Inhibitors of sterol synthesis. Chemical synthesis, structure, and biological activities of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one, a metabolite of 3β-hydroxy-5α-cholest-8(14)-en-15-one

Hong-Seok Kim, William K. Wilson, Dolores H. Needleman, Frederick D. Pinkerton, David K. Wilson, Florante A. Quiocho, and George J. Schroepfer, Jr.[1]

Departments of Biochemistry and Chemistry, Rice University, Houston, TX 77251, and Howard Hughes Medical Institute and the Department of Biochemistry, Physiology, and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030

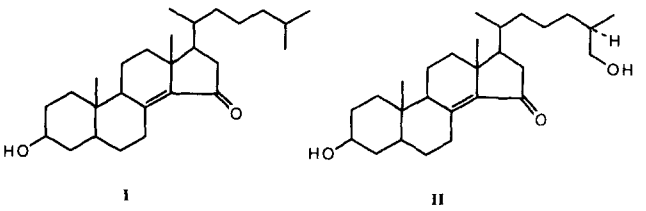
Abstract 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I**) is a potent inhibitor of sterol synthesis with significant hypocholesterolemic activity. (25R)-3β,26-Dihydroxy-5α-cholest-8(14)-en-15-one (**II**) has been shown to be a major metabolite of **I** after incubation with rat liver mitochondria. Described herein is the chemical synthesis of **II** from diosgenin. As part of this synthesis, improved conditions are described for the conversion of diosgenin to (25*R*)-26-hydroxycholesterol. Benzoylation of the latter compound gave (25*R*)-cholest-5-ene-3β,26-diol 3β,26-dibenzoate which, upon allylic bromination followed by dehydrobromination, gave (25*R*)-cholesta-5,7-diene-3β,26-diol 3β,26-dibenzoate. Hydrogenation–isomerization of the $\Delta^{5,7}$-3β,26-dibenzoate to (25*R*)-5α-cholest-8(14)-ene-3β,26-diol 3β,26-bis(cyclohexanecarboxylate) followed by controlled oxidation with CrO_3-dimethylpyrazole gave (25*R*)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one 3β,26-bis(cyclohexanecarboxylate). Acid hydrolysis of the $\Delta^{8(14)}$-15-ketosteryl diester gave **II**. ^{13}C NMR assignments are given for all synthetic intermediates and several major reaction byproducts. The structure of **II** was unequivocally established by X-ray crystal analysis. **II** was found to be highly active in the suppression of the levels of 3-hydroxy-3-methylglutaryl coenzyme A reductase in cultured mammalian cells and to inhibit oleoyl coenzyme A-dependent esterification of cholesterol in jejunal microsomes.—**Kim, H-S., W. K. Wilson, D. H. Needleman, F. D. Pinkerton, D. K. Wilson, F. A. Quiocho, and G. J. Schroepfer, Jr.** Inhibitors of sterol synthesis. Chemical synthesis, structure, and biological activities of (25R)-3β,26-dihydroxy- 5α-cholest-8(14)-en-15-one, a metabolite of 3β-hydroxy-5α-cholest-8(14)-en-15-one. *J. Lipid Res.* 1989. **30:** 247–261.

Supplementary key words 15-ketosterol • ACAT • diosgenin • HMG-CoA reductase • hypocholesterolemic activity • X-ray crystal analysis

3β-Hydroxy-5α-cholest-8(14)-en-15-one (**I; Fig. 1**) is a novel regulator of cholesterol metabolism. The 15-ketosterol is highly active as an inhibitor of sterol synthesis in mammalian cells in culture and lowers the level of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (1–4) and of cytosolic acetoacetyl-CoA thiolase (2, 4) and HMG-CoA synthase (2, 4) activity. Dietary administration of **I** to rats is associated with a marked inhibition of the intestinal absorption of exogenous cholesterol (5, 6). Thus, the 15-ketosterol affects the two ultimate sources of cholesterol in higher animals, i.e., the de novo synthesis of cholesterol and the absorption of cholesterol. Compound **I** has been shown to serve as an alternative substrate for acyl CoA:cholesterol acyltransferase (ACAT) of microsomes of rat liver and jejunum and to inhibit the oleoyl-CoA-dependent esterification of cholesterol in hepatic and jejunal microsomes (7). Dietary administration of **I** to rats has been shown to cause a marked reduction of the levels of ACAT activity of jejunal microsomes without affecting microsomal cholesterol levels (8). Thus, the 15-ketosterol not only affects the levels of activity of key regulatory enzymes involved in the biosynthesis of cholesterol, but also affects ACAT, an enzyme intimately involved in the regulation of the intracellular metabolism of cholesterol. The 15-ketosterol has significant hypocholesterolemic activity upon oral administration to rodents (9) and nonhuman primates (10, 11). The reduction of total serum cholesterol levels induced by **I** in rhesus monkeys has been shown to

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; ACAT, acyl coenzyme A:cholesterol acyltransferase; LDL, low density lipoprotein; HDL, high density lipoprotein; MP, melting point; UV, ultraviolet; IR, infrared; MS, mass spectra; NMR, nuclear magnetic resonance; DEPT, distortionless enhancement by polarization transfer; HETCOR, ^{13}C-^{1}H shift-correlated 2D NMR; COSY, ^{1}H-^{1}H shift-correlated 2D NMR; TLC, thin-layer chromatography; MPLC, medium pressure liquid chromatography; HPLC, high pressure liquid chromatography; BSA, bovine serum albumin; PBS, phosphate-buffered saline; SC, side chain; TBDMS, *tert*-butyldimethylsilyl.

[1]To whom inquiries should be directed at Department of Biochemistry, Rice University, Post Office Box 1892, Houston, Texas 77251.



I II

Fig. 1. Structures of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) and (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**).

be associated with a lowering of the levels of low density lipoprotein (LDL) cholesterol and LDL protein, an elevation of the levels of high density lipoprotein (HDL) cholesterol and HDL protein, and a shift in the HDL profile to one in which the HDL_2 species predominates (11). All of the effects of **I** noted above are believed to be beneficial for potential use in the prevention and/or treatment of atherosclerosis.

The 15-ketosterol, an inhibitor of cholesterol biosynthesis, is convertible to cholesterol, a transformation that has been demonstrated in vitro in rat liver subcellular preparations (12, 13) and in vivo after intravenous and oral administration to rats and baboons (6, 14–18). Cholesterol and cholesteryl esters have been found to be the major metabolites found in blood and tissues at 48 hr after the intravenous administration of **I** to rats (17). However, a quantitatively more important fate of **I** under these conditions is very rapid metabolism to polar metabolites which are excreted in bile (17). In initial studies of the nature of potential polar metabolites of **I**, we have shown that (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**) is the major metabolite of **I** detected after its incubation with mitochondria of rat liver (19). The purpose of this report is to describe the chemical synthesis of **II**, the X-ray crystal structure of **II**, and the effect of **II** on the levels of HMG-CoA reductase activity in CHO-K1 cells and on ACAT activity of jejunal microsomes.

EXPERIMENTAL

Materials and methods

Melting points (MP) were determined using a Thomas-Hoover apparatus in sealed, evacuated capillary tubes. Ultraviolet (UV) spectra were recorded on an IBM 9430 spectrophotometer using methanol as the solvent. Infrared (IR) spectra were obtained using a Beckman 4230 spectrophotometer with KBr pellets. Low resolution mass spectra (MS) were recorded on a Shimadzu QP-1000 quadrupole spectrometer with electron energy of 20 eV and direct inlet sample introduction. High resolution MS were recorded on a JEOL HX110HF spectrometer. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured on an IBM AF300 spectrometer in $CDCl_3$ solution in 5-mm tubes. ^1H 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. As described previously (20), ^{13}C NMR peak assignments were determined from 90° and 135° DEPT (distortionless enhancement by polarization transfer), HETCOR (^{13}C–^1H shift- correlated 2D NMR), and COSY (^1H–^1H shift-correlated 2D NMR) spectra obtained using standard IBM-Bruker software.

Thin-layer chromatography (TLC) was carried out on high performance TLC plates (10 cm × 20 cm) coated with silica gel 60 (EM Science, Cherry Hill, NJ) for synthetic intermediates, on Whatman LK5D silica gel TLC plates for assays of HMG-CoA reductase activity, and on silica gel G TLC plates from Analtech (Newark, DE) for assays of ACAT activity. Components on the plates were visualized by spraying the dried developed plates with 5% ammonium molybdate(VI) in 10% sulfuric acid, followed by heating (5 min) in an oven (100–120°C) (21). Solvents systems for TLC were as follows: SS-1, 50% ethyl acetate in hexane; SS-2, 10% ethyl acetate in hexane; SS-3, 5% ethyl acetate in hexane; SS-4, benzene; SS-5, 50% ether in benzene; SS-6, 5% ether in benzene; SS-7, 5% ethyl acetate in benzene; SS-8, hexane–ethyl acetate–$CHCl_3$ 3:4:3; SS-9, 30% ethyl acetate in hexane; and SS-10, 50% acetone in benzene. Medium pressure liquid chromatography (MPLC) was carried out using columns of alumina–$AgNO_3$ (22) or silica gel (32–63 microns) eluted at 4–10 ml/min. Analytical high performance liquid chromatography (HPLC) was carried out using a Waters Model 6000 unit equipped with a U6K injector, a Shimadzu SPD-6A UV detector, and a Spherisorb ODS-II column (5 μm, 4.6 mm × 250 mm; Custom LC, Houston, TX). Diosgenin was purchased from Steraloids, Inc. (Wilton, NH).

Dithiothreitol, bovine serum albumin (BSA; essentially fatty acid-free), oleoyl CoA, cholesteryl oleate, and all reagents for the assay of HMG-CoA reductase were obtained from Sigma Chemical Company (St. Louis, MO). Compound **I** was prepared by a modification (23) of a procedure described previously (1, 24) and showed a single component on TLC (SS-4, SS-9). The preparation and purity of an authentic sample of the oleate ester of **I** have been described previously (7). [1-^{14}C]CoA (11,000 dpm per nmol after dilution with unlabeled oleoyl CoA), (3*RS*)-[3-^{14}C]HMG-CoA (56 mCi per mmol), and (3*RS*)-[2-^3H]mevalonolactone (176 mCi per mmol) were obtained from the Amersham Corporation (Arlington Heights, IL). [1,2,6,7-^3H(N)]Cholesteryl oleate (65.8 mCi per mmol) was purchased from New England Nuclear (Boston, MA). Trypsin was purchased from Gibco Laboratories (Grand Island, NY) and Lux tissue culture plastic ware was obtained from Miles Scientific (Elkhart, IN). Powdered Ham's F12 medium (25) 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 purchased from Irvine Scientific (Irvine, CA). Fetal calf serum was obtained from Whittaker M.A. Bioproducts (Elkhart, IN). For cell culture studies, measured amounts of ethanolic solutions of **I** or **II** were added to Ham's F12 medium supplemented with 5% delipidated (26) fetal calf serum (lipid-deficient medium) and allowed to equilibrate for at least 6 hr at room temperature prior to storage at 4°C. Chinese hamster ovary cells (CHO-K1) were obtained from the American Type Culture Collection (Rockville, MD). Male Sprague-Dawley rats (150–250 g) were maintained on a light–dark cycle (light, 6:00 AM to 6:00 PM) and fed Purina Formulab 5008 chow.

Radioactivity was assayed in Packard 4640 and Beckman 9800 liquid scintillation counters using 2,5-diphenyloxazole (0.4%) in toluene or toluene–ethanol 2:1 as the scintillation fluid. Protein in detergent-solubilized extracts of cultured cells was assayed by the method of Lowry et al. (27) after precipitation with trichloroacetic acid, and the protein of jejunal microsomes was assayed by a modification (28) of the method of Lowry et al.

Studies of the effects of **I** and **II** on HMG-CoA reductase activity were made in CHO-K1 cells. The cells were maintained in Ham's F12 medium supplemented with 5% fetal calf serum (lipid-rich medium) in a humidified atmosphere of 5% CO_2–95% air at 37°C. Each experiment was initiated by inoculating 3.75×10^5 cells into 100-mm dishes containing lipid-rich medium (10 ml), followed by incubation for 48 hr. The medium was aspirated and, after rinsing the plates with PBS (10 ml), lipid-deficient medium (10 ml) was added to each plate, and the cells were incubated further for 18 hr. The lipid-deficient medium was removed and fresh lipid-deficient medium containing **I** or **II** (or the appropriate quantities of ethanol in the cases of controls) was added to the plates, and the incubation was continued for 4 hr. Cells were harvested by scraping, and detergent-solubilized cell preparations were obtained for the assay of HMG-CoA reductase activity using the method of Brown, Dana, and Goldstein (29). Replicate assays (n = 3) were carried out as described by Pinkerton et al. (3) except that the specific activity of the (3*RS*)-[3-^{14}C]HMG-CoA was 20,000 dpm per nmol.

Studies of the effects of **I** and **II** on ACAT activity in jejunal microsomes were made by assay of the incorporation of [1-^{14}C]oleoyl CoA into cholesteryl esters. Rat jejunal microsomes were prepared by a modification of the method of Suckling, Stange, and Dietschy (30). The small intestine was removed (8:00 AM to 10:00 AM) and the intestinal contents were removed by thorough rinsing with ice-cold physiological saline. Jejunal mucosa, removed by scraping with a microscope slide, was disrupted by vortexing in 25 ml of potassium phosphate buffer (0.05 M; pH 7.2) containing sucrose (0.1 M), KCl (0.05 M), and dithiothreitol (3 mM). The dispersed cells were collected by centrifugation (500 *g* for 10 min) and washed two more times with the same buffer (25 ml). The washed mucosal cells were resuspended in buffer (10 ml), treated with solid NaF (to give a final concentration of 150 mM), and then sonicated for 2.0 min at 4°C at 20 KHz (Model W375 sonicator; Heat Systems + Ultrasonics, Inc.). The resulting homogenate was centrifuged for 10 min at 700 *g* and the resulting supernatant fraction was recentrifuged at 8,500 *g* for 10 min. The resulting supernatant fluid was then centrifuged at 100,000 *g* for 60 min and the isolated microsomes were washed by resuspension in the same buffer and recentrifugation. The final microsomal pellet was resuspended in potassium phosphate buffer (0.1 M; pH 7.4; 1 ml). The ACAT activity of jejunal microsomes was assayed using minor modifications of the conditions described by Helgerud, Haugen, and Norum (31). The assay mixture consisted of microsomal protein (20 μg) in potassium phosphate buffer (100 mM; pH 7.4) containing BSA (6 mg per ml) and dithiothreitol (1 mg per ml). Compounds **I** and **II** were added as solutions in dimethylsulfoxide (5 μl). Control assays (no added sterol) contained the same volume of dimethylsulfoxide. All assays were done in replicate (n = 4). The assay tubes were preincubated for 6 min at 37°C in a shaking water bath. The reaction was initiated by the addition of [1-^{14}C]oleoyl CoA (11,000 dpm per nmol) to give a final concentration of 40 μM. The assay was terminated after 2 min of incubation at 37°C by the addition of 5 ml of $CHCl_3$–methanol 2:1. An internal standard of [^{3}H]cholesteryl oleate (10,000 dpm) was added, followed by unlabeled cholesteryl oleate (80 μg), triolein (40 μg), and the oleate ester of **I** (40 μg). An additional 10 ml of $CHCl_3$–methanol 2:1 and 2.5 ml of acidified water (pH 3) saturated with $CHCl_3$ were added, and the mixture was thoroughly vortexed. The $CHCl_3$ phase was evaporated to dryness at 40°C under a stream of nitrogen. The residue was dissolved in $CHCl_3$ (125 μl) and spotted on a silica gel G plate which was developed with SS-4. This system permitted the resolution of oleic acid (R_f 0.00–0.07), the oleate ester of **I** (R_f 0.34), triolein (0.43), and cholesteryl oleate (R_f 0.69). Material with the mobility of cholesteryl oleate was assayed for radioactivity.

Chemical syntheses

(25R)-Cholest-5-ene-3β,16β,26-triol (**IV**). To a refluxing mixture of diosgenin (**III**; 2.0 g; 4.8 mmol), zinc amalgam (60 g; freshly prepared from $HgCl_2$ (1 g) and mossy zinc (60 g, Aldrich) in water (150 ml)) in 95% ethanol (200 ml) was added concentrated hydrochloric acid (60 ml) over 2 hr, and heating was then continued for 15 min. The reaction mixture was decanted from the zinc, cooled to room temperature, diluted with water, and thoroughly extracted with $CHCl_3$. The combined extracts were washed with water, dried over Na_2SO_4, and evaporated to a white solid. This solid was subjected to MPLC (silica gel, elution with

ethyl acetate–hexane–$CHCl_3$ 4:3:3 followed by a 3:1:1 mixture of the same solvents. Evaporation of fractions 85–150 (20 ml fraction volumes) gave **IV** (0.90 g; 45% yield), which showed a single component on TLC in three solvent systems (SS-1, R_f 0.22; SS-5, R_f 0.29; SS-8, R_f 0.18). Crystallization from acetone afforded an analytical sample: MP 182.0–182.6°C (lit., 182–183°C (32), 176–178°C (33), 177–179°C (34), and 178–179°C (35)); IR ν_{max} 3380, 2980–2800, 1460, 1375, 1048, 1035, and 1015 cm^{-1}; high resolution MS on ion at *m/z* 418, 418.3439 (calc. for $C_{27}H_{46}O_3$: 418.3447).

(25R)-3β,26-Bis(tert-butyldimethylsilyloxy)cholest-5-en-16β-ol (**V**). To a solution of *tert*-butyldimethylsilyl chloride (1.51 g; 10.0 mmol) in dry dimethylformamide (30 ml) was added imidazole (2.73 g; 40.0 mmol), and the mixture was stirred for 15 min at room temperature under nitrogen. Compound **IV** (1.34 g; 3.21 mmol) was added, and the resulting mixture was heated for 90 min at 70°C. The reaction mixture was diluted with water (100 ml) and extracted with ethyl acetate, and the combined extracts were washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue was subjected to MPLC (silica gel, 3.0 cm × 100 cm column, elution with ethyl acetate–hexane 1:19, 20-ml fraction volumes). Evaporation of fractions 21–45 gave **V** (1.94 g; 93% yield), which showed a single component on TLC in three solvent systems (SS-2, R_f 0.62; SS-3, R_f 0.35; SS-4, R_f 0.44). An analytical sample was obtained by crystallization from methanol–CH_2Cl_2: MP 123–124°C; IR ν_{max} 3615, 2960, 2930, 2890, 2860, 1465, 1380, 1365, 1250, 1090, 1070, 1020, 985, 880, 865, 830, and 770 cm^{-1}; high resolution MS on ion at *m/z* 646, 646.5156 (calc. for $C_{39}H_{74}O_3Si_2$: 646.5177.

(25R)-3β,26-Bis(tert-butyldimethylsilyloxy)cholest-5-en-16-one (**VI**). Pyridinium chlorochromate (0.4 g; 1.86 mmol; 1.2 equiv.) was added to a solution of **V** (1.00 g; 1.55 mmol) in dry CH_2Cl_2 (5 ml). After stirring at room temperature for 18 hr under nitrogen, the solution was diluted with ether (50 ml), and the resulting mixture was filtered through a short pad of Florisil, using ether to wash the insoluble residue and the Florisil pad. After evaporation of the solvent, the crude product was subjected to MPLC (silica gel, 2.0 cm × 100 cm column, elution with ethyl acetate–hexane 8:92, 20-ml fraction volumes). Evaporation of fractions 17–22 gave **VI** (881 mg; 88% yield), which showed a single component on TLC in three solvent systems (SS-2, R_f 0.85; SS-3, R_f 0.56; SS-4, R_f 0.68). Crystallization from methanol–CH_2Cl_2 gave an analytical sample: MP 81.5–82.5°C; IR ν_{max} 2955, 2930, 2890, 2860, 1735 (C=O), 1460, 1380, 1360, 1250, 1095, 1000, 880, 860, 797, and 767 cm^{-1}; high resolution MS on ion at *m/z* 644, 644.4993 (calc. for $C_{39}H_{72}O_3Si_2$: 644.5020).

(25R)-3β,26-Dihydroxycholest-5-en-16-one (**VII**). To a solution of **VI** (644 mg; 1.0 mmol) in acetone (20 ml) was added *p*-toluenesulfonic acid monohydrate (0.5 g). After stirring at room temperature for 24 hr, water (100 ml) and ethyl acetate (50 ml) were added, and the resulting mixture was thoroughly extracted with ethyl acetate. The combined extracts were washed with saturated $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated to dryness. The crude product was subjected to MPLC (silica gel, 2.0 cm × 100 cm column, elution with ethyl acetate–hexane 1:1, 8-ml fraction volumes). Evaporation of fractions 43–65 gave **VII** (390 mg; 94% yield), which showed a single component on TLC in three solvent systems (SS-1, R_f 0.27; SS-5, R_f 0.37; SS-8, R_f 0.27). Crystallization from methanol gave an analytical sample: MP 169–170°C (lit., 170°C (32), 167–169°C (33), and 155–160°C (34)); IR ν_{max} 3360, 2960–2820, 1730 (C=O), 1465, 1445, 1380, and 1050 cm^{-1}; high resolution MS on ion at *m/z* 416, 416.3321 (calc. for $C_{27}H_{44}O_3$: 416.3290).

(25R)-Cholest-5-ene-3β,26-diol (**VIII**). A mixture of **VII** (250 mg; 0.60 mmol), hydrazine hydrochloride (465 mg), and 85% hydrazine hydrate (2.4 g) in diethylene glycol (15 ml) was heated at 135°C for 90 min. After the addition of KOH (1.185 g), the resulting mixture was heated at 220°C for 3.5 hr with removal of water by distillation. After cooling to room temperature, the mixture was diluted with water and extracted with $CHCl_3$. The extracts were washed with water, dried over Na_2SO_4, and evaporated to dryness. The crude product was subjected to MPLC (silica gel, 2.0 cm × 100 cm column, elution with ethyl acetate–hexane 1:1, 7-ml fraction volumes). Evaporation of fractions 23–70 gave **VIII** (201 mg; 83% yield), which showed a single component on TLC in three solvent systems (SS-1, R_f 0.52; SS-5, R_f 0.59; SS-8, R_f 0.51). Crystallization from methanol gave an analytical sample: MP 177–178°C (lit., 176°C (32), 176–178°C (36), 178–179°C (37), 175–177°C (33), 172–173°C (38), and 177–178°C (34)); IR ν_{max} 3330, 2960, 2920, 2900, 2870, 1460, 1370, 1350, 1052, and 1020 cm^{-1}; high resolution MS on ion at *m/z* 402, 402.3503 (calc. for $C_{27}H_{46}O_2$: 402.3498). None of the 25*S*-epimer (32) was detected by ^{13}C NMR (detection limit, 10%).

(25R)-Cholest-5-ene-3β,26-diol 3β,26-dibenzoate (**IX**). To **VIII** (201 mg; 0.50 mmol) in dry pyridine (10 ml) was added benzoyl chloride (0.5 ml). After stirring for 2 hr at room temperature, methanol was added, and the resulting mixture was extracted with ethyl acetate. The extract was washed with 10% HCl, water, saturated $NaHCO_3$, and water, and then dried over Na_2SO_4 and evaporated to dryness. The crude product was subjected to MPLC (silica gel, 2.0 cm × 100 cm column, elution with ethyl acetate–hexane 1:19, 8-ml fraction volumes). Evaporation of fractions 25–41 gave **IX** (290 mg; 95% yield), which showed a single component on TLC in three solvent systems (SS-2, R_f 0.59; SS-3, R_f 0.38; SS-4, R_f 0.77). An analytical sample was obtained by crystallization from methanol–CH_2Cl_2: MP 136.5–137.5°C; IR ν_{max} 2970- 2840, 1715 (C=0), 1460, 1445, 1310, 1266, 1245, 1105, 1060, 1020, and 702 cm^{-1}; high resolution MS on ion at *m/z* 488, 488.3685 (calc.

for $C_{34}H_{48}O_2$: 488.3654).

(25R)-Cholesta-4,6-diene-3β,26-diol 3β,26-dibenzoate (**X**) *and (25R)-cholesta-5,7-diene-3β,26-diol 3β,26-dibenzoate* (**XI**). To a solution of **IX** (183 mg; 0.30 mmol) in a 1:4 mixture of benzene and hexane (15 ml) was added 1,3-dibromo-5,5-dimethylhydantoin (51 mg; 0.18 mmol). After heating under reflux for 2 min, the reaction mixture was cooled to room temperature, filtered, and evaporated to dryness. The residue was dissolved in *o*-xylene (15 ml) and triethyl phosphite (0.8 ml), and the resulting mixture was heated at 160°C for 3 hr. After evaporation of the solvent under high vacuum, the resulting yellow residue was subjected to MPLC (alumina-$AgNO_3$ column, elution with hexane–benzene 3:7, 8-ml fraction volumes).

Evaporation of fractions 18–24 gave the $\Delta^{4,6}$ isomer **X** (63 mg; 35% yield), which showed a single component on TLC in three solvent systems (SS-2, R_f 0.55; SS-3, R_f 0.35; and SS-4, R_f 0.74). Crystallization from acetone-CH_2Cl_2 gave an analytical sample: MP 148.5–149.5°C; IR ν_{max} 3035, 2980–2840, 1715 (C=O), 1598, 1580, 1465, 1448, 1310, 1265, 1170, 1105, 1060, 1018, 965, 950, 920, and 702 cm^{-1}; UV λ_{max} 231 nm (ε 38,900); high resolution MS on ion at *m/z* 486; 486.3480 (calc. for $C_{34}H_{46}O_2$: 486.3498).

Evaporation of fractions 28–53 gave the $\Delta^{5,7}$ isomer **XI** (80 mg; 44% yield) which showed a single component on TLC in three solvent systems (SS-2, R_f 0.53; SS-3, R_f 0.34; SS-4, R_f 0.74). Crystallization from acetone-CH_2Cl_2 gave an analytical sample: MP 130.0–130.5°C; IR ν_{max} 2960, 2940, 2870, 2850, 1715 (C=O), 1600, 1465, 1450, 1312, 1273, 1253, 1170, 1110, 1020, and 704 cm^{-1}; UV λ_{max} 229 (ε 27,600), 272 (14,400), 281 (14,400), and 293 nm (7,600); high resolution MS on ion at *m/z* 486, 486.3513 (calc. for $C_{34}H_{46}O_2$: 486.3498).

(25R)-5α-Cholest-8(14)-ene-3β,26-diol 3β,26-bis(cyclohexanecarboxylate) (**XII**). Compound **XI** (100 mg; 0.165 mmol) was dissolved in a 19:1 mixture of ethyl acetate and acetic acid (10 ml) and hydrogenated at one atmosphere at room temperature in the presence of Adams catalyst (PtO_2, 55 mg). After removal of insoluble material by filtration through a pad of Celite, the solvent was evaporated to give a solid (102 mg) which, upon recrystallization from methanol-CH_2Cl_2, gave **XII** (99 mg; 97% yield): single component on TLC in three solvent systems (SS-2, R_f 0.68; SS-3, R_f 0.43; SS-4, R_f 0.66); MP 113.5–114.5°C; IR ν_{max} 2930, 2855, 1728 (C=O), 1445, 1370, 1305, 1240, 1185, 1165, 1125, and 1030 cm^{-1}; high resolution MS on ion at *m/z* 622, 622.5007 (calc. for $C_{41}H_{66}O_4$: 622.4961).

(25R)-3β,26-Dihydroxy-5α-cholest-8(14)-en-15-one 3β,26-bis(cyclohexanecarboxylate) (**XIII**), *(25R)-3β,26-dihydroxy-8α,14α-epoxy-5α-cholestan-15-one 3β,26-bis(cyclohexanecarboxylate)* (**XIVa**), *and (25R)-3β,26-dihydroxy-8α,14α-epoxy-5α-cholestan-7-one 3β,26-bis(cyclohexanecarboxylate)* (**XVa**). 3,5-Dimethylpyrazole (197 mg; 2.0 mmol) was added to a suspension of chromium trioxide (200 mg; 2.0 mmol; dried over P_2O_5 in vacuo) in dry CH_2Cl_2 (5 ml) at – 20°C under nitrogen, and the mixture was stirred at – 20°C for 30 min. To the resulting dark red solution was added **XII** (62.2 mg) in one portion, and the resulting mixture was stirred at – 20°C for 1 hr. A 5 N NaOH solution (5 ml) was added, and the reaction mixture was stirred for 30 min at 0°C. The resulting mixture was extracted twice with CH_2Cl_2 (20-ml portions), and the extracts were washed with 10% HCl and water, dried over Na_2SO_4, and evaporated to dryness. The yellow residue (52 mg) was subjected to MPLC (silica gel, 1.0 cm × 50 cm column, elution with ethyl acetate–hexane 8:92, 7-ml fraction volumes).

Evaporation of fractions 8–11 gave ketodiester **XIII** (20 mg; 30% yield) which showed a single component on TLC in three solvent systems (SS-2, R_f 0.53; SS-6, R_f 0.39; SS-7, R_f 0.54). Crystallization from methanol gave an analytical sample: MP 124.5–125.5°C; IR ν_{max} 2930, 2850, 1728 (ester C=O), 1698 (conjugated C=O), 1618, 1445, 1305, 1242, 1190, 1170, 1128, and 1030 cm^{-1}; UV λ_{max} 259 nm (ε 14,700); high resolution MS on ion at *m/z* 636, 636.4739 (calc. for $C_{41}H_{64}O_5$: 636.4753).

Evaporation of fractions 13–20, gave 15-keto-epoxide **XIVa** (5 mg; 8%), which showed a single component on TLC in three solvent systems (SS-2, R_f 0.46; SS-6, R_f 0.25; SS-7, R_f 0.46). Crystallization from methanol-CH_2Cl_2 gave an analytical sample: MP 165.5–166.5°C; IR ν_{max} 2935, 2860, 1732 (C=O), 1728 (ester C=O), 1445, 1240, 1165, 1125, and 1030 cm^{-1}; high resolution MS on ion at *m/z* 652, 652.4720 (calc. for $C_{41}H_{64}O_6$: 652.4703).

Evaporation of fractions 23–40 gave 7-keto-epoxide **XVa** (11 mg; 17%): single component on TLC in three solvent systems (SS-2, R_f 0.37; SS-6, R_f 0.09; SS-7, R_f 0.28); MP 133.0–133.5°C; IR ν_{max} 2930, 2855, 1728, (C=O), 1462, 1445, 1308, 1242, 1168, 1129, and 1032 cm^{-1}; high resolution MS on ion at *m/z* 652, 652.4719 (calc. for $C_{41}H_{64}O_6$: 652.4703).

(25R)-3β,26-Dihydroxy-5α-cholest-8(14)-en-7-one 3β,26-bis(cyclohexanecarboxylate) (**XVIa**). To **XVa** (7 mg) in acetic acid (3 ml) was added zinc dust (10 mg). The reaction mixture was heated under reflux for 30 min, diluted with ethyl acetate, and filtered. The filtrate was diluted with water, extracted with ethyl acetate, and the extracts were washed with saturated $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated to dryness. The crude product was subjected to MPLC (silica gel, 1.0 cm × 50 cm column, elution with ethyl acetate–hexane 8:92, 8-ml fraction volumes). Evaporation of fractions 8–12 gave **XVIa** (5.8 mg; 85% yield) which showed a single component on TLC in three solvent systems (SS-2, R_f 0.52; SS-6, R_f 0.34; SS-7, R_f 0.51). Crystallization from methanol-CH_2Cl_2 provided an analytical sample: MP 130–131°C; IR ν_{max} 2930, 2850, 1728 (ester C=O), 1670 (conjugated C=O), 1595, 1465, 1305, 1260, 1190, 1130, and 1030 cm^{-1}; UV λ_{max} 262 nm

(ε 11,000); high resolution MS on ion at *m/z* 636, 636.4747 (calc. for $C_{41}H_{64}O_5$: 636.4753).

(25R)-3β,26-Dihydroxy-5α-cholest-8(14)-en-15-one (**II**). Compound **XIII** (7 mg) in a 19:1 mixture of methanol and water (5 ml) was cooled to 5°C. Concentrated H_2SO_4 (5 drops) was added, and the resulting mixture was heated at 85°C for 6 hr under nitrogen. Water and ethyl acetate were added, and the resulting mixture was extracted with ethyl acetate. The combined extracts were washed with saturated $NaHCO_3$ and water, dried over Na_2SO_4, and evaporated to dryness. The crude product was subjected to MPLC (silica gel, 1.0 cm × 50 cm column, elution with ethyl acetate–hexane 1:1, 8-ml fraction volumes). Evaporation of fractions 18–27 gave **II** (4.1 mg; 90% yield), which showed a single component on TLC in three solvent systems (SS-1, R_f 0.21; SS-5, R_f 0.25; SS-8, R_f 0.22). Crystallization from methanol afforded an analytical sample: MP 197–198°C; IR ν_{max} 3430, 3390, 2970, 2935, 2860, 1687 (conjugated C=O), 1613, 1583, 1465, 1377, 1325, 1228, 1215, 1120, 1085, and 1040 cm^{-1}; UV λ_{max} 259 nm (ε 14,300); high resolution MS on ion at *m/z* 416, 416.3274 (calc. for $C_{27}H_{44}O_3$: 416.3291).

X-ray crystal structure determination of II

A crystal of **II** of dimensions 0.2 × 0.2 × 0.1 mm was obtained by recrystallization from methanol. Diffraction data were collected on a Rigaku AFC5R diffractometer using graphite monochromated CuKα radiation. Systematic extinctions indicated a $P2_12_12_1$ unit cell with edges of 11.428 (2) Å, 20.209 (2) Å, and 10.458 (2) Å. A 2 θ/ω scan was used to collect 1721 reflections (maximum 2θ of 119.8° (0.89 Å)). Three check reflections were monitored every 100 reflections. Less than 2% decay was observed in the intensities of the monitor reflections during the course of data collection. After elimination of replicate reflections, 1661 independent observable reflections remained and were used for refinement.

The direct methods option of the TEXSAN software package (Molecular Structure Corp., College Station, TX) yielded a partially interpretable E-map. Successive difference Fourier maps were used to locate the remaining nonhydrogen atoms. Atom positions with isotropic temperature factors were refined by least squares techniques. A second cycle of least squares refinements using anisotropic temperature factors, followed by a final cycle of refinements using hydrogens placed at idealized positions gave the final structure. The final unweighted R value was 0.065 (weighted R 0.087). The largest electron density observed in the final difference Fourier map was 0.22 electrons / $Å^3$.

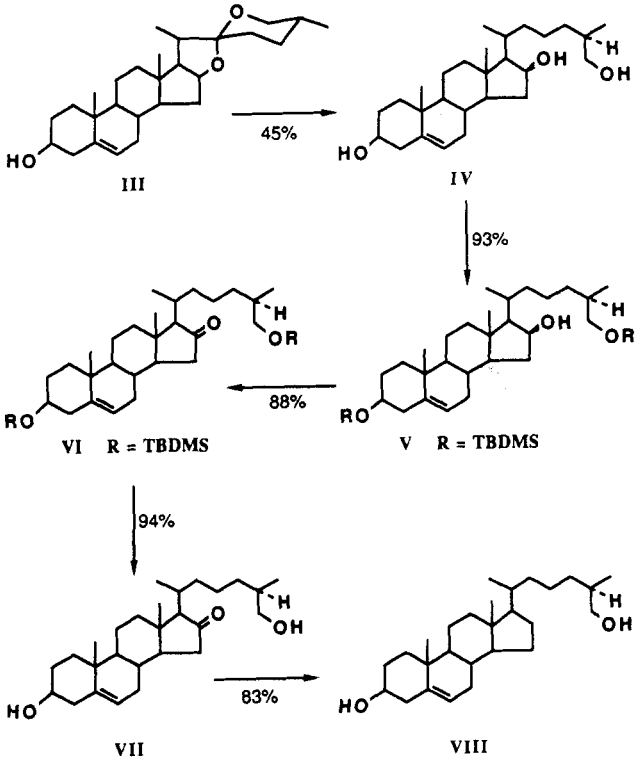


Fig. 2. Synthesis of (25R)-26-hydroxycholesterol from diosgenin.

RESULTS AND DISCUSSION

For the synthesis of (25*R*)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**) we chose diosgenin (**III**) as the starting material because it is commercially available in high purity as the 25*R* isomer. Our synthetic strategy for the synthesis of **II** involved conversion of diosgenin to 26-hydroxycholesterol (**VIII**) (**Fig. 2**), transformation of the Δ^5 double bond of **VIII** via a $\Delta^{5,7}$ intermediate to the $\Delta^{8(14)}$ position, followed by an allylic oxidation at C(15) while maintaining the 25*R* configuration during each step. The structure and stereochemistry of each synthetic intermediate were confirmed by ^1H and ^{13}C NMR (**Table 1** and **Table 2**) and MS (**Table 3**).

Clemmensen reduction (35) of diosgenin gave (25*R*)-cholest-5-ene-3β,16β,26-triol (**IV**) in 40–45% yield. In order to remove the 16β-hydroxy group, we needed to selectively protect the 3β- and 26-hydroxy groups. We were unable to prepare the 3β,26-diacetate of **IV** (35% yield) or the 3β,26-dibenzoate of **IV** (74% yield) without forming significant amounts of the triester. Previously reported preparations of the benzoate (68% yield (40), 34% yield (41)) and *p*-nitrobenzoate esters (37% yield (32)) of the 3β,16β,26-triol were evidently accompanied by similar problems. Consequently, we turned to the bulky *tert*-butyldimethylsilyl (TBDMS) group (42). We succeeded in preparing the easily chromatographed 3β,26-bis(TBDMS) ether **V** in 93% yield. In practice, we avoided the tedious isolation of **IV** by recrystallizing the crude product of the Clemmensen reduction, silylating this ~90% pure

TABLE 1. ^{1}H NMR chemical shifts[a-d]

Sterol	3α-H	4α,4β-H or 4-H	6α,6β-H or 6-H	7β-H or 7-H	16α,16β-H	18-H_3	19-H_3	21-H_3	26-H_2	27-H_3
IV	3.51	2.29, 2.24	5.35		4.35	0.889	1.019	0.983	3.45, 3.48	0.910
V	3.48	2.26, 2.17	5.31		4.35	0.882	1.007	0.981	3.36, 3.43	0.863
VI	3.47	2.27, 2.18	5.32			0.832	1.030	0.970	3.35, 3.44	0.862
VII	3.53		5.35			0.835	1.040	0.972	3.43, 3.50	0.914
VIII	3.52	2.29, 2.24	5.35			0.678	1.008	0.914	3.42, 3.50	0.914
IX	4.86	2.50, 2.44	5.42			0.686	1.070	0.930	4.12, 4.21	1.022
X	5.62	5.42	5.93	5.67		0.729	1.057	0.926	4.12, 4.21	1.021
XI	4.97	2.65, 2.52	5.61	5.41		0.628	1.002	0.956	4.12, 4.21	1.026
XII	4.71					0.837	0.703	0.928	3.84, 3.94	0.914
XIII	4.72			4.12	2.34, 2.05	0.970	0.730	0.998	3.84, 3.93	0.914
XIVa	4.70				2.45, 1.98	0.885	0.954	1.010	3.84, 3.94	0.925
XVa[e]	4.71		2.37, 2.28			0.954	1.114	0.911	3.84, 3.93	0.911
XVIa	4.71					0.905	0.838	0.948	3.85, 3.94	0.916
II	3.65			4.13	2.35, 2.06	0.972	0.715	1.001	3.42, 3.49	0.912

[a]300.1 MHz spectra. Chemical shifts (δ) in ppm relative to $(CH_3)_4Si$ in $CDCl_3$ solution.

[b]Multiplicities: 3α-H: m. 4-H: **IV-IX**, AB portion of ABX system (denoting 4β-H as A), J_{AB} = 13 Hz, J_{AX} = 12 Hz, J_{BX} = 5 Hz; **X**, s; **XI**: AB quartet, outer peaks not observed. 6-H: **IV-IX**: d, J = 5.1 ± 0.1 Hz; **X**: dd, J = 9.9, 2.5 Hz; **XI**: dd, J = 5.7, 2.3 Hz; **XVIa**: AB portion of ABX system (denoting 6β-H as A): J_{AB} = 16.8 Hz, J_{AX} = 13.1 Hz, J_{BX} = 4.5 Hz. 7-H: **X**: d, J = 10.8 Hz; **XI**: dd, J = 5.6, 2.8 Hz; **XIII, II**, ddd, J = 14.0, 3.7, 1.9 Hz. 16α-H: **IV**: ddd, J = 8.0, 7.2, 4.5 Hz; **V**: m; **XIII, XIVa, II**: AB portion of ABX system: J_{AB} = 19.0 ± 0.5 Hz, J_{AX} = 12.1 ± 0.1 Hz (16β-H-17α-H), J_{BX} = 8.1 ± 0.3 Hz (16α-H-17α-H). 18-H_3: s. 19-H_3: s. 21-H_3: d, J = 6.5 ± 0.2 Hz. 26-H_2: AB portion of ABX system (denoting the upfield proton of the AB pair as A): J_{AB} = 10.5 ± 0.5 Hz, J_{AX} = 6.4 ± 0.3 Hz, J_{BX} = 5.9 ± 0.3 Hz. 27-H_3: d, J = 6.7 ± 0.1 Hz.

[c]TBDMS ethers **IV** and **V** gave additional singlets at δ 0.035 (CH_3Si), 0.059 (CH_3Si), and 0.891 $(CH_3)_3C$).

[d]Benzoate esters **IX, X,** and **XI** also showed multiplets at δ 7.44 (meta-H), 7.54 (para-H), and 8.05 (ortho-H).

[e]Also for **XVIa,** δ 2.52 (15-H), dddd, J = 20.5, 10.3, 3.7, 1.9 Hz; 3.09 (15-H), dddd, J = 20.4, 9.0, 9.0, 2.8 Hz.

TABLE 2. ^{13}C NMR assignments[a,b]

Atom	IV	V[c]	VI[c]	VII	VIII	IX[d]	X[d]	XI[d]	XII[e]	XIII[e]	XIVa[e]	XVa[e]	XVIa[e]	II
1	37.15	37.30	37.06	36.91	37.23	36.99	33.31	37.92	36.26	36.30	36.64	36.07	35.62*	36.51
2	31.59	32.03	31.96	31.47	31.64	27.84	24.73	28.19	27.57	27.24	26.84	26.65	27.42	31.14
3	71.75	72.59	72.46	71.59	71.78	74.53	71.46	73.35	73.05	72.59	72.61	71.74	72.12	70.91
4	42.24	42.76	42.72	42.14	42.27	38.17	121.66	36.71	34.13	33.70	33.48	33.41	33.70	37.75
5	140.85	141.66	141.72	140.96	140.75	139.61	146.56	138.49	44.07	43.95	43.89	40.44	41.03	44.10
6	121.45	120.92	120.45	120.94	121.68	122.73	127.73	120.28	28.76	29.06	26.46	44.65	44.32	29.13
7	31.78	31.82	31.81	31.74	31.90	31.88	132.11	116.32	29.54	27.52	28.14	204.78	200.89	27.58
8	31.42	31.47	30.74	30.71	31.88	31.83	37.32	141.53	126.25	150.42	70.74*	66.81	126.07	150.74
9	50.05	50.16	49.92	49.79	50.10	49.97	51.18	46.00	49.19	50.80*	49.64	46.43	49.50	50.84
10	36.49	36.58	36.67	36.56	36.48	36.61	35.05	37.10	36.77	38.72	37.40**	36.58	35.04	38.73
11	20.67	20.67	20.50	20.51	21.06	21.01	20.63	21.01	19.90	19.54	16.16	18.49	19.74	19.56
12	39.82	39.86	38.93	38.87	39.76	39.68	39.73	39.10	37.24	36.95	38.56	30.69	35.62	36.95
13	42.18	42.18	42.97	42.94	42.31	42.28	43.38	42.88	42.71	42.57	38.50**	42.52	45.60	42.56
14	54.45	54.52	50.93	50.84	56.74	56.62	53.91	54.42	142.65	140.33	69.83*	81.40	166.86	140.23
15	36.60	36.48	38.91	38.87	24.27	24.24	23.74	22.98	25.78	207.89	214.27	25.75	29.90	208.07
16	72.44	72.48	218.74	218.98	28.24	28.23	28.21	28.08	27.04	42.43	40.26	23.76	27.29	42.46
17	61.46	61.37	67.93	67.92	56.11	56.01	55.97	55.78	56.82	50.75*	50.62	52.24	55.15	50.80
18	13.02	13.00	13.66	13.55	11.85	11.82	11.94	11.80	18.21	18.79	15.32	14.90	19.47	18.78
19	19.37	19.41	19.41	19.37	19.38	19.35	17.85	16.21	12.71	12.85	12.46	14.02	11.62	12.92
20	29.63	29.75	31.25	31.11	35.70	35.68	35.67	36.03	34.34	34.47	34.95	35.00	34.01	34.45
21	18.15	18.18	18.66	18.54	18.66	18.66	18.58	18.79	19.04	19.18	18.91	18.64	19.01	19.17
22	35.93	36.28	35.91	35.79	36.13	36.00	35.97	35.95	35.85	35.74	35.16	35.42	35.73*	35.81
23	23.70	23.74	24.61	24.41	23.39	23.26	23.30	23.32	23.13	22.99	23.06	23.04	23.19	23.18
24	33.30	33.59	33.31	33.06	33.52	33.88	33.85	33.88	33.86	33.70	33.61	33.63	33.70	33.43
25	35.60	35.78	35.75	35.65	35.78	32.68	32.66	32.69	32.61	32.58	32.58	32.51	32.56	35.74
26	68.43	68.49	68.49	68.40	68.52	69.98	69.94	69.94	69.11	68.96	68.88	69.03	69.05	68.37
27	16.63	16.68	16.69	16.53	16.49	16.96	16.94	16.96	16.85	16.83	16.84	16.73	16.80	16.47

[a]75.5 MHz; chemical shifts (δ) in ppm referenced to $CDCl_3$ at 77.0 ppm in $CDCl_3$ solution (0.01-0.1 M).

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

[c]Chemical shifts for TBDMS groups of **V** and **VI**: δ 25.96 and 25.91 ($(\underline{C}H_3)_3C$), 18.35 and 18.24 ($(CH_3)_3\underline{C}$), −4.60 and −5.35 (CH_3Si, diastereotopic pair).

[d]Chemical shifts for benzoate groups of **IX-XI**: δ 166.6, 166.0 (C=O); 132.8, 132.7 (para); 130.7, 130.5 (quaternary); 129.6, 129.5 (ortho); 128.3, 128.2 (meta).

[e]Chemical shifts for cyclohexanecarboxylate groups of **XII-XVIa**: 176.2, 175.7 (C=O); 43.4, 43.3 (C(1)); 29.05, 29.00 (C(2)); 25.45, 25.40 (C(3)); 25.8 (C(4)).

TABLE 3. Mass spectral data[a]

Sterol R	IV H	V $(Me_3C)Me_2Si$	VI $(Me_3C)Me_2Si$	VII H	VIII H	IX C_6H_5CO
M	418 (4)	646 (4)	644 (0.2)	416 (5)	402 (61)	
M-Me		631 (3)	629 (2)	401 (31)	387 (26)	
M-C_4H_9		589 (73)	587 (100)			
M-ROH	400 (34)	514 (1)			384 (63)	488 (48)
M-ROH-Me	385 (36)	499 (17)	497 (17)	383 (27)	369 (40)	
M-ROH-ROH	382 (38)			367 (6)		366 (38)
M-SC	289 (44)	403 (1)	401 (1)	287 (4)	273 (28)	
M-SC-ROH	271 (100)	271 (4)	269 (4)		255 (44)	255 (12)
M-SC-ROH-H_2O	253 (47)	253 (19)	253 (12)			
ABC[b]	213 (72)	213 (38)	213 (54)	213 (18)	213 (79)	213 (13)
Other ions	367 (36)[c]	571 (25)[d]	455 (39)[g]	371 (6)	291 (54)[i]	351 (6)
	315 (22)	457 (92)[e]	363 (9)	315 (14)[h]	282 (24)	253 (16)
	300 (14)	439 (17)[f]	345 (9)	273 (100)[h]	231 (27)[i]	158 (36)
	159 (84)	365 (23)	75 (58)	255 (4)	107 (100)	105 (100)
	69 (83)	255 (25)				
		75 (100)				

Sterol R	X C_6H_5CO	XI C_6H_5CO	XII $C_6H_{11}CO$	XIII $C_6H_{11}CO$	XIVa $C_6H_{11}CO$	XVa $C_6H_{11}CO$	XVIa $C_6H_{11}CO$	II H
M	608 (5)	608 (0.5)	622 (5)	636 (65)	652 (11)	652 (5)	636 (21)	416 (58)
M-Me					637 (11)		621 (2)	401 (16)
M-H_2O				618 (3)		634 (3)		398 (13)
M-ROH	486 (23)	486 (53)	494 (100)	508 (24)	524 (10)		508 (10)	(401)
M-ROH-Me	471 (7)	471 (17)	479 (34)	493 (12)	509 (15)		493 (2)	383 (26)
M-ROH-H_2O				490 (7)				
M-ROH-ROH-Me	349 (3)	349 (6)	351 (16)	365 (10)				365 (10)
M-SC		375 (1)	383 (6)	397 (11)	413 (15)	413 (9)	397 (20)	287 (19)
M-SC-H_2O				379 (28)	395 (8)	395 (29)		269 (94)
M-SC-ROH	253 (11)	253 (32)	255 (48)	269 (16)	285 (9)	285 (5)	269 (7)	(269)
M-SC-ROH-H_2O				251 (41)	267 (6)	267 (8)	251 (5)	251 (30)
R	105 (100)	105 (100)	111 (28)	111 (27)	111 (33)	111 (33)	111 (26)	
C_6H_5			83 (94)	83 (100)	83 (100)	83 (100)	83 (100)	
Other ions	381 (7)	364 (10)[k]	213 (54)	490 (7)[m]	358 (17)	542 (3)	526 (6)	297 (6)
	367 (41)	251 (23)		526 (13)	335 (8)	385 (6)	380 (4)[k]	292 (5)[n]
	251 (5)	211 (14)		383 (20)	231 (11)	346 (14)	344 (26)	277 (10)[n]
	211 (7)	158 (35)		365 (10)	239 (5)	333 (37)		259 (11)
				344 (4)		320 (8)		55 (100)

[a]Suggested assignments of major ions at 70 eV (**II, IX, XII–XVIa**) or 20 eV: *m/z* (relative intensity, %).
[b]Fragment d_5 of reference 39, p. 97.
[c]M-ROH-ROH-Me.
[d]M-C_4H_9-H_2O.
[e]M-ROH-C_4H_9-Me.
[f]M-ROH-C_4H_9-Me-H_2O.
[g]M-ROH-C_4H_9.
[h]Fragments analogous to structures a_{10} and d_{10} of reference 39, p. 22.
[i]Fragments analogous to structures b_5 and h_5 of reference 39, p. 97.
[k]M-ROH-ROH.
[m]M-ROH-H_2O.
[n]Analogous to CD fragments of reference 20.

material, and chromatographing silyl ether **V** to 99% purity. Oxidation of **V** with pyridinium chlorochromate (43) in CH_2Cl_2 gave 16-keto sterol **VI**, which upon hydrolysis in acetone/*p*-toluenesulfonic acid afforded 3β,26-dihydroxycholest-5-en-16-one (**VII**). Wolff-Kishner reduction (Huang-Minlon modification) (32, 34) of **VII** gave 26-hydroxycholesterol (**VIII**) in 83% yield. By the above sequence the 25*R* isomer of 26-hydroxycholesterol was obtained from diosgenin in 29% overall yield, an improvement over previous yields (7–20%) (32, 33, 40, 41)) of **VIII** from diosgenin or diosgenin acetate.

For the conversion of the B ring functionality of (25*R*)-26-hydroxycholesterol from Δ^5 to $\Delta^{5,7}$, the TBDMS ether protecting group was replaced with the benzoate group because the latter group gave better yields in the allylic bromination–dehydrobromination (44, 45) reactions. Benzoylation of **VIII** gave cholest-5-ene-3β,26-diol dibenzoate (**IX**) in 95% yield. Allylic bromination of **IX** with 1,3-dibromo-5,5-dimethylhydantoin, followed by dehydrobromination with triethyl phosphite yielded a 55:45 mixture of the $\Delta^{5,7}$ and $\Delta^{4,6}$ sterols **XI** and **X**, which were readily separated by alumina-$AgNO_3$ MPLC (22) using

a benzene–hexane eluent. The two dienes were easily identified and differentiated from their UV and ^1H and ^{13}C NMR spectra and by comparison with spectra of cholestadienol benzoate standards (23, 46).

Because the ratio of the desired $\Delta^{5,7}$ steryl product to the $\Delta^{4,6}$ byproduct was low and also somewhat capricious, the allylic bromination–dehydrobromination reaction sequence was tested with other Δ^5 substrates. The best results were obtained with the benzoate of **VII**: a 5:3 ratio of $\Delta^{5,7}$:$\Delta^{4,6}$ dienes was observed, and the $\Delta^{5,7}$ sterol was isolated in 61% yield. However, this route was abandoned because at this stage we could not remove the 16-keto group in good yield. The silyl ether of **VIII** gave ~1:1 ratio of the silyl ethers of the $\Delta^{5,7}$ and $\Delta^{4,6}$ sterols, but the isolated yields were only 33% each, possibly due to partial hydrolysis of the silyl ether groups. The diacetate of **VIII** was also employed as a protecting group and gave yields of the $\Delta^{5,7}$ sterol which were comparable to those observed for the benzoate. However, separation of the diene diacetates by alumina–$AgNO_3$ MPLC required an eluting solvent of high polarity such as ethyl acetate, which gradually deactivated the stationary phase.

Hydrogenation–isomerization of diene **XI** with PtO_2 in a mixture of acetic acid–ethyl acetate, according to the procedure reported for converting 7-dehydrocholesteryl benzoate to the $\Delta^{8(14)}$ benzoate (47, 48), led to the $\Delta^{8(14)}$ sterol **XII**. The spectral data indicated that the aromatic ring had been hydrogenated to a cyclohexane ring, thus providing an explanation for the unexpectedly large quantity of PtO_2 required in this reaction. Reinspection of the IR spectrum in one of the original reports (48) indicated that the aromatic ring was hydrogenated in that case also. The 5α-configuration of **XII** was proved by the similarity of its ^{13}C NMR spectrum with that of 3β-benzoyloxy-5α-cholest-8(14)-ene (49).

Allylic oxidation (50, 51) of $\Delta^{8(14)}$-sterol **XII** with a 20-fold excess of chromium trioxide/3,5-dimethylpyrazole complex in CH_2Cl_2 at −20°C gave, after chromatography, $\Delta^{8(14)}$-15-one **XIII** in 30% yield, along with two side products; 8α,14α-epoxy-15-one **XIVa** (8% yield) and 8α,14α-epoxy-7-one **XVa** (17% yield). Diester **XIII** showed IR (1698 cm^{-1}) and UV (λ_{max} 259 nm (ε 14,700)) absorptions characteristic of the $\Delta^{8(14)}$-15-one functionality. Hydrolysis of diester **XIII** in methanol–sulfuric acid afforded the desired (25*R*)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**), the structure of which was confirmed by its IR, UV, MS, ^1H and ^{13}C NMR spectra. Thus, **II** was synthesized from diosgenin in ten steps in 3% yield (**Fig. 3**).

The structures of epoxide byproducts **XIVa** and **XVa** were determined by a combination of spectral and chemical methods (**Fig. 4**). Epoxide **XVa** was reduced in high yield with zinc dust in acetic acid (52) to $\Delta^{8(14)}$-7-one **XVIa** which was identified by the similarity of its ^1H and ^{13}C NMR spectra with those of **XVIb**[2]. Further support for

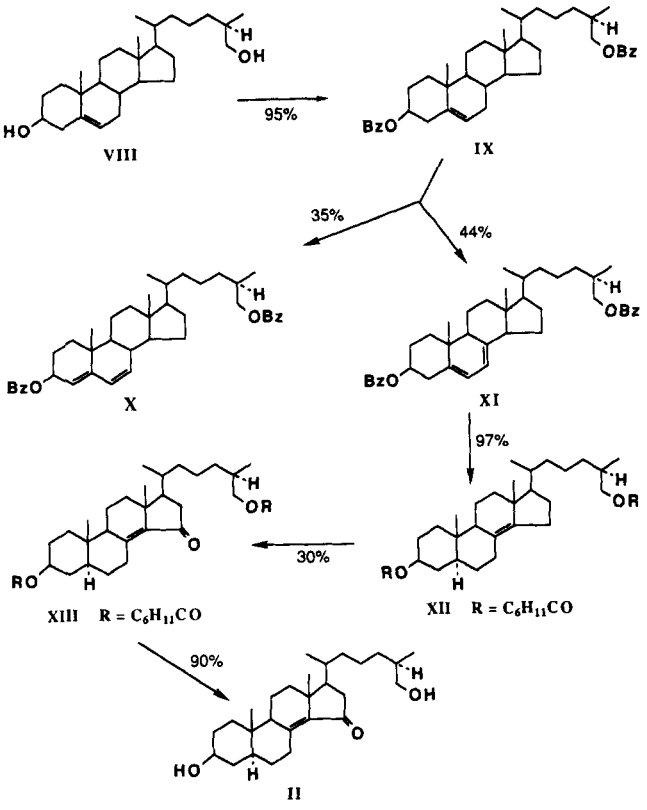


Fig. 3. Synthesis of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one from (25R)-26-hydroxycholesterol.

the structure **XVIa** was derived from IR (carbonyl absorption bands at 1670 cm^{-1} and 1728 cm^{-1}), UV (λ_{max} 262 nm, ε 11,000, characteristic of a $\Delta^{8(14)}$-7-one chromophore (50)), and high resolution MS ($C_{41}H_{64}O_5$) data and from the similarity of the ^{13}C NMR spectra of **XVa** and **XVb**. Likewise, epoxide **XIVa** was identified by the similarity of the ^{13}C NMR spectra of **XIVa** and **XIVb** and by the reduction of **XIVa** to a conjugated ketone whose spectral data and TLC mobility were identical to those of **XIII**. Epoxides **XVa** and **XIVa** were assigned the 8α,14α stereochemistry based on literature precedent (50, 52) and on comparison of the ^{13}C NMR spectra with those of olefins **XVIa** and **XIII** (49, 53).

In order to unequivocally confirm the 25*R* stereochemistry of **II**, we determined the X-ray crystal structure of **II**. The fractional atomic coordinates for the non-hydrogen atoms of **II** are presented in **Table 4**. The bond lengths, valency angles, and torsion angles for **II** are given in **Table 5**, **Table 6**, and **Table 7**, respectively. A stereo view of **II** is shown in **Fig. 5**.

[2]The 7-keto acetate **XVIb** and 7-keto-epoxide acetate **XVb** were isolated from oxidation of 3β-acetoxy-5α-cholest-8(14)-ene (50). The 15-keto-epoxide acetate **XIVb** was also isolated from this reaction. The ^{13}C NMR chemical shifts of **XIVa–XVIa** differed from those of **XIVb–XVIb** by less than 0.2 ppm in rings B, C, and D.

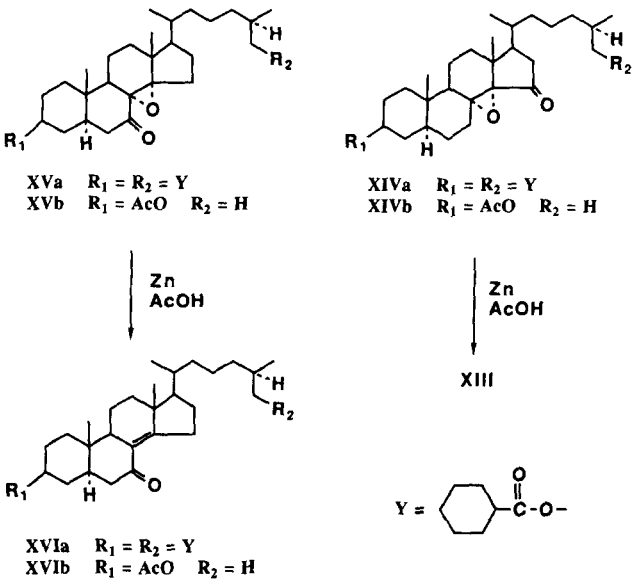


Fig. 4. Reduction of epoxyketones to α,β-unsaturated ketones.

The signs of the torsion angles centered on the bonds common to the fused rings indicated (54, 55) that the A–B ring junction is *trans* and that the B–C and C–D ring junctions are quasi-*trans*. The twist of the sterol (the C(19)–C(10)–C(13)–C(18) pseudo-torsion angle) is −9.8°. Based on calculations of asymmetry parameters and least squares planes (54, 55), the conformation of ring A is a symmetrical chair, and that of ring B is a chair slightly distorted by the presence of an sp^2 atom (C-8). The conformation of ring C is intermediate between an 11β,12α-half chair and a 12α-sofa, and that of ring D is intermediate between a 13β-envelope and a 13β,17α-half chair. The pseudo-rotation parameters (56) Δ and ϕ_m are 54.9 and 36.4. The averages of the absolute values of the endocyclic torsion angles in rings A, B, C, and D are 54.3°, 51.8°, 29.7°, and 23.6°, respectively. The α,β unsaturated carbonyl system deviates noticeably from planarity: C(8) is 0.49 Å below the plane containing C(14), C(15), and O(15), and torsion angle C(8)–C(14)–C(15)–O(15) is −27°. As in the crystal structure of (25*R*)-cholest-5-ene-3β,26-diol (**VIII**) (57), the sterol side chain (including C(26) and O(26)) is in a fully extended conformation, and C(21) and C(27) are on opposite sides of this chain. Inspection of a Dreiding model constructed according to the side-chain torsion angles listed in Table 7 indicated the 25*R* configuration for **II**. The intermolecular distances O(3)...O(26) of 2.76 (1) Å and O(3)...O(15) of 2.90 (1) Å indicate the presence of hydrogen bonding. No intramolecular hydrogen bonding was observed.

TABLE 4. Fractional atomic coordinates and temperature factors for **II** (estimated standard deviations in parentheses)

Atom	x	y	z	B_{eq}
C(1)	0.6690 (6)	0.3227 (2)	0.1163 (7)	4.2 (3)
C(2)	0.6358 (6)	0.3968 (2)	0.1164 (6)	3.9 (3)
C(3)	0.6354 (5)	0.4246 (2)	−0.0153 (8)	4.3 (3)
C(4)	0.5600 (6)	0.3854 (2)	−0.1047 (7)	4.3 (3)
C(5)	0.5935 (5)	0.3118 (2)	−0.1055 (6)	2.8 (3)
C(6)	0.5218 (7)	0.2720 (2)	−0.1989 (6)	4.6 (3)
C(7)	0.5640 (7)	0.2001 (3)	−0.2073 (7)	5.2 (4)
C(8)	0.5797 (4)	0.1672 (2)	−0.0816 (6)	2.8 (2)
C(9)	0.6417 (4)	0.2081 (2)	0.0200 (6)	2.6 (2)
C(10)	0.5911 (4)	0.2802 (2)	0.0286 (6)	2.6 (2)
C(11)	0.6516 (5)	0.1725 (2)	0.1456 (6)	3.5 (3)
C(12)	0.6799 (5)	0.0985 (2)	0.1309 (7)	4.2 (3)
C(13)	0.5835 (4)	0.0629 (2)	0.0562 (6)	2.8 (2)
C(14)	0.5534 (4)	0.1023 (2)	−0.0650 (6)	2.8 (2)
C(15)	0.5102 (5)	0.0554 (2)	−0.1625 (7)	3.4 (3)
C(16)	0.5461 (5)	−0.0128 (2)	−0.1222 (6)	3.0 (2)
C(17)	0.6249 (4)	−0.0045 (2)	−0.0055 (6)	3.0 (3)
C(18)	0.4727 (6)	0.0523 (3)	0.1340 (6)	3.6 (3)
C(19)	0.4678 (5)	0.2766 (3)	0.0849 (7)	3.9 (3)
C(20)	0.6273 (5)	−0.0668 (2)	0.0787 (6)	3.2 (3)
C(21)	0.6971 (7)	−0.0576 (3)	0.1983 (8)	5.6 (4)
C(22)	0.6750 (5)	−0.1257 (2)	0.0033 (7)	3.8 (3)
C(23)	0.6471 (5)	−0.1928 (2)	0.0617 (7)	3.8 (3)
C(24)	0.7106 (5)	−0.2510 (3)	−0.0008 (8)	3.9 (3)
C(25)	0.6898 (5)	−0.3181 (2)	0.0649 (6)	3.5 (3)
C(26)	0.7730 (5)	−0.3697 (3)	0.0140 (10)	6.4 (4)
C(27)	0.5652 (5)	−0.3407 (2)	0.0554 (7)	4.3 (3)
O(3)	0.5956 (4)	0.4926 (2)	−0.0098 (5)	5.5 (2)
O(15)	0.4540 (4)	0.0674 (2)	−0.2595 (5)	5.3 (2)
O(26)	0.7719 (4)	−0.4289 (2)	0.0882 (6)	6.9 (3)

TABLE 5. Bond lengths (Å) for II with standard deviations in parentheses

Ring A		Ring B	
C(1)-C(2)	1.546 (7)	C(5)-C(6)	1.508 (8)
C(2)-C(3)	1.488 (10)	C(6)-C(7)	1.535 (8)
C(3)-C(4)	1.497 (8)	C(7)-C(8)	1.484 (9)
C(4)-C(5)	1.536 (7)	C(8)-C(9)	1.521 (7)
C(5)-C(10)	1.541 (8)	C(9)-C(10)	1.570 (6)
C(10)-C(1)	1.540 (8)	C(10)-C(5)	1.541 (8)
Ring C		Ring D	
C(8)-C(9)	1.521 (7)	C(13)-C(14)	1.536 (8)
C(9)-C(11)	1.502 (8)	C(14)-C(15)	1.477 (8)
C(11)-C(12)	1.538 (7)	C(15)-C(16)	1.499 (7)
C(12)-C(13)	1.531 (7)	C(16)-C(17)	1.525 (8)
C(13)-C(14)	1.536 (8)	C(17)-C(13)	1.579 (7)
C(14)-C(8)	1.355 (6)		
C(10)-C(19)	1.528 (7)	C(24)-C(25)	1.538 (7)
C(13)-C(18)	1.519 (8)	C(25)-C(27)	1.498 (8)
C(17)-C(20)	1.538 (7)	C(25)-C(26)	1.510 (8)
C(20)-C(21)	1.495 (9)	O(3)-C(3)	1.449 (6)
C(20)-C(22)	1.528 (7)	O(15)-C(15)	1.224 (7)
C(22)-C(23)	1.522 (7)	O(26)-C(26)	1.428 (8)
C(23)-C(24)	1.529 (8)		

Compound I has been shown to be a potent inhibitor of sterol synthesis in cultured mammalian cells and to lower the levels of HMG-CoA reductase activity in these cells (1-4). In the present study we have found that II is also highly active in the reduction of HMG-CoA reductase activity in CHO-K1 cells. II blocked the rise in the levels of activity of this enzyme, which is induced by transfer of the cells from media containing serum to media containing delipidized serum. II also reduced the existing elevated levels of HMG-CoA reductase activity in-

TABLE 6. Valency angles (°) for II with standard deviations in parentheses

Ring A		Ring B	
C(1)-C(2)-C(3)	111.5 (3)	C(5)-C(6)-C(7)	111.8 (6)
C(2)-C(3)-C(4)	112.4 (5)	C(6)-C(7)-C(8)	114.3 (5)
C(3)-C(4)-C(5)	111.8 (5)	C(7)-C(8)-C(9)	115.5 (4)
C(4)-C(5)-C(10)	113.1 (5)	C(8)-C(9)-C(10)	111.9 (4)
C(5)-C(10)-C(1)	107.4 (4)	C(9)-C(10)-C(5)	109.1 (5)
C(10)-C(1)-C(2)	113.4 (5)	C(10)-C(5)-C(6)	111.0 (4)
Ring C		Ring D	
C(8)-C(9)-C(11)	112.7 (4)	C(13)-C(14)-C(15)	108.1 (4)
C(9)-C(11)-C(12)	113.2 (5)	C(14)-C(15)-C(16)	107.7 (5)
C(11)-C(12)-C(13)	110.9 (4)	C(15)-C(16)-C(17)	106.6 (4)
C(12)-C(13)-C(14)	109.7 (4)	C(16)-C(17)-C(13)	104.2 (4)
C(13)-C(14)-C(8)	123.9 (5)	C(17)-C(13)-C(14)	100.2 (4)
C(14)-C(8)-C(9)	122.6 (5)		
C(4)-C(5)-C(6)	112.6 (5)	C(17)-C(20)-C(21)	112.8 (4)
C(7)-C(8)-C(14)	121.3 (5)	C(17)-C(20)-C(22)	110.4 (5)
C(10)-C(9)-C(11)	114.9 (5)	C(21)-C(20)-C(22)	109.8 (5)
C(1)-C(10)-C(9)	109.8 (4)	C(20)-C(22)-C(23)	114.4 (5)
C(12)-C(13)-C(17)	113.5 (4)	C(22)-C(23)-C(24)	114.5 (5)
C(8)-C(14)-C(15)	127.3 (5)	C(23)-C(24)-C(25)	114.4 (5)
C(12)-C(13)-C(18)	113.1 (5)	C(24)-C(25)-C(27)	112.7 (5)
C(14)-C(13)-C(18)	109.2 (4)	C(24)-C(25)-C(26)	110.6 (5)
C(17)-C(13)-C(18)	110.3 (4)	C(27)-C(25)-C(26)	111.4 (5)
C(1)-C(10)-C(19)	109.2 (5)	O(3)-C(3)-C(2)	108.8 (5)
C(5)-C(10)-C(19)	112.8 (4)	O(3)-C(3)-C(4)	110.2 (5)
C(9)-C(10)-C(19)	108.5 (4)	O(15)-C(15)-C(14)	128.4 (5)
C(13)-C(17)-C(20)	118.5 (5)	O(15)-C(15)-C(16)	123.9 (5)
C(16)-C(17)-C(20)	112.3 (4)	O(26)-C(26)-C(25)	112.3 (7)

TABLE 7. Torsion angles (°) for **II** with standard deviations in parentheses

Ring A		**Ring B**	
C(10)-C(1)-C(2)-C(3)	- 55.0 (7)	C(10)-C(5)-C(6)-C(7)	- 57.3 (7)
C(1)-C(2)-C(3)-C(4)	53.6 (7)	C(5)-C(6)-C(7)-C(8)	49.2 (8)
C(2)-C(3)-C(4)-C(5)	- 54.2 (7)	C(6)-C(7)-C(8)-C(9)	- 44.5 (8)
C(3)-C(4)-C(5)-C(10)	55.5 (7)	C(7)-C(8)-C(9)-C(10)	47.1 (7)
C(4)-C(5)-C(10)-C(1)	- 53.9 (6)	C(5)-C(10)-C(9)-C(8)	- 53.4 (5)
C(2)-C(1)-C(10)-C(5)	53.7 (7)	C(6)-C(5)-C(10)-C(9)	59.4 (6)
Ring C		**Ring D**	
C(11)-C(9)-C(8)-C(14)	- 9.6 (7)	C(17)-C(13)-C(14)-C(15)	32.4 (5)
C(8)-C(9)-C(11)-C(12)	39.7 (7)	C(13)-C(14)-C(15)-C(16)	- 17.1 (6)
C(9)-C(11)-C(12)-C(13)	- 61.0 (7)	C(14)-C(15)-C(16)-C(17)	- 6.6 (6)
C(11)-C(12)-C(13)-C(14)	47.9 (7)	C(15)-C(16)-C(17)-C(13)	26.7 (5)
C(8)-C(14)-C(13)-C(12)	- 19.3 (7)	C(14)-C(13)-C(17)-C(16)	- 35.4 (5)
C(9)-C(8)-C(14)-C(13)	- 0.5 (8)		
C(11)-C(12)-C(13)-C(18)	- 74.3 (6)	C(2)-C(1)-C(10)-C(19)	- 68.9 (7)
C(8)-C(14)-C(13)-C(18)	105.2 (6)	C(4)-C(5)-C(10)-C(19)	66.5 (6)
C(15)-C(14)-C(13)-C(18)	- 83.5 (5)	C(6)-C(5)-C(10)-C(19)	- 61.2 (6)
C(16)-C(17)-C(13)-C(18)	79.6 (5)	C(8)-C(9)-C(10)-C(19)	69.8 (6)
C(20)-C(17)-C(13)-C(18)	- 46.0 (6)	C(11)-C(9)-C(10)-C(19)	- 60.4 (6)
C(1)-C(10)-C(5)-C(6)	178.4 (5)	C(12)-C(13)-C(17)-C(20)	82.1 (6)
C(1)-C(10)-C(9)-C(8)	- 170.9 (5)	C(14)-C(13)-C(17)-C(20)	- 161.0 (4)
C(1)-C(10)-C(9)-C(11)	58.9 (6)	C(15)-C(16)-C(17)-C(20)	156.2 (5)
C(2)-C(1)-C(10)-C(9)	172.2 (5)	C(13)-C(17)-C(20)-C(21)	- 54.2 (7)
C(3)-C(4)-C(5)-C(6)	- 177.7 (5)	C(16)-C(17)-C(20)-C(21)	- 175.8 (5)
C(4)-C(5)-C(6)-C(7)	174.7 (6)	C(13)-C(17)-C(20)-C(22)	- 177.4 (4)
C(4)-C(5)-C(10)-C(9)	- 172.9 (4)	C(16)-C(17)-C(20)-C(22)	61.0 (6)
C(5)-C(10)-C(9)-C(11)	176.4 (4)	C(17)-C(20)-C(22)-C(23)	- 162.2 (5)
C(6)-C(7)-C(8)-C(14)	143.4 (6)	C(21)-C(20)-C(22)-C(23)	72.8 (6)
C(7)-C(8)-C(9)-C(11)	178.4 (5)	C(20)-C(22)-C(23)-C(24)	- 170.7 (5)
C(7)-C(8)-C(14)-C(13)	171.0 (5)	C(22)-C(23)-C(24)-C(25)	175.5 (5)
C(7)-C(8)-C(14)-C(15)	1.5 (10)	C(23)-C(24)-C(25)-C(27)	65.5 (7)
C(8)-C(14)-C(13)-C(17)	- 138.9 (5)	C(23)-C(24)-C(25)-C(26)	- 169.0 (6)
C(8)-C(14)-C(15)-C(16)	153.8 (6)		
C(9)-C(8)-C(14)-C(15)	- 170.1 (5)	O(3)-C(3)-C(2)-C(1)	175.9 (5)
C(10)-C(9)-C(8)-C(14)	- 141.0 (5)	O(3)-C(3)-C(4)-C(5)	- 175.7 (5)
C(10)-C(9)-C(11)-C(12)	169.5 (4)	O(15)-C(15)-C(14)-C(8)	- 26.8 (10)
C(11)-C(12)-C(13)-C(17)	159.0 (5)	O(15)-C(15)-C(14)-C(13)	162.3 (6)
C(12)-C(13)-C(14)-C(15)	152.0 (5)	O(15)-C(15)-C(16)-C(17)	174.0 (6)
C(12)-C(13)-C(17)-C(16)	- 152.3 (5)	O(26)-C(26)-C(25)-C(24)	168.9 (6)
		O(26)-C(26)-C(25)-C(27)	- 64.9 (8)

TABLE 8. Effects of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) and (25*R*)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**) on levels of HMG-CoA reductase in CHO-K1 cells

	HMG-CoA Reductase Activity (% of control activity)			
	Suppression of Rise Induced by Transfer of Cells to Lipid-Deficient Media		Suppression of Elevated Levels Induced by Transfer of Cells to Lipid-Deficient Media	
Sterol Concentration	**I**	**II**	**I**	**II**
nM				
0	100.0[a]	100.0[a]	100.0[b]	100.0[b]
50	79.1 ± 4.9[c]	71.2 ± 7.5[c]	79.1 ± 4.0[c]	99.9 ± 17.6[c]
100	54.2 ± 2.7	51.5 ± 8.2	57.3 ± 3.5	46.1 ± 2.2
250	36.6 ± 4.5	42.6 ± 4.8	45.7 ± 4.8	51.6 ± 5.0
500	23.7 ± 2.0	41.6 ± 2.2	32.6 ± 1.7	25.6 ± 3.3
1000	15.4 ± 1.4	12.1 ± 0.5	26.5 ± 2.5	21.2 ± 3.6

[a]Mean value for controls was 4,118 pmol/min per mg protein.
[b]Mean value for controls was 1,363 pmol/min per mg protein.
[c]Variation is expressed as ± SD of replicate (n = 3) assays for the experimental values.

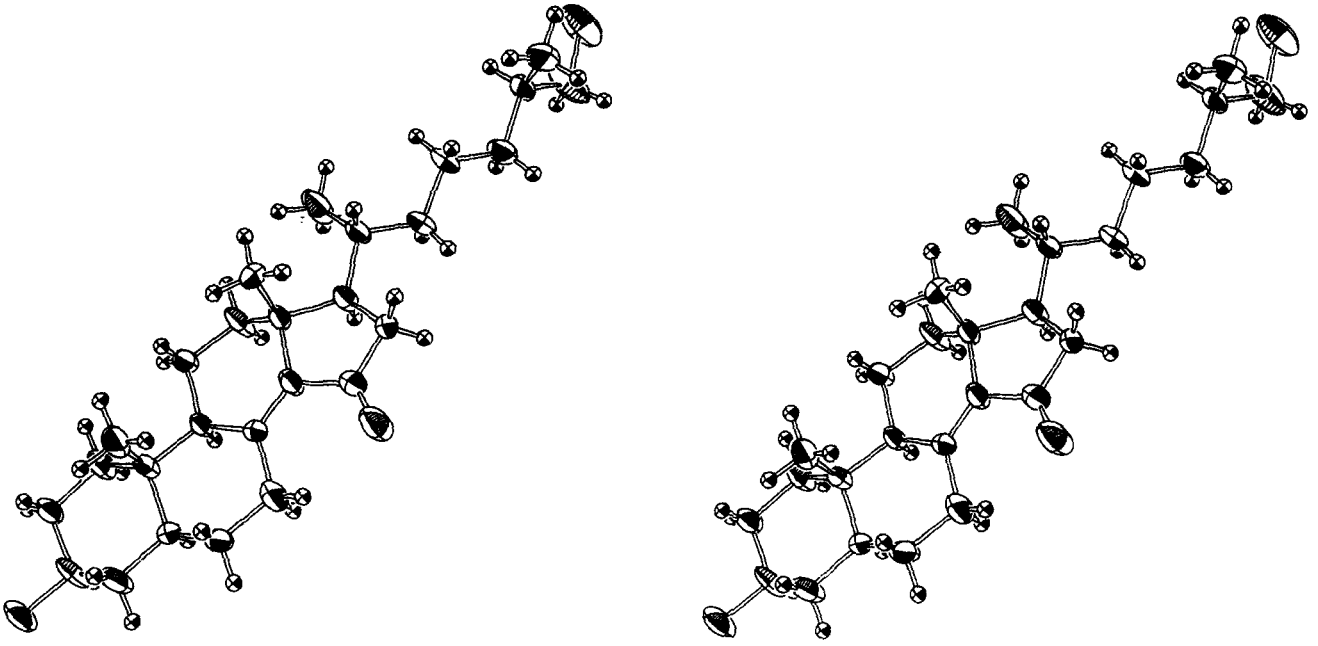


Fig. 5. Stereoscopic view of a single molecule of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**).

duced by transfer of the cells to delipidized media. In both cases, the potency of **II** was essentially the same as that of **I** (**Table 8**), with 50% suppression of the levels of HMG-CoA reductase activity at ~0.1 μM. These findings raise the possibility that **II** might be the active species responsible for the effects of **I** on HMG-CoA reductase in CHO-K1 cells. However, the results of a recent study (4) of the metabolism of **I** in these cells indicate little, if any, metabolism of **I** to materials more polar than **I** (or to material with what we now know to be the chromatographic behavior of **II**). The only significant metabolism of **I** detected in the CHO-K1 cells was conversion of **I** to its fatty acid esters (4).

TABLE 9. Effect of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**I**) and (25*R*)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**II**) on the oleyl CoA-dependent esterification of cholesterol in rat jejunal microsomes

Sterol Concentration	Formation of Cholesteryl Oleate	
	I	**II**
μM	*pmol/min per mg protein ± SEM (n = 4)*	
0.0	1,740 ± 25	1,740 ± 25
0.5	1,440 ± 30 (−17%)[a]	1,500 ± 63 (−14%)[a]
1.0	1,260 ± 76 (−28%)	1,430 ± 52 (−18%)
2.0	825 ± 26 (−53%)*	1,300 ± 36 (−25%)**
5.0	428 ± 8 (−75%)*	1,105 ± 39 (−36%)**
10.0	155 ± 15 (−91%)*	787 ± 12 (−55%)*

[a]Percent inhibition relative to control value (no added sterol). *$P < 0.001$; **$P < 0.01$.

Compound **I** has been found to serve as an alternative substrate for ACAT activity of hepatic and jejunal microsomes and to inhibit the oleoyl CoA-dependent esterification of cholesterol (7). The results presented in **Table 9** show that **II** also inhibits the oleoyl CoA-dependent esterification of cholesterol. The potency of **II** in the inhibition of ACAT activity was less than that of **I**. Compound **I** caused a 53% inhibition of cholesteryl oleate formation at 2 μM, whereas a comparable (−55%) inhibition of cholesterol esterification by **II** was observed at a much higher concentration, i.e., 10 μM.

Thus, **II**, a major mitochondrial metabolite of **I**, has been prepared by chemical synthesis and shown to be not only highly active in the suppression of the levels of a key regulatory enzyme in the biosynthesis of cholesterol but also to inhibit an enzyme of considerable importance in the intestinal absorption of cholesterol and in the general intracellular metabolism of cholesterol. The combined findings indicate that a complete definition of the biological actions of **I** must consider the activities of **II** and other potential metabolites of **I**.

Portions of this research (synthetic plus ACAT studies and cell culture plus spectral studies) were supported by grants HL-22532 and HL-15376, respectively, from the National Institutes of Health. The support of the Ralph and Dorothy Looney Endowment Fund and the American Cyanamid Company is also gratefully acknowledged. We thank Drs. C. C. Sweeley and J. T. Watson for the high resolution mass spectral measurements.

Manuscript received 27 June 1988.

REFERENCES

1. Schroepfer, G. J., Jr., E. J. Parish, H. W. Chen, and A. A. Kandutsch. 1977. Inhibition of sterol biosynthesis in L cells and mouse liver cells by 15-oxygenated sterols. *J. Biol. Chem.* **252:** 8975–8980.
2. Miller, L. R., F. D. Pinkerton, and G. J. Schroepfer, Jr. 1980. 5α-Cholest-8(14)-en-3β-ol-15-one, a potent inhibitor of sterol synthesis, reduces the levels of activity of enzymes involved in the synthesis and reduction of 3-hydroxy-3-methylglutaryl coenzyme A in CHO-K1 cells. *Biochem. Internat.* **1**: 223–228.
3. Pinkerton, F. D., A. Izumi, C. M. Anderson, L. R. Miller, A. Kisic, and G. J. Schroepfer, Jr. 1982. 14α-Ethyl-5α-cholest-7-ene-3β,15α-diol, a potent inhibitor of sterol biosynthesis, has two sites of action in cultured mammalian cells. *J. Biol. Chem.* **257:** 1929–1936.
4. Pajewski, T. N., F. D. Pinkerton, L. R. Miller, and G. J. Schroepfer, Jr. 1988. Inhibitors of sterol synthesis. Studies of the metabolism of 5α-cholest-8(14)-en-3β-ol-15-one in Chinese hamster ovary cells and its effects on activities of early enzymes in cholesterol biosynthesis. *Chem. Phys. Lipids.* **48:** 153–168.
5. Schroepfer, G. J., Jr., A. Christophe, D. H. Needleman, A. Kisic, and B. C. Sherrill. 1987. Inhibitors of sterol synthesis. Dietary administration of 5α-cholest-8(14)-en-3β-ol-15-one inhibits the intestinal absorption of cholesterol. *Biochem. Biophys. Res. Commun.* **146:** 1003–1008.
6. Brabson, J. S., and G. J. Schroepfer, Jr. 1988. Inhibitors of sterol synthesis. The effects of dietary 5α-cholest-8(14)-en-3β-ol-15-one on the fate of [4-^{14}C]cholesterol and [2,4-^{3}H]5α-cholest-8-(14)-en-3β-ol-15-one after intragastric administration to rats. *Chem. Phys. Lipids.* **47:** 1–20.
7. Miller, L. R., D. H. Needleman, J. S. Brabson, K-S. Wang, and G. J. Schroepfer, Jr. 1987. 5α-Cholest-8(14)-en-3β-ol-15-one. A competitive substrate for acyl coenzyme A:cholesterol acyl transferase. *Biochem. Biophys. Res. Commun.* **148:** 934–940.
8. Needleman, D. H., K. Strong, K. A. Stemke, J. S. Brabson, A. Kisic, and G. J. Schroepfer, Jr. 1987. Inhibitors of sterol synthesis. Effect of dietary 5α-cholest-8(14)-en-3β-ol-15-one on ACAT activity of jejunal microsomes of the rat. *Biochem. Biophys. Res. Commun.* **148:** 920–925.
9. Schroepfer, G. J., Jr., D. Monger, A. S. Taylor, J. S. Chamberlain, E. J. Parish, A. Kisic, and A. A. Kandutsch. 1977. Inhibitors of sterol synthesis. Hypocholesterolemic action of dietary 5α-cholest-8(14)-en-3β-ol-15-one in rats and mice. *Biochem. Biophys. Res. Commun.* **78:** 1227–1233.
10. Schroepfer, G. J., Jr., E. J. Parish, A. Kisic, E. M. Jackson, C. M. Farley, and G. E. Mott. 1982. 5α-Cholest-8(14)-en-3β-ol-15-one, a potent inhibitor of sterol biosynthesis, lowers serum cholesterol and alters the distribution of cholesterol in lipoproteins in baboons. *Proc. Natl. Acad. Sci. USA.* **79:** 3042–3046.
11. Schroepfer, G. J., Jr., B. C. Sherrill, K-S. Wang, W. K. Wilson, A. Kisic, and T. B. Clarkson. 1984. 5α-Cholest-8(14)-en-3β-ol-15-one lowers serum cholesterol and induces profound changes in the levels of lipoprotein cholesterol and apoproteins in monkeys fed a diet of moderate cholesterol content. *Proc. Nat. Acad. Sci. USA.* **81:** 6861–6865.
12. Monger, D. J., E. J. Parish, and G. J. Schroepfer, Jr. 1980. 15-Oxygenated sterols. Enzymatic conversion of [2,4-^{3}H]5α-cholest-8(14)-en-3β-ol-15-one to cholesterol in rat liver preparations. *J. Biol. Chem.* **255:** 11122–11129.
13. Monger, D. J., and G. J. Schroepfer, Jr. 1988. Inhibitors of cholesterol biosynthesis. Further studies of the metabolism of 5α-cholest-8(14)-en-3β-ol-15-one in rat liver preparations. *Chem. Phys. Lipids.* **47:** 21–46.
14. Brabson, J. S. 1981. In vivo conversion of 5α-cholest-8(14)-en-3β-ol-15-one to cholesterol in rats. *Fed. Proc.* **40:** 1680.
15. Brabson, J. S., and G. J. Schroepfer, Jr. 1988. Inhibitors of sterol synthesis. Studies of the distribution and metabolism of [2,4-^{3}H]5α-cholest-8(14)-en-3β-ol-15-one after intragastric administration to rats. *Steroids.* In press.
16. Schroepfer, G. J., Jr., T. N. Pajewski, M. Hylarides, and A. Kisic. 1987. 5α-Cholest-8(14)-en-3β-ol-15-one. In vivo conversion to cholesterol upon oral administration to a nonhuman primate. *Biochem. Biophys. Res. Commun.* **146:** 1027–1032.
17. Schroepfer, G. J., Jr., A. J. Chu, D. H. Needleman, A. Izumi, P. T. Nguyen, K-S. Wang, J. M. Little, B. C. Sherrill, and A. Kisic. 1988. Inhibitors of sterol synthesis. Metabolism of 5α-cholest-8(14)-en-3β-ol-15-one after intravenous administration to bile duct-cannulated rats. *J. Biol. Chem.* **263:** 4110–4123.
18. Schroepfer, G. J., Jr., A. Kisic, A. Izumi, K-S. Wang, K. D. Carey, and A. J. Chu. 1988. Inhibitors of sterol synthesis. Metabolism of [2,4-^{3}H]5α-cholest-8(14)-en-3β-ol-15-one after intravenous administration to a nonhuman primate. *J. Biol. Chem.* **263:** 4098–4109.
19. Schroepfer, G. J., Jr., H-S. Kim, J. L. Vermilion, T. W. Stephens, F. D. Pinkerton, D. H. Needleman, W. K. Wilson, and J. St. Pyrek. 1988. Enzymatic formation and chemical synthesis of an active metabolite of 3β-hydroxy-5α-cholest-8(14)-en-15-one, a potent regulator of cholesterol metabolism. *Biochem. Biophys. Res. Commun.* **151:** 130–136.
20. St. Pyrek, J., W. K. Wilson, and G. J. Schroepfer, Jr. 1987. Inhibitors of sterol synthesis. Spectral characterization of derivatives of 5α-cholest-8(14)-en-3β-ol-15-one. *J. Lipid Res.* **28:** 1296–1307.
21. Knapp, F. F., Jr., and G. J. Schroepfer, Jr. 1975. Chemical synthesis, spectral properties and chromatography of 4α-methyl and 4β-methyl isomers of (24R)-24-ethyl-cholestan-3β-ol and (24S)-24-ethyl-cholesta-5,22-dien-3β-ol. *Steroids.* **26:** 339–357.
22. Pascal, R. A., Jr., C. L. Farris, and G. J. Schroepfer, Jr. 1980. Sterol synthesis. Medium pressure chromatography of C_{27} sterol precursors of cholesterol on alumina silver nitrate columns. *Anal. Biochem.* **101:** 15–22.
23. Wilson, W. K., K-S. Wang, A. Kisic, and G. J. Schroepfer, Jr. 1988. Concerning the synthesis of 3β-hydroxy-5α-cholest-8(14)-en-15-one, a novel regulator of cholesterol metabolism. *Chem. Phys. Lipids.* **47:** 273–282.
24. Parish, E. J., T. E. Spike, and G. J. Schroepfer, Jr. 1977. Sterol synthesis. Chemical synthesis of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene. A key intermediate in the synthesis of 15-oxygenated sterols. *Chem. Phys. Lipids.* **18:** 233–239.
25. Ham, R. G. 1965. Clonal growth of mammalian cells in a chemically defined, synthetic medium. *Proc. Natl. Acad. Sci. USA.* **53:** 288–293.
26. Cham, B. E., and B. R. Knowles. 1976. A solvent system for delipidation of plasma or serum without protein precipitation. *J. Lipid Res.* **17:** 176–181.
27. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
28. Peterson, G. L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal. Biochem.* **83:** 346–356.

29. Brown, M. S., S. E. Dana, and J. L. Goldstein. 1974. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured human fibroblasts. *J. Biol. Chem.* **249:** 789–796.
30. Suckling, K. E., E. F. Stange, and J. M. Dietschy. 1983. Dual modulation of hepatic and intestinal acyl-CoA:cholesterol acyltransferase activity by (de-) phosphorylation and substrate supply in vitro. *FEBS Lett.* **151:** 111–116.
31. Helgerud, P., R. Haugen, and K. R. Norum. 1982. The effect of feeding and fasting on the activity of acyl-CoA:cholesterol acyltransferase in rat small intestine. *Eur. J. Clin. Invest.* **12:** 493–500.
32. Seo, S., Y. Yoshimura, T. Satoh, A. Uomori, and K. Takeda. 1986. Synthesis of (25S)-[26-$^{2}H_{1}$]cholesterol and ^{1}H n.m.r. signal assignments of the pro-*R* and pro-*S* methyl groups at C-25. *J. Chem. Soc. Perkins Trans. 1.* 411–414.
33. Arunachalam, T., P. J. Mackoul, N. M. Green, and E. Caspi. 1981. Synthesis of 26-halo-, 26-(phenylseleno)-, and 26- indolylcholesterol analogues. *J. Org. Chem.* **46:** 2966–2968.
34. Scheer, R. E., M. J. Thompson, and E. Mosettig. 1956. 5-Cholestene-3β,26-diol. *J. Am. Chem. Soc.* **78:** 4733–4736.
35. Marker, R. E., and D. L. Turner. 1941. Sterols. CXV. Sapogenins. XLIV. The relation between diosgenin and cholesterol. *J. Am. Chem. Soc.* **63:** 767–771.
36. Midland, M. M., and Y. C. Kwon. 1985. Stereospecific synthesis of (25R)-26-hydroxycholesterol via [2,3] sigmatropic rearrangement. A new stereoselective two-carbon homologation of 20-keto steroids to the 23-aldehyde. *Tetrahedron Lett.* 5021–5024.
37. Kluge, A. F., M. L. Maddox, and L. G. Partridge. 1985. Synthesis of (20*R*,25*R*)-cholest-5-ene-3β,26-diol and the occurrence of base-catalyzed 1,5-hydride shift in a steroidal 1,5-ketone. *J. Org. Chem.* **50:** 2359–2365.
38. Varma, R. K., M. Koreeda, B. Yagen, K. Nakanishi, and E. Caspi. 1975. Synthesis and C-25 chirality of 26-hydroxycholesterols. *J. Org. Chem.* **40:** 3680–3686.
39. Zaretskii, Z. 1976. Mass Spectrometry of Steroids. John Wiley & Sons, New York
40. Tschesche, R., and H. R. Brennecke. 1979. Partialsynthese von (25R)-26-Aminocholesterol und (25R)-26-Amino-5-cholesten-3β,16β-diol aus Diogenin. *Chem. Ber.* **112:** 2680–2691.
41. Noam, M., I. Tamir, E. Breuer, and R. Mechoulam. 1981. Conversion of ruscogenin into 1α- and 1β-hydroxycholesterol derivatives. *Tetrahedron.* **37:** 597–604.
42. Corey, E. J., and A. Venkateswarlu. 1972. Protection of hydroxyl groups as *tert*-butyldimethylsilyl derivatives. *J. Am. Chem. Soc.* **94:** 6190–6191.
43. Piancatelli, G., A. Scettri, and M. D'Auria. 1982. Pyridinium chlorochromate: a versatile oxidant in organic synthesis. *Synthesis.* 245–258.
44. Garry, A. G., J. M. Midgley, W. B. Whalley, and B. J. Wilkins. 1977. Unsaturated steroids. Part 3. Synthesis of steroidal 22,24 (28)-dienes, ergosta-5,7,22,24(28)-tetraen-3β-ol, and cholesta-5,7,22-trien-3β-ol. *J. Chem. Soc. Perkin Trans. 1.* 809–812.
45. Acklin, G., and W. Graf. 1979. The course of the catalytic hydrogenation/isomerization reaction of steroidal ring B olefins in the 13β- and 13α-series. *Helv. Chim. Acta.* **62:** 2732–2753.
46. Schaltegger, H. 1950. Die direkte Photobromierung der Cholesterylester in der Allylstellung (C_7-Position). *Helv. Chim. Acta.* **33:** 2101–2110.
47. Lee, W-H., R. Kammereck, B. N. Lutsky, J. A. McCloskey, and G. J. Schroepfer, Jr. 1969. Studies on the mechanism of the enzymatic conversion of Δ^{8}-cholesten-3β-ol to Δ^{7}-cholesten-3β-ol. *J. Biol. Chem.* **244:** 2033–2040.
48. Lee, W-H. 1968. Studies on the biosynthesis of cholesterol. Ph.D. dissertation, University of Illinois.
49. Tsuda, M., and G. J. Schroepfer, Jr. 1979. Carbon-13 nuclear magnetic resonance studies of C_{27} sterol precursors of cholesterol. *J. Org. Chem.* **44:** 1290–1293.
50. Chorvat, R. J., and B. N. Desai. 1979. Facile synthesis of 3β-hydroxy-5α-cholest-8(14)-en-15-one 3-acetate. *J. Org. Chem.* **44:** 3974–3976.
51. Wintersteiner, O., and M. Moore. 1943. Oxidation products of α-cholestenyl acetate. *J. Am. Chem. Soc.* **65:** 1513–1516.
52. Anastasia, M., A. Fiecchi, and A. Scala. 1976. A ready synthesis of 5α,14β-cholest-7-en-3β-ol. *J. Chem. Soc. Perkin Trans. 1.* 378–380.
53. Schneider, H-J., and P. K. Agrawal. 1986. ^{13}C NMR and lanthanide-induced shifts in epoxides of terpenes and related compounds. *Magn. Reson. Chem.* **24:** 718–722.
54. Duax, W. L., and D. A. Norton, editors. 1975. Atlas of Steroid Structure. Vol. 1. Plenum, New York. 16–25.
55. Griffin, J. F., W. L. Duax, and C. M. Weeks, editors. 1984. Atlas of Steroid Structure. Vol. 2. Plenum, New York. 7–20.
56. Altona, C., H. J. Geise, and C. Romers. 1968. Conformation of non-aromatic ring compounds. XXV. Geometry and conformation of ring D in some steroids from X-ray structure determinations. *Tetrahedron.* **24:** 13–32.
57. Kirfel, A., G. Will, R. Brennecke, and R. Tschesche. 1977. (25R)-Cholest-5-en-3β,26-diol. *Acta Crystallogr.* **B33:** 895–897.