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INHIBITORS OF STEROL SYNTHESIS: 3β-HYDROXY-25,26,26,26,27,27,27-HEPTAFLUORO-5α-CHOLESTAN-15-ONE, AN ANALOG OF A POTENT HYPOCHOLESTEROLEMIC AGENT IN WHICH ITS MAJOR METABOLISM IS BLOCKED

Shankar Swaminathan[a], Abdul U. Siddiqui[a], Frederick D. Pinkerton[a], Nicolas Gerst[a], William K. Wilson[a], and George J. Schroepfer, Jr.[a,b]*

Departments of [a] Biochemistry and Cell Biology and [b] Chemistry
Rice University, P.O. Box 1892, Houston, TX 77251

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Summary: The chemical synthesis of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one (**IV**) has been pursued to provide an analog of the potent hypocholesterolemic agent 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) in which its major metabolism is blocked. Reduction of 3β-acetoxy-5α-chola-8(14),23-dien-15-one with lithium in liquid ammonia gave 3β-hydroxy-5α-chol-23-en-15-one (**VI**). Addition of $(CF_3)_2CFI$ to **VI** in the presence of triethylborane gave 3β-hydroxy-23R-iodo-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one, which was reduced to **IV** with tributyltin hydride. **IV** was found to be highly active in lowering the levels of HMG-CoA reductase activity in CHO-K1 cells, in lowering acyl coenzyme A:cholesterol acyltransferase activity in jejunal microsomes, and in lowering serum cholesterol levels in rats.

3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) (Figure 1) is a potent hypocholesterolemic agent upon oral administration to rodents (1) and nonhuman primates (2,3). **I** is highly active as an inhibitor of sterol synthesis in cultured mammalian cells and lowers the levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (4,5). **I** is also a potent inhibitor of cholesterol absorption in animals (6) and inhibits acyl coenzyme A:cholesterol acyltransferase (ACAT) activity in jejunal and hepatic microsomes (7).

I is convertible to cholesterol upon incubation with rat liver homogenate preparations (8,9) and upon oral and intravenous administration to rats and baboons (10-13). A quantitatively much more important fate of **I** in rats is conversion to polar metabolites which are excreted in bile and of which a substantial fraction undergoes enterohepatic circulation (11). The bulk of the formation of the polar metabolites appears to be initiated by side-chain oxidation at C-26 (14,15). To evaluate the effect of blockage of the side-chain oxidation of **I**, a fluorinated analog, 3β-hydroxy-

*To whom correspondence should be directed.

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ACAT, acyl coenzyme A:cholesterol acyltransferase; IR, infrared; MS, mass spectra; NMR, nuclear magnetic resonance; TLC, thin layer chromatography; HPLC, high performance liquid chromatography; AIBN, 2,2'-azobisisobutyronitrile.

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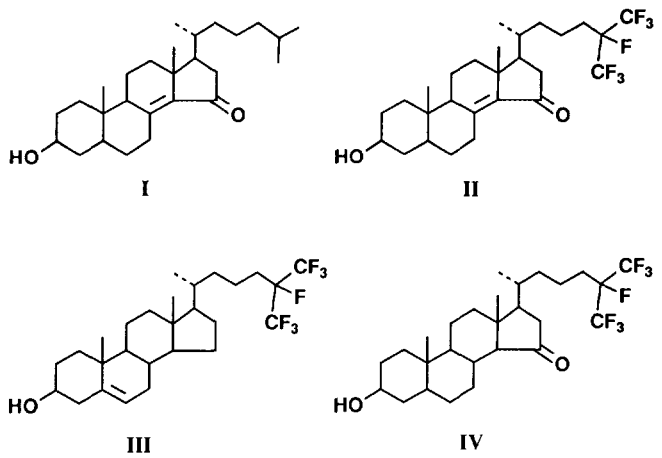

Figure 1. Structures of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**), 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**II**), 25,26,26,26,27,27,27-heptafluorocholesterol (**III**), and 3β-hydroxy-25,26,26,26,27,27-heptafluoro-5α-cholestan-15-one (**IV**).

25,26,26,26,27,27,27-heptafluoro-5α-cholest-8(14)-en-15-one (**II**), was prepared by chemical synthesis (16). **II** has been shown to be highly active in lowering HMG-CoA reductase activity in cultured mammalian cells (16). In contrast to **I**, **II** lowered serum cholesterol levels in rats without suppression of food consumption (17). A potentially undesirable effect observed after oral administration of **II** was the accumulation of 25,26,26,26,27,27,27-heptafluorocholesterol (**III**) in blood and liver. This finding indicated the conversion of **II** to **III**, presumably by the same series of reactions delineated for the conversion of **I** to cholesterol (9). To eliminate this conversion to **III**, we have prepared the saturated analog of **II**, i.e., 3β-hydroxy-25,26,26,26,27,27,27-5α-cholestan-15-one (**IV**) (Figure 2). **IV** has been shown to be highly active in lowering the levels of HMG-CoA reductase activity in CHO-K1 cells, in inhibiting ACAT activity in jejunal microsomes, and in lowering serum cholesterol levels in rats.

MATERIALS AND METHODS

The recording of melting points (m.p.), infrared (IR) spectra, and ^{1}H and ^{13}C NMR spectra were carried out as described in detail previously (16). Mass spectra (MS) were recorded on a Kratos MS-50DA spectrometer at the Midwest Center for Mass Spectrometry (Lincoln, NE). Thin layer chromatography (TLC) was carried out on silica gel G plates (Analtech; Newark, DE) except that Whatman LK5D plates (Whatman, Inc., Clinton, NJ) were used in assays of HMG-CoA reductase. Solvent systems used for TLC were: SS-1, 40% ethyl acetate in hexane; SS-2, 50% ether in benzene. High performance liquid chromatography (HPLC) was carried out using a 5 μm Customsil ODS reversed phase column (250 mm × 4.6 mm; Custom LC; Houston, TX) using 5% water in methanol as solvent at 1.0 ml per min.

Triethylborane and tributyltin hydride 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). 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) (18) and 3β-acetoxy-5α-chola-8(14),23-dien-15-one (**V**) (16) were prepared as described previously. **V**, with a m.p. of 156-

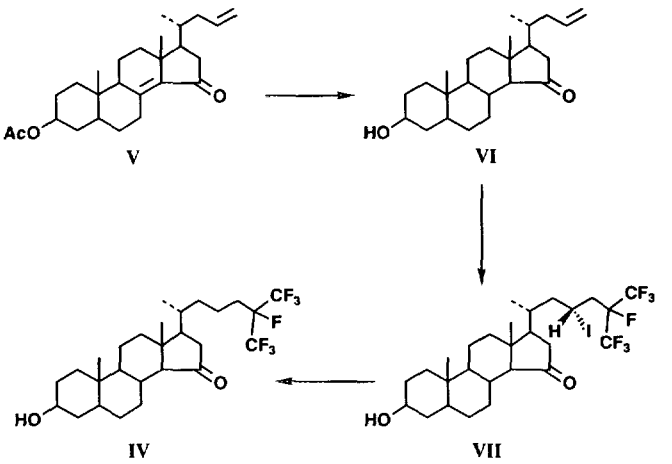

Figure 2. Chemical synthesis of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one (**IV**).

157°C, showed a single component on TLC in two solvent systems (SS-1 and SS-2) and a purity of >99% by ^{1}H NMR at 500 MHz.

The effect of **IV** on HMG-CoA reductase activity was studied in CHO-K1 cells as described previously (16). The effect of **IV** on the levels of ACAT activity in rat jejunal microsomes, isolated by a modification of the method of Suckling *et al.* (19), was assayed using minor modifications of the conditions described by Helgerud *et al.* (20). The effect of **IV**, prepared by a modification of the method described below, on serum cholesterol levels was studied in male Sprague-Dawley rats as described in detail previously (17). Two control groups, rats fed Purina Formulab 5008 diet either *ad libitum* or pair-fed to experimental animals, were employed as described previously (1,17).

3β-Hydroxy-5α-chol-23-en-15-one (VI)

3β-Acetoxy-5α-chola-8(14),23-dien-15-one (**V**; 504 mg) was dissolved in ether and cooled in a dry ice-acetone bath. Dry liquid ammonia (~100 ml) was condensed in the flask followed by the addition, in one portion, of lithium (260 mg). After stirring for 15 min, *tert*-butanol (20 ml) was added and the resulting mixture was cautiously poured onto ice. After extraction with ethyl acetate, the organic phase was evaporated to dryness and subjected to silica gel column (15 cm × 1 cm) chromatography using 10% ethyl acetate in hexane (250 ml) and 15% ethyl acetate in hexane (250 ml) as the eluting solvents. Fractions 22 ml in volume were collected. The contents of fractions 14-22 were combined and evaporated to dryness to give **VI** (180 mg): m.p. 153-154°C; single component on TLC in two solvent systems (SS-1 and SS-2); IR, ν_{max} 3350, 2926, 2857, 1734, 1640, 1449, 1383, 1132, 1076, 1045, 995, 910 cm^{-1}; MS, 358 (82; M^+), 343 (5; M–CH_3), 325 (4; M–H_2O–CH_3), 317 (11; M–C_3H_5), 299 (7; M–C_3H_5–H_2O), 261 (100; M–SC–C_2H_4); high resolution MS, 358.2862 (calcd. for $C_{24}H_{38}O_2$: 358.2870); ^{1}H NMR, δ 0.75 (s), 0.81 (s), 1.01 (d, 6.2 Hz), 1.67 (d, ~10.6 Hz), 3.59 (tt, ~5, 11.1 Hz), 5.01 (m), 5.76 (dddd, 6.0, 8.5, 10.4, 16.7 Hz); ^{13}C NMR, δ 13.0 (C-18), 65.8 (C-14), 71.2 (C-3), 215.9 (C-15).

3β-Hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one (IV)

To **VI** (50 mg) in hexane (12 ml) was added sufficient 2-iodoheptafluoropropane (0.3 ml) to dissolve the sterol. Triethylborane (0.1 ml; 1 M solution in hexanes) was added, and the resulting mixture was stirred for 2 h at room temperature. The mixture was passed through a column (7 cm × 0.5 cm, i.d.) of silica gel using hexane (50 ml) and 50% ethyl acetate in hexane as the eluting solvents. Fractions 50 ml in volume were collected. Evaporation of the contents of fraction 4 gave a product composed predominantly of 3β-hydroxy-23R-iodo-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one (**VII**; ~70 mg) by ^{1}H NMR and showing a single component on TLC in two solvent systems (SS-1 and SS-2). To a solution of the iodide (~70 mg) and AIBN (8 mg) in dry

tetrahydrofuran (10 ml) was added tributyltin hydride (0.46 ml) under argon. After 6 h, the solvent was evaporated and the resulting residue was dissolved in CH_2Cl_2 (1 ml) and subjected to silica gel column (19 cm × 1 cm i.d.) chromatography, with successive elution with hexane (300 ml), 5% ethyl acetate in hexane (500 ml), and 10% ethyl acetate in hexane. Fractions 20 ml in volume were collected. The contents of fractions 70-77 were pooled (48 mg) and recrystallized twice from methanol to give **IV** (21 mg): m.p. 146-147°C; single component on TLC in two solvent systems (SS-1 and SS-2) and on HPLC (t_R 6.9 min); IR, ν_{max} 3400, 2924, 2849, 1736, 1317, 1223, 1157, 1132, 1042, 939, and 719 cm^{-1}; MS, 528 (100; M^+), 513 (12; M–CH_3), 510 (15; M–H_2O), 495 (9; M–H_2O–CH_3), 477 (3; M–$2H_2O$–CH_3), 456 (6), 438 (3), 335 (55), 319 (7), 295 (2), 289 (13; M–SC), 276 (9), 271 (6; M–SC–H_2O), 266 (7), 261 (93; M–SC–C_2H_4), 259 (4), and 253 (4; M–SC–$2H_2O$); high resolution MS, 528.2837 (calcd. for $C_{27}H_{39}O_2F_7$: 528.2838); ^1H NMR, δ 0.75 (s), 0.81 (s), 1.01 (d, 6.2 Hz), 1.68 (d, 10.4 Hz), 3.59 (tt, 4.6, 11.1 Hz); ^{13}C NMR, δ 13.0 (C-18), 65.8 (C-14), 71.1 (C-3), 91.7 (C-25), 121.0 (C-26, C-27), 215.5 (C-15).

RESULTS AND DISCUSSION

Our recent demonstration of a remarkably efficient and specific oxidation of the side chain of the acetate derivative of **I** (21) provided a facile route (16) for the preparation of the C_{24} steroidal starting material (**V**) for the chemical synthesis of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one (**IV**). Reduction of **V** with lithium in liquid ammonia gave, after silicic acid chromatography, 3β-hydroxy-5α-chol-23-en-15-one (**VI**). The saturated F_7-15-ketosterol **IV** was constructed from **V** by the successful adaptation of reactions (16) developed for the chemical synthesis of the F_7-$\Delta^{8(14)}$-15-ketosterol (**II**). Thus, treatment of **VI** with 2-iodoheptafluoropropane in the presence of triethylborane gave the 23-iodo-F_7-15-ketosterol (**VII**) which, upon reduction with tributyltin hydride, provided the desired saturated F_7-15-ketosterol **IV**.

The saturated F_7-15-ketosterol **IV** was highly active in the lowering of HMG-CoA reductase activity in CHO-K1 cells (Table 1). The potency of **IV** was essentially the same as that of **I**. The activity of **IV** (IC_{50} ~3.6 μM) in the inhibition of the oleoyl-CoA-dependent esterification of

Table 1. Effect of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one (**IV**) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) on the levels of HMG-CoA reductase activity in CHO-K1 cells

Sterol (μM)	HMG-CoA Reductase Activity (% of Control)	
	I[a]	IV[b]
0.0	100.0	100.0
0.1	62.5 ± 2.8	71.5 ± 10.1
0.25	45.8 ± 2.0	47.3 ± 4.2
0.50	36.6 ± 1.6	43.7 ± 7.6
1.0	28.8 ± 1.4	36.1 ± 3.9
2.5	23.6 ± 1.6	28.1 ± 3.8

[a] Mean ± S.E.M. of 40 independent experiments in which triplicate determinations of enzyme activity were made at each concentration.

[b] Mean ± S.E.M. of 3 independent experiments in which triplicate determinations of enzyme activity were made at each concentration.

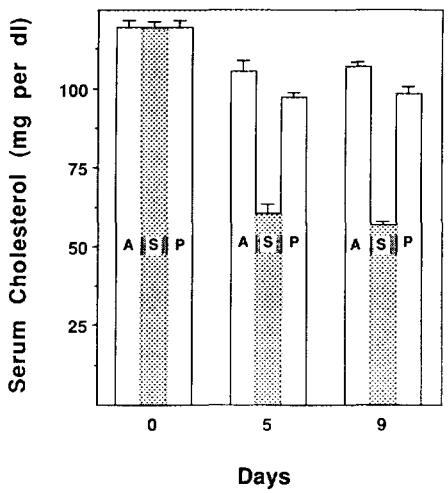

Figure 3. Effect of dietary administration of 3β-hydroxy-25,26,26,26,27,27,27-heptafluoro-5α-cholestan-15-one (**IV**; 0.125% by weight in diet; 2.37 μmoles per g of diet) on serum cholesterol levels in male Sprague-Dawley rats. A, *ad libitum* controls; S, experimental animals treated with **IV**; P, pair-fed controls (N = 8 for each group; results presented as mean ± S.E.M.). Day 0 values used for matching of animals were determined by enzymatic assay, whereas those on days 5 and 9 were determined by capillary GC analysis.

cholesterol by rat jejunal microsomes was similar to that of **I** (IC_{50} ~2.7 μM). Dietary administration of **IV**, at a concentration of 0.125% by weight in diet, resulted in a moderate suppression of food consumption (with an average reduction of 16% on days 2-10), very considerably less than the marked suppression of food consumption (average of –48% over the same period) caused by **I** at an equimolar level in diet (17). **IV** had significant hypocholesterolemic action upon dietary administration to rats (Figure 3). After administration of **IV** (0.125% by weight in diet) for 9 days, serum cholesterol levels were reduced 47% and 43% (p=0.0001) relative to *ad libitum* and pair-fed control animals, respectively (Figure 3). In contrast to the case of administration of the F_7-15-ketosterol **II** to rats (17), no accumulation of F_7-cholesterol (**III**) in serum or liver was detected after dietary administration of **IV**.

The saturated F_7-15-ketosterol **IV** was constructed so as to block the major metabolism of the hypocholesterolemic agent **I**. The results presented herein indicate that this design provides a potentially promising analog of **I**.

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