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Synthesis of (25*R*)-26-hydroxycholesterol

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Abstract

We describe the synthesis of (25*R*)-cholest-5-en-3β,26-diol ((25*R*)-26-hydroxycholesterol) from diosgenin in four steps in 58% overall, yield via a modified Clemmensen reduction followed by a Barton deoxygenation reaction.

Keywords: Oxysteroids; 26-Hydroxycholesterol; Diosgenin; Modified Clemmensen reduction; Barton deoxygenation reaction; Synthesis

1. Introduction

The role of oxysteroids including (25*R*)-cholest-5-en-3β,26-diol ((25*R*)-26-hydroxycholesterol) (**1**) in the regulation of cholesterol homeostasis has been critically examined [1]. (25*R*)-26-Hydroxycholesterol is an intermediate in the metabolic pathway from cholesterol to the bile acids [2]. It is a potent inhibitor of cholesterol biosynthesis in vitro as it is an effective inhibitor of HMG-CoA reductase [3], but there is little direct evidence that it is important under in vivo conditions. (25*R*)-26-Hydroxycholesterol has also been shown to be an inhibitor of DNA synthesis [4].

(25*R*)-26-Hydroxycholesterol has been synthesized from two readily available natural products, kryptogenin [5–8], and diosgenin [9–11] and by addition of a side chain building block to the steroid backbone [12].

We now describe in Fig. 1, a convenient and higher yielding method, using Barton's dithiocarbonate deoxygenation procedure [13], for the synthesis of (25*R*)-26-hydroxycholesterol from diosgenin in four steps in 58% overall yield.

2. Experimental

Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Mattson 4040 FT-IR spectrometer and the data reported in wavenumbers (cm^{-1}). The ^{1}H NMR and ^{13}C NMR spectra were recorded on a GE QE 300 (at 300 and 75.48 MHz, respectively) spectrometer. Spectra were recorded in $CDCl_3$ with chemical shift values (in ppm) relative to the solvent peak (7.26 ppm for ^{1}H and 77.0 ppm for ^{13}C). Coupling constants are in units of hertz (Hz). High resolution mass spectra (HRMS) were obtained on a Fissons ZAB HF double-focusing mass spectrometer at Drexel University, Philadelphia, PA.

Analytical thin layer chromatography (TLC) was conducted using Analtech silica gel GF plates (250 μm) containing a fluorescent indicator. Detection was performed by observation under UV light or by iodine staining. Preparative TLC was carried out using 20 cm^2 silica gel GF plates (1000 μm). Flash column chromatography was performed using Merck silica gel 50 (230–400 mesh). Elemental analyses were performed by Galbraith Laboratories, Inc. Knoxville, TN.

2.1. (25R)-Cholest-5-en-3β,16β,26-triol (2)

To a 100 ml three neck flask was added sequentially diosgenin (0.22 g, 0.53 mmol), zinc dust (4.5 g, 68.8 mmol), and 50 ml absolute alcohol. After the mixture was stirred, heated to reflux, and 40 ml of concentrated HCl was added dropwise during a 30-min period. The reaction was refluxed for an additional 30 min, then filtered to remove zinc dust. The solution was collected and distilled water was added until a precipitate appeared. The solution was heated until transparent, then slowly cooled. The precipitate, which formed, was collected by suction filtration and washed three times with cold water. The crystals were then dried to yield the triol **2** as a white solid (0.18 g, 85%): mp = 172–174 °C, lit. [9] mp = 176–178 °C. IR (KBr) (cm^{-1}): 3377, 2934, 1464, 1378, 1043, 827. ^{1}H NMR ($CDCl_3/CD_3OD$ (2:1)), δ (ppm): 0.82 (s, 3H, Me-18), 0.83 (d, J = 7.0 Hz, 3H, Me-27),

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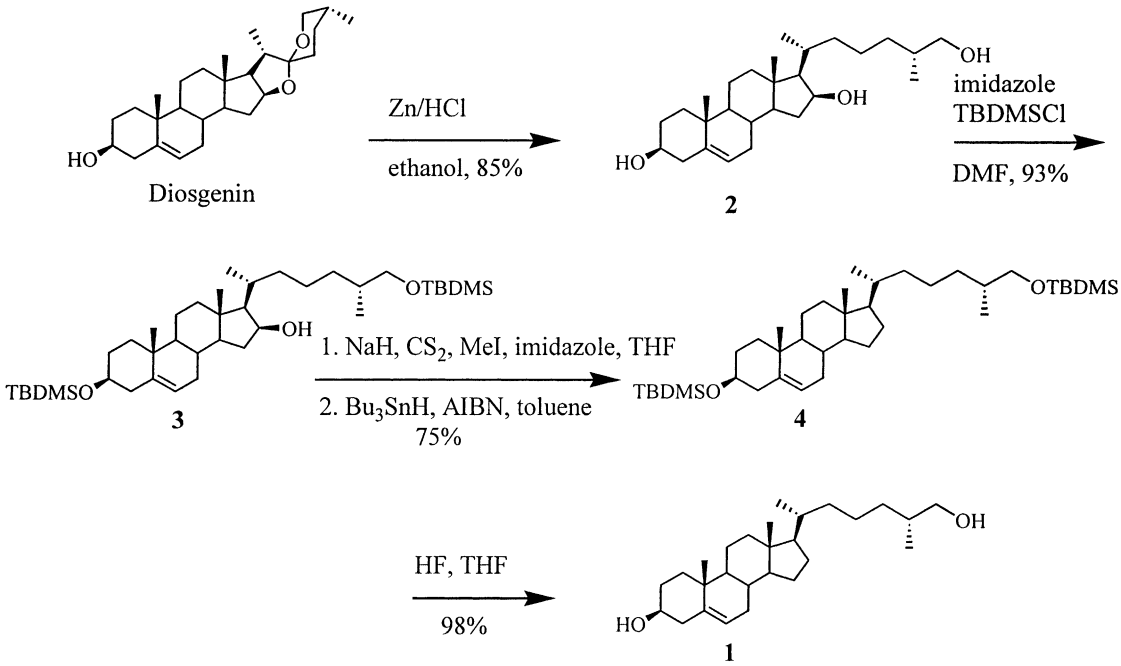

Fig. 1. Synthesis of (25*R*)-26-hydroxycholesterol (**1**) from diosgenin.

0.91 (d, J = 6.5 Hz, 3H, Me-21), 0.95 (s, 3H, Me-19), 3.33–3.56 (m, 3H, H-3α and H-26), 4.28 (m, 1H, H-16β), 5.28 (m, 1H, H-6). ^{13}C NMR ($CDCl_3/CD_3OD$ (2:1)), δ (ppm): 13.40(C18), 17.01(C27), 18.63(C21), 19.98(C19), 21.25(C11), 23.97(C23), 30.29(C20), 31.67(C2), 32.04(C8), 32.04(C7), 32.35(C24), 35.89(C25), 36.44(C22), 37.06(C10), 37.12(C15), 37.75(C1), 40.42(C12), 42.74(C4), 42.38(C13), 50.66(C9), 54.97(C14), 61.99(C17), 68.23(C26), 71.77(C3), 72.50(C16), 121.83(C6), 141.55(C5).

2.2. (25R)-3β,26-Bis[(tert-butyldimethylsilyl)oxy]-cholest-5-en-16β-ol (3)

To a solution of *tert*-butyldimethylsilyl chloride (0.9 g, 6 mmol) in 10 ml of dry DMF was added imidazole (0.8 g, 12 mmol) and the mixture was stirred at room temperature for 15 min under nitrogen **2** (0.5 g, 1.2 mmol) was added and the mixture stirred at room temperature for 16 h. A white precipitate was obtained by filtration, which was purified by flash chromatography (20:1, hexane:ethyl acetate) to afford **3** as a white solid (0.64 g, 93%): mp = 122–124 °C, lit. [10] mp = 123–124 °C. IR (KBr) (cm^{-1}): 3629, 2956, 1470, 1384, 1253, 1080, 837. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.04 (s, 6H, $(CH_3)_2$–Si), 0.06 (s, 6H, $(CH_3)_2$–Si), 0.86 (s, 3H, Me-18), 0.891 (s, 9H, $(CH_3)_3$–Si), 0.896 (s, 9H, $(CH_3)_3$–Si), 0.98 (d, J = 6.5 Hz, 3H, Me-21), 1.01 (s, 3H, Me-19), 3.33–3.56 (m, 3H, H-3α and H-26), 4.33 (m, 1H, H-16β), 5.34 (m, 1H, H-6). ^{13}C NMR ($CDCl_3$), δ (ppm): −4.62(CH_3Si), −3.86(CH_3Si), 13.71(C18), 17.45(C27), 18.87((CH_3)$_3$CSi), 18.92((CH_3)$_3$CSi), 19.02(C21), 20.12(C19), 21.38(C11), 24.47(C23), 30.41(C20), 26.64((CH_3)$_3$CSi), 26.69((CH_3)$_3$-CSi), 32.17(C8), 32.54(C7), 32.75(C2), 34.27(C24), 36.46-(C25), 36.96(C22), 37.23(C15), 37.33(C10), 38.01(C1), 40.60(C12), 43.50(C4), 42.86(C13), 50.86(C9), 55.26(C14), 62.13(C17), 69.11(C26), 72.96(C3), 73.22(C16), 121.61 (C6), 141.19(C5).

2.3. (25R)-3β,26-Bis[(tert-butyldimethylsilyl)oxy]-cholest-5-ene (4)

To a solution of **3** (30 mg, 0.046 mmol) in 5 ml THF were added 40 mg (1 mmol) NaH (65% suspension in mineral oil) and 4 mg imidazole. The mixture was refluxed under argon for 30 min, then 0.4 ml (4.7 mmol) CS_2 was added. The mixture was refluxed for another hour, then 0.2 ml (3 mmol) of methyl iodide was added. The mixture was refluxed for an additional 30 min. Then the mixture was poured into 10 ml of water, and extracted with ether (2 × 10 ml). The ether extracts were washed with brine, dried (Na_2SO_4) and the solvent was removed in vacuo to give a yellow oil. The oil was refluxed in toluene (10 ml) containing a trace of AIBN, under argon. A solution of tributylstannane (0.2 ml, 0.75 mmol) in toluene (3 ml) was added over 30 min. The mixture was refluxed overnight. The reaction was quenched with aqueous saturated $NaHCO_3$, and the aqueous layer was extracted with diethyl ether. The combined organic phases were dried (Na_2SO_4), and the solvent was evaporated in vacuo. The residue was purified by flash chromatography (10:1 hexane:ethyl acetate) to yield **4** as a white solid (22 mg, 75%): mp = 98–100 °C. IR (CH_2Cl_2) (cm^{-1}): 2933, 2857, 1464, 1254, 1097, 836,

774. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.04 (s, 6H, $(CH_3)_2$–Si), 0.06 (s, 6H, $(CH_3)_2$–Si), 0.67 (s, 3H, Me-18), 0.85 (d, $J = 3.5$ Hz, 3H, Me-27), 0.889 (s, 9H, $(CH_3)_3$–Si), 0.895 (s, 9H, $(CH_3)_3$–Si), 0.90 (d, $J = 6.5$ Hz, 3H, Me-21), 1.00 (s, 3H, Me-19), 3.33–3.56 (m, 3H, H-3α and H-26), 5.34 (m, 1H, H-6). ^{13}C NMR ($CDCl_3$), δ (ppm): −4.91(CH_3Si), −4.16(CH_3Si), 12.27(C18), 17.11(C27), 18.67(($CH_3)_3C$Si), 18.77(($CH_3)_3C$Si), 19.12(C21), 19.85(C19), 21.49(C11), 23.78(C23), 24.71(C15), 26.38(($CH_3)_3$CSi × 2), 28.67(C16), 32.32(C2), 32.36(C8), 32.51(C7), 34.00(C24), 36.15(C20), 36.15(C25), 36.61(C22), 37.00(C10), 37.81(C1), 40.23(C12), 42.74(C4), 43.25(C13), 50.63(C9), 56.57(C17), 57.22(C14), 68.96(C26), 73.05(C3), 121.58(C6), 141.94(C5).

2.4. (25R)-Cholest-5-ene-3β,26-diol (*1*)

In a plastic tube was placed the disilyl ether **4** (0.064 g, 0.1 mmol), THF (2 ml), and 49% aqueous HF (1 ml). The solution was allowed to stir for 20 h. Water was added and the solution extracted with ethyl acetate (3 × 10 ml). The organic layers were combined and washed to neutrality with a saturated $NaHCO_3$ solution, then with water and finally dried (Na_2SO_4) and the solvent removed in vacuo. Recrystallization from ethyl acetate yielded 26-hydroxycholesterol (**4**) (40 mg, 98%), mp = 168–170 °C, lit. [6] mp = 172–173 °C. IR (KBr) (cm^{-1}): 3341, 2932, 1466, 1378, 1055. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.61 (s, 3H), 0.85 (d, $J = 6.43$ Hz, 3H), 0.90 (d, $J = 6.5$ Hz, 3H), 0.98 (s, 3H), 3.33–3.56 (m, 3H), 5.30 (m, 1H). ^{13}C NMR ($CDCl_3$ + CD_3OD, two drops), δ (ppm): 12.17(C18), 16.80(C27), 18.98(C21), 19.7(C19), 21.41(C11), 23.78(C23), 24.62(C15), 28.58(C16), 31.63(C2), 32.24(C7), 32.24(C8), 33.92(C24), 36.05(C20), 36.09(C25), 36.52(C22), 36.85(C10), 37.59(C1), 40.13(C12), 42.31(C4), 42.66(C13), 50.48(C9), 56.48(C17), 57.11(C14), 68.46(C26), 71.78(C3), 121.96(C6), 141.19(C5).

3. Results and discussion

Diosgenin was chosen as the starting material for the synthesis of (25*R*)-26-hydroxycholesterol (**1**) since it is commercially available in high purity. Clemmensen reduction of diosgenin has been reported to yield (25*R*)-cholest-5-en-3β,16β,26-triol (**2**) in a variety of yields: 50–60% [9], 45% [10] and **2** and recovered diosgenin ∼75% [11]. The yield for this reaction was improved to 85% by removing the mercury and just using zinc dust in ethanol and adding concentrated HCl acid dropwise. The product was then pure enough to crystallize from aqueous ethanol instead of being extracted with chloroform and chromatographed [10].

In order to remove the C-16β-hydroxy group, we needed to selectively protect the C-3β- and C-26-hydroxy groups. This was achieved by reaction of the triol **2** with *tert*-butyldimethylsilyl chloride in dry DMF using imidazole as a catalyst to afford the 3β,26-bis-silyl ether **3** in 93% yield. Next, we needed a reaction for the deoxygenation of a secondary alcohol to a hydrogen without the possibility of a rearrangement that could occur if the reaction proceeded via an ionic process of any type. We chose the Barton deoxygenation reaction which proceeds via a radical mechanism [13]. The C-16 alcohol in **3** was converted to the intermediate dithiocarbonate using sodium hydride, carbon disulfide, and methyl iodide and then reduced with *n*-Bu_3SnH and AIBN to the 3β,26-bis-silyl ether **4** in 75% yield. Desilylation of **4** using 49% aqueous HF [14] afforded 26-hydroxycholesterol in 98% yield. Using this sequence the 25*R* isomer of 26-hydroxycholesterol was synthesized from diosgenin in 58% overall yield, an improvement over the best previous yield of 45% [11].

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