Notes

Selenium Dioxide Oxidation of 3β-Benzoyloxy-5α-cholest-8(14)-en-15-one: Chemical Synthesis of 3β-Hydroxy-5α-cholest-8(14),16-dien-15-one

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Introduction

3β-Hydroxy-5α-cholest-8(14),16-dien-15-one (**1**) and 3β-hydroxy-5α-cholest-8(14)-en-15-one (**2**) have been shown to exhibit the inhibition of cholesteryl ester transfer protein.[1a] 3β-Hydroxy-5α-cholest-8(14)-en-15-one (**2**) has been known to show a number of biological activities. **2** is a highly active inhibitor of sterol synthesis and lowers the levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-Co A) reductase activity in cultured mammalian cells, and also a potent inhibitor of cholesterol absorption and inhibits the oleoyl-CoA-dependent esterification of cholesterol in hepatic and jejunal microsomes.[2]

We have previously reported a six-step synthesis of **1** from diosgenin in 4.3% overall yield.[1] However, some of the step are inefficient for multigram preparation. Oxidation of 3β-benzoyloxy-5α,14α-cholest-16-en-15-one to 3β-benzoyloxy-5α-cholest-8(14),16-dien-15-one (**4**) with selenium dioxide results in modest yields (36%). Previously Schroepfer *et al.* reported large-scale synthesis of 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (**3**) from 7-dehydrocholesterol by three sequential steps.[3] In continuation of our study on the synthesis and evaluation of biological activity of 15-oxysterols, we examined an alternate synthesis of **1** starting from **3**. Described herein is a synthesis of **1** by oxidation of **3** with selenium dioxide.

Experimental Section

General experimental procedures for melting points, FT-IR spectra, UV spectra, NMR spectra, mass spectrometry, and high resolution MS have been described previously.[4] ^{1}H and ^{13}C NMR assignments were made from DEPT, COSY, HETCOR and by comparison with spectra of similar sterols.[1,5,6] Elemental analyses were performed by CSI at Kyungpook National University. TLC analyses were carried out on precoated 0.2 mm HPTLC silica gel 60 plates; substances were visualized by spraying with 5% ammonium molybdate in 10% H_2SO_4 followed by heating. TLC solvent systems were: (SS-1), EtOAc : hexane 1 : 2; (SS-2), EtOAc: hexane 1 : 4; (SS-3), EtOAc : hexane 1 : 19; (SS-4), Et_2O: benzene 1 : 1; (SS-5), Et_2O : benzene 1 : 4. Radial chromatography was performed on a Harrison Research Chromatotron, by using Merck silica gel 60 PF_{254}. All reactions were performed under argon. Selenium dioxide (SeO_2) and 2-methyl-2-propanol were purchased from Aldrich. 2-Methyl-2-propanol was dried over CaH_2 and distilled prior to use. 3β-Hydroxy-5α-cholest-8(14),16-dien-15-one (**1**).[1] 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (**3**)[1,3] and 3β-benzoyloxy-5α-cholest-8(14),16-dien-15-one (**4**) were prepared previously.[1]

Oxidation of 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (3) with selenium dioxide. A mixture of 3β-benzoyloxy-5α-cholest-8(14)-en-15-one **3** (504 mg, 0.10 mmol) and SeO_2 (275 mg, 0.25 mmol) in 2-methyl-2-propanol (6 mL) was heated for 3 h. The reaction mixture was diluted with brine and extracted with ethyl acetate. The combined extracts were washed with a saturated solution of sodium bicarbonate and brine, and dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was chromatographed on a short path silica gel column to give 467 mg of crude product. The crude product was further purified on chromatotron (EtOAc : hexane 1 : 9, v/v). The first fraction gave 3β-benzoyloxy-5α-cholest-8(14),16-dien-15-one (**4**) (406 mg, 81%). Mp. 164-165 °C (CH_2Cl_2-MeOH) (lit.[1] 164-165 °C). Single component on TLC in three solvent systems, R_f 0.71 (SS-2), 0.52 (SS-3, developed 2 times), 0.79 (SS-5); MS *m/z*: 502 (70, M^+); HRMS *m/z*: 502.3440 for $C_{34}H_{46}O_3$ requires 502.3447.

Further elution with same solvent gave 3β-benzoyloxy-5α-cholest-8(14),16-dien-15-one 16-selenenic acid (**5**) (45 mg, 7%) as a yellowish solid. Mp. 240 °C (CH_2Cl_2-hexane). Single component on TLC in three solvent systems: R_f 0.67 (SS-2), 0.17 (SS-3, developed 2 times), 0.88 (SS-5). FT-IR (KBr): 3554, 2933, 2863, 1717, 1690, 1644, 1454, 1273, 1111, 713 cm^{-1}; ^{1}H NMR: δ 0.85 (*d*, 3H, *J* = 6.4 Hz, 26, 27-CH_3), 0.86 (*s*, 3H, 19-CH_3), 0.98 (*d*, 3H, *J* = 6.8 Hz, 21-CH_3), 1.15 (*s*, 3H, 18-CH_3), 2.45 (*m*, 1H, 20-H), 4.12 (*bd*, 1H, *J* = 12.5 Hz, 7β-H), 5.01 (*m*, 1H, 3α-H), 7.43 (*dd*, *J* = 7.4 Hz, 2H, *m* of Ph), 7.55 (*dd*, *J* = 7.4 Hz, 1H, *p* of Ph), 8.04 (*d*, *J* = 7.4 Hz, 2H, *o* of Ph); ^{13}C NMR: δ 193.1 (C-15), 185.0 (C-17), 166.1 (C=O), 145.8 (C-8), 135.9 (C-14), 132.8 (C4

of Ph), 130.8 (C1 of Ph), 129.9 (C-16), 129.5 (C2 of Ph), 128.3 (C3 of Ph), 73.8 (C-3), 51.3 (C-9), 46.3, 44.5, 38.9, 38.8, 38.1, 36.2, 34.3, 33.7, 30.6, 29.2, 27.8, 27.6, 27.4, 26.1, 24.4 (C-18), 22.6 (C-26, 27), 19.6, 18.3 (C-21), 12.9 (C-19); UV λ_{max}: 211 (log ε 4.49), 228 (log ε 4.40), 267 nm (log ε 4.21); MS *m/z*: 597 (17, M-H), 584 (31), 583 (65), 582 (56, M-CH_3), 581 (47, M-OH), 580 (43, M-H_2O), 579 (41), 578 (40), 577 (17), 576 (19), 501 (29, M-SeOH), 486 (45), 469 (26), 467 (20), 388 (21), 362 (19), 347 (18), 345 (22), 266 (15), 105 (100, $C_6H_5CO^+$); HRMS on ion at *m/z* 581(M-OH): 581.2559 for $C_{34}H_{45}O_3Se$ requires 581.2534. Anal. Calcd for $C_{34}H_{46}O_4Se$: C, 68.32; H, 7.76; Found C, 68.81; H, 8.01.

Third fraction gave 3β-benzoyloxy-9α-hydroxy-5α-cholest-6,8(14),16-trien-15-one (**6**) (19 mg, 4%). Mp. 141-142 °C (CH_2Cl_2-MeOH). Single component on TLC in three solvent systems: R_f 0.33 (SS-2), 0.08 (SS-3, developed 2 times), 0.42 (SS-5). FT-IR (KBr): 3456, 2931, 2865, 1715, 1672, 1456, 1275, 1115, 754, 714 cm^{-1}; ^1H NMR: δ 0.87 (*d*, 6H, *J* = 6.6 Hz, 26, 27-CH_3), 0.97 (*s*, 3H, 19-CH_3), 1.12 (*d*, 3H, *J* = 6.8 Hz, 21-CH_3), 1.23 (*s*, 3H, 18-CH_3), 2.43 (*q*, 1H, *J* = 6.8 Hz, 20-H), 5.49 (*m*, 1H, 3α-H), 5.93 (*s*, 1H, 16-H), 5.82 (*d*, 1H, *J* = 9.9 Hz, 6-H), 7.41 (*m*, 2H, *m* of Ph), 7.54 (*m*, 1H, *p* of Ph), 7.60 (*d*, 1H, *J* = 9.9 Hz, 7-H), 8.02 (*m*, 2H, *o* of Ph); ^{13}C NMR: δ 196.1 (C-15), 187.2 (C-17), 166.0 (C=O), 140.4 (C-8), 137.0 (C-14), 136.4 (C-7), 132.7 (C4 of Ph), 130.8 (C1 of Ph), 129.5 (C2 of Ph), 128.5 (C3 of Ph), 127.2 (C-16), 125.4 (C-6), 73.3 (C-9), 71.0 (C-3), 45.7, 40.8, 40.7, 39.0, 37.8, 36.9, 33.1, 29.9, 28.5, 27.9, 26.9 (C-18), 26.6, 26.6, 25.4, 22.6 (C-26, 27), 21.8 (C-21), 15.8 (C-19); UV λ_{max}: 206 (log ε 4.30), 230 (log ε 4.34), 305 nm (log ε 4.16); MS *m/z*: 516 (22, M^+), 501 (24, M-CH_3), 498 (29, M-H_2O), 394 (32, M-C_6H_5COOH), 379 (34), 376 (81), 281 (16), 266 (43), 263 (29), 105 (100, $C_6H_5CO^+$); HRMS *m/z*: 516.3232 for $C_{34}H_{44}O_4$ requires 516.3240. Anal. Calcd for $C_{34}H_{44}O_4$: C, 79.03; H, 8.58; Found C, 79.20; H, 8.75.

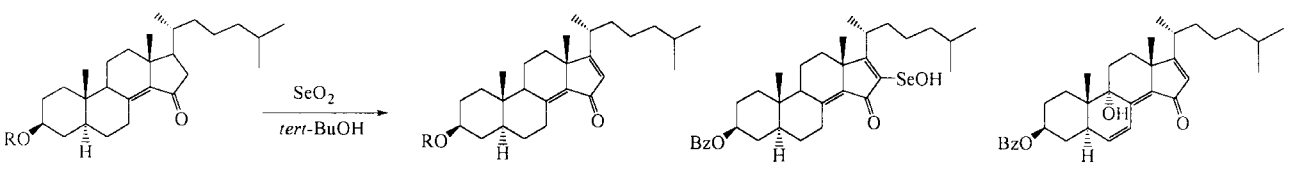
3β-Hydroxy-5α-cholest-8(14),16-dien-15-one (1). Compound **4** (80 mg, 0.159 mmol) in 1 M 5% KOH in ethanol solution (10 mL) was stirred at room temperature for 2 h. The reaction mixture was diluted with brine (30 mL) and then extracted twice with ethyl acetate (30 mL). The combined extracts were washed sequentially with 2% HCl and water, dried over anhydrous sodium sulfate, and concentrated to dryness. The crude product was chromatographed on silica gel (EtOAc : hexane 1 : 2, v/v) and afforded a white solid **1** (59 mg, 0.148 mmol, 93%). Mp. 127-128.5 °C (H_2O-MeOH) (lit.[1] 127-129 °C). Single component on TLC in three solvent systems: R_f 0.40 (SS-1), 0.13 (SS-2), 0.53 (SS-4). UV: λ_{max} 259 nm (log ε 4.05); MS *m/z*: 398 (100, M); HRMS *m/z*: 398.3178 for $C_{27}H_{40}O_2$ requires 398.3185.

Results and Discussion

Reaction of 3β-benzoyloxy-5α-cholest-8(14)-en-15-one (**3**) with selenium dioxide in 2-methyl-2-propanol resulted in products that were a mixture of $\Delta^{8(14),16}$-15-one **4** (81%) along with the $\Delta^{8(14),16}$-15-one 16-selenenic acid **5** (7%) and the $\Delta^{6,8(14),16}$-9α-ol-15-one **6** (4%), which were easily isolated by silica gel chromatography (Scheme 1). Structure of compound **4** was confirmed by comparison with the known analytical data and m.p., and R_f value in TLC.[1] FT-IR spectrum showed the characteristic conjugated carbonyl absorption and ester carbonyl stretching absorption bands at 1680 and 1717 cm^{-1}, respectively. UV spectrum revealed the absorptions at λ_{max} 233 nm (log ε 4.29) and 259 nm (log ε 4.23) for the characteristics of $\Delta^{8(14),16}$-15-one.

Selenenic acid **5** was characterized by ^1H and ^{13}C NMR data (chemical shifts, coupling constants, homonuclear and heteronuclear shift correlations), FT-IR, UV, MS and high resolution MS. In the ^{13}C NMR spectrum, existence of the selenenic acid group at C-16 caused the anticipated downfield shift at C-16 (2.1 ppm) and upfield shifts at C-14 (2.0 ppm), C-15 (4.4 ppm), C-17 (1.9 ppm) and C-21 (3.8 ppm). Due to the deshielding by the carbonyl group at C-15, the ^1H NMR signal of 7β-H appears at δ 4.12 (*J* = 12.5 Hz). Infrared stretching bands at 3554 and 1690 cm^{-1}, and UV absorption at λ_{max} 267 nm (log ε 4.21) confirms the existence of $\Delta^{8(14),16}$-15-one 16-selenic acid. The presence of one selenium atom in a molecule could be immediately recognizable from the characteristic isotopic pattern. Selenium has six stable isotopes: ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se and ^{80}Se is the major one.[7] Based on this isotopic complexity, the ion at *m/z* 597 (17% relative abundance) represents M-H. The set of ions from *m/z* 576 to 584 may represent overlapping ions such as M-CH_3, M-OH and M-H_2O.

The structure of **6** was determined based on the various spectroscopic data. For instance, ^1H NMR spectrum showed 6, 7 and 16-protons at δ 5.82 (*J* = 9.9 Hz), 7.60 (*J* = 9.9 Hz) and 5.93,[5] and one quaternary carbon at δ 73.3 (C-9) and two olefinic carbons appeared at δ 125.4 (C-6) and 136.4 (C-7) on ^{13}C NMR spectrum.[6] The extension of the double bond



3: R = COC_6H_5
2: R = H

4: R = COC_6H_5
1: R = H

5

6

Scheme 1. Synthetic scheme for the preparation of 3β-hydroxy-5α-cholest-8(14),16-dien-15-one (1).

was confirmed by the bathochromic shift from **4** (λ_{max} 259 nm; log ε 4.23) to **6** (λ_{max} 305 nm; log ε 4.16).[1] Infrared stretching bands at 3546 and 1672 cm^{-1} also confirms the existence of $\Delta^{8(14),16}$-9α-ol-15-one. The mass spectrum displayed the molecular ion at *m/z* 516 (22% relative abundance).

Hydrolysis of **4** in 1 M ethanolic potassium hydroxide solution gave **1** in 93% yield. The structure and stereochemistry of **1** were confirmed by comparing the ^1H and ^{13}C-NMR and MS values of **1** reported in the literature.[1]

In summary, practical synthesis of 3β-hydroxy-5α-cholest-8(14),16-dien-15-one (**1**) has been presented. Two side products also have been isolated, identified and characterized by spectroscopic methods. The stable selenenic acid bearing a steroid skeleton was also prepared.

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