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FURTHER STUDIES ON THE INHIBITION OF STEROL BIOSYNTHESIS IN ANIMAL CELLS BY 15-OXYGENATED STEROLS

GEORGE J. SCHROEPFER, JR., EDWARD J. PARISH, and ANDREW A. KANDUTSCH

Departments of Biochemistry and Chemistry, Rice University, Houston, TX 77001, and The Jackson Laboratory, Bar Harbor, ME 04609 (U.S.A.)

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The chemical syntheses of a number of C_{27} 15-oxygenated sterols and their derivatives have been pursued to permit evaluation of their activity in the inhibition of sterol biosynthesis in animals cells in culture. Described herein are chemical syntheses of 3α-benzoyloxy-5α-cholest-8(14)-en-15-one, 5α-cholest-8(14)-en-3α-ol-15-one, 5α-cholest-8-(14)-en-15-one-3β-yl pyridinium sulfate, 5α-cholest-8(14)-en-15-one-3β-yl potassium sulfate (monohydrate), 5α-cholest-8(14)-en-15-one-3α-yl pyridinium sulfate, 5α-cholest-8(14)-en-3α-yl potassium sulfate (monohydrate), 5α-cholest-8(14)-en3,7,15-trione, 5α-cholest-8(14)-en-15α-ol-3-one, 5α,14α-cholestan-3β,15β-diol diacetate, 5α,14β-cholestan-3β,15β-diol diacetate, 5α,14α-cholestan-3β,15α-diol, 5α,14α-cholestan-15α-ol-3-one, 5α,14β-cholestan-3β,15β-diol, 5α,14α-cholestan-3,15-dione, and 5α,14β-cholestan-3,5-dione. The effects of 8 of the above compounds and of 5α-cholesta-6,8(14)-dien-3β-ol-15-one, 3β-hemisuccinoyloxy-5α-cholest-8(14)-en-15-one, 3β-hexadecanoyloxy-5α-cholest-8(14)-en-15-one, 5α-cholest-8(14)-en-3,15-dione, 5α-cholesta-6,8(14)-dien-3,15-dione, 5α-cholest-8-en-3β,15α-diol, 5α-cholest-7-en-3β,15α-diol, 5α-cholest-8(14)-en-15α-ol-3-one, 5α-cholest-8-en-15α-ol-3-one, and 5α-cholest-7-en-15α-ol-3-one on the synthesis of digitonin-precipitable sterols and on levels of HMG-CoA reductase activity have been investigated and compared with previously published data on 7 other C_{27} 15-oxygenated sterols.

Introduction

A number of 15-oxygenated sterols have recently been shown to be potent inhibitors of sterol biosynthesis in animal cells in culture [1–7]. Moreover, several of these 15-oxygenated sterols have been shown to possess significant hypocholesterolemic activity in animals [8–11]. As an extension of our initial studies of the effects of 15-oxygenated sterols on sterol biosynthesis in animal cells in culture, we have prepared a number of new 15-oxygenated sterols and have investigated the effects of these sterols and a number of other 15-oxygenated sterols on sterol biosynthesis and on the activity of HMG-CoA reductase in mouse L cells grown in a serum-free medium.

Experimental procedures and results

General methods and materials

Procedures for the recording of melting points (m.p.) [2], optical rotations (in $CHCl_3$) [5], infrared spectra [2], low resolution mass spectra (MS) [12,13], ultraviolet (in ethanol) spectra [14], and nuclear magnetic resonance (NMR) spectra [5] have been described previously. Chemical shifts for the C-18 and C-19 methyl resonances were calculated according to the method of Zurcher [15]. High resolution mass spectral measurements were made using the peak matching technique on a Varian CH-5 spectrometer (courtesy of Dr. C.C. Sweeley). Gas-liquid chromatographic analyses were made on 3% OV-1 and 3% OV-17 columns (270°C) as described previously [16]. Trimethylsilyl derivatives were prepared as described previously [17]. Thin layer chromatographic (TLC) analyses were made on plates of silica gel G (E. Merck, Darmstadt) or, in the case of the isolation of mevalonolactone, silica gel 60 (E. Merck, Darmstadt). The solvent systems used were as follows: SS-1, 25% ethyl acetate in methanol; SS-2, 25% ethyl acetate in ethanol; SS-3, benzene; SS-4, 10% ether in benzene; SS-5, 10% ether in hexane; SS-6, 5% acetone in $CHCl_3$; SS-7, 35% ethyl acetate in $CHCl_3$; SS-8, 50% ethyl acetate in toluene; SS-9, ethyl acetate; SS-10, 10% ethyl acetate in hexane; SS-11, 10% ethyl acetate in toluene; SS-12, 50% ethyl acetate in hexane; SS-13, 5% ethyl acetate in toluene; SS-14, 50% ether in hexane; SS-15, 20% ethyl acetate in hexane; SS-16, $CHCl_3$; and SS-17, 10% ether in toluene. Components on the plates were visualized after spraying with molybdic acid [18]. The preparation of 5α-cholest-8(14)-en-3β-ol-15-one (*I*) has been described previously [2,8] as have the syntheses of 5α-cholest-8(14)-en-3β,15α-diol (*II*; [19,20]), 5α-cholest-8(14)-en-3,15-dione (*III*; [20]), 5α-cholest-8(14)-en-3β-ol (*IV*; [21]), 5α-cholesta-6,8(14)-dien-3,15-dione (*V*; [14]), 5α-cholesta-6,8(14)-dien-3β-ol-15-one (*VI*; [14]), 3β-hexadecanoyloxy-5α-cholest-8(14)-en-15-one (*VII*; [9]), 3β-hemisuccinoyloxy-5α-cholest-8(14)-en-15-one (*VIII*; [9]), 5α-cholest-7-en-3β,15α-diol (*IX*; [14]), 5α-cholest-8-en-3β,15α-diol (*X*; [14]), 5α-cholest-8-en-15α-ol-3-one (*XI*; [14]), 5α-cholest-7-en-15α-ol-3-one (*XII*; [14]), and 5α-cholest-8(14)-en-3β,7α,15α-triol (*XIII*; [2,22]). A 1 M solution of borane-tetrahydrofuran complex in tetrahydrofuran (stabilized with 5 mol% sodium borohydride) was purchased from Aldrich Chemical Company (Milwaukee, WI). Silver carbonate-celite was prepared according to Fetizon and Goldfier [23]. [1,2-^{3}H]Cholesterol and [5-^{3}H]mevalonic acid (purchased in the form of the lactone) were obtained from the New England Nuclear Corporation (Boston, MA). Bovine serum albumin (Pentex; crystallized) was purchased from Miles Laboratories, Inc. (Elkhart, IN).

*5α-Cholest-8(14)-en-15-one-3β-yl pyridinium sulfate (*XIV*)*

Compound *XIV* (Fig. 1) was prepared from the corresponding 3β-hydroxysterol by a modification of the method of McKenna and Norymberski [24]. Compound *I* (2.0 g; 5.0 mmol) in dry $CHCl_3$ (50 ml; ethanol-free; dried over Linde molecular sieve, type 3A). Pyridine-sulfur trioxide (5.0 g) was added and the resulting mixture

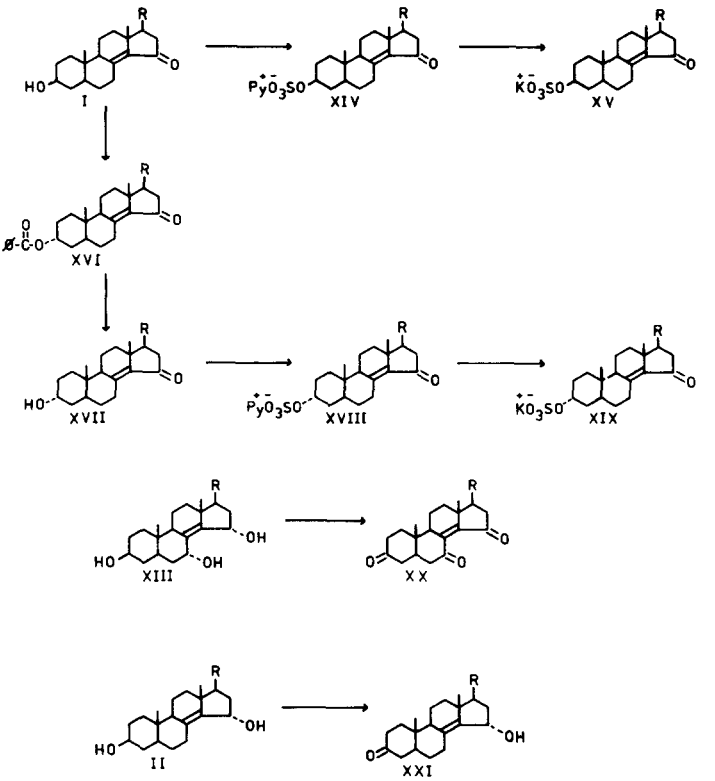


Fig. 1. Synthetic schemes for the preparation of 5α-cholest-8(14)-en-3α-ol (*XVI*) and the potassium sulfate esters of this compound and its 3β-hydroxy epimer (*I*) and for the preparation of 5α-cholest-8(14)-en-3,7,15-dione (*XX*) and 5α-cholest-8(14)-15α-ol-3-one (*XXI*).

was stirred at room temperature for 3 h. The excess reagent was removed by filtration, washed with a small volume of $CHCl_3$, and the filtrate was cooled to −40°C (dry ice/acetone bath) and filtered through Hyflo Super-Cel (Johns-Manville Corp.). Hexane was added to the $CHCl_3$ filtrate until a cloudiness appeared whereupon cooling to 0°C gave a white precipitate. This material was collected by filtration and placed in a vacuum desiccator for 100 h at room temperature to remove all traces of solvent. The product, *XIV* (2.4 g; 86% yield), melted at 185.5–186.5°C; infrared, ν_{max} 3100, 1708, 1650, 1545, 1240, 1061, 690, and 612 cm^{-1}; NMR, 0.07 (s, 3H, C–19–CH_3), 0.98 (s, 3H, C–18–CH_3), 4.25 (m, 2H, C–7β–H and C–3–H), and 8.6 (m, 5H aromatic); MS, 382 (100%; M–H_2SO_4 · pyridine), 367 (46%; M–CH_3–H_2SO_4 · pyridine), 364 (4%; M–H_2O–H_2SO_4 · pyridine), 328 (10%), 269 (20%; M–side chain–H_2SO_4 · pyridine), and 251 (39%; M–H_2O–side chain-H_2SO_4 · pyridine); high resolution MS on ion at *m/e* 382, 382.3239 (calc. for $C_{27}H_{42}O$: 382.3236). The product showed a single component on TLC (SS-1, R_F 0.66; SS-2, R_F 0.64).

5α-Cholest-8(14)-en-15-one-3β-yl potassium sulfate (monohydrate) (XV)

Compound *XV* (Fig. 1) was prepared from the corresponding steryl pyridium sulfate by the following modification of the procedure of McKenna and Norymberski [24]. Compound *XIV* (1.0 g; 1.79 mmol) was dissolved in distilled water (50 ml) at 25°C and an aqueous saturated solution of KCl was slowly added to the stirred mixture. After 15 min the resulting suspension was filtered, washed with water, and dried in a vacuum desiccator for several hours and the resulting white residue (0.85 g; 89%), *XVI* melted at 231.5–232.5°C; infrared, ν_{max} 3550, 1708, 1620, 1240, 1061, 1002, 828, and 612 cm^{-1}; NMR, 0.82 (s, 3H, C–19–CH_3), 0.92 (s, 3H, C–18–CH_3), 4.25 (m, 2H, C–7β–H and C–3–H); MS, 400 (13%; M–$KHSO_4$), 382 (100%; M–H_2O–$KHSO_4$), 367(61%; M–CH_3–H_2O–$KHSO_4$), 364 (7%; M–H_2O–H_2O–$KHSO_4$), 287 (49%; M–side chain–$KHSO_4$), 269 (33%; M–H_2O–side chain–$KHSO_4$), 251 (39%; M–H_2O–H_2O–side chain–$KHSO_4$); high resolution MS on ion at *m/e* 400, 400.3342 (calc. for $C_{27}H_{44}O_2$: 400.3341).

3α-Benzoyloxy-5α-cholest-8(14)-en-15-one (XVI)

Compound *XVI* (Fig. 1) was prepared from corresponding 3β-hydroxysterol by a modification of the method of Bose et al. [25]. To a stirred solution of *I* (1.00 g; 2.5 mmol), triphenyl phosphine (1.97 g; 7.5 mmol), and benxoic acid (0.61 g; 5.0 mmol) in dry tetrahydrofuran (30 ml) was added dropwise a solution of diethyl azodicarboxylate (0.87 g; 5.0 mmol) in tetrahydrofuran (5 ml). After stirring for 14 h, the mixture was poured into water and thoroughly extracted with ether containing CH_2Cl_2 (5%). The combined extracts were dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The resulting light yellow solid was subjected to silica gel (100 g; 60–300 mesh) column (60 cm × 2.0 cm) chromatography. Using benzene as the eluting solvent, fractions 24 ml in volume (flow rate, 1.5 ml/min) were collected. The contents of fractions 40–95 were pooled and, after evaporation of the solvent under reduced pressure, crystallized from acetone/water to give *XVI* (1.15 g; 91% yield) which melted at 128.5–129.5°C; infrared, ν_{max} 1720, 1630, 1280, 1125, and 718 cm^{-1}; NMR, 0.76 (s, 3H, C–19–CH_3), 0.98 (s, 3H, C–18–CH_3), 4.22 (m, 1 H, C–7β–H), 5.37 (m, 1 H,C–3–H), 7.90 (m, 5H, aromatic); MS, 504 (85%; M), 489 (6%; M–CH_3), 382 (90%; M–benzoic acid), 380 (37%), 374 (7%), 367 (100%; M–CH_3–benzoic acid), 365 (30%), 269 (90%; M–side chain–benzoic acid), 251 (88%; M–side chain–benzoic acid–CH_3); high resolution MS, 504.3614 (calc. for $C_{34}H_{48}O_3$: 504.3603); $[\alpha]_D$ +111.4°. The compound showed a single component on TLC (SS-3, R_F 0.05; SS-4 R_F 0.67; SS-5, R_F 0.15; SS-6, R_F 0.51; and SS-7, R_F 0.76).

5α-Cholest-8(14)-en-3α-ol-15-one (XVII)

To *XVI* (500 mg; 1.00 mmol) in absolute ethanol (175 ml) was added water (10 ml) and concentrated H_2SO_4 (30 ml). After heating the mixture under reflux for 24 h in an atmosphere of nitrogen, the volume was reduced to approx. 1/2 of its initial value under reduced pressure and poured into water. The mixture was thor-

oughly extracted with ether containing CH_2Cl_2 (5%) and the combined extracts were evaporated to dryness under reduced pressure. The resulting light yellow residue was crystallized twice from acetone/water. The crystals were dissolved in acetone and warmed in the presence of decolorizing carbon (Norite A) for 15 min. The solution was filtered through Hyflo Super-Cel (Johns-Manville Corp.) and the Super-Cel was washed with methanol. The acetone and methanol solutions were combined and, after the addition of water, the crystalline product which formed was collected and dried in vacuo to give *XVII* (326 mg; 82% yield; Fig. 1) melting at 148–149°C; infrared, ν_{max} 3310, 1708, and 1015 cm^{-1}; NMR, 0.69 (s, 3H, C–19–CH_3), 0.97 (s, 3H, C–18–CH_3), 4.08 (m, 1H, C–3–H), and 4.15 (m, 1H, C–7β–H); ultraviolet, λ_{max} 261 nm (ϵ=13 300); MS, 400 (90%; M), 385 (21%; M–CH_3), 382 (80%; M–H_2O), 367 (92%; M–CH_3–H_2O), 287 (27%), 276 (21%), 269 (100%; M–H_2O–side chain), and 261 (34%); high resolution MS, 400.3346 (calc. for $C_{27}H_{44}O_2$: 400.3341); $[\alpha]_D$ + 139.3°. The compound showed a single component on TLC (SS-4, R_F 0.08; SS-6, R_F 0.15; and SS-7, R_F 0.49) and on GLC (OV-1 and OV-17).

5α-Cholest-8(14)-en-15-one-3α-yl pyridinium sulfate (XVIII)

Compound *XVIII* (Fig. 1) was prepared in 72% yield from *XVII* (200 mg) by the precedure described above for the case of the preparation of *XIV*. Compound *XVIII* melted at 175–177°C; infrared, ν_{max} 3103, 1707, 1649, 1543, 1065, and 690 cm^{-1}; NMR, 1.20 (m, methylene envelope), 4.23 (m, 1H, C–7β–H), 4.85 (m, 1H, C–3–H), and 8.6 (m, 5H; aromatic); MS, 382 (100%; M–H_2SO_4 · pyridine), 367 (43%; M–CH_3–H_2SO_4 · pyridine), 364 (2%; M–H_2O–H_2SO_4 · pyridine), 328 (7%), 269 (19%; M–side chain–H_2SO_4 · pyridine), 251 (25%; M–H_2O–side chain–H_2SO_4 · pyridine); high resolution MS on ion at *m/e* 382, 382.3226 (calc. for $C_{27}H_{42}O$: 382.3246). The compound showed a single component on TLC (SS-1, SS-2).

5α-Cholest-8(14)-en-15-one-3α-yl potassium sulfate monohydrate (XIX)

Compound *XIX* (Fig. 1) was prepared in 76% yield from *XVIII* (100 mg) by the procedure described above for the case of the preparation of *XIV*. The compound melted at 163.0–164.5°C; infrared, ν_{max} 3552 (H_2O), 1709, 1623, 1063, 1005, 831, and 6.6 cm^{-1}; NMR, 1.20 (m, methylene envelope), 4.25 (m, 2H, C–7β–H and C–3–H); MS, 400 (10%; M–$KHSO_4$), 385 (3%; M–CH_3–$KHSO_4$), 382 (100%; M–H_2O–$KHSO_4$), 367 (55%; M–CH_3–H_2O–$KHSO_4$), 269 (33%; M–H_2O–side chain-$KHSO_4$), 251 (31%), and 241 (5%); high resolution MS on ion at *m/e* 400, 400.3343 (calc. for $C_{27}H_{44}O_2$: 400.3341).

5α-Cholest-8(14)-en-3,7,15-trione (XX)

To *XIII* (1.00 g; 2.39 mmol) in dry CH_2Cl_2 (200 ml) was added sodium acetate (0.60 g) and pyridinium chlorochromate (3.50 g; 16.2 mmol). After stirring for 30 min at 25°C under nitrogen, the mixture was poured into ether and thoroughly washed with a saturated NaCl solution. The resulting ether solution was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness under reduced pressure to

give a brown residue which was subjected to chromatography on a silica gel (60–200 mesh) column (60 cm × 2.0 cm). Using 10% ethyl acetate in $CHCl_3$ as the eluting solvent (flow rate, 5 ml per min), fractions 20 ml in volume were collected. The contents of fractions 54–74 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized twice from tetrahydrofuran/hexane (1:9) at −40°C to give *XX* (0.61; 62% yield; Fig. 1) melting at 155.0–156.5°C (literature: 154–155°C [2]); infrared ν_{max} 1725, 1700, 1642, and 1220 cm^{-1}; ultraviolet, λ_{max} 257 nm (ϵ=9700); NMR, 1.04 (s, 3H, C–19–CH_3; calc., 1.06), 1.17 (s, 3H, C–18–CH_3), and no downfield resonances; MS, 412 (35%; M), 397 (84%; M–CH_3), 301 (10%), 299 (18%; M–side chain), 286 (6%), 272 (100%), 246 (10%), and 162 (78%. The compound showed a single component on TLC (SS-7, SS-8, SS-9) and on GLC (OV-1 and OV-17). In contrast to our previously reported synthesis of this compound [2], the above procedure leads to a product of much higher priority.

5α-Cholest-8(14)-en-15α-ol-3-one (XXI)

To component 1 (180 ml) of the Biodynamics (BMC Division) Cholesterol Auto Test was added 'cholesterol oxidase' (5.5 ml; component 3 of the Cholesterol Auto Test) and the resulting mixture was diluted with distilled water (540 ml). Compound *II* (105 mg; 0.26 mmol) in isopropanol (40 ml) was added and the resulting mixture was incubated with shaking for 8 h at 37°C. A saturated solution (200 ml) of NaCl was added and the mixture was extracted 5 times with 100-ml portions of $CHCl_3$. The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The resulting residue was subjected to silica gel (150 g; 70–325 mesh) column (90 cm × 2.0 cm) chromatography using 10% ether in benzene as the eluting solvent (flow rate, 5 ml/min). Fractions 20 ml in volume were collected. The contents of fractions 25–45 were pooled and, after evaporation of the solvent under reduced pressure, crystallized from acetone/water to give *XXI* (88 mg; 85% yield; Fig. 1) melting at 134.5–135.5°C; infrared, ν_{max} 3360, 1725 (C=O stretch in 6-membered ring), and 1042 cm^{-1}; NMR, 0.79 (s, 3H, C–19–CH_3), 0.88 (s, 3H, C–18–CH_3), 4.74 (m, 1H, C–15–H); MS, 400 (11%; M), 385 (20%; M–CH_3), 382 (100%; M–H_2O), 367 (50%; M–CH_3–H_2O), 287 (9%; M–side chain), 269 (92%; M–H_2O–side chain), and 255 (56%); high resolution MS., 400.3354 (calc. for $C_{27}H_{44}O_2$: 400.3341). The compound showed a single component on TLC (SS-4, R_F 0.12; SS-7, R_F 0.58; SS-6, R_F 0.53) and a purity in excess of 98% on GLC (OV-1 and OV-17).

5α,14α-Cholestan-3β,15α-diol Diacetate (XXII) *and 5α,14β-Cholestan-3β,15β-diol Diacetate* (XXIII)

To *IV* (5.00 g; 12.9 mmol) was added a 1 M solution (150 ml) of borane-tetrahydrofuran complex in tetrahydrofuran and the resulting solution was stirred at 45–55°C for 6 h under nitrogen. After cooling to 4°C, ice was cautiously added to decompose the excess reagent. Water and ether (1000 ml) were added and the separated organic phase was washed with water and evaporated to dryness under

reduced pressure. The resulting residue was dissolved in tetrahydrofuran (200 ml) and, after cooling to 4°C, a 20% NaOH solution (75 ml) and a 30% hydrogen peroxide solution (75 ml) were slowly added. The resulting mixture was stirred for 1 h at 4°C and then for 2 h at 25°C. Ether (1000 ml) was added and the separated organic phase was successively washed with a 10% sodium sulfite solution and a saturated NaCl soltuion, dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure. Analysis by GLC (OV-17) indicated the presence of two components; a major component (76%) and a more polar component (24%). Attempts to resolve the mixture by TLC were unsuccessful and purification in the form of the acetate derivatives was pursued. The crude mixture (4.76 g) was dissolved in pyridine (75 ml) and acetic anhydride (75 ml) was added. After standing at 25°C overnight the mixture was poured into ice water and extracted with ether (1000 ml). The extract was washed successively with water, a cold 5% HCl solution, a 5% Na_2CO_3 solution, and water, dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure to yield a colorless oil. Analysis by TLC. (SS-5) showed a major component (R_F 0.38) and a minor component (R_F 0.30). The crude product was subjected to medium pressure (413 kPa) liquid chromatography on a silica gel (0.032–0.063 mm; ICN Pharmaceuticals, Cleveland, OH) column (100 cm × 2.5 cm). Using 10% ether in hexane (flow rate, 5 ml/min), fractions 20 ml in volume were collected. The contents of fractions 73–95, corresponding to the minor component detected by TLC, were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from acetone/water to yield *XXII* (1.15 g; 18% yield; Fig. 2) melting at 147.5–148.5°C (literature: 144–146°C [26]); infrared, ν_{max} 1748, 1255, and 1034 cm^{-1}; NMR, 0.72 (s, 3H, C–18–CH_3; calc., 0.72), 0.83 (s, 3H, C–19–CH_3; calc., 0.83), 1.97 (s, 6H, methyl of acetoxy functions), and 4.75 (m, 2H, C–3–H and C–15–H); MS, 428 (93%; M–CH_3COOH), 413 (41%; M–CH_3–CH_3COOH), 368 (35%; M–CH_3COOH–CH_3COOH), 353 (43%; M–CH_3COOH–CH_3COOH–CH_3), 315 (100%; M–side chain–CH_3COOH), 255 (89%; M–side chain–CH_3COOH–CH_3COOH), and 213 (29%); high resolution MS on ion at *m/e* 428, 428.3655 (calc. for $C_{29}H_{48}O_2$: 428.3671); $[\alpha]_D$ + 50.7° (*c*, 0.41) (literature: + 45° [26]). The product showed a single component on TLC (SS-5, SS-10, and SS-11) and on GLC (OV-1 and OV-17).

The contents of fractions 105–170, corresponding to the major component detected by TLC, were combined and, after evaporation of the solvent under reduced pressure, yielded *XXIII* as a colorless oil which formed a clear glassy solid (4.14 g; 66% yield; Fig. 2) on standing; infrared, ν_{max} 1750, 1255, 1035, and 610 cm^{-1}; NMR, 0.79 (s, 3H, C–18–CH_3), 0.89 (s, 3H, C–19–CH_3), 1.97 (s, 6H, methyls of acetoxy functions), 4.67 (m, 1H, C–3–H), and 5.12 (m, 1H, C–15–H); MS, 428 (14%; M–CH_3COOH), 413 (10%; M–CH_3–CH_3COOH), 368 (18%; M–CH_3COOH–CH_3COOH), 353 (10%; M–CH_3–CH_3COOH–CH_3COOH), 315 (100%; M–side chain–CH_3COOH), and 255 (78%; M–CH_3COOH–CH_3COOH–side chain); high resolution MS on ion at *m/e* 428, 428.3663 (calc. for $C_{29}H_{48}O_2$: 428.3655); $[\alpha]_D$–19.8° (*c*, 0.57). The compound showed a single component on TLC (SS-5),

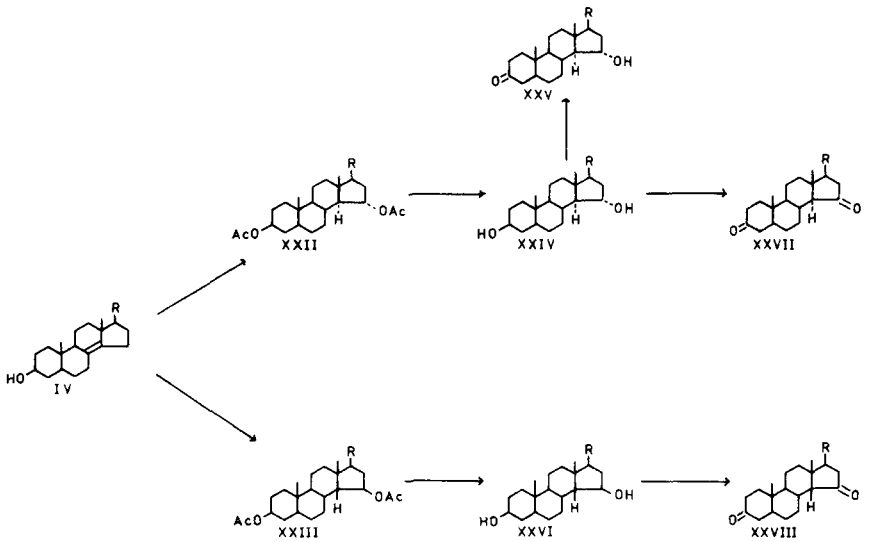


Fig. 2. Synthetic schemes for the preparation of 5α,14α-cholestan-3β,15α-diol (*XXIV*), 5α,14α-cholestan-15α-ol-3-one (*XXV*), and 5α,14β-cholestan-3β,15β-diol (*XXVI*).

SS-10, and SS-11) and on GLC (OV-1 and OV-17).

The yield of *XXII* from *IV* was increased from 18% to 78% by the following modification of the reaction condition. Compound *IV* (5.00 g; 12.9 mmol) was treated with a 1 M solution (150 ml) of borane-tetrahydrofuran complex as described above. The organo-borane residue was dissolved in xylene (75 ml) and heated at 130°C for 6 h. The xylene was removed under reduced pressure and the resulting residue was dissolved in tetrahydrofuran (200 ml) and oxidized with alkaline hydrogen peroxide as described above. Analysis by GLC (3% OV-17; 270°C) showed a minor component (22%) and a major, polar component (78%). The residue (4.74 g) was converted to the diacetate as described above and recrystallized twice from acetone/water at 4°C and twice from acetone/water at −15°C to yield *XXII* (4.68 g; 74% yield) melting at 147.5–148.5°C; $[\alpha]_D$ + 49.4° (*c*, 0.38). The infrared, NMR, and MS spectra were essentially identical to those obtained upon *XXII* as described above. The product showed a single component on TLC and GLC when analyzed as described above.

5α,14α-Cholestan-3β,15α-diol (XXIV)

To *XXII* (1.00 g; 2.05 mmol) in ethanol (100 ml) added KOH (5 g) in water (5 ml). After standing at 25°C for 24 h, the volume of the mixture was reduced to approx. 1/5 of its initial value under reduced pressure. Water was added and the resulting mixture was thoroughly extracted with ether containing CH_2Cl_2 (10%).

The residue obtained upon evaporation of the solvent under reduced pressure was recrystallized from acetone/water to yield *XXIV* (0.78 g; 94% yield; Fig. 2) melting at 191.5–192.5°C (literature: 191–193°C [26]); infrared, ν_{max} 3400, and 1048 cm^{-1}; NMR, 0.67 (s, 3H, C–18–CH_3: calc., 0.68), 0.81 (s, 3H, C–19–CH_3; calc., 0.82), 3.50 (m, 1H, C–3–H), and 3.90 (m, 1H, C–15–H); MS, 404 (10%; M), 386 (100%; M–H_2O), 371 (61%; M–CH_3–H_2O), 353 (11%; M–CH_3–H_2O), 291 (5%; M–side chain), 273 (47%; M–H_2O–side chain), 255 (27%; M–H_2O–H_2O–side chain), and 217 (75%); high resolution MS, 404.3663 (calc. for $C_{27}H_{48}O_2$: 404.3655); $[\alpha]_D$ + 49.7° (*c*, 0.40) (literature: + 60° [26]). The compound showed a single component on TLC (SS-7, SS-8, and SS-12) and on GLC (OV-1 and OV-17); of the free sterol and its trimethylsilyl derivative.

5α-14α-Cholestan-15α-ol-3-one (XXV)

To *XXIV* (500 mg; 1.24 mmol) in toluene (55 ml) was added silver carbonate-celite (3.30 g) and the resulting mixture was heated to reflux. After approx. 10 ml had distilled off the mixture was heated under reflux for 4 h. The mixture was filtered and evaporated to dryness under reduced pressure. The resulting white residue (451 mg) was subjected to medium pressure (689 kPa) liquid chromatography on a silica gel (0.032–0.063 mm) column (118 cm × 1.5 cm). Using 20% ethyl acetate in toluene (5 ml per min) as the eluting solvent, fractions 20 ml in volume were collected. The contents of fractions 14–28 were combined and, after evaporation of the solvent under reduced pressure, gave *XXV* as a white crystalline solid (438 mg: 88% yield; Fig. 2) which, upon recrystallization from acetone/water, melted at 127.5–128.5°C; infrared, ν_{max} 3470, 1718, 1079, and 1049 cm^{-1}; NMR, 0.64 (s, 3H, C–18–CH_3; calc., 0.63), 1.20 (s, 3H, C–19–CH_3; calc., 1.20), and 3.98 (m, 1H, C–15–H); $[\alpha]_D$ + 68.8° (*c*, 0.37); MS, 402 (12%; M), 384 (100%; M–H_2O), 369 (31%; M–CH_3–H_2O), 289 (4%; M–side chain), 271 (69%; M–H_2O–side chain), 261 (44%), 233 (59%), and 218 (40%); high resolution MS, 402.3499 (calc. for $C_{27}H_{46}O_2$: 402.3498). The compound showed a single component on TLC (SS-7, SS-13, and SS-14) and on GLC (OV-1 and OV-17).

5α,14β-Cholestan-3β,15β-diol (XXVI)

Compound *XXIII* (1.00 g; 2.05 mmol) was saponified and the reaction mixture was processed as described above for the case of *XXIV*. The crude product was recrystallized from acetone/water to yield *XXVI* (0.77 g; 93% yield; Fig. 2) melting at 166–167°C; infrared, ν_{max} 3360, 1054, and 1036 cm^{-1} NMR, 0.78 (s, 3H, C–19–CH_3), 0.99 (s, 3H, C–18–CH_3), 3.60 (m, 1H, C–3–H), and 4.25 (m, 1H, C–15–H); MS, 404 (1%; M), 386 (55%; M–H_2O), 371 (16%; M–CH_3–H_2O), 368 (4%; M–H_2O–H_2O), 353 (4%; M–CH_3–H_2O–H_2O), 291 (7%; M–side chain), 273 (52%; M–side chain–H_2O–H_2O), and 217 (100%); high resolution MS, 404.3655 (Calc. for $C_{27}H_{48}O_2$: 404.3655); $[\alpha]_D$ + 28.9° (*c*, 0.25). The compound showed a single component on TLC (solvent systems, same as in case of *XXIV* above) and on GLC (OV-1 and OV-17) of the free sterol and its trimethylsilyl derivative.

5α,14α-Cholestan-3,15-dione (XXVII)

To compound *XXIV* (250 mg; 0.62 mmol) in glacial acetic acid (5 ml) was added a solution of chromium trioxide (175 mg) in 80% acetic acid/water (8 ml). After standing overnight at 25°C, the mixture was poured into cold water and the resulting precipitate was collected by filtration and purified by silica gel (60–200 mesh) column (60 cm × 1.5 cm) chromatography. Using 20% ethyl acetate in hexane as the eluting solvent, fractions 20 ml in volume were collected. The contents of fractions 28–37 were pooled and, after evaporation of the solvent under reduced pressure, recrystallized from acetone/water to give *XXVII* (218 mg; 88% yield; Fig. 2); infrared, ν_{max} 1742 (ketone in 5-membered ring), 1725 (ketone in 6-membered ring), and 1233 cm^{-1}; NMR, 0.76 (s, 3H, C–18–CH_3; calc.*, 0.76), 1.02 (s, 3H, C–19–CH_3; calc.*, 1.03), and no downfield protons; MS, 400 (61%; M), 385 (9%; M–CH_3), 382 (2%; M–H_2O), 367 (2%; M–CH_3–CH_3–H_2O), 287 (19%; M-side chain), 273 (24%), 259 (65%), 232 (98%), 217 (100%), and 209 (68%). The product showed a single component on TLC (SS-6, SS-15, SS-16, SS-17) with the same mobility as 5α-14β-cholestan-3,15-dione (*XXIX*; see below). Analyses by GLC (OV-1 and OV-17) indicated the presence of a less polar component (approx. 13%) which had the same chromatographic mobility as that of *XXVIII*.

5α,14β-Cholestan-3,15-dione (XXVIII)

Compound *XXVI* (250 mg; 0.62 mmol) was oxidized with chromium trioxide and the reaction mixture was processed as described above for the case of the preparation of *XXVII*. After silica gel column chromatography the contents of fractions 29–36 were combined and, after evaporation of the solvent under reduced pressure, recrystallized from acetone/water to give *XXVIII* (213 mg; 86% yield; Fig. 2); infrared, ν_{max} 1740 (ketone in a 5-membered ring), 1718 (ketone in a 6-membered ring), and 1382 cm^{-1}; NMR, 0.96 (s, 3H, C–18–CH_3; calc.*, 0.97), 1.18 (s, 3H, C–19–CH_3; calc.*, 1.18), and no downfield protons; MS, 400 (15%; M), 385 (4%; M–CH_3), 382 (2%; M–H_2O), 367 (2%; M–CH_3–H_2O), 287 (30%), 273 (4%), 259 (9%), 232 (17%), 217 (29%), and 209 (100%). The product showed a single component on TLC (SS-6, SS-15, SS-16, SS-17) with the same mobility as *XXVII*. Analyses by GLC (OV-1 and OV-17) showed the presence of a more polar component (approx. 23%) which had the same chromatographic behavior as that of the *trans*–C–D isomer (*XXVII*).

Cell cultures

Mouse L cell cultures were grown as monolayers in serum-free, chemically defined media in 75 cm^{-2} culture flasks as previously described [30,31].

*The NMR calculations for the C-18 and C-19 methyl resonances were made using published data of others [27–29].

Assays for suppression of HMG-CoA reductase activity and inhibition of sterol synthesis*

Methods for the measurement of DNA and protein were described previously [30]. Procedures for assaying rates of conversion of [1-^{14}C]acetate into digitonin-precipitable sterols and fatty acids were the same as described previously [30,31] except that following the incubation of the cultures with the labeled acetate, [1,2-^{3}H]cholesterol (40 000 dpm; 0.3 pmol) was added to each flask to permit estimation of [^{14}C]sterol recoveries. Steroids were prepared for addition to the cultures by dissolving them in redistilled absolute ethanol and diluting the ethanol solution with 9 vols. of a 5% solution (w/v) of bovine serum albumin. The resulting mixture (0.5 ml) was then diluted with 4.5 ml of culture medium and the steroid-containing medium was added to a previously drained culture. Control flasks were fed with all of the components present in the experimental flasks with the exception of the test steroid. The final concentrations of bovine serum albumin (0.5%) and ethanol (1%) were identical in all experimental and control flasks. As a preliminary test for inhibitory activity, L cell cultures were incubated with at least 4 concentrations of steroid in the range from 0.1 to 20 μM for 4 h at 37°C on a gyrating table (50 rev./min). Sodium [1-^{14}C]acetate (20 μCi; 20 μmol) in 0.14 N NaCl solution (50 μl) was then added to each culture flask, the flasks were capped with septum stoppers fitted with plastic caps, and the incubation was continued for an additional 2 h, after which [^{14}C]fatty acids and [^{14}C]sterols were determined. We have previously established that, under these conditions, the rates of acetate metabolism to sterols, fatty acids, and CO_2 in control cultures are essentially constant for at least 12 h [30]. Steroids that did not depress the ratio of sterol synthesis to fatty acid synthesis to a level equal to or less than 50% of the control at a concentration of 11 μM were not tested further. Steriods that were highly inhibitory at the lowest concentrations used in the preliminary test were retested over successively lower ranges of concentration until the concentration required for 50% inhibition of sterol synthesis could be estimated from a well-defined plot of activity versus concentration. Studies of rates of acetate metabolism to fatty acids were also made so as to detect any possible effects of the inhibitors of sterol synthesis on general metabolism. In the present series of tests, none of the compounds examined showed any consistent effects on the rates of fatty acid production. Minor variations in rates of fatty formation in experimental flasks as well as in control flasks were, therefore, considered to be due to technical error and to variations in the metabolic characteristics of individual cultures in a similarly prepared and similarly treated experimental group. In an effort to correct for the effects of these sources of variation upon estimates of inhibitory potency, concentrations required to inhibit sterol synthesis by 50% were estimated from plots of the ratio of [^{14}C]sterols to [^{14}C]fatty acids as a function of the concentration of the inhibitor [30].

The concentrations of steroids required to depress HMG-CoA reductase activity

*The abbreviation used is: HMG, 3-hydroxy-3-methylglutaric acid.

in L cells to 50% of the control value were determined in experiments similar to those carried out to establish concentrations required to inhibit sterol synthesis from acetate except that enzyme activity was determined after incubating the cells for 5 h with the steroid-containing medium. The cells were then scraped from the flasks in 5 ml of phosphate-buffered saline (pH 7.4), sedimented by centrifugation at 800 *g* for 5 min, and then frozen in liquid nitrogen. Under these conditions HMG-CoA reductase activity was stable for at least one month. The cells were thawed by the addition of a cold buffer solution (0.5 ml/mg protein) containing 50 mM potassium phosphate buffer (pH 7.4), 5 mM dithiothreitol and 1 mM EDTA, sonicated for 2 s and then preincubated at 37°C for 15 min to activate any inactive HMG-CoA reductase present and to eliminate interference by HMG-CoA lyase [32]. Following preincubation, aliquots (50 μl; containing approx. 100 μg of protein) were added to an incubation mixture containing 2.5 mM NADPH, 20 mM glucose 6-phosphate, 0.7 units of glucose-6-phosphate dehydrogenase, 80 μM D,L-[3-^{14}C] HMG-CoA (10 Ci/mol), 2.5 mM dithioerythritol and 0.1 M potassium phosphate buffer (pH 7.5) in a final volume of 0.2 ml. The mixtures were shaken in a water bath at 37°C for 30 min and the reaction was stopped by adding 25 μl of 12 N HCl. [5-^{3}H]Mevalonic acid (50 000 dpm), was added and the mixtures were allowed to stand for at least 30 min at room temperature. Na_2SO_4 (0.3 g) was added and the mixtures were extracted by shaking for 5 min with 5 ml of diethyl ether (freshly distilled over a solution of 60 g $FeSO_4 \cdot 7H_2O$ and 6 ml concentrated H_2SO_4 in 110 ml H_2O into a flask containing sodium diethyl dithiocarbonate (20 mg/100 g of ether). The ether layer was removed and evaporated to dryness under nitrogen. The resulting residue was dissolved in 50 μl of toluene containing mevalonolactone at a concentration of 20 mg/ml. The toluene solution was streaked on a thin layer (0.25 mm thick) of silica gel 60 and developed with ethyl acetate. Visualization of the mevalonolactone band and assay of the band for ^{14}C and ^{3}H have been described [30].

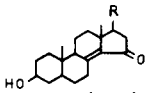
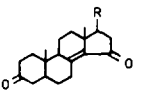
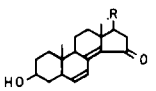
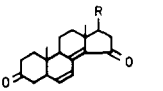
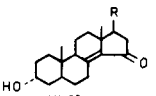
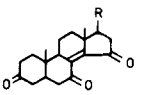
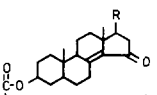
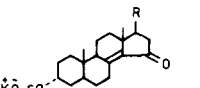
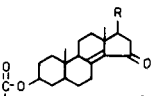
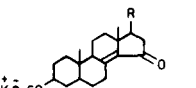
Inhibition of sterol synthesis and reduction of HMG-CoA reductase activity in L cells by 15-oxygenated sterols

The effects of ten C_{27} 15-ketosterols and their derivatives on sterol synthesis and on the levels of HMG-CoA reductase activity on L cells are presented in Table I. With the exception of the palmitate and sulfate esters of 5α-cholest-8(14)-en-3β-ol-15-one and the sulfate ester of 5α-cholest-8(14)-en-3α-ol-15-one, all of the new compounds tested were effective as inhibitors of steol synthesis. However, none of new 15-ketosterols tested were more effective in suppressing sterol synthesis in the L cells than 5α-cholest-8(14)-en-3β-ol-15-one (*I*). In general, the concentrations of the 15-ketosterols required to cause a 50% inhibition of sterol synthesis were similar to the concentrations required to cause a 50% reduction in the levels of HMG-CoA reductase activity in the same cells. The largest discrepancy on this point was the case of 5α-cholest-8(14)-en-3α-ol-15-one, in which the concentration required to cause a 50% reduction in sterol synthesis was 6-times lower than that

TABLE I

INHIBITION OF STEROL SYNTHESIS AND REDUCTION OF LEVELS OF HMG-CoA REDUCTASE ACTIVITY IN L CELLS BY C_{27} $\Delta^{8(14)}$-15-KETOSTEROLS AND THEIR DERIVATIVES.

CONCENTRATIONS (μM) REQUIRED FOR 50 % INHIBITION

INHIBITOR	STEROL SYNTHESIS	HMG-CoA REDUCTASE	INHIBITOR	STEROL SYNTHESIS	HMG-CoA REDUCTASE
 I [ref. 2]	0.1	0.3	 III	0.4	0.4
 VI	1.0	0.3	 V	0.8	0.3
 XVII	0.5	3.0	 XX	0.4	1.0
 VIII	3.2	3.0	 XIX	>11	—
 VII	>16	—	 XV	>11	—

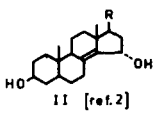
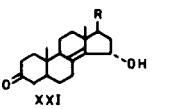
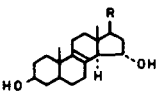
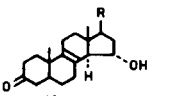
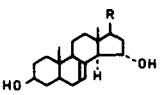
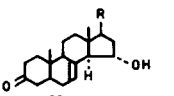
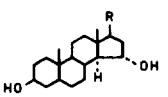
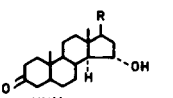
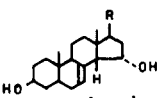
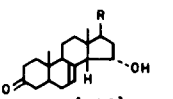
required to cause a 50% reduction in the levels of HMG-CoA reductase activity.

Presented in Table II is a condensed summary of the results of studies of the effects of ten C_{27} 15α-hydroxysterols. With the exception of the 3-keto derivative of 5α-cholest-8(14)-en-3β,15α-diol, all of the new 15α-hydroxysterols tested were more potent than 5α-cholest-8(14)-en-3β,15α-diol in the inhibition of sterol synthesis and in the reduction of the levels of HMG-CoA reductase activity in the L cells. In general, the concentrations of the C_{27} 15α-hydroxysterols required to cause a 50% inhibition of sterol synthesis were similar to the concentrations required to cause a 50% reduction in the levels of HMG-CoA reductase in the same cells. The largest discrepancy was in the case of 5α-cholestan-15α-ol-3-one, in which the concentration required to cause a 50% inhibition of sterol synthesis was 4 times lower than that required to cause a comparable reduction of the level of HMG-CoA reductase.

Similar data for four C_{27} 15β-hydroxysterols are presented in Table III.

TABLE II

INHIBITION OF STEROL SYNTHESIS AND REDUCTION OF LEVELS OF HMG-CoA REDUCTASE ACTIVITY IN L CELLS BY C_{27} 15α-HYDROXYSTEROLS

	CONCENTRATIONS (μM) REQUIRED FOR 50% INHIBITION				
INHIBITOR	STEROL SYNTHESIS	HMG-CoA REDUCTACE	INHIBITOR	STEROL SYNTHESIS	HMG-CoA REDUCTASE
 II [ref.2]	3.7	8.8	 XXI	5.8	6.0
 X	0.3	0.3	 XI	0.6	0.3
 IX	0.3	0.5	 XII	0.3	0.4
 XXIV	0.2	0.5	 XXV	0.2	0.8
 XXIX [ref.2]	3.2	6.7	 XXX [ref.5]	2.0	0.3

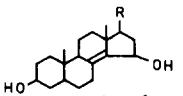
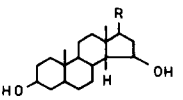
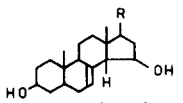
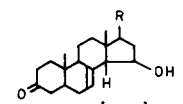
Discussion

In this and a previous study [14] we have prepared a number of other C_{27} 15-oxygenated sterols to attempt to determine which structural features of the C_{27} 15-oxygenated sterols are important in the inhibitory action of these compounds on sterol synthesis in L cells in culture. 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one (*XVI*) was prepared from the corresponding 3β-hydroxysterol (*I*) in 91% yield by a modification of the approach introduced by Bose et al. [25]. Compound *XVI* was characterized by its m.p. and by the results of infrared, NMR, and by the results of high and low resolution MS analyses. Acid hydrolysis of *XVI* gave 5α-cholest-8-(14)-en-3α-ol-15-one (*XVII*) in 82% yield. The latter compound was characterized

TABLE III

INHIBITION OF STEROL SYNTHESIS AND REDUCTION OF LEVELS OF HMG-CoA REDUCTASE ACTIVITY IN L CELLS BY C_{27} 15β-HYDROXYSTEROLS

CONCENTRATIONS (μM) REQUIRED FOR 50% INHIBITION

INHIBITOR	STEROL SYNTHESIS	HMG-CoA REDUCTASE
 XXXI [ref. 2]	1.8	2.5
 XXVI	0.6	4.0
 XXXII [ref. 2]	1.0	4.5
 XXXIII [ref. 5]	0.3	0.3

by its m.p. and optical rotation and by the results of infrared, NMR, ultraviolet, and high and low resolution MS analyses.

The potassium salts of the sulfate esters of the $\Delta^{8(14)}$-15-keto 3β-hydroxy-sterol (*I*) and its corresponding 3α-hydroxy epimer (*XVII*) were prepared from the corresponding free sterols (*I* and *XVII*, respectively) via the corresponding intermediary pyridinium sulfate esters (*XIV* and *XVIII*, respectively) by a modification of the approach of McKenna and Norymberski [24].

Selective oxidation of the 3β-hydroxyl function of 5α-cholest-8(14)-en-3β,15α-diol (*II*) was effected through the use of cholesterol oxidase to give 5α-cholest-8-(14)-en-15α-ol-3-one (*XXI*) in 85% yield. The product waa characterized by its m.p. and by the results of infrared, NMR, and high and low resolution MS analyses. The location of the ketone function at C-3 in *XXI* was established by the absence, in its NMR spectrum, of the 3α-proton resonance at 3.64 ppm [20] of the starting material *II* and by the location of the carbonyl absorption at 1725 cm^{-1} (indicative

of a ketone function in a 6-membered ring as contrasted to an α,β-unsaturated ketone a 5-membered ring [22]) in the infrared spectrum of *XXI*. We have previously used cholesterol oxidase for the selective oxidation of the 3β-hydroxyl function of 3β,15-dihydroxy sterols [4,22].

As part of our investigations of the effect of 15-oxygenated sterols on sterol biosynthesis we sought the preparation of C_{27} 15-oxygenated sterols with a saturated sterol nucleus. Mincione and Feliziani [33,34] have reported that hydroboration of 5α-ergost-8(14)-en-3β-ol at 40–60°C gave, after oxidation with hydrogen peroxide and esterification with acetic anhydride, a mixture of 5α,14α-ergostan-3β,15α-diol diacetate (75%) and 5α,14β-ergostan-3β,15β-diol diacetate (15%). They further reported that if the mixture of the organoboranes, obtained from 5α-ergost-8(14)-en-3β-ol, was heated at 130°C and subsequently oxidized with hydrogen peroxide and esterified with acetic anhydride the formation of 5α,14α-ergostan-3β-15α-diol diacetate was favored (76% yield). Anastasia et al. [29] have utilized the approach of Mincione and Feliziani [33,34] to prepare 5α,14β-cholestan-3β-ol by hydroboration of 5α-cholest-8(14)-en-3β-ol followed by protonolysis of the organoborane. We have applied the approach of Mincione and Feliziani to the case of the hydroboration of 5α-cholest-8(14)-en-3β-ol (*IV*). Treatment of *IV* with borane-tetrahydrofuran complex in tetrahydrofuran for 6 h at 45–55°C followed by treatment of the resulting organoborane with alkaline hydrogen peroxide gave, after standard workup, a mixture of 5α,14β-cholestan-3β,15β-diol (*XXVI*; 76%) and 5α,14α-cholestan-3β,15α-diol (*XXIV*; 24%) which were separable by GLC but not by TLC. Acetylation of the crude product gave a mixture of the corresponding diacetates which were separable by medium pressure liquid column chromatography to give 5α,14β-cholestan-3β,15β-diol diacetate (*XXIII*; 66% yield) and 5α,14α-cholestan-3β,15α-diol diacetate (*XXII*; 18% yield). The latter compound was characterized by its m.p. and optical rotation and by the results of infrared, NMR, and high and low resolution MS analyses. The values of the m.p. and optical rotation were in reasonable agreement with those reported for 5α,14α-cholestan-3β,15α-diol diacetate, formed upon acetylation of 5α,14α-cholestan-3β,15α-diol which had been obtained by hydroboration of 5α-cholest-14-en-3β-ol followed by treatment of the resulting organoborane with alkaline hydrogen peroxide [26]. Compound *XXII* was characterized by its optical rotation and by the results of infrared, NMR, and high and low resolution MS analyses. The yield of *XXII* from *IV* was increased from 18% to 74% by hydroboration of *IV* as described above followed by heating of the crude organoborane at 130°C for 6 h to give, after treatment of the organoborane with alkaline hydrogen peroxide, acetylation, and recrystallization, *XXII* which was indistinguishable from the sample whose preparation is described above.

Saponification of *XXIII* gave, in 93% yield, 5α,14β-cholestan-3β,15β-diol (*XXVI*) which was characterized by its m.p. and optical rotation and by the results of infrared, NMR, and high and low resolution MS analyses. Oxidation of *XXVI* with chromium trioxide gave 5α,14β-cholestan-3,15-dione (*XXVIII*) which was contaminated to the extent of approx. 23% with the corresponding *trans*–C–D isomer

(*XXVII*). Saponification of *XXII* gave, in 94% yield, 5α,14α-cholestan-3β,15α-diol (*XXIV*) which was characterized by its m.p. and optical rotation and by the results of infrared, NMR, and high and low resolution MS analyses. The m.p. was essentially the same as that reported by Nussim et al. [26] for the same compound prepared by an alternative route (see above). The specific rotation (+ 49.7°) was less than that (+ 46°) reported by Nussim et al. [26]. Oxidation of *XXIV* with chromium trioxide gave 5α,14α-cholestan-3,15-dione (*XXVII*) which was contaminated to the extent of approx. 13% with the corresponding *cis*–C–D isomer (*XXVIII*).

Apart from the analogy with the results of Mincione and Feliziani [32, 33] and Anastasia et al. [29], evidence for the C–D ring juncture in *XXVI* can be derived from considerations of the C-18 and C-19 angular methyl resonances in the spectra of *XXVI* and related compounds. Using published NMR data for 5α,14β-cholest-7-ene [35] and 3β-acetoxy-5α,14β-cholestane [28], values for the contribution of the Δ^7 double bond to the chemical shifts of the C-18 and C-19 methyl resonances in the 5α,14β-cholestane system can be calculated to be 0.052 and 0.060 ppm, respectively. The observed C-18 and C-19 methyl resonances in *XXVI* were 0.99 and 0.78 ppm, respectively. From the values we can calculate the expected values for the chemical shifts of the C-18 and C-19 methyl resonances, in the corresponding 5α,14β-Δ^7-3β,15β-diol as 0.94 (0.99 – 0.05) and 0.72 (0.78 – 0.06), respectively. These values are in close agreement with the corresponding observed values of 0.94 and 0.71 ppm in 5α,14β-cholest-7-en-3β,15β-diol whose structure has been unambiguously established by X-ray crystal analysis of a suitable derivative [36–38].

The optical rotation of 5α,14β-cholestan-3β,15β-diol is also worthy of note. In steroids with the 'normal' *trans*–C–D ring juncture it has been observed that the 15β-hydroxy derivatives are more levoratatory than the corresponding 15α-hydroxy derivatives [39–41]. The same relationship has also recently been observed in the case of the 15α-hydroxy and 14α-methyl-, 14α-ethyl-, 14α-*n*-propyl-, and 14α-*n*-butyl-Δ^7-cholesten-3β-hydroxysterols [6]. In the case of the 14α-methyl- and 14α-ethyl-Δ^7-cholestenols, the *trans*–C–D ring juncture and the absolute configurations of 15-hydroxy functions have been unequivocally established by the results of X-ray crystallographic analyses of suitable derivatives [42,43]. In the case of the 15α-hydroxy- and 15β-hydroxy-epimers of 5α-cholest-8(14)-en-3β-ol, in which the configurations of the hydroxyl functions at C-15 have been established by X-ray crystallographic analysis [44,45], the 15β-hydroxy-epimer was more levoratatory than its 15α-hydroxy-epimer [46]. The same relationship has also been observed in the case of the 15α-hydroxy- and 15β-hydroxy-epimers of 5α,14β-cholest-7-en-3β-ol in which the 'unnatural' *cis*–C–D ring juncture and the configurations of the 15-hydroxy functions have been unequivocally established by X-ray crystallographic analysis of a suitable derivative [36–38]. using the reported [28] value of –6.21° for $[\alpha]_D$ for 5α,14β-cholest-7-en-3β-ol, the calculated rotational differences ($\Delta[M]_D$) for 5α,14β-cholest-7-en-3β,15α-diol and 5α,14β-cholest-7-en-3β,15β-diol were +106.8° and –17.8°, respectively [37]. Similar considerations of the case of 5α,14β-cholestan-3β,15β-diol are complicated by the absence of a published rotation

for the parent sterol, 5α,14β-cholestan-3β-ol. However, the specific rotation (+38°) of the 3,5-dinitro-benzoate ester of this compound had been reported [28]. Using reported values for the specific rotations of the 3,5-dinitrobenzoate ester of 14β-cholest-5-en-3β-ol (+32°) [29] and of 14β-cholest-5-en-3β-ol (+29°) [29] a dextrorotatory effect of the dinitrobenzoate function can be noted. The calculated rotational difference ($\Delta[M]_D$) for the Δ^5 dinitrobenzoate versus the free sterol was +44.2°. The calculated expected molecular rotation ($[M]_D$) for 5α,14β-cholestan-3β-ol was +142.0°. Using this value the calculated rotational difference ($\Delta[M]_D$) for the 5α,14β-cholestan-3β,15β-diol versus the parent 5α,14β-cholestan-3β-ol is −25.2°. This value is in close agreement, albeit perhaps fortuitously, with the corresponding value of −17.8° for the case of 5α,14β-cholest-7-en-3β,15β-diol versus 5α,14β-cholest-7-en-3β-ol.

Using a mixture of silver carbonate and celite, prepared according to Fetizon and Goldfier [23], selective oxidation of the 3β-hydroxyl function of 5α,14α-cholestan-3β,15α-diol (*XXIV*) was effected in 88% yield to give 5α,14α-cholestan-15α-ol-3-one (*XXV*). Compound *XXV* was characterized by its m.p. and optical rotation and by the results of infrared, NMR, and high and low resolution MS analyses. The location of the ketone at C-3 in *XXV* was indicated by the absence, in its NMR spectrum, of the 3α-proton resonance at 3.50 ppm of the starting material *XXIV*, by the retention of the 15β–H resonance of the starting material, and by the location of the carbonyl absorption at 1718 cm^{-1} (indicative of a ketone function in a 6-membered ring as contrasted to a ketone in a 5-membered ring [47]) in the infrared spectrum of *XXV*. The reagent of Fetizon and Goldfier [23] has previously been shown to be useful for the selective oxidation of the 3-hydroxyl function of a number of bile acids [48] and androstane derivatives [49,50]. The results presented herein, coupled with our previous report on the selective oxidation of 3β-hydroxyl function of 5α-cholest-7-en-3β,15α-diol and 5α-cholest-8-en-3β,15α-diol [14], indicate the utility of this reagent for the selective oxidation of the 3β-hydroxyl function of 3β,15α-dihydroxysterols.

We have previously reported that a number of C_{27} 15-oxygenated sterols are potent inhibitors of sterol synthesis in animal cells in culture [1,2]. The most potent of these C_{27} 15-oxygenated sterols in the inhibition of sterol synthesis in L cells was 5α-cholest-8(14)-en-3β-ol-15-one (*I*), which caused a 50% inhibition of sterol synthesis at 1×10^{-7} M [2]. This same compound has also been found to have very significant hypocholesterolemic activity upon oral or subcutaneous administration to intact animals [8,10,11]. In the present study we have prepared a number of new $\Delta^{8(14)}$-15-ketosterols and their derivatives (Table I). The hemisuccinate ester of *I* was found to be moderately active in the inhibition of sterol synthesis and in reduction of the levels of HMG-CoA reductase activity in L cells. However, the compound was less active than the corresponding free sterol. The hemisuccinate ester has previously been found to have significant hypocholesterolemic activity upon subcutaneous administration to rats [9]. Of considerable interest is the observation that the palmitate ester of *I*, previously demonstrated to be ef-

fective in reducing the serum cholesterol levels of rats upon subcutaneous administration [9], had little or no effect on sterol synthesis in the L cells. The 3α-hydroxy epimer (*XVII*) of *I* was less active than *I* in the inhibition of sterol synthesis and in the reduction of the levels of HMG-CoA reductase activity in the L cells. The potassium sulfate esters of *I* and *XVII* were essentially inactive as inhibitors of sterol synthesis in the L cells under the conditions employed. Introduction of a Δ^6 double bond into *I* (compound *VI*) had no effect (relative to *I*) on the reduction of the levels of HMG-CoA reductase activity in the L cells but appeared to be less active than *I* in the inhibition of sterol synthesis. Similar findings were made in the case of the $\Delta^{6,8(14)}$-3,15-diketone (V). The $\Delta^{8(14)}$-3,7,15-triketone (*XX*) was also very active in the inhibition of sterol synthesis. The results indicate that the 3β-hydroxyl function is not required for the inhibitory activities of *I* but can be replaced by 3-keto or 3α-hydroxyl functions. Extending the conjugation with the α,β-unsaturated $\Delta^{8(14)}$-3-ketone function of *I* by the introduction of a Δ^6 double bond did not result in an enhancement of inhibitory activity.

We have previously reported on the inhibition of sterol biosynthesis by two C_{27} 3β,15α-dihydroxysterols (Table II). These two sterols, 5α-cholest-8(14)-3β,15α-diol (*II*) and 5α,14β-cholest-7-en-3β,15α-diol (*XXIX*) had comparable inhibitory action on sterol synthesis and on the levels of HMG-CoA reductase activity in L cells [2]. The three new C_{27} 3β,15α-dihydroxysterols, 5α-cholest-8-en-3β,15α-diol (*X*), 5α-cholest-7-en-3β,15α-diol (*IX*), and 5α-cholestan-3β,15α-diol (*XXIV*) – all with the *trans*–C–D ring junction, were significantly more potent in the inhibition of sterol synthesis and in the reduction of the levels of HMG-CoA reductase activity in L cells. The same findings were made in the case of the 3-keto derivatives (*XII* and *XXV*) of the latter two compounds.

We have previously reported on the inhibition of sterol biosynthesis by three 15β-hydroxysterols (Table *III*), 5α-cholest-8(14)-en-3β,15β-diol (*XXXI*), 5α,14β-cholest-7-en-3β,15β-diol (*XXXII*) and 5α,14β-cholest-7-en-15β-ol-3-one (*XXIII*). The new 15β-hydroxysterol, 5α,14β-cholestan-3β,15β-diol (*XXVI*) was approximately as potent as the other C_{27} 3β,15β-dihydroxysterols.

In our previous reports on the inhibition of sterol biosynthesis by 15-oxygenated sterols, all of the latter sterols have possessed Δ^7 or $\Delta^{8(14)}$-nuclear double bonds [1–5]. The results presented herein demonstrate that several C_{27} 15-oxygenated sterols lacking any nuclear double bond (*XXIV*, *XXV*, and *XXVI*) are also potent inhibitors of sterol biosynthesis in animal cells in culture. We have recently observed that another 15-oxygenated sterol lacking a double bond, 14α-methyl-5α-cholestan-3β,7α,15α-triol, also is a potent inhibitor of sterol synthesis in L cells*.

In the three cases of C_{27} 15-hydroxysterols in which comparisons can be made (*XXIX* versus *XXXII*, *XXX* versus *XXXIII*, and *II* versus *XXXI*), the 15β-hydroxysterols appeared to be slightly more potent in the inhibition of sterol synthesis in the L cells than the corresponding 15α-hydroxysterols. The fact that a number of

*G.J. Schroepfer, Jr., E.J. Parish, R.A. Pascal, Jr., and A.A. Kandutsch, report in preparation.

15-oxygenated sterols with the 'unnatural' *cis* C–D ring juncture (*XXVI, XXIX, XXX, XXXII,* and *XXXIII*) serve as potent inhibitors of sterol synthesis is also worthy of note.

The results of the present study represent an extension of our knowledge concerning the effects of variations in structure of C_{27} 15-oxygenated sterols on sterol biosynthesis and on the levels of HMG-CoA reductase activity in L cells grown in chemically-defined media. Further studies of the metabolism, biological effects, and mechanisms of action of the new C_{27} 15-oxygenated sterols described herein are in progress.

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