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# Synthesis of (25*R*)-26-hydroxy-15-ketosterols☆

Hong-Seok Kim*, Dong-Il Kim[1]

*Department of Industrial Chemistry, Kyungpook National University, 1370 Sankyuk-dong, Pook-Ku, Taegu 702-701, South Korea*

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**Abstract**

(25*R*)-3$\beta$,26-Dihydroxy-5$\alpha$-cholest-8(14)-en-15-one (**1**) and (25*R*)-3$\beta$,26-dihydroxy-5$\alpha$,14$\beta$-cholest-16-en-15-one (**2**) were synthesized from (25*R*)-3$\beta$,26-dibenzoyloxy-5$\alpha$,14$\alpha$-cholest-16-ene (**4**). Oxidation of **4** with $CrO_3$-3,5-dimethylpyrazole at −20°C gave (25R)-3$\beta$,26-dibenzoyloxy-5$\alpha$,14$\alpha$-cholest-16-en-15-one (**5**) along with (25*R*)-3$\beta$,26-dibenzoyloxy-5$\alpha$-cholest-16$\alpha$,17$\alpha$-epoxide (**6**). Oxidation of **5** with selenium dioxide afforded (25*R*)-3$\beta$,26-dibenzoyloxy-5$\alpha$-cholest-8(14),16-dien-15-one (**7**) and (25*R*)-3$\beta$,26-dibenzoyloxy-5$\alpha$,14$\beta$-cholest-16-en-15-one (**8**). Selective hydrogenation of **7** followed by hydrolysis in alcoholic potassium hydroxide yielded (25*R*)-3$\beta$,26-dihydroxy-5$\alpha$-cholest-8(14)-en-15-one (**1**). Hydrolysis of **5** and **8** in alcoholic potassium hydroxide provided (25*R*)-3$\beta$,26-dihydroxy-5$\alpha$,14$\beta$-cholest-16-en-15-one (**2**). 

*Keywords:* 15-Ketosterol; Allylic oxidation; Selenium dioxide; Selective hydrogenation; Hydrolysis

## 1. Introduction

(25*R*)-3$\beta$,26-Dihydroxy-5$\alpha$-cholest-8(14)-en-15-one (**1**) has been shown to be a major metabolite of 3$\beta$-hydroxy-5$\alpha$-cholest-8(14)-en-15-one (**3**) after incubation with rat liver mitochondria [1,2] (Fig. 1). Thus, **1**, a major mitochondrial metabolite of **3**, was found to be highly active in the suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in cultured mammalian cells and in inhibiting oleoyl coenzyme A-dependent esterification of cholesterol in jejunal microsomes [2]. In continuation of our interest in the synthesis of 15-ketosterols, we investigated an alternative synthesis of **1**. Although compound **1** has already been synthesized from 3$\beta$,26-dibenzoyloxy-5$\alpha$-cholest-8(14)-ene[2] and 3$\beta$,26-diacetoxy-5$\alpha$-cholest-8,14-diene [3], this compound and its analog **2** are needed for biological evaluation. Herein, we wish to report the synthesis of two (25*R*)-26-hydroxy-15-ketosterols, **1** and **2**, from the same starting material, **4**. Sterol **2** shows close structural similarity to **1**, the difference lying in the position of the double bond.

## 2. Experimental

Melting points were measured by using a Thomas–Hoover (Thomas Scientific, USA) melting point apparatus and are uncorrected. IR spectra were recorded on a Matton GL-6030E spectrophotometer. Ultraviolet (UV) spectra were determined in methanol on a Shimadzu (Shimadzu Scientific Instrument, Kyoto, Japan) UV-2100 spectrophotometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AM-300 instrument (Bruker, Billerica, MA, USA); unless otherwise stated, all NMR were performed in $CDCl_3$ solution. The chemical shifts of $^1$H NMR spectra are given in ppm downfield from tetramethylsilane, and $^{13}$C NMR spectra were referenced to $CDCl_3$ at 77.0 ppm. $^1$H and $^{13}$C NMR assignments were made from distortionless enhancement by polarization (DEPT), $^1$H-$^1$H shift-correlated 2D NMR (COSY), $^{13}$C-$^1$H shift-correlated 2D NMR (HETCOR), and by comparison with spectra of similar sterols [2,3]. Low-resolution MS were recorded on a Shimadzu QP-1000 spectrometer with electron energy of 70 eV and direct sample introduction. High-resolution MS were measured on a JEOL KMS-DX 303 spectrometer. Elemental anal-

* Corresponding author. Tel.: +82-53-950-5588; fax: +82-53-950-6594.

*E-mail address*: kimhs@kyungpook.ac.kr (H.-S. Kim)

☆Dedicated to the memory of Prof. George J. Schroepfer Jr., who died December 11, 1998.

[1] Present address: Household & Personal Care Products R&D Institute, LG Chem./Research Park (W), Taejon, Korea.

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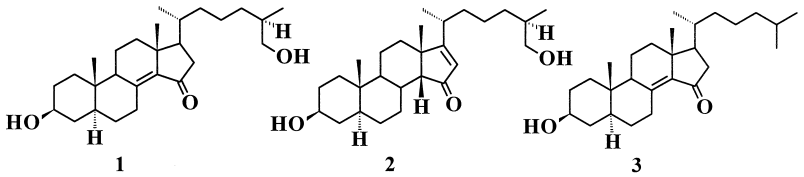


Fig. 1. Structures of 3$\beta$,26-dihydroxy-5$\alpha$-cholest-8(14)-en-15-one (**1**), 3$\beta$,26-dihydroxy-5$\alpha$,14$\beta$-cholest-16-en-15-one (**2**), and 3$\beta$-hydroxy-5$\alpha$-cholest-8(14)-en-15-one (**3**).

yses were performed by CSI at Kyungpook National University. TLC analyses were performed on precoated 0.2 mm HPTLC silica gel 60 plates (Merck, Darmstadt, Germany); substances were visualized by spraying with 5% ammonium molybdate in 10% $H_2SO_4$ followed by heating. Radial chromatography was performed on a Harrison chromatotron (Harrison Research, Palo Alto, CA, USA), by using Merck silica gel 60 $PF_{254}$. For routine column chromatography, Merck silica gel (70–230 mesh) was used as an adsorbent. Solvents were distilled before use and were dried, as necessary, by procedures in the literature [4]. Solutions were dried over anhydrous sodium sulfate. 3,5-Dimethylpyrazole (3,5-DMP), $CrO_3$, imidazole, and $SeO_2$ were purchased from Aldrich (Milwaukee, WI, USA) and used as received. Preparation of (25*R*)-3$\beta$,26-dibenzoyloxy-5$\alpha$-cholest-16-ene **4** will be described elsewhere.

### *2.1. (25*R*)-3$\beta$,26-Dibenzoyloxy-5$\alpha$,14$\alpha$-cholest-16-en-15-one (**5**)*

3,5-Dimethylpyrazole (1.34 g, 13.97 mmol) was added to a suspension of chromium trioxide (1.40 g, 13.97 mmol) in dry $CH_2Cl_2$ (5 ml) at −20°C under nitrogen, and the resulting mixture was stirred at −20°C for 30 min. A solution of **4** (300 mg, 0.49 mmol) in dry $CH_2Cl_2$ (2 ml) was added to the dark red solution. The resulting mixture was stirred at −20°C for 1 h. A 5 N sodium hydroxide solution (2 ml) was subsequently added to the reaction mixture and stirred at 0°C for 30 min. The resulting mixture was extracted twice with ethyl acetate (30 ml), and the extracts were washed sequentially with 10% HCl and water and then dried. The solvent was evaporated, and the crude product was chromatographed on silica gel (ethyl acetate/hexane 5:95, v/v). The first fraction gave (25*R*)-3$\beta$,26-dibenzoyloxy-5$\alpha$,14$\alpha$-cholest-16$\alpha$,17$\alpha$-epoxide (**6**) (76 mg, 0.12 mmol, 24%). m.p. 120.5–121.5°C (dichloromethane-methanol). Single component on TLC in two solvent systems: $R_f$ 0.63 (ethyl acetate/hexane 1:4), 0.43 (ethyl acetate/hexane 1:9). FT-IR (KBr): 2937, 2859, 1717, 1454, 1273, 1111, 714 $cm^{-1}$; $^1$H NMR: $\delta$ 8.03 (2H, m, *o* of Ph), 7.25–7.57 (3H, m, *m*, *p* of Ph), 4.93 (1H, m, 3$\alpha$-H), 4.19 (1H, dd, $J$ = 10.7, 6.0 Hz, 26-$H_a$), 4.12 (1H, dd, $J$ = 10.7, 6.5 Hz, 26-$H_b$), 3.25 (1H, s, 16$\beta$-H), 1.013 (3H, d, $J$ = 6.8 Hz, 21-$CH_3$), 0.908 (3H, d, $J$ = 6.8 Hz, 27-$CH_3$), 0.874 (3H, s, 18-$CH_3$), 0.778 (3H, s, 19-$CH_3$); $^{13}$C NMR: $\delta$ 166.6 and 166.0 (C = O), 132.8 and 132.6 (C4 of Ph), 131.0 and 130.5 (C1 of Ph), 129.5 (C2 of Ph), 128.3 and 128.2 (C3 of Ph), 74.2 (C-3), 73.4 (C-17), 69.7 (C-26), 60.0 (C-16), 54.6 (C-9), 45.0 (C-14), 44.8 (C-5), 42.6 (C-13), 36.7 (C-11), 35.7 (C-10), 35.7 (C-1), 34.1 (C-8), 33.9 (C-22), 33.7 (C-4), 33.5 (C-24), 33.0 (C-25), 31.6 (C-15), 29.2 (C-20), 28.5 (C-7), 27.5 (C-6), 27.4 (C-2), 24.9 (C-23), 20.9 (C-11), 17.0 (C-21), 16.9 (C-27), 16.1 (C-18), 12.2 (C-19); MS *m/z*: 626 (38, $M^+$), 611 (6, M-$CH_3$), 608 (5, M-$H_2O$), 504 (23, M-$C_6H_5CO_2H$), 489 (11, M-$C_6H_5CO_2H$-$CH_3$), 486 (8, M-$C_6H_5CO_2H$-$H_2O$), 393 (79, M-SC), 367 (10, M-2$C_6H_5CO_2H$-$CH_3$), 271 (16, M-SC-$C_6H_5CO_2H$), 253 (11, M-SC-$C_6H_5CO_2H$-$H_2O$); high-resolution MS *m/z*: 626.4004 for $C_{41}H_{54}O_5$ requires 626.3971. Anal. Calcd for $C_{41}H_{54}O_5$: C, 78.56; H, 8.68; Found C, 78.40; H, 8.72.

Further elution with the same solvent gave **5** (148 mg, 0.24 mmol, 49%) as a white solid. m.p. 49.5–50.5°C (dichloromethane-methanol). Single component on TLC in two solvent systems: $R_f$ 0.45 (ethyl acetate/hexane 1:4), 0.24 (ethyl acetate/hexane 1:9). FT-IR (KBr): 2937, 2859, 1713, 1455, 1273, 1107, 710 $cm^{-1}$; $^1$H NMR: $\delta$ 8.03 (2H, m, *o* of Ph), 7.26–7.57 (3H, m, *m*, *p* of Ph), 5.62 (1H, s, 16-H), 4.95 (1H, m, 3$\alpha$-H), 4.19 (1H, dd, $J$ = 10.5, 6.0 Hz, 26-$H_a$), 4.12 (1H, dd, $J$ = 10.5, 6.5 Hz, 26-$H_b$), 2.74 (1H, d, $J$ = 12.9 Hz, 7$\beta$-H), 2.42 (1H, q, $J$ = 6.8 Hz, 20-H), 1.105 (3H, d, $J$ = 6.7 Hz, 21-$CH_3$), 1.009 (3H, d, $J$ = 6.8 Hz, 27-$CH_3$), 0.993 (3H, s, 18-$CH_3$), 0.927 (3H, s, 19-$CH_3$); $^{13}$C NMR: $\delta$ 207.5 (C-15), 188.3 (C-17), 166.5 and 166.0 (C = O), 132.8 and 132.6 (C4 of Ph), 130.9 and 130.5 (C1 of Ph), 129.5 (C2 of Ph), 128.3 and 128.2 (C3 of Ph), 124.4 (C-16), 74.0 (C-3), 69.6 (C-26), 63.8 (C-14), 54.9 (C-9), 47.0 (C-13), 44.9 (C-5), 36.6 (C-12), 36.2 (C-1), 35.9 (C-10), 34.0 (C-4), 33.5 (C-24), 33.1 (C-8), 32.7 (C-22), 32.6 (C-25), 32.4 (C-20), 30.4 (C-7), 28.2 (C-6), 27.5 (C-2), 24.9 (C-23), 23.6 (C-21), 21.2 (C-18), 20.5 (C-11), 16.9 (C-27), 12.3 (C-19); UV $\lambda_{max}$: 229 nm (log $\epsilon$ 4.19); MS *m/z*: 624 (65, $M^+$), 609 (9, M-$CH_3$), 606 (2, M-$H_2O$), 502 (53, M-$C_6H_5CO_2H$), 487 (10, M-$C_6H_5CO_2H$-$CH_3$), 484 (2, M-$C_6H_5CO_2H$-$H_2O$), 391 (6, M-SC), 365 (4, M-2$C_6H_5CO_2H$-$CH_3$), 269 (13, M-SC-$C_6H_5CO_2H$), 251 (3, M-SC-$C_6H_5CO_2H$-$H_2O$); high-resolution MS *m/z*: 624.3792 for $C_{41}H_{52}O_5$ requires 624.3814. Anal. Calcd for $C_{41}H_{52}O_5$: C, 78.81; H, 8.39; Found C, 78.54; H, 8.30.

*2.2. (25R)-3β,26-Dibenzoyloxy-5α-cholest-8(14),16-dien-15-one (**7**)*

A mixture of **5** (98 mg, 0.157 mmol) and selenium oxide (87 mg, 0.78 mmol) in 2-methyl-2-propanol (5 ml) was refluxed for 5 h under nitrogen. After removal of insoluble material by filtration through a pad of celite, the filtrate was diluted with water (20 ml) and extracted with ethyl acetate (30 ml). The combined extracts were washed with brine, dried, and concentrated to give a yellow residue (109 mg). The crude product was chromatographed on silica gel (ethyl acetate/hexane 1:4). The first fraction gave 3β,26-dibenzoyloxy-5α,14β-cholest-16-en-15-one (**8**) (22 mg, 0.035 mmol, 22%) as a viscous oil. Single component on TLC in two solvent systems: $R_f$ 0.41 (ethyl acetate/hexane 1:4), 0.24 (ethyl acetate/hexane 1:9). IR (neat): 2933, 2867, 1717, 1609, 1455, 1316, 1273, 1111, 713 $cm^{-1}$; $^1H$ NMR: δ 8.03 (2H, m, *o* of Ph), 7.26–7.58 (3H, m, *m*, *p* of Ph), 5.93 (1H, 16-H), 4.92 (1H, m, 3α-H), 4.19 (1H, dd, $J$ = 10.7, 6.0 Hz, 26-$H_a$), 4.11 (1H, dd, $J$ = 10.7, 6.4 Hz, 26-$H_b$), 2.36 (1H, q, $J$ = 6.6 Hz, 20-H), 2.33 (1H, 7β-H), 1.181 (3H, s, 18-$CH_3$), 1.106 (3H, d, $J$ = 6.7 Hz, 21-$CH_3$), 1.016 (3H, d, $J$ = 6.8 Hz, 27-$CH_3$), 0.839 (3H, s, 19-$CH_3$); $^{13}C$ NMR: δ 210.3 (C-15), 191.6 (C-17), 166.6 and 166.0 (C=O), 132.8 and 132.6 (C4 of Ph), 131.0 and 130.5 (C1 of Ph), 129.5 (C2 of Ph), 128.5 (C-16), 128.3 and 128.2 (C3 of Ph), 74.2 (C-3), 69.7 (C-26), 57.2 (C-14), 48.4 (C-13), 44.6 (C-5), 44.0 (C-9), 38.1 (C-12), 36.8 (C-10), 36.1 (C-1), 34.0 (C-8), 34.0 (C-4), 33.9 (C-22), 33.5 (C-24), 32.7 (C-25), 32.6 (C-20), 29.7 (C-6), 28.9 (C-7), 27.2 (C-2), 24.9 (C-23), 24.3 (C-18), 21.1 (C-21), 19.2 (C-11), 16.9 (C-27), 10.9 (C-19); MS *m/z*: 624 (3, $M^+$), 502 (17, M-$C_6H_5CO_2H$), 487 (6, M-$C_6H_5CO_2H$-$CH_3$), 391 (3, M-SC), 365 (2, M-2$C_6H_5CO_2H$-$CH_3$), 269 (8, M-SC-$C_6H_5CO_2H$), 251 (2, M-SC-$C_6H_5CO_2H$-$H_2O$), 105 (100); high-resolution MS *m/z*: 624.3841 for $C_{41}H_{52}O_5$ requires 624.3814.

Further elution with the same solvent gave **7** (44 mg, 0.071 mmol, 45%) as a white solid. m.p. 158–159°C (dichloromethane-methanol). Single component on TLC in two solvent systems: $R_f$ 0.38 (ethyl acetate/hexane 1:4), 0.16 (ethyl acetate/hexane 1:9). FT-IR (KBr): 2944, 2867, 1717, 1679, 1636, 1601, 1451, 1316, 1277, 1176, 1111, 714 $cm^{-1}$; $^1H$ NMR: δ 8.03 (2H, m, *o* of Ph), 7.26–7.58 (3H, m, *m*, *p* of Ph), 5.93 (1H, 16-H), 5.00 (1H, m, 3α-H), 4.19 (1H, dd, $J$ = 10.7, 5.9 Hz, 26-$H_a$), 4.12 (1H, dd, $J$ = 10.7, 6.3 Hz, 26-$H_b$), 4.08 (1H, dd, $J$ = 9.9, 2.0 Hz, 7β-H), 2.39 (1H, q, $J$ = 6.8 Hz, 20-H), 1.155 (3H, s, 18-$CH_3$), 1.102 (3H, d, $J$ = 6.8 Hz, 21-$CH_3$), 1.013 (3H, d, $J$ = 6.8 Hz, 27-$CH_3$), 0.856 (3H, s, 19-$CH_3$); $^{13}C$ NMR: δ 197.3 (C-15), 186.5 (C-17), 166.6 and 166.0 (C=O), 145.2 (C-8), 137.9 (C-14), 132.8 and 132.7 (C4 of Ph), 130.8 and 130.5 (C1 of Ph), 129.5 (C2 of Ph), 128.3 and 128.2 (C3 of Ph), 127.5 (C-16), 73.8 (C-3), 69.7 (C-26), 51.2 (C-9), 45.4 (C-13), 44.4 (C-5), 38.9 (C-10), 37.0 (C-12), 36.2 (C-1), 33.7 (C-4), 33.7 (C-22), 33.5 (C-24), 32.8 (C-25), 32.6 (C-20), 30.5 (C-7), 29.3 (C-6), 27.4 (C-2), 25.0 (C-23), 24.8 (C-21), 21.7 (C-18), 19.7 (C-11), 16.9 (C-27), 12.8 (C-19); UV $\lambda_{max}$: 203 nm (log ε 4.26), 229 nm (log ε 4.49), 265 nm (log ε 4.23); MS *m/z*: 622 (100, $M^+$), 607 (3, M-$CH_3$), 500 (21, M-$C_6H_5CO_2H$), 485 (12, M-$C_6H_5CO_2H$-$CH_3$), 389 (4, M-SC), 363 (3, M-2$C_6H_5CO_2H$-$CH_3$), 267 (6, M-SC-$C_6H_5CO_2H$); high-resolution MS *m/z*: 622.3682 for $C_{41}H_{50}O_5$ requires 622.3658. Anal. Calcd for $C_{41}H_{50}O_5$: C, 79.07; H, 8.09; Found C, 79.46; H, 8.21.

*2.3. (25R)-3β,26-Dibenzoyloxy-5α-cholest-8(14)-en-15-one (**9**)*

Compound **7** (50 mg, 0.080 mmol) was dissolved in ethyl acetate (5 ml) and hydrogenated at 1 atmosphere of $H_2$ at room temperature for 3 h in the presence of 5% Pt/C (25 mg). After removal of insoluble material by filtration through a short pad of celite, the solvent was evaporated to give a solid, which was chromatographed on silica gel (ethyl acetate/hexane 1:5) and yielded a white solid **9** (36 mg, 0.058 mmol, 73%). m.p. 165–166°C (dichloromethane-methanol). Single component on TLC in two solvent systems: $R_f$ 0.52 (ethyl acetate/hexane 1:4), 0.32 (ethyl acetate/hexane 1:9). FT-IR (KBr): 2937, 2871, 1713, 1621, 1455, 1277, 1176, 1115, 714 $cm^{-1}$; $^1H$ NMR: δ 8.03 (2H, m, *o* of Ph), 7.26–7.58 (3H, m, *m*, *p* of Ph), 4.99 (1H, m, 3α-H), 4.21 (1H, dd, $J$ = 10.7, 5.9 Hz, 26-$H_a$), 4.12 (1H, dd, $J$ = 10.7, 6.5 Hz, 26-$H_b$), 4.15 (1H, d, $J$ = 16.4 Hz, 7β-H), 1.021 (3H, d, $J$ = 6.6 Hz, 21-$CH_3$), 1.013 (3H, d, $J$ = 6.7 Hz, 27-$CH_3$), 0.983 (3H, s, 18-$CH_3$), 0.784 (3H, s, 19-$CH_3$); $^{13}C$ NMR: δ 207.8 (C-15), 166.6 and 166.1 (C=O), 150.2 (C-8), 140.4 (C-14), 132.8 and 132.7 (C4 of Ph), 130.8 and 130.5 (C1 of Ph), 129.5 (C2 of Ph), 128.3 and 128.2 (C3 of Ph), 73.8 (C-3), 69.8 (C-26), 50.9 (C-9), 50.8 (C-17), 44.1 (C-5), 42.6 (C-13), 42.4 (C-16), 38.8 (C-10), 37.0 (C-12), 36.4 (C-1), 35.8 (C-22), 34.5 (C-20), 33.9 (C-4), 33.8 (C-24), 32.7 (C-25), 29.1 (C-6), 27.6 (C-7), 27.4 (C-2), 23.2 (C-23), 19.6 (C-11), 19.2 (C-21), 18.8 (C-18), 17.0 (C-27), 12.9 (C-19); UV $\lambda_{max}$: 202 nm (log ε 4.14), 229 nm (log ε 4.47), 259 nm (log ε 4.25); MS *m/z*: 624 (100, $M^+$), 609 (2, M-$CH_3$), 606 (5, M-$H_2O$), 502 (22, M-$C_6H_5CO_2H$), 487 (13, M-$C_6H_5CO_2H$-$CH_3$), 484 (3, M-$C_6H_5CO_2H$-$H_2O$), 391 (2, M-SC), 365 (9, M-2$C_6H_5CO_2H$-$CH_3$), 269 (4, M-SC-$C_6H_5CO_2H$), 251 (10, M-SC-$C_6H_5CO_2H$-$H_2O$); high-resolution MS *m/z*: 624.3843 for $C_{41}H_{52}O_5$ requires 624.3814. Anal. Calcd for $C_{41}H_{52}O_5$: C, 78.81; H, 8.39; Found C, 78.80; H, 8.30.

*2.4. (25R)-3β,26-Dihydroxy-5α-cholest-8(14)-en-15-one (**1**)*

Compound **9** (118 mg, 0.189 mmol) in 1 M 95% ethanolic potassium hydroxide solution (10 ml) was refluxed for 10 min. The reaction mixture was diluted with water (30 ml) and then extracted with chloroform (30 ml). The combined extracts were washed sequentially with 2% HCl and water, dried, and concentrated to give a solid residue (75 mg),

which was further purified with chromatotron (1 mm thick disk, ethyl acetate/hexane 3:7) and afforded a white solid **1** (71 mg, 0.171 mmol, 90%). m.p. 197–198°C ($H_2O$-methanol) (lit. [2] 197–198°C). Single component on TLC in three solvent systems: $R_f$ 0.30 (ethyl acetate/hexane 1:1), 0.22 (ethyl acetate/hexane/chloroform 4:3:3), 0.25 (diethyl ether/benzene 1:1); MS *m/z*: 416 (100, M); high-resolution MS *m/z*: 416.3318 for $C_{27}H_{44}O_3$ requires 416.3290.

### *2.5. (25*R*)-3β,26-Dihydroxy-5α,14β-cholest-16-en-15-one (**2**)*

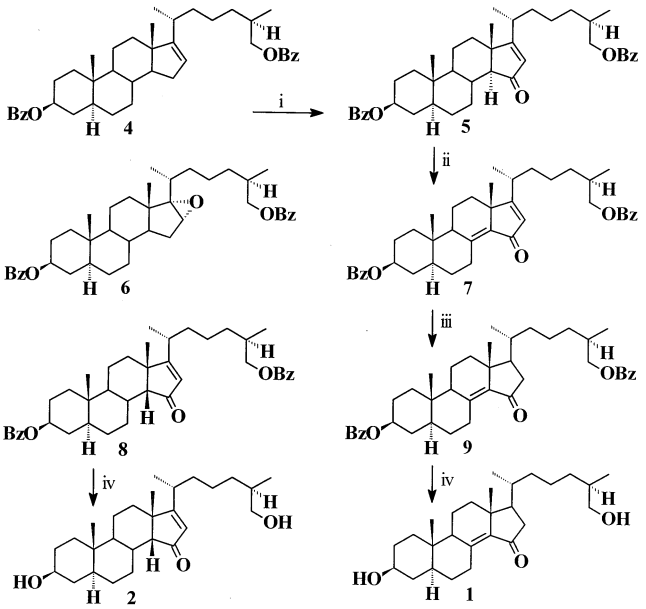
Compound **5** (39 mg, 0.063 mmol) in 1 M 95% ethanolic potassium hydroxide solution (5 ml) was refluxed for 10 min. The reaction mixture was diluted with water (20 ml) and then extracted with ethyl acetate (20 ml). The combined extracts were washed sequentially with 2% HCl and water, dried, and concentrated to give a viscous residue, which was further purified with chromatotron (1 mm thick disk, ethyl acetate/hexane 1:2) and afforded a viscous oil **2** (25 mg, 0.060 mmol, 95%). Single component on TLC in two solvent systems: $R_f$ 0.40 (ethyl acetate/hexane 2:1), 0.15 (diethyl ether/benzene 3:1). FT-IR ($CHCl_3$): 3401, 2937, 1685, 1453, 1376, 1268, 1051 $cm^{-1}$; $^1H$ NMR: δ 5.92 (1H, s, 16-H), 3.55 (1H, m, 3α-H), 3.47 (1H, dd, $J$ = 10.5, 6.0 Hz, 26-$H_a$), 3.40 (1H, dd, $J$ = 10.5, 6.3 Hz, 26-$H_b$), 1.184 (3H, s, 18-$CH_3$), 1.095 (3H, d, $J$ = 6.8 Hz, 21-$CH_3$), 0.901 (3H, d, $J$ = 6.6 Hz, 27-$CH_3$), 0.769 (3H, s, 19-$CH_3$); $^{13}C$ NMR: δ 211.1 (C-15), 192.4 (C-17), 128.2 (C-16), 70.1 (C-3), 68.0 (C-26), 57.1 (C-14), 48.3 (C-13), 44.5 (C-5), 44.0 (C-9), 38.0 (C-12), 37.9 (C-4), 36.7 (C-10), 36.6 (C-22), 36.1 (C-1), 35.5 (C-25), 33.8 (C-8), 33.1 (C-24), 32.3 (C-20), 30.8 (C-2), 29.6 (C-6), 28.9 (C-7), 24.9 (C-23), 24.3 (C-18), 20.9 (C-21), 19.0 (C-11), 16.5 (C-27), 10.8 (C-19); MS *m/z*: 416 (4, $M^+$), 288 (100, M-SC), 269 (13), 105 (22), 91 (25), 69 (51), 55 (55); high-resolution MS *m/z*: 416.3287 for $C_{27}H_{44}O_3$ requires 416.3290.

Compound **8** (12 mg, 0.019 mmol) in 1 M 95% ethanolic potassium hydroxide solution (3 ml) when reacted similarly provided **2** (6.4 mg, 0.015 mmol, 79%) as a viscous oil.

## 3. Results and discussion

Our interest in the side chain (25*R*)-26-hydroxy group in compound **1** and **2** led us to choose (25*R*)-3β,26-dibenzoyloxy-5α-cholest-16-ene **4** as our starting compound. This compound has a quite similar, yet slightly modified structure compared with **1** and **2**. The synthesis of **1** and **2** involved the Clemmensen reduction of diosgenin, hydrogenation of $\Delta^5$ of the resulting cholest-5-ene-3β,16β,26-triol, selective protection of the 3β,26-hydroxyl groups, and the elimination of the 16-hydroxyl group [5]. The resulting compound, in turn, may be transformed to our target molecule by introduction of the required functionality in the C and D rings via allylic oxidation of $\Delta^{16}$.

The synthesis of **1** described in this report is based on the well-established synthesis of the parent 15-ketosterol **3** from 5α-cholest-16-ene [6,7]. This route consists of allylic oxidation to $\Delta^{16}$-15-one, formation of the $\Delta^{8(14),16}$-15-one, selective hydrogenation of $\Delta^{16}$, and hydrolysis to the desired 15-ketosterol (Scheme 1). Thus, allylic oxidation of the




Scheme 1. Synthetic scheme for the preparation of 3β,26-dihydroxy-5α-cholest-8(14)-en-15-one (**1**) and 3β,26-dihydroxy-5α,14β-cholest-16-en-15-one (**2**).

5α-$\Delta^{16}$ **4** with $CrO_3$-3,5-dimethylpyrazole in dichloromethane at −20°C gave a white solid 14α-$\Delta^{16}$-15-one **5** along with the 16α,17α-epoxide **6** in a 2:1 ratio. Compound **5** shows IR (1713 $cm^{-1}$) and UV [$\lambda_{max}$ 229 nm (log ε 4.19)] absorptions characteristic of $\Delta^{16}$-15-one functionality. Due to the deshielding by the carbonyl group at the 15 position, the $^1H$ NMR signal of 7β-H appears at δ 2.74.

Selenium dioxide oxidation of 14α-$\Delta^{16}$-15-one **5** in 2-methyl-2-propanol yielded products that were a mixture of $\Delta^{8(14),16}$-15-one **7** (45%) and a viscous oil 14β-$\Delta^{16}$-15-one **7** (22%), which were isolated by silica gel chromatography. Oxidized compound **5** was easily isomerized to **8**. The 14-H configurations of compounds **5** and **8** were determined based on $^{13}C$ NMR data: i.e. the chemical shift of 18-$CH_3$ for **5** was at δ 21.2, and that for **8** was at δ 24.3. These values are in good agreement with those reported for the corresponding compound [6,7]. Some characteristic features of compound **7** included the appearance of infrared stretching bands at 1679 and 1636 $cm^{-1}$ for the conjugated carbonyl group as well as the $^{13}C$ NMR peaks at δ 197.3, 186.5, 145.2, 137.9, and 127.5 assignable to C-15, 17, 8, 14, and 16 atoms, respectively. The addition of the double bond

in **7** was confirmed by the bathochromic shift found in **7** ($\lambda_{max}$ 265 nm; log $\epsilon$ 4.23) compared with that in **5** ($\lambda_{max}$ 229 nm; log $\epsilon$ 4.19).

Catalytic hydrogenation of $\Delta^{8(14),16}$-15-one **7** with 5% platinum on carbon under one atmospheric pressure of hydrogen resulted in $\Delta^{8(14)}$-15-one **9** in 73% yield. Confirmation of the 17-H configuration of the latter was made by comparing the $^{13}$C NMR spectrum with that of the value reported in the literature [6,7]. The hydrogenation preferentially took place in the less-hindered $\alpha$-direction. Various spectroscopic data were consistent with the structure of **9**. For instance, $^{1}$H NMR showed one deshielded 7$\beta$-proton at $\delta$ 4.15, and two olefinic carbons appeared at $\delta$ 140.4 (C-14) and 150.2 (C-8) on $^{13}$C NMR spectrum. The absorption at 259 nm (log $\epsilon$ 4.25) confirms the existence of $\Delta^{8(14)}$-15-one. The mass spectrum also revealed a peak at *m/z* 624 corresponding to the molecular ion.

Hydrolysis of **9** in 1 M ethanolic potassium hydroxide solution gave 3$\beta$,26-dihydroxy-5$\alpha$-cholest-8(14)-en-15-one **1** in 90% yield. The structure and stereochemistry of **1** were confirmed by comparing the $^{1}$H, $^{13}$C NMR, and MS values reported in the literature [2,3].

To obtain (25*R*)-3$\beta$,26-dihydroxy-5$\alpha$,14$\alpha$-cholest-16-en-15-one, compound **5** was hydrolyzed in 1 M ethanolic potassium hydroxide solution giving (25*R*)-3$\beta$,26-dihydroxy-5$\alpha$,14$\beta$-cholest-16-en-15-one (**2**) in 95% yield. Under basic conditions, 14$\alpha$-H in **5** easily epimerized to 14$\beta$-H. Confirmation of the 14$\beta$ configuration of **2** was made by comparing the $^{13}$C NMR spectrum with that of compound **8**. The chemical shifts of 18-$CH_3$ for both **2** and **8** appeared at exactly same position, $\delta$ 24.3. Compound **2** also was prepared by hydrolysis of **8** under same conditions as for **5** in 73% yield.

In summary, in this work, we prepared two (25*R*)-26-hydroxy-15-ketosterols. Studies on the biologic activities of **1** and **2** are now in progress.

## Acknowledgment

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