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Oxysterols: 27-hydroxycholesterol and its radiolabeled analog☆

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Abstract

We describe a convenient and stereoselective route to the synthesis of 27-hydroxycholesterol. Also its radiolabeled analog, 22, 23 di [^{3}H]-27-hydroxycholesterol with high specific radioactivity (55 Ci/mmol) was synthesized by this method. Julia condensation of steroidal 22-sulfone with aldehyde, led to the addition of the 23–27 carbon side chain building block to the steroid backbone. Formed in this reaction β-hydroxysulfone moiety was reduced by sodium amalgam generate 22–23 unsaturated bond. Further reduction either by hydrogen or tritium furnished substrates for the synthesis of title compounds.

Keywords: Oxysteroids; Hydroxycholesterol; Kryptogenin; Diosgenin; Condensation; Reductive elimination

1. Introduction

Due to their unique properties, oxysteroids attract much attention in their preparation and biologic evaluation. The title[2,3] compound **1** is the intermediate in the metabolic pathway from cholesterol to the bile acids [3]. In addition to its role as a metabolic intermediate, other important properties have been described. 27-Hydroxycholesterol (**1**) was found to be an effective inhibitor of HMG-CoA reductase [4,5], an inhibitor of DNA synthesis [6] and was shown to have good anitumor properties (D'Ambra et al., in preparation). Recent data also suggest important role of this type of compounds in the development of atherosclerosis [7] (Fig. 1).

For our study of cholesterol metabolism, the chemically and optically pure **1** and its radiolabeled (tritiated) analog were required.

Because the side chain of **1** is degraded during the metabolic process to the shorter 24 carbons metabolite, tritium should be introduced at positions before C-24.

The metabolically modified C-2, C-3, C-5, C-6, and C-7 positions should also be excluded. Previous routes to the synthesis of **1** utilized the two readily available natural isoprenoids, kryptogenin (**2**), and diosgenin (**3**). In the first approach kryptogenin (**2**) was converted [8–10] to the desired 27-hydroxycholesterol (**1**) through the two-step reduction of the C-16 and C-22 oxo functions. Overall yield was reported as high as 40%. The second method, starting from diosgenin (**3**) [11–14], utilized the Clemmensen, Wolf–Kishner reduction sequence. However this approach requires blocking of the C-3 and C-26 hydroxyls. Also this method allowed preparation [15] of the 25S epimer starting from yamogenin (**4**), which is present in natural material in concentration of up to 15%.

These past approaches suffer from several faults. The most important is the potential for epimerization at the C-25 stereocenter, which leads to the contamination of the final product. It has been shown [16–18], by HPLC analysis, that samples of 27-hydroxycholesterol (**1**) previously claimed as 'pure' contained significant amounts of the 25S epimer. The mechanism for such an epimerization is the steroidal [1,5]-H shift [9,19].

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[3] According to the recent nomenclature (25R)-26-hydroxycholesterol is referred as 27-hydroxycholesterol, whereas 25S isomer as a 26-hydroxycholesterol, see ref [2].

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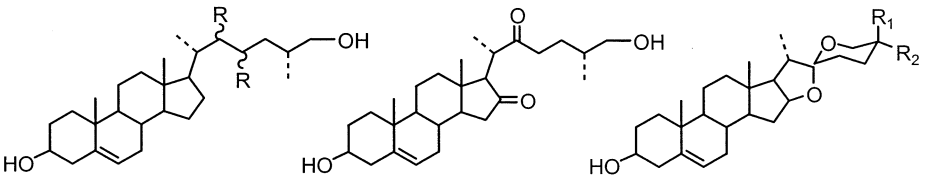

1 R = H
5 R = ^{3}H

2

3 R_1=H, R_2=CH_3;
4 R_1=CH_3; R_2=H;

Fig. 1.

In addition to the use of **2** and **3** other approaches to **1** [20,21] and its 25S epimer [22,23] have been described. However, none of these methods were deemed suitable for the synthesis of the radiolabeled analog of **1** under the requirements described above[4].

Herein we described simple and convenient synthesis of optically and chemically pure **1**, as well as its radiolabeled analog **5**.

2. Experimental

All non-aqueous reactions were performed under a dry atmosphere of nitrogen. Solvents were dried by reflux over drying agent (CaH_2 for methylene chloride, $LiAlH_4$ for tetrahydrofuran and toluene) and distilled just before use. Pyridine and other solvents were used without purification. All the commercial reagents were purchased from Aldrich (Milwaukee, WI, USA), Fluka (Buchs, Switzerland), or Sigma (St. Louis, MO, USA). TLC analyses were performed on pre-coated silica gel plates (Merck, West Point, PA, USA) and visualized by spraying with a solution of ammonium molybdate (48 g) and cerium sulfate (2 g) in 10% H_2SO_4 (1-l) and heating to about 200°C. Column chromatography was done using silica gel (Merck or Whatman). Solutions were dried using anhydrous Na_2SO_4 or anhydrous $MgSO_4$. Amberlite IRN-78 (Alfa) was used as a basic resin. All products that contain THP group were stabilized with pyridine (10^{-4}%) during storage. ^{1}H NMR spectra were recorded with Bruker AC 300 spectrometer, with TMS as an internal standard with the following frequencies: 300 MHz for ^{1}H resonance, and 75 MHz for ^{13}C resonance. DEPT 135, DEPT 90, DEPT 45, and HETCOR techniques were used for necessary structure determination and signals assignment. Abbreviations used for ^{13}C NMR spectra descriptions: (CH_3)-primary carbon, (CH_2)-secondary carbon, (CH)-tertiary carbon, (C)-quaternary carbon. IR spectra were obtained on a Perkin Elmer 1000 FT Infrared Spectrophotometer. Mass spectroscopic analyses were performed on a Shimadzu QP-5000 GC/mass spectrometer (CI, methane). Melting points were obtained with an Electrochemical Melting point apparatus and are uncorrected. Optical rotations were taken at the D-Line of sodium on a Perkin-Elmer 243B Polarimeter at room temperature (23°C). The tritiated analog was purified by HPLC using column Zorbax ODS (250 × 9.4 mm) column with methanol-water mixture as a mobile phase, with detection at 205 nm. Radiochemical purity was determined by TLC. Specific activity was determined by mass spectral analysis.

*2.1. (2S, 2′RS) 2-Methyl-3-(2-tetrahydropyranyloxy)-propanol **(9)***

To a mixture of the hydroxyester **7** (4.8 g, 40.6 mmol), dihydropyran (4.8 ml, 52.8 mmol), and CH_2Cl_2 (40 ml), 10-camphorsulfonic acid (cat. amount) was added and the resultant solution was stirred at room temperature overnight. After dilution with CH_2Cl_2 (100 ml) the reaction mixture was washed with aqueous $NaHCO_3$, dried and evaporated. The residue was dissolved in hexane (1 l) and filtered through a bed of silica gel (50 g). The filtrate was evaporated to give crude THP derivative **8** (9.0 g) as a slightly yellow oil. This oil was dissolved in THF (300 ml), cooled to 0°C, and slowly added to a stirred at 0°C solution of $LiAlH_4$ (5.46 g, 0.144 mol) in THF (100 ml). After complete addition the cooling bath was removed and the reaction mixture was stirred at room temperature for 1 h, then refluxed for 0.5 h. The reaction mixture was cooled to 5°C and the following were added: wet ether (115 ml), H_2O (6 ml), 15% NaOH (20 ml), and, finally, H_2O (20 ml). A white precipitate formed and was filtered off and washed with ether (2 × 200 ml). Combined filtrates were dried and evaporated. The crude product **9** was purified by distillation under reduced pressure. With a temperature of 86–88°C/0.5 Torr. Yield 5.8 g (82%) of colorless oil.

^{1}H NMR ($CDCl_3$), δ, ppm: 0.90, 0.91 (2d, J = 6.9 Hz, 3H, CH_3), 1.46–2.12 (m, 6H), 2.71 (br.s., 1H), 3.35 (dd, J = 7.6 Hz, J = 9.2 Hz, 1H), 3.45–3.74 (m, 5H), 3.81–3.96 (m, 1H), 4.56–4.64 (m, 1H); **^{13}C NMR** ($CDCl_3$), δ, ppm: 13.67, 13.76 (CH_3); 19.74, 25.47, 30.72 (CH_2); 35.65, 35.89 (CH); 62.59, 67.02, 71.89 (CH_2); 99.20, 99.45 (CH); **IR** (film) cm^{-1}: 3422, 2942, 1454, 1120; **$[\alpha]_D$** = +8.18° (c = 1, $CHCl_3$); **MS**, *m/z*: 175 $[M+H]^+$.

[4] For syntheses of isotope labeled 27-hydroxycholesterol see ref. [24] and references cited therein.

*2.2. (2S, 2′RS) 2-Methyl-3-(2-tetrahydropyranyloxy)propyl 4-toluenesulfonate **(10)***

To a stirred solution of alcohol **9** (5.3 g, 30.4 mmol) in pyridine (50 ml) at 0°C, a solution of 4-toluenesulfonyl chloride (7.5 g, 39.5 mmol) in pyridine (70 ml) was added followed by a catalytic amount of DMAP. The cooling bath was removed and reaction mixture was stirred at room temperature for 4 h. A reaction was quenched with water (5 ml), and at the end of the exothermic reaction (20 min) hexane (200 ml) was added. The mixture was washed with water and aqueous $CuSO_4$ until the blue-violet color disappeared. Drying and evaporation of solvent afforded tosylate **10** (9.5 g, 95%) as a colorless oil. An analytical sample was purified by column chromatography using hexane/ethyl acetate (95:5 v/v) as an eluent.

^{1}H NMR ($CDCl_3$), δ, ppm: 0.94, 0.95 (2d, J = 6.9 Hz, 3H), 1.38–1.80 (m, 6H), 2.02–2.15 (m, 1H); 2.45 (s, 3H), 3.17–3.29 (m, 1H), 3.42–3.64 (m, 2H), 3.69–3.81 (m, 1H), 3.92–4.10 (m, 2H), 4.40–4.50 (m, 1H), 7.30–7.40 (m, 2H), 7.74–7.84 (m, 2H); **^{13}C NMR** ($CDCl_3$), δ, ppm: 13.73, 13.80 (CH_3); 19.43, 19.56 (CH_2); 21.75 (CH_3); 25.60, 30.60 (CH_2); 33.65, 33.79, 62.10, 62.30, 68.12, 68.56 (CH); 72.38 (CH_2); 98.74, 99.26, 128.05, 129.93 (CH); 133.28, 144.78 (C); **IR** (film), cm^{-1}: 2943, 1598, 1454, 1361, 1177; **$[\alpha]_D$** = +4.39° (c = 1, $CHCl_3$); **MS** *m/z*: 329 $[M+H]^+$.

*2.3. (2′RS, 3R) 3-Methyl-4-(2-tetrahydropyranyloxy)butyronitrile **(11)***

A mixture of tosylate **10** (3.3 g, 10 mmol), KCN (3.9 g, 60 mmol), MeOH (240 ml), DMF (100 ml), and HMPA (20 ml) was heated at 80°C for 12 h. Methanol was evaporated under reduced pressure leaving a residue, which was dissolved in ether (200 ml) and washed several times with water and aqueous $CuSO_4$. Drying and evaporation of the solvents afforded crude product, which was purified by short column chromatography. Yield 1.49 g (81%) of the colorless oil.

^{1}H NMR ($CDCl_3$), δ, ppm: 1.09, 1.10 (2d, J = 6.8 Hz, 3H), 1.45–1.90 (m, 4H), 2.08–2.24 (m, 1H), 2.32–2.58 (m, 2H), 3.21 (dd, J = 8.1 Hz, J = 9.8 Hz, 1H), 3.38 (dd, J = 4.8 Hz, J = 9.9 Hz, 1H), 3.49–3.61 (m, 2H), 3.72–3.92 (m, 2H), 4.56–4.61 (m, 1H); **^{13}C NMR** ($CDCl_3$), δ, ppm: 16.10, 16.18 (CH_3); 19.23, 19.39, 21.27, 25.28, 30.38, 30.95 (CH_2); 61.95, 62.23 (CH); 70.06, 70.51 (CH_2); 98.44, 98.17 (CH); 118.56 (C); **IR** (neat) cm^{-1}: 2245; **MS**, *m/z*: 184 $[M+H]^+$; **$[\alpha]_D$** = +38.9° (c = 1, $CHCl_3$).

*2.4. (2′RS, 3R) 3-Methyl-4-(2-tetrahydropyranyloxy)butanal **(12)***

To a stirred solution of cyanide **11** (0.73 g, 4.0 mmol) in toluene (20 ml) at −70°C, 1.5 M DIBAL in toluene (2.8 ml, 4.2 mmol) was slowly added over 0.5 h and the reaction mixture was stirred an additional 0.5 h at the same temperature. Water (3 ml) was added to quench the reaction, and the resulting slurry was stirred overnight at room temperature. A white precipitate formed and was filtered off and washed with ether (2 × 15 ml). Combined filtrates and washings were dried and evaporated. The resulting colorless oily residue was purified by column chromatography. Yield of **12**, 0.65 g, (89%), colorless oil.

^{1}H NMR ($CDCl_3$), δ, ppm: 1.00 (d, J = 6.4 Hz, 3H), 1.43–1.89 (m, 4H), 2.20–2.65 (m, 4H), 3.14 (dd, J = 7.7 Hz, J = 9.6 Hz, 1H), 3.34 (dd, J = 4.8 Hz, J = 9.4 Hz, 1H), 3.45–3.60 (m, 2H), 3.68–3.71 (m, 2H), 4.54–4.65 (m, 1H), 9.75, 9.80 (2t, J = 5.1 Hz, 1H); **^{13}C NMR** ($CDCl_3$), δ, ppm: 17.20 (CH_3); 19.29, 19.52, 25.55 (CH_2); 29.23, 29.37 (CH); 30.60, 48.68, 48.83, 62.30, 63.34, 72.00, 72.47, 98.64, 99.25 (CH_2); 202.50 (CH); **IR** (neat) cm^{-1}: 2942, 1726, 1455, 1122, 1034; **MS**, *m/z*: 187 $[M+H]^+$; **$[\alpha]_D$** = +21.9° (c = 1, $CHCl_3$).

*2.5. (2′RS, 22RS, 23RS, 25R) 6β-Methoxy-22-phenylsulfonyl-26-(2-tetrahydropyranyloxy)-3a,5-cyclo-5a-cholestan-23-ol **(14)***

To the stirred −70°C solution of sulfone **13** (205 mg, 0.43 mmol) in THF (6 ml) 1.6 M BuLi in hexane (0.27 ml, 0.43 mmol) was slowly added. After 15 min, a −70°C solution of the aldehyde **12** (54 mg, 0.29 mmol) in THF (2 ml) was added dropwise. The reaction mixture was stirred for an additional 15 min and sat. aqueous NH_4Cl (2 ml) was added, and the reaction mixture was extracted with Et_2O (3 × 10 ml). Combined extracts were dried and evaporated. The isomeric mixture of hydroxysulfones **14** (0.120 g, 65%) was isolated by column chromatography. Analytical sample was purified by column chromatography. Colorless foam. Mixture of the isomers.

^{1}H NMR ($CDCl_3$), δ, ppm: 0.42 (dd, J = 5.2 Hz, J = 7.8 Hz, 1H), 0.57 (d, J = 4.6 Hz, 3H), 0.64 (t, J = 4.7 Hz, 1H), 0.70–2.12 (m, 37H), 2.18–2.33 (m, 1H), 2.70–2.77 (m, 1H), 3.30 (s, 3H), 3.04–3.36 (m, 2H), 3.92–3.95 (m, 4H), 4.30–4.43 (m, 1H), 4.51–4.60 (m, 1H), 7.52–7.67 (m, 3H), 7.88–7.95 (m, 2H); **^{13}C NMR** ($CDCl_3$), δ, ppm: 12.06 (CH_3), 13.29 (CH_2), 15.00, 15.15, 18.68 (CH), 19.42 (CH_3), 19.77 (CH_2), 21.62 (CH_3), 22.90, 24.05, 25.14, 25.66, 28.31 (CH_2), 30.46 (CH_3), 30.74 (CH), 30.90, 33.55 (CH_2), 35.17 (C), 35.39, 40.32, 41.98, 42.31 (CH_2), 43.36, 43.55 (C), 46.40 (CH_2), 48.07, 54.27, 56.64 (CH), 56.76 (CH_3), 62.49 (CH_2), 67.43, 71.62 (CH), 72.09, 72.56 (CH_2), 82.45, 99.25, 99.54, 128.23, 128.74, 129.21, 129.51, 133.60 (CH), 140.55, 140.79, 140.92 (C). **IR** (KBr) cm^{-1}: 2942, 1447, 1295, 1141, 1080, 1032; **MS**, *m/z*: 657 $[M+H]^+$; **$[\alpha]_D$** = +17.6° (c = 1, $CHCl_3$).

*2.6. (2′RS, 25R) 6β-Methoxy-26-(2-tetrahydropyranyloxy)-3α,5-cyclo-5α-cholest-22-(E)-ene **(15)***

To a stirred mixture of hydroxysulfones **14** (320 mg, 0.49 mmol), THF (10 ml), and saturated solution of Na_2HPO_4 in MeOH (10 ml) at 0°C, 5% sodium amalgam

[25] (2.25 g, 4.9 mmol) was added in small portions at 10 min intervals. When TLC showed no substrate liquid was removed and mercury was washed with ether (2 × 20 ml). To the combined liquids, ether (40 ml), followed by water (30 ml) were added. Layers were separated. Organic was dried ($MgSO_4$) and evaporated. Residue was chromatographed to afford **15** (124 mg, 51%) as a colorless oil. Mixture of isomers.

^{1}H NMR ($CDCl_3$), δ, ppm: 0.42 (dd, J = 5.9 Hz, J = 8.8 Hz, 1H), 0.66 (t, J = 4.4 Hz, 1H), 0.74, 0.76 (2s, 3H), 0.90, 0.92 (2d, J = 6.2 Hz, 3H), 1.00, 1.02, 1.04 (3s, 3H), 0.76–2.20 (m, 31H), 2.75–2.81 (m, 1H), 3.16–3.27 (m, 2H), 3.33 (s, 3H), 3.46–3.64 (m, 2H), 3.82–3.94 (m, 1H), 4.54–4.62 (m, 1H), 5.17–5.34 (m, 2H); **^{13}C NMR** ($CDCl_3$), δ, ppm: 12.67 (CH_3); 13.29 (CH_2); 17.09,19.51 (CH_3); 19.76 (CH_2); 20.86; 21.02 (CH); 21.72 (CH_3); 22.99; 24.42; 25.19; 25.79; 28.99 (CH_2); 30.71 (CH); 30.95; 33.60 (CH_2); 34.04, 34.15 (CH); 35.30 (CH_2); 35.55 (C); 36.94, 40.41 (CH_2); 42.93 (C); 43.64 (C); 48.27, 56.22, 56.77 (CH); 56.86 (CH_3); 62.29, 72.74 (CH_2); 82.65, 99.03, 99.16, 125.52, 125.62, 138.95 (CH); **MS**, *m/z*: 499 $[M+H]^+$.

*2.7. (2′RS, 25R) 6β-Methoxy-26-(2-tetrahydropyranyloxy)- 3α,5-cyclo-5α-cholestan **(16)***

The mixture of olefins **15** (3.45 g, 6.92 mmol), 10% Pd-C (300 mg), ethyl acetate (50 ml), and triethylamine (0.1 ml) was stirred in a H_2 atmosphere for 4 h. Catalyst was filtered off, filtrate was evaporated and dried in vacuo to give **16** (3.39 g, 98%) as a white foam.

^{1}H NMR ($CDCl_3$) δ, ppm: 0.39 (t, J = 5 Hz, 1H), 0.62 (t, J = 5 Hz, 1H), 0.69 (s, 3H, CH_3), 0.75–2.06 (m, 45 H), 0.88, 0.89 (2d, J = 6.2 Hz, 3H), 0.99 (s, 3H, CH_3), 2.70–2.80 (m, 1H), 3.06–3.28 (m, 2H), 3.29 (s, 3H, OCH_3), 3.41–3.52 (m, 2H), 3.78–3.90 (m, 1H), 4.52–4.58 (m, 1H); **^{13}C NMR** ($CDCl_3$), δ, ppm: 12.65 (CH_3); 13.29 (CH_2); 17.32, 19.44 (CH_3); 19.80 (CH_2); 20.86, 21.12 (CH); 21.83 (CH_3); 23.05, 23.95, 24.40, 25.31, 25.70, 28.80 (CH_2); 30.91 (CH); 30.99 (CH_2); 33.22 (CH_2); 33.99, 34.20 (CH); 35.20 (CH_2); 35.55 (C); 36.88, 37.15 (CH_2); 40.41 (CH_2); 43.10 (C); 43.84 (C); 49.07, 56.31, 56.76 (CH); 56.90 (CH_3); 63.11, 71.94, 72.46 (CH_2); 82.60, 99.20, 99.22 (CH); **MS**, *m/z*: 501 $[M+H]^+$.

*2.8. (25R) Cholest-5-ene-3β,26-diol, diacetate **(17)***

The mixture of **i**-steroids **16** (2.4 g, 4.79 mmol), $ZnCl_2$ dihydrate (1.58 g, 7.2 mmol), acetic anhydride (5 ml), and acetic acid (30 ml) was heated at reflux for 2 h. The solvents were evaporated; the residue was portioned between water and CH_2Cl_2. The organic layer was separated, washed with $NaHCO_3$ aq., dried and evaporated. The residue was dissolved in a mixture of hexane/methylene chloride (8:1 v/v), filtered through the bed of silica gel and eluted with mixture of hexane/methylene chloride (30:1 v/v). Eluted fractions were evaporated and the residue was recrystallized from hexane/methylene chloride to give 1.81 g (81%) of colorless crystals. **m.p.** = 128–130°C; **^{1}H NMR** (δ, ppm): 0.67 (s, 3H), 0.91 (d, J = 6.6 Hz, 3H), 1.01 (s, 3H), 0.95–2.10 (m, H), 2.03 (s, 3H), 2.05 (s, 3H), 2.31 (d, J = 7.8 Hz, H), 3.80–3.96 (m, 2H), 4.53–4.67 (m, 1H), 5.34–5.40 (m, 1H); **^{13}C NMR** ($CDCl_3$) δ, ppm: 12.14, 17.06, 18.93, 19.57 (CH_3); 21.24, 21.28, 21.68, 23.49, 24.53, 28.01, 28.49 (CH_2); 32.09, 32.12 (CH_3); 32.73, 35.92, 36.26 (CH); 36.60 (C); 36.81, 37.22, 39.94 (CH_2); 42.45 (C); 42.53 (CH_2); 50.22, 56.27, 56.81 (CH); 69.76 (CH_2); 74.12, 122.68 (CH); 139.66, 170.48, 171.26 (C); **IR** (KBr) cm^{-1}: 2943, 1737, 1239, 1034; **MS**, *m/z*: 487 $[M+H]^+$; $[\alpha]_D = -41.1^o$ (c = 1, $CHCl_3$).

*2.9. (25R) Cholest-5-ene-3β, 26-diol **(1)***

Diacetate **17** (265 mg, 0.54 mmol), methanol (15 ml) and basic resin (50 mg) were stirred at room temperature, overnight. The resin was filtered off and washed with methanol. Filtrate and washings were evaporated to give 27-hydroxycholesterol (**1**) (195 mg, 89%) as a white solid. **m.p.** = 172–174°C; $[\alpha]_D = -37.7^o$ (c = 1, $CHCl_3$).

*2.10. (25R) [22,23 -^{3}H]-Cholest-5-ene-3β, 26-diol **(5)***

A mixture of the olefin **15** (25 mg, 0.05 mmol), anhydrous 1,4-dioxane (2.0 ml), and Pd/C (25 mg) was stirred in tritium atmosphere for 2 h. After the catalyst was filtered off, the filtrate was diluted with anhydrous 1,4-dioxane to 30 ml of final volume, to give 1800 mCi of tritium labeled crude product (**18**). To 2.0 ml (120 mCi) of above solution, p-TsOH·H_2O (cat. amount) was added and resulted solution was heated at 80°C for 5 h. At the end reaction mixture was evaporated. The residue was dissolved in Et_2O (5 ml) and washed with $NaHCO_3$ aq. and water. Further drying and evaporation gave 100 mCi of **5**, which was found to have 95% of the radiochemical purity. Final purification (50 mCi scale) was done using HPLC.

3. Discussion

In 1994 Schroepfer's group reported [24] the preparation of 22, 23 di-(^{3}H)-27-hydroxycholesterol (**5**) from compound

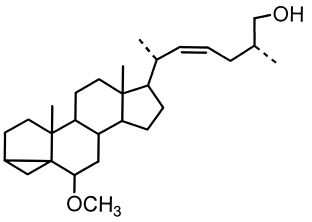

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Fig. 2.

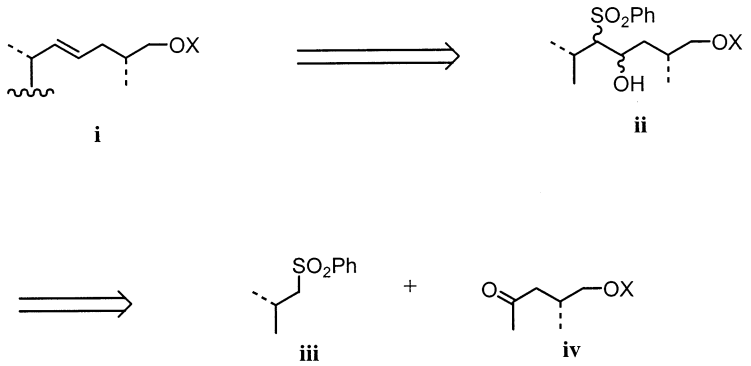

Fig. 3.

6. The olefin **6** was obtained in low yield from a byproduct formed during preparation of **1** from **3** (Fig. 2).

Methods for introducing the 22,23 double bond by partial synthesis are well described [26]. The method developed by Julia and co-workers [27] utilized the readily available sulfones and carbonyl compounds (aldehydes or ketones).

Based on this approach we designed synthesis of the key intermediate **6**. Main features of our project are outlined in Fig. 3.

Condensation of aldehyde **iv** with sulfone **iii** will effect the formation of the hydroxysulfone **ii**. Subsequent reductive elimination will provide the desired 22, 23-unsaturation.

For the synthesis of the C-23 through C-27 fragment, we started with the commercially (Aldrich) available methyl (S) (+) 3-hydroxy-2-methyl propionate (**7**). The alcohol function in **7** was protected as THP acetals (equimolar mixture of diastereomers), then the resulting crude mixture **8** was subjected to $LiAlH_4$ reduction to afford a mixture of the two diastereomeric alcohols **9** in high overall yield (82%)[5] (Fig. 4).

One carbon homologation was achieved by replacement of alcohol function by cyano group.

Reaction of **9** with TsCl in pyridine provided the mixture of diastereomeric tosylates **10** in excellent yield (95%). Heating of **10** with excess of KCN in MeOH-DMF-HMPA solution gave a mixture of cyanides **11** in 81% yield. The IR spectrum of **11** showed an absorption corresponding to a CN group at 2245 cm^{-1}. Reduction of the cyanide **11** by DIBAL-H provided mixture of the two aldehydes **12** (Fig. 5).

As a steroidal building block, we used sulfone **13** that was prepared by known procedure from ergosterol [23,29].

To give the mixture of steroidal hydroxysulfones **14** in 65% yield, **13** was condensed with aldehydes **12** under Julia conditions. ^1H as well as ^{13}C NMR spetra of **14** indicated complex mixture of the products. Theoretically 8 diastereomers could be expected to form. TLC analysis showed three main products. This mixture was partially resolved by chromatography and was shown (^1H NMR, CI MS) to be in each case a mixture of diastereomers. The main part of this mixture was fully characterized (see Section 2). Reductive elimination of the β-hydroxysulfone moiety in **14** by sodium amalgam afforded a mixture of two olefins[6] **15** in moderate yield (51%); the absorption corresponding to the formed double bond was observed as a multiplets at 5.17–5.24 ppm.

[5] Opposite enantiomers of compounds **8–11** were described, see ref. [28].

[6] Such reductive elimination reactions of β-hydroxysulfones have been known to produce predominantly or exclusively *trans*-olefin, see Ref. [30].

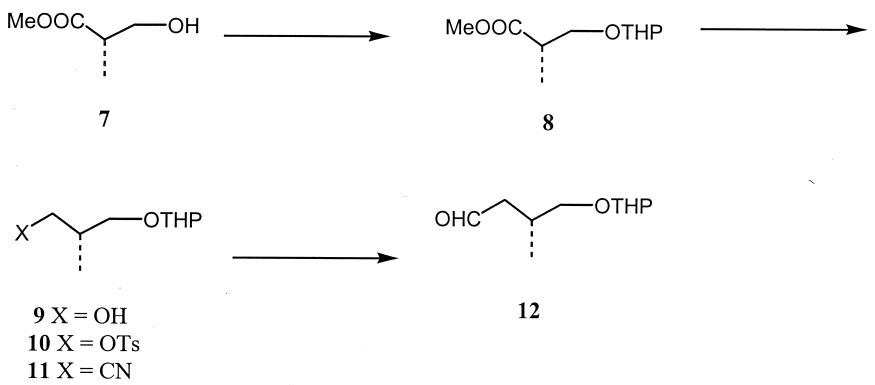

Fig. 4.

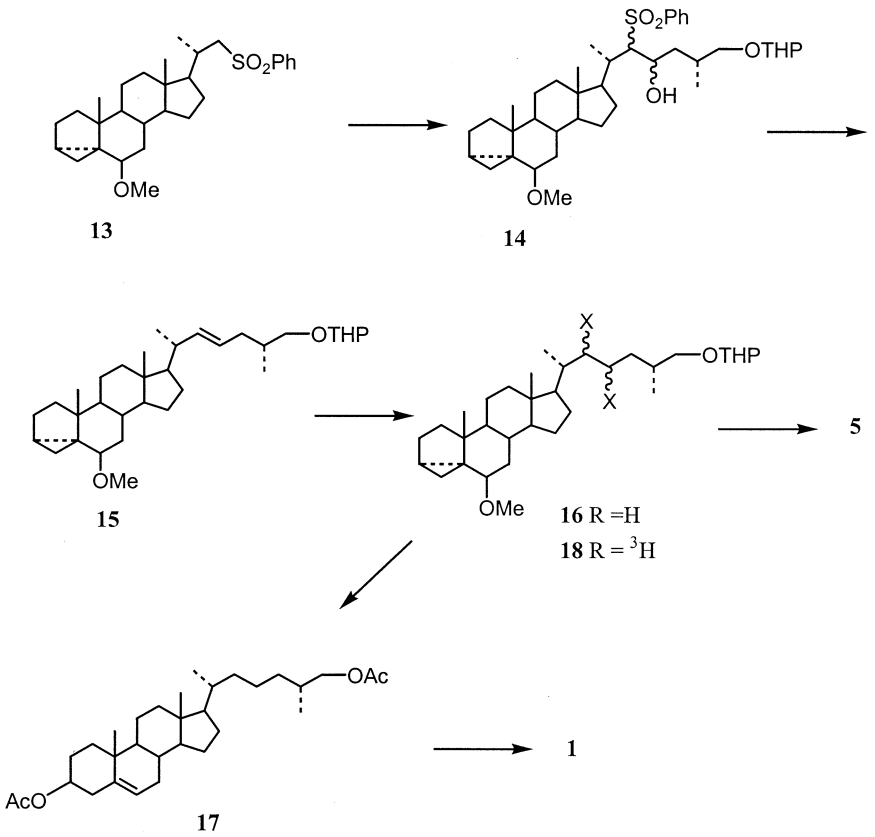

Fig. 5.

The synthesis of **1** from **15** was accomplished in three steps. The alkene **15** was efficiently reduced by hydrogen with Pd-C as a catalyst, in the presence of triethylamine, to afford i-steroid **16**, as a mixture of the THP acetals (98% yield). Subsequent acid catalyzed ($ZnCl_2$) ring opening of **16** restored the original cholesterol policyclic ring system to give diacetate **17** in 81% yield. Base catalyzed (basic resin) methanolysis of **17** afforded **1** in 89% yield.

Spectrochemical data for (25R)-cholest-5-ene-3β, 26-diol (**1**) obtained in this manner were identical in all respects with those reported previously [8,24].

Substitution of the tritium for the reduction of **15**, followed by acid catalyzed hydrolysis of the resulting cyclosteroid **18** afforded the tritiated analog **5**. This tritiated analog exhibited very high specific activity (55 Ci/mmol). Distribution of tritium was determined by ^3H NMR. Spectrum showed the following broad signals (relative intensities estimated by integration): δ 0.87 (7.0%, ^3H-21, ^3H-26), 1.05 (28%, ^3H-24), 1.24 (13%, ^3H-23), 1.32 (42.4%, ^3H-22, ^3H-23, ^3H-24, ^3H-25), 1.56 (5.6%, ^3H-7, ^3H-20), 3.32 (1.8%, ^3H-27), and 2.7% of ^3H in other positions. These data are in agreement with those reported by Ni et al. [24].

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