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Synthesis of 7α-hydroxy derivatives of regulatory oxysterols☆

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Abstract

7α-Hydroxy derivatives of oxysterols are of considerable interest because of their possible involvement in regulation of cholesterol metabolism. This paper describes stereoselective syntheses and complete characterization of the 7α-hydroxy derivatives of four key oxysterols: 25-hydroxycholesterol, 27-hydroxycholesterol, 24(*S*)-hydroxycholesterol, and 24(*S*),25-epoxycholesterol.

Keywords: Oxysterols; 7α-Hydroxyoxysterols; Synthesis

1. Introduction

Recent evidence indicates that oxysterols are involved in regulation of cholesterol 7α-hydroxylase, the enzyme that catalyzes the rate-limiting step in the conversion of cholesterol to bile acids. Mangelsdorf's group [1] reported that specific oxysterols activate transcription of an LXRE-luciferase construct through the orphan nuclear transcription factor LXRα, and suggested that this might be part of a feedback regulation of crucial metabolic pathways, such as steroid hormone or bile acid biosynthesis. Shortly thereafter, Lehmann et al. [2], reported that the receptors LXRα and LXRβ are activated by specific oxysterols, and that they induce transcription of a cholesterol 7α-hydroxylase promoter, implicating oxysterols as regulators of this key enzyme. Mangelsdorf et al. have also described an extended investigation of structural requirements for oxysterol ligands for the LXRα and LXRβ receptors [3]. It has been observed that some of these same oxysterols are also subject to enzymatic 7α-hydroxylation [4]. It has, in fact, been suggested that such 7α-hydroxylation of 25-hydroxycholesterol (**1**) (Scheme 1) may be important to inactivate **1** as a regulator of HMG-CoA reductase and the LDL receptor [5], in another putative regulatory role for oxysterols. In support of this possibility, it has been reported that the 7α-hydroxy derivative of 27-hydroxycholesterol (**2**) has no inhibitory effect on cholesterol biosynthesis [6]. However, it has also been reported that in human diploid fibroblasts the 7α-hydroxy derivatives of both **1** and **2** do repress HMG-CoA reductase activity [7].

Further studies of the effects of 7α-hydroxylation of oxysterols on different aspects of cholesterol regulation are clearly needed. As part of such studies, it is obviously important to have available samples of the pertinent 7α-hydroxylated oxysterols. This paper describes the synthesis and complete characterization of the 7α-hydroxy derivatives of four key oxysterols: 25-hydroxycholesterol (**1**), 27-hydroxycholesterol (**2**), 24(*S*)-hydroxycholesterol (**3**), and 24(*S*),25-epoxycholesterol (**4**). These were selected on the basis of their previous implication as possible regulators of cholesterol metabolism. 25-Hydroxycholesterol (**1**) is one of the most potent oxysterols in HMG-CoA reductase repression assays [8,9] and because it is commercially available, it has been by far the most widely used "representative" oxysterol. Observations consistent with a regulatory role for **1** have recently been reported [10,11]. 27-Hydroxycholesterol (**2**) has been identified in mouse liver [12], and has been proposed as a regulator of cholesterol metabolism [13–15], although not without dispute [16]. 24(*S*)-Hydroxycholesterol (**3**) and 24(*S*),25-epoxycholesterol (**4**) appeared to be particularly promising candidate oxysterols on the basis of our earlier investigations [17,18] and the recent finding of their high affinities as ligands for the LXR receptors [1,2].

The 7α-hydroxy derivative **5** (Scheme 2) of 25-hydroxycholesterol (**1**) has been previously prepared by a different route from that described herein, requiring isolation from a mixture with its 7β-isomer [19]. The 7α-hydroxy derivative

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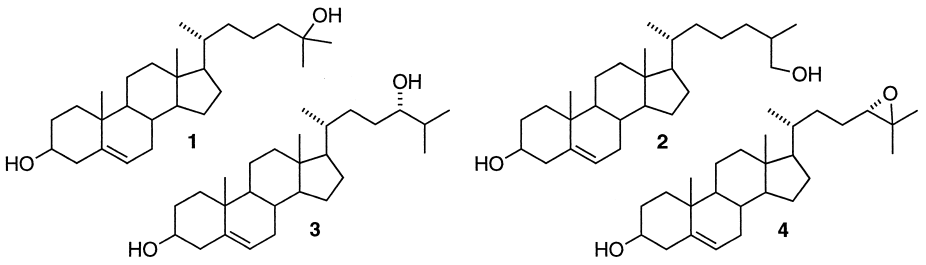


Scheme 1.

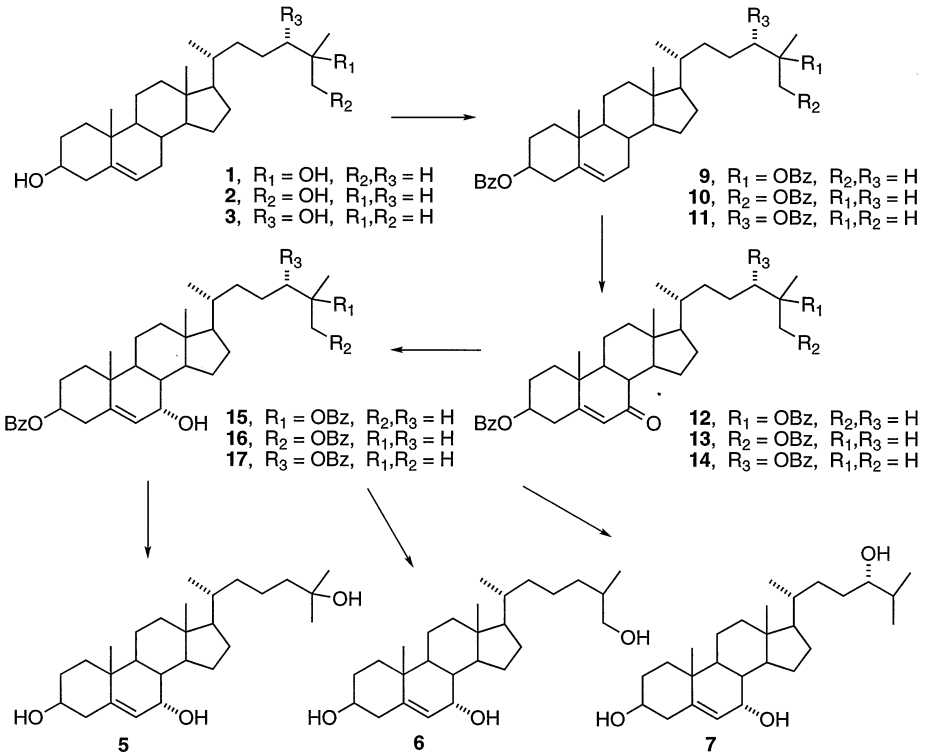
6 of 27-hydroxycholesterol (**2**) has also been reported, but without complete separation from its 7β-isomer and complete characterization [20]. During the course of this work, Corey and Grogan [21] reported preparation of 7α-hydroxy-24(*S*),25-epoxycholesterol (**8**) (Scheme 3), by a synthesis slightly different from that reported herein. This paper presents full details of stereoselective syntheses of the 7α-hydroxy derivatives **5–8** of all four key oxysterols **1–4**.

2. Experimental

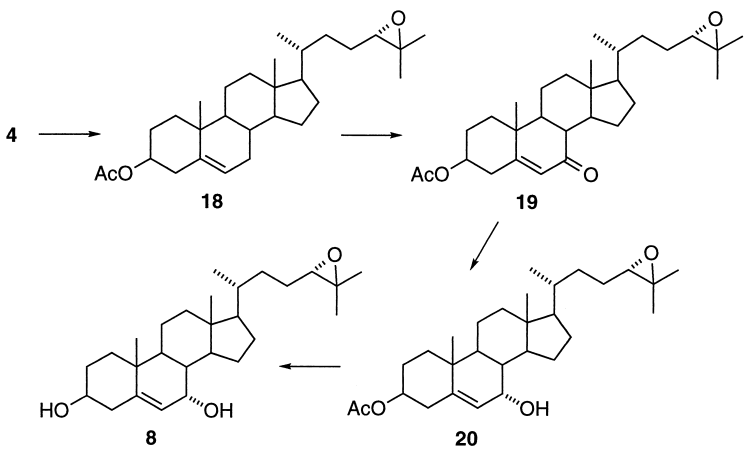
2.1. General

NMR spectra, unless otherwise noted, were taken in $CDCl_3$ on a 300-MHz Varian spectrometer. The chemical shifts are reported in units of δ. Melting points (m.p.) were determined using a Thomas-Hoover apparatus in capillary tubes and are uncorrected. Thin-layer chromatography (TLC) was carried out on EM plastic sheets pre-coated with silica gel 60 F-254 (Whatman, Tewksbury, MA, USA). Visualization was obtained by exposure to 5% phosphomolybdic acid in 2-propanol. Flash chromatography was carried out on EM Reagent silica gel 60 (230–400 mesh), unless otherwise noted. $MgSO_4$ was used to dry all organic layers.

CH_2Cl_2 was distilled from calcium hydride. Tetrahydrofuran and ether were distilled from sodium/benzophenone. Pyridine was distilled from calcium hydride onto 3Å molecular sieves. Acetonitrile was dried over 3Å molecular sieves for 24 h and then distilled onto 3Å molecular sieves. CrO_3 was dried in vacuo for 12 h over KOH before use. All



Scheme 2.



Scheme 3.

reagents, unless otherwise noted, were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

2.2. Cholest-5-ene-3β,25-diol 3β,25-Dibenzoate (*9*)

According to a procedure by Kim et al. [22], to a solution of 43.6 mg (0.108 mmol) of **1** (Steraloids Inc.) in 2.0 ml of dry pyridine was added 0.5 ml (4 mmol) of benzoyl chloride. The mixture was stirred at room temperature for 3 h under N_2, diluted with 10 ml of MeOH and 20 ml of hexane, washed with 10 ml each of 10% HCl, H_2O, saturated $NaHCO_3$ solution and H_2O, dried, filtered, evaporated to give 65.4 mg (98%) of **9**: m.p. 100–103°C (lit. [23] m.p. 100–102°C); ^{1}H NMR 8.03 (m, 4H), 7.54 (m, 2H), 7.45 (m, 4H), 5.43 (m, 1H), 4.87 (m, 1H), 1.26 (s, 6H), 1.07 (s, 3H), 0.93 (d, J = 6.6 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR 166.1, 165.8, 139.8, 132.9, 132.6, 132.2, 131.0, 129.7, 129.6, 128.4, 128.3, 122.9, 83.5, 74.7, 56.8, 56.3, 50.2, 42.5, 41.7, 39.9, 38.4, 37.2, 36.8, 36.4, 35.8, 32.1, 32.0, 28.4, 28.1, 26.3, 26.3, 24.5, 21.2, 20.6, 19.6, 18.8, 12.1.

2.3. 3β,25-Dihydroxycholest-5-en-7-one 3β,25-Dibenzoate (*12*)

According to a procedure by Parish and Wei [24], to a solution of 26.3 mg (0.0431 mmol) of **9** in 5 ml of dry benzene were added 35.0 mg of 3A molecular sieves and 232 mg (1.08 mmol) of PCC. After the mixture was refluxed for 24 h under N_2, 10 ml of brine was added carefully and the resulting mixture was extracted with 3 × 10 ml of CH_2Cl_2. The combined organic layers were evaporated to give a brown residue, which was taken up in 50 ml of ether and filtered. The filtrate was dried, filtered, and evaporated to give 28.2 mg of residue that was chromatographed (1:9 EtOAc:hexane) to give 23.4 mg (87%) of **12**. Recrystallization from CH_2Cl_2-hexane gave colorless **12**: m.p. 148.9–149.4°C; ^{1}H NMR 8.02 (m, 4H), 7.56 (m, 2H), 7.44 (m, 4H), 5.75 (m, 1H), 4.98 (m, 1H), 1.27 (s, 6H), 1.07 (s, 3H), 0.93 (d, J = 6.6 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR 202.2, 166.1, 166.0, 164.1, 133.3, 132.7, 132.3, 130.5, 129.9, 129.6, 128.6, 128.5, 127.1, 83.6, 73.1, 55.1, 50.2, 50.1, 45.7; 43.4, 41.8, 38.9, 38.7, 38.1, 36.5, 36.3, 35.9, 28.8, 27.8, 26.6, 26.4, 26.4, 21.4, 20.7, 19.1, 17.6, 12.3. Analysis Calculated for $C_{41}H_{52}O_5$: C, 78.80; H, 8.39. Found: C, 78.58; H, 8.55.

2.4. Cholest-5-ene-3β,7α,25-triol 3β,25-Dibenzoate (*15*)

According to a modification of a procedure by Kumar et al. [25], to a solution of 18 mg (0.035 mmol) of **12** in 1 ml of dry THF at −78°C was added 0.1 ml of 1 M L-Selectride in THF. The resulting solution was stirred at −78°C for 5 h under N_2 and 0.1 ml each of water, 6 N NaOH and 30% H_2O_2 were added. Most of the THF was evaporated, 10 ml of water was added, and the mixture was extracted with 3 × 10 ml of CH_2Cl_2. The organic layers were dried, filtered, and evaporated to give 17.0 mg of residue, which was chromatographed (3:17 EtOAc:hexane) to afford 12.8 mg (71%) of **15**. Recrystallization from ether-hexane gave colorless **15**: m.p. 136.5–137.2°C; ^{1}H NMR 8.03 (m, 4H), 7.55 (m, 2H), 7.44 (m, 4H), 5.69 (d, J = 5.1 Hz, 1H), 4.93 (m, 1H), 3.87 (m, 1H), 1.26 (s, 6H), 1.07 (s, 3H), 0.94 (d, J = 6.6 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR 166.1, 165.9, 145.4, 133.0, 132.6, 132.3, 130.9, 129.8, 129.6, 128.5, 128.4, 125.1, 83.5, 74.2, 65.5, 56.1, 49.6, 42.4, 42.4, 41.7, 39.4, 38.2, 37.8, 37.7, 37.0, 36.4, 35.9, 28.5, 27.8, 26.4, 24.5, 20.9, 20.6, 18.9, 18.5, 11.9. Analysis Calculated for $C_{41}H_{54}O_5$: C, 78.54; H, 8.69. Found: C, 78.46; H, 8.62.

2.5. Cholest-5-ene-3β,7α,25-triol (*5*)

A solution of 12.8 mg (0.0204 mmol) of **15** in 10 ml of MeOH containing 0.5 g of KOH was heated at reflux for 1 h. Most of MeOH was evaporated, 10 ml of H_2O was added, and the resulting mixture was extracted with 3 × 10 ml of CH_2Cl_2. The combined organic layers were washed

with 10 ml of brine, dried, filtered, and evaporated to give a yellow residue, which was chromatographed (4:1 EtOAc: hexane) twice to give 10 mg of white solid. Further purification by HPLC on a 5-μm Ultrasphere ODS semi-preparative HPLC column (10 × 300 mm, Beckman) with 1:9 H_2O:MeOH as efluant at a flow rate of 3 ml/min and recrystallization from CH_2Cl_2-acetone afforded 4.3 mg (51%) of **5**: m.p. 234 to 235°C (lit. [19] m.p. 234.5–235.5°C); ^{1}H NMR (CD_3OD) 5.53 (d, J = 5.1 Hz, 1H), 3.75 (m, 1H), 3.45 (m, 1H), 1.16 (s, 6H), 0.99 (s, 3H), 0.96 (d, J = 6.3 Hz, 3H), 0.71 (s, 3H). (lit. [19] ^{1}H NMR (pyridine-d_5) 5.57 (m, 1H), 5.3–4.5 (m, 1H), 3.92 (m, 1H), 1.28 (s, 6H), 0.93 (s, 3H), 0.61 (s, 3H)).

*2.6. (25*R*)-Cholest-5-ene-3β,26-diol 3β,26-Dibenzoate (**10**)*

As in the preparation of **9** from **1**, 76.4 mg (0.190 mmol) of **2**, prepared from diosgenin by the method of Kim et al. [22], was converted to crude **10**, which was chromatographed (1:19 EtOAc:hexane) to give 102 mg (87%) of **10**. Recrystallization from methanol-CH_2Cl_2 gave colorless **10**: m.p. 136–137°C (lit. [22] m.p. 136.5–137.5°C); ^{1}H NMR 8.07 (m, 4H), 7.56 (m, 2H), 7.46 (m, 4H), 5.43 (m, 1H), 4.87 (m, 1H), 4.13 (m, 2H), 1.08 (s, 3H), 1.04 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.3 Hz, 3H), 0.70 (s, 3H) (lit. [22] ^{1}H NMR 8.05 (m, 4H), 7.54 (m, 2H), 7.44 (m, 4H), 5.42 (m, 1H), 4.86 (m, 1H), 4.21 (m, 1H), 4.12 (m, 1H), 1.07 (s, 3H), 1.02 (s, 3H), 0.93 (d, J = 6.5 Hz, 3H), 0.69 (s, 3H)); ^{13}C NMR 166.8, 166.1, 139.8, 132.9, 132.9, 131.0, 130.7, 129.7, 128.5, 128.4, 122.9, 74.7, 70.1, 56.8, 56.2, 50.1, 42.5, 39.9, 38.4, 37.2, 36.8, 36.2, 35.9, 34.1, 32.9, 32.1, 32.0, 28.4, 28.1, 24.4, 23.5, 21.2, 19.5, 18.9, 17.2, 12.0; (lit. [22] ^{13}C NMR 166.6, 166.0, 139.6, 132.8, 132.7, 130.7, 130.5, 129.6, 129.5, 128.3, 128.2, 122.7, 74.5, 70.0, 56.6, 56.0, 50.0, 42.2, 39.7, 38.2, 37.0, 36.6, 36.0, 35.7, 33.9, 32.7, 31.9, 28.2, 27.8, 24.2, 23.3, 21.0, 19.4, 18.7, 17.0, 11.8.)

*2.7. (25*R*)-3β,26-Dihydroxycholest-5-en-7-one 3β,26-Dibenzoate (**13**)*

According to a procedure by Blair et al. [26], to a solution of 101.5 mg (0.163 mmol) of **10** in 0.6 ml of dry pyridine and 20 ml of dry CH_2Cl_2 was added 0.6 g of CrO_3. The mixture was heated at reflux overnight under N_2, cooled, and decanted. After addition of 3 × 10 ml of CH_2Cl_2 and 10 ml of ether, the resulting mixture was washed with 4 × 7 ml of saturated $NaHCO_3$ solution and the combined aqueous layers were extracted with 15 ml of ether. The combined organic layers were washed with 2 × 10 ml of 5% HCl, 10 ml of saturated $NaHCO_3$ solution and 10 ml of brine, dried, filtered, and evaporated to give 215.6 mg of brown residue, which was chromatographed (1:9 EtOAc:hexane) to afford 56.0 mg (54%) of **13**: m.p. 167.5–168.3°C; ^{1}H NMR 8.06 (m, 4H), 7.57 (m, 2H), 7.46 (m, 4H), 5.76 (m, 1H), 4.98 (m, 1H), 4.17 (m, 2H), 1.27 (s, 3H), 1.03 (d, J = 6.9 Hz, 3H), 0.94 (d, J = 6.3 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR 202.1, 166.9, 166.0, 164.0, 133.2, 133.0, 130.7, 130.5, 129.8, 129.7, 128.6, 128.5, 127.0, 73.0 70.1, 54.9, 50.1, 50.0, 45.6, 43.3, 38.9, 38.6, 38.0, 36.2, 35.9, 34.1, 32.9, 29.9, 28.8, 27.7, 26.5, 23.5, 21.4, 19.0, 17.5, 17.5, 12.2. FAB-HRMS (M-H$^+$) Analysis Calculated for $C_{41}H_{51}O_5$: 623.3737. Found: 623.3735. Analysis Calculated for $C_{41}H_{52}O_5$: C, 78.80; H, 8.39. Found: C, 78.83; H, 8.38.

*2.8. (25*R*)-Cholest-5-ene-3β,7α,26-triol 3β,26-Dibenzoate (**16**)*

As in the preparation of **15** from **12**, 38.6 mg (0.0619 mmol) of **13** was converted to 45.0 mg of crude **16**, which was recrystallized from acetone-CH_2Cl_2 three times to give 35.0 mg (93%) of **16**: m.p. 143.4–144.4°C; ^{1}H NMR 8.06 (m, 4H), 7.55 (m, 2H), 7.46 (m, 4H), 5.69 (d, J = 5.1 Hz, 1H), 4.92 (m, 1H), 4.17 (m, 2H), 3.87 (m, 1H), 1.07 (s, 3H), 1.03 (d, J = 6.9 Hz, 3H), 0.95 (d, J = 6.3 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR 166.9, 166.1, 145.4, 133.0, 130.8, 130.7, 129.8, 129.7, 128.5, 128.5, 125.1, 74.2, 70.2, 65.5, 56.0, 49.6, 42.4, 42.3, 39.3, 38.2, 37.7, 37.7, 37.0, 36.2, 35.9, 34.1, 32.9, 28.5, 27.8, 24.5, 23.4, 20.9, 19.0, 18.5, 17.2, 11.9. Analysis Calculated for $C_{41}H_{54}O_5$: C, 78.54; H, 8.68. Found: C, 78.03; H, 8.78.

*2.9. (25*R*)-Cholest-5-ene-3β,7α,26-triol (**6**)*

As in the preparation of **5** from **15**, 39.1 mg (0.0625 mmol) of **16** was converted to crude **6**, which was chromatographed (7:3 EtOAc:hexane) to give 20.9 mg (80%) of **6**. Recrystallization from CH_2Cl_2-acetone gave colorless **6**: m.p. 214–216°C; ^{1}H NMR (CD_3OD) 5.52 (d, J = 5.4 Hz, 1H), 3.74 (m, 1H), 3.47 (m, 1H), 3.34 (m, 2H), 0.99 (s, 3H), 0.94 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (CD_3OD) 146.8, 125.1, 72.2, 68.7, 66.0, 57.6, 50.8, 43.5, 43.4, 43.0, 40.8, 39.1, 38.6, 37.6, 37.3, 37.0, 34.5, 32.3, 30.9, 29.6, 25.3, 24.7, 22.0, 19.4, 18.8, 17.2, 12.3. Analysis Calculated for $C_{27}H_{46}O_3$: C, 77.45; H, 11.08. Found: C, 77.20; H, 11.06.

*2.10. Cholest-5-ene-3β,24(*S*)-diol 3β,24-Dibenzoate (**11**)*

To a solution of 21.6 mg (0.0537 mmol) of **3**, prepared by the procedures of Zhang Z, Li D, Blanchard DE, Lear SE, Erickson SK, Spencer TA (being prepared for publication), in 1.0 ml of dry pyridine was added 0.5 ml (4 mmol) of benzoyl chloride. After the mixture was stirred at room temperature overnight, 5 ml of MeOH was added carefully, and the resulting mixture was extracted with 2 × 20 ml of EtOAc. The combined organic layers were washed with 10 ml each of 10% HCl, H_2O, saturated $NaHCO_3$ solution, and H_2O, dried, filtered, and evaporated to give 145 mg of yellow solid, which was chromatographed (1:19 EtOAc: hexane) to give 28.3 mg (86%) of **11**: m.p. 183.5–184.1°C (lit. [27] m.p. 182–184°C); ^{1}H NMR 8.06 (m, 4H), 7.56 (m,

2H), 7.46 (m, 4H), 5.42 (m, 1H), 4.96 (m, 1H), 4.87 (m, 1H), 1.07 (s, 3H), 1.00 (d, $J = 6.9$ Hz, 3H), 0.98 (d, $J = 6.6$ Hz, 3H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.66 (s, 3H); ^{13}C NMR 166.7, 166.3, 139.9, 133.0, 133.0, 131.1, 130.9, 129.8, 128.6, 128.5, 123.0, 80.0, 74.8, 56.9, 56.0, 50.3, 42.6, 40.0, 38.5, 37.3, 36.9, 36.0, 32.1, 31.9, 31.5, 28.4, 28.1, 27.8, 24.5, 21.3, 19.7, 19.2, 19.0, 17.6, 12.1 (lit. [28] ^{13}C NMR (25.2 MHz) 165.5, 139.4, 132.7, 130.8, 129.6, 128.3, 122.5, 79.6, 74.5, 56.6, 55.6, 50.0, 42.2, 39.7, 38.2, 37.0, 36.6, 35.6, 31.8, 31.8, 31.2, 31.1, 28.1, 27.9, 27.3, 24.3, 21.1, 19.3, 18.9, 18.7, 17.3, 11.8.

*2.11. 3β,24(S)-Dihydroxycholest-5-en-7-one 3β,24-Dibenzoate (**14**)*

According to a procedure by Ratcliffe and Rodehorst [29], to a solution of 16.0 mg (0.0256 mmol) of **11** in 0.3 ml of pyridine and 10 ml of dry CH_2Cl_2 was added 0.3 g of CrO_3. The mixture was heated at reflux overnight under N_2, decanted and evaporated to give a black residue, which was taken up in 50 ml of ether and filtered. The filtrate was washed with 10 mL of 5% $NaHCO_3$ solution and 10 ml of brine, dried, filtered, and evaporated to give 38.0 mg of yellow oil which was chromatographed (1:9 EtOAc:hexane) to afford 12.7 mg (78%) of **14**: m.p. 143–147°C. Recrystallization from CH_2Cl_2-hexane three times gave **14**: m.p. 151.6 to 152.4°C; ^{1}H NMR 8.05 (m, 4H), 7.56 (m, 2H), 7.45 (m, 4H), 5.75 (s, 1H), 4.96 (m, 2H), 1.27 (s, 3H), 1.00 (d, $J = 6.9$ Hz, 3H), 0.98 (d, $J = 6.9$ Hz, 3H), 0.95 (d, $J = 6.3$ Hz, 3H), 0.67 (s, 3H); ^{13}C NMR 202.2, 166.7, 166.0, 164.1, 133.3, 133.0, 131.1, 130.5, 129.9, 129.8, 128.6, 128.6, 127.1, 80.0, 73.1, 54.6, 50.2, 50.0, 45.7, 43.4, 38.9, 38.7, 38.1, 36.3, 35.9, 31.8, 31.4, 28.7, 27.8, 27.7, 26.5, 21.4, 19.2, 19.2, 17.6, 17.6, 12.2. Analysis Calculated for $C_{41}H_{52}O_5$: C, 78.80; H, 8.39. Found: C, 78.56, H, 8.37.

*2.12. Cholest-5-ene-3β,7α,24(S)-triol 3β,24-Dibenzoate (**17**)*

As in the preparation of **15** from **12**, except for a reaction time of three rather than 5 h, 34.9 mg (0.0558 mmol) of **14** was converted to 34.6 mg of crude **17**, which was chromatographed (3:17 EtOAc:hexane) to give 24.2 mg (69%) of **17**: m.p. 171–173°C; ^{1}H NMR 8.05 (m, 4H), 7.56 (m, 2H), 7.45 (m, 4H), 5.67 (d, $J = 5.1$ Hz, 1H), 4.95 (m, 2H), 3.86 (m, 1H), 1.06 (s, 3H), 1.00 (d, $J = 6.9$ Hz, 3H), 0.97 (d, $J = 6.6$ Hz, 3H), 0.95 (d, $J = 5.7$ Hz, 3H), 0.67 (s, 3H); ^{13}C NMR 166.6, 166.1, 145.4, 133.0, 132.9, 131.1, 130.8, 129.8, 129.7, 128.5, 125.1, 80.0, 74.2, 65.4, 55.6, 49.6, 42.4, 42.3, 39.3, 38.2, 37.7, 37.7, 37.0, 35.8, 31.8, 31.4, 28.4, 27.8, 27.6, 24.4, 20.9, 19.1, 19.0, 18.5, 17.5, 11.8. FAB-HRMS (M-H$^+$) Analysis Calculated for $C_{41}H_{53}O_5$: 625.3893. Found: 625.3894.

*2.13. Cholest-5-ene-3β,7α,24(S)-triol (**7**)*

As in the preparation of **5** from **15**, 17.5 mg (0.0280 mmol) of **17** was converted to crude **7**, which was chromatographed (4:1 EtOAc:hexane) to give 11.4 mg (97%) of colorless **7** that was homogeneous by TLC. Recrystallization twice from acetone-hexane gave **7**: m.p. 203–204°C; ^{1}H NMR (CD_3OD) δ 5.53 (d, $J = 5.4$ Hz, 1H), 3.76 (m, 1H), 3.47 (m, 1H), 3.21 (m, 1H), 1.00 (s, 3H), 0.97 (d, $J = 6.6$ Hz, 3H), 0.91 (d, $J = 6.0$ Hz, 3H), 0.89 (d, $J = 6.0$ Hz, 3H), 0.72 (s, 3H). ^{13}C NMR (CD_3OD) 146.8, 125.1, 78.3, 72.2, 66.0, 57.5, 50.8, 43.5, 43.4, 43.0, 40.8, 39.1, 38.6, 38.2, 37.6, 34.7, 33.8, 32.3, 31.8, 29.5, 25.2, 22.0, 19.7, 19.6, 18.8, 17.7, 12.3; EI-HRMS (M^+-H_2O) Analysis Calculated for $C_{27}H_{42}O$: 400.3341. Found: 400.3349.

*2.14. 24(S),25-Epoxycholesteryl-3β-acetate (**18**)*

To a solution of 40.4 mg (0.101 mmol) of **4**, prepared as previously described [30], in 1.5 ml of dry pyridine was added 0.7 ml of acetic anhydride. The resulting mixture was stirred under N_2 for 12 h, 10 ml of saturated $NaHCO_3$ solution was added, and the mixture was extracted with 3×10 ml of CH_2Cl_2. The combined organic layers were dried, filtered, and evaporated to give 109.7 mg of residue, which was chromatographed on aluminum oxide (active neutral, gamma, −60 mesh, AlfaAesar; 1:19 EtOAc:hexane) to give 36.7 mg (82%) of **18**. Recrystallization from CH_2Cl_2-hexane afforded colorless **18**: m.p. 113 to 115°C (lit. [31] m.p. 114.5–116.5°C); ^{1}H NMR 5.38 (m, 1H), 4.60 (m, 1H), 2.68 (m, 1H), 2.03 (s, 3H), 1.30 (s, 3H), 1.27 (s, 3H), 1.02 (s, 3H), 0.94 (d, $J = 6.3$ Hz, 3H), 0.70 (s, 3H); ^{13}C NMR 170.7, 139.8, 122.8, 74.2, 65.1, 58.3, 56.9, 56.2, 50.2, 42.6, 39.9, 38.3, 37.2, 36.8, 35.9, 32.8, 32.0, 29.9, 28.4, 28.0, 25.9, 25.2, 24.5, 21.7, 21.2, 19.5, 18.9, 18.8, 12.1.

*2.15. 24(S),25-Epoxycholest-5-en-3β-ol-7-one, 3β-Acetate (**19**)*

According to a procedure by Salvador et al. [32], to a solution of the crude product (40.6 mg) containing **18**, prepared as described above from 28.5 mg (0.0713 mmol) of **4**, and 8.1 mg of CuI in 2 ml of dry CH_3CN was added 0.2 ml (5.0–6.0 M solution in decane, 1.0–1.2 mmol) of *t*-BuOOH. The resulting mixture was stirred at 50°C for 25 h under N_2, 10 ml of 10% Na_2SO_3 was added, and the mixture was extracted with 3×10 ml of CH_2Cl_2. The combined organic layers were washed with 10 ml of saturated $NaHCO_3$ solution, 10 ml of H_2O, and 10 ml of brine, dried, filtered, and evaporated to give 60.4 mg of yellow residue that was crystallized twice from CH_2Cl_2-hexane to give 22.3 mg (69% from **4**) of **19**: m.p. 175.5–177.5°C; ^{1}H NMR 5.70 (s, 1H), 4.72 (m, 1H), 2.69 (m, 1H), 2.05 (s, 3H), 1.31 (s, 3H), 1.27 (s, 3H), 1.21 (s, 3H), 0.95 (d, $J = 6.6$ Hz, 3H), 0.69 (s, 3H); ^{13}C NMR 202.2, 170.6, 164.2, 127.0, 72.5, 65.1, 54.8, 50.2, 50.0, 45.7, 43.4, 38.9, 38.6, 38.0,

36.3, 35.8, 32.8, 28.8, 27.6, 26.6, 25.9, 25.2, 21.5, 21.4, 19.0, 18.9, 17.5, 12.2. Analysis Calculated for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.40; H, 9.63.

*2.16. 24(*S*),25-Epoxycholest-5-ene-3β,7α-diol 3β-Acetate (**20**)*

As in the preparation of **15** from **12**, 24.3 mg (0.0533 mmol) of **19** was converted to 20.4 mg of crude **20**, which was crystallized from ether-hexane to afford 17.3 mg (71%) of **20**: m.p. 185 to 186°C; ^{1}H NMR 5.63 (d, J = 4.8 Hz, 1H), 4.65 (m, 1H), 3.85 (bs, 1H), 2.69 (m, 1H), 2.04 (s, 3H), 1.31 (s, 3H), 1.27 (s, 3H), 1.01 (s, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.69 (s, 3H); ^{13}C NMR 170.7, 145.4, 124.9, 73.6, 65.4, 65.2, 58.4, 55.9, 49.6, 42.4, 39.3, 38.1, 37.7, 37.7, 37.0, 35.9, 32.8, 28.5, 27.7, 25.8, 25.2, 24.5, 21.6, 20.9, 18.9, 18.8, 18.4, 11.9. Analysis Calculated for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.75; H, 10.08.

*2.17. 24(*S*),25-Epoxycholest-5-ene-3β,7α-diol (**8**)*

According to a modification of a procedure by Corey and Grogan [21], a solution of 17.3 mg of **20** in 4 ml of 1:4 THF-MeOH containing 0.04 g of NaOH was stirred at rt for 2.5 h, 10 ml of water was added, and the resulting mixture was extracted with 3 × 10 ml of CH_2Cl_2. The combined organic layers were washed with 10 ml of brine, dried, filtered and evaporated to give 12.6 g of yellow residue that was chromatographed on aluminum oxide (active neutral, gamma, 60 mesh, AlfaAesar; 6:4 EtOAc:hexane) to give 8.3 mg (54%) of colorless **8** that was homogeneous by TLC: m.p. 149.4–150.5°C; ^{1}H NMR 5.61 (d, J = 4.8 Hz, 1H), 3.86 (bs, 1H), 3.59 (m, 1H), 2.69 (m, 1H), 1.31 (s, 3H), 1.27 (s, 3H), 1.00 (s, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR 146.5, 124.1, 71.6, 65.6, 65.2, 58.4, 56.0, 49.7, 42.5, 42.4, 42.3, 39.4, 37.8, 37.7, 37.3, 36.0, 32.8, 31.7, 28.6, 25.9, 25.2, 24.6, 21.0, 19.0, 18.9, 18.5, 11.9; EI-HRMS (M^+-H_2O) Analysis Calculated for $C_{27}H_{40}O$: 398.3184. Found: 398.3184.

3. Results and discussion

25-Hydroxycholesterol (**1**) is commercially available, but adequate amounts of the other three oxysterols had to be synthesized for conversion to their 7α-hydroxy derivatives. 27-Hydroxycholesterol (**2**) was prepared from diosgenin by the method reported by Schroepfer and co-workers [22]. 24(*S*)-Hydroxycholesterol (**3**) was prepared from stigmasterol by our recently developed route (by the procedures of Zhang, Li, Blanchard, Lear, Erickson, Spencer, unpublished results) and 24(*S*),25-epoxycholesterol (**4**) was synthesized using procedures we have recently reported [30].

The strategy for the preparation of the 7α-hydroxy derivatives is illustrated for diols **1** to **3** in Scheme 2. Initial protection of the free hydroxyl groups was accomplished by conversion to the known dibenzoates **9** to **11** [22,25,27] Allylic oxidation at C7 can be effected to afford 7-hydroxy derivatives directly, but produces a mixture of 7α and 7β isomers [20]. Alternatively, oxidation can be effected to form a 7-keto derivative which can be stereoselectively reduced to the axial 7α-hydroxy compound by use of a hindered complex metal hydride, such as L-selectride [25]. Accordingly, each of **9** to **11** was subjected to allylic oxidation (CrO_3/pyridine for **9** [24] and **11** [29]; PCC for **10**) [27] to afford enones **12** to **14** in good yield, and these were in turn reduced using L-selectride to yield the 7α-hydroxy steroids **15** to **17** with complete stereoselectivity. The ^{1}H NMR spectra of each of the crude hydride reduction products showed only the relatively deshielded doublet for a 6β-H and none of the relatively shielded singlet expected for a 6α-H [25]. Removal of the dibenzoate protecting groups from **15** to **17** by treatment with KOH in MeOH then produced the desired triols **5** to **7** in excellent yield.

Synthesis of 7α-hydroxy-24(*S*),25-epoxycholesterol (**8**) (Scheme 3) required a modified reaction sequence because of the acid liability of the epoxide function. The known [31] acetate **18** of 24(*S*),25-epoxycholesterol (**4**) was in this case used as the protected substrate for allylic oxidation. Several methods, including those used for the conversion of **9** to **11** to **12** to **14**, caused destruction of the epoxide group as well as allylic oxidation. Attempted direct allylic oxidation of **4** was also unsuccessful. The conversion of **18** to **19** was achieved, however, by the procedure of Salvador et al. [32], with *t*-BuOOH and CuI and CH_3CN, as had been employed by Corey and Grogan [21] for the analogous reaction on 24(*S*),25-epoxycholesteryl benzoate. Reduction with L-selectride yielded **20** and cleavage of the acetate protecting group then afforded **8**. Thus, all four 7α-hydroxylated oxysterols **5** to **8** are now available as reference standards and procedures have been developed for their synthesis in larger amounts as required for further experimentation.

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