Synthesis of the marine epoxy sterol 9α,11α-epoxy-5α-cholest-7-ene-3β,5,6β-triol

Anna Migliuolo, Giacomo Notaro, Vincenzo Piccialli, and Donato Sica

Dipartimento di Chimica Organica e Biologica, Università di Napoli, Napoli, Italy

*The synthesis of 9α,11α-epoxy-5α-cholest-7-ene-3β,5,6β-triol (**1**), a highly oxygenated marine sterol containing a 9,11-epoxide moiety in the nucleus, is described. Epoxy sterol **1** was synthesized from cholesta-5,7-dien-3β-ol. Oxidation of this sterol with* m-*chloroperbenzoic acid followed by hydrolysis and acetylation furnished 5α-cholest-7-ene-3β,5,6α-triol 3,6-diacetate (**2**). Mercuric acetate dehydrogenation of diacetate **2**, followed by oxidation with manganese dioxide and epoxidation with* m-*chloroperbenzoic acid, afforded 9α,11α-epoxy-3β,5-dihydroxy-5α-cholest-7-en-6-one (**5**). Reduction of **5** with lithium aluminum hydride gave the desired compound **1**. The structures of all synthetic intermediates were confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. A reassignment of resonances for carbons 1, 8, and 15 in the ^{13}C NMR spectrum of **1**, based on 2D-NMR correlation spectroscopy, has been accomplished. (Steroids* **56:**154–158, 1991)

Keywords: 9α,11α-epoxy-5α-cholest-7-ene-3β,5,6β-triol; synthesis; 2D-NMR; sterols; 9,11-epoxy marine sterol; 9α,11α-epoxy-5α-cholest-7-ene-3β-5-diol-6-one; 9α,11α-epoxy-5α-cholest-7-ene-3β,5,6α-triol; 5α-cholesta-7,9(11)-diene-3β,5-diol-6-one

Introduction

A polyhydroxylated epoxy sterol has been recently isolated from the marine gastropod *Planaxis sulcatus* and has been assigned the structure 9α,11α-epoxy-5α-cholest-7-ene-3β,5,6β-triol.[1] This sterol may be an intermediate in the biosynthesis of some highly oxygenated Δ^7-sterols, all having a common 3β,5α,6β-hydroxylation pattern, recently found in marine organisms[2–6] and, likely, the immediate precursor of 5α-cholest-7-ene-3β,5,6β,9α-tetrol, a sterol that we recently isolated from the sponge *Spongia officinalis*.[7] To verify such a hypothesis, radioactively labeled **1** is needed. We report here a synthesis of **1** that lends itself to the incorporation of radiolabels in the nucleus.

Experimental

Proton nuclear magnetic resonance (^{1}H NMR) and ^{13}C NMR spectra were recorded on Bruker Instrument Inc. WM 200, WM 270, and WM 400 spectrometers in $CDCl_3$, CD_3OD, or C_5D_5N solutions. ^{1}H Nuclear magnetic resonance spectra were recorded at 270 MHz unless otherwise mentioned. Proton chemical shifts were referenced to the residual $CHCl_3$, CH_3OH, and C_5H_5N signals (7.26, 3.31, and 8.71 ppm, respectively). Coupling constants are given in Hz. ^{13}C Nuclear magnetic resonance chemical shifts were referenced to the solvents ($CDCl_3$: 77.0 ppm; C_5D_5N: 135.5 ppm).

The multiplicity of ^{13}C NMR resonances was determined by DEPT experiments[8] that were performed using polarization transfer pulses of 90 and 135 degrees; in the first case, only signals for CH groups were obtained and, in the second case, positive signals for CH and CH_3 and negative signals for CH_2 were obtained. Bruker Aspect 3000 2D-NMR programs have been used for recording the 2D spectra. The ^{1}H-^{1}H shift correlation experiment was performed with a COSY 45 sequence.[9,10] The ^{1}H-^{13}C shift correlation experiment via ^{1}J coupling[11] was carried out, adjusting the time for the development of the polarization transfer to give maximum enhancement for J_{CH} = 135 Hz.

High resolution electron impact mass spectra (MS) were recorded on a Kratos Analytical Instruments AEI MS 902 spectrometer. Fourier transform infrared (FTIR) spectra were obtained with a Perkin-Elmer Corp. 1760-X FTIR spectrophotometer and were taken as films. Ultraviolet (UV) spectra were recorded with a

Address reprint requests to Dr. Donato Sica, Dipartimento di Chimica Organica e Biologica, Università di Napoli, Via Mezzocannone 16, I-80134, Napoli, Italy.
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Perkin-Elmer Model 550S spectrophotometer in $CHCl_3$ solutions.

High-performance liquid chromatography (HPLC) analyses were performed using a Varian Associates Inc. 2510 pump equipped with a Waters Associates dual cell refractometer using Hibar LiChrosorb Si-60 (250 × 10 mm) and Hibar Superspher RP-18 (250 × 4 mm) columns. Melting points were determined on a Reichert Thermovar type 300429 Kofler hot stage melting apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter in $CHCl_3$ solutions. Column chromatography was carried out on Merck Silica gel 40 (70-230 mesh) and 60 230-400 mesh). Thin-layer chromatography (TLC) analyses were carried out on precoated silica gel F_{254} plates (0.25 thick, Merck). Cholesta-5,7-dien-3β-ol was purchased from Fluka AG (Buchs, Switzerland).

5α-Cholest-7-ene-3β,5,6α-triol 3,6-diacetate **(2)**

Cholesta-5,7-diene-3β-ol (9.0 g) was treated with *m*-chloroperbenzoic acid (6.0 g) in chloroform (180 ml) at 4 C for 2 hours. The reaction mixture was washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated. The crude residue was hydrolyzed by heating under reflux in 5% methanolic potassium hydroxide (150 ml) for 1 hour. The crystalline material, obtained after the work-up, was chromatographed on a silica gel column using increasing amounts of methanol in chloroform as the eluent. Chloroform/methanol (96 : 4 v/v) eluted 5α-cholest-7-ene-3β,5,6α-triol (4.0 g): mp, 231 to 232 C (MeOH); $[\alpha]_D^{20}$ = +33.3° (c = 0.5) (reported[12]: mp, 231 to 232 C; $[\alpha]_D^{20}$ = +26.4° [$CHCl_3$]); FTIR, ν_{max} 3,390 cm^{-1}; ^1H NMR (C_5D_5N) δ 0.62 (3H, s, 18-CH_3), 0.87 (6H, d, J = 6.8 Hz, 26-CH_3 and 27-CH_3), 0.96 (3H, d, J = 6.4 Hz, 21-CH_3), 1.10 (3H, s, 19-CH_3), 2.98 (1H, dd, J = 12.8 and 4.7 Hz, 4-H_{eq}), 4.36 (1H, bs, $W_{1/2}$ = 7.7 Hz, 6β-H), 4.64 (1H, m, 3α-H), and 5.41 (1H, bs, $W_{1/2}$ = 5.1 Hz, 7-H).

5α-Cholest-7-ene-3β,5,6α-triol (4.0 g) was acetylated overnight at room temperature with pyridine/acetic anhydride (2 : 1 v/v). The usual work-up and purification on a silica gel column with light petroleum/ethyl acetate (86 : 14 v/v) as eluent gave pure **2** (4.1 g): mp, 196 to 198 C (MeOH); $[\alpha]_D^{20}$ = +57.7° (c = 1.0) (reported[12]: mp, 188 to 189 C [MeOH]; $[\alpha_D^{20}$ = +58.0° [$CHCl_3$]); FTIR, ν_{max} 3,456, 1,725, 1,709, 1,258, and 1,240 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.54 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.4 Hz, 26-CH_3 and 27-CH_3), 0.92 (3H, d, J = 6.0 Hz, 21-CH_3), 1.02 (3H, s, 19-CH_3), 2.02 (3H, s, acetate), 2.12 (3H, s, acetate), 4.82 (1H, bs, $W_{1/2}$ = 5.5 Hz, 6β-H), 5.09 (1H, m, 3α-H), and 5.24 (1H, bs, $W_{1/2}$ = 5.5 Hz, 7-H).

5α-Cholesta-7,9(11)-diene-3β,5,6α-triol **(3)**

5α-Cholest-7-ene-3β,5,6α-triol 3,6-diacetate **2** (2.0 g), dissolved in chloroform (17 ml), was added to a solution of mercuric acetate (3.0 g) in acetic acid (32 ml); the mixture was stirred for 22 hours at room temperature. The mixture was filtered, and the filtrate was concentrated to a small volume at reduced pressure, dissolved in ether, washed with saturated aqueous $NaHCO_3$ solution and brine, dried (Na_2SO_4), and evaporated. The crude product was hydrolyzed by treatment with 5% methanolic potassium hydroxide (100 ml) under reflux for 1 hour and worked up in the usual manner to give a product that was purified by chromatography on a silica gel column under a slight N_2 pressure with chloroform/methanol (9 : 1 v/v) as the eluent to give 5α-cholesta-7,9(11)-diene-3β,5,6α-triol **(3)** (475 mg): mp, 199 to 200 C (acetone); $[\alpha]_D^{20}$ = +90.0° (c = 0.4) (reported[12]: mp, 201 to 202 C; $[\alpha]_D^{20}$ = +100.0° [$CHCl_3$]); FTIR, ν_{max} 3,400 cm^{-1}; UV, λ_{max} 237 (ε 14,800), 243 (ε 16,100), and 252 nm (ε 9,025); ^1H NMR ($CDCl_3$) δ 0.53 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.4 Hz, 26-CH_3 and 27-CH_3), 0.91 (3H, d, J = 6.4 Hz, 21-CH_3), 1.10 (3H, s, 19-CH_3), 2.15 (1H, bd, J = 17.5 Hz, 12-H_a), 2.37 (1H, dd, J = 17.5 and 6.4 Hz, 12-H_b), 4.00 (2H, overlapping multiplets, 3α-H and 6β-H), 5.16 (1H, bs, $W_{1/2}$ = 5.1 Hz, 7-H), and 5.67 (1H, bd, J = 6.4 Hz, 11-H); MS *m/z* 416.3299 (M^+, calculated for $C_{27}H_{44}O_3$ 416.3279, 78%), 398.3225 ($C_{27}H_{42}O_2$, M^+-H_2O, 100%), 383.2909 ($C_{26}H_{39}O_2$, M^+-H_2O-CH_3, 13%), 380.3044 ($C_{27}H_{40}O$, M^+-$2H_2O$, 62%), 267.1723 ($C_{19}H_{23}O$, M^+-$2H_2O$-C_8H_{17}, 24%), and 249.1624 ($C_{19}H_{21}$, M^+-$3H_2O$-C_8H_{17}, 33%).

3β,5-Dihydroxy-5α-cholesta-7,9(11)-dien-6-one **(4)**

5α-Cholesta-7,9(11)-diene-3β,5,6α-triol **3** (500 mg) was stirred with a suspension of manganese dioxide (3.75 g) in chloroform (40 ml) at room temperature for 4 hours. The mixture was filtered, and the filtrate was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by chromatography on a silica gel column with chloroform/methanol (99 : 1 v/v) as the eluent to give 3β,5-dihydroxy-5α-cholesta-7,9(11)-dien-6-one **(4)** (76.1 mg): mp, 179 to 181 C ($CHCl_3$/MeOH 1 : 1); $[\alpha]_D^{20}$ = +148.4° (c = 0.5); FTIR, ν_{max} 3,390, 1,674, and 1,622 cm^{-1}; UV λ_{max} 292 nm (ε 23,700); ^1H NMR ($CDCl_3$) δ 0.61 (3H, s, 18-CH_3), 0.87 (6H, d, J = 6.8 Hz, 26-CH_3 and 27 CH_3), 0.93 (3H, d, J = 6.0 Hz, 21-CH_3), 1.13 (3H, s, 19-CH_3), 2.25 (1H, bd, J = 18.3 Hz, 12-H_a), 2.54 (1H, dd, J = 18.3 and 7.7 Hz, 12-H_b), 4.07 (1H, m, 3α-H), 5.67 (1H, bs, $W_{1/2}$ = 5.1 Hz, 7-H), and 6.05 (1H, bd, J = 7.3 Hz, 11-H); MS *m/z* 414.3064 (M^+, calculated for $C_{27}H_{42}O_3$ 414.3123, 49%), 386.3218 ($C_{26}H_{42}O_2$, M^+-CO, 100%), 378.2944 ($C_{27}H_{38}O$, M^+-$2H_2O$, 4%), 371.2928 ($C_{25}H_{39}O_2$, M^+-CO-CH_3, 44%), 368.3068 ($C_{26}H_{40}O$, M^+-CO-H_2O, 31%), 353.2842 ($C_{25}H_{37}O$, M^+-CO-H_2O-CH_3, 2%), 273.1832 ($C_{18}H_{25}O_2$, M^+-CO-C_8H_{17}, 4%), and 255.1669 ($C_{18}H_{23}O$, M^+-CO-H_2O-C_8H_{17}, 9%).

9α,11α-Epoxy-3β,5-dihydroxy-5α-cholest-7-en-6-one **(5)**

A solution of *m*-chloroperbenzoic acid (65 mg) in chloroform was added to a solution of 3β,5-dihydroxy-5α-cholesta-7,9(11)-dien-6-one **(4)** (90 mg) in $CDCl_3$ (25

ml) in an ice bath, and the mixture was stirred at 0 C. When the starting product disappeared (after approximately 6 hours, ^{1}H NMR monitoring), the reaction mixture was taken to dryness and washed with a saturated aqueous $NaHCO_3$ solution and brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by chromatography on a silica gel column using chloroform/methanol (99.5 : 0.5 v/v) as the eluent, to give 9α,11α-epoxy-3β,5-dihydroxy-5α-cholest-7-en-6-one **(5)** (70 mg): mp, 201 to 202 C; $[\alpha]_D^{20}$ = +121.5° (c = 0.3); FTIR, ν_{max} 3,510, 3,450, 3,350, 3,250, 1,681, and 1,632 cm^{-1}; UV, λ_{max} 252 (ε 12,040), 244 (ε 12,320), and 238 nm (ε 14,440); ^{1}H NMR (CD_3OD) δ 0.67 (3H, s, 18-CH_3), 0.88 (6H, d, J = 6.8 Hz, 26-CH_3 and 27-CH_3), 0.95 (3H, d, J = 6.0 Hz, 21-CH_3), 1.13 (3H, s, 19-CH_3), 1.52 (1H, dd, J = 14.1 and 11.5 Hz, 4-H_{ax}), 1.82 (1H, bd, J = 14.1 Hz, 2-H_{eq}), 1.88 (1H, d, J = 14.9 Hz, 12-H_{ax}), 2.05 (1H, ddd, J = 14.1, 14.1, and 4.3 Hz, 1-H_{ax}), 2.13 (1H, ddd, J = 14.1, 5.1, and 2.1 Hz, 4-H_{eq}), 2.27 (1H, dd, J = 14.9 and 6.0 Hz, 12-H_{eq}), 2.57 (1H, ddd, J = 8.1, 8.1, and 1.7 Hz, 14-H), 3.33 (1H, d, J = 6.0 Hz, 11-H), 3.91 (1H, m, 3α-H), and 5.85 (1H, d, J = 1.7 Hz, 7-H); MS *m/z* 430.3080 (M^+, calculated for $C_{27}H_{42}O_4$ 430.3072, 100%), 369.2840 ($C_{25}H_{37}O_2$, M^+-CO-H_2O-CH_3, 4%), and 317.1760 ($C_{19}H_{25}O_4$, M^+-C_8H_{17}, 18%).

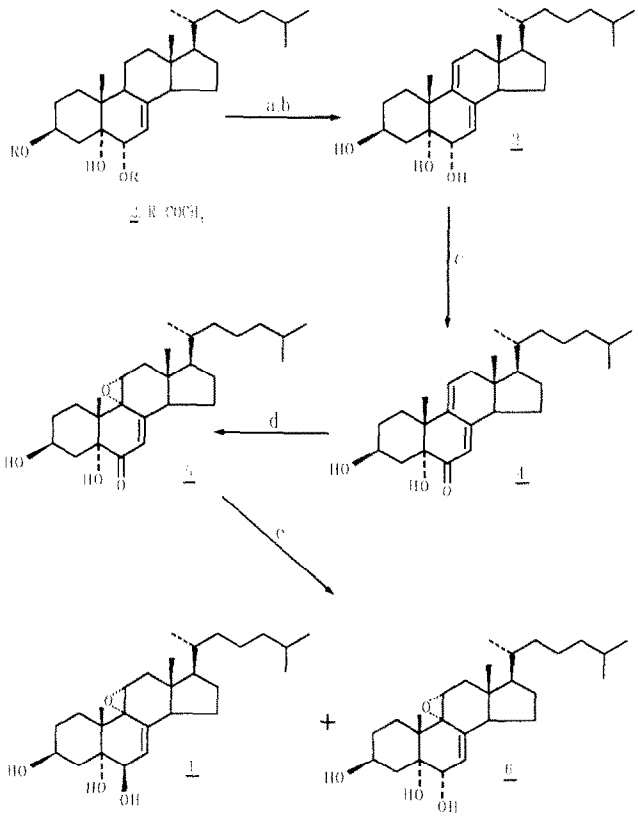
9α,11α-Epoxy-5α-cholest-7-ene-3β,5,6β-triol **(1)** *and its 6α-epimer* **(6)**

9α,11α-Epoxy-3β,5-dihydroxy-5α-cholest-7-en-6-one **5** (11.3 mg) in anhydrous ether (5 ml) was treated with excess lithium aluminum hydride at room temperature. When no starting material remained (5 minutes, TLC monitoring), water was added cautiously until a white precipitate was formed. The ether layer was decanted, washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure. Fractionation by HPLC on a Hibar LiChrosorb Si-60 (250 × 10 mm) column using chloroform/methanol (96 : 4 v/v) as eluent gave 9α,11α-epoxy-5α-cholest-7-ene-3β,5,6β-triol **(1)** (5.5 mg) and its 6α-epimer (**6**, 1.5 mg). Compound **1** had the following values: mp, 237 to 239 C (acetone) (reported[1]: mp, 250 to 252 C [$CHCl_3$/hexane 1 : 2]); $[\alpha]_D^{20}$ = −29.1° (c = 0.3, $CHCl_3$); FTIR, ν_{max} 3,390 cm^{-1}; ^{1}H NMR (C_5D_5N) see Table 1; ^{1}H NMR ($CDCl_3$) δ 0.63 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.4 Hz, 26-CH_3 and 27-CH_3), 0.90 (3H, d, J = 6.4 Hz, 21-CH_3), 1.31 (3H, s, 19-CH_3), 2.19 (1H, dd, J = 11.9 and 5.5 Hz, 12β-H), 2.33 (1H, dddd, J = 9.8, 9.8, 2.1, and 2.1 Hz, 14-H), 3.16 (1H, d, J = 5.5 Hz, 11-H), 3.91 (1H, ddd, J = 5.5, 5.5, and 2.1 Hz, 6α-H), 4.10 (1H, m, 3α-H), and 5.73 (1H, dd, J = 4.7 and 2.1 Hz, 7-H); δ ^{13}C NMR (C_5D_5N) see Table 1; MS *m/z* 432.3305 (M^+, calculated for $C_{27}H_{44}O_4$ 432.3269, 100%), 417.3006 ($C_{26}H_{41}O_4$, M^+-CH_3, 3%), 414.3143 ($C_{27}H_{42}O_3$, M^+-H_2O, 8%), 399.2985 ($C_{26}H_{39}O_3$, M^+-H_2O-CH_3, 7%), 398.3175 ($C_{27}H_{42}O_2$, M^+-O-H_2O, 3%), 396.3100 ($C_{27}H_{40}O_2$, M^+-2H_2O, 3%), 380.2995 ($C_{27}H_{40}O$, M^+-O-2H_2O, 2%), 378.2865 ($C_{27}H_{38}O$, M^+-3H_2O, 2%), 362.3029 ($C_{27}H_{38}$, M^+-O-3H_2O, 2%), 301.1840 ($C_{19}H_{25}O_3$, M^+-C_8H_{17}-H_2O, 3%), 283.1721 ($C_{19}H_{23}O_2$, M^+-C_8H_{17}-2H_2O, 2%), 265.1498 ($C_{19}H_{21}O$, M^+-C_8H_{17}-3H_2O, 3%), and 223.1085 ($C_{16}H_{15}O$, M^+-C_8H_{17}-3H_2O-42, 2%).

6α-Epimer (**6**) had the following values: mp, 216 to 218 C (H_2O-acetone); $[\alpha]_D^{20}$ = +21.8° (c = 0.4); FTIR, ν_{max} 3,430 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.58 (3H, s, 18-CH_3), 0.86 (6H, d, J = 6.8 Hz, 26-CH_3 and 27-CH_3), 0.89 (3H, d, J = 6.8 Hz, 21-CH_3), 1.19 (3H, s, 19-CH_3), 1.85 (1H, d, J = 14.9 Hz, 12-Hax), 2.17 (1H, dd, J = 14.9 and 5.5 Hz, 12-Heq), 2.31 (1H, dddd, J = 10.2, 10.2, 1.7, and 1.7 Hz, 14-H), 3.19 (1H, d, J = 5.5 Hz, 11-H), 4.00 (2H, overlapped multiplets, 3α-H and 6β-H), and 5.48 (1H, dd, J = 1.7 and 1.7 Hz, 7-H); MS *m/z* 432.3252 (M^+, calculated for $C_{27}H_{44}O_4$ 432.3269, 100%), 417.3030 ($C_{26}H_{41}O_4$, M^+-CH_3, 5%), 414.3221 ($C_{27}H_{42}O_3$, M^+-H_2O, 13%), 398.3214 ($C_{27}H_{42}O_2$, M^+-H_2O-O, 3%), 396.3020 ($C_{27}H_{40}O_2$, M^+-2H_2O, 5%), 380.3088 ($C_{27}H_{40}O$, M^+-2H_2O-O, 2%), 378.2906 ($C_{27}H_{38}O$, M^+-3H_2O, 3%), 363.2765 ($C_{26}H_{35}O$, M^+-3H_2O-CH_3, 2%), 301.1844 ($C_{19}H_{25}O_3$, M^+-C_8H_{17}-H_2O, 5%), 283.1678 ($C_{19}H_{23}O_2$, M^+-C_8H_{17}-2H_2O, 3%), and 265.1497 ($C_{19}H_{21}O$, M^+-C_8H_{17}-3H_2O, 4%).

Results and discussion

The synthesis of **1** was achieved as outlined in Scheme 1. 5α-Cholest-7-ene-3β,5,6α-triol was prepared by per-



a. $Hg(OAc)_2$, $CHCl_3$/HOAc; b. KOH/MeOH; c. MnO_2; d. mCPBA; e. $LiAlH_4$

Scheme 1

Table 1 ^{13}C nuclear magnetic resonance data for compounds **1** through **5**[a] and natural **1** and ^{1}H nuclear magnetic resonance data for compound **1**[b]

	δ_C							δ_H (m, J)
Position	**2** (R=H) (C_5D_5N)	**3** ($CDCl_3$)	**4** ($CDCl_3$)	**5** (C_5D_5N)	**1** ($CDCl_3$)	Natural **1**[c] ($CDCl_3$)	**1** (C_5D_5N)	**1** (C_5D_5N)
1	32.3[d]	30.1[f]	29.0[j]	24.1[l]	25.9	36.0	26.9	H_{ax} 2.64 (ddd, 14.0, 14.0, 3.7); H_{eq} 1.21[q]
2	32.6[d]	30.6[f]	30.0[j]	31.0	30.2	31.9	31.9	H_{ax} 2.04 (m); H_{eq} 2.27 (bd, 12.2)
3	67.2	67.0	67.1	66.6	67.2	67.3	67.2	4.80 (m)
4	41.0	42.4	42.4	37.3	40.1	41.15	41.2	H_{ax} 2.92 (dd, 12.8, 12.8); H_{eq} 2.56 (dd, 12.8, 4.3)
5	75.9	76.2	77.1	77.1	76.2	76.33	76.3	
6	70.6	70.5	199.1	199.5	72.8	73.41	73.4	4.52 (dd, 4.9, 1.9)
7	125.6	124.1[g]	118.0	125.0	125.0	126.49	127.2	6.08 (dd, 4.9, 1.7)
8	141.1	137.2[h]	145.5	158.6	138.7	135.61	137.3	
9	43.7	139.3[h]	140.8	63.8	62.7	64.01	64.0	
10	39.2	41.0	41.6	40.5	38.3	39.7	39.3	
11	21.7	121.2[g]	131.2	55.6	54.6	54.1	54.1	3.27 (d, 5.5)
12	40.0	37.5	34.8	40.2	39.4	40.8	40.8	H_{ax} 1.88 (d, 14.6); H_{eq} 2.14 (dd, 14.6, 5.5)
13	43.9	42.6	43.4	45.5	43.4	43.9	43.9	
14	55.1	51.0	52.2	48.0	46.7	47.2	47.2	2.51 (bdd, 11.0, 11.0)
15	23.2	23.1	22.7	22.0	22.5	24.2	22.8	H_a, H_b 1.5–1.65 (m)[q]
16	28.3	28.3	28.0	27.9	27.9	28.2	28.2	H_a 1.77 (m); H_b 1.15[q]
17	56.4	56.2	56.3	56.8	56.5	56.8	56.8	1.15[q]
18	12.3	11.3	11.4	14.2	13.7	13.9	13.9	0.69 (s)
19	17.9	24.3	23.9	20.3	21.5	22.0	22.0	1.72 (s)
20	36.49	35.9	35.8	35.8	35.7	36.0	36.0	1.29[q]
21	19.1	18.4	18.4	18.5	18.4	18.6	18.6	0.88 (d, 6.7)
22	36.55	36.0	35.9	36.0	35.8	36.20	36.2	H_a 1.11[q]; H_b 0.95 (m)
23	24.4	23.9	23.9	24.7[l]	23.8	24.17	24.2	H_a 1.28[q]; H_b 1.13[q]
24	39.8	39.4	39.4	39.6	38.4	39.72	39.7	H_a, H_b 1.10[q]
25	28.3	28.0	27.9	28.2	28.0	28.2	28.2	1.49 (m)
26	23.0[e]	22.5[i]	22.5[k]	22.6[m]	22.4[n]	22.7[o]	22.7[p]	0.85 (d, 6.7)
27	22.8[e]	22.8[i]	22.8[k]	22.9[m]	22.8[n]	23.0[o]	23.0[p]	0.85 (d, 6.7)

[a] ^{13}C nuclear magnetic resonance spectra were recorded for compounds **2** and **4** at 67.9 MHz, for **3** at 50.3 MHz, and for **5** and **1** at 100.1 MHz. Assignment of nuclear carbons for compound **2** is based on comparison with its 6β-epimer[5]; nuclear carbon assignments for **3**, **4**, and **5** are aided by literature data.[17,18] Side chain carbon assignments for compounds **2** through **5** are by analogy with cholesta-5-en-3β-ol.[17] The assignment of all protonated carbons in the ^{13}C NMR spectrum of compound **1** recorded in C_5D_5N is based on the 2D heterocorrelated spectrum via $^{1}J_{C\text{-}H}$, whereas quaternary carbons are assigned on comparison with (22E,24R)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol[5] (C-5, C-10, C-13) and literature data[17,18] (C-9). Assignments of carbons in the ^{13}C NMR spectrum of **1** recorded in $CDCl_3$ are based on comparison with the ^{13}C NMR spectrum of **1** recorded in C_5D_5N.

[b] The ^{1}H NMR spectrum of **1** was recorded at 400 MHz. Assignments are based on the ^{1}H-^{1}H COSY spectrum and decoupling experiments.

[c] Values reported in ref. 1.

[d–p] Values with identical superscripts within each column may be interchanged.

[q] Overlapped with other signals.

acid oxidation of commercially available cholesta-5,7-dien-3β-ol followed by hydrolysis as described for its Δ^{22} C-24 methyl homolog.[13,14] Subsequent acetylation with acetic anhydride in pyridine at room temperature overnight gave 5α-cholest-7-ene-3β,5,6α-triol 3,6-diacetate (**2**). Mercuric acetate dehydrogenation of diacetate **2** in chloroform/acetic acid for 22 hours at room temperature[15] produced 5α-cholesta-7,9(11)-diene-3β,5,6α-triol 3,6-diacetate (**3**; 3,6-diacetate). Hydrolysis at reflux temperature with methanolic potassium hydroxide, followed by oxidation with a suspension of manganese dioxide at room temperature,[16] afforded 3β,5-dihydroxy-5α-cholesta-7,9(11)-dien-6-one (**4**). Treatment of **4** in chloroform with *m*-chloroperbenzoic acid in an ice bath gave the keto epoxide **5**. Subsequent reaction of this product with $LiAlH_4$ at room temperature for 5 minutes gave compound **1** and its 5α-epimer **6** in the ratio of 4 : 1; these compounds were separated by HPLC. Compound **1** labeled with tritium at C-6 could be obtained from the $LiAlT_4$ reduction of the keto epoxide **5**.

The structures of compounds **1** through **6** were determined on the basis of high-resolution mass spectrometry, infrared, ^{1}H NMR, and ^{13}C NMR spectra. Further-

more, the structure of **1** was unambiguously confirmed through 2D (^{1}H-^{1}H COSY and HETCOR via $^1J_{CH}$) and homonuclear spin decoupling experiments. A complete assignment of both ^{1}H and ^{13}C NMR spectra for compound **1**, accomplished on the basis of the above experiments, is presented in Table 1, which also shows a comparative picture of ^{13}C NMR data of synthetic compounds **2** through **5** along with the ^{13}C NMR values reported[1] for the natural compound **1**.* The ^{1}H NMR values for synthetic **1** agree with those reported for the natural compound.[1] We recorded the ^{13}C NMR spectrum of **1** both in $CDCl_3$ and C_5D_5N solutions. Surprisingly, our ^{13}C NMR values from the spectrum recorded in C_5D_5N are in good agreement with those reported[1] for the ^{13}C NMR spectrum of **1** recorded in $CDCl_3$, except for carbons 1, 8, and 15. On the contrary, greater differences are observed when comparing the ^{13}C NMR spectra of synthetic and natural **1**, both recorded in $CDCl_3$ (see Table 1 for comparison). These observations can be rationalized only by assuming that the ^{13}C NMR values reported for natural **1** refer to a spectrum recorded in C_5D_5N solution and not in $CDCl_3$.

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* We could not perform a comparison with the natural product since we were unsuccessful in our efforts to reach Dr. Alam. Therefore, our comparison is based on reported data.[1]