Inhibitors of sterol synthesis. Chemical synthesis and spectral properties of 3β-hydroxy-5α-cholesta-8(14),24-dien-15-one, 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one, and 3β,24-dihydroxy-5α-cholest-8(14)-en-15-one and their effects on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in CHO-K1 cells

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Abstract Side-chain functionalized $\Delta^{8(14)}$-15-ketosterols have been synthesized from 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VI**) as part of a program to prepare potential metabolites and analogs of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**), a potent regulator of cholesterol metabolism. Oxidation of **VI** to the 24-aldehyde **VII**, followed by Wittig olefination with isopropyltriphenylphosphonium iodide gave 3β-acetoxy-5α-cholesta-8(14),24-dien-15-one (**VIII**), which was hydrolyzed to the free sterol **IX**. Oxymercuration of **VIII** followed by hydrolysis of the 3β-acetate gave 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (**IV**). Hydroboration-oxidation of **VIII** followed by hydrolysis of the 3β-acetate gave 3β,24-dihydroxy-5α-cholest-8(14)-en-15-one (**V**) as a 5:4 mixture of the 24R and 24S epimers. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) assignments and mass spectral fragmentation patterns, supported by high-resolution measurements, are presented for **IV** and its 3β-acetate, **V, VII, VIII,** and **IX**. Characterization of **IV** by NMR and of trimethylsilyl ethers of **IV** and **V** by gas chromatography–mass spectrometry was compatible with spectral data for samples of **IV** and **V** isolated previously after incubation of **I** with rat liver mitochondria in the presence of NADPH. Sterols **IV, V,** and **IX** were very potent in lowering of the level of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in Chinese hamster ovary cells; their potency was comparable to that of **I**. —**Swaminathan, S., F. D. Pinkerton, S. Numazawa, W. K. Wilson, and G. J. Schroepfer, Jr.** Inhibitors of sterol synthesis. Chemical syntheses and spectral properties of 3β-hydroxy-5α-cholesta-8(14),24-dien-15-one, 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one, and 3β,24-dihydroxy-5α-cholest-8(14)-en-15-one and their effects on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in CHO-K1 cells. *J. Lipid Res.* 1992. **33:** 1503–1515.

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3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) is a novel regulator of cholesterol metabolism. **I** is a potent inhibitor of sterol biosynthesis and lowers the level of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in cultured mammalian cells (1–4). In addition, dietary administration of **I** to rats results in a marked inhibition of the absorption of cholesterol (5, 6). The 15-ketosterol serves as a substrate for acyl coenzyme A:cholesterol acyltransferase (ACAT) and inhibits the oleoyl-CoA-dependent esterification of cholesterol in hepatic and jejunal microsomes (7). Oral administration of **I** to rats has been shown to result in a reduction of ACAT activity of jejunal microsomes (8). The 15-ketosterol has been shown to lower serum cholesterol levels upon oral administration to animals (9–11).

An understanding of the metabolism of **I** is critical to considerations of its actions. **I** is convertible to cholesterol,

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; CHO, Chinese hamster ovary; DEPT, distortionless enhancement by polarization transfer; GC, gas chromatography; HETCOR, ^{1}H-^{13}C shift-correlated spectroscopy; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IR, infrared; MS, mass spectrometry or mass spectrum; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; SC, side chain; TLC, thin-layer chromatography; TMS, trimethylsilyl or trimethylsilyloxy.

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a transformation that has been demonstrated in vitro in rat liver subcellular preparations (12, 13) and in vivo after oral or intravenous administration to rats and baboons (14–19). 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 (16). However, a quantitatively more important fate of **I** under these conditions is very rapid conversion to polar metabolites that are excreted in bile (16) and of which a significant fraction undergoes enterohepatic circulation (16). In our initial studies of the chemical nature of these polar metabolites of **I,** we have shown that (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**) is the major metabolite of **I** formed upon its incubation with rat liver mitochondria in the presence of NADPH (20–22). **II,** prepared by chemical synthesis, was shown to be very potent in lowering the levels of HMG-CoA reductase activity in Chinese hamster ovary (CHO-K1) cells (20, 23). Other metabolites detected after incubation of **I** with rat liver mitochondria included (25S)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one, 3β-hydroxy-15-keto-5α-cholest-8(14)-en-26-oic acid (**III**), 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (**IV**), and 3β,24-dihydroxy-5α-cholest-8(14)-en-15-one (**V**) (21, 22) (**Fig. 1**).

We have recently found that oxidation of the 3β-acetate derivative of **I** with a mixture of trifluoroacetic anhydride, hydrogen peroxide, and sulfuric acid gives 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VI**) in remarkably high yield (24, 25). The availability of **VI,** selectively protected at C-3, provided a key intermediate for the chemical syntheses of 3β-hydroxy-5α-cholesta-8(14),24-dien-15-one (**IX**), 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (**IV**), and 3β,24-dihydroxy-5α-cholest-8(14)-en-15-one (**V**) (**Fig. 2**). Described herein are the syntheses and characterization of **IV, V,** and **IX** and their effects on the level of HMG-CoA reductase activity in CHO-K1 cells. A preliminary account of a portion of these results has been presented (26).

EXPERIMENTAL PROCEDURES AND RESULTS

Materials and methods

Procedures and instrumentation for nuclear magnetic resonance (NMR), mass spectrometry (MS) by direct inlet, infrared (IR) spectroscopy, thin-layer chromatography (TLC), and assay of radioactivity have been described previously (25). Materials used in the cell culture studies and in assays of HMG-CoA reductase activity have also been described previously (25). Whatman LK5D TLC plates were used for assays of HMG-CoA reductase activity; otherwise, aluminum-backed silica gel 60 plates (EM Sciences) were used. Gas chromatography–mass spectrometry (GC–MS) was carried out at 70 eV on an Extrel ELQ-400 quadruple GC–MS system interfaced with a Varian chromatograph (model 3400) containing a DB-5 capillary column (J&W Scientific, Folsom, CA), 15 m, 0.25 mm ID, 0.1 μm film thickness, bonded stationary phase of 5% diphenyl–95% dimethyl polysiloxane. The GC column was held at 200°C for 2 min and then increased to 290°C at a rate of 10°C per min. The temperature of the injector and the transfer line was 290°C. Samples were injected along with n-alkane standards (C_{32-38} or C_{30-40}). High resolution MS were measured by

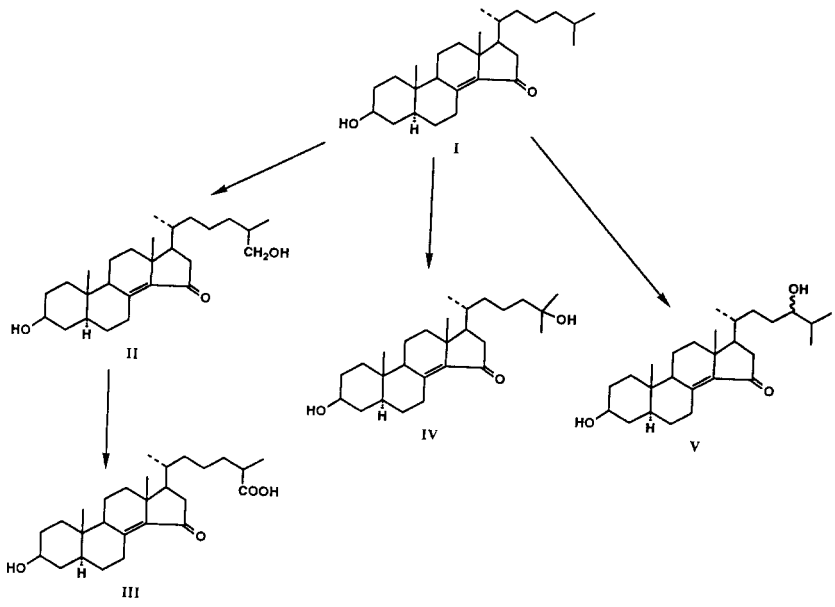

Fig. 1. Metabolism of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) upon incubation with rat liver mitochondria in the presence of NADPH.

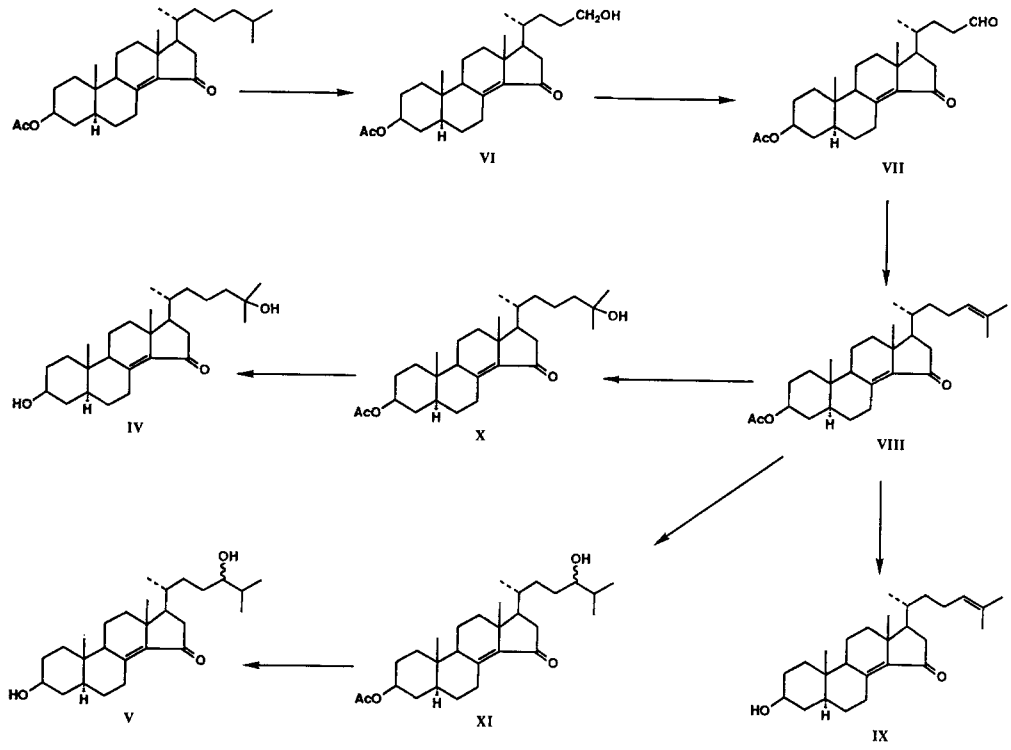

Fig. 2. Chemical syntheses of Δ^{24}, 25-hydroxy, and (24RS)-24-hydroxy derivatives of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**).

electron impact on a Kratos MS-50DA spectrometer at the Midwest Center for Mass Spectrometry (Lincoln, NE).

^{1}H and ^{13}C NMR assignments were made from a combination of DEPT and HETCOR spectra in conjunction with ^{1}H and ^{13}C chemical shift comparisons and substituent increments, as described previously (27). In addition, stereochemical assignments for the H-16 (δ 2.1, 2.4) signals of $\Delta^{8(14)}$-15-ketosterols were made by nuclear Overhauser effect (NOE) difference spectroscopy. NOE difference spectra (300 MHz, 2.5-s acquisition time, 0.5-s relaxation delay, irradiation at ~1 milliwatt for 0.7 s or 1 s, 90° read pulse, ~300 scans, zero-filling to 32k points, 1.5-Hz line broadening, identical phasing corrections applied to all spectra) were measured on nondegassed solutions of **I** and **VI** (~0.2 M in $CDCl_3$) at ambient temperature (~22°C). Molecular mechanics structures (PC Model; Serena Software, Bloomington, IN) of **I** and **VI** showed protons at positions 11β, 16β, 19, 20, and 21 to be within 2.7 Å of protons at H-18. In NOE difference spectra of **I**, irradiation of H-18 (irradiation power of 50 dB below 2 watts) led to enhancement of H-12β (δ 2.11, dt), H-20 (δ 1.57, m), and signals at δ 2.06 (dd) and δ 1.5 (m); no signals were enhanced at δ 2.36 or δ 1.6–1.7.[3] These combined results indicate that the assignments for H-16α, H-16β are δ 2.4, 2.1 and suggest tentative assignments for H-11α, H-11β as δ 1.65, 1.5. The latter assignments are supported by comparison with H-11 assignments for other steroids (28).

For cell culture studies, the sterols were added as ethanolic solutions to Ham's F12 medium (29) supplemented with 5% delipidated (30) fetal calf serum (lipid-deficient medium) and allowed to equilibrate for at least 6 h at room temperature prior to storage at 4°C. Protein in detergent solubilized extracts of cultured cells was assayed by the Peterson modification (31) of the method of Lowry et al. (32).

Studies of the effects of the oxygenated sterols on HMG-CoA reductase activity were made in CHO-K1 cells. The cells were maintained in Ham's F12 medium supplemented with 5% fetal calf serum (lipid-rich medium) in a humidified atmosphere of 5% CO_2–95% air at 37°C. Each experiment was initiated by inoculating 3.75×10^5 cells into 100-mm dishes containing the lipid-rich medium (10 ml), followed by incubation for 48 h. The medium was aspirated and, after rinsing of the plates with phosphate-buffered saline (10 ml), the cells were incubated for 18 h in lipid-deficient media (10 ml). The cells were then incubated with fresh lipid-deficient media con-

[3]Potential misinterpretations arising from irradiation of the H-18 singlet of **I** at δ 0.972 affecting the H-21 doublet at δ 0.998 or the H-22 multiplet at δ 1.08 were excluded by an additional NOE experiment in which irradiation of H-21 showed no enhancement of the H-16 doublet of doublets at δ 2.06 or any signal at δ 1.5. Similar results were obtained from NOE difference experiments with 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VI**).

taining various concentrations (from 0.0 μM to 2.5 μM) of the oxygenated sterols. Cells were harvested by scraping, and detergent-solubilized cell preparations were obtained for assay of HMG-CoA reductase activity using the method of Brown, Dana, and Goldstein (33). Replicate assays (n = 3) were carried out as described by Pinkerton et al. (3), except that the specific activity of the (3RS)-[3-^{14}C]HMG-CoA was 20,000 dpm per nmol.

3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) was prepared as described previously (34) and showed a single component on analysis by TLC. 3β-Acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VI**) was prepared from 3β-acetoxy-5α-cholest-8(14)-en-15-one as described previously (24, 25).[4] Periodinane was prepared according to Dess and Martin (35). Borane–dimethylsulfide complex (neat) was purchased from Aldrich Chemical Company (Milwaukee, WI), and isopropyltriphenylphosphonium iodide was obtained from Lancaster Synthesis (Windham, NH).

3β-Acetoxy-15-keto-5α-chol-8(14)-en-24-al (VII)

A mixture of **VI** (565 mg; 1.36 mmol) and periodinane (1.26 g; 2.99 mmol) in CH_2Cl_2 (10 ml) was stirred at room temperature for 3 h under argon. The mixture was diluted with ether (25 ml) and poured into a saturated solution of $NaHCO_3$ (40 ml) containing a sevenfold excess of sodium thiosulfate. After 10 min of occasional swirling, the layers were separated and the organic phase was washed with 10% $NaHCO_3$. The residue (600 mg) obtained upon evaporation of the solvent was subjected to chromatography on a silica gel (10 g) column (15 cm × 1 cm). Using 10% ethyl acetate in hexane as the eluting solvent, fractions 50 ml in volume were collected. The contents of fractions 3–7 were combined and, after evaporation of the solvent, gave **VII** (502 mg; 89% yield) melting at 162–164°C; IR, ν_{max} 2961, 2944, 2920, 2857, 1723, 1697, 1628, 1250, 1230, 1221, and 1032 cm^{-1}; single component (>99%) on TLC (solvent, 40% ethyl acetate in hexane; R_f 0.63). The MS data are presented in **Table 1,** and ^{13}C and ^{1}H NMR spectral assignments are presented in **Table 2** and **Table 3,** respectively.

3β-Acetoxy-5α-cholesta-8(14),24-dien-15-one (VIII)

To a cold slurry (0°C) of isopropyltriphenylphosphonium iodide (839 mg; 1.94 mmol) in tetrahydrofuran (5 ml) was added n-butyllithium (1.27 mmol) under argon. The red solution was stirred for 15 min and then added dropwise to a solution of **VII** (502 mg; 1.21 mmol) in tetrahydrofuran (4 ml) at −78°C. After washing of the flask with tetrahydrofuran to ensure a complete transfer of the ylide, the reaction mixture was stirred for 2 h at 0°C. The mixture was poured into water and extracted with ether. The ether solution was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to give a residue (700 mg) which was subjected to chromatography on a silica gel (10 g) column (15 cm × 1 cm). Using 4% ethyl acetate in hexane as the eluting solvent, fractions 50 ml in volume were collected. The contents of fractions 1–6 were combined and, after evaporation of the solvent, gave **VIII** (400 mg; 75% yield) melting at 129–130°C; IR, ν_{max} 2959, 2934, 2866, 1738, 1699, 1624, 1258, and 1030 cm^{-1}; single component (>99%) on TLC (solvent, 40% ethyl acetate in hexane; R_f 0.86). The MS data are presented in Table 1, and the ^{13}C and ^{1}H NMR spectral assignments are presented in Tables 2 and 3, respectively.

3β-Hydroxy-5α-cholesta-8(14),24-dien-15-one (IX)

To a solution of **VIII** (150 mg; 0.341 mmol) in a mixture of tetrahydrofuran (1 ml) and methanol (2 ml) was added K_2CO_3 (89 mg; 0.65 mmol). After stirring for 4 h at room temperature, the mixture was poured into water and extracted with ether. The residue (141 mg) obtained upon evaporation of the solvent was subjected to chromatography on a silica gel (4.2 g) column (10 cm × 0.8 cm). Using 16% ethyl acetate in hexane as the eluting solvent, fractions 9 ml in volume were collected. The contents of fractions 15–26 were combined and, after evaporation of the solvent, gave **IX** (100 mg; 74% yield). Crystallization from methanol gave needles melting at 98–100°C; IR, ν_{max} 3441, 2932, 2859, 1699, 1620, 1451, 1379, 1125, and 1090 cm^{-1}. The MS data are presented in Table 1, and the ^{13}C and ^{1}H NMR spectral assignments are presented in Tables 2 and 3, respectively.

3β-Acetoxy-25-hydroxy-5α-cholest-8(14)-en-15-one (X)

To a solution of mercuric acetate (147 mg) in a 1:1 mixture (0.6 ml) of tetrahydrofuran and water was added a solution of **VIII** (131 mg) in tetrahydrofuran (0.6 ml). After stirring at 0°C for 4 h and then at room temperature for 5 h, 3 N NaOH (0.15 ml) was added followed by the addition of sodium borohydride (550 mg) in 3 N NaOH (2.5 ml) at 10°C. After 5 min, TLC analysis (solvent, 50% ethyl acetate in hexane) indicated completion of the reaction with a single major component at R_f 0.75. The reaction mixture was poured into water (10 ml) and extracted 3 times with ether (5-ml portions). The combined ether extracts were dried over anhydrous sodium sulfate, and the residue (140 mg) obtained upon evaporation of the solvent was subjected to chromatography on a silica gel column (12.5 cm × 0.8 cm). Using 16% ethyl acetate in hexane as the eluting solvent, fractions 40 ml in volume were collected. The contents of fractions 6–14 were combined and, after evaporation of the solvent, gave **X** (119 mg; 87% yield), melting at 151.0–152.5°C; IR,

[4]This procedure has consistently given **VI** in yields of 64–66% provided that vigorous mechanical stirring was maintained. Less vigorous mechanical stirring resulted in yields of 47–56%.

TABLE 1. Ion abundances in the mass spectra of $\Delta^{8(14)}$-15-ketosteroids[a,b]

Suggested Assignment[c]	VII 3β-OAc 24-al	VIII 3β-OAc Δ^{24}	IX 3β-OH Δ^{24}	X 3β-OAc 25-OH	IV 3β-OH 25-OH	V 3β-OH 24-OH
M^+	414* (37)	440* (32)	398* (39)	458* (51)	416* (64)	416* (100)
$M-CH_3$	399* (4)	425* (12)	383* (16)	443* (30)	401* (34)	401* (4)
$M-H_2O$	396* (6)	422* (5)	380* (5)	440* (100)	398* (100)	398* (10)
$M-CH_3-H_2O$	381 (3)	407 (3)	365* (22)	425* (32)	383* (45)	383* (57)
$M-CH(CH_3)_2$						373* (7)
$M-H_2O-H_2O$				422* (18)	380* (22)	
$M-H_2O-H_2O-CH_3$			347 (2)	407 (8)	365* (68)	365* (31)
$M-CH_3COOH$	354* (3)	380* (3)[d]				
$M-CH_3COOH-CH_3$	339* (30)[e]	365* (21)		383 (3)		
$M-CH_3COOH-H_2O$	336* (2)	362 (1)		380* (8)		
$M-CH_3COOH-CH_3-H_2O$	321* (6)	347 (2)		365* (64)		
Ion $D-H_2O$		339 (3)	297 (5)	339* (7)	297* (9)	297* (5)
Ion $D-H_2O-2H$		337 (2)	295 (3)	337* (14)	295* (12)	295 (4)
M-SC	329 (5)	329* (6)	287* (11)	329* (43)	287* (51)	287* (9)
$M-SC-H_2O$	311* (19)	311 (2)	269* (25)	311* (31)	269* (81)	269* (25)
Ion C	301* (3)	301 (2)	259 (12)	301* (85)	259* (99)	259* (9)[f]
Ion A	259* (7)	285 (3)	285* (5)	303 (8)[g]		
Ion B		274 (4)[h]	274* (3)			
$M-SC-CH_3COOH$	269 (11)	269* (16)		269* (32)		
$M-SC-H_2O-H_2O$			251* (11)		251* (24)	251* (8)
$M-SC-CH_3COOH-H_2O$	251* (28)	251* (12)		251* (31)		
Other ions	175 (15)	199 (9)	165 (100)	400 (7)[i]	358 (6)[i]	347 (2)
	169 (11)	175 (12)	159 (31)[j]	355 (6)	347 (5)	271 (30)
	159 (30)[j]	165 (100)	143 (23)	347 (7)	337 (5)	161 (12)
	145 (28)	159 (22)[j]	109 (49)	285 (4)	313 (7)	159 (12)[j]
	143 (33)	145 (18)	107 (90)	275 (9)	309 (4)	147 (12)
	107 (100)	109 (30)		274 (6)	285 (13)	145 (10)
	105 (94)	107 (71)		267 (9)	275 (11)	121 (10)
				165 (21)	274 (11)	119 (17)
				159 (21)[j]	267 (13)	117 (11)
				107 (43)	165 (27)	107 (26)
					159 (29)[j]	105 (20)
					107 (63)	

[a]Major ions above *m/z* 100 in mass spectra acquired at 70 eV by direct-probe. Relative intensities as % of base peak.
[b]Ions also observed in the high resolution mass spectrum are indicated by an asterisk. Unless otherwise noted, all such ions were compatible (± 3.0 millimass units) with the suggested assignments.
[c]SC, side chain; see ref. 25 for definition of ions A, B, C, and D.
[d]The high resolution MS showed a second ion in very low abundance at *m/z* 380.3114.
[e]The ions at *m/z* 339, corresponding to $M-CH_3COOH-CH_3$ and to ion $D-H_2O$, both had same elemental composition ($C_{23}H_{31}O_2$) and could not be distinguished by high resolution MS.
[f]The high resolution MS showed a second ion in very low abundance at *m/z* 259.2067 corresponding to $C_{18}H_{27}O$ (+0.5 millimass units).
[g]The ion of very low abundance observed at *m/z* 303 in the high resolution MS did not correspond to the elemental composition ($C_{20}H_{31}O_2$) of ion A.
[h]The very low abundance ion observed at *m/z* 274.1912 corresponded to $C_{18}H_{26}O_2$ (*m/z* 274.1933) and not to ion B ($C_{19}H_{30}O$, *m/z* 274.2297).
[i]The ions at *m/z* 400 and 358 in the MS of **X** and **IV**, respectively, correspond to M-58 (compatible with $M-(CH_3)_2CO$).
[j]The high resolution MS showed two significant ions at *m/z* 159; the ion of major abundance was compatible with the composition $C_{12}H_{15}$.

ν_{max} 3439, 2969, 2940, 2866, 1736, 1701, 1626, and 1258 cm^{-1}; single component (>99%) on TLC (solvent, 50% ethyl acetate in hexane; R_f 0.75). The MS data are presented in Table 1, and the ^{13}C and ^1H NMR spectral assignments are presented in Tables 2 and 3, respectively.

3β,25-Dihydroxy-5α-cholest-8(14)-en-15-one (IV)

To a solution of **X** (30 mg) in methanol (2 ml) was added potassium carbonate (20 mg). After stirring 4 h at room temperature, TLC analyses (solvent systems, 70% ethyl acetate in hexane and 40% acetone in benzene) indicated completion of the reaction with a single component (R_f values of 0.38 and 0.55 in the two solvent systems, respectively). The reaction mixture was poured into water (10 ml) and extracted 3 times with ether (5-ml portions). The combined ether extracts were dried over anhydrous sodium sulfate, and the residue (30 mg) obtained upon evaporation of the solvent was subjected to chromatography on a silica gel (1.5 g) column (6.5 cm × 0.8 cm). The column was eluted with 20% ethyl acetate (100 ml) followed by 30% ethyl acetate in hexane (40 ml). Fractions 40 ml in volume were collected. The contents of fractions 4–7 were pooled and, after evaporation of solvent, gave **IV** (26.6 mg; 98% yield), melting at 177–179°C; IR, ν_{max}

TABLE 2. ^{13}C NMR chemical shifts of $\Delta^{8(14)}$-15-ketosteroids[a]

	24-al 3β-OAc VII	Δ24 3β-OH IX	Δ24 3β-OAc VIII	25-OH 3β-OH IV	25-OH 3β-OAc X	24(R)-OH 3β-OH V[b]	24(S)-OH 3β-OH V[b]
C-1	36.14	36.45	36.20	36.47	36.17	36.48	
C-2	27.08	31.02	27.15	31.07	27.12	31.06	
C-3	73.03	70.75	73.14	70.82	73.12	70.78	
C-4	33.50	37.64	33.56	37.69	33.53	37.69	
C-5	43.78	44.02	43.86	44.05	43.82	44.06	
C-6	28.90	29.08	28.97	29.10	28.94	29.10	
C-7	27.37	27.52	27.42	27.55	27.41	27.55	
C-8	150.57	150.75	150.21	150.86	150.35	150.79[c]	150.88[c]
C-9	50.56	50.77	50.65	50.80	50.62	50.79	
C-10	38.58	38.68	38.62	38.71	38.60	38.71	
C-11	19.38	19.49	19.46	19.52	19.43	19.52	
C-12	36.77	36.84	36.83	36.91	36.82	36.90	
C-13	42.42	42.50	42.51	42.53	42.48	42.50	
C-14	139.92	140.16	140.32	140.16	140.25	140.11[c]	140.16[c]
C-15	207.06	208.14	207.97	208.06	207.91	207.95[c]	207.98[c]
C-16	42.07	42.33	42.33	42.41	42.35	42.37	
C-17	50.59	50.69	50.71	50.69	50.65	50.74	50.55
C-18	18.72	18.72	18.73	18.75	18.71	18.75	
C-19	12.72	12.86	12.78	12.89	12.75	12.87	
C-20	33.97	34.19	34.23	34.45	34.43	34.45	34.57
C-21	18.80	19.06	19.09	19.14	19.10	19.10	19.25
C-22	27.41	35.60	35.63	36.02	35.99	31.78[d]	31.87[d]
C-23	40.60	24.37	24.39	20.44	20.42	30.28[d]	30.19[d]
C-24	202.24	124.47	124.49	44.20	44.17	76.80	77.09
C-25		131.35	131.39	70.96	70.84	33.56	33.21
C-26		25.64	25.67	29.27*	29.23*	17.16	16.75
C-27		17.58	17.61	29.19*	29.18*	18.82	18.94
Acetate	170.51		170.60		170.60		
	21.31		21.37		21.35		

[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. Assignments marked by asterisks may be interchanged within a column.

[b]Analyzed as a mixture of C-24 epimers; the 24R and 24S epimers showed ~5:4 ratio of ^{13}C signal intensities (see discussion).

[c]Major and minor component signals could not be unequivocally differentiated in these cases.

[d]The signal assignments for C-22 and C-23 were derived from chemical shift comparisons (ref. 43) and have not been confirmed by HETCOR analysis.

3495, 3451, 2938, 2861, 1701, 1683, 1622, 1607, 1379, 1244, 1154, and 1092 cm^{-1}; single component (>99%) on TLC in two solvent systems (70% ethyl acetate in hexane, R_f 0.38; and 40% acetone in benzene, R_f 0.55). The MS data are presented in Table 1, and the ^{13}C and ^1H NMR spectral assignments are presented in Tables 2 and 3, respectively.

(24RS)-3β,24-Dihydroxy-5α-cholest-8(14)-en-15-one (V)

To a solution of **VIII** (220 mg) in tetrahydrofuran (3 ml) was added borane–dimethyl sulfide (0.125 ml) dropwise at 0°C. After standing overnight at −20°C, 3 N sodium acetate (0.4 ml) was added followed by 30% aqueous H_2O_2 (0.4 ml) at room temperature. After stirring for 3 h at room temperature, the reaction mixture was poured into water (10 ml) and extracted 3 times with ether (5-ml portions). The combined ether extracts were washed with water (5 ml), dried over anhydrous sodium sulfate, and evaporated to dryness. The resulting oily residue was subjected to silica gel column (15 cm × 0.8 cm) chromatography. Using 16% ethyl acetate in hexane as the eluting solvent, fractions 8 ml in volume were collected. The contents of fractions 64–99 were pooled and, after evaporation of the solvent, gave **XI** (66 mg) as an oil to which, after dissolving in methanol (3 ml), potassium carbonate (40 mg) was added. After stirring for 4 h at room temperature, TLC analyses (solvent systems, 70% ethyl acetate in hexane and 50% acetone in benzene) indicated completion of the reaction with one major product (with R_f values of 0.51 and 0.27 in the two solvent systems, respectively). The reaction mixture was poured into water (10 ml), extracted 3 times with ether (5-ml portions), and the combined ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residue (55 mg) was subjected to chromatography on a silica gel (4 g) column (10 cm × 0.8 cm). Using 30% ethyl acetate in hexane as the eluting solvent, fractions 8 ml in volume were collected. The contents of fractions 11–17 were combined and, after evaporation of the solvent, gave **V** (21 mg; 10% yield). The MS data are presented in Table

TABLE 3. ^{1}H NMR chemical shifts of $\Delta^{8(14)}$-15-ketosteroids[a-e]

	24-al 3β-OAc VII	Δ24 3β-OH IX	Δ24 3β-OAc VIII	25-OH 3β-OH IV	25-OH 3β-OAc X	24(R)-OH 3β-OH V[f]	24(S)-OH 3β-OH V[f]
H-1α	1.25	1.19	1.25		1.24		
H-1β	1.75	1.72	1.75		1.74		
H-2α	1.86	*1.84*	1.86		1.86		
H-2β	1.42	1.33	1.42		*1.43*		
H-3α	4.73	3.64	4.73	3.64	4.73	3.64	
H-4α	1.71	1.68	1.71		1.72		
H-4β	1.35	1.25	1.34		1.35		
H-5α	1.48	1.41	1.47	1.41	1.47	1.41	
H-6	*1.38*	*1.29*	*1.34*		*1.38*		
	1.50	1.48	1.49		*1.50*		
H-7α	1.57	1.57			1.58		
H-7β	4.13	4.13	4.13	4.13	4.13	4.13	
H-9α	1.89	1.86	1.88	1.86	1.88	1.86	
H-11	*1.67*	*1.68*	*1.68*		*1.64*		
	1.57	*1.55*	*1.56*		*1.55*		
H-12α	1.26	1.24	1.25		1.25		
H-12β	2.10	2.11	2.10	2.10	2.10		
H-16α	2.38	2.37	2.37	2.35	2.36	2.40	2.36
H-16β	2.10	2.06	2.05	2.05	2.05	2.07	2.08
H-17α	1.48	1.47	1.48	1.47	1.48	1.48	1.48
H-18	0.983	0.971	0.972	0.973	0.973	0.978	
H-19	0.734	0.714	0.733	0.714	0.732	0.714	
H-20	1.64	1.59	1.59	1.61	1.61	1.61	1.61
H-21	1.011	1.017	1.019	1.026	1.018	1.013	1.021
H-22	1.37	1.11	1.11		1.10		
	1.81	1.40	1.40		1.38		
H-23	*2.40*	1.88	1.88		1.25		
	2.46	2.03	2.02		1.45		
H-24		5.07	5.07		*1.40*	3.30[g]	3.30[g]
					1.48		
H-25						1.65	1.65
H-26		1.681	1.683	1.212	1.213	0.908	0.893
H-27		1.596	1.597	1.212	1.213	0.915	0.919
Acetate	2.028		2.029		2.029		

[a]Data obtained at 300.1 MHz in $CDCl_3$ solution at a concentration of 0.02–0.02 M. Chemical shifts referenced to $Si(CH_3)_4$ signal.

[b]Chemical shifts of methylene protons between δ 0.9 and 2.0, obtained from HETCOR spectra, are generally accurate to ±0.02 ppm except for values in italics (±0.1 ppm).

[c]No stereochemical assignments are given for the protons of C-6, C-11, C-22, C-23, and C-24.

[d]Selected ^{1}H coupling constants in Hz (average of observed values, individual deviations generally ≤0.1 Hz): H-3α, tt, 11.1, 4.9 (acetates) or 10.8, 4.9 (free sterols); H-7β, distorted ddd, −14.0, 3.9, 1.9; H-9α, br dd, 10.5, 7.4; H-12β, ddd, −12.5, 3.4, 3.4; H-16α, dd, −18.5, 7.7; H-16β, dd, −18.5, 12.2; H-18 (s); H-19 (s); H-21, d, 6.5.

[e]Additional couplings in Hz: H-1β (**IX**), ddd, −12.8, 3.3, 3.3; H-24 (**VIII** and **IX**), triplet (7.1) of septet (1.3); H-26 and H-27 (**V**), d, 6.7; H-26 and H-27 (**IV, VIII, IX, X**), s.

[f]Chemical shifts derived from ^{1}H and HETCOR spectra of a 5:4 mixture of 24R and 24S epimers. The signals for the two epimers were distinguished by signal intensities in the ^{1}H spectrum (H-16α, H-16β), correlations to ^{13}C signals that are assigned in Table 3 (H-17, H-20), or a combination of these methods (methyl resonances).

[g]The combined H-24 signal consisted of a multiplet 19 Hz wide centered at δ 3.30.

1, and the ^{13}C and ^{1}H NMR spectral assignments are presented in Tables 2 and 3, respectively.

GC–MS of trimethylsilyl derivatives of II, IV, and V

Trimethylsilyl (TMS) ether derivatives of **II, IV,** and **V** were prepared by treating the sterols (10 μg) with a mixture of pyridine (50 μl) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA; 50 μl) followed by standing at room temperature (condition A) or at 60°C (condition B) or 100°C (condition C) for 20 h. Silylation of **II** under conditions A and C gave 3β,26-bis(trimethylsilyloxy)-5α-cholest-8(14)-en-15-one (**IIb**) and 3β,15,26-tris(trimethylsilyloxy)-5α-cholesta-7,14-diene (**IIc**), respectively. Silylation of **IV** under condition A gave a 32:68 mixture of 3β-trimethylsilyloxy-25-hydroxy-5α-cholest-8(14)-en-15-one (**IVa**) and 3β,25-bis(trimethylsilyloxy)-5α-cholest-8(14)-

TABLE 4. Ion abundances in the mass spectra of trimethysilyl ether derivatives of 24-, 25-, and 26-hydroxy derivatives of 3β-hydroxy-5α-cholest-8(14)-en-15-one[a]

	Sterol TMS Ether Derivative			
Suggested Assignment[b]	3β-OTMS 25-OH **IVa**	3β-OTMS 24-OTMS **Vb**	3β-OTMS 25-OTMS **IVb**	3β-OTMS, 26-OTMS **IIb**
M^+	488 (25)	560 (63)	560 (2)	560 (100)
$M-CH_3$	473 (16)	545 (15)	545 (17)	545 (14)
$M-H_2O$	470 (100)	542 (3)		542 (6)
$M-H_2O-CH_3$	455 (16)			527 (1)
$M-2H_2O$	452 (4)			
$M-2H_2O-CH_3$	437 (5)			
$M-CH(CH_3)_2$		517 (13)		
M-TMSOH		470 (25)	470 (100)	470 (7)
$M-TMSOH-CH_3$		455 (6)	455 (7)	455 (17)
$M-TMSOH-H_2O$	380 (9)	452 (4)	452 (4)	452 (2)
$M-TMSOH-CH_3-H_2O$	365 (28)	437 (1)	437 (1)	
$M-TMSOH-CH_3-2H_2O$	347 (5)			
M-2TMSOH		380 (6)	380 (5)	
$M-2TMSOH-CH_3$		365 (13)	365 (13)	365 (7)
$M-2TMSOH-CH_3-H_2O$		347 (4)	347 (4)	347 (8)
$M-CH(CH_3)_2$-TMSOH		427 (8)		
$M-CH(CH_3)_2$-2TMSOH		337 (8)		
M-129	359 (10)	431 (7)	431 (21)	
Ion A	303 (3)			375 (5)
Ion A-H_2O	285 (8)	285 (1)	285 (2)	
Ion D-TMSOH	297 (1)	297 (1)		
Ion D-TMSOH-H_2O	279 (1)	279 (2)		
Ion B-CH_3				349 (5)
Ion B-CH_3-H_2O	259 (2)			
Ion B-TMSOH		274 (2)		
Ion C	331 (23)	331 (100)	331 (56)	331 (6)
M-SC	[359]	359 (6)	359 (2)	359 (3)
M-SC-H_2O	341 (19)	341 (10)	341 (4)	341 (13)
M-SC-TMSOH	269 (10)	269 (6)	269 (7)	269 (5)
M-SC-TMSOH-H_2O	251 (19)	251 (8)	251 (4)	251 (10)
m/z 267	267 (5)	267 (8)	267 (2)	267 (1)
$CH(=OTMS)CH(CH_3)_2$		145 (27)		
$(CH_3)_2C=OTMS$			131 (86)	
Other ions	430 (5)[c]		502 (7)[c]	
	274 (6)		385 (5)	

[a]Major ions above *m/z* 100 in mass spectra acquired at 70 eV by GC-MS. Relative intensities as % of base peak.
[b]SC, side chain; see ref. 25 for definition of ions A, B, C and D.
[c]The ions at *m/z* 430 and 502 in the MS of **IVa** and **IVb**, respectively, correspond to M-58 (compatible with $M-(CH_3)_2CO$).

en-15-one (**IVb**).[5] In contrast, silylation of **IV** under condition B gave a 94:6 mixture of **IVb** and 3β,15,25-tris(trimethylsilyloxy)-5α-cholesta-7,14-diene (**IVc**). Silylation of **IV** under condition C gave a 95:5 mixture of **IVc** and 3β,15,25-tris(trimethylsilyloxy)-5α-cholesta-8(14),15-diene. Silylation of **V** under condition A gave almost exclusively 3β,24-bis(trimethylsilyloxy)-5α-cholest-8(14)-en-15-one (**Vb**), whereas silylation under condition C gave exclusively 3β,15,24-tris(trimethylsilyloxy)-5α-cholesta-7,14-diene (**Vc**). The silylation products were analyzed by GC-MS. The mass spectra of **IIb, IVa, IVb,** and **Vb** are presented in **Table 4,** and those of **IIc, IVc,** and **Vc** are given in **Table 5.** The following retention times (methylene units) were observed: **IIb,** 12.93 min (37.24), **IIc,** 11.02 min (34.83), **IVa,** 11.98 min (36.14), **IVb,** 12.27 min

[5]Silylation of the metabolic samples of **IV** gave, in addition to the 3β-TMS and 3β,25-bis-TMS ethers and tris-TMS dienol ethers, minor amounts of 25-hydroxy-3β,15-bis-TMS dienol ethers. The 3β,15-bis-TMS ethers of **IV** were distinguished from the 3β,25-bis-TMS ether by their MS fragmentation patterns and GC retention times. Relative to the 3β,15-bis-TMS ethers, the 3β,25-bis-TMS ether showed much higher abundance of ions *m/z* 331 and 131 and much lower abundance of ion *m/z* 455. Upon GC analysis on a DB-5 column, the 3β,15-bis-TMS dienol ethers of **IV** were eluted by GC before the 3β-TMS ether, whereas the 3β,25-bis-TMS ether was eluted later. The spectral data of a 3β,15-bis-TMS dienol ether were inadvertently presented for the 3β,25-bis-TMS ether of **IV** in ref. 21 (Table VI, entry **IVc**). The spectral data shown in Table 5 for the 3β,25-bis-TMS ether of **IV** are similar to those observed for the 3β,25-bis-TMS ether of **IV** obtained by incubation of **I** with rat liver mitochondria: *m/z* 545 (16), 470 (100), 455 (13), 431 (10), 380 (10), 365 (32), 359 (4), 357 (4), 347 (7), 341 (8), 331 (27), 269 (10), 267 (5), 251 (22), 241 (18), and 215 (14) (ions above 200 amu).

TABLE 5. Ion abundances in the mass spectra of the trimethylsilyl dienol ethers of 24-, 25-, and 26-hydroxy derivatives of 3β-hydroxy-5α-cholest-8(14)-en-15-one[a]

	Sterol Derivative		
Suggested Assignment	3β,15,24-tris-OTMS $\Delta^{7,14}$ **Vc**	3β,15,25-tris-OTMS $\Delta^{7,14}$ **IVc**	3β,15,26-tris-OTMS $\Delta^{7,14}$ **IIc**
M^+	632 (100)	632 (100)	632 (100)
$M-CH_3$	617 (85)	617 (74)	617 (95)
$M-CH(CH_3)_2$	589 (19)		
$M-CH_2=Si(CH_3)_2$	560 (2)		560 (2)
M-TMSOH	542 (6)	542 (5)	542 (4)
$M-TMSOH-CH_3$	527 (10)	527 (8)	527 (10)
$M-CH(CH_3)_2$-TMSOH	499 (9)		
$M-CH_2=Si(CH_3)_2$-TMSOH	470 (1)	470 (3)	470 (1)
M-2TMSOH	452 (1)	452 (2)	452 (2)
$M-2TMSOH-CH_3$	437 (4)	437 (2)	437 (6)
M-SC	431 (9)	431 (7)	431 (10)
$M-CH(CH_3)_2$-2TMSOH	409 (2)		
$M-CH_2=Si(CH_3)_2$-2TMSOH-CH_3		365 (1)	365 (1)
M-SC-TMSOH	341 (5)	341 (5)	341 (15)
m/z 309		309 (1)	309 (5)
M-SC-2TMSOH	251 (7)	251 (5)	251 (12)
$CH(=OTMS)CH(CH_3)_2$	145 (15)		
$(CH_3)_2C=OTMS$		131 (49)	

[a]Major ions above *m/z* 100 in mass spectra acquired at 70 eV after GC separation of the major TMS ether product obtained by silylation of the free sterols under condition C (see text). Relative intensities as % of base peak.

(36.62), **IVc,** 10.75 min (34.34), **Vb,** 12.30 min (36.55), and **Vc,** 10.65 min (34.12). The 24R and 24S epimers were not resolved under these conditions.

Effects of 3β-hydroxy-5α-cholest-8(14)-en-15-one (I), 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (IV), (24RS)-3β,24-dihydroxy-5α-cholest-8(14)-en-15-one (V), and 3β-hydroxy-5α-cholesta-8(14),24-dien-15-one (IX) on the levels of HMG-CoA reductase activity in CHO-K1 cells

The effects of the 15-ketosterol **I** and its 25-hydroxy-, (24RS)-24-hydroxy-, and Δ^{24}-analogs (**IV, V,** and **IX**) on HMG-CoA reductase activity were studied in CHO-K1 cells (**Table 6**). Each of the analogs of the 15-ketosterol was highly active in lowering the levels of HMG-CoA reductase activity.

DISCUSSION

The major metabolite of the 15-ketosterol **I** upon its incubation with rat liver mitochondria in the presence of NADPH is (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**) (20–22). Other metabolites formed from **I** in significant amounts under these conditions were the (25S)-isomer of **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**) (21, 22). 3β,24-Dihydroxy-5α-cholest-8(14)-en-15-one (**V**) was also detected as a minor component (21, 22).

One major goal of this study was the chemical synthesis of **IV,** the 25-hydroxy analog of **I.** The elaboration of an efficient synthesis of **IV,** requiring the introduction of the $\Delta^{8(14)}$-15-keto system in the sterol nucleus and the introduction of the 25-hydroxyl function in the sterol side chain, presented a significant challenge. Two simple, extreme cases were considered: *a*) introduction of the $\Delta^{8(14)}$-15-keto system into a 25-hydroxysterol and *b)* introduction of a 25-hydroxyl function into a $\Delta^{8(14)}$-15-ketosterol. The former approach finds analogy in our previous synthesis of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**) from (25R)-26-hydroxycholesterol (20, 23). However, this approach would require the synthesis of significant amounts of 25-hydroxycholesterol and several subsequent steps for its conversion to **IV.** The latter approach, i.e., direct 25-hydroxylation of **I,** represents a case of oxidation at an unactivated carbon atom in the sterol side chain, for which the development of reactions providing specificity and high yields remains a continuing challenge in synthetic chemistry. A number of approaches for direct hydroxylation at C-25 have been described (36–40) but were not pursued here because of reported low yields and/or incompatibility with the $\Delta^{8(14)}$-15-ketosterol functionality.

Our approach to this problem has involved exploitation of our recent demonstration of a specific, high yield oxidation of the side chain of **I** (24, 25), for which an efficient synthesis from 7-dehydrocholesterol has been described (34). Oxidation of the acetate of **I** with a mixture of trifluoroacetic anhydride, sulfuric acid, and hydrogen perox-

TABLE 6. Effects of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**), 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (**IV**), (24RS)-3β,24-dihydroxy-5α-cholest-8(14)-en-15-one (**V**), and 3β-hydroxy-5α-cholesta-8(14),24-dien-15-one (**IX**) on the levels of HMG-CoA reductase activity in CHO-K1 cells

Sterol Concentration μM	HMG-CoA Reductase Activity (% of Control Activity)[a]			
	Suppression of Elevated Levels Induced by Transfer of Cells to Lipid-deficient Media			
	I	**IV**	**V**	**IX**
0.0	100.0 ± 1.4[b]	100.0 ± 2.8[c]	100.0 ± 2.0[d]	100.0 ± 2.1[e]
0.1	61.9 ± 1.2	66.4 ± 4.7	63.4 ± 0.2	60.5 ± 2.3
0.25	52.1 ± 1.3	56.2 ± 2.0	33.5 ± 1.0	67.0 ± 3.0
0.5	42.2 ± 2.0	50.2 ± 2.0	32.2 ± 0.9	51.1 ± 0.6
1.0	35.8 ± 0.6	34.8 ± 2.3	34.2 ± 2.8	40.0 ± 1.4
2.5	24.4 ± 0.8	23.7 ± 1.9	21.5 ± 1.2	35.2 ± 1.8

[a]Variation is expressed as ± SD of replicate (n = 3) assays of HMG-CoA reductase activity.
[b–e]Mean values for controls were 854, 1128, 1265, and 877 pmol/min per mg protein, respectively.

ide followed by treatment of the crude product with methanol and triethylamine gave 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**VI**) in 61% yield (24, 25). The availability of **VI** provided a key intermediate for the chemical synthesis of the 25-hydroxy derivative **IV.** Oxidation of the 24-hydroxyl group of **VI** with Dess-Martin reagent (35) gave the aldehyde **VII** in 89% yield. Wittig olefination of **VII** with isopropyltriphenylphosphonium iodide gave the desired Δ^{24} analog (**VIII**) of the acetate of **I.** Oxymercuration (41) of **VIII** proceeded in high yield to give the 25-hydroxy derivative **X.** Mild alkaline hydrolysis of **VIII** and **X** gave the corresponding free sterols, 3β-hydroxy-5α-cholesta-8(14),24-dien-15-one (**IX**) and 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (**IV**), respectively. Hydroboration of **VIII** with borane–dimethylsulfide gave, after hydrolysis and extensive chromatography, 3β,24-dihydroxy-5α-cholest-8(14)-en-15-one (**V**) as a mixture of C-24 epimers.

The ^{1}H and ^{13}C NMR and HETCOR spectra of **V** showed splitting of signals corresponding to side-chain carbons and various other carbon and hydrogen atoms. For each pair of split ^{13}C signals, one peak was consistently ~25% higher than the other. Owing to the anticipated similarity of relaxation times and nuclear Overhauser effects for corresponding carbon atoms of each C-24 epimer, these minor differences in signal intensities were deemed to be significant. Diagnostic ^{13}C signals (C-24, C-25, C-26, C-27) corresponding to the major and minor epimers matched those reported for (24R)- and (24S)-24-hydroxycholesterol (42, 43); small discrepancies in the chemical shift comparisons of the side-chain carbons were attributable to effects of the $\Delta^{8(14)}$-15-keto functionality and to instrumental differences (temperature, digital resolution, and magnet field strength). These results indicate that a small degree of asymmetric induction occurred in the hydroboration of **VIII.**

Each of the above sterols was characterized by IR, low and high resolution MS (Table 1) and ^{13}C and ^{1}H NMR (Tables 2 and 3). In addition, compounds **IV** and **V,** along with (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one, were studied by GC–MS in the form of trimethylsilyl ether derivatives (Tables 4 and 5).

The low resolution and high resolution MS data for the $\Delta^{8(14)}$-15-ketosteroids **VII, VIII, IX, X, IV,** and **V** (Table 1) were fully compatible with the assigned structures. The suggested fragment ion assignments (Table 1) are in accord with those made previously for the 15-ketosterol **I** and its side-chain oxygenated derivatives (20–23, 25, 44). Fragmentations of $\Delta^{8(14)}$-15-ketosteroids and definitions of ions A, B, C, and D have been presented previously (21, 25, 44). The 3β,25-dihydroxysterol **IV** and its 3β-acetate derivative **X** showed ions of very high abundance corresponding to M–H_2O and ion C. The 3β,24-dihydroxysterol **V** showed an ion of significant abundance at *m/z* 373 corresponding to M–$CH(CH_3)_2$, a fragmentation not observed in the cases of the 25-hydroxy- or Δ^{24}-sterols or their 3β-acetoxy derivatives. With a few exceptions (see Table 1), the results of high resolution MS measurements were compatible with the suggested fragment ion assignments. The Δ^{24}-sterol (**IX**) and its 3β-acetate derivative (**VIII**) showed an ion of very high abundance (base peak) at *m/z* 165. This ion was also present, albeit at much lower abundance, in the spectra of the 25-hydroxysterol **IV** and its 3β-acetoxy derivative **X.** Sterols **IV** and **X** could be expected to lose water readily to yield a Δ^{24}-sterol, a process that may account for the presence of the ion at *m/z* 165 in the spectra of **IV** and **X.** High resolution mass measurements for the ion at *m/z* 165 for **IV, VIII, IX** and **X** indicated the formula $C_{11}H_{17}O$ (all ± 0.2 millimass units), which is compatible with an ion composed of the side chain plus C_{15}, C_{16}, and C_{17} and their substituents. As other $C_{11}H_{17}O$ ions can be formu-

lated, further studies will be required to determine the exact origin of this ion.

Trimethylsilyl ether derivatives of **I** and its metabolites have proven useful in studies of these compounds by GC–MS (20–23, 44, 45). Silylation of various $\Delta^{8(14)}$-15-ketosterols under mild conditions leads to derivatization of primary alcohol groups and of unhindered secondary alcohol functions (21, 22). Under these conditions, tertiary hydroxyl groups, such as the 25-hydroxyl function in **IV,** are not silylated (21, 22). However, under forcing conditions of silylation, tertiary hydroxyls can be derivatized (21, 22). Under these forcing conditions, i.e., BSTFA and pyridine at 100°C for 20 h, $\Delta^{8(14)}$-15-ketosterols give $\Delta^{7,14}$-dienol TMS derivatives accompanied by minor amounts of $\Delta^{8(14),15}$-dienol ethers (22). The utility of the $\Delta^{7,14}$-dienol TMS ether derivatives in studies of a variety of $\Delta^{8(14)}$-15-ketosterols has been presented previously (22). In the present study we have compared the GC–MS properties of various TMS derivatives of (24RS)-24-hydroxy-5α-cholest-8(14)-en-15-one (**V**), 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (**IV**), and (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**), all prepared by chemical synthesis. The GC retention data from analyses conducted with a DB-5 capillary GC column showed that, for the $\Delta^{7,14}$-dienol tris-TMS derivatives of the 24-, 25-, and 26-hydroxy analogs of **I,** the order of elution was 24-hydroxy < 25-hydroxy < 26-hydroxy. In the case of the bis-TMS ether derivatives, a useful separation of the 24-hydroxy and 25-hydroxy compounds was not observed. As noted previously (22), the retention times of the $\Delta^{7,14}$-dienol tris-TMS derivatives of the dihydroxy-$\Delta^{8(14)}$-15-ketosterol were shorter than those of the corresponding bis-TMS ether derivatives.

The MS of the bis-TMS ethers of the synthetic samples of **II, IV,** and **V** and the MS of the 3β-TMS derivative of **IV** showed complex fragmentation (Table 4). However, assignments for almost all ions above *m/z* 250 could be suggested on the basis of previous studies of **I,** its analogs, and deuterium-substituted derivatives of **I** (22). The diagnostic value of silylation of the 25-hydroxyl function of **IV** is illustrated by the presence in high abundance of the ion at *m/z* 131, resulting from cleavage of the C24–C25 bond to give the ion $(CH_3)_2COSi(CH_3)_3$. This ion is characteristic of silyl derivatives of 25-hydroxysterols (21, 22, 46).

The mass spectra of the $\Delta^{7,14}$-dienol TMS ethers (Table 5) are considerably simpler than spectra of the corresponding $\Delta^{8(14)}$-15-ketosterols and are dominated by M^+ and $M-CH_3$ ions. These patterns have been noted previously for $\Delta^{7,14}$-dienol TMS ethers (22). The mass spectrum of the dienol tris-TMS ether derivative of the 24-hydroxysterol **V** was readily distinguished from spectra of the corresponding derivatives of the 25-hydroxysterol **IV** and the 26-hydroxysterol **II** by the presence of ions showing loss of $CH(CH_3)_2$ (*m/z* 589, 499, and 409). As expected, the ion at *m/z* 131, characteristic of TMS derivatives of 25-hydroxysterols, was much more prominent in the spectrum of the dienol tris-TMS ether of the 25-hydroxysterol **IV** than in the spectra of the derivatives of the 24-hydroxysterol **V** and the 26-hydroxysterol **II.**

Both the 25-hydroxy and 24-hydroxy 15-ketosterols **IV** and **V** have been observed as metabolic products in the incubation of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) with rat liver mitochondria in the presence of NADPH (21, 22). The metabolic sample of **IV** was characterized by ^{1}H NMR as the free sterol and 3β-acetate, and the metabolic samples of **IV** and **V** were characterized by GC–MS as free sterols and various TMS ethers (21, 22). The ^{1}H NMR chemical shifts presented here for the synthetic samples of **IV** and its acetate **X** agree (±0.01 ppm) with those reported (21) for the metabolic samples of **IV** and **X.** The mass spectra of **IV** and **V** (Table 1), the $\Delta^{7,14}$-tris-TMS dienol ethers of **IV** and **V** (Table 5), the 3β,24-bis-TMS ether of **V,** and the 3β-TMS ether of **IV** (Table 4) were compatible with spectra reported for the corresponding metabolic samples (obtained after incubation of **I** with rat liver mitochondria (21, 22)), allowing for consideration of differences in instrumentation and operating conditions.[5,6]

The 25-hydroxy-, (24RS)-24-hydroxy-, and Δ^{24}-analogs of **I** were each found to be highly active in lowering the levels of HMG-CoA reductase activity in CHO-K1 cells (Table 6). The 24-hydroxy analog of **I** appeared to be more potent than **I.** This synthetic sample was, by NMR analysis (see above), a mixture of the 24R and 24S isomers of the 24-hydroxysterol, slightly enriched in the 24R isomer. Further studies are planned to determine the potencies of the individual isomers of the 24-hydroxysterol.

We have previously identified (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**), the 25-hydroxy analog (**IV**) of **I,** and a 24-hydroxy analog (**V**) of **I** as mitochondrial metabolites of the 15-ketosterol **I** (20–22). It should be noted that **I,** 26-hydroxycholesterol, 25-hydroxycholesterol, and 24-hydroxycholesterol are among the most potent oxysterols in lowering of HMG-CoA reductase activity in cultured mammalian cells (1–4, 47, 48). The results presented in this study, coupled with those described previously (20, 23), demonstrate that hydroxylation of **I** at C-26, C-25, or C-24 leads to metabolites of very high potency. These findings point out the importance of these metabolites in considerations of the overall actions of **I** in intact animals or in cells in which they are formed.

[6]Differences in tuning, ionization energy, conditions in the ion source, and other instrumental parameters affect relative intensities and probably account for observed variations in ion abundances, such as the much higher abundance of the *m/z* 380 ion for the free sterols **IV** and **V** reported in ref. 21.

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