138334-74-4; **2y**·HCl, 138353-24-9; **3v**, 60326-45-6; **4** (X = 4-Et), 138334-75-5; **4** (X = 4-OMe), 7443-25-6; **5** (X = 4′-OMe, Y = 7-Cl), 138334-76-6; **5** (X = 3′-OMe, Y = 7-Cl), 138334-77-7; **5** (X = 2′-OMe, Y = 7-Cl), 138334-78-8; **5** (X = 4′-OMe, Y = H), 129151-87-7; **5** (X = 4′-OMe, Y = 6-Cl), 138334-79-9; **5** (X = 4′-OMe, Y = 6-Me), 138334-80-2; **5** (X = 4′-OMe, Y = 6-CF_3), 138334-81-3; **5** (X = 4′-OMe, Y = 6-CN), 138334-82-4; **5** (X = 4′-OMe, Y = 6-NO_2), 138334-83-5; **5** (X = 4′-OMe, Y = 6-OMe), 138334-84-6; **5** (X = 4′-OMe, Y = 6-CO_2Et), 138334-85-7; **5** (X = 4′-OMe, Y = 7-OBn), 138334-86-8; **5** (X = 4′-OMe, Y = 7-S-*t*-Bu), 138352-85-9; **5** (X = 4′-OMe, Y = 7-OCF_2H), 138334-87-9; **5** (X = 4′-OMe, Y = 7-SPh), 138334-88-0; **5** (X = 4′-OMe, Y = 7-OPh), 138334-89-1; **5** (X = 4′-OMe, Y = 7-CF_3), 138334-90-4; **5** (X = 4′-OMe, Y = 6-OMe, 7-Br), 138334-91-5; **5** (X = 4′-SMe, Y = 6-CF_3), 138384-04-0; **5** (X = 4′-Et, Y = 6-CF_3), 138384-05-1; **5** (X = 3′,4′-$(OMe)_2$, Y = 6-CF_3), 138334-92-6; **6g**, 138383-10-5; **6v**, 138334-93-7; **7** (X = 4′-OMe, Y = 7-SHgSPh, R = OH), 138334-94-8; **7a**, 138334-95-9; **7b**, 138334-96-0; **7c**, 138334-97-1; **7d**, 129151-91-3; **7e**, 128574-37-8; **7f**, 138334-98-2; **7g**, 133963-42-5; **7h**, 138334-99-3; **7i**, 138335-00-9; **7j**, 138335-01-0; **7k**, 138335-02-1; **7o**, 138335-03-2; **7s**, 138335-04-3; **7t**, 138335-05-4; **7u** free base, 128510-87-2; **7v**, 138335-06-5; **7w**, 138335-07-6; **7x**, 138335-08-7; **7y**, 138335-09-8; **8x**, 138335-10-1; **9v**, 138335-11-2; **10d**, 138352-86-0; **11a**, 128510-83-8; **11c**, 138335-12-3; **11d**, 138335-13-4; **11e**, 138335-14-5; **11g**, 138335-15-6; *cis*-**11h**, 111605-16-4; *trans*-**11h**, 138383-11-6; *cis*-**11i**, 138335-16-7; *trans*-**11i**, 138335-17-8; *cis*-**11k**, 119217-65-1; *trans*-**11k**, 138383-12-7; **11n**, 138335-18-9; **11o**, 138335-19-0; **11p**, 138335-20-3; **11q**, 129151-99-1; **11r**, 119217-62-8; **12a** free base, 138335-21-4; **12a**·fumarate, 138335-22-5; **12b** free base, 138335-23-6; **12b**·2HCl, 138335-24-7; **12c** free base, 138335-25-8; **12c**·HCl, 138335-26-9; **12d** free base, 138335-27-0; **12d**·HCl, 138335-28-1; **12e** free base, 138335-29-2; **12e**·HCl, 138335-30-5; **12f** free base, 138335-31-6; **12f**·HCl, 138335-32-7; **12g** free base, 138353-25-0; **12g**·HCl, 138335-33-8; **12h** free base, 119217-15-1; **12h**·HCl, 119217-31-1; **12i** free base, 119217-13-9; **12i**·HCl, 119217-30-0; **12j** free base, 119217-14-0; **12j**·HCl, 119217-29-7; **12k** free base, 138383-13-8; **12k**·fumarate, 138456-70-9; **12l** free base, 138335-34-9; **12l**·HCl, 138335-35-0; **12m** free base, 138335-36-1; **12m**·HCl, 138335-37-2; **12n** free base, 138335-38-3; **12n**·HCl, 138335-39-4; **12o** free base, 138335-40-7; **12o**·HCl, 138335-41-8; **12p** free base, 138335-42-9; **12p**·HCl, 138335-43-0; **12q** free base, 119217-37-7; **12q**·HCl, 119217-36-6; **12r** free base, 119217-39-9; **12r**·HCl, 119217-38-8; **12s** free base, 138335-44-1; **12s**·HCl, 119217-40-2; *cis*-**14**, 138335-45-2; *trans*-**14**, 138335-46-3; **15a** free base, 132201-65-1; **15a**·HCl, 129524-09-0; **15b** free base, 138335-47-4; **15b**·fumarate, 138383-14-9; **15c** free base, 138335-48-5; **15c**·fumarate, 138383-15-0; **15d** free base, 138335-49-6; **15e** free base, 138335-50-9; **15f** free base, 138335-51-0; **15f**·HCl, 138383-16-1; **15g** free base, 138335-52-1; **15h** free base, 138335-53-2; **15h**·HCl, 138383-17-2; **15i** free base, 138335-54-3; **15i**·HCl, 138383-18-3; **15j** free base, 138335-55-4; **15j**·HCl, 138383-19-4; **15k** free base, 138335-56-5; **15l** free base, 128573-80-8; **15l**·HCl, 128509-61-5; **15m** free base, 128573-81-9; **15m**·HCl, 128656-27-9; **15n** free base, 128573-82-0; **15n**·HCl, 128509-64-8; **15n** (R″ = CH(Me)CN) free base, 128510-18-9; **15n** (R″ = CH(Me)CH_2NH_2) free base, 138383-20-7; **15o** free base, 138383-21-8; **15o**·HCl, 128573-83-1; **15o** (R″ = CH(Me)CN) free base, 128574-18-5; **15o** (R″ = CH(Me)CH_2NH_2) free base, 138383-22-9; **15p** free base, 138335-57-6; **15p**·HCl, 138335-58-7; **15q** free base, 138335-59-8; **15q**·HCl, 138335-60-1; **15r** free base, 119217-19-5; **15r**·HCl, 119217-35-5; **15s** free base, 138335-61-2; **15s**·fumarate, 138335-62-3; **15t** free base, 138335-63-4; **15t**·HCl, 138335-64-5; **15u** free base, 138335-65-6; **15u**·HCl, 138383-23-0; **15u**·oxalate, 138383-24-1; $Me_2N(CH_2)_2Cl$, 107-99-3; $MeCH(NMe_2)CH_2Cl$, 53309-35-6; thiophenol, 108-98-5; 2-nitro-5-chlorotoluene, 5367-28-2; [1-(4-methoxyphenyl)-2-[2-amino-5-(phenylthio)phenyl]-ethyl]propanedioic acid, dimethyl ester, 138335-66-7; 1,3,4,5-tetrahydro-7-(phenylthio)-3-hydroxy-3-(methoxycarbonyl)-4-(4-methoxyphenyl)-2*H*-1-benzazepin-2-one, 138335-67-8; 2-nitro-6-(trifluoromethyl)toluene, 6656-49-1; [1-methyl-1-(4-ethylphenyl)-2-[2-nitro-6-(trifluoromethyl)phenyl]ethyl]propanedioic acid, dimethyl ester, 138335-68-9; [1-methyl-1-(4-ethylphenyl)-2-[2-amino-6-(trifluoromethyl)phenyl]ethyl]propanedioic acid, dimethyl ester, 138353-26-1.

Communications to the Editor

Inhibitors of Sterol Synthesis. 3β,25-Dihydroxy-5α-cholest-8(14)-en-15-one, an Active Metabolite of 3β-Hydroxy-5α-cholest-8(14)-en-15-one

Oxygenated sterols are potent regulators of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in mammalian cells.[1,2] 15-Oxygenated sterols are particularly active in the regulation of HMG-CoA reductase activity and of cholesterol biosynthesis.[1-7] One 15-oxygenated sterol, 3β-hydroxy-5α-cholest-8(14)-en-15-one (**1**), is highly active in lowering not only the levels of HMG-CoA reductase activity in cultured mammalian cells but also that of two other key enzymes involved in the formation of mevalonic acid, i.e., cytosolic acetoacetyl-CoA thiolase and HMG-CoA synthase.[5] In addition to its inhibitory action on cholesterol biosynthesis, **1** has been shown to be a potent inhibitor of cholesterol absorption in intact rats.[8,9] The 15-ketosterol serves as a substrate

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for acyl coenzyme A:cholesterol acyltransferase (ACAT) and inhibits the oleoyl-CoA-dependent esterification of cholesterol in hepatic and jejunal microsomes.[10] Oral administration of 1 to rats has been shown to cause a reduction of ACAT activity of jejunal microsomes.[11] The 15-ketosterol has been shown to lower serum cholesterol levels upon oral administration to animals.[12-14]

Delineation of the metabolism of 1 is critical to an understanding of its actions. 1 is convertible to cholesterol upon incubation with rat liver subcellular preparations[15,16] and upon oral or intravenous administration to rats and baboons,[9,17-20] and a pathway for the overall conversion of 1 to cholesterol has been presented.[16] Cholesterol and its esters have been shown to be the major metabolites of 1 found in tissues and blood after its intravenous administration to bile duct-cannulated rats.[17] However, a quantitatively more important fate of 1 under these conditions is very rapid conversion to polar metabolites which are excreted in bile[17,19] and of which a significant fraction undergoes enterohepatic circulation.[17] In initial studies of the nature of the polar metabolites of 1, we have shown that hydroxylation at C-26 and C-25 occurs upon its incubation with rat liver mitochondria in the presence of NADPH.[21] (25*R*)-3β,26-Dihydroxy-5α-cholest-8(14)-en-15-one, prepared by chemical synthesis, was shown to be highly active in lowering the levels of HMG-CoA reductase activity in CHO-K1 cells.[22]

The purposes of the present study were to synthesize 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (2) and to evaluate its action on HMG-CoA reductase activity in cultured mammalian cells.

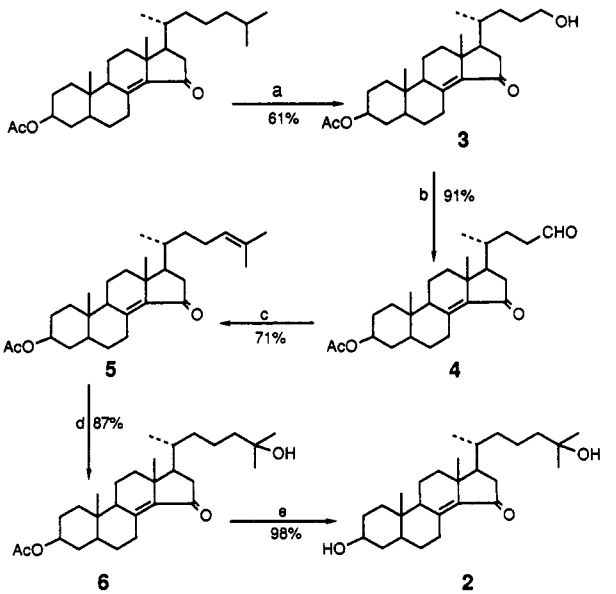

Figure 1. Conversion of 3β-acetoxy-5α-cholest-8(14)-en-15-one to 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one: (a) $(CF_3CO)_2O$, H_2O_2, H_2SO_4; triethylamine, CH_3OH (ref 28); (b) periodinane; (c) isopropyltriphenylphosphonium iodide, butyllithium; (d) $Hg(OAc)_2$; $NaBH_4$; (e) K_2CO_3; CH_3OH.

Table I. Effects of 3β,25-Dihydroxy-5α-cholest-8(14)-en-15-one (2) and 3β-Hydroxy-5α-cholest-8(14)-en-15-one (1) on the Levels of HMG-CoA Reductase Activity in CHO-K1 Cells

sterol concentration, μM	HMG-CoA reductase activity (% of control activity)[a]	
	2	1
0.0	100.0 ± 2.0[b]	100.0 ± 1.4[c]
0.1	63.4 ± 0.2	61.9 ± 1.2
0.25	33.5 ± 1.0	52.1 ± 1.3
0.5	32.2 ± 0.9	42.2 ± 2.0
1.0	34.2 ± 2.8	35.8 ± 0.6
2.5	21.5 ± 1.2	24.4 ± 0.8

[a] Variation is expressed as SD of triplicate assays for the experimental values. [b,c] Mean values for controls were 1265 and 854 pmol min^{-1} mg^{-1} protein, respectively.

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The development of an efficient chemical synthesis of 2 presents a significant challenge. The realization of this goal requires the construction of two functional domains, i.e., the $\Delta^{8(14)}$-15-ketone system and the 25-hydroxy-substituted sterol side chain. Two approaches can be considered: (a) introduction of the $\Delta^{8(14)}$-15-ketone functionality into a 25-hydroxysterol such as 25-hydroxycholesterol, and (b) introduction of the 25-hydroxyl group into a $\Delta^{8(14)}$-15-ketosterol. The former approach, for which analogy can be found in our previous synthesis of (25*R*)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one from (25*R*)-26-hydroxycholesterol,[22] would be limited by the need to prepare significant amounts of 25-hydroxycholesterol and the multiple steps required for its conversion to 2. The latter approach, direct hydroxylation of 1, represents a case of specific oxidation at an unactivated carbon atom of the sterol side chain, a continuing challenge in synthetic organic chemistry. Several approaches[23-27] for direct hydroxylation at C-25 have been described but these were not pursued because of reported low yields and/or unsuitability to the case of a $\Delta^{8(14)}$-15-ketosterol. Our current effort concentrated on exploitation of our recent demonstration of a specific, very high yield side-chain oxidation of 1,[28] for which an efficient synthesis has been described.[29] Oxidation of the acetate of 1 with a mixture of trifluoroacetic anhydride, hydrogen peroxide, and sulfuric acid, followed by treatment of the crude product with triethylamine and methanol, provided 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (3) in 61% yield.[28]

The availability of 3, selectively protected at C-3, provided a key intermediate for the chemical synthesis of 2. Oxidation of the 24-hydroxyl function of 3 with Dess–Martin reagent[30] gave the aldehyde 4[31] in 91% yield. Wittig olefination of 4 with isopropyltriphenylphosphonium iodide gave the desired Δ^{24} analogue 5[32] of the acetate of 1. Oxymercuration, following the procedure of Morisaki et al.,[33] proceeded in high yield to give the 25-hydroxy derivative 6[34] despite the presence of the $\Delta^{8(14)}$-15-ketone functionality. Mild alkaline hydrolysis[35] of 6 gave the desired 3β,25-dihydroxy-5α-cholest-8(14)-en-15-one (2).[36] The overall yield of 2 from the acetate of 1 was 36%.

The 3β,25-dihydroxy-15-ketosterol 2 was highly active in lowering the levels of HMG-CoA reductase activity in CHO-K1 cells (Table I).[37] It should be noted that 1, 26-hydroxycholesterol, and 25-hydroxycholesterol are among the most potent of oxysterols in the lowering of HMG-CoA reductase activity in cultured mammalian cells.[6] The results presented herein, coupled with those described previously,[22] demonstrate that hydroxylation of 1 at C-26 or C-25 leads to metabolites of very high activity, findings which indicate the importance of these metabolites in considerations of the overall actions of 1 in intact animals or in cells in which they are formed.

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(31) Oxidation of 3 (565 mg; 1.36 mmol) in CH_2Cl_2 (10 mL) with periodinane[30] (1.26 g; 2.99 mmol) for 3 h at 25 °C gave, after silica gel column chromatography (solvent, 10% ethyl acetate in hexane), 3β-acetoxy-15-oxo-5α-chol-8(14)-en-24-al (4) in 91% yield: mp 162–164 °C; IR (KBr) 1723, 1697, 1628 cm^{-1}; MS 414 (37%; M$^+$) calcd for $C_{28}H_{38}O_4$ 414.2770, found 414.2757; ^{13}C NMR δ 73.0 (C-3), 40.6 (C-23), 202.2 (C-24); single component on TLC (solvent, 40% ethyl acetate in hexane).

(32) 3β-Acetoxy-5α-cholesta-8(14),24-dien-15-one (5) was prepared in 71% yield by condensation of 4 (502 mg; 1.21 mmol) with the ylide prepared from isopropyltriphenylphosphonium iodide (839 mg; 1.99 mmol) and butyllithium (1.27 mmol) in THF at −78 °C for 15 min followed by stirring at 0 °C for 2 h and silica gel column chromatography (solvent, 4% ethyl acetate in hexane): mp 129–130 °C; IR (KBr) 1738, 1699, 1624 cm^{-1}; MS 440 (32%; M$^+$) calcd for $C_{29}H_{44}O_3$ 440.3291, found 440.3275; ^{13}C NMR δ 24.4 (C-23), 124.5 (C-24), 131.4 (C-25), 25.6 (C-26), 17.6 (C-27); single component on TLC (solvent, 40% ethyl acetate in hexane).

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(34) Mercuric acetate (147 mg; 0.461 mmol) in a 1:1 mixture (0.6 mL) of THF and water was added to 5 (131 mg; 0.297 mmol) in THF (0.6 mL). After stirring at 0 °C for 4 h and then at 25 °C for 5 h, the mixture was treated with $NaBH_4$ (550 mg) in 3 N NaOH for 5 min, and, after standard workup, subjected to silica gel column chromatography (solvent, 16% ethyl acetate in hexane) to give 3β-acetoxy-25-hydroxy-5α-cholest-8-(14)-en-15-one (6) in 87% yield: mp 151.0–152.5 °C; IR (KBr) 1736, 1701, 1626 cm^{-1}; MS 458 (51%; M) calcd for $C_{29}H_{46}O_4$ 458.3396, found 458.3393; ^{13}C NMR δ 73.1 (C-3), 44.2 (C-24), 70.8 (C-25); single component on TLC (solvent, 50% ethyl acetate in hexane).

(35) K_2CO_3 (20 mg) in methanol (2 mL); 4 h at 25 °C.

(36) 2: mp 177–179 °C; IR (KBr) 1701, 1683, 1622, 1607 cm^{-1}; MS 416 (64%; M$^+$) calcd for $C_{27}H_{44}O_3$ 416.3291, found 416.3303; ^{13}C NMR δ 70.8 (C-3), 37.7 (C-4), 31.1 (C-2), 71.0 (C-25); single component on TLC (solvents, 70% ethyl acetate in hexane and 40% acetone in benzene).

(37) The effects of 1 and 2 on the elevated levels of HMG-CoA reductase activity induced by transfer of the cells to lipid-deficient media were assayed as described previously.[7]

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