PREPARATION OF 25,26,26,26,27,27,27-HEPTAFLUORO-15-KETOSTEROLS LABELED AT C-23 WITH DEUTERIUM OR TRITIUM

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Summary

Side-chain fluorinated 15-ketosterols are potent regulators of sterol synthesis. For investigations of their metabolism, we have prepared 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one and its 7α-methyl and 8(14)-saturated derivatives with one deuterium or tritium atom at C-23. The isotopic hydrogen was introduced by reduction of 23-iodo-15-ketosterols with tributyltin deuteride or tritide. Mass spectral analyses of the deuterated sterols showed incorporation of one deuterium in the side chain, and ^{2}H and ^{13}C NMR showed that deuterium was present only at C-23. The tritiated sterols had specific activities of 82-179 mCi/mmol and showed high radiochemical purities.

Key words: 15-oxygenated sterols, deiodination, stereoselectivity, mechanism

Introduction

3β-Hydroxy-5α-cholest-8(14)-en-15-one (**1**; 15-ketosterol) is a potent regulator of cholesterol metabolism. The actions and metabolism of **1** have recently been reviewed (1). This oxygenated sterol is highly active not only in the inhibition of sterol synthesis in cultured mammalian cells, but also in the inhibition of the intestinal absorption of cholesterol in intact animals. Extensive studies of the metabolism of **1** by rat liver subcellular fractions

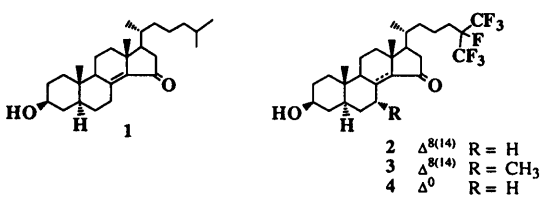

Figure 1. 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**1**) and side-chain fluorinated analogs (**2**, **3**, and **4**).

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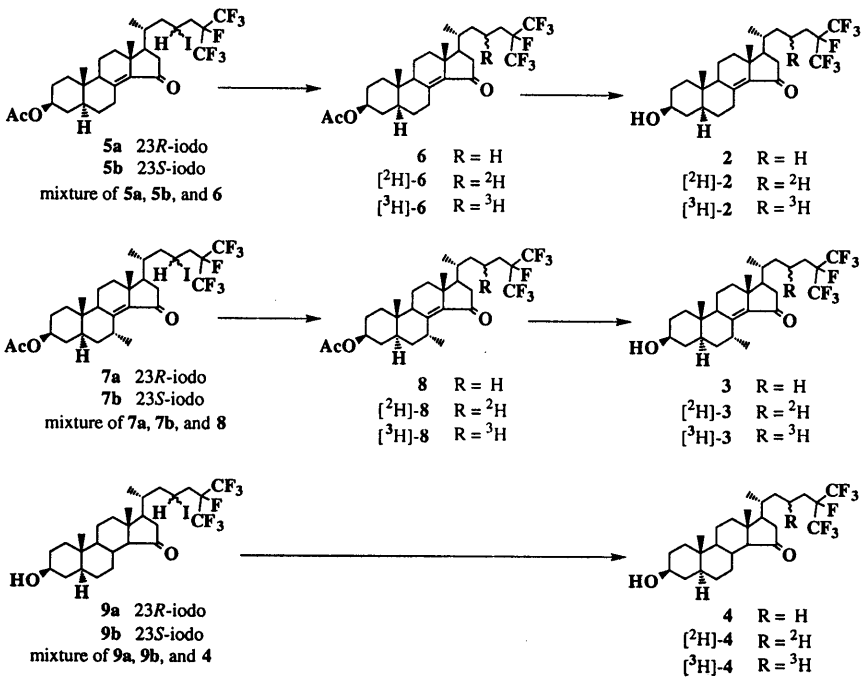
and by intact rats and nonhuman primates have indicated conversion of **1** to: (1) polar metabolites which are rapidly excreted in bile and (2) cholesterol. The very substantial formation of the polar metabolites of **1** appears to be initiated by oxidation at C-26 of the sterol side chain. In order to block the side-chain oxidation and/or the conversion of **1** to cholesterol, side-chain fluorinated derivatives of the 15-ketosterol have been prepared (2-5). Three such F_7-15-ketosterols (**2**, **3**, and **4**) are shown in Figure 1. Each of these F_7-15-ketosterols has been shown to lower the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured mammalian cells and to lower serum cholesterol levels upon oral administration to rats (2-4, 6). Isotopically labeled forms of these sterols are required for detailed studies of their metabolism. Described herein are efficient chemical syntheses of the F_7-15-ketosterols **2**, **3**, and **4** labeled with either deuterium or tritium at carbon atom 23.

Results and Discussion

Syntheses of deuterium- and tritium-labeled F_7-15-ketosterols **2**, **3**, and **4** are shown in Scheme 1. The isotopic hydrogen was introduced by reduction of 23-iodo-F_7-15-ketosterols with tributyltin deuteride or tritide, reagents that are easily prepared by treatment of tributyltin chloride with isotopically labeled sodium borohydride (7). Each of the three F_7-15-ketosterols was prepared as the deuterated and tritiated derivative.



Scheme 1. Syntheses of isotopically labeled F_7-15-ketosterols from 23-iodides.

The isotopic hydrogen was introduced in either the final or the penultimate step of the reported (2, 3, 5) syntheses of the F_7-15-ketosterols. In the preparation of F_7-15-ketosterol **4**, the reduction was performed on the free sterol because of likely epimerization at C-14 under conditions needed to remove an acetate group (5). For syntheses of F_7-15-ketosterols **2** and **3**, reductions were carried out on 3β-acetate derivatives, followed by removal of the acetate group by methanolysis. Because no significant steroidal byproducts were observed in labeled or unlabeled reductions (8), the methanolysis could be carried out on the crude tritiated reduction products. Consequently, chromatographic purification and other manipulations of radiolabeled sterols were minimal.

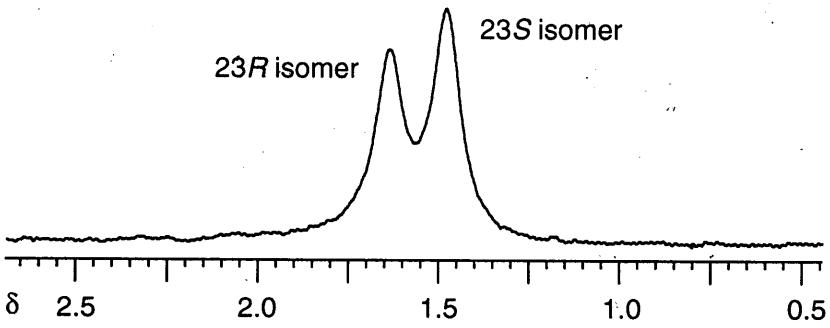


Figure 2. ^2H NMR spectrum of F_7-15-ketosterol [^2H]-**2**, which is a 5:4 mixture of the 23*S*- and 23*R*-deuterio isomers. Assignment of stereochemistry is based upon ^1H NMR signal assignments established by us for the side-chain protons of F_7-15-ketosterols (2, 3, 5).

All five deuterated sterols were characterized by melting point, thin-layer chromatography (TLC), mass spectrometry (MS), and ^1H, ^2H, and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. ^{13}C NMR chemical shifts of each sterol closely matched those of the protio analogs. In every case, the signal for C-23 was either absent or observed as a broad, weak multiplet, and signals for C-20, C-22, and C-24 were split (owing to deuterium isotope effects or long-range ^2H-^{13}C couplings). ^2H NMR spectra of each sterol showed two signals indicating that deuterium was distributed over both C-23 positions, with a modest excess of the 23*S*-deuterio isomer (Figure 2). No deuterium was observed by ^2H or ^{13}C NMR at any other position. ^1H NMR spectra indicated ≥98% purity of each deuterated sterol and corresponded to spectra of the undeuterated analogs (except for diminished intensity of H-23*R* and H-23*S* signals and changes in coupling patterns of the C-22 and C-24 proton signals). Melting points and TLC data also indicated high purity. Electron-impact mass spectra were similar to those of the unlabeled sterols except that ions containing the sterol side chain showed an additional mass unit. The spectra of the 23-deuterio-F_7-15-ketosterols were compatible with all suggested and implied ion assignments described

previously (2, 3, 5). The combined analytical data indicated high chemical purity and the presence of deuterium only at C-23. The level of deuterium incorporation was ~95% d_1 for sterols synthesized with commercial tributyltin deuteride and somewhat lower for sterols made with tributyltin deuteride prepared in our laboratory.

Tritiated intermediates and products were analyzed for radiochemical purity by radio-TLC on silica gel and by reversed phase radio-HPLC. As shown in Figure 3, each of the three final products ([^{3}H]-**2**, [^{3}H]-**3**, [^{3}H]-**4**) corresponded to a single component by radio-HPLC except for a minor contaminant in [^{3}H]-**4** (9). Each final product was also a single component by radio-TLC. On both TLC and HPLC (UV detection at 210 and/or 259 nm), ^{3}H radioactivity had the same chromatographic mobility as the unlabeled F_7-15-ketosterols.

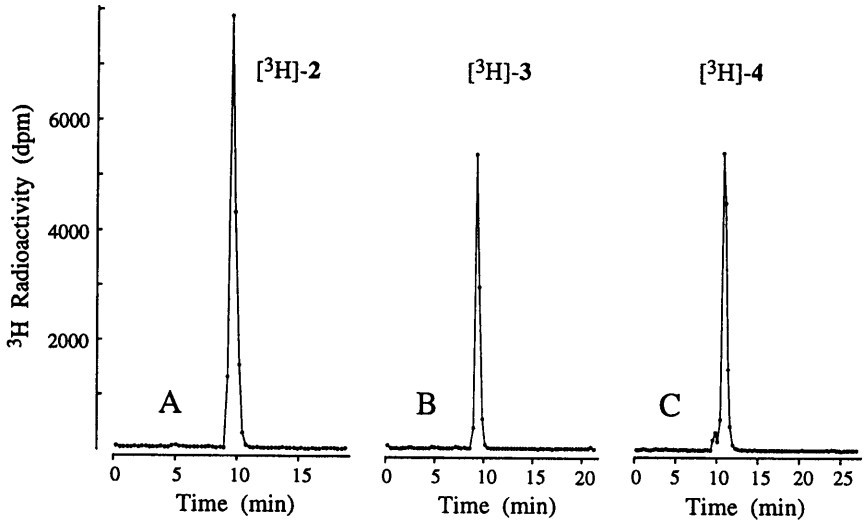


Figure 3. Radio-HPLC analyses of tritium-labeled F_7-15-ketosterols: F_7-$\Delta^{8(14)}$-15-ketosterol [^{3}H]-**2** (A), 7α-methyl-$\Delta^{8(14)}$-15-ketosterol [^{3}H]-**3** (B), and F_7-15-ketosterol [^{3}H]-**4** (C). Conditions: 5-μm Spherisorb ODS-II column (250 × 4.6 mm i.d.); elution with water-methanol 1:9 at 1 ml/min. Fractions collected at 20-s intervals were analyzed for tritium by scintillation counting.

The 23-iodo-F_7-15-ketosterol starting materials were mixtures that were resistant to purification by recrystallization or chromatography on silica gel (2-5). These mixtures consisted of the 23*R*-iodide accompanied by about 15% of the 23*S*-iodide and variable amounts (2-30%) of the corresponding sterol containing hydrogen in place of iodide, i.e. the 23-H_2 sterols **4**, **6**, and **8** (5). Presence of a 23-H_2 sterol is inconsequential in syntheses of

the protio compound but limits the isotopic enrichment attainable in the preparation of deuterated F_7-15-ketosterols. For example, an iodide mixture containing 30% of the 23-H_2 sterol might give at best a deuterated product of 70% d_1 and 30% d_0. After becoming aware of this problem (5), we chose iodide mixtures containing low levels of the 23-H_2 sterol for deuterium and tritium labeling (10).

The 23-H_2 byproducts arose because a C-23 free radical intermediate abstracted hydrogen from the reaction medium instead of reacting with C_3F_7I in the chain-propogating step to form an iodide (Figure 4A). The same free radical intermediate is formed in the iodide reductions with tributyltin deuteride and normally abstracts deuterium in the chain-propogating step (Figure 4B). However, the radical might alternatively react with other species present in the reaction mixture. In related reductions with tributyltin deuteride, hydrogen abstraction has been reported to occur intramolecularly from NH of a thiocarbamate and intermolecularly from ethyl radicals from a triethylborane initiator (11). Hydride reductions in the present work were carried out only with 2,2'-azobisisobutyronitrile (AIBN) as the initiator, and intramolecular abstraction of hydrogen appears unlikely for the F_7-15-ketosterols. The lower level of deuterium incorporation into sterols made using tributyltin deuteride prepared by us relative to that using the commercial reagent might be attributable to the presence of an additional hydrogen source, such as residual ethanol solvent used in the preparation of tributyltin deuteride.

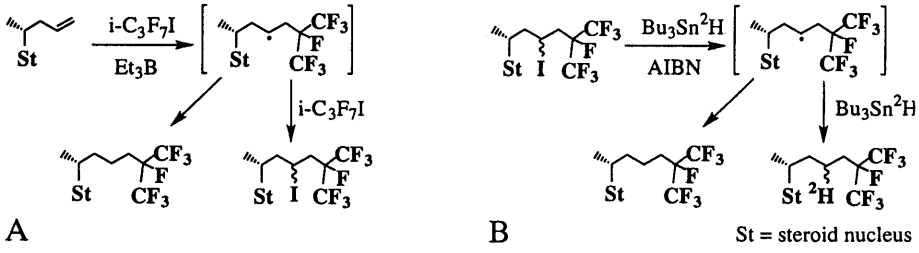


Figure 4. A free radical at C-23 normally abstracts iodine in a reaction with heptafluoroisopropyl iodide (A, see ref. 5) or deuterium in reductions of iodides with tributyltin deuteride (B, described herein). A side product of both reactions may arise if the radical abstracts hydrogen from the reaction medium.

The free radical mechanism normally observed for tributyltin hydride reductions of alkyl halides leads to loss of any existing stereochemical identity at the central carbon atom (12). Any stereoselectivity (observable in reductions with tributyltin deuteride) is attributable to steric differences in the approach of the tributyltin hydride to the radical center (11, 13). As noted above, ^2H NMR analysis of the deuterated sterols indicated a modest preference for

the 23*S*-deuterio epimers over the 23*R* products (14). Interestingly, formation of the 23-iodides by free-radical addition of heptafluoroisopropyl iodide to a C_{24}-Δ^{23} steroid, gave predominantly the 23*R*-iodide. These strikingly different results might be explained by proposing that heptafluoroisopropyl iodide and tributyltin deuteride approach the C-23 radical from opposite sides because of unusual differences in steric requirements. A more plausible explanation is that formation of the 23*S* isomer is kinetically favored in both reactions but the reaction is reversible only in the case of the iodide. Thus, tributyltin deuteride reduction gives an excess of the kinetically favored product (23*S*) whereas the iodide addition reaction is reversible and leads mainly to the thermodynamically favored product (23*R*). In support of this explanation, we have previously suggested that the iodide addition reaction is reversible (5) and that the best conformations of the 23*S*-iodides have unfavorable *gauche* interactions not present in the prevailing side-chain conformation of the 23*R*-iodide (2).

In summary, we have described an efficient method for incorporating deuterium or tritium into fluorinated 15-ketosterols. The isotopic hydrogen was introduced by tributyltin deuteride or tritide reduction of an iodide in the presence of multiple functional groups. The location of the ^3H at C-23 of the three fluorinated 15-ketosterols provides labeled compounds for which stability during their metabolism can be anticipated for many potential *in vitro* and *in vivo* investigations. To our knowledge, tributyltin tritide has been employed only three times previously (7a, 15, 16) for the preparation of ^3H-labeled organic compounds and only once previously for the synthesis of a tritiated steroid (7a).

Experimental

TLC was performed using precoated silica gel G plates (0.25-mm layer thickness; Analtech, Newark, DE). Solvent systems for TLC were: SS-1, ethyl acetate-hexane 1:9; SS-2, ethyl acetate-hexane 2:8; SS-3, ethyl acetate-hexane 4:6; SS-4, ethyl acetate-hexane 1:1; SS-5, ethyl acetate-hexane 6:4; SS-6, ether-benzene 5:95; SS-7, ether-benzene 1:1; SS-8, ether-benzene 6:4; SS-9, acetone-toluene 3:7; SS-10, acetone-toluene 1:3. TLC plates were charred by spraying with 5% ammonium molybdate(VI) in 10% sulfuric acid followed by heating. Radio TLC analyses were carried out by scraping 1-cm sections of the developed TLC plate directly into a 7-ml vial for scintillation counting (10 min). Medium-pressure liquid chromatography (MPLC) was done on a column (500 × 10 mm i.d. or 700 × 10 mm i.d.) dry packed with silica gel (230-400 mesh; EM Science, Gibbstown, NJ). Unless specified otherwise, fraction volumes were 20 ml. High performance liquid chromatography (HPLC) was performed at 1 ml/min on a 5-μm Spherisorb ODS-II column (250 × 4.6 mm i.d.; Custom LC, Houston, TX) with a Waters 510 pump, Rheodyne 7125 injector, and UV detection at 259 or 210 nm.

Radioactivity was measured on a Packard model 1500 or model 4640 liquid scintillation analyzer using 0.4% 2,5-diphenyloxazole in toluene or ScintiVerse from Fisher

Scientific (Fair Lawn, NJ) as scintillation fluid. ^{1}H and ^{13}C NMR spectra were measured on an AMX-500 spectrometer as described previously (5). ^{2}H NMR spectra were acquired unlocked in $CHCl_3$ solution at 77 MHz with proton decoupling during acquisition and referenced to $CDCl_3$ at δ 7.26; 1.1-s acquisition time, 0.8-s relaxation delay, 90° pulse, 2-Hz exponential line broadening. Mass spectra (electron impact) were recorded by direct probe on a ZAB-HF sector instrument and/or a Shimadzu QP-1000 quadrupole instrument or by GC-MS on an Extrel ELQ-400 quadrupole instrument. Levels of deuterium incorporation were estimated from the intensity of M^+ relative to its isotope peaks.

Tri-*n*-butyltin tritide was prepared as described previously (7a) by adding tri-*n*-butyltin chloride to a suspension of sodium borotritide and sodium borohydride in absolute ethanol. A white precipitate formed instantaneously. The mixture was stirred under nitrogen for 1 h at 23°C and evaporated under nitrogen at 50°C to a cloudy oil, which was dried in vacuo for 10 min. Tri-*n*-butyltin deuteride was prepared analogously or purchased in 97% isotopic purity from Aldrich Chemical Company (Milwaukee, WI). The following reagents were obtained commercially: AIBN from Janssen Chimica (San Diego, CA); tri-*n*-butyltin chloride from Acros Organics (Pittsburgh, PA); sodium borohydride and sodium borodeuteride from Aldrich; and sodium borotritide (17) from Amersham (Arlington Heights, IL). Iodide preparations **5a** and **5b** (2), **7a** and **7b** (3), and **9a** and **9b** (4, 5) were synthesized as described previously.

Syntheses of deuterium-labeled 15-ketosterols

[23*RS*-^{2}H]3β-Acetoxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one ([^{2}H]-6). To a solution of $NaBD_4$ (7.8 mg, 0.188 mmol) in ethanol (0.5 ml) was added tributyltin chloride (51 μl, 0.188 mmol). The suspension was stirred under argon for 1 h, evaporated in a stream of argon, and dried in vacuo for 5 min. A solution of **5a**, **5b**, and **6** (88:9:3 mixture; 50 mg, 0.072 mmol) and AIBN (5 mg) in tetrahydrofuran (1 ml) was added under argon, and the mixture was stirred for 4 h. The reaction was poured into water and extracted with ethyl acetate (2 × 25 ml). The combined organic extracts were washed with water (3 × 25 ml), dried over sodium sulfate, and evaporated to a white solid (44 mg). Column chromatography (4 g silica gel, 70-230 mesh; elution with ethyl acetate-hexane 5:95) followed by evaporation of fractions 12-19 gave [^{2}H]-**6** (36 mg): mp 187-188°C (lit. mp for unlabeled **6**, 187-188°C (2)); TLC, single component in SS-2 (R_f 0.64) and SS-6 (R_f 0.59); >99% purity by ^{1}H NMR (containing 0.3% 3β-acetoxy-5α-chola-8(14),24-dien-15-one); ^{13}C NMR signals matched those of unlabeled **6** within 0.1 ppm; ^{2}H NMR showed a 5:4 ratio of singlets at δ 1.48 (H-23*S*) and 1.64 (H-23*R*); GC-MS showed 29% d_0, 71% d_1.

Similar reduction of the same iodide preparation (50 mg; 0.072 mmol) with commercial tributyltin deuteride (51 μl, 0.188 mmol) and AIBN (2 mg) in tetrahydrofuran (1 ml) gave [^{2}H]-**6** of similar purity: mp 187-189°C (after recrystallization from methanol);

TLC, single component in SS-2 (R_f 0.64) and SS-6 (R_f 0.59); MS and GC-MS indicated 5% d_0, 95% d_1; ^1H, ^2H, and ^{13}C NMR essentially identical to [^2H]-**6** described above.

[23*RS*-^2H]3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one ([^2H]-2). A solution of the acetate [^2H]-**6** (30 mg) in degassed methanol (1 ml) and degassed tetrahydrofuran (0.5 ml) was stirred with potassium carbonate (29 mg) for 3 h at room temperature. The reaction was poured into water and extracted with ethyl acetate (2 × 25 ml). The combined organic layers were washed with water (3 × 25 ml), dried over sodium sulfate, and evaporated to a white solid (28 mg). Column chromatography (3 g silica gel, 70-230 mesh; elution with hexane (100 ml), ethyl acetate-hexane 5:95 (100 ml), and ethyl acetate-hexane 1:9) followed by evaporation of fractions 14-20 gave [^2H]-**2** (25 mg). An analytical sample was obtained by recrystallization from methanol: mp 177-178 (lit. mp for unlabeled **2**, 177-179 °C (2)); TLC, single component in SS-3 (R_f 0.39) and SS-7 (R_f 0.36); ≥99% purity by ^1H NMR; ^{13}C NMR shieldings matched those of unlabeled **2** within 0.1 ppm; GC-MS (trimethylsilyl ether derivative) indicated 6% d_0, 94% d_1; ^2H NMR showed a 5:4 ratio of singlets at δ 1.49 (H-23*S*) and 1.64 (H-23*R*).

[23*RS*-^2H]3β-Acetoxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one ([^2H]-8). Reduction of a mixture of 7α-methyl-15-ketosterols **7a**, **7b**, and **8** (82:11:7; 100 mg, 0.142 mmol) with tributyltin deuteride (prepared from $NaBD_4$ (15.5 mg) and tributyltin chloride (100 µl) in ethanol (1 ml)) as described above gave [^2H]-**8** as a white solid (85 mg). An analytical sample was obtained by recrystallization from methanol: mp 127-128 °C (lit. mp for unlabeled **8**, 128-129 °C (3)); TLC, single component in SS-2 (R_f 0.68) and SS-6 (R_f 0.72); ≥99% purity by ^1H NMR (excluding minor organotin contaminants); ^{13}C NMR signals matched those of **8** within 0.12 ppm; GC-MS indicated 15% d_0, 85% d_1; ^2H NMR showed a 5:4 ratio of singlets at δ 1.48 (H-23*S*) and 1.65 (H-23*R*).

Similar reduction the same 7α-methyl-iodide preparation (50 mg; 0.071 mmol) with commercial tributyltin deuteride (49.8 µl, 0.184 mmol) and AIBN (2 mg) in tetrahydrofuran (1 ml) gave [^2H]-**8** (41 mg) of similar purity: 127-129 °C; TLC, single component in SS-2 (R_f 0.68) and SS-6 (R_f 0.72); GC-MS indicated 7% d_0, 93% d_1; ^1H, ^{13}C, and ^2H NMR essentially identical to [^2H]-**8** described above.

[23*RS*-^2H]3β-Hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one ([^2H]-3). Hydrolysis as above of a solution of [^2H]-**8** (50 mg) gave [^2H]-**3** as a white solid (42 mg): mp 152-153 °C (lit. mp for unlabeled **3**, 153.5-154.5°C (3)); TLC, single component in SS-3 (R_f 0.48) and SS-7 (R_f 0.42); ~99% purity by ^1H NMR; ^{13}C NMR signals matched those of **3** within 0.1 ppm; GC-MS (trimethylsilyl ether) indicated 8% d_0, 92% d_1; ^2H NMR showed a 4:3 ratio of singlets at δ 1.47 (H-23*S*) and 1.64 (H-23*R*).

[23*RS*-^2H]3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one ([^2H]-4). Reduction of a mixture of **9a**, **9b**, and **4** (13:3:4 ratio; 50 mg, 0.077 mmol)

with tributyltin deuteride (prepared from $NaBD_4$ (8.4 mg) and tributyltin chloride (54 μl) in ethanol (0.5 ml)) as described above gave a crude product (44 mg) that was chromatographed on silica gel (4 g, 70-230 mesh; elution with hexane (100 ml), ethyl acetate-hexane 5:95 (100 ml), ethyl acetate-hexane 1:9 (250 ml), and ethyl acetate-hexane 15:85). Evaporation of fractions 21-29 gave [^{2}H]-**4** (40 mg); recrystallization from methanol furnished an analytical sample: mp 146-147 °C (lit. mp for unlabeled **4**, 146-147°C (5)); TLC, single component in SS-3 (R_f 0.47) and SS-7 (R_f 0.40); 98% purity by ^{1}H NMR (containing 1% (23*E*)-3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholesta-8(14),23-dien-15-one); ^{13}C NMR signals matched those of **4** within 0.1 ppm; MS and GC-MS (trimethylsilyl ether) indicated 42% d_0, 54% d_1; ^{2}H NMR showed a 3:2 ratio of singlets at δ 1.47 (H-23*S*) and 1.66 (H-23*R*).

Syntheses of tritium-labeled 15-ketosterols

[23-^{3}H]3β-Hydroxy-7α-methyl-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one ([^{3}H]-3). To [^{3}H]tributyltin hydride (prepared from sodium borotritide (500 mCi, 16.3 Ci/mmol), sodium borohydride (10.4 mg, 0.28 mmol), and tributyltin chloride (111 mg, 0.31 mmol in ethanol (1.2 ml)) were added an 18:3:1 mixture of **7a**, **7b**, and **8** (98.8 mg; 0.139 mmol), AIBN (4.5 mg) and anhydrous tetrahydrofuran (2.4 ml). The reaction mixture was stirred at room temperature under nitrogen for 6 h and then partitioned between brine (40 ml) and ether (40 ml). The aqueous layer was further extracted with ether (2 × 40 ml), and the combined organic phase was washed with brine (50 ml) and dried over sodium sulfate. Radio-TLC of the crude [^{3}H]-**8** (67 mCi) on a silica gel G in SS-1 showed that 90% of the radioactivity comigrated with unlabeled **8** (R_f 0.48). To half of crude [^{3}H]-**8** (34 mCi; evaporated to an oily residue) were added degassed methanol (3 ml), degassed tetrahydrofuran (1.5 ml), and potassium carbonate (20 mg). The mixture was stirred at room temperature under nitrogen for 4.3 h and then partitioned between brine (50 ml) and ethyl acetate (50 ml). The aqueous layer was further extracted with ethyl acetate (50 ml), and the combined organic phase was washed with water (50 ml) and dried over sodium sulfate. Radio-TLC of the crude [^{3}H]-**3** on silica gel G (5 × 20 cm plate; elution with ethyl acetate-hexane 3:7) showed a 3:1 mixture of radioactivity that comigrated with unlabeled free sterol **3** (R_f 0.43) and unlabeled acetate **8** (R_f 0.91). Evaporation of the solvent under nitrogen and in vacuo gave an oily product, which was adsorbed onto silica gel and subjected to MPLC on silica gel (500 × 10 mm i.d. column; elution with ether-hexane 6:94 (1200 ml), ethyl acetate-hexane 6:94 (1000 ml), ethyl acetate-hexane 1:9 (500 ml), and ethyl acetate-hexane 15:85). Fraction volumes were 15 ml. Fractions 32-53 contained material (5.6 mg; 1.5 mCi) corresponding to unreacted acetate. Fractions 150-176 were evaporated to give [^{3}H]-**3** (21.2 mg; 179 mCi/mmol): single radioactive component comigrating with unlabeled **3** by TLC and radio-TLC in SS-5 (R_f 0.44), SS-8 (R_f 0.46), and SS-9 (R_f 0.31); single component that comigrated with unlabeled **3** (t_R 9.3 min) on radio-HPLC (Figure 3B).

[23-^{3}H]3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one ([^{3}H]-2). As described above, reaction of tributyltin tritide (prepared as above from 250 mCi of sodium borotritide, 8.8 Ci/mmol) with a 88:9:3 mixture of **5a**, **5b**, and **6** (50 mg, 0.072 mmol), AIBN (2.8 mg), and anhydrous tetrahydrofuran (2.0 ml) for 6 h gave a crude product that was hydrolyzed for 6 h with potassium carbonate (25 mg) in degassed methanol (4 ml) and degassed tetrahydrofuran (3 ml). Radio-TLC in SS-4 showed a 4:1 mixture of materials that comigrated with [^{3}H]-**2** (R_f 0.33) and [^{3}H]-**6** (R_f 0.73). The crude product was adsorbed onto silica gel (280 mg) and subjected to MPLC (700 × 10 mm i.d. column; elution with ether-hexane 6:94 (1400 ml), ethyl acetate-hexane 9:91 (2000 ml), ethyl acetate-hexane 12:88 (1000 ml), ethyl acetate-hexane 16:84 (1000 ml), and ethyl acetate-hexane 20:80). Evaporation of fractions 139-188 gave a white solid (11.2 mg; 139 mCi/mmol): single component comigrating with unlabeled **2** on TLC in SS-5 (R_f 0.44), SS-8 (R_f 0.46), and SS-9 (R_f 0.33); single component comigrating with unlabeled **2** (t_R 9.0 min) on radio-HPLC (Figure 3A).

[23-^{3}H]3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one ([^{3}H]-4). Reaction of a 13:3:4 mixture of **9a**, **9b**, and **4** (50 mg, 0.076 mmol) with tributyltin tritide (prepared as above from 250 mCi of sodium borotritide, 8.8 Ci/mmol) and AIBN (2.5 mg) in anhydrous tetrahydrofuran (2.0 ml) for 6 h as described above gave a crude product that was adsorbed onto silica gel (300 mg) and subjected to MPLC (700 × 10 mm i.d. column; elution with ether-hexane 5:95 (2000 ml) and ethyl acetate-hexane 9:91 (2000 ml)). Evaporation of fractions 86-122 gave a white solid (36 mg; 82 mCi/mmol): single component comigrating with unlabeled **4** by TLC in SS-4 (R_f 0.42), SS-8 (R_f 0.39), and SS-10 (R_f 0.46); major component comigrating with unlabeled **4** (t_R 10.4 min; 94%) accompanied by minor component (t_R 9.9 min; 4%) by radio-HPLC (Figure 3C). The latter may correspond to the 14β isomer of [^{3}H]-**4**.

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References and Notes

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7. (a) Parnes H. and Pease J. – J. Org. Chem. 44: 151-152 (1979). (b) Tributyltin deuteride is also commercially available in 97% isotopic purity. (c) The specific activity attainable for tributyltin tritide is limited by the specific activity of sodium borotritide (commercially available at levels up to ~15 Ci/mmol). Tributyltin tritide of high specific activity can also be obtained either by quenching Bu_3SnLi with tritiated water (7a) or by treating tributyltin chloride with Li^3H prepared from carrier-free tritium gas (7d). (d) Jaiswal D.K., Andres H., Morimoto, H., and Williams, P.G. – J. Chem. Soc., Chem. Commun. 907-909 (1993).

8. (23*E*)-3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholesta-8(14),23-dien-15-one and analogous Δ^{23} sterols were observed as trace (≤1%) contaminants in some reduction products, as noted previously (5).

9. This contaminant may be the 14β isomer, which can be removed by recrystallization (5). Recrystallization also removes traces of organotin contaminants, which may be present in unrecrystallized material (5). In the case of the other F_7-15-ketosterols, any organotin contaminants are removed during the acetate hydrolysis (or methanolysis) and the subsequent chromatographic purification. If the saturated 15-ketosterol **4** were subjected to these conditions, extensive epimerization would occur at C-14 (5). In order to minimize the levels of organotin contaminants in [^{3}H]-**4**, a large volume of weak solvent was passed through the MPLC column prior to elution of [^{3}H]-**4**.

10. Iodide preparations may also be contaminated by traces of the C_{24}-Δ^{23} steroid starting material (e.g. 3β-acetoxy-5α-chola-8(14),23-dien-15-one). The three C_{24}-Δ^{23} steroids are inert to tributyltin hydride and have chromatographic properties almost identical to those of the corresponding F_7-15-ketosterols, both as acetates and free sterols (5).

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14. Relative amounts of 23*R* and 23*S* epimers were estimated from ^{2}H NMR spectra, which show chemical shifts very similar to those in ^{1}H NMR spectra. Assignment of 23*R* and 23*S* configuration was based upon ^{1}H NMR assignments of the pro-*R* and pro-*S* hydrogens at C-23 of unlabeled **2**, **3**, and **4** (2, 3, 5). Stereochemical assignments of the 23-iodides are based upon analysis of NMR shieldings and coupling constants for the 23*R* epimer in conjunction with conformational analysis grounded in molecular modeling (2). Iodide and deuterium designated as 23*R* in the RS nomenclature system have the same stereochemical orientation as substituents at C-23.

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17. The specific activities and levels of ^{3}H of the sodium borotritide used in these studies were those provided by the commercial supplier.

