column (solvent, methanol–water 9:1; 3 ml per min). The material with the mobility of **X** was subjected to GC–MS in the form of its TMS derivative. The retention time and MS (Table 5) were essentially the same as those of an authentic sample of **X.** Independent GC analysis on a DB-5 column (using an internal standard of the TMS ether of cholesterol) indicated a level of **X** of 26.3 μg per g of liver.

The livers from two additional experimental animals were analyzed in an identical manner. The mean level of total **X** in the livers of the three rats was 85 ± 5 nmol per g, approximately 1.4% of the mean level of total cholesterol. The mean levels of **X** in the free and esterified states were 59 ± 6 and 26 ± 2 nmol per g, respectively.

The levels of **X** in the small intestine of three rats, that received **X** at a level of 0.15% in diet for 10 days, were also determined by the same methodology described above. Identification of **X** was based upon comparison of its chromatographic behavior on reversed-phase HPLC and capillary GC (TMS ether) and its MS (TMS ether, Table 5) with those of an authentic sample of **X.** The mean level of total **X** in the small intestines of the three rats was 172 ± 40 nmol per g, approximately 3.8% of the level of total cholesterol. The mean levels of free and esterified **X** were 150 ± 33 and 22 ± 9 nmol per g, respectively.

The nature of sterols in serum was studied as follows. From the serum sample obtained at time of death (day 10) from each of the animals treated with **X** (0.15% in diet), an aliquot (200 μl) was taken and combined to give a pooled sample that was subjected to mild alkaline hydrolysis as described above. A small aliquot of the free sterols, obtained by extraction with hexane (3 × 10 ml), was analyzed by GC on a DB-5 column. Clear resolution of the TMS derivative of the F_7-7α-methyl-15-ketosterol **X** (12.66 min; 5.8%), cholesterol (13.93 min; 91.5%), campesterol (15.53 min; 1.7%) and β-sitosterol (17.30 min; 1.1%) was achieved. Independent GC–MS analyses indicated that the MS of each of the four components was compatible with the assigned structures. As noted previously, campesterol and β-sitosterol were significant sterols present in the chow diet used in these experiments.

The remainder of the nonsaponifiable lipids recovered from the pooled serum sample was subjected to reversed-phase HPLC on a semi-preparative C_{18} column using methanol–water 95:5 for 17 min and methanol for 25 min as the elution solvents. The material with the retention time (7.85 min) of **X** was analyzed by GC–MS in the form of its TMS derivative. A single component was noted with essentially the same retention time (8.0 min) and MS (Table 5) as that of the TMS derivative of an authentic sample of **X.** The concentration of **X** in serum was determined to be 25.8 μg per ml by an independent GC analysis on a DB-5 column using an internal standard of the TMS derivative of cholesterol (20 μg).

Capillary GC analyses of total (free plus esterified) **X** in serum obtained on days 5 and 9 indicated little effect of either duration of treatment or dosage. The mean levels (μM) on days 5 and 9 were 83 ± 5 and 77 ± 4 at 0.05%, 92 ± 4 and 88 ± 4 at 0.075%, 86 ± 4 and 97 ± 3 at 0.10%, 82 ± 5 and 85 ± 4 at 0.125%, and 73 ± 6 and 83 ± 3 at 0.15% by weight in diet.

Effects of dietary administration of 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (X) on serum cholesterol levels in male Sprague-Dawley rats

Administration of the F_7-7α-methyl-15-ketosterol **X** resulted in significant lowering of serum cholesterol levels at all dosages tested (**Table 8**). The magnitude of the cholesterol lowering by **X** was related to dosage over the range from 0.05% to 0.10% in diet. The extents of cholesterol lowering at dosages of 0.10%, 0.125%, and 0.15% were similar. The magnitudes of the reduction of serum cholesterol by **X** on days 5 and 9 were comparable at all dosages studied. The F_7-7α-methyl-15-ketosterol **X** was very considerably more potent in lowering serum cholesterol levels than was the 7α-methyl-15-ketosterol **IX** lacking the fluorine substitution. For example, **IX** had no effect at a dosage of 2.42 μmol per g of diet whereas **X,** at a dosage of 0.93 μmol per g of diet caused an approximately 30% lowering of serum cholesterol. Furthermore, roughly comparable levels of hypocholesterolemic action were caused by **IX** at a dosage of 4.84 μmol per g of diet and by **X** at a level of only 1.39 μmol per g of diet. Whereas **X** caused a 30% lowering of serum cholesterol at 0.93 μmol per g of diet, **I,** at a higher dosage (i.e., 1.25 μmol per g of diet), had no effect on serum cholesterol (Table 8).

DISCUSSION

The strategy selected for the construction of the desired F_7-7α-methyl-15-ketosterol **X** involved degradation of the 7α-methyl-15-ketosterol **IX** to a C_{24} steroid, which was then utilized to introduce the F_7-isopropyl function (Fig. 3). This approach was notably successful for the chemical synthesis of the F_7 analog of the 15-ketosterol **I** (32). A

TABLE 8. Comparison of the effects of dietary administration of 3β-hydroxy-7α-methyl-5α-cholest-8(14)-en-15-one (**IX**), 3β-hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**X**), and 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) on the levels of serum cholesterol in male Sprague-Dawley rats

		Concentration in Diet		Serum Cholesterol (mg per dl)			Cholesterol Lowering (% change from ad libitum controls)	
Experiment	Group	% by Weight	μmol/g Diet	Day 0	Day 5	Day 9	Day 5	Day 9
1	Control (ad libitum)			125.3 ± 3.0	104.9 ± 3.2	102.1 ± 1.9		
	Compound **X**	0.050	0.93	124.9 ± 2.7	74.8 ± 3.8	71.9 ± 3.3	−28.7[d]	−29.6[d]
	Compound **X**	0.075	1.39	126.3 ± 2.5	67.6 ± 1.6	62.7 ± 2.7	−35.6[d]	−38.6[d]
	Compound **X**	0.100	1.85	126.3 ± 2.1	56.5 ± 1.1	54.8 ± 1.2	−46.1[d]	−46.3[d]
	Compound **X**	0.125	2.31	126.3 ± 2.0	52.6 ± 2.2	50.4 ± 1.7	−49.1[d]	−50.3[d]
2	Control (ad libitum)			105.1 ± 1.8	96.8 ± 3.6	95.4 ± 3.8[b]		
	Compound **X**	0.150	2.78	105.1 ± 1.6	49.2 ± 1.7	50.3 ± 2.1	−49.2[d]	−47.3[d]
	Control (pair-fed)			105.4 ± 1.9	93.5 ± 4.0[b]	92.0 ± 3.7[c]		
3	Control (ad libitum)			110.2 ± 2.0	103.9 ± 3.6	103.3 ± 3.0		
	Compound **I**	0.050	1.25	110.6 ± 1.9	96.9 ± 3.3	92.0 ± 3.7	−6.7[ns]	−10.9[ns]
4[a]	Control (ad libitum)			108.5 ± 2.5	103.3 ± 1.7	95.6 ± 2.6		
	Compound **IX**	0.100	2.42	108.5 ± 2.1	99.1 ± 2.0	97.3 ± 1.4	−4.1[ns]	+1.8[ns]
	Control (pair-fed)			108.2 ± 2.1	98.4 ± 1.5	92.8 ± 1.9		
5[a]	Control (ad libitum)			125.7 ± 3.2	102.5 ± 2.2	96.9 ± 2.6		
	Compound **IX**	0.150	3.62	125.7 ± 3.0	86.6 ± 2.9	81.7 ± 2.5	−15.5[e]	−15.7[f]
	Compound **IX**	0.200	4.84	126.5 ± 2.6	69.2 ± 1.4	68.3 ± 2.8	−32.5[d]	−35.7[d]

[a] Results from ref. 34.
[b] $n = 7$.
[c] $n = 6$.
[d] $P = 0.0001$.
[e] $P = 0.0044$.
[f] $P = 0.0033$.
ns, Not significant ($P > 0.05$).

critical reaction in this synthetic scheme is the transformation of a C_{27} sterol with a C_8H_{17} side chain into a C_{24} steroid bearing a 24-hydroxyl group (31). Although the acetate of **I** undergoes this oxidative side-chain cleavage in remarkably high yield (~65%), much lower yields are reported for similar trifluoroperacetic acid oxidations of other sterols (31). In view of the unknown scope of this reaction, we were gratified to observe a ~68% yield in the conversion of the acetate derivative of **IX** to 3β-acetoxy-7α-methyl-24-hydroxy-5α-chol-8(14)-en-15-one (**XII**). Treatment of **XII**, selectively protected at C-3, with *ortho*-nitrophenyl selenocyanate (35) and tributylphosphine (50) furnished the crude *ortho*-nitrophenyl selenide **XIII**, which, upon treatment with hydrogen peroxide, gave the Δ^{23}-olefin **XIV**. The Δ^{23}-olefin **XIV** was condensed with 2-iodoheptafluoropropane in a triethylborane-induced radical addition (51) to give chiefly (32) the 23R-iodo-F_7 isomer **XV**, which was selectively reduced with tributyltin hydride in the presence of AIBN to give **XVI**. Mild alkaline hydrolysis of **XVI** gave the desired F_7-7α-methyl-15-ketosterol **X**. This synthesis of **X** from **IX** proceeded in 28% overall yield based on the procedure bypassing the isolation of selenide **XIII**.

The F_7-7α-methyl-15-ketosterol **X** and synthetic intermediates were characterized by IR, ^1H and ^{13}C NMR, MS, and high-resolution MS. The mass spectral fragmentation patterns of the 7α-methyl-15-ketosterols in Table 4 were similar to those of the corresponding unsubstituted 15-ketosterols (31, 32, 52) except that the 7α-methyl derivatives showed ions incorporating loss of H_2O in higher abundance and ion B (loss of ring A and C-6) and ion B − CH_3 in lower abundance. Ion B of the 7α-methyl derivatives was too weak (1–2% intensity) to be reported, as had been the case for M–SC–H_2O–14 of the unsubstituted 15-ketosterols. The 7α-methyl substitution may facilitate loss of H_2O from the $\Delta^{8(14)}$-15-keto moiety by stabilizing a proposed initial $\Delta^{7,14}$ intermediate (52), and the 7α-methyl substitution can be expected to affect the abundance of ion B, which is formed after cleavage of the C6–C7 bond. The high and low mass spectral results for the 7α-methyl-15-ketosterols are fully in accord with assignments presented previously (24, 28, 29, 31, 32, 52) for fragment ions of 15-ketosterols.

Complete ^1H, ^{13}C, and ^{19}F NMR signal assignments (Tables 1 and 3) and an extensive tabulation of ^1H-^1H coupling constants (Table 2) are presented for the 7α-methyl-15-ketosteroids **X**–**XVI**. Critical to the determination of many ^1H-^1H coupling constants in Table 2 was the use of saturation difference spectroscopy (47, 48), a sensitive technique for isolating a partially overlapped multiplet by irradiation of a single line. The usefulness of saturation difference spectroscopy and the less sensitive NOE difference experiment is illustrated in Fig. 4, which shows the almost complete isolation of four severely over-

lapped resonances corresponding to side-chain protons of selenide **XIII.** Coupling constants can be measured readily from line positions in these difference spectra. By contrast, accurate determination of couplings from cross-sections of 2D spectra normally requires tedious line-shape analysis.

Application of a Karplus relationship (49) to the ^1H-^1H coupling constants in Table 2 permitted extensive conformational analysis of the side chain of 7α-methyl-15-ketosteroids. Couplings for the bile alcohol **XII** showed the side chain to be mainly in the extended conformation except for the presence of multiple rotamers about the C23–C24 bond. In the case of selenide **XIII,** the couplings indicated some preference for selenium *anti* to C-22, corresponding to a distribution of C23–C24 rotamers intermediate between that of the bile alcohol **XII** and the predominantly extended conformation of F_7-sterols. NMR results also shed light on the population distribution of C20–C22 and C17–C20 rotamers. In each of the C_{24} and C_{27} sterols, the *anti* $J_{H20\text{-}H22}$ coupling[2] was ~8.6 Hz, a value significantly lower than that (12.3 Hz) predicted from a Karplus relationship. The reduced $J_{H20\text{-}H22}$ value is compatible with a ~40% population of the *+gauche* C17–C20–C22–C23 rotamer, which is energetically as favorable as the extended conformer by molecular modeling (53) and force-field calculations.[6] This significant population of the *+gauche* rotamer, which has been noted previously for C_{27} sterols (54), is also supported by NOE difference experiments showing correlations between H-16α and both C-22 protons.[7] The $J_{H17\text{-}H20}$ value of ~9.7 Hz similarly suggests a significant (albeit smaller) population of an additional conformer, the *+gauche* C13–C17–C20–C22 rotamer.[6] ^{13}C chemical shift comparisons point to analogous conformations for sterols with a C_8H_{17} side chain, for which conformational analysis using the above methodology is frequently precluded by strong coupling effects at currently available field strenghts.

The foregoing conformational analyses together with conformational preferences described recently for other C_{24} steroids (44) and C_{27} sterols (32, 34, 54) permit the following generalizations. The population distribution of C17–C20, C20–C22, and C22–C23 rotamers is essentially identical for cholesterol, 15-ketosterols, F_7-15-ketosterols, and a variety of C_{24} steroids. The ratio of *+gauche:anti* conformers appears to be roughly 1:9 for C17–C20 rotamers and 4:6 for C20–C22 rotamers. The Δ^{23}-C_{24} steroid **XIV** and the 23-iodide **XV** deviate slightly from this norm, as judged by their ^{13}C shieldings and $J_{H17\text{-}H20}$ and $J_{H20\text{-}H22}$ values. Excluding **XIV** and **XV,** the C22–C23 rotamers are chiefly *anti* for both C_{24} steroids and C_{27} sterols, with *gauche* rotamers representing ~20% of the total population. The C23–C24 rotamers are likewise mainly *anti* for C_{27} sterols but vary among C_{24} steroids depending on the C-24 substituent. Regarding the C24–C25 rotamers, the likelihood of a C-25 substituent to be *anti* to C-23 is: C-26 ~ C-27 > H-25 or F-25. These generalizations apply to both C_8H_{17} and $C_8H_{10}F_7$ side chains (excluding iodide **XV**). The absence of line broadening for any ^1H or ^{13}C signals of the C_{24} or C_{27} steroids indicates that the side-chain conformers interconvert readily at room temperature.

Comparison of the ^{13}C NMR spectra of 7α-methyl-15-ketosterols (Table 1 and ref. 34) with those of the corresponding unsubstituted 15-ketosterols (31, 32, 34) showed the expected downfield shifts for β-carbons C-6 and C-8 and upfield shifts for γ-carbons C-5 and C-9.[8] In the ^1H NMR spectra, introduction of the 7α-methyl group caused the anticipated (34) downfield shifts (~0.25 ppm) for H-5α, H-6β, H-7β, and H-9α and an upfield shift (~0.3 ppm) for H-6α.[8] These NMR chemical shift comparisons and analogous coupling constant comparisons are compatible with a previous conclusion (34) that introduction of the 7α-methyl group has little effect on the conformation of 15-ketosterols.

The 15-ketosterol **I** has been shown to be among the most potent oxysterols in the lowering of HMG-CoA reductase activity and in the inhibition of sterol synthesis in cultured mammalian cells (1–4, 55). We have previously shown that its F_7-analog (**VIII**) is also highly active in the suppression of reductase activity in cultured cells (32). That this action of **VIII** was not due to the F_7-substitution was shown by the equivalency of the activities of **I** and **VIII** in lowering reductase activity and by the

[6]Force-field calculations were carried out with PC Model for compounds **IX, X, XII,** and **XIV** (all as free sterols). Relative to the fully extended side chain form, the *+gauche* C17-C20-C22-C23 rotamer was 0.3–0.5 kcal/mol lower in energy, and the *+gauche* C13-C17-C20-C22 rotamer was 1.3 kcal/mol higher in energy. The relative energies for the corresponding conformers of cholesterol were 0.2 kcal/mol lower and 1.6 kcal/mol higher, respectively.

[7]The following signals were enhanced in NOE experiments: **XIII,** irradiation of H-22S: H-16α, H-16β (weak), H-17α (weak), H-20, H-22R, H-24R, H-24S; **XIV,** irradiation of H-23: H-16α, H-17α, H-20; irradiation of H-16α: H-16β, H-22R, H-22S; irradiation of H-17α: H-12α, H-22R, H-23; irradiation of H-22R: H-16α, H-17α, H-22R, H-24; irradiation of H-22S: H-16α, H-17α, H-20, H-22R.

[8]Substituent-induced shifts for the 7α-methyl group, expressed as δ(7α-methyl derivative) – δ(unsubstituted 15-ketosterol), were calculated as the average of the chemical shift differences for eight 7α-methyl-15-ketosterols (**IX**-XVI). Standard deviations were ≤ 0.06 ppm for ^{13}C and < 0.02 ppm for ^1H); only ^1H chemical shifts known to an accuracy of ± 0.02 ppm were used. The following ^{13}C-induced shifts > 0.1 ppm were observed: C-5, −6.05 ppm; C-6, 5.72 ppm; C-8, 5.77 ppm; C-9, −4.92 ppm; C-11, −0.22 ppm; C-14, −0.47 ppm; C-15, −0.42 ppm; C-16, 0.20 ppm; C-17, −0.25 ppm; C-18, 0.26 ppm; C-19, −0.46 ppm. The induced shift for C-7 was only 0.08 ppm, although a 1.9-ppm shift was noted in the case of the $\Delta^{8(14)}$-3β,15α-diols. The following ^1H-induced shifts > 0.02 ppm were observed: H-4α, −0.08 ppm; H-4β, −0.05 ppm, H-5α, 0.23 ppm; H-6α, −0.29 ppm; H-6β, 0.21 ppm; H-7β, 0.25 ppm; H-9α, 0.26 ppm; H-12α, −0.03 ppm; H-17α, −0.04 ppm.

lack of effect of F_7-cholesterol on this enzyme activity (32). In the present study, we have shown that the F_7-7α-methyl-15-ketosterol is also very potent in the lowering of reductase activity in CHO-K1 cells (Table 6). The demonstration that **X** was as potent as **I** and its 7α-methyl analog **IX** (34) indicates that neither the 7α-methyl substitution nor the F_7 substitution affects the action of the parent 15-ketosterol **I** in lowering reductase activity in these cells.

The 15-ketosterol **I** has been shown to act as an alternative substrate for ACAT and to inhibit the oleoyl-CoA-dependent esterification of cholesterol in rat liver and jejunal microsomes (11). In the present study we have shown that the 7α-methyl-F_7 analog (**X**) of **I** also shows high potency in the inhibition of ACAT activity in rat jejunal microsomes, with a potency similar to that of **I** (Table 6). It is assumed that **X** also acts as a substrate for ACAT; however, direct demonstration of the esterification of **X** under the assay conditions will await the chemical synthesis of **X** in labeled form.

Dietary administration of **X** to male Sprague-Dawley rats resulted in significant lowering of serum cholesterol levels at each of the dosage levels studied (from 0.05% to 0.15%) (Table 8). **X** was considerably more potent than the corresponding 7α-methyl-15-ketosterol **IX** lacking the F_7 substitution in the side chain. For example, whereas **IX** had no effect on serum cholesterol at a dosage of 2.42 μmol per g of diet (34), **X**, at a comparable dosage (i.e., 2.31 μmol per g of diet), caused a 50% lowering of serum cholesterol. Furthermore, significant hypocholesterolemic action of **X** was observed at a very low dosage level, i.e., 0.93 μmol per g of diet. Thus, the combined results indicate that the introduction of the F_7 substitution in the 7α-methyl-15-ketosterol is associated with a very considerable increase in potency with respect to its hypocholesterolemic action. It is also important to note that **X** had little or no effect on food consumption at the dosages studied (i.e., 0.93 to 2.78 μmol per g of diet), whereas the parent 15-ketosterol **I** markedly suppressed food consumption when administered to rats at a level of 0.10% by weight in diet (2.5 μmol per g of diet) (6–8, 33). In addition, **X** was more potent than **I** in reducing serum cholesterol levels in rats under the conditions studied. For example, whereas **X** at dosages of 0.93 and 1.39 μmol per g of diet reduced serum cholesterol levels by 30% and 39%, respectively, **I** at a dosage of 1.25 μmol per g of diet had no effect on serum cholesterol (Table 8).

Also in marked contrast to **I** (6–8, 33), **X** had little or no effect on the growth of animals, as measured by changes in body weight. The major effect of **X** was on the weight of small intestine, which showed a dose-dependent increase at dosages between 1.39 to 2.78 μmol per g of diet (Table 7). The effect of **X** on small intestinal weight was considerably less than that of **I**. It is important to note that the effect of **I** on small intestinal weight of rats, for which detailed morphological analyses have been reported (8), appears to be a rodent-specific phenomenon as a similar effect was not observed upon oral administration of **I** to nonhuman primates (8). The clear absence of any effects of **X** on food consumption at dosages of 0.93 and 1.39 μmol per g of diet has very significant practical consequences inasmuch as it obviates the need for the inclusion of the costly pair-fed control animals in the exploration of the actions, toxicology, and metabolism of **X** in rats. It is important to note that the hypocholesterolemic action of **X** was observed at a dosage (0.93 μmol per g of diet) at which it had no effect on food consumption, growth, or the weight of small intestine. Similar observations have not been made with **I**.

Upon dietary administration of **X** to rats, low levels of **X** were observed in serum. No relationship between dosage and the levels of **X** in serum was observed. The presence of other metabolites of **X** in serum was not detected. Detailed analyses of the sterols of the livers and small intestines of rats fed the F_7-7α-methyl-15-ketosterol **X** (0.15% in diet for 10 days) indicated the presence of low levels of **X** as the free sterol and as its fatty acid esters. No other metabolites of **X** were detected in liver or small intestine.

The levels of **IX** and **X** in serum of treated rats were not equivalent. The mean levels of **X** in serum on day 9 were higher (at all dosages studied, i.e., from 0.93 to 2.78 μmol per g of diet) than those observed in pooled samples of serum obtained on day 10 from rats treated with **IX** at levels of 2.42, 3.62, and 4.84 μmol per g of diet. For example, pooled sera from rats treated with **IX** at a dosage of 2.42 μmol per g of diet contained 19.8 μM **IX** (34), whereas animals fed **X** at 2.31 μmol per g diet for 9 days showed 85 $\pm$ 4 μM **X** in serum. Similarly, the levels of **IX** and **X** in livers of treated rats were not equivalent. For example, the mean concentration of **X** in livers of rats fed **X** at 2.78 μmol per g of diet for 10 days was 85 $\pm$ 5 nmol per g of liver, whereas the mean level of **IX** in livers of animals fed **IX** at a much higher dose, i.e., 4.84 μmol per g of diet, for the same period of time was only 16.3 $\pm$ 1.3 nmol per g of liver (34). The higher levels of the F_7-7α-methyl-15-ketosterol **X** in serum and liver (relative to animals fed **IX**) are likely caused by a blockage of metabolism of **X** involving oxidation at C-26 (or C-25), thereby preventing the formation of the major polar metabolites and their subsequent rapid excretion in bile as observed with the parent 15-ketosterol **I** (19, 21).

The results of our previous studies (33) have indicated that introduction of the F_7 substitution into the parent 15-ketosterol provides favorable features, presumably due to a blockage of side chain oxidation of **I**. The F_7-15-ketosterol **VIII** showed significant hypocholesterolemic activity at dosage levels considerably less than that required for **I** (33). Moreover, **VIII**, in contrast to **I**, had little or no effect on food consumption upon dietary ad-

ministration to rats. A potentially undesirable feature of **VIII** was its metabolism to F_7-cholesterol, presumably by the same pathway as that formulated for the overall conversion of **I** to cholesterol (16) (Fig. 1). A major goal of the present study was to prepare an analog of **VIII** in which this metabolism does not occur. This goal appears to have been achieved with the 7α-methyl analog of **VIII.** After administration of either the 7α-methyl-15-ketosterol **IX** (34) or the F_7-7α-methyl-15-ketosterol **X** to rats, only low levels of **IX** and **X** were observed in blood and in liver. No material with the expected properties of F_7-7-methylcholesterol (or potential intermediates in its possible formation from **X**) was observed. These findings indicate that no metabolism analogous to that involved in the conversion of **I** to cholesterol or of **VIII** to F_7-cholesterol was detected. Definitive studies of this matter await the preparation of **X** in labeled form for use in metabolic studies. The marked differences in the potencies of **IX** and **X** with respect to hypocholesterolemic action demonstrate the beneficial effect of the F_7 substitution, presumably by blocking the rapid side-chain oxidation of the 15-ketosterol and subsequent excretion of polar metabolites.

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