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Inhibitors of sterol synthesis. An improved chemical synthesis of 26-oxygenated $\Delta^{8(14)}$-15-ketosterols having the 25R configuration

Abdul U. Siddiqui, William K. Wilson, George J. Schroepfer, Jr.*

Departments of Biochemistry and Cell Biology and of Chemistry, Rice University, P.O. Box 1892, Houston, Texas 77251-1892, USA

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Abstract

(25R)-3β,26-Dihydroxy-5α-cholest-8(14)-en-15-one (**I**) was synthesized in four steps from (25R)-3β,26-diacetoxycholesta-5,7-diene (**III**) in 30% overall yield. Isomerization of **III** with HCl in chloroform-dichloromethane at −60°C gave (25R)-3β,26-diacetoxy-5α-cholesta-7,14-diene together with the 5α-$\Delta^{8,14}$ and 5β-$\Delta^{8,14}$ isomers in a 5:1:1 ratio. Epoxidation of the crude diene mixture with *m*-chloroperbenzoic acid, followed by hydrolysis in acetone containing concentrated $HClO_4$ (0.1%) gave (25R)-3β,26-diacetoxy-5α-cholest-8(14)-en-15-one (**VIII**), accompanied by numerous minor byproducts, including the 5α,14β-Δ^7, 5α, 14β-Δ^8 and 5β,14β-Δ^8 isomers of **VIII**. All four 15-ketosterol esters were isolated by chromatography and fully characterized by mass spectrometry and ^1H and ^{13}C nuclear magnetic resonance. Treatment of **VIII** with potassium carbonate in degassed methanol gave **I**.

Keywords: 15-oxygenated sterols; Diene isomerization; Epoxide hydrolysis; Mass spectrometry; ^1H and ^{13}C-NMR

1. Introduction

(25R)-3β,26-Dihydroxy-5α-cholest-8(14)-en-15-one (**I**, Fig. 1) is a major mitochondrial metabolite of 3β-hydroxy-5α-cholest-8(14)-en-15-one (**II**) [1,2], a potent regulator of cholesterol metabolism. **II** is a highly active inhibitor of sterol synthesis and lowers the levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity in cultured mammalian cells [3–7]. **II** is also a potent inhibitor of cholesterol absorption [8,9] and inhibits the oleoyl-CoA-dependent esterification of cholesterol in hepatic and jejunal microsomes [10]. The 15-ketosterol **II** has been shown to have significant hypocholesterolemic action upon administration to rats [11–13], mice [11], baboons [14] and rhesus monkeys [15].

The (25R)-3β,26-dihydroxy-15-ketosterol **I** has been prepared by chemical synthesis and shown to have high potency, equivalent to **II**, in lowering HMG-CoA reductase activity in CHO-K1 cells [1,16]. **I** also inhibited acyl coenzyme A:cholesterol acyltransferase activity in jejunal micro-

* Corresponding author.

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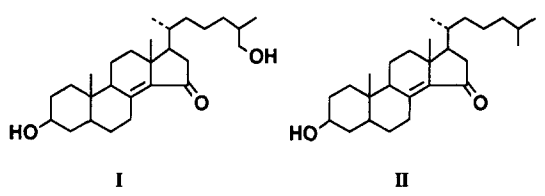

Fig. 1. Structures of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (I) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (II).

somes, but was less active than II [1,16]. I may contribute to the actions of II observed in animals. To explore these matters, an improved chemical synthesis of I was required.

We have previously presented a ten-step synthesis of I from diosgenin in 3% overall yield [16] (Fig. 2). With the aid of recent insights into the Clemmensen reduction of diosgenin [17], this synthesis represents an efficient route to (25R)-26-hydroxycholesterol. However, some of the subsequent steps are inefficient for multigram prepara-

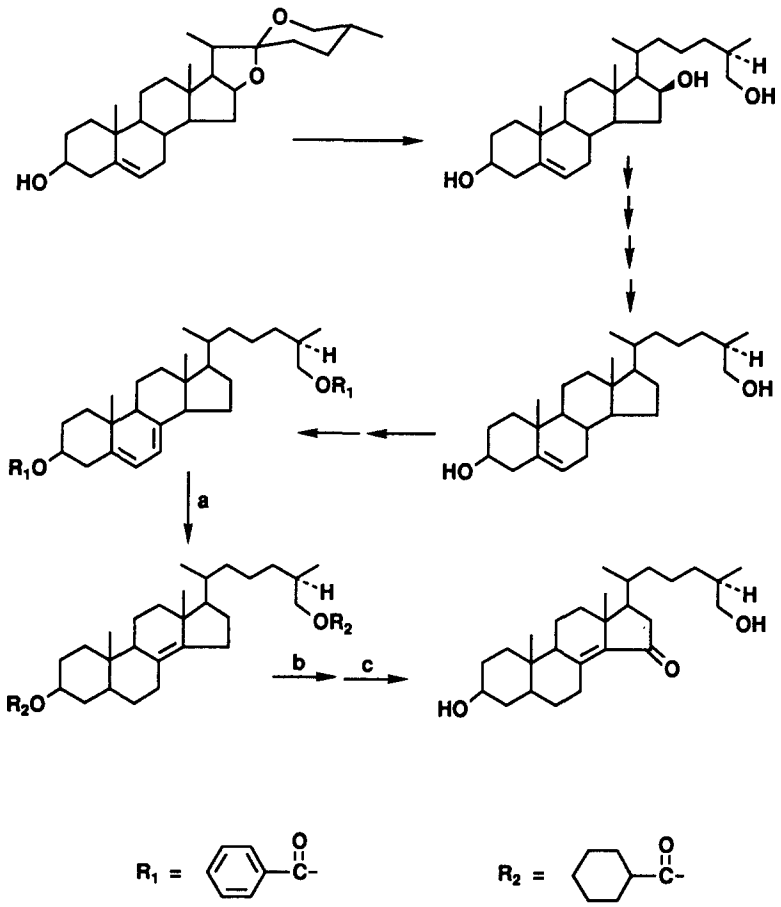

Fig. 2. Summary of earlier chemical synthesis of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (I) described in Ref. 16. Reagents for the final three steps were: (a) H_2, PtO_2, AcOH–EtOAc; (b) CrO_3-3,5-dimethylpyrazole, CH_2Cl_2, −20°C; (c) H_2SO_4, $CH-H_2O$, 85°C.

tions of I. For example, conversion of (25R)-26-hydroxycholesterol dibenzoate to the corresponding $\Delta^{5,7}$ compound and allylic oxidation of the $\Delta^{8(14)}$-sterol to a $\Delta^{8(14)}$-15-ketosterol results in only modest yields (44% and 30%, respectively). Our attempts to scale up the latter reaction beyond ~200 mg have resulted in significantly lower yields. Additionally, reduction of the $\Delta^{5,7}$ dibenzoate to the $\Delta^{8(14)}$ species with concomitant hydrogenation of the benzoate groups consumes significant quantities of platinum oxide. Some of these difficulties were overcome by an improved method for converting Δ^5 sterols to $\Delta^{5,7}$ dienes [18] and by the use of acetate rather than benzoate protecting groups [19], but the inefficient allylic oxidation step remained a problem.

Preparation of multigram quantities of I necessitated either significant modifications to the existing synthesis of I [16] or the design of a new synthesis. One interesting alternative strategy, entailing conversion of a Δ^5-16-hydroxysterol to a $\Delta^{8(14)}$-15-ketosterol [20], would appear to be similar in length to the existing synthesis of I. Another strategy, consisting of elaboration of the side chain of a C_{22}- or C_{24}-$\Delta^{8(14)}$-15-ketosterol, was useful for preparing some side-chain oxygenated sterols [21,22], but in the present case difficulties can be expected in separating mixtures of C-25 epimers and in efficiently protecting the $\Delta^{8(14)}$-15-ketone moiety. These considerations and our ongoing interest in improving the existing synthesis of I discouraged us from either of the former strategies. We have now found that the most inefficient steps of our previously published synthesis of I [16] can be replaced with reactions used to prepare the parent 15-ketosterol II from 7-dehydrocholesterol [23]. Described herein is an improved synthesis of (25R)-3β,26-diacetoxy-5α-cholest-8(14)-en-15-one from (25R)-3β,26-diacetoxycholesta-5,7-diene (III) suitable for work on a multigram scale.

2. Experimental procedures and results

2.1. Materials and methods

Instrumentation for measuring melting points (m.p.), optical rotations ($CHCl_3$ solution, ~22°C), infrared (IR) spectra and low-resolution mass spectra (MS) have been described previously [19]; all IR spectra showed absorptions at 2980–2840, 1460, 1380 and 1360 cm^{-1}. High-resolution MS were measured by electron impact on a Kratos MS-50DA or VG ZAB-SE spectrometer. Nuclear magnetic resonance (NMR) spectra were acquired in $CDCl_3$ solution in 5-mm tubes on an IBM AF300 spectrometer (300 MHz for ^1H and 75.5 MHz for ^{13}C, ~22°C) or a Bruker AMX500 spectrometer (500 MHz for ^1H, 27°C, inverse-detection probe) and referenced to internal tetramethylsilane (^1H) or $CDCl_3$ at 77.0 ppm (^{13}C). Standard Bruker software was used to acquire DEPT (distortionless enhancement by polarization transfer), COSYDEC (ω_1-decoupled ^1H-^1H correlation spectroscopy; 0.2-s fixed evolution period τ_e, δ ~0.6–2.5, 256 increments) (Ref. 24 and references therein), HETCOR (^1H-^{13}C shift correlated spectroscopy; ~50 increments, δ_H ~0.6–2.6), HMQC (heteronuclear multiple-quantum coherence), HSQC (heteronuclear single-quantum coherence) and nuclear Overhauser enhancement (NOE) difference spectra. Many overlapped multiplets in congested regions of ^1H spectra were isolated by saturation difference spectroscopy [25,26]; a single line of a multiplet was irradiated using conditions for NOE difference spectroscopy: 500-MHz, low-power irradiation for 0.7 s, 90° read pulse, 2.7-s acquisition time, 16 scans per cycle, non-degassed sample, no spinning. Coupling constants were derived from line spacings of resolution-enhanced 1D spectra. PCMODEL (Macintosh version 4.4, Serena Software, Bloomington, IN) was used for modeling of sterol structures by molecular mechanics and for predicting vicinal ^1H-^1H-NMR coupling constants. ^{13}C-NMR assignments were made chiefly from DEPT and HETCOR spectra in conjunction with ^1H and ^{13}C chemical shift comparisons with spectra of 26-hydroxysterols [16,18,19,27] and corresponding sterols lacking a 26-hydroxyl group [23,28,29]. ^1H stereochemical assignments were based on chemical shift and coupling constant comparisons [18,19,30], NOE difference experiments and comparisons of observed and predicted ^1H-^1H coupling constants.

Analytical thin-layer chromatography (TLC)

was performed using precoated 0.25-mm silica gel G plates (Analtech, Newark, DE); substances were visualized by spraying with 5% ammonium molybdate(VI) in 10% sulfuric acid followed by heating. High-performance liquid chromatography (HPLC) was performed isocratically with a Waters liquid chromatograph (U6K injector, model 510 pump and model 481 variable wavelength detector). Analytical HPLC was done with a 5-μm Spherisorb ODS-II column (250 mm × 4.6 mm i.d.; Custom LC, Houston, TX) at 1.0 ml/min. Preparative HPLC was done with a 5-μm Custom-sil C_{18} column (250 mm × 9.4 mm i.d.; Custom LC) or an 8-μm C_{18} Dynamax column (250 mm × 21.4 mm i.d.; Rainin Instrument Company, Woburn, MA). Medium-pressure liquid chromatography (MPLC) was carried out with a reversed-phase Lobar RP-18 column (310 × 25

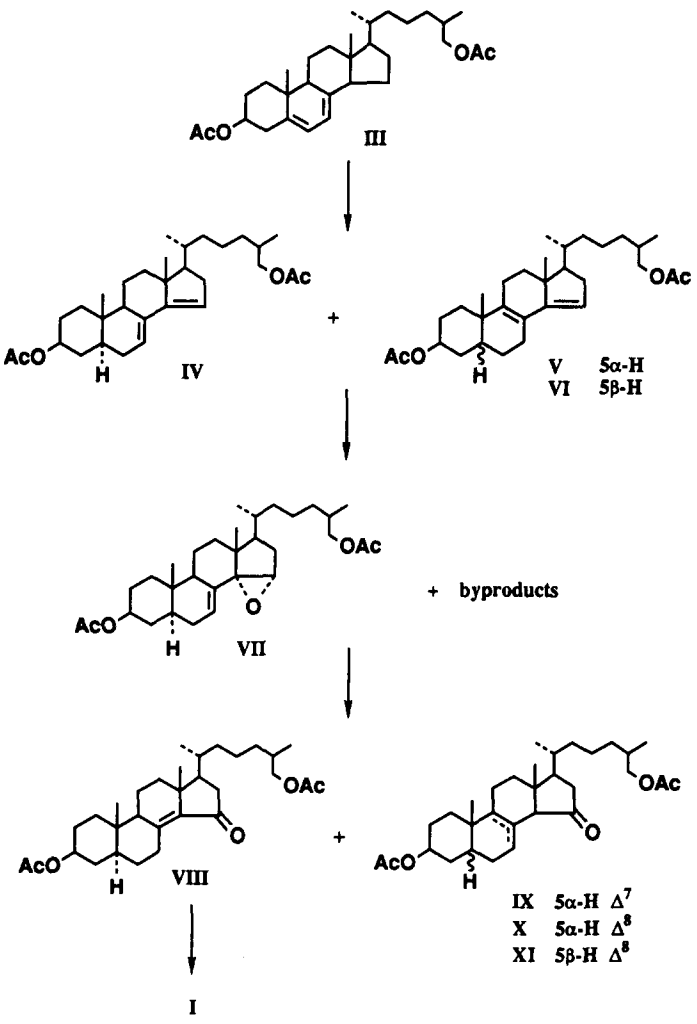

Fig. 3. Chemical synthesis of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**I**) from (25R)-3β,26-diacetoxycholesta-5,7-diene (**III**).

mm i.d.; EM Science, Gibbstown, NJ) or a 1000 × 10 mm i.d. column packed with silica gel (230–400 mesh). Fraction volumes for MPLC and HPLC were 20 ml. Sterol samples were adsorbed onto a stationary phase by rotary evaporation of a sterol solution containing silica gel or C_{18} material (~5 g per g of sterol), placed in a small column and eluted onto the main MPLC column.

Structures of new sterols were established by comparison of ^1H and ^{13}C-NMR chemical shifts with those reported for similar sterols [16,28,29]. Progress of reactions was monitored by TLC or HPLC. Reaction products were identified by ^1H and (in some cases) ^{13}C-NMR. 3β-Benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene (**XII**, m.p. 208–210°C, lit. 210–211°C) [23] and (25R)-3β,26-diacetoxycholesta-5,7-diene (**III**) [18] were prepared as described previously. Structures of **III–XI** and **XII–XIV** are shown in Figs. 3 and 4, respectively. *m*-Chloroperbenzoic acid (80–85%; Aldrich Chemical Company, Milwaukee, WI) and concentrated $HClO_4$ (70%) were used as received.

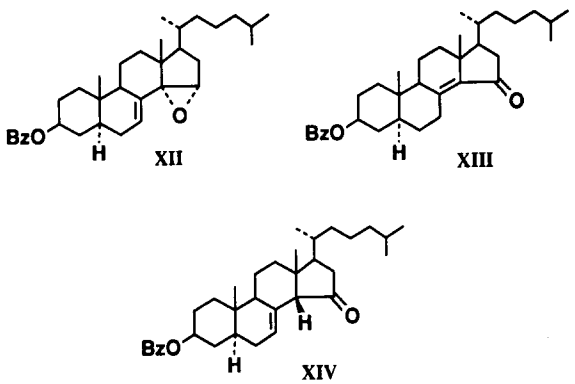

Fig. 4. Structures of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene (**XII**), 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (**XIII**) and 3β-benzoyloxy-5α,14β-cholest-7-en-15-one (**XIV**).

2.2. Isomerization of **III** *to a 5:1:1 mixture of (25R)-3β,26-diacetoxy-5α-cholesta-7,14-diene (***IV***), (25R)-3β,26-diacetoxy-5α-cholesta-8,14-diene (***V***) and (25R)-3β,26-diacetoxy-5β-cholesta-8,14-diene (***VI***)*

A solution of $\Delta^{5,7}$ diacetate **III** (2.60 g, 5.36 mmol) in a mixture of $CHCl_3$ (30 ml) and CH_2Cl_2 (10 ml) was prepared in a 100-ml three-necked flask fitted with a thermometer and a gas dispersion tube. The flask was rapidly cooled to −55°C, HCl gas was bubbled through the solution for 15 min, and the reaction mixture was stirred at −60°C for an additional 20 min. The cold reddish-brown solution was poured into cold concentrated NH_4OH solution (25 ml), and the organic phase was washed with water (3 × 100 ml). Pyridine (12 drops) was added to the lower phase, which was concentrated to a brown oil. Trituration with methanol (25 ml) gave a white solid (1.769 g), shown by ^{1} H-NMR analysis to be a 5:1:1 mixture of **IV**, **V** and **VI**.

In a similar experiment using 200 mg of **III**, the crude product (~60% **IV** by HPLC analysis) was subjected to reversed-phase MPLC (elution with water-methanol, 5:95). Evaporation of fractions 47–56 gave **IV** (116 mg, 84% purity by HPLC), which was again purified by reversed-phase MPLC (elution with methanol). Evaporation of fractions 26–27 gave **IV** (35 mg, 95% purity). Attempts to purify **IV** by recrystallization from methanol were unsuccessful, as the mother liquor and crystals both showed 95% purity by ^1H-NMR. The $\Delta^{7,14}$ diacetate **IV** (95:5 mixture of **IV** and **V** by ^1H-NMR analysis) was characterized as follows: m.p. 98–99°C; $[\alpha]_D^{23}$ −150.7° (*c*, 0.6); TLC, single component in 1:9 ethyl acetate-hexane (R_f 0.55) and 5:95 ether-benzene (R_f 0.68); single component on HPLC; IR, ν_{max} 3034, 3016, 1738, 1238, 1030 cm^{-1}; MS, *m/z* 484 (29, M^+), 469 (20, M−CH_3), 424 (12, M−CH_3COOH), 409 (16 M−CH_3COOH−CH_3), 341 (5), 339 (4), 313 (100, M−SC), 253 (23, M−SC−CH_3COOH); high-resolution MS, calcd. for $C_{31}H_{48}O_4$ 484.3553, obsd. 484.3548; ^1H and ^{13}C-NMR, Tables 1 and 2.

Evaporation of MPLC fractions 22–23 gave a 5:1 mixture of the 5β isomer **VI** (15 mg) and an unidentified 3β-acetoxy-5α-sterol diene: partial ^{13}C-NMR: δ_C 163.6 (C), 158.5 (C), 121.1 (CH), 117.7 (CH), 73.5 (CH), 57.2 (CH), 53.4 (C), 44.4 (CH), 12.2 (CH_3). ^1H and ^{13}C-NMR assignments for **VI** are given in Tables 1 and 2.

Table 1
^{1}H-NMR chemical shifts of **I** and 3β,26-diacetate intermediates in its synthesis [a]

	5α-Δ^{7,14} **IV**	5β-Δ^{8,14} **VI**[b]	5α,14β-Δ^7 15-keto **IX**	5α,14β-Δ^8 15-keto **X**	5β,14β-Δ^8 15-keto **XI**	5α-Δ$^{8(14)}$ 15-keto **VIII**[c]	5α-Δ$^{8(14)}$ 15-keto **I**
H-1α	1.12	1.63‡	1.08	1.20	1.68†	1.25	1.20
H-1β	1.86	1.63‡	1.79	1.82	1.44†	1.74	1.73
H-2α	1.84	1.57‡	1.83	1.88	1.21‡	1.86	1.85
H-2β	1.49	1.57‡	1.45	1.54	1.68†	1.46	1.37
H-3α	4.69	4.94	4.67	4.70	4.98	4.73	3.64
H-4α	1.75	1.72‡	1.75	1.70	1.47	1.71	1.68
H-4β	1.33	1.72‡	1.33	1.41†	1.55	1.34	1.27
H-5α	1.48	1.68†	1.58	1.43†	1.73	1.48	1.42
H-6α	1.86	1.57‡*	1.95	1.47	1.36†	1.48	1.48
H-6β	1.77	1.80‡*	1.74	1.37	1.92†	1.34	1.35
H-7α	5.74	2.40‡*	5.41	2.18	2.17	1.59	1.58
H-7β		1.98‡*		1.88	1.80	4.13	4.14
H-9α	1.75		1.47			1.87	1.85
H-11α	1.60	2.03‡	1.51	2.08†	2.06‡	1.65	1.65
H-11β	1.48	2.22‡	1.32†	2.04†	2.06‡	1.53	1.54
H-12α	1.32	1.39†	1.27†	1.90	1.86	1.25	1.25
H-12β	2.03	2.02†	1.44	1.29	1.33	2.10	2.10
H-14			2.68	2.30	2.32		
H-15	5.50	5.38					
H-16α	2.31	2.37†	2.44	2.41	2.39	2.34	2.35
H-16β	1.91	2.06†	2.25	1.98	1.99†	2.05	2.05
H-17α	1.58	1.53	1.79	1.99	1.99†	1.46	1.46
H-18	0.827	0.828	1.128	0.970	0.987	0.974	0.974
H-19	0.801	1.125	0.771	0.963	1.075	0.732	0.717
H-20	1.59	1.63	1.93	1.54	1.59†	1.58	1.59
H-21	0.925	0.943	0.894	0.990	0.989	1.001	1.002
H-22R	1.37	1.38‡	1.31	1.33‡	1.33‡	1.33	1.32†
H-22S	1.07	1.08‡	1.00	1.04	1.03	1.09	1.10
H-23	1.26	1.25‡	1.27†	1.25	1.26	1.27	1.27†
H-23	1.34	1.32‡	1.38†	1.37†	1.34	1.32	1.32†
H-24	1.14	1.15‡	1.14	1.13	1.14†	1.13	1.10
H-24	1.32	1.35‡	1.32	1.31†	1.32†	1.32	1.32†
H-25	1.77	1.78	1.75	1.77	1.76	1.76	1.61
H-26	3.849	3.849	3.846	3.847	3.849	3.840	3.425
H-26	3.944	3.945	3.929	3.935	3.931	3.928	3.495
H-27	0.922	0.923	0.915	0.919	0.916	0.917	0.913
3-Ac	2.026	2.040	2.015	2.017	2.051*	2.025	
26-Ac	2.054	2.054	2.052	2.049	2.057*	2.056	

[a]Data from ^{1}H spectra at 300 and 500 MHz in $CDCl_3$ solution at a concentration of 0.01–0.2 M or from HETCOR spectra. Estimated accuracy of chemical shifts: ±0.01 ppm except for values marked by † (±0.02 ppm) or ‡ (±0.05 ppm). Asterisks indicate that stereochemical or other assignments are uncertain. Coupling constants in Hz (average values, deviations ≤0.2 Hz): H-1α (**I**, **IV**, **IX**, **X**) td, 13.7, 3.7; H-1β (**I**, **IV**, **IX**, **X**) dt, 13.2, 3.5 (**XI**) td, ~13.1, ~2.3; H-2β (**IV**) dddd, 13.9, 12.3, 11.7, 3.7; H-3α (**I**, **IV**, **IX**, **X**) tt, 11.3, 4.7 (**XI**) quintet, 3.5; H-4α (**I**, **IV**) dddd, 12.4, 4.9, 3.6, 2.2 (**XI**) ddd, 14.4, 11.3, 3.2; H-4β (**IV**, **IX**) td, 12.4, 11.6 (**XI**) dtd, 14.5, 4.2, 1.5; H-5α (**I**) tt, 12.2, 3.0 (**XI**) dq, 11.4, 3.9; H-6α (**IX**) dtd, 17.6, 5.0, 1.7 (**XI**) ddt, ~13.9, ~6.5, ~3.2; H-6β (**IX**) dddd, 17.4, 11.7, 3.7, 2.0; H-7 (**IV**, **IX**) dt, 5.2, ~2.3; H-7β (**I**) ddd, 14.3, 4.2, 2.1 (**XI**) dt, ~15.6, 1.5; H-9α (**I**) dd, ~10.5, 7.1; H-11α (**I**) ddt, 14.0, 7.1, 3.5; H-11β (**IV**) br qd, 13.1, 3.1; H-12α (**X**) ddd, 13.7, 5.2, 3.4 (**XI**) dt, 13.5, 4.7; H-12β (**II**, **IV**) dt, 12.8, 3.4; H-14β (**IX**, **X**, **XI**) br s; H-15 (**IV**) dd, 3.4, 1.9; H-16α (**I**) dd, 18.4, 7.8 (**IV**) ddd, 15.8, 7.2, 3.5 (**IX**) ddd, 20.0, 2.7, 1.6 (**XI**) dd, ~17.7, ~7.6; H-16β (**I**) dd 18.4, 12.5 (**IV**) dd, ~15.8, ~8.7 (**IX**) dd, 20.0, 10.1; H-17α (**IX**) dt, 10.1, 2.9; H-18 (s); H-19 (s); H-21 (**I**, **IX**, **X**, **XI**) d, 6.7 (**IV**) d, 6.2; H-26 (upfield signal) dd, 10.7, 6.7; H-26 (downfield signal) dd, 10.7, 6.0; H-27, d, 6.8.

[b]Chemical shifts of relatively low precision, derived only from HETCOR spectrum and 1D spectrum at 300 MHz.

[c]Chemical shifts from an HSQC spectrum of an authentic sample of **VIII**.

2.3. (25R)-3β,26-Diacetoxy-5α-cholest-8(14)-en-15-one (VIII), (25R)-3β,26-diacetoxy-5α,14β-cholest-7-en-15-one (IX), (25R)-3β,26-diacetoxy-5α,14β-cholest-8-en-15-one (X) and (25R)-3β,26-diacetoxy-5β,14β-cholest-8-en-15-one (XI)

A solution of the crude $\Delta^{7,14}$ diacetate **IV** (1.769 g; 3.65 mmol; 85% purity by HPLC) in *tert*-butyl methyl ether (50 ml) was cooled to 0°C, and a cooled solution of *m*-chloroperbenzoic acid (1.259 g; 7.3 mmol) in *tert*-butyl methyl ether (5 ml) containing $NaHCO_3$ (613 mg; 7.3 mmol) was added with stirring. The reaction mixture was stirred for 40 min under nitrogen, followed by washing with 10% Na_2SO_3 solution (2 × 50 ml) until the organic phase tested negative to starch-iodide paper. The organic phase was washed with 5% $NaHCO_3$ (50 ml) and water (3 × 150 ml) and dried over Na_2SO_4. Evaporation gave (25R)-3β,26-diacetoxy-14α,15α-epoxy-5α-cholest-7-ene (**VII**) as a colorless oil (1.788 g), which was immediately hydrolyzed with $HClO_4$ (see below). The crude epoxide product of ~70% purity from a separate experiment was characterized by ^{13}C-NMR (Table 2) and ^{1}H-NMR: δ 5.59 (br s, H-7), 4.68 (br tt, ~11, ~5 Hz, H-3α), 3.93 (dd, 10.7, 6.1 Hz, H-26), 3.84 (dd, 10.7, 6.8 Hz, H-26), 3.68 (s, H-15), 2.05 (s, Ac), 2.02 (s, Ac), 0.914 (d, 6.7 Hz, H-27), 0.858 (s, H-19), 0.754 (s, H-18).

Acetone (50 ml) containing concentrated $HClO_4$ (50 μl) was added to crude epoxide **VII** (1.78 g), and the resulting solution was stirred under nitrogen at room temperature for 2 h. The organic phase was diluted with ethyl acetate (100 ml), washed with 5% $NaHCO_3$ (100 ml) and water (3 × 100 ml), dried (Na_2SO_4) and evaporated to a colorless oil (1.772 g). The crude product was adsorbed onto 5 g of silica gel and subjected to MPLC on silica gel (elution with ethyl acetate-hexane, 6:94). Evaporation of fractions 91–168 gave $\Delta^{8(14)}$-15-keto-diacetate **VIII** as a white solid (880 mg, 33% yield from **III**) showing >99% purity by ^{1}H-NMR: m.p. 110.5–111.5°C, lit. m.p. 110.5–111.5°C [19]; IR, ν_{max} 1738, 1699, 1624, 1260, 1085, 1031 cm^{-1}; TLC, single component in 2:8 ethyl acetate-hexane (R_f 0.46) and 3:7 ethyl acetate-hexane (R_f 0.70); HPLC (1:9 water-methanol, UV detection at 259 nm), t_R 18.2 min, 99% purity; high-resolution MS, calcd. for $C_{31}H_{48}O_5$, 500.3502, found, 500.3497; MS, Table 3; ^{1}H-NMR chemical shifts (Table 1) essentially identical to those reported previously [2,19].

Evaporation of fractions 188–230 gave an oil (151 mg), which was found to be >95% **IX** by ^{1}H and ^{13}C-NMR. Preparative HPLC (250 mm × 21.4 mm i.d. C_{18} column, elution with 1:9 water-methanol) of a portion of this material (90 mg) gave, in addition to fractions of lower purity, an analytical sample of the 14β-Δ^7-15-keto-diacetate **IX** as an oil (15 mg): $[\alpha]_D^{23}$ −4.1° (*c*, 0.7); IR, ν_{max} 3055, 1736, 1244, 1032, 737 cm^{-1}; TLC, single component in 2:8 ethyl acetate-hexane (R_f 0.46) and 5:95 ether-benzene (R_f 0.30); HPLC (1:9 water-methanol; UV detection at 210 nm), t_R 14.0 min, >99% purity; high-resolution MS, calcd. for $C_{31}H_{48}O_5$, 500.3502, found, 500.3488; ^{1}H and ^{13}C-NMR and MS, Tables 1–3.

Evaporation of fractions 69–85 gave a white solid (180 mg) that was shown by ^{1}H and ^{13}C-NMR to be a 4:6 mixture of the 5α,14β-Δ^8-15-keto-diacetate **X** and the 5β,14β-Δ^8-15-keto-diacetate **XI**. Preparative HPLC (250 mm × 9.4 mm i.d. C_{18} column, elution with 18:82 water-methanol) of 90 mg of this mixture gave analytical samples of **X** (10 mg) and **XI** (5 mg), each of >99% purity by ^{1}H-NMR.

Compound **X** was characterized as an oil: $[\alpha]_D^{23}$ −51.6° (*c*, 0.8); IR, ν_{max} 1736, 1699, 1245, 1095, 1030 cm^{-1}; TLC, single component in 2:8 ethyl acetate-hexane (R_f 0.44) and 5:95 ether-benzene (R_f 0.28); HPLC (1:9 water-methanol; UV detection at 210 nm), t_R 15.1 min, >99% purity; high-resolution MS, calcd. for $C_{31}H_{48}O_5$, 500.3502, found, 500.3495; ^{1}H and ^{13}C-NMR and MS, Tables 1–3.

Compound **XI** was characterized as an oil: $[\alpha]_D^{23}$ −45.2° (*c*, 0.7); IR, ν_{max} 1734, 1699, 1240, 1028 cm^{-1}; TLC, single component in 2:8 ethyl acetate-hexane (R_f 0.44) and 5:95 ether-benzene (R_f 0.26); HPLC (1:9 water-methanol, UV detection at 210 nm), t_R 14.1 min, 99% purity; high-resolution MS, calcd. for $C_{31}H_{48}O_5$, 500.3502, found, 500.3483; ^{1}H and ^{13}C-NMR and MS, Tables 1–3.

The following additional fractions were analyzed by ^{1}H and ^{13}C-NMR: fractions 20–25 (67 mg),

Table 2
^{13}C-NMR chemical shifts of 3β,26-diacetate intermediates and byproducts in the synthesis of **I**[a,b]

	5α-Δ^{7,14} **IV**	5β-Δ^{8,14} **VI**	5α,14α-Δ^{7} epoxide **VII**	5α,14β-Δ^{7} 15-keto **IX**	5α,14β-Δ^{8} 15-keto **X**	5β,14β-Δ^{8} 15-keto **XI**
C-1	36.43	30.96	36.14	36.07	33.89	30.21
C-2	27.48	27.31	27.21	27.22	27.19	27.39
C-3	73.25	70.54	73.51	73.19	73.27	70.21
C-4	33.67	32.74[b]	33.68	33.45	33.72	31.10[b]
C-5	39.41	38.71[b]	39.08	39.31	40.88	35.96[b]
C-6	30.08	24.21[b]	29.05	29.76	24.98	22.96[b]
C-7	119.97	24.21[b]	122.35	126.57	29.03	25.15[b]
C-8	134.31	123.74	134.10	132.06	121.75	123.14
C-9	49.47	138.07[b]	47.78	47.09	138.77	134.06
C-10	33.78	36.88	33.73	33.76	36.96	37.37
C-11	20.82	22.54	20.21	20.37	21.08	21.36
C-12	39.99	36.77	33.39	37.51	31.98	32.22
C-13	46.37	44.99	40.24	42.09	40.82	40.81
C-14	151.83	150.60	72.92	64.32	65.47	65.06[b]
C-15	119.44	117.48	58.74	218.92	217.82	217.75
C-16	35.09	35.78	31.76	36.83	40.62	40.20
C-17	58.55	57.05	47.23	48.97	42.25	43.14
C-18	16.43	15.33	14.45	19.65	22.39	21.86
C-19	12.20	25.77[b]	12.53	12.61	17.24	28.43[b]
C-20	33.97	33.83	33.55	33.16	34.51	34.38
C-21	18.79	18.73	18.57	19.14	18.84	18.87
C-22	35.84	35.85	35.28	31.58	35.41	35.11
C-23	23.13	23.05	22.86	24.82	23.75	23.70
C-24	33.64	33.65	33.51	33.45	33.51	33.50
C-25	32.39	32.39	32.24	32.45	32.37	32.43
C-26	69.51	69.46	69.30	69.28	69.43	69.37
C-27	16.70	16.68	16.54	16.63	16.71	16.67
3β-Ac	170.58	170.57	170.97	170.50	170.61	170.74
	21.39	21.40	21.19	21.35	21.41	21.48
26-Ac	171.23	171.15	170.26	171.21	171.28	171.29
	20.93	20.88	20.75	20.89	20.97	20.97

[a]Chemical shifts referenced to the $CDCl_3$ signal at 77.0 ppm. Data obtained at 75 MHz in $CDCl_3$ solution at a concentration of 0.05–0.2 M.

[b]Signals broadened in room temperature spectra, reflecting large chemical shift differences between conformers A and B.

4:1 mixture of Δ^{7,14} diacetate **IV** and its Δ$^{6,8(14)}$ isomer (identified by comparison with ^{1}H and ^{13}C-NMR data for 3β-benzoyloxy-5α-cholesta-6,8(14)-diene [28]); fractions 26–31 (34 mg), mixture of two unidentified diacetates (δ_H 5.39 (br s), 5.19 (br s), δ_C 153.2, 148.8, 120.7, 117.9, 61.9, 58.5, 57.8, 52.6, 51.3, 12.6, 11.7); fractions 50–68 (97 mg), 1:1:1 mixture of **X**, **XI** and an unidentified sterol (δ_H 5.62 (br s), δ_C 137.6, 119.3, 109.9, 97.0, 14.6, 12.9); fractions 86–90 (43 mg), 9:1 mixture of **VIII** and **XI**. After fraction 230, elution with 1:1 ethyl acetate-hexane gave an unidentified hydroxy-keto-aldehyde diacetate (307 mg; impure) with the following distinctive NMR signals: δ_H

Table 3
Ion abundances in the mass spectra of unsaturated 15-keto 3β,26-diacetates with suggested assignments[a]

	5α-$\Delta^{8(14)}$ VIII	5α,14β-Δ^{7} IX	5α,14β-Δ^{8} X	5β,14β-Δ^{8} XI
$M^+(C_{31}H_{48}O_5)$	500* (100)	500* (100)	500* (100)	500* (2)
$M-CH_3$	485* (10)	485* (17)	485* (16)	485 (0.4)
$M-H_2O$	482* (6)	482 (1)	482 (1)	
$M-CH_3COOH$	440* (9)	440* (70)	440* (78)	440* (100)
$M-CH_3COOH-CH_3$	425* (21)	425* (77)	425* (90)	425* (18)
$M-CH_3COOH-H_2O$	422* (6)	422* (5)	422* (5)	422 (5)
$M-CH_3COOH-H_2O-CH_3$	407* (7)	407* (27)	407* (31)	407* (9)
m/z 399, $C_{26}H_{39}O_3$		399 (4)	399* (4)	399* (3)
m/z 398	398 (1)	398 (4)	398 (5)	398 (3)
m/z 397	397 (2)	397 (3)	397 (4)	397 (2)
RDA		386* (13)	386* (17)	386* (53)
m/z 383, $C_{26}H_{39}O_2$	383* (11)	383* (5)	383* (7)	383* (3)
$RDA-H_2O$	368 (1)	368 (3)	368 (4)	368* (18)
$M-2CH_3COOH-CH_3$	365* (15)	365* (12)	365* (16)	365* (4)
$M-2CH_3COOH-H_2O-CH_3$	347* (3)	347* (4)	347* (5)	347 (2)
Ion B	334* (3)			
M–SC	329* (10)	329* (7)	329* (8)	
Ion $B-CH_3$	319* (4)			
$M-SC-H_2O$	311* (42)	311* (4)	311* (3)	311 (1)
Ion C	301* (8)			
m/z 275, $C_{17}H_{23}O_3$	275 (1)	275* (4)	275* (6)	
$M-SC-CH_3COOH$	269* (19)	269* (24)	269* (35)	269* (29)
*m/z*267, $C_{16}H_{27}O_3$	267 (5)	267* (7)	267* (9)[b]	267* (20)[b]
m/z 259, $C_{17}H_{23}O_2$	259 (2)	259* (5)	259* (8)	
m/z 253, $C_{18}H_{21}O$	253* (8)	253* (6)	253* (9)	253 (3)
$M-SC-CH_3COOH-H_2O$	251* (47)	251* (11)	251* (15)	251* (9)
m/z 107	107 (39)	107 (26)	107 (34)	107 (10)
m/z 105	105 (33)	105 (49)	105 (89)	105 (25)

[a]Major ions above *m/z* 100; mass spectra acquired at 70 eV by direct probe. Relative intensities as percentage of base peak. Notation for suggested assignments: SC, side chain; ion B, loss of ring A and C-6; ion C, $M-SC-CC_2H_4$; ion D, loss of side chain excluding C-20 and C-21; RDA, retro-Diels-Alder cleavage in ring A. Exact definitions of ions A, B, C and D are given in Refs. 2 and 29. Asterisks denote ions also observed in the high-resolution mass spectrum and compatible (3.5 millimass units) with the suggested assignment or formula.

[b]Additional *m/z* 267 ion corresponding to $C_{19}H_{23}O$ observed in the high-resolution mass spectrum.

9.84 (d, 1.9 Hz), 3.08 (dd, 13.0, 1.9 Hz); δ_C 221.7, 205.4 (CH), 85.4 (C), 66.5 (CH), 62.9 (CH), 60.3 (CH), 13.7 (CH_3).

2.4. *(25R)-3β,26-Dihydroxy-5α-cholest-8(14)-en-15-one* (I)

To a solution of diacetate **VIII** (300 mg; 0.599 mmol) in degassed methanol (12 ml) was added K_2CO_3 (331 mg, 2.4 mmol). The mixture was stirred under nitrogen for 3 h, diluted with ethyl acetate (50 ml) and washed with water (3 × 100 ml). The organic phase was dried (Na_2SO_4) and evaporated to a white solid (241 mg) that was purified on a silica gel column (10 g, 70–230 mesh; elution with ethyl acetate-hexane, 35:65). Evaporation of fractions 26–60 gave **I** as a white solid (226 mg; 91% yield; ≥ 99% purity by HPLC

and ^{1}H-NMR): m.p. 196–198°C, lit. 197–198°C [16]; TLC, single component in 1:1 ethyl acetate-hexane (R_f 0.26) and in 1:1 ether-benzene (R_f 0.21); HPLC (15:85 water-methanol; UV detection at 259 nm), t_R 8.0 min, 99% purity; ^{1}H-NMR, Table 1.

2.5. Hydrolysis of 3β-benzoyloxy-14α,15α-epoxy-5α-cholest-7-ene (XII)

To **XII** (1 g) was added acetone (50 ml) containing concentrated $HClO_4$ (50 μl). The reaction was stirred under nitrogen at room temperature for 2.5 h and poured into ice-water (200 ml). The precipitate that formed was filtered, washed with 2% $NaHCO_3$ solution (2 × 100 ml) and water (500 ml) and dried in vacuo. The crude product (995 mg) was adsorbed onto silica gel (3 g) and subjected to normal-phase MPLC (elution with 4% ethyl acetate in hexane). The products were analyzed by ^{1}H and ^{13}C-NMR. Fractions 11–13 were evaporated to a complex mixture of unidentified materials (31 mg) showing numerous olefinic ^{13}C signals; fractions 14–25 gave 768 mg of 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (**XIII**), and fractions 32–41 gave 101 mg of 3β-benzoyloxy-5α,14β-cholest-7-en-15-one (**XIV**). Elution of the column with ethyl acetate gave an additional fraction (84 mg) that consisted mainly of an unidentified hydroxy-keto-aldehyde benzoate having distinctive NMR signals similar to those given above for the analogous 3β,26-diacetate by-product.

2.6. Stability of 15-ketosterol esters XIII and XIV to $HClO_4$ in acetone or K_2CO_3 in methanol

A sample of $\Delta^{8(14)}$-15-ketosterol benzoate **XIII** (1 g) in acetone (75 ml) containing concentrated $HClO_4$ (75 μl) was stirred under nitrogen at room temperature for 3 h. The reaction mixture was poured into ice-water. The resulting precipitate was washed with 5% sodium bicarbonate (100 ml) and water (500 ml) and dried in vacuo to a white solid (988 mg). A similar reaction done using 750 μl of $HClO_4$ gave 984 mg of white solid. Both products consisted solely of **XIII** as judged by TLC and ^{1}H and ^{13}C-NMR. The estimated detection limit for minor sterol components was ~1%.

Acetone (5 ml) containing concentrated $HClO_4$ (5 μl) was added to 14β-Δ^7-15-ketosterol benzoate **XIV** (5 mg), and the reaction mixture was stirred at room temperature. After 3 h, an aliquot was removed, diluted with ethyl acetate, washed with sodium bicarbonate and water, dried and evaporated to an oil. ^{1}H-NMR showed **XIV** and **XIII** (11:1 ratio). After stirring overnight, the remainder of the reaction mixture was diluted with ethyl acetate (25 ml), washed with 5% $NaHCO_3$ (10 ml) and water (3 × 25 ml), dried, and evaporated to an oil containing **XIV** and **XIII** (6:4 ratio) by ^{1}H-NMR analysis.

A mixture of **XIV** (5 mg), methanol (1 ml, degassed), tetrahydrofuran (0.5 ml, degassed) and K_2CO_3 (5.5 mg) was stirred overnight under argon, poured into water and extracted with ethyl acetate (25 ml). The organic extracts were dried and evaporated to an oil (4 mg) that was shown by ^{1}H-NMR analysis to be a 1:1 mixture of **XIII** and **II**.

3. Discussion

The improved synthesis of **I** featured herein is based on methodology used in an established, efficient synthesis of the parent 15-ketosterol **II** from 7-dehydrocholesterol [23,31]. This route consists of acid isomerization to the $\Delta^{7,14}$ diene, formation of the Δ^7-14α,15α-epoxide and hydrolysis to the desired 15-ketosterol. Thus, isomerization of the $\Delta^{5,7}$ diacetate **III** with HCl in chloroform-dichloromethane at −60°C gave a crystalline product consisting of the desired $\Delta^{7,14}$ diene **IV** together with the 5α-$\Delta^{8,14}$ and 5β-$\Delta^{8,14}$ isomers **V** and **VI** in a 5:1:1 ratio. Although **IV** can be partially purified by reversed-phase MPLC, this separation is impractical on a large scale. Epoxidation of the crude diene mixture with *m*-chloroperbenzoic acid gave a product composed mainly of the Δ^7-14α,15α-epoxide **VII** as judged by ^{1}H and ^{13}C-NMR analysis. In the synthesis of **II** [23], recrystallization of the epoxide intermediate is a crucial step that removes impurities derived from 5α-$\Delta^{8,14}$ and 5β-$\Delta^{8,14}$ byproducts of the diene isomerization and thereby simplifies the purification of the final $\Delta^{8(14)}$-15-ketosterol product. The epoxide purifica-

tion was not possible in the present case, because neither diacetate **VII** nor the corresponding dibenzoate[1] could be induced to crystallize. Hydrolysis of the crude epoxide **VII** gave a complex mixture consisting of the desired diacetate **VIII** together with many polar and nonpolar byproducts, including the 5α,14β-Δ^7 (**IX**), 5α,14β-Δ^8 (**X**) and 5β,14β-Δ^8 (**XI**) isomers of **VIII**. Fortunately, isolation of **VIII** from this potentially intractable mixture was readily achieved on normal-phase MPLC. However, isomers **IX**, **X** and **XI** were obtained as mixtures, and their isolation required reversed-phase chromatography. It is noteworthy that no isolated component or mixture of 15-keto isomers showed NMR signals expected for a 5β-$\Delta^{8(14)}$-15-ketosterol.[2]

In an earlier synthesis of the parent 15-ketosterol **II** [23], the epoxide function and benzoate ester were hydrolyzed concurrently by refluxing with a mixture of sulfuric acid and ethanol for 24 h. Saponification of the benzoate ester was avoided because **II** had been found to be unstable to basic conditions [32]. Subsequent experiments showed that bulk amounts[3] of $\Delta^{8(14)}$-15-ketosterols are relatively stable to base in the absence of oxygen [33], and numerous $\Delta^{8(14)}$-15-ketosterol esters have been saponified in good yield [16,19,21,31]. In the present synthesis, the acetate groups were cleaved with 4 equivalents of potassium carbonate in degassed methanol to give **I** in 91% yield. The overall yield of **I** from the $\Delta^{5,7}$ starting material **III** was 30%.

Because $\Delta^{8(14)}$-15-ketosterols are known to undergo partial deconjugation in refluxing methanol containing $HClO_4$ [29], we considered the possibility that the β,γ-unsaturated 15-ketosterol esters **IX** and **X** might have arisen partially from deconjugation of **VIII** under the hydrolysis conditions (0.1% $HClO_4$ in acetone, room temperature, 2 h). However, no traces of β,γ-unsaturated 15-ketosterol esters were detected by ^{1}H-NMR when $\Delta^{8(14)}$-15-ketosterol benzoate **XIII** of high purity was subjected to these hydrolysis conditions. Model reactions were also carried out with epoxide **XII** of high purity. Hydrolysis of **XII** with dilute $HClO_4$ in acetone for 2.5 h gave the $\Delta^{8(14)}$-15-ketosterol benzoate **XIII**, 14β-Δ^7-15-ketosterol benzoate **XIV** and an unidentified hydroxy-keto-aldehyde benzoate in an 80:11:9 ratio, isolated products that accounted for 97% of all sterol material.[4] These results indicate that, under the conditions employed, the Δ^8-15-ketosterol esters **X** and **XI** arose from epoxidation-hydrolysis of $\Delta^{8,14}$ byproducts originating in the diene isomerization of $\Delta^{5,7}$-diacetate **III**.

Analysis of the MPLC fractions from the hydrolysis of crude epoxide **VII** show that the desired $\Delta^{8(14)}$-15-ketosterol ester **VIII** represented ~50% of the product and that the 14β-Δ^7 and 14β-Δ^8 isomers **IX** and **X** were each approximately 6–9%. The overall yield in the synthesis of **II** would be markedly increased if conditions could be found to isomerize these β,γ-unsaturated ketones to the more stable $\Delta^{8(14)}$-15-keto isomer **VIII**. Model reactions with 14β-Δ^7-15-ketosterol benzoate **XIV** indicate that the 14β-Δ^7 isomer is remarkably stable to conjugation under the acidic hydrolysis conditions (0.1% $HClO_4$ in acetone). However, **XIV** was readily isomerized to the $\Delta^{8(14)}$-15-keto isomer **XIII** with K_2CO_3 in methanol, conditions that also partially hydrolyzed the benzoate group. Thus, the yield of **II** can probably be increased by methanolysis of the 14β-Δ^7 isomer **IX**, which is easily isolated by MPLC. Conversion of the 14β-Δ^8 isomer **X** to **VIII** or **II** is more problematical, because it is obtained from MPLC as a mixture that includes the 5β,14β-Δ^8 isomer **XI** and an additional minor component. However, treatment of a mixture of **X** and **XI** with K_2CO_3 in methanol

1. The dibenzoate analog of **VIII** was prepared from the dibenzoate analog of **III** [16] by the same sequence of reactions described here. The formation of dibenzoate analogs of **VII**, **VIII**, **IX** and **X** was observed by ^{1}H and ^{13}C-NMR. However, the benzoate analog of epoxide **VII** could not be induced to crystallize, and the benzoate protecting groups offered no advantages over the diacetate protection described here.

2. Force-field calculations indicate that the 5β,14β-Δ^8-15-ketosterol is more stable than the 5β-$\Delta^{8(14)}$-15-ketosterol by ~2 kcal/mol.

3. The observed instability of microgram quantities of 15-ketosterols to basic conditions may be due to the difficulty of completely excluding oxygen from solvents.

4. ^{1}H-NMR analysis of crude products from other hydrolyses of **XII** showed much smaller amounts of the hydroxy-keto-aldehyde benzoate and the presence of an additional sterol, possibly the 14α-Δ^7 isomer of **XIII**.

may be expected to give the 5α-$\Delta^{8(14)}$-15-ketosterol **II** and its 5α,14α-Δ^{8} isomer,[5] a mixture that may be separable by recrystallization. The presence of numerous non-polar and polar components in addition to unsaturated 15-ketosterols cautions against the otherwise promising strategy of postponing purification until after the methanolysis step.

The four unsaturated 15-ketosterol esters **VIII–XI** were characterized by TLC, HPLC, IR, optical rotation, MS, high-resolution MS and ^{1}H and ^{13}C-NMR. Two of these unsaturated 15-ketosterol esters were easily identified by their mass spectral fragmentation patterns. The spectrum of $\Delta^{8(14)}$-15-ketosterol ester **VIII** was distinguished by the absence of a retro-Diels-Alder fragment, low abundance of $M-CH_3COOH$ and $M-CH_3COOH-CH_3$ ions and high abundance of $M-CH-_2O$ and $M-SC-CH_2COOH_3COOH$ ions. The 5β-Δ^{8}-15-ketosterol ester **XI** was recognizable from its mass spectrum showing an abundant retro-Diels-Alder fragment and by the low abundance of its molecular ion. The Δ^{7} and Δ^{8}-15-ketosterol esters **IX** and **X** had essentially identical fragmentation patterns and could not be distinguished from each other. Mass spectra of Δ^{7} and Δ^{8}-15-ketosterols with a C_8H_{17} side chain have also been found to be indistinguishable [29]. Most ions of the 15-ketosterol esters of 5α configuration were analogous to those observed for the corresponding sterols having a C_8H_{17} side chain [29,33].

^{1}H and ^{13}C-NMR data (chemical shifts, coupling constants, homonuclear and heteronuclear shift correlations and NOE results) were fully compatible with the structures shown in Fig. 3 and the signal assignments presented in Tables 1 and 2. Precise chemical shifts and stereochemical assignments not previously available for ^{1}H resonances of $\Delta^{7,14}$ sterols and unsaturated 15-ketosterols[6] were established by chemical shift comparisons, COSYDEC, NOE and saturation difference spectroscopy and HSQC or HMQC. However, even these powerful experiments were insufficient to establish accurate ^{1}H chemical shifts and stereochemical assignments for the 5β-$\Delta^{8,14}$ diacetate **VI**. Normally, 5β sterols exist as a mixture of two conformers, designated A and B [34]. In conformer A, the usual conformation for 5β sterols, ring A is a chair with the 3β substituent in the axial position, and ring B is a 5α-sofa. In conformer B, ring B is a 5β,6α half-chair, and ring A is a chair with an equatorial 3β substituent. In rings A and B, equatorial substituents in conformer A become axial in going to conformer B, and axial substituents become equatorial. The two conformers interconvert rapidly on the NMR time scale at room temperature, making NOE results difficult to interpret. When the population of the two conformers is roughly equal, the usual distinctions between axial and equatorial protons are blurred, and geminal protons have similar chemical shifts and coupling constants. This situation results in networks of strongly coupled protons, further complicating the spectral analysis. Because 5β-$\Delta^{8,14}$ diacetate **VI** appears to have similar populations of conformers A and B, ^{1}H-NMR assignments for **VI** (Table 1) are rather imprecise. In the case of 5β-Δ^{8}-15-ketosterol ester **XI**, conformer A predominates, as indicated by the symmetrical quintet with $J = 3.7$ Hz for H-3α. The predominance of conformer A in **XI** permitted ready identification of protons as axial or equatorial, and stereochemical assignments could be deduced from the coupling constants and chemical shifts.

In summary, an efficient, practical synthesis of (25R)-3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**I**) has been presented. This work also represents a synthesis of the diacetate derivative **VIII**, which is a key intermediate in the preparation of several 26-oxygenated $\Delta^{8(14)}$-15-ketosterols having the 25R configuration [19,35]. Potential impurities in **VIII** have been isolated, identified and characterized by spectroscopic and chromatographic methods. Analytical techniques to detect such contaminants are important for ensuring the purity of the 15-oxygenated sterols from syntheses in which diene isomerization byproducts are not removed at an intermediate step [36,37].

5. See note 2.

6. The ^{1}H stereochemical assignments and coupling constants are compatible with force-field calculations indicating that ring C of Δ^{8}-15-ketosterol esters **X** and **XI** is approximately a 12β-sofa and that the C13-C17-C20-C22 dihedral angle is *+gauche* in the 14β-Δ^{7}-15-ketosterol ester **IX**. Conformational analyses based on NMR data for other Δ^{7} and Δ^{8}-15-ketosterols of 14β configuration will be presented elsewhere.

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