Inhibitors of sterol synthesis. A highly efficient and specific side-chain oxidation of 3β-acetoxy-5α-cholest-8(14)-en-15-one for construction of metabolites and analogs of the 15-ketosterol

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Abstract As part of a program directed towards the chemical syntheses of potential metabolites and analogs of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**), a potent regulator of cholesterol metabolism, several routes have been explored for the preparation of 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (**IV**). These investigations led to a remarkably specific and efficient side-chain oxidation of **I**. For example, treatment of the acetate of **I** with a mixture of trifluoroacetic anhydride, hydrogen peroxide, and sulfuric acid for 3.5 h at −2°C gave a crude product consisting of 3β-acetoxy-24-trifluoroacetoxy-5α-chol-8(14)-en-15-one (**XI**), 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**XII**), and 3β, 24-diacetoxy-5α-chol-8(14)-en-15-one (**XIII**) in yields of 58%, 8%, and 3%, respectively, by HPLC analysis. **XI** was readily hydrolyzed to **XII** upon treatment with triethylamine in methanol at room temperature. Oxidation of **XII** with Jones reagent gave 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oic acid (**XVIII**) from which its methyl ester (**IX**) was prepared by treatment with diazomethane. Mild alkaline hydrolysis of **XVIII** gave the 3β-hydroxy-$\Delta^{8(14)}$-15-keto C_{24} acid (**IV**). Hydrolysis of the crude product of the side-chain oxidation with K_2CO_3 in methanol gave 3β,24-dihydroxy-5α-chol-8(14)-en-15-one (**XIV**) which was oxidized with Jones reagent to yield 3,15-diketo-5α-chol-8(14)-en-24-oic acid (**XV**). Treatment of **XV** with diazomethane gave its methyl ester (**XVI**) which, upon controlled reduction with $NaBH_4$, yielded methyl 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oate (**XVII**). Compound **IX** was also prepared by an independent route. Full ^1H and ^{13}C NMR assignments are presented for 12 new compounds. **IV** caused a ~56% reduction of the level of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in CHO-K1 cells at a concentration of 2.5 μM. In contrast, the corresponding 3,15-diketo acid **XV** had no detectable effect on reductase activity under the same conditions.—**Herz, J. E., S. Swaminathan, F. D. Pinkerton, W. K. Wilson, and G. J. Schroepfer, Jr.** Inhibitors of sterol synthesis. A highly efficient and specific side-chain oxidation of 3β-acetoxy-5α-cholest-8(14)-en-15-one for construction of metabolites and analogs of the 15-ketosterol. *J. Lipid Res.* 1992. **33:** 579–598.

Supplementary key words ^1H and ^{13}C NMR spectroscopy • mass spectrometry • 15-oxygenated bile acid

The chemical oxidation of the saturated side chain of sterols constitutes an important approach for structure elucidation and also for the synthesis of analogs with side chains of varying lengths and substituents. Low yields and/or low degrees of specificity represent limitations of this approach. We now report a case of notable success in the specific oxidation of the saturated side chain of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) (**Fig. 1**).

The $\Delta^{8(14)}$-15-ketosterol **I** is a novel regulator of cholesterol metabolism. **I** is a potent inhibitor of sterol biosynthesis in cultured mammalian cells and decreases the levels of activity of key regulatory enzymes involved in the biosynthesis of cholesterol at the level of the enzymatic formation of mevalonic acid (1–4). In addition, **I** serves as an alternative substrate for acyl coenzyme A:cholesterol acyltransferase (ACAT) and inhibits the oleoyl coenzyme A-dependent esterification of cholesterol in hepatic and jejunal microsomes (5). Moreover, oral administration of **I** to rats lowers the levels of ACAT activity in jejunal microsomes (6) and inhibits the intestinal absorption of cholesterol (7, 8). **I** has been shown to have significant hypocholesterolemic action upon oral administration to ani-

Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; CHO, Chinese hamster ovary; DEPT, distortionless enhancement by polarization transfer; HETCOR, ^1H-^{13}C heteronuclear shift-correlated spectroscopy; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HPLC, high performance liquid chromatography; IR, infrared; MP, melting point; MS, mass spectrometry or mass spectrum; MPLC, medium pressure liquid chromatography; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; SC, side chain; TLC, thin-layer chromatography; UV, ultraviolet (spectrum).

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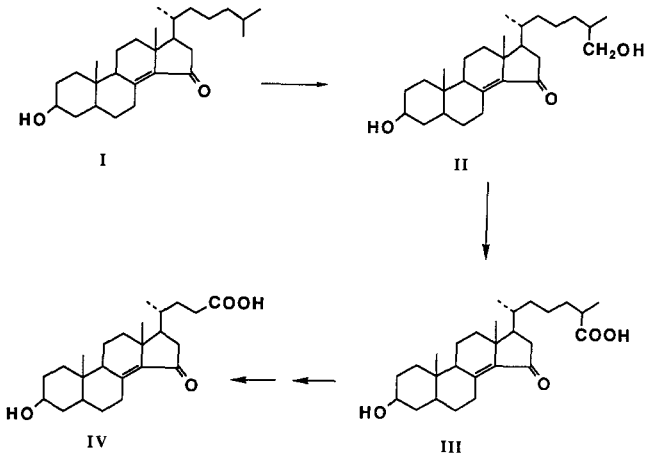

Fig. 1. Metabolism of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**).

mals (8–13) and, in the case of rhesus monkeys, to lower the levels of low density lipoprotein cholesterol, and to increase the level of high density lipoprotein cholesterol (13).

A detailed delineation of the metabolism of **I** is critical to an understanding of its actions. The 15-ketosterol is metabolized to cholesterol in in vitro systems (14, 15) and in intact animals (9, 16–21), and a scheme has been presented to account for the overall metabolism of **I** to cholesterol (15). In addition, very substantial metabolism of **I** to polar metabolites has been demonstrated in rat liver mitochondria (22, 23), cultured human hepatoma (HepG2) cells (J. S. Pyrek, F. D. Pinkerton, and G. J. Schroepfer, Jr., unpublished data), and intact animals (18, 21) (J. S. Pyrek, S. Numazawa, and G. J. Schroepfer, Jr., unpublished data). The formation of these polar metabolites from **I** appears to be initiated by oxidation to give the corresponding C_{27} alcohol (**II**) and carboxylic acid (**III**) followed by further metabolism to give the corresponding C_{24} bile acid (**IV**) (Fig. 1).

The chemical synthesis of the metabolites of **I** is required not only for their complete characterization but also for exploration of their biological activities and metabolism. Pursuit of this matter was markedly stimulated by the demonstration that metabolite **II** was highly active in reducing 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in cultured mammalian cells and inhibited ACAT activity in jejunal microsomes (22, 24). A multistep synthesis of **II** from diosgenin has been presented (24). Using the same approach employed for the conversion of (25R)-26-hydroxycholesterol to **II** (24), we have prepared the 3β-acetate derivative of the methyl ester of **IV** from methyl 3β-acetoxychol-5-en-24-oate (**V**) (**Fig. 2**).

Consideration of alternative approaches led to exploration of direct oxidation of the side chain of **I**. We now report that treatment of the acetate ester of **I** with a mixture of sulfuric acid, hydrogen peroxide, and trifluoroacetic anhydride resulted in remarkably efficient oxidation at C-24 to give a mixture of **XI**, **XII**, and **XIII** (**Fig. 3**), with estimated overall yields of 58%, 8%, and 3%, respectively. This ~69% yield of C_{24} oxygenated products (based upon HPLC analyses of the crude reaction product) was confirmed by selective hydrolysis of the trifluoroacetoxy function of **XI** of the crude reaction product followed by column chromatography to give isolated yields of **XII** and **XIII** of 64% and 3%, respectively. The high yield preparation of the monoacetate **XII** provided a selectively protected (at C-3) 24-hydroxy-C_{24}-$\Delta^{8(14)}$-15-keto intermediate for the chemical synthesis of $\Delta^{8(14)}$-15-keto metabolites and analogs of **I**. A preliminary account of a portion of these results has been presented (25).

EXPERIMENTAL

Materials and methods

Melting points (MP) were measured in sealed, evacuated capillary tubes using a Thomas-Hoover apparatus. Ultraviolet (UV) spectra were recorded on an IBM 9430 spectrophotometer using ethanol as the solvent. Infrared (IR) spectra were obtained on a Mattson Galaxy 6020 Fourier-transform IR spectrometer with KBr pellets. Optical rotations were measured at room temperature (23°C) at 589 nm on a JASCO DIP-4 digital polarimeter using $CHCl_3$ as solvent unless specified

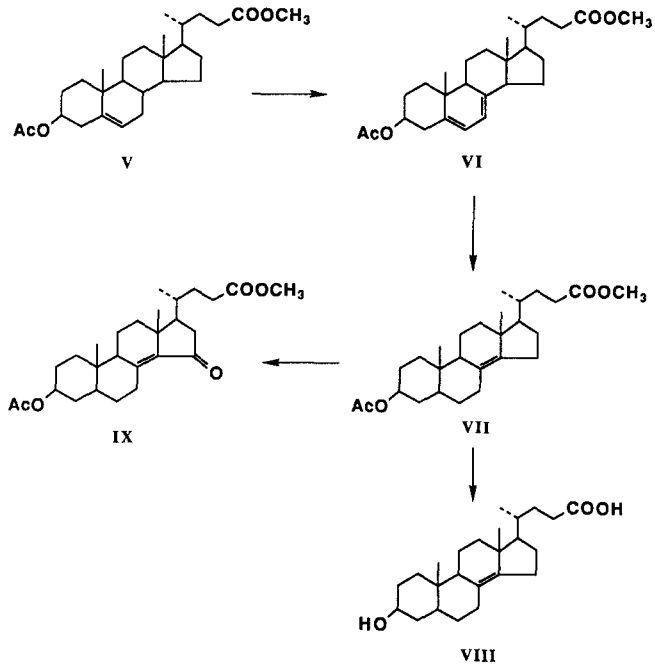

Fig. 2. Chemical synthesis of methyl 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oate (**IX**) from methyl 3β-acetoxychol-5-en-24-oate. (**V**).

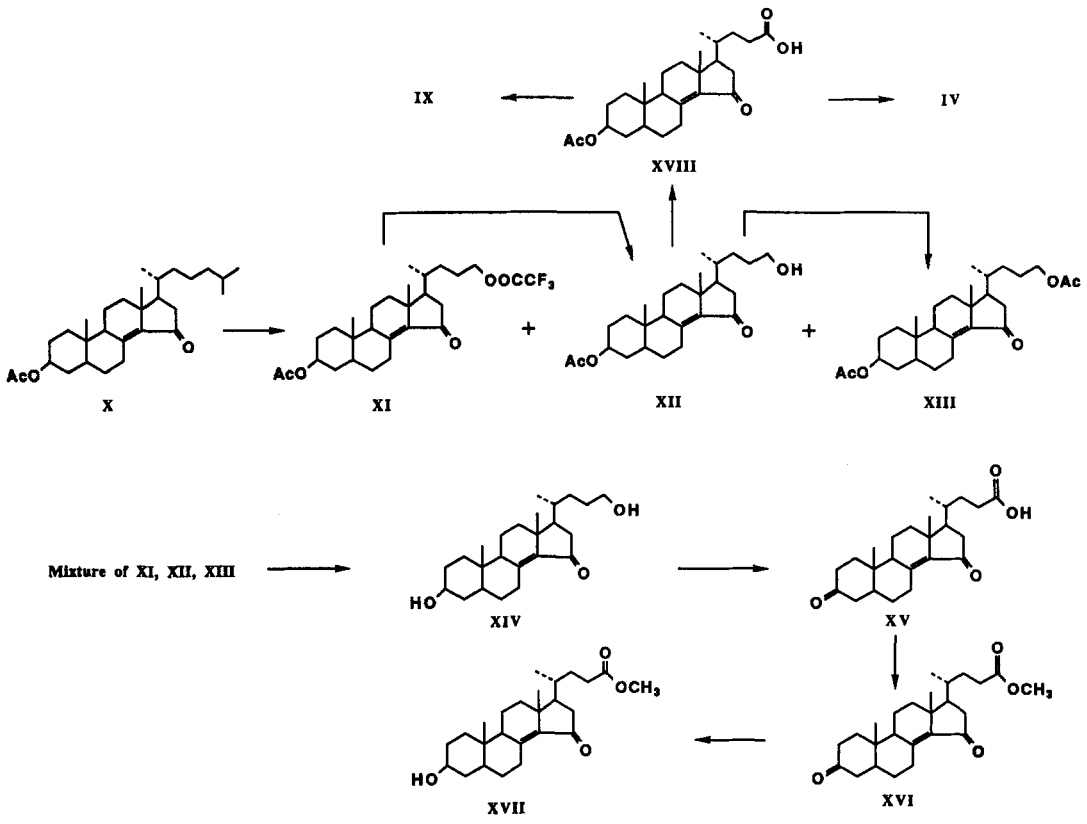

Fig. 3 Side-chain oxidation of 3β-acetoxy-5α-cholest-8(14)-en-15-one (**X**) and conversion of its products to 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (**IV**) and related products.

otherwise. Low resolution mass spectra (MS) were recorded on a Shimadzu QP-1000 quadrupole spectrometer with an electron energy of 70 eV and direct inlet sample introduction or, in the case of **VII**, by gas chromatography-MS on an Extrel ELQ-400 quadrupole instrument. High resolution MS were recorded on a Finnigan MAT90 spectrometer with an ionizing energy of 70 eV. Radioactivity was assayed in a Packard 4640 liquid scintillation spectrometer using Scintisol (Isolab, Inc.; Akron, OH) as the scintillation fluid.

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ solution in 5-mm tubes on an IBM AF300 spectrometer. ^{1}H NMR spectra (300.1 MHz) were referenced to tetramethylsilane, and ^{13}C NMR spectra (75.5 MHz) were referenced to $CDCl_3$ at 77.0 ppm. ^{1}H and ^{13}C NMR assignments were made from DEPT (distortionless enhancement by polarization transfer), ^{1}H–^{13}C shift-correlated spectroscopy (HETCOR), and by comparison with spectra of similar sterols. DEPT and HETCOR (~50 increments, δ 0.6–2.6 window in the ^{1}H dimension) experiments were done with standard Bruker Aspect 3000 software.

Thin-layer chromatography (TLC) was performed using precoated silica gel G plates or silica gel GF plates (Analtech; Newark, DE). Components on the plates were visualized after spraying with 5% ammonium molybdate(VI) in 10% sulfuric acid followed by heating in an oven (100–120°C) for 5 min or after illumination with a UV lamp. Preparative TLC was carried out on tapered plates (0.3–1.7 mm layer) with a preadsorbent layer (Uniplate-T; Analtech) or on 0.5-mm plates (Uniplate; Analtech). Analytical high performance liquid chromatography (HPLC) was performed isocratically with a Water U6K injector, a Model 6000 pump, and a Shimadzu SPD variable wavelength detector. UV detection was done at 260 nm for $\Delta^{8(14)}$-15-ketosteroids, 279 nm for $\Delta^{5,7}$ steroids, and 210 nm for other samples, unless otherwise stated. A 5-μm C_{18} Microsorb column (4.6 mm × 250 mm; Rainin instruments; Woburn, MA) or a 5 μm Spherisorb ODS-II column (4.6 mm × 250 mm; Custom LC, Houston, Texas) was used for reversed phase analytical HPLC, and an 8-μm Dynamax 60A C_{18} column (21.4 mm × 250 mm; Rainen Instruments) was used for preparative HPLC. Silica gel (70–230 mesh) for routine column chromatography was obtained from Aldrich Chemical Company (Milwaukee, WI) or Mallinckrodt, Inc. (Paris, KY). Silica gel (230–400 mesh) was obtained from Aldrich. Medium pressure liquid chromatography (MPLC) was carried out with Lobar columns (37 mm × 440 mm; EM Science; Cherry Hill, NJ) containing silica gel 60 (40–63 μm).

3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) was prepared as described previously (26) and showed a purity in excess of 99% as judged by TLC. 3β-Acetoxy-5α-cholest-8(14)-en-15-one (**X**), prepared by treatment of **I** with acetic anhydride and pyridine, melted at 136.0–136.5°C [lit. 135.0–135.5°C (27)] and showed a purity in excess of 99% as judged by TLC. [2,4-^{3}H]-**I** (sp act, 13.5 mCi per mmol) was prepared by a modification of a procedure described previously (28). [2,4-^{3}H]-3β-Acetoxy-**I** was prepared from [2,4-^{3}H]-**I** by treatment with acetic anhydride and pyridine. Methyl 3β-acetoxychol-5-en-24-oate (**V**), melting at 154–156°C [lit. 155–156°C (29)] was prepared from hyodeoxycholic acid (mp 201.5–203°C [lit. 196–197°C (30)]) according to the procedures of Vargha and Rados (29) and Ziegler and Bharucha (31). 3β-Acetoxy-5α-cholestane (MP, 110–111°C [lit. 109–110°C (32); 110–111°C (33)]) was prepared by treatment of 5α-cholestan-3β-ol (Sigma Chemical Company; St. Louis, MO) with acetic anhydride and pyridine. Trifluoroacetic anhydride was obtained from PCR, Inc. (Gainesville, FL). ^{1}H and ^{13}C NMR analyses of trifluoroacetic anhydride (380 mg) showed no impurities corresponding to acetic anhydride or acetic acid. Spectra acquired after addition of acetic anhydride (11 mg) to the sample indicated that the detection limit by ^{1}H NMR was ~2×10^{-5} mole acetic anhydride per mole of trifluoroacetic anhydride. Other chemicals were purchased from Aldrich.

Chinese hamster ovary (CHO-K1) cells were obtained from the American Type Collection (Rockville, MD). (3RS)-[3-^{14}C]HMG-CoA (56 mCi per mmol) and (3RS)-[2-^{3}H]mevalonolactone (176 mCi per mmol) were purchased from Amersham Corporation (Arlington Heights, IL). Lux tissue culture plasticware was from Miles Scientific (Elkhart, IN). Trypsin was obtained from Gibco Laboratories (Grand Island, NY) and Ham's F12 medium (34) and phosphate-buffered saline (PBS; KCl, 2.7 mM; KH_2PO_4, 1.2 mM; NaCl, 137 mM; and Na_2HPO_4, 8.1 mM) were obtained from Irvine Scientific (Irvine, CA). Fetal calf serum was purchased from Whittaker M.A. Bioproducts (Elkhart, IN).

For cell culture experiments, the sterols and C_{24} acids were added as ethanolic solutions to Ham's F12 medium supplemented with 5% delipidated (35) 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 (36) of the method of Lowry et al. (37).

The CHO-K1 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 PBS (10 ml), the cells were incubated for 18 h in lipid-deficient media (10 ml). The cells then were incubated with fresh lipid-deficient media (10 ml) containing various concentrations of the oxygenated sterols (from 0.0 μM to 2.5 μM) for 4 h. 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 (38). 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.

Methyl 3β-acetoxychola-5,7-dien-24-oate (VI)

Compound **VI** was prepared from methyl 3β-acetoxychol-5-en-24-oate (**V**) by the following adaption of the procedure of Confalone, Kulesha, and Uskokovic (39).

To a solution of **V** (3.0 g) in a mixture of benzene (50 ml) and hexane (70 ml) (both dried over molecular sieve 3A) was added 1,3-dibromo-5,5-dimethylhydantoin (1.7 g) and anhydrous $NaHCO_3$ (3.42 g). After heating under reflux for 30 min, the mixture was passed through a glass filter and evaporated to dryness under reduced pressure at 35°C. The resulting residue was dissolved in dry toluene (25 ml), cooled to 0°C, and a solution of anhydrous LiBr (1.2 g) in dry acetone (16 ml) was added with stirring. After agitation of the mixture for 2 h at 0°C, benzenethiol (1.0 ml) and triethylamine (1.32 ml) were added, and the agitation was continued at 25°C for 75 min. The reaction mixture was taken up in ethyl acetate, washed with 1 N HCl and water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting residue was dissolved in ethyl acetate (40 ml) and a solution of *m*-chloroperbenzoic acid (1.57 g) in ethyl acetate (10 ml) was added dropwise over 60 min at 0°C. After stirring at 0°C for an additional 60 min, the reaction mixture was washed with 10% $NaHCO_3$ and water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting residue, an orange-colored oil, was dissolved in toluene (60 ml), and triethylamine (2.2 ml) was added. After 28 h at 71°C, the reaction mixture was diluted with ethyl acetate, washed with 1 N HCl and water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting orange-colored oily residue was subjected to silica gel (90 g) column (36 × 100 mm) chromatography using CH_2Cl_2 as solvent (fraction volume, 20 ml). A portion (1.05 g) of the contents of fractions 6–10 (1.59 g) was rechromato-

graphed on a silica gel (27 g) column (24 × 180 mm) using CH_2Cl_2 as the eluting solvent (fraction volume, 10 ml). The contents of fractions 15–21 (710 mg) were combined and subjected to MPLC using a mixture of hexane and ethyl acetate (9:1) as the eluting solvent (fraction volume, 10 ml). The contents of fractions 89–91 (149 mg) showed a purity of over 94% by reversed phase analytical HPLC, and the contents of fractions 84–88 and 92–95 (66 mg) showed a purity of ~74% by HPLC. A portion (100 mg) of the contents of fractions 89–91 was dissolved in methanol (10 ml), and aliquots (0.5 ml) were subjected to preparative reversed phase HPLC (using methanol as the eluting solvent with UV detection at 279 nm) to give 26 mg pure **VI** melting at 130–131°C; $[\alpha]_D$ –77.9° (c, 0.6); UV, λ_{max} 262 nm (ε 7,640), 271 nm (ε 11,000), 282 nm (ε 11,500), and 294 nm (ε 6,500); IR, 2989, 1732, 1377, 1252, and 1036 cm^{-1}; MS, 428 (6, M^+), 368 (100; M–CH_3COOH), 353 (52, M–CH_3COOH–CH_3), 337 (11; M–CH_3COOH–OCH_3), 253 (88; M–SC–CH_3 COOH), 211 (28), 199 (42), 158 (39), 143 (62), and 128 (12) (the latter three ions reported (40) to be characteristic of the $\Delta^{5,7}$-diene system); high resolution MS, ion at *m/z* 428, 428.2922 (calc. for $C_{27}H_{40}O_4$: 428.2926); ion at *m/z* 368, 368.2695 (calc. for $C_{25}H_{36}O_2$: 368.2713); ion at *m/z* 353, 353.2495 (calc. for $C_{24}H_{33}O_2$: 353.2479); ion at *m/z* 337, 337.2518 (calc. for $C_{24}H_{33}O$: 337.2530); and ion at *m/z* 253, 253.1962 (calc. for $C_{19}H_{25}$: 253.1955); ^{13}C and ^1H NMR (**Table 1** and **Table 2**).

Methyl 3β-acetoxy-5α-chol-8(14)-en-24-oate (VII)

The $\Delta^{5,7}$-diene **VI** (99 mg; purity 94%) was dissolved in a mixture of ethyl acetate (3 ml) and acetic acid (3 ml) and hydrogenated (one atm.) for 20 h over a platinum oxide catalyst (100 mg; Adams catalyst;

TABLE 1. ^{13}C NMR chemical shifts for C_{24} acids and alcohols and their derivatives[a,b]

					$\Delta^{8(14)}$-15-Ketosterols									
3-Substituent 24-Function Atom	Δ^5 3β-OH CO_2Me[d]	Δ^5 3β-OAc CO_2Me **V**	$\Delta^{5,7}$ 3β-OAc CO_2Me **VI**	$\Delta^{8(14)}$ 3β-OAc CO_2Me **VII**	3β-OAc CH_2OR[c] **XI**	3β-OAc OH **XII**[c]	3β-OAc CH_2OAc **XIII**[c]	3β-OH CH_2OH **XIV**	3-Keto CO_2H **XV**	3-Keto CO_2Me **XVI**	3β-OH CO_2Me **XVII**	3β-OAc CO_2H **XVIII**	3β-OAc CO_2Me **IX**[f]	3β-OH CO_2H **IV**
C-1	37.17	36.88	37.80	36.13	36.18	36.14	36.21	36.48	37.86	37.82	36.43	36.18	36.20	36.49
C-2	31.52	27.65	27.98	27.43	27.13	27.09	27.15	31.08	37.74	37.71	31.00	27.12	27.15	31.08
C-3	71.65	73.85	72.64	73.54	73.10	73.11	73.13	70.85	211.33	211.08	70.71	73.17	73.13	70.94
C-4	42.18	38.00	36.53	33.97	33.54	33.50	33.56	37.70	44.13	44.09	37.62	33.53	33.56	37.69
C-5	140.70	139.52	138.46	43.93	43.83	43.78	43.85	44.06	45.91	45.86	43.99	43.82	43.85	44.07
C-6	121.57	122.50	120.10	28.61	28.94	28.91	28.97	29.10*	29.26	29.21	29.05	28.95	28.95	29.10
C-7	31.80	31.75	116.35	29.40	27.43	27.39	27.45	27.57	27.19	27.10	27.51	27.47	27.44	27.60
C-8	31.79	31.73	141.11	126.27	150.70	150.45	150.56	150.93	149.50	149.21	151.03	150.89	150.55	151.18
C-9	49.97	49.86	45.87	49.02	50.62	50.59	50.64	50.81	50.22	50.16	50.73	50.63	50.62	50.81
C-10	36.41	36.47	36.95	36.63	38.63	38.58	38.65	38.72	38.74	38.68	38.69	38.65	38.64	38.76
C-11	20.99	20.91	20.88	19.77	19.42	19.40	19.46	19.53	19.62	19.57	19.45	19.43	19.44	19.54
C-12	39.64	39.57	38.99	37.08	36.83	36.79	36.86	36.91	36.77	36.70	36.82	36.81	36.82	36.91
C-13	42.28	42.25	42.81	42.60	42.47	42.43	42.49	42.52	42.52	42.45	42.45	42.50	42.49	42.55
C-14	56.64	56.53	54.28	142.26	139.97	140.17	140.14	140.11	140.57	140.53	139.88	140.02	140.06	139.96
C-15	24.18	24.15	22.88	25.63	207.21	207.83	207.61	207.92	207.54	207.31	207.49	207.67	207.39	207.61
C-16	28.05	28.01	27.86	26.80	42.07	42.24	42.26	42.35	42.05	42.01	42.10	42.07	42.10	42.16
C-17	55.67	55.64	55.37	56.42	50.52	50.63	50.59	50.70	50.61	50.55	50.56	50.60	50.62	50.65
C-18	11.79	11.77	11.72	18.12	18.70	18.70	18.75	18.78	18.80*	18.74*	18.72	18.77	18.75	18.82*
C-19	19.34	19.22	16.05	12.58	12.75	12.72	12.78	12.90	12.05	12.00	12.84	12.77	12.78	12.92
C-20	35.30	35.27	35.59	33.87	34.04	34.27	34.12	34.32	34.05	33.99	33.99	34.03	34.03	34.07
C-21	18.24	18.22	18.33	18.57	18.92	19.08	19.01	19.14	18.73*	18.71*	18.70	18.71	18.75	18.76*
C-22	30.92	30.90	30.81	30.68	31.23	31.49	31.61	31.54	30.29	30.46	30.48	30.28	30.52	30.55
C-23	30.97	30.93	30.93	30.84	24.59	29.00	24.94	29.06*	30.72	30.76	30.78	30.77	30.82	30.67
C-24	174.79	174.66	174.53	174.60	68.32	63.02	64.68	63.18	178.72	174.13	174.22	179.11	174.20	178.34
3-Acetate		170.44	170.37	170.57	170.60	170.62	170.63					170.74	170.63	
3-Acetate		21.35	21.30	21.31	21.33	21.32	21.39					21.37	21.37	
24-Ester					157.42[g]		171.17							
24-Ester					114.42[h]		20.97							
CH_3O	51.48	51.40	51.36	51.35						51.53	51.54		51.55	

[a]Chemical shifts referenced to $CDCl_3$ signal at 77.0 ppm. Data obtained at 75 MHz in $CDCl_3$ solution at a concentration of 0.02–0.2M

[b]Assignments marked with an asterisk may be interchanged.

[c]R = $OCCF_3$.

[d]Methyl 3β-hydroxychol-5-en-24-oate.

[e]Data from samples prepared by process B; values from the corresponding samples from process A differ by ≤ 0.03 ppm (≤ 0.12 ppm for sp^2 carbons and C-24) after small reference adjustments.

[f]Data from sample prepared by oxidation of **VII** (Fig. 2). The sample prepared by methylation of **XVIII** (Fig. 3) gave ^{13}C NMR chemical shifts identical within ± 0.03 ppm after application of a 0.11 ppm referencing adjustment.

[g]Quartet, J_{CF} = 42 Hz.

[h]Quartet, J_{CF} = 285 Hz.

TABLE 2. ^{1}H NMR chemical shifts for C_{24} acids and alcohols and their derivatives[a]

3-Substituent 24-Function Atom	Δ^5 3β-OH CO_2Me [d]	Δ^5 3β-OAc CO_2Me V	$\Delta^{5,7}$ 3β-OAc CO_2Me VI	$\Delta^{8(14)}$ 3β-OAc CO_2Me VII[b]	$\Delta^{8(14)}$ 3β-OH CO_2H VIII	$\Delta^{8(14)}$-15-Ketosterols 3β-OAc $CH_2O_2CCF_3$ XI	3β-OAc CH_2OH XII[c]	3β-OAc CH_2OAc XIII[c]	3β-OH CH_2OH XIV	3-Keto CO_2H XV	3-Keto CO_2Me XVI	3β-OH CO_2Me XVII	3β-OAc CO_2H XVIII	3β-OAc CO_2Me IX[c]	3β-OH CO_2H IV
1α	1.07	1.13	1.36	1.14		1.25	1.25	*1.24*	*1.2*		1.58	1.21	1.25	1.25	
1β	1.84	1.86	1.89	1.71		1.75	1.75	*1.75*	*1.73*		2.03	1.73	1.74	1.76	1.72
2α	1.84	1.85	*1.92*	1.85		1.89	*1.88*	1.88	*1.87*		*2.34*	1.86	*1.89*	1.87	
2β	1.51	1.58	*1.5*	*1.4*		*1.44*	1.46	*1.43*			*2.34*	1.37	*1.45*	*1.42*	
3α[e]	3.52	4.60	4.69	4.70	3.62	4.73	4.73	4.73	3.64			3.64	4.73	4.73	3.66
4α	*2.27*	*2.31*	2.49[f]	1.68		1.72	1.72	*1.72*	*1.69*		*2.17**	1.69	*1.74*	*1.74*	
4β	*2.27*	*2.31*	2.35[f]	1.33		1.35	1.36	1.35	*1.28*		*2.27**	1.28	*1.32*	*1.35*	
5				1.31		1.48	1.47	1.48	1.41		1.83	1.41	1.48	1.48	
6	5.35[g]	5.37[g]	5.55[f]	*1.3*		*1.32*	*1.35*	*1.3*	*1.35*		*1.4*	*1.3*	*1.3*	*1.34*	
				1.36		1.49	*1.5*	*1.5*	*1.5*		*1.6*	*1.5*	*1.5*	*1.49*	
7α	*1.52*	*1.53*	5.37[f]	1.72		1.60	1.59	1.59			*1.61*	*1.6*	*1.58*	*1.58*	
7β	1.97	1.97		2.36	2.37	4.13[h]	4.12[h]	4.13[h]	4.13[h]	4.17	4.17[h]	4.12[h]	4.12[h]	4.13[h]	4.12[h]
8β	1.47	1.45													
9α	0.93	0.95	1.99	1.65		1.91	1.88[i]	1.88[i]	1.86		1.97[i]	1.86	1.89	1.89[i]	1.86
11	*1.48*	*1.47*	*1.55*	*1.5*		*1.47*	*1.55*	*1.5*	*1.5*		1.62	*1.5*	*1.5*	*1.5*	
	1.48	*1.47*	*1.65*	*1.5*		*1.68*	*1.65*	*1.65*	*1.65*		1.72	*1.65*	*1.65*	*1.7*	
12α	*1.18*	1.16	1.24	1.10		1.28	1.25	1.27	1.2		1.29	1.25	*1.25*	1.26	
12β[j]	1.99	2.00	2.08	1.92		2.10	2.10	2.10	2.10		2.14	2.09	2.09	2.10	2.09
14α	0.99	1.01	1.89												
15α	*1.61*	1.59	*1.74*	*2.22*	*2.22*										
15β	*1.07*	1.08	*1.38*	*2.22*	*2.22*										
16α	*1.87*	*1.86*	*1.95*	*1.84*		2.34[k]	2.37[k]	2.36[k]	2.37[k]		2.41[k]	2.39[k]	2.40[k]	2.39[k]	2.40[k]
16β	*1.25*	*1.30*	*1.33*	*1.40*		2.05[k]	2.06[k]	2.05[k]	2.06[k]		2.11[k]	2.09[k]	2.10[k]	2.09[k]	2.10[k]
17α	1.10	1.10	1.23	1.12		1.47	1.48	1.48	1.48		1.50	1.47	1.48	1.47	
18[l]	0.677	0.677	0.619	0.840	0.846	0.982	0.977	0.979	0.978	1.020	1.020	0.978	0.980	0.980	0.981
19[l]	1.004	1.016	0.948	0.701	0.687	0.735	0.732	0.735	0.715	0.930	0.936	0.714	0.731	0.733	0.715
20	1.42	1.42	1.44	1.50		1.63	1.62	1.62	1.62		1.65	1.62	1.65	1.63	
21[m]	0.924	0.925	0.953	0.939	0.954	1.036	1.022	1.022	1.022	1.033	1.023	1.008	1.020	1.011	1.019
22	1.33	1.33	1.36	1.40		1.15	1.14	1.13	*1.11*		1.39	1.38		*1.37*	
	1.79	1.79	1.82	1.83		1.49	1.49	1.47	*1.48*		1.82	*1.82*	1.82	1.80	
23	2.22[n]	2.22[n]	2.22[n]	*2.2*	2.27[n]	1.68	*1.45*	*1.5*	*1.5*		2.27	2.26[n]	2.30[n]	2.26[n]	2.29[n]
	2.36[n]	2.36[n]	2.36[n]	2.35	2.40[n]	1.86	*1.59*	*1.65*	*1.6*		2.37	2.37[n]	2.41[n]	2.37[n]	2.41[n]
24						4.33[o]	3.60[o]	4.017[o]	3.605[o]						
						4.33[o]	3.62[o]	4.049[o]	3.624[o]						
Methoxy[l]	3.663	3.662	3.664	3.665							3.671	3.669		3.668	
Acetate[f]		2.031	2.040	2.025		2.029		2.029 2.047					2.032	2.028	

[a]Chemical shifts referenced to $Si(CH_3)_4$ signal at 0 ppm. Data obtained at 300.1 MHz in $CDCl_3$ solution at a concentration of 0.02–0.2 M. Chemical shifts of methylene protons between δ 0.9 and 2.4, obtained from HETCOR data, are generally accurate to ± 0.02 ppm. Values in italics are of lower accuracy (± 0.1 ppm). Asterisks indicate assignment of α and β stereochemistry may be interchanged. No stereochemical assignments are given for H-6α and H-6β, H-11α and H-11β, and for side chain protons H-22, H-23, and H-24.

[b]Data from sample prepared by oxidation of **VII**; methyl signals agree ± 0.003 ppm with those for sample prepared by methylation of **XVIII**.

[c]Data obtained on sample from process B; products worked up by process A showed the same ^{1}H NMR spectrum (± 0.003 ppm for methyl resonances).

[d]Methyl 3β-hydroxychol-5-en-24-oate.

[e]H-3α; tt, 11.1 ± 0.2, 4.7 ± 0.6 Hz (free sterol of **V**, tdd, 10.9, 5.6, 4.2 Hz)

[f]**VI**: H-4α: ddd, ~14.4, 5.0, 2.2 Hz; H-4β: br dd, ~14, 13 Hz; H-6, dd, 5.7, 2.4 Hz; H-7: ddd, 5.6, 2.7, 2.7 Hz.

[g]H-6: br d, ~5 Hz.

[h]H-7β: distorted ddd, −14.0 ± 0.1, 4.0 ± 0.2, 1.9 ± 0.2 Hz.

[i]H-9α: dd, 10.3 ± 0.2, 7.1 ± 0.1 Hz.

[j]H-12β: ddd, −12.5, 3.4, 3.4 Hz (representative values).

[k]H-16α and H-16β (tentative stereochemical assignments based on comparison of observed and calculated J values): ABX system, $J_{16\alpha\text{-}16\beta}$ = −18.5 ± 0.1 Hz; $J_{16\alpha\text{-}17\alpha}$ = 7.7 ± 0.1 Hz; $J_{16\beta\text{-}17\alpha}$ = 12.3 ± 0.3 Hz.

[l]H-18, H-19, methoxyl, and acetate methyl: singlets.

[m]H-21: d, 6.3–6.4 Hz.

[n]H-23 (upfield): ddd, −15.7 ± 0.1, 9.3 ± 0.1, 6.5 ± 0.2, H-23(downfield): ddd, −15.7 ± 0.1, 9.7 ± 0.5, 6.0 ± 0.8.

[o]H-24: ABXY system with J_{AB} = −10.5 ± 0.2 Hz (J_{AB} not observed for **XI**) and vicinal couplings of 6.6 ± 0.1 Hz.

Bishop and Company, Platinum Works; Malvern, PA). TLC on a silica gel GF plate (solvent, 1% methanol in $CHCl_3$) showed one principal spot (R_f 0.48). The crude product (92 mg) was subjected to preparative reversed phase HPLC (solvent, methanol) to give **VII** (50 mg) melting at 86–87°C; single component on TLC in three solvent systems (10% acetone in benzene, R_f 0.64; 20% ethyl acetate in hexane, R_f 0.31; and 30% ethyl acetate in hexane R_f 0.62); $[\alpha]_D$ +5.1° (c, 0.6); IR, 2949, 2911, 2883, 1732, 1449, 1377, 1263, 1169, and 1034 cm^{-1}; MS, 430 (100, M^+), 415 (49, M–CH_3), 399 (4, M–OCH_3), 370 (73, M–CH_3COOH), 355

(75; M–CH_3COOH–CH_3), 323 (5), 315 (18, M–SC), 273 (4), 255 (64, M–SC–CH_3COOH), 229 (24), 213 (98), and 107 (70); high resolution MS, ion at *m/z* 430, 430.3068 (calc. for $C_{27}H_{42}O_4$: 430.3081); ^{13}C and ^1H NMR (Tables 1 and 2).

3β-Hydroxy-5α-chol-8(14)-en-24-oic acid (VIII)

Compound **VII** (23 mg) and LiOH-H_2O (14 mg) in methanol (1.2 ml) and water (0.35 ml) was stirred at room temperature for 23 h in a sealed vial under nitrogen. The mixture was neutralized with 1 N HCl, and ether and water were added. The ether phase was washed with water until the washes were neutral, and the ether solution was filtered and evaporated to dryness under reduced pressure to give **VIII** (19 mg) melting at 214.5–215.5°C; single component on reserved phase HPLC (solvent, 20% water in methanol) and on TLC analyses in three solvent systems (isooctane–ethyl acetate–acetic acid 5:5:1, R_f 0.65; hexane–$CHCl_3$-acetic acid 7:2:1, R_f 0.41; and $CHCl_3$–acetone–methanol 7:5:1, R_f 0.36); IR, 3320, 2967, 2932, 2807, 1690, 1470, 1291, 1265, 1088, and 1036 cm^{-1}; MS, 374 (100, M^+), 359 (25; M–CH_3), 356 (5, M–H_2O), 341 (10, M–H_2O–CH_3), 273 (12, M–SC), 255 (6, M–H_2O–SC), 231 (10), 213 (19), and 107 (41); high resolution MS, ion at *m/z* 374, 374.2811 (calc. for $C_{24}H_{38}O_3$: 374.2821); ion at *m/z* 359, 359.2593 (calc. for $C_{23}H_{35}O_3$: 359.2584); ion at *m/z* 356, 356.2713 (calc. for $C_{24}H_{36}O_2$: 356.2713); ion at *m/z* 341, 341.2507 (calc. for $C_{23}H_{33}O_2$: 341.2479); ion at *m/z* 273, 273.2219 (calc. for $C_{19}H_{29}O$: 273.2217); and ion at *m/z* 255, 255.2107 (calc. for $C_{19}H_{27}$: 255.2111); ^1H NMR (Table 2).

Methyl 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oate (IX)

To a solution of CrO_3 (800 mg) and 3,5-dimethylpyrazole (800 mg) in dry CH_2Cl_2 (25 ml) at –20°C was added a precooled (–20°C) solution of **VII** (174 mg) in dry CH_2Cl_2 (8 ml). (The chromium trioxide and the 3,5-dimethylpyrazole were separately dried over P_2O_5 under high vacuum and, in addition, in the case of the chromium trioxide, by heating with a hot-air pistol set to 510°C in a vial and sealed.) The mixture was stirred for 3.5 h at –20°C under nitrogen. A 20% NaOH solution (20 ml) was added, and stirring was continued for 30 min at 0°C. The layers were separated and the aqueous phase was extracted several times with CH_2Cl_2. The combined CH_2Cl_2 solutions were washed with 1 N HCl and water and then dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure to give 190 mg of a dark oil. A portion (81 mg) of the crude product was subjected to preparative TLC (solvent, 10% acetone in benzene) on a 0.5-mm Uniplate. The material with R_f of 0.46 was eluted with ethyl acetate (18 mg of crystalline product) and subjected again to preparative TLC (Uniplate-T; solvent, 10% acetone in benzene) to yield **IX** (12 mg) melting at 130–132°C; single component on TLC in three solvent systems (10% acetone in benzene, R_f 0.58; $CHCl_3$-acetone-methanol 7:5:1, R_f 0.63; 30% ethyl acetate in hexane, R_f 0.49); UV, λ_{max} 258 nm (ε 14,400); IR, 2946, 2863, 1740, 1699, 1624, 1246, 1173, 1125, and 1028 cm^{-1}; MS (**Table 3**); high resolution MS (**Table 4**); ^{13}C and ^1H NMR (Tables 1 and 2).

Side chain oxidation of 3β-acetoxy-5α-cholest-8(14)-en-15-one (X) with a mixture of trifluoroacetic anhydride, sulfuric acid, and hydrogen peroxide

The $\Delta^{8(14)}$-15-ketosteryl acetate (**X**) was oxidized by the following modification and adaptation of the approach introduced by Deno and Meyer (41) for the side chain oxidation of a cholesterol derivative. **Caution: Although the following procedure has been carried out numerous times without incident, this reaction should be conducted in a vented reaction flask behind a safety shield.**

To a mechanically stirred mixture of trifluoroacetic anhydride (100 ml) and sulfuric acid (40.8 ml; 96%) maintained at –10°C was added a solution of hydrogen peroxide (9.88 ml; 30%) dropwise over a period of 30 min. During the addition, the temperature of the mixture varied from –4°C to –8°C. The $\Delta^{8(14)}$-15-ketosteryl acetate (5.65 g; **X**) was, with continued vigorous stirring, added in one portion and the temperature of the reaction mixture was increased to –2°C. Within 1 h the mixture turned to a thick slurry which, with continued vigorous stirring, changed to a clear, light yellow colored, mobile solution after ~3.5 h. TLC (solvent, 30% ethyl acetate in hexane) of an ethyl acetate extract of an aliquot of the reaction mixture indicated completion of the reaction as judged by consumption of almost all of the starting material (**X**; R_f 0.86) and the presence of a major component with an R_f of 0.67 with minor components with R_f values of 0.60, 0.19, and 0.00.

The crude reaction mixture was processed as follows.

Process A

The reaction mixture was poured onto ice (1000 g), and the resulting white precipitate was collected on a Buchner funnel fitted with polypropylene filter cloth. The solid was dissolved in a mixture (300 ml) of tetrahydrofuran and ethyl acetate (1:4) and passed through a plug of silica gel (30 g) which was then washed with ethyl acetate (600 ml). Evaporation of the

TABLE 3. Ion abundances in the mass spectra of $\Delta^{8(14)}$-15-keto C_{24} acids and alcohols[a]

C-3 Substituent C-24 Functionality Suggested Assignment	**XI** 3β-OAc CH_2OOCCF_3	**XII**[b] 3β-OAc CH_2OH	**XIII**[b] 3β-OAc CH_2OAc	**XIV** 3β-OH CH_2OH	**XV** 3-Keto COOH	**XVI** 3-Keto $COOCH_3$	**XVII** 3β-OH $COOCH_3$	**XVIII** 3β-OAc COOH	**IX**[c] 3β-OAc $COOCH_3$	**IV** 3β-OH COOH
M^+	512(83)	416(83)	458(86)	374(85)	386(100)	400(100)	402(100)	430(82)	444(71;88)	388(99)
$M-CH_3$	497(7)	401(20)	443(10)	359(25)	371(52)	385(65)	387(8)	415(11)	429(14;16)	373(21)
$M-H_2O$	494(11)	398(10)	440(8)	356(16)	368(15)	382(17)	384(20)	412(12)	426(10;11)	370(20)
$M-H_2O-CH_3$	479(3)	383(40)	425(4)	341(93)	353(42)	367(30)	369(42)	397(7)	411(6;7)	355(59)
M–ROH	452(10)	356(6)	398(9)	[356]	[371]	[382]	[387]	370(8)	384(7;7)	[370]
$M-ROH-CH_3$	437(61)	341(65)	383(44)	[341]	[353]	[367]	[384]	355(59)	369(43;50)	[355]
$M-ROH-H_2O$	434(5)	338(4)	380(13)	338(2)	350(4)		366(3)	352(7)	366(9;10)	352(3)
$M-ROH-H_2O-CH_3$	419(9)	323(12)	365(15)	323(10)	335(15)	349(2)	351(13)	337(26)	351(27;31)	337(19)
Ion $D-H_2O$[d]	339(3)	339(7)	339(6)	297(9)	295(10)	295(22)	297(8)	339(7)	339(8;9)	297(4)
M–SC[d]	329(12)	329(14)	329(14)	287(16)	285(47)	285(52)	287(12)	329(10)	329(11;12)	287(14)
$M-H_2O-SC$	311(51)	311(47)	311(60)	269(93)	267(94)	267(78)	269(70)	311(60)	311(40;50)	269(97)
Ion C[d]	301(6)	301(7)	301(7)	259(8)	257(11)	257(13)	259(7)	301(7)	301(8;9)	259(7)
Ion A[d]	357(12)	261(8)	303(10)	261(8)	275(3)	289(2)	289(4)	275(9)	289(7;8)	275(7)
Ion B[d]	346(7)	250(4)	292(5)	250(10)	264(1)		278(7)	264(4)	278(4;4)	264(11)
M–ROH–SC	269(14)	269(27)	269(28)	[269]	[267]	[267]	[269]	269(22)	269(23;24)	[269]
IonB–CH_3	331(18)	235(13)	277(9)	235(21)	[249]	263(5)	263(12)	249(12)	263(9;10)	249(20)
$M-ROH-H_2O-SC$	251(42)	251(46)	251(78)	251(31)	249(14)	249(14)	251(26)	251(54)	251(54;62)	251(33)
Other ions	409(3)	313(16)	401(4)	271(14)	317(5)	369(10)[e]	337(7)[e]	213(13)	353(8;7)[e]	213(11)
	355(5)	213(11)	341(27)	213(10)	311(6)	353(13)	319(7)[e]	199(15)	337(8;8)[e]	199(14)
	343(6)	199(16)	305(9)	199(15)	215(16)	351(6)[e]	213(9)	107(100)	319(7;6)[e]	107(100)
	199(11)	107(100)	213(14)	107(100)	197(11)	350(8)[e]	199(12)	105(81)	213(11;11)	105(74)
	107(100)	105(78)	199(18)	105(78)	107(50)	335(19)[e]	107(84)		199(16;16)	
	105(76)		107(100)		105(73)	317(7)[e]	105(69)		107(100;100)	
			105(78)			215(16)			105(78;75)	
						107(57)				
						105(79)				
ROH =	AcOH	AcOH	AcOH	H_2O	H_2O[f]	H_2O[f]	H_2O	AcOH	AcOH	H_2O

[a]Major ions above *m/z* 100 in direct-probe mass spectra acquired at 70 eV. Relative intensities as % of base peak.
[b]Data from material prepared by process B; material from process A gave the same ions with comparable intensities.
[c]Ion abundances for samples of **IX** prepared from **VI** and from **XVIII**, respectively.
[d]Ion A, ion B, ion C, ion D, and M–SC are defined in Fig. 5.
[e]These peaks may be ascribed to losses of methoxyl radical or methanol either alone or accompanied by other losses.
[f]Suggested losses of water from keto groups.

TABLE 4. High resolution mass spectral data for $\Delta^{8(14)}$15-keto-C_{24} acids and their derivatives[a]

C-3 Substituent C-24 Functionality	**IV** 3β-OH COOH	**IX** 3β-OAc $COOCH_3$	**XV** 3-Keto COOH	**XVI** 3-Keto $COOCH_3$	**XVII** 3β-OH $COOCH_3$	**XVIII** 3β-OAc COOH
M^+	388.2603 (–0.9)	444.2876 (+0.2)	386.2451 (–0.4)	400.2629 (+1.7)	402.2765 (–0.3)	430.2713 (–0.4)
$M-CH_3$	373.2371 (–0.6)	429.2596 (–4.3)	371.2217 (–0.4)	385.2363 (–1.4)	387.2519 (–1.4)	415.2482 (–0.1)
$M-H_2O$	370.2484 (–2.2)	426.2737 (–3.1)	368.2344 (–0.6)	382.2476 (–3.0)	384.2643 (–2.0)	412.2640 (+2.8)
$M-H_2O-CH_3$	355.2274 (+0.2)		353.2106 (–0.9)	367.2289 (+1.7)	369.2419 (–0.9)	
M–ROH		384.2670 (+0.7)				370.2478 (–2.8)
$M-ROH-CH_3$		369.2431 (+0.3)				355.2268 (–0.4)
$M-ROH-H_2O$		366.2556 (–0.1)				
$M-ROH-H_2O-CH_3$	337.2165 (–0.1)	351.2332 (+1.0)	335.2000 (–1.0)			
Ion $D-H_2O$			295.2078 (+1.7)	295.2089 (+2.7)		337.2216 (0.0)
M–SC	287.2005 (–0.5)	329.2098 (–1.7)	285.1841 (–1.2)	285.1880 (+2.7)	287.2009 (–0.1)	
$M-SC-H_2O$	269.1906 (+0.2)	311.2031 (+2.1)	267.1742 (–0.6)	267.1748 (0.0)	269.1907 (+0.3)	311.1992 (–1.8)
Ion C	259.1664 (–3.3)	301.1826 (+2.4)	257.1529 (–1.1)			
Ion A	275.1659 (+1.3)	289.1846 (+4.4)				
Ion B	264.1700 (–2.4)	278.1892 (+1.1)			278.1884 (+0.3)	
M–ROH–SC		269.1909 (+0.5)				269.1923 (+1.9)
Ion B–CH_3	[b]	263.1640 (–0.6)		263.1679 (–3.3)	263.1649 (+0.3)	
$M-ROH-H_2O-SC$[b]	251.1802 (+0.3)	251.1807 (+0.8)	[b]	[b]	251.1812 (+1.3)	251.1791 (–0.8)

[a]Values in parentheses are the differences (in millimass units) between the observed masses and the calculated values for the elemental composition of the ions shown in the left column (corresponding to the ions presented in Table 3 and in Fig. 5).
[b]Exact masses were not determined below *m/z* 250.

solvent under reduced pressure gave a white solid (4.42 g). Reversed phase HPLC (UV detection at 259 nm) showed that the major component corresponded to 3β-acetoxy-24-trifluoroacetoxy-5α-chol-8(14)-en-15-one (**XI**; 83%) (**Table 5**). Also present were 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**XII**; 11%) and 3β,24-diacetoxy-5α-chol-8(14)-en-15-one (**XIII**; 4%). The crude product contained only 1.3% of the unreacted 15-ketosteryl acetate (**X**). The HPLC data indicated overall yields of the C_{24} oxygenated compounds **XI, XII,** and **XIII** from **X** of 58%, 8%, and 3%, respectively. The HPLC analyses further indicated that the 24-trifluoroacetate **XI**, upon standing in methanol solution at room temperature, was gradually hydrolyzed to the corresponding 24-hydroxy compound (**XII**) (Table 5).

To obtain an analytical sample of **XI**, a portion (200 mg) of the crude product was subjected to rapid silica gel (4.0 g; 230–400 mesh) column (1 × 11 cm) chromatography using 10% ethyl acetate in hexane as the eluting solvent (fraction volume, 20 ml). Fraction 3 contained **XI** (30 mg) which, by TLC, was free of **XII, XIII,** and **X**. **XI** melted at 162.0–162.5°C; IR, 2934, 2864, 1784, 1738, 1697, 1624, 1375, 1358, 1260, 1231, 1157, and 1032 cm^{-1}; MS (Table 3); high resolution MS, ion at *m/z* 512, 512.2740 (calc. for $C_{28}H_{39}O_5F_3$: 512.2747); ^{13}C and ^1H NMR (Tables 1 and 2).

Another portion (2.03 g) of the crude product was stirred with a mixture of methanol (50 ml), triethylamine (0.40 ml), and tetrahydrofuran (10 ml) for 1 h at room temperature. Evaporation of the solvent under reduced pressure yielded a white solid (1.80 g) which was applied to a silica gel (34 g; 230–400 mesh) column (2.5 × 30 cm) by the addition of the product preadsorbed on silica gel (5 g; 70–230 mesh). Fractions 22 ml in volume were collected. The column was successively eluted with 8% ethyl acetate in hexane (500 ml), 16% ethyl acetate in hexane (500 ml), 24% ethyl acetate in hexane (1000 ml) and 28% ethyl acetate in hexane (250 ml), and finally with methanol (150 ml). The chromatography was monitored by TLC and appropriate fractions were pooled and evaporated to dryness under reduced pressure.

The major product (1.554 g), corresponding to an overall yield of 64% from the starting material (**X**), was eluted in fractions 51–112 and was characterized as 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (**XII**) by its melting point (146.0–147.5°C) and by the results of low and high resolution MS (Table 3 and **Table 6**) and ^{13}C and ^1H NMR (Tables 1 and 2). The IR spectrum showed absorbances at 3468, 2953, 2942, 2861, 1736, 1699, 1624, 1379, 1360, 1258, 1125, and 1015 cm^{-1}. Fractions 6–10 from the silica gel column chromatography contained the 15-ketosteryl acetate **X** (20 mg; 0.8% of the original starting material) which, after recrystallization from methanol, melted at 135–136°C (lit., 135.0–135.5° (27)). Fractions 24–34 from the silica gel column chromatography contained 3β,24-diacetoxy-5α-chol-8(14)-en-15-one (**XIII**; 76 mg, corresponding to an overall yield of 2.7% from the 15-ketosteryl acetate (**X**)) which, after recrystallization from methanol, melted at 171–172°C; IR, 2957, 2930, 2897, 1730, 1697, 1618, 1244, 1177, 1042, and 1026 cm^{-1}; single component on TLC (solvent, 30% ethyl acetate in hexane); ^{13}C and ^1H NMR (Tables 1 and 2), and MS (Table 3). Elution of the silica gel column with methanol gave, upon evaporation of the solvent, 141 mg of material which was not characterized.

TABLE 5. Reversed phase HPLC analyses (Spherisorb ODS-II column; UV detection at 259 nm)[a] of crude products obtained upon side-chain oxidation of 3β-acetoxy-5α-cholest-8(14)-en-15-one (**X**)

Compound	Substituents		Percentage Composition			
			Process A			Process B[b]
	C-3	C-24	t = 0 h[b]	t = 0.75 h[b,c]	t = 3 h[d,e]	
XI	CH_3COO	CF_3COO	83	71	50	68
XII	CH_3COO	OH	11	23	44	27
XIII	CH_3COO	CH_3COO	4	3.5	2.3	4
XIV	OH	OH	≤ 0.1	≤ 0.1	n.d.[f]	≤ 0.5
X	CH_3COO	$CH(CH_3)_2$	n.d.	n.d.	1.3	n.d.
I	OH	$CH(CH_3)_2$	n.d.	n.d.	< 0.1	n.d.
Unknown			2	1.5	n.d.	n.d

[a]Very similar results were observed with UV detection at 210 nm.
[b]Solvent, water–methanol (1:7) at a flow rate of 1.0 ml per min. The retention times were identical (± 0.1 min) with those of authentic samples. The observed retention times were: **XI**, 15.9 min; **XII**, 8.1 min; **XIII**, 13.5 min; **XIV**, 4.3 min; **X**, ~30 min; and unknown, 11.6 min.
[c]Sample stood in methanol solution for 45 min prior to injection.
[d]Solvent, methanol at a flow rate of 1.0 ml per min. The retention times were identical (± 0.1 min) with those of authentic samples. The observed retention times were **XI**, 4.1 min; **XII**, 3.7 min; **XII**, 4.5 min; **X**, 6.2 min; and **I**, 9.4 min.
[e]Sample stood in methanol for 3 h prior to injection.
[f]n.d., Not determined under these HPLC conditions.

TABLE 6. High resolution mass spectral data for $\Delta^{8(14)}$-15-keto-C_{24} alcohols and their derivatives[a]

C-3 Substituent C-24 Functionality	XII 3β-OAc CH_2OH	XIII 3β-OAc CH_2OAc	XIV 3β-OH CH_2OH
M^+	416.2933 (+0.9)	458.3044 (+1.4)	374.2825 (+0.6)
$M-CH_3$	401.2727 (+3.7)	443.2818 (+2.3)	359.2588 (+0.4)
$M-H_2O$	398.2820 (−2.5)	440.2931 (+0.7)	356.2709 (−0.4)
$M-H_2O-CH_3$	383.2601 (+1.7)		341.2496 (+1.7)
M–ROH	356.2683 (−3.0)	398.2816 (−0.3)	
$M-ROH-CH_3$	341.2476 (−0.3)	383.2587 (+0.3)	
$M-ROH-H_2O$		380.2687 (−2.6)	
$M-ROH-H_2O-CH_3$	323.2395 (+2.0)	365.2493 (+1.4)	323.2381 (+0.8)
Ion $D-H_2O$	393.2304 (−1.8)		297.2226 (+0.9)
M–SC	329.2113 (−0.2)	329.2127 (+1.2)	287.2021 (+1.1)
$M-SC-H_2O$	311.2024 (+1.4)	311.2001 (−0.9)	269.1902 (−0.2)
Ion C	301.1790 (−1.2)	301.1811 (+0.9)	259.1660 (−3.7)
Ion A	261.1832 (−2.1)	303.1944 (−1.5)	261.1811 (−4.2)
Ion B	250.1932 (+0.1)		250.1932 (+0.1)
M–ROH–SC		269.1915 (+1.1)	
Ion $B-CH_3$	[b]	277.1833 (+3.1)	[b]
$M-ROH-H_2O-SC$	251.1797 (−0.2)	251.1804 (+0.5)	251.1836 (+3.7)

[a]Values in parentheses are the differences (in millimass units) between the observed masses and the calculated values for the elemental composition of the ions shown in the left column (corresponding to the ions presented in Table 3 and in Fig. 5).
[b]Not determined (below *m/z* 250).

Process B

The reaction mixture (from a 5.50-g scale reaction) was poured onto ice (2500 g) containing sodium sulfite (20 g), and the precipitate that formed was collected on a polypropylene filter cloth on a Buchner funnel with suction filtration for ~2 h. The solid was washed overnight with water (2000 ml) until the filtrate was neutral. The filter cake and material recovered from the filter cloth were dried in vacuo to give a crude product (3.88 g) that by reversed phase HPLC analysis (Table 5) consisted of **XI**, 68%; **XII**, 27%; and **XIII**, 4%.

A portion (3.82 g) of the crude product from above was dissolved in degassed methanol (100 ml) and triethylamine (0.32 ml), and the resulting solution was stirred at room temperature for 3 h under nitrogen. Ethyl acetate (250 ml) was added, and the resulting mixture was washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure to give a white solid (3.48 g) which was subjected to chromatography on a silica gel (96 g) column (40 × 220 mm; fraction volume, 20 ml). The column was eluted with ethyl acetate–hexane 5:95 which, at fraction 78, was changed to ethyl acetate–hexane 1:9. At fraction 95 the elution solvent was changed to ethyl acetate–hexane 13:87; at fraction 146 to ethyl acetate–hexane 1:4; at fraction 159 to ethyl acetate–hexane 35:65; and at fraction 187 to ethyl acetate–hexane 1:1. Upon evaporation of the solvent, the following materials were recovered: fractions 50–59, unreacted starting material **X** (36 mg); fractions 78–94, unidentified material (20 mg); fractions 128–134, the diacetate **XIII** (176 mg); and fractions 191–208, the monoacetate **XII** (2.30 g). The yields of **XII** and **XIII** from **X** were 45.1% and 3.1%, respectively (based upon isolated mass).

The reaction mixture (from a 6.00-g scale reaction) was processed in a similar fashion to yield 5.50 g of crude product. A portion (3.00 g) of this material in methanol (75 ml) and triethylamine (0.20 ml) was stirred for 1 h under nitrogen. After evaporation of the solvent under reduced pressure at room temperature, a portion (2.3 g) of the resulting residue (2.6 g) was subjected to silica gel (70 g) column (34 × 180 mm) chromatography using 2.5% acetone in benzene as the eluting solvent (fraction volume, 20 ml). Fractions 10–12 contained the starting material **X** (74 mg) (identification by NMR), and fractions 30–39 contained the diacetate **XIII** (59 mg; 2% yield from **X**). The 3,24-diol 3-acetate **XII** (1.774 g; 65% yield from **X**) was recovered in fractions 90–120. A portion (100 mg) of this material was further purified by preparative TLC (Uniplate-T; solvent 5% methanol in $CHCl_3$) to give an analytical sample of **XII** (70 mg) which, after recrystallization from ether–hexane, melted at 147.5–148.0°C; single component on TLC in three solvent systems (5% methanol in $CHCl_3$, R_f 0.52; 10% acetone in benzene, R_f 0.21; and 30% ethyl acetate in hexane, R_f 0.13) and a purity in excess of 99% on reversed phase HPLC (solvent, 20% water in methanol); $[\alpha]_D + 114°$ (c, 0.69); UV, λ_{max} 258 nm (ε 14,500); IR, 3440, 2938, 2866, 1738, 1699, 1624, 1258,

1175, and 1030 cm^{-1}; MS (Table 3); high resolution MS (Table 6); ^{13}C and ^{1}H NMR (Tables 1 and 2).

The side chain oxidation of **X** gave consistently good yields. Another reaction (from 5.00 g of **X**) yielded a white solid (3.8 g) which, upon selective hydrolysis of the crude product with triethylamine–methanol, gave **XII** as the major component (2.87 g; 61% yield) along with small amounts of the diacetate **XIII** (156 mg; 3% yield) and starting keto-acetate **X** (25 mg).

These combined results indicate that treatment of **X** with trifluoroacetic anhydride, sulfuric acid, and hydrogen peroxide under the conditions described above gave a crude product, easily isolated by collection of the precipitate formed upon addition of the reaction mixture to ice, in which the major component was the 3β-acetoxy-24-trifluoroacetoxy-C_{24}-$\Delta^{8(14)}$-15-ketone (**XI**) accompanied by small and variable amounts of the 3β-acetoxy-24-hydroxy-$\Delta^{8(14)}$-15-ketone (**XII**), the 3β,24-diacetate (**XIII**), and an unidentified component(s) of high polarity. In addition the crude product contained trace quantities of unreacted starting material (**X**) and the $\Delta^{8(14)}$-15-keto-C_{24}-3β,24-diol (**XIV**). These components of the crude product accounted for ~65–70% of the total reaction mixture. To study the optimum method of processing of the reaction mixture and determination of the nature of the remaining 30–35% of the crude reaction mixture, the side chain oxidation of [2,4-^{3}H]-**X** (2.80 g; 10 μCi) was also explored. The crude reaction mixture obtained after 3 h showed disappearance of **X** on TLC analysis (solvent, 10% ethyl acetate in hexane). The crude solid product (1.96 g) obtained according to process B contained 69% of the recovered ^{3}H, and the combined filtrate and washes of the solid product contained 31% of the recovered ^{3}H. The chemical nature of the ^{3}H-labeled material in the filtrate and washes was not established.

The reaction conditions used for the side chain oxidation of **X** were also applied to 3β-acetoxy-5α-cholestane. To a vigorously stirred mixture of trifluoroacetic anhydride (51.5 ml) and sulfuric acid (21.2 ml; 96%), cooled to –10°C, was added hydrogen peroxide (5.2 ml; 30%) dropwise. 3β-Acetoxy-5α-cholestane (3.0 g) was added in one portion with vigorous mechanical stirring at –3°C. The sterol did not dissolve, and stayed mainly on the walls of the flask. After 3 h, TLC analysis (solvent, 10% ethyl acetate in hexane) showed only the unreacted starting material. A second experiment under the same conditions gave an identical result.

3β,24-Diacetoxy-5α-chol-8(14)-en-15-one (XIII) from 3β-acetoxy-24-hydroxy-5α-chol-8(14)-en-15-one (XII)

Compound **XII** (50 mg) was dissolved in a mixture of dry pyridine (0.5 ml) and acetic anhydride (0.5 ml). After standing for 24 h at room temperature, the mixture was poured onto ice and the resulting precipitate was collected and dissolved in ethyl acetate. The solution was washed with 1 N HCl and water and then dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure to give crude **XIII** (45 mg) which was recrystallized from ethyl acetate to give an analytical sample (28 mg) melting at 174–175°C; single component on TLC in two solvent systems (10% acetone in benzene, R_f 0.78; 30% ethyl acetate in hexane, R_f 0.45); $[\alpha]_D$ +100.6° (c, 0.65); UV, λ_{max} 258 nm (ε 13,700); IR, 2953, 2862, 1738, 1699, 1620, 1246 and 1030 cm^{-1}; MS (Table 3); high resolution MS (Table 6); ^{13}C and ^{1}H NMR (Tables 1 and 2).

3β,24-Dihydroxy-5α-chol-8(14)-en-15-one (XIV)

The crude product (2.65 g) consisting of **XI, XII,** and **XIII** (see above) from a side chain oxidation of **X** was stirred in methanol (20 ml) containing anhydrous K_2CO_3 (1.0 g) under nitrogen at room temperature in a sealed vial for 2 h. After the addition of acetic acid (1 ml), the mixture was evaporated to dryness under reduced pressure. The resulting residue was taken up in ethyl acetate and water, and the separated organic phase was washed several times with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The resulting residue (2.03 g) was subjected to silica gel (60 g) column chromatography using 5% methanol in $CHCl_3$ (fraction volume, 20 ml). The contents (1.03 g) of fractions in 15–22 were pooled and a portion (100 mg) was subjected to preparative TLC (Uniplate-T; solvent, $CHCl_3$–acetone–methanol 7:5:1) to give **XIV** (52 mg) melting at 178–181°C; single component on TLC in three solvent systems (5% methanol in $CHCl_3$, R_f 0.22; 10% methanol in $CHCl_3$, R_f 0.46; $CHCl_3$–acetone–methanol 7:5:1, R_f 0.67) and a purity of 99.8% on reversed phase HPLC (solvent, 20% water in methanol); $[\alpha]_D$ +139.1° (c, 0.72), UV, λ_{max} 259 nm (ε 13,600); IR, 3280, 2978, 2957, 2924, 1697, 1616, 1088, 1047, and 1022 cm^{-1}; MS (Table 3); high resolution MS (Table 6); ^{13}C and ^{1}H NMR (Tables 1 and 2).

3,15-Diketo-5α-chol-8(14)-en-24-oic acid (XV)

To the $\Delta^{8(14)}$-3β,24-diol **XIV** (190 mg) in acetone (35 ml) was added 8 N Jones reagent dropwise with stirring at room temperature until the orange color of the reagent persisted. The excess of CrO_3 was destroyed by the addition of 2-propanol (1 ml) and the precipitated chromium salts were removed by filtration through a layer of Celite. The filtrate was evaporated to dryness under reduced pressure at 35°C, and the residue was taken up in ethyl acetate and water. The separated organic phase was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. A portion (100 mg) of the

resulting residue (113 mg) was subjected to preparative TLC (Uniplate-T; solvent, 10% methanol in $CHCl_3$) and preparative reversed phase HPLC (solvent, 70% water in methanol) to give **XV** (29 mg) melting at 212–213°C; single component on TLC in three solvent systems (20% methanol in $CHCl_3$, R_f 0.36; isooctane–ethyl acetate–acetic acid 5:5:1, R_f 0.53; and 20% 2-propanol in $CHCl_3$, R_f 0.27) and a purity of 99.7% on reversed phase HPLC (solvent, 30% methanol in water); $[\alpha]_D$ +151.4° (c, 0.46); UV, λ_{max} 258 nm (ε 13,400); IR, 3240, 2959, 2942, 1736, 1711, 1684, 1608, 1223, 1188, and 1167 cm^{-1}; MS (Table 3); high resolution MS (Table 4); ^{13}C and ^1H NMR (Tables 1 and 2).

Methyl 3,15-diketo-5α-chol-8(14)-en-24-oate (XVI)

The diketo acid **XV** (112 mg) was suspended in benzene (10 ml) containing a few drops of methanol and treated with excess diazomethane in ether. After decomposition of the excess reagent by the addition of a few drops of acetic acid, the solution was evaporated to dryness to give the crude ester (112 mg) which was purified by preparative TLC (Uniplate-T; solvent, 10% acetone in benzene) to give **XVI** (57 mg) melting at 148.5–150.0°C; single component on TLC in three solvent systems (10% methanol in $CHCl_3$, R_f 0.45; isooctane–ethyl acetate–acetic acid 5:5:1, R_f 0.55; and $CHCl_3$–acetone–methanol 7:5:1, R_f 0.88); $[\alpha]_D$ +154° (c, 0.6); UV, λ_{max} 258 nm (ε 14,000); IR, 2974, 2947, 1736, 1713, 1701, 1620, 1248, and 1233 cm^{-1}; MS (Table 3); high resolution MS (Table 4); ^{13}C and ^1H NMR (Tables 1 and 2).

Methyl 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oate (XVII)

The methyl ester of the $\Delta^{8(14)}$-3,15-diketo acid **XVI** (100 mg) in a 1:1 mixture (6 ml) of CH_2Cl_2 and methanol, cooled to –65° to –70°C in a dry ice-acetone bath, was added to sodium borohydride (97 mg) in the same solvent mixture (14 ml) at the same temperature. The mixture was stirred at this temperature for 75 min under nitrogen. After decomposition of the excess hydride with acetone (5 ml), the mixture was diluted with ethyl acetate and washed with 10% NaOH and water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The crude product (94 mg), which showed one principal component on TLC (solvent, 10% acetone in benzene), was purified by preparative TLC (Uniplate-T; solvent, 20% acetone in benzene) and recrystallization from ethyl acetate to give **XVII** (54 mg) melting at 134–136°C; single component on TLC in three solvent systems (10% acetone in benzene, R_f 0.20; 20% acetone in benzene, R_f 0.39; and 30% ethyl acetate in hexane, R_f 0.11); $[\alpha]_D$ +134.6° (c, 0.8); UV, λ_{max} 257 nm (ε 14,100); IR, 3470, 2961, 2924, 1703, 1620, 1269, 1257, 1119, 1092, 1055, and 1005 cm^{-1}; MS (Table 3); high resolution MS (Table 4); ^{13}C and ^1H NMR (Tables 1 and 2).

3β-Acetoxy-15-keto-5α-chol-8(14)-en-24-oic acid (XVIII)

To compound **XII** (1.0 g) in acetone (50 ml) an 8 N solution of Jones reagent was added dropwise with stirring at room temperature until the orange color of the reagent persisted. 2-Propanol (1 ml) was added, and the reaction mixture was filtered through a sintered glass filter and evaporated to dryness under reduced pressure. The residue was dissolved in ethyl acetate, and the organic solution was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure to give **XVIII** (1.0 g) of over 98% purity as judged by ^1H NMR. A portion (50 mg) of this material was purified by preparative TLC (Uniplate-T; solvent, 5% methanol in $CHCl_3$) to give pure **XVIII** (40 mg) melting at 194.5–196.0°C; single component on TLC in three solvent systems (20% methanol in $CHCl_3$, R_f 0.64; isooctane–ethyl acetate–acetic acid 5:5:1, R_f 0.56; and $CHCl_3$–acetone–methanol 7:5:1, R_f 0.18); $[\alpha]_D$ +114.2° (c, 0.67); UV, λ_{max} 258 nm (ε 13,300); IR, 3440, 2938, 2866, 1738, 1701, 1626, 1257, and 1030 cm^{-1}; MS (Table 3); high resolution MS (Table 4); ^{13}C and ^1H NMR (Tables 1 and 2).

Methyl 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oate (IX) by esterification of 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oic acid (XVIII)

The 3-acetate-24-acid **XVIII** (200 mg) was suspended in ether (20 ml) containing several drops of methanol and methylated by treatment with diazomethane as described above for the preparation of **XVI**. The crude product was purified by preparative TLC (Uniplate-T; solvent, 30% ethyl acetate in hexane). The material of R_f 0.49 was recovered by extraction with ethyl acetate to yield **IX** (111 mg) melting at 132.0–134.5°C; single component on TLC in three solvent systems (10% acetone in benzene, R_f 0.58; $CHCl_3$-acetone-methanol 7:5:1, R_f 0.63; 30% ethyl acetate in hexane, R_f 0.49); $[\alpha]_D$ +110.7° (c, 0.67); UV, λ_{max} 258 nm (ε 14,700); IR, 2945, 2863, 1740, 1699, 1624, 1246, 1175, 1125, and 1028 cm^{-1}; MS (Table 3); ^{13}C and ^1H NMR (Tables 1 and 2).

3β-Hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (IV) from 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oic acid (XVIII)

Compound **XVIII** (300 mg) and anhydrous K_2CO_3 (350 mg) in methanol (40 ml) were stirred at room temperature for 5 h under nitrogen in a sealed vial.

After the addition of 1 N HCl (6 ml), the mixture was evaporated to dryness under reduced pressure. Ethyl acetate and water were added, and the separated organic phase was washed with water to neutrality. After evaporation of the solvent under reduced pressure, a portion (100 mg) of the crude product (273 mg) was subjected to preparative TLC (Uniplate-T; solvent, 10% acetic acid in $CHCl_3$), and the product (42 mg) was further purified by preparative reversed phase HPLC (solvent, 20% methanol in water) to remove minor impurities. After evaporation of the solvent, the residue was dissolved in 2-propanol and passed through a small column (6 × 90 mm) of Amberlyst (H^+) to give, after evaporation of the solvent, **IV** melting at 224–226°C; single component on TLC in three solvent systems (10% acetic acid in $CHCl_3$, R_f 0.83; isoocctane–ethyl acetate–acetic acid 5:5:1, R_f 0.41; and hexane-$CHCl_3$-acetic acid 7:2:1, R_f 0.12); $[\alpha]_D$ +136.7° (c, 0.6; 3.3% methanol in $CHCl_3$); UV, λ_{max} 258 nm (ε 13,200); IR, 3320, 2967, 2932, 2863, 1692, 1626, 1265, 1252, 1230, 1125, 1086, and 1032 cm^{-1}; MS (Table 3); high resolution MS (Table 4); ^{13}C and ^1H NMR (Tables 1 and 2).

Effects of 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (IV), 3,15-diketo-5α-chol-8(14)-en-24-oic acid (XV), and 3β-hydroxy-5α-cholest-8(14)-en-15-one (I) on the levels of HMG-CoA reductase activity in CHO-K1 cells

The effects of the $\Delta^{8(14)}$-15-keto-acid **IV**, the $\Delta^{8(14)}$-3,15-diketo-acid **XV**, and the 15-ketosterol **I** on HMG-CoA reductase activity were studied in CHO-K1 cells. The $\Delta^{8(14)}$-15-keto-acid (**IV**) lowered HMG-CoA reductase activity with a 50% reduction occurring between 1.0 to 2.5 μM (**Table 7**). The potency of **IV** was considerably less than that of the 15-ketosterol **I** which, under the same conditions, lowered HMG-CoA reductase activity by ~50% at 0.1 μM. While the $\Delta^{8(14)}$-15-keto-acid **IV** lowered HMG-CoA reductase activity, the $\Delta^{8(14)}$-3,15-diketo-acid **XV** had no effect on reductase activity under the conditions studied (**Table 8**).

TABLE 8. Effects of 3,15-diketo-5α-chol-8(14)-en-24-oic acid (**XV**) and of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) on the levels of HMG-CoA reductase activity in CHO-K1 cells

Sterol Concentration (μM)	HMG-CoA Reductase Activity (% of Control Activity)[a]	
	Compound **XV**	Compound **I**
0.0	100.0[b]	100.0[c]
0.1	104.5 ± 0.1	80.6 ± 2.1
0.25	90.2 ± 2.4	42.7 ± 2.6
0.5	95.3 ± 1.0	44.8 ± 2.1
1.0	95.2 ± 2.6	43.5 ± 1.3
2.5	96.4 ± 1.5	26.2 ± 1.4

[a]Variation expressed as ± SD of replicate (n = 3) assays of HMG-CoA reductase activity.
[b,c]Mean values for controls were 749 and 1097 pmol per min per mg protein, respectively.

DISCUSSION

One important goal of this research was to prepare 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (**IV**) which, on the basis of GC-MS studies, has been found to be a metabolite of **I** after its intravenous or intraduodenal administration to bile duct-cannulated rats (J. S. Pyrek, S. Numazawa, and G. J. Schroepfer, Jr., unpublished data) and after incubation with HepG2 cells (J. S. Pyrek, F. D. Pinkerton, and G. J. Schroepfer, Jr., unpublished data). Our initial approach to this problem is outlined in Fig. 2 and represents a modification of the general approach used previously for the conversion of (25R)-26-hydroxycholesterol to (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (22, 24). The starting material for this work

TABLE 7. Effects of 3β-hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (**IV**) and of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) on the levels of HMG-CoA reductase activity in CHO-K1 cells

Sterol Concentration (μM)	HMG-CoA Reductase Activity (% of Control Activity)[a]			
	Compound **IV**		Compound **I**	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
0.0	100.0[b]	100.0[c]	100.0[d]	100.0[e]
0.1	69.8 ± 2.0	88.5 ± 2.7	45.7 ± 2.7	57.3 ± 1.6
0.25	70.0 ± 1.3	77.3 ± 7.8	27.1 ± 0.7	27.5 ± 1.4
0.5	53.1 ± 0.8	69.9 ± 6.3	25.1 ± 1.1	25.3 ± 1.1
1.0	60.5 ± 1.6	65.0 ± 2.5	17.9 ± 2.4	21.7 ± 0.6
2.5	42.8 ± 0.7	45.6 ± 0.6	15.7 ± 1.8	16.5 ± 1.2

[a]Variation expressed as ± SD of replicate (n = 3) assays of HMG-CoA reductase activity.
[b–e]Mean values for controls were 970, 931, 1155, and 949 pmol per min per mg protein, respectively.

was methyl 3β-acetoxychol-5-en-24-oate (**V**), which was prepared from hyodeoxycholic acid according to the approach described by Vargha and Rados (29) and Ziegler and Bharucha (31). Compound **V** was converted to the corresponding $\Delta^{5,7}$-diene **VI** by an adaptation of the procedure developed by Confalone et al. (39) for the synthesis of 7-dehydrocholesterol. The $\Delta^{5,7}$ diene **VI** was converted to methyl 3β-acetoxy-5α-cholest-8(14)-en-24-oate (**VII**) by hydrogenation-isomerization with PtO_2 in a mixture of ethyl acetate and acetic acid as utilized previously for the formation of $\Delta^{8(14)}$-sterols from the corresponding $\Delta^{5,7}$-dienes (22, 24, 32). Compound **VII** was characterized as such and after its hydrolysis with LiOH in methanol to give the corresponding $\Delta^{8(14)}$-carboxylic acid (**VIII**). Allylic oxidation (22, 24, 42, 43) of **VII** with CrO_3-3,5-dimethylpyrazole in CH_2Cl_2 at −20°C gave, after extensive chromatographic purification, methyl 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oate (**IX**).

Although this approach (Fig. 2) permitted the successful preparation of the desired methyl 3β-acetoxy-15-keto-chol-8(14)-en-24-oate (**IX**), the overall yield for the conversion of **V** to **IX** was disappointing, a situation resulting largely from difficulties encountered in the conversion of the Δ^5-acid (**V**) to the $\Delta^{5,7}$-acid (**VI**) and in the allylic oxidation of **VII** to give the $\Delta^{8(14)}$-15-ketone (**IX**). Consideration of alternative approaches for the preparation of **IV** and its 3β-acetoxy-methyl ester derivative (**IX**) led to exploration of direct side-chain oxidation of **I**.

The oxidation of the saturated side chain of sterols has been a subject of continuing scientific and commercial interest. Early uses of this approach, chiefly in the form of chromic acid oxidations, not only gave key information regarding the structure of sterols and steroid hormones but also provided for the commercial preparation of steroid hormones from cholesterol and its derivatives. An excellent summary of this early work was presented by Fieser and Fieser (44). More recently, renewed interest in chemical oxidations of the side chain of sterols has been stimulated by the demonstration of the importance of side-chain oxygenated derivatives of vitamin D in the expression of its activities and by the recognition of the very high potency of sterols with oxygen functions in the side chain in the regulation of HMG-CoA reductase activity and in the control of sterol and isoprenoid biosynthesis.

As noted above, the oxidation of the saturated side chain of sterols with chromic acid constituted an important early approach. For example, Wallis and Fernholtz (45) reported that oxidation of the 5,6-dibromide derivative of cholesteryl acetate gave, after treatment of the crude product with zinc in acetic acid, dehydroepiandrosterone acetate which was isolated as the semi-carbazone in 1.8% yield and 3β-hydroxychol-5-en-24-oic acid which was recovered in crude form in ~4% yield. Other studies, summarized by Fieser and Fieser (44), indicated the formation of a number of other side-chain oxygenated products. Maas and de Heus (46) studied the CrO_3 oxidation of 5α- and 5β-cholestanyl acetates and of a number of halogenated cholestane derivatives. The highest reported yield (~15%) of the 17-ketosteroid (isolated as the semicarbazone) was with the oxidation of 5β,6α-dibromo-cholestan-3β-yl acetate.

More recently, several other approaches to the side-chain oxidation of sterols have been described. One method involved the photolysis of a sterol solution in the presence of peracetic acid (47). Oxidation of 5α-cholestan-3β-yl acetate gave the corresponding 25-hydroxy (~ 15% yield) and 5α-hydroxy (~12% yield) derivatives along with other oxygenated products (47). Similar oxidation of cholestane-3β,5α-diol 3-acetate gave the 25-hydroxy product in ~19% yield. Another oxidation method involved the treatment of the sterol, preadsorbed on silica gel, with dry ozone at low temperature. Using this approach, Cohen and Mazur (48) reported selective formation of 25-hydroxylated sterols with yields varying from 5.5% for the oxidation of 3β-acetoxy-5α-cholestane to 15.6% for the oxidation of 3β-acetoxy-6β,7α-dibromo-5α-cholestane. Groves and Neumann (49) reported a selective hydroxylation of cholesterol at C-25 (2% yield) using a steroid manganese(III) porphyrin complex in a synthetic phospholipid bilayer. Barton and his associates have conducted extensive studies of the selectivity and mechanisms involved in the oxidation of saturated hydrocarbons by various Gif systems (ref. 50 and references cited therein). In one study (51), the oxidation of cholest-4-en-3-one by the Gif^{IV} system was studied at various temperatures. Oxidation at −30°C gave 25-hydroxycholest-4-en-3-one in 1.3% yield (with no detectable formation of progesterone). In contrast, oxidation at 20°C gave progesterone in 2.2% yield (with no detectable formation of the 25-hydroxysterol). Oxidations at intermediate temperatures (0°C, −15°C, and −25°C) gave mixtures of progesterone and the 25-hydroxysterol. In an extension and modification of reactions developed by Breslow (52), Orito, Satoh, and Suginome (53) have reported a long-range intramolecular functionalization at C-25 of the sterol side chain by irradiation of 7α-derivatives of 5α-cholestane. The resulting macrocyclic ether lactones were formed in ~4% yield and then reduced to 5α-cholestane-7α,25-diol with sodium in liquid ammonia.

In 1979 Deno and Meyer (41) described the oxidation of the side chain of a cholesterol derivative with trifluoroperoxyacetic acid–sulfuric acid and reported a multistep conversion of cholesterol to chol-5-ene-

3β,24-diol in an overall yield of 14%. Prior to oxidation, the hydroxyl group of cholesterol was protected by acetylation, and the Δ^5 double bond was protected by conversion of the acetate to 6β-acetamido-5α-cholestan-3β-yl acetate. The latter derivative was formed from the Δ^5-steryl acetate via the 6-nitro derivative which was reduced to the 6β-acetamido-derivative and acetylated. Treatment of the acetamido-steryl acetate with a mixture of trifluoroacetic acid, sulfuric acid, and hydrogen peroxide for 4 h at 0°C gave a crude product (not characterized) which was heated with a mixture of acetic anhydride and 5-sulfosalicylic acid in toluene to give a crude product which, after purification by silica column chromatography and recrystallization, gave the diacetate derivative of chol-5-ene-3β,24-diol (23% yield from the acetamido derivative). Reduction of the diacetate with lithium aluminum hydride gave the 3β,24-Δ^5-diol.[2] It should be noted that, in contrast to the synthesis reported in the present work, the reactions utilized by Deno and Meyer gave the Δ^5-3β,24-diol which would require selective protection of the 3β-hydroxyl group for further modifications of the side chain.

Manley et al. (54) reported the side-chain oxidation of cholest-4-en-3-one with trifluoroperoxyacetic acid–sulfuric acid to give 24-hydroxychol-4-en-3-one (as its 24-acetate derivative) in 17% yield of crystalline product. Treatment of the Δ^4-3-one with a mixture of trifluoroacetic acid, sulfuric acid, and hydrogen peroxide for 4 h at 0°C gave a crude product (not characterized) which was refluxed for 18 h with a mixture of acetic acid, water, and sodium acetate to yield, after purification by silica gel column chromatography and crystallization from methanol, 24-acetoxychol-4-en-3-one (17% yield). Saponification with ethanolic KOH gave 24-hydroxychol-4-en-3-one in 89% yield.

Rosenfeld, Paul, and Zumoff (55) applied the procedure of Manley et al. (54) to the oxidation of [^{3}H]cholest-4-en-3-one (derived from [^{3}H]cholesterol which had been labeled by tritiation of the Δ^{24}-double bond of 6-methoxy-3α,5α-cyclocholest-24-ene). The labeled cholest-4-en-3-one (619 mg) was oxidized with a mixture of trifluoroacetic acid, sulfuric acid, and hydrogen peroxide, and the crude product was treated with a mixture of acetic acid, water, and sodium acetate according to Manley et al. (54) to give, after alumina column chromatography, starting material (74 mg) and the 24-acetate derivative of 24-hydroxychol-4-en-3-one (24% yield). Nicotra et al. (56) also applied the conditions of Manley et al. (54) for the conversion of [23,23,25-$^3H_3$,4-^{14}C]cholest-4-en-3-one to [23,23-$^3H_2$,4-^{14}C]24-hydroxychol-4-en-3-one. In contrast to the latter study in which the nature of the crude reaction products was not studied, Nicotra et al (56) found that, after flash chromatography of the crude product, the 24-trifluoroacetoxy derivative of the doubly labeled 24-hydroxychol-4-en-3-one[3] was obtained in 16% yield. Also recovered were starting material (150 mg) and more polar products that were not studied further. The trifluoroacetate derivative, which was characterized by melting point, IR, ^{1}H NMR, and MS, was converted to 24-hydroxychol-4-en-3-one in 87% yield by saponification with ethanolic KOH. The latter compound, upon treatment with pyridinium dichromate in N,N-dimethylformamide and chromatography of the crude reaction mixture, gave the corresponding [22,23-^{3}H,4-^{14}C]3-ketochol-4-en-24-oic acid in 66% yield (56).

Takano, Sato, and Ogasawara (57) reported a modification of the same approach for the conversion of 7-ketocholesteryl acetate to 3β,24-dihydroxychol-5-en-7-one in 19% yield. 7-Ketocholesteryl acetate was treated with a mixture of trifluoroacetic anhydride, sulfuric acid, and hydrogen peroxide for 4.5 h at 0°C. After the addition of triethylamine at –15°C, crude, 3β-acetoxy-24-trifluoroacetoxychol-5-en-7-one was obtained which was directly saponified with potassium carbonate in methanol to give the C_{24} 7-keto-3β,24-diol. The authors also reported that, after treatment of the initial crude reaction mixture with triethylamine as described above, 3β-acetoxy-24-trifluoroacetoxychol-5-en-7-one was isolated in pure form in 29% yield by chromatography. However, no characterization of this product was presented other than its melting point.

In the present study, we have applied the general approach introduced by Deno and Meyer (41) to the side-chain oxidation of the $\Delta^{8(14)}$-15-ketosteryl acetate (**X**). Using modifications of the conditions described by Deno and Meyer (41), Manley et al. (54), and Takano et al. (57), we have observed remarkably high yields of C_{24} oxygenated products upon treatment of **X** with a mixture of trifluoroacetic anhydride, sulfuric acid, and hydrogen peroxide for ~3 h at –2°C. For example, oxidation of **X** (5.65 g) gave, according to HPLC analysis of the crude product, 3β-acetoxy-24-trifluoroacetoxy-5α-chol-8(14)-en-15-one (**XI**) in 58% yield, along with small amounts of 3β-acetoxy-24-

[2]The reported yield of 14% for the overall conversion of cholesterol was apparently based upon the assumption of yields of 100%, 90% (actually 0–90%), 70%, 23%, and 100%, respectively, for the five individual steps.

[3]It is noteworthy that the trifluoroacetate derivative was obtained by crystallization from methanol (details not given) whereas in the present study the trifluoroacetoxy C_{24} compound **XI** was readily hydrolyzed to the corresponding C_{24} alcohol upon standing in methanol at room temperature.

hydroxy-5α-chol-8(14)-en-15-one (**XII**; 8% yield), and 3β,24-diacetoxy-5α-chol-8(14)-en-15-one (**XIII**; 3% yield). Thus, under the conditions described herein, the total estimated yield of C_{24} oxidation products (**XI, XII,** and **XIII**) from **X** was 69%. It is important to note that, under these conditions, only trace quantities of starting material remained after ~3 h. The remainder of the product consisted of very polar material which was not studied further. The ~69% of C_{24} oxygenated products was confirmed by selective hydrolysis of the trifluoroacetate function of **XI** followed by chromatography to give isolated yields of **XII** and **XIII** of 64% and 3% respectively.

Progress in the development of optimal conditions for the side chain oxidation of **X** was facilitated by following the oxidation of [2,4-^{3}H]-**X** and by analyses of reaction mixtures by TLC, HPLC, and NMR. The major product of the side-chain oxidation of **X**, the trifluoroacetate **XI**, was characterized by IR, MS, and ^{1}H and ^{13}C NMR. Compound **XII** was characterized as a minor product of the side-chain oxidation and also after selective hydrolysis of the trifluoroacetate function of **XI**.

The detailed mechanisms involved in the side-chain oxidation of **X** have not been established in the present study. However, a probable reaction course, based upon the results presented here and those reported previously for similar oxidations of other compounds (41, 54–60), can be suggested (**Fig. 4**). The overall reaction can be envisioned as being initiated by the formation of the C-25 hydroperoxide or, more likely under the reaction conditions employed, the trifluoroacyl peroxide. Cleavage of the O–O bond under the strongly acidic conditions followed by rearrangement (as in the cases of *tert*-pentyl hydroperoxide or the corresponding peroxy ester (61)) to form the carboxonium ion which, under the acidic conditions, would yield acetone and the 3β-acetoxy-24-ol **XII**. Although acetone and other methyl alkyl ketones usually do not undergo Baeyer-Villiger oxidation (61), such oxidation of these compounds has been observed under strongly acidic conditions (61–64). Thus, under the conditions employed in this study, the formation of methanol and acetic acid would be anticipated. Trifluoroacetylation of **XII** would yield **XI**. Similar esterification of the methanol under the reaction conditions would be expected, although no attempt was made to isolate the ester in this study.

This proposal also provides an explanation for the observation that small amounts of the diacetate **XIII** were formed in the side-chain oxidation of **X**. Analyses of the trifluoroacetic anhydride used in this study established the absence of either acetic anhydride or acetic acid. One other possibility for the origin of the

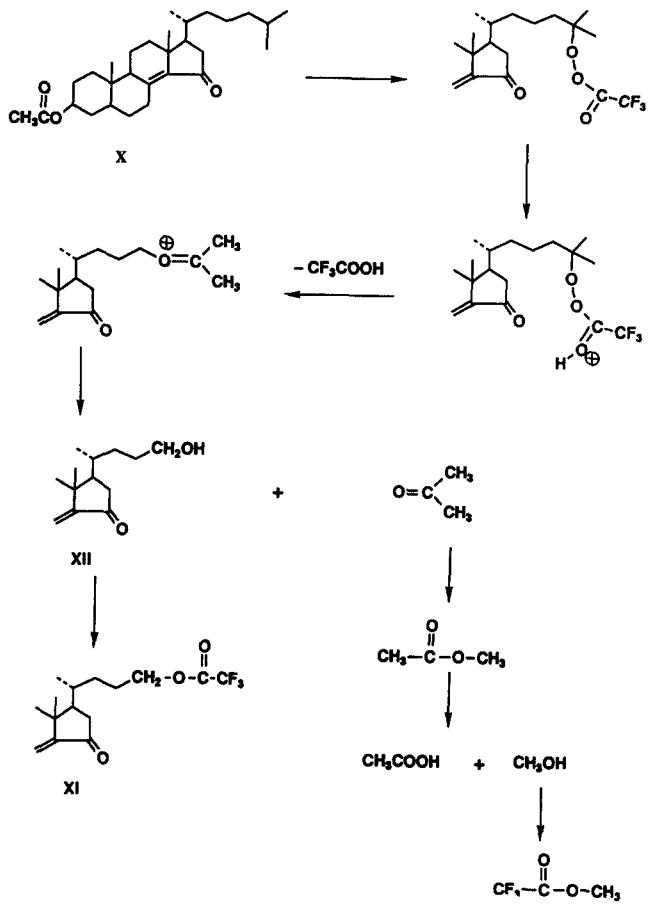

Fig. 4. Possible mechanism for the side-chain oxidation of the $\Delta^{8(14)}$-15-ketosterol acetate **X** by sulfuric acid, hydrogen peroxide, and trifluoroacetic anhydride.

acetoxy moiety at C-24 in **XIII** could be from an intermolecular transfer of acetate from one molecule of **X**, **XI**, or **XII** to C-24 of another molecule of **XII**. This possibility appears to be very unlikely in view of the very low levels of 3β,24-dihydroxy-5α-chol-8(14)-en-15-one (**XIV**) and of the free sterol **I** in the reaction mixture, and more importantly, by the results of the side-chain oxidation of the pivaloate ester of **I**. In this case, formation of the 3β-pivaloate-24-acetoxy-derivative of **XIV** (without formation of the 3β,24-dipivaolate ester of **XIV**) was observed (S. Swaminathan and G. J. Schroepfer, Jr., unpublished data). The combined results and considerations of the scheme presented in Fig. 4 strongly suggest that the origin of the acetoxy function of C-24 in **XIII** is from acetate derived from the Baeyer-Villiger oxidation of acetone generated in the side chain oxidation of **X**, followed by either acetylation of **XII** or, more likely, the displacement of the trifluoroacetate function of **XI** in a transesterification reaction. Further studies will be required to confirm these suggestions.

Treatment of 3β-acetoxy-5α-cholestane with the mixture of trifluoroacetic anhydride, sulfuric acid, and hydrogen peroxide (under the conditions that gave high yields of C_{24} oxygenated products upon oxidation of **X**) gave essentially no products with the expected properties of oxygenated products analogous to those observed in the oxidation of **X**. Under the conditions employed, only the unreacted starting material was noted.

Apart from the very high yields of 24-oxygenated products, the oxidation of **X** gave **XI** as the major product along with small amounts of **XII**. Since the trifluoroacetate function of **XI** was very readily hydrolyzed to give **XII**, the facile formation of **XII** from **X** provides an extremely useful intermediate permitting further modification of the side chain without need for selective protection of the hydroxyl function at C-3. This feature is nicely illustrated in the oxidation of **XII** to give 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oic acid (**XVIII**) which was then converted to the corresponding methyl ester (**IX**) which was shown to be identical to **IX** prepared from methyl 3β-acetoxychol-5-en-24-oate by an independent route (Fig. 2). Compound **XII** has also been used as the starting material for the chemical synthesis of the 25-aza analog of **I** (25, 65).

Hydrolysis of the crude reaction product (composed of a mixture of **XI**, **XII**, and **XIII**) of the side-chain oxidation of **X** with potassium carbonate gave the $\Delta^{8(14)}$-15-keto-3β,24-diol (**XIV**), which was oxidized with Jones reagent to give the desired $\Delta^{8(14)}$-3,15-diketo-acid (**XV**). Treatment of **XV** with diazomethane gave methyl ester **XVI** which was reduced with sodium borohydride at low temperature to the 3β-hydroxy-$\Delta^{8(14)}$-15-keto-C_{24} methyl ester (**XVII**). Thus, the mild alkaline hydrolysis of the crude product from the side-chain oxidation of **X** gave the $\Delta^{8(14)}$-15-keto-3β,24-diol (**XIV**) which, by CrO_3 oxidation, provided a ready access to the desired $\Delta^{8(14)}$-3,15-diketo-acid (**XV**).

All new compounds **XIV, XV, XVI,** and **XVII** were characterized by their melting points and optical rotations, and by the results of UV, IR, MS, and NMR studies. A summary of the optical rotations and UV spectral data of the new $\Delta^{8(14)}$-15-keto C_{24} acids and alcohols and their derivatives is presented in **Table 9**. All of the new $\Delta^{8(14)}$-15-keto C_{24} compounds were strongly dextrorotatory, an observation compatible with the high dextrorotation ($[\alpha]_D$ +139° (26)) of the 15-ketosterol **I**. In the cases of the $\Delta^{8(14)}$-15-keto C_{24} acids, the values of the optical rotation for the free acids (**IV** and **XV**) were essentially the same as those for the corresponding methyl esters (**XVII** and **XVI**). The values of the optical rotation for the $\Delta^{8(14)}$-3,15-diketo acid (**XV**) and its methyl ester (**XVI**) were higher than those observed for the corresponding 3β-hydroxy compounds (**IV** and **XVII**, respectively), observations in accord with the increased dextrorotation observed for the transition from 5α-cholestan-3β-ol ($[\alpha]_D$ + 24°) to 5α-cholestan-3-one ($[\alpha]_D$ + 41°) (30). The value of the optical rotation for the 3β-acetate derivative (**XII**) of the C_{24} $\Delta^{8(14)}$-15-keto-3β,24-diol (**XIV**) was less than that of the free diol (**XIV**), a finding in accord with the increased dextrorotation observed on the transition from 3β-acetoxy-5α-cholestane ($[\alpha]_D$ + 13°) to 5α-cholestan-3β-ol ($[\alpha]_D$ + 24°) (30).

All of the new $\Delta^{8(14)}$-15-keto C_{24} acids and alcohols and their derivatives showed the UV properties expected for the $\Delta^{8(14)}$-15-ketone system in ethanol solution, i.e., absorbance maximum at 258 ± 1 nm and ε values between 13,200 and 14,700.

The low resolution MS data on new $\Delta^{8(14)}$-15-keto C_{24} acids and alcohols and their derivatives are presented in Table 3. In each case, the observed MS data were fully compatible with the assigned structures for the new compounds. The mass spectra of the two samples of the methyl 3β-acetoxy-15-keto-5α-chol-8(14)-en-24-oate (**IX**), prepared by oxidation of **VII** (Fig. 2) or by methylation of **XVIII** (derived from the side-chain oxidation of **X**; (Fig. 3)) were essentially identical. The suggested fragment ion assignments (Table 3 and Fig. 5) are in accord with those made previously for the 15-

TABLE 9. Optical rotations and ultraviolet spectral data of $\Delta^{8(14)}$-15-keto-C_{24} acids and alcohols

Compounds	Substituent at C-3	C-24 Functionality	Optical Rotation $[\alpha]_D$	Ultraviolet	
				λmax	ε
IV	3β-OH	COOH	+137°	258	13,200
XVII	3β-OH	$COOCH_3$	+135°	257	14,100
XVIII	3β-OAc	COOH	+114°	258	13,300
IX	3β-OAc	$COOCH_3$	+111°	258[a]	14,400[a]
				258[b]	14,700[b]
XIV	3β-OH	CH_2OH	+139°	259	13,600
XII	3β-OAc	CH_2OH	+114°	258	14,500
XIII	3β-OAc	CH_2OAc	+101°	258	13,700
XV	3-keto	COOH	+151°	258	13,400
XVI	3-keto	$COOCH_3$	+154°	258	14,000

[a]From oxidation of **VII**.
[b]From methylation of **XVIII**.

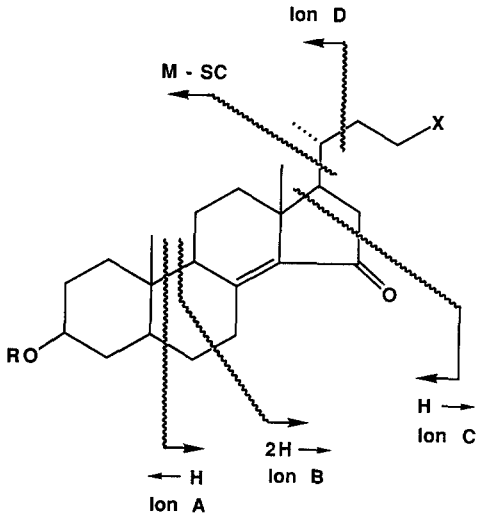

Fig. 5. Major mass spectral fragmentation processes for $\Delta^{8(14)}$-15-keto C_{24} acids and alcohols and their derivatives.

ketosterol (**I**) and its side-chain oxygenated derivatives (23, 26). Moreover, the results of high resolution mass spectral measurements (Tables 4 and 6) were compatible with the suggested fragment ion assignments.

3β-Hydroxy-15-keto-5α-chol-8(14)-en-24-oic acid (**IV**) has been found to be a significant metabolite of the 15-ketosterol (**I**) after intravenous or intraduodenal administration of **I** to bile duct-cannulated rats (J. S. Pyrek, S. Numazawa, and G. J. Schroepfer, Jr., unpublished data) and after incubation with HepG2 cells (J. S. Pyrek, F. D. Pinkerton, and G. J. Schroepfer, Jr., unpublished data). In the present study we have shown that the $\Delta^{8(14)}$-15-keto acid **IV** lowered HMG-CoA reductase activity upon incubation with CHO-K1 cells. In two experiments, **IV**, at a concentration of 2.5 μM in the culture medium, reduced the level of reductase activity by 57% and 54%. Despite the fact that **IV** was less potent than the 15-ketosterol (**I**) in lowering reductase activity in these cells, the activity of **IV** may be of importance in the regulation of HMG-CoA reductase activity in the intact animal and may contribute to the lowering of serum cholesterol levels observed after administration of the 15-ketosterol to rodents (8, 9, 12, 13) and nonhuman primates (10, 11). It is important to note that after intravenous administration of labeled **I** or chylomicroms containing fatty acid esters of labeled **I** to bile duct-cannulated rats, a major fraction of the administered radioactivity was excreted in bile in the form of polar metabolites of **I** (18, 21, 23). The $\Delta^{8(14)}$-15-keto-C_{24} acid **IV** represented a significant in vivo metabolite of **I** in the rat (J. S. Pyrek, S. Numazawa, and G. J. Schroepfer, Jr., unpublished data). We have also shown that a significant fraction of the polar biliary metabolites of **I** undergoes enterohepatic circulation (18). Further studies will be required to determine the extent of the intestinal absorption of **IV** and its further metabolism in the intact animal.

The results of GC–MS studies of the polar metabolites formed from the 15-ketosterol upon its incubation with rat liver mitochondria (23), HepG2 cells (J. S. Pyrek, F. D. Pinkerton, and G. J. Schroepfer, Jr., unpublished data), or after its administration to bile duct-cannulated rats (J. S. Pyrek, S. Numazawa, and G. J. Schroepfer, Jr., unpublished data) also indicated the formation of polar metabolites with 3α-hydroxy functions and that their formation involved loss of the 3α-hydrogen of **I** (J. S. Pyrek, F. D. Pinkerton, S. Numazawa, and G. J. Schroepfer, Jr., unpublished data). Thus, the 3,15-diketo-acid **XV** represents a potential metabolite of **I**, and the availability of synthetic **XV**, as reported herein, should facilitate investigations of the possible importance of **XV** in the overall metabolism of **I** and **IV**. It is noteworthy that, while **IV** lowered HMG-CoA reductase activity in CHO-K1 cells, the 3,15-diketo-acid **XV** had no detectable effect on reductase activity under the conditions used (i.e., up to 2.5 μM in the culture medium). These observations suggest the possibility that significant reduction of **XV** to **IV** does not occur in the CHO-K1 cell under conditions studied. Further studies will be required to clarify this matter.

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