Studies of the synthesis of biomarkers. VII. Synthesis of 5α-(17R,20R)-14,15-secocholestane

Tong-Shuang Li,* Yu-Lin Li,* and Xiao-Tian Liang†

**Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, People's Republic of China; and †Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, People's Republic of China*

5α-(17R,20R)-14,15-Secocholestane ***(12)*** *was synthesized from cholesterol* ***(1)*** *in 12 steps. The key intermediate, 5α-cholest-14-en-3β-yl acetate* ***(4)****, underwent ozonization, reduction, hydrolysis, and oxidation to provide 5α-14,15-secocholesta-3,14,15-trione* ***(8)****. One of the Clemmensen reduction products of* ***8*** *is 5α(17R,20R)-14,15-secocholest-15-ol* ***(11)****; treatment of the alcohol* ***(11)*** *with tosyl chloride and subsequent reduction with lithium aluminum hydride yielded the target molecule* ***(12)****.* (*Steroids* **55**:263–265, 1990)

Keywords: steroids; secocholestane synthesis; biomarkers; 14,15-secocholestane; sterols

Introduction

Yang and Yang reported that a novel secosterane was uncovered in crude oils from the Kalamayi oilfield of China.[1] Judging from the mass spectrum, they suggested that the structure was a 13,17-secosterane. It has been shown[2–4] that C14 or C15 can be preferentially functionalized under some reaction conditions. Therefore, it is possible that the 14,15-secosterane is more likely to be formed than the 13,17-secosterane. The mass spectrum reported could be seen to be-more consistent with a 14,15-secosterane structure. To ascertain the structure of the secosterane of Yang and Yang, we decided to synthesize an authentic sample of 5α-(17R,20R)-14,15-secocholestane **(12)**.

Experimental

All melting points (mp) were uncorrected. Infrared spectra were recorded on a Nicolet FT-IR-5DX spectrometer. ^{1}H Nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were measured on Varian FT-80A and Bruker AM-400 spectrometers, using $CDCl_3$ solvent and tetramethylsilane as internal references. Mass spectra were obtained on a Finnigan-4021C spectrometer (EI, 70 eV) or on a MAT-44S spectrometer (CI) using isobutane as reaction gas. Gas chromatography was performed on a Shimadzu GC-9A instrument, SE-54 quartz glass capillary column, 280° C.

3β-Acetoxy-5α-cholestan-7β-ol (3)

3β-Acetoxycholest-5-en-7-one **(2)**[5] (1.00 g) was hydrogenated over Raney nickel (2 g) in absolute ethanol (150 ml) at room temperature for 2 hours. Removal of the catalyst and solvent yielded **3** (1.01 g), mp 73° C (colorless needless from methanol). ν_{max}: 3360, 1736, 1038 cm^{-1} δ_H: 2.01 (3H, s, CH_3CO–), 3.31 (1H, m, W1/2 = 20 Hz, 7α-H), 4.67 (1H, m, W1/2 = 24 Hz, 3α–H) ppm.

*3β-Acetoxy-5α-cholest-14-ene ozonides (***5a** *and* **5b***)*

A solution of 200 mg of 3β-acetoxy-5α-cholest-14-ene **(4)**[6,7] in 10 ml of dichloromethane was cooled to −20° C and ozonized until the reaction was complete. After removing the solvent, a white crystalline substance was obtained (218 mg). It was a mixture of **5a** and **5b** (4 : 1) as indicated by ^{1}H NMR (400 MHz). ν_{max} (mixture): 1,735, 1,246, 1,075, 1,030 cm^{-1}. δ_H **(5a)**: 0.83 (3H, s, 19-CH_3), 0.86 (6H, d, J = 6.6 Hz, 26,27-CH_3), 0.91 (3H, d, J = 6.9 Hz, 21-CH_3), 0.95 (3H, s, 18-CH_3), 2.03 (3H, s, CH_3CO—), 4.69 (1H, m, 3α-H), 5.77 (1H, d, J = 6.5 Hz, 15-H) ppm. δ_H **(5b)**: 0.99 (3H, s, 18-CH_3), 2.02 (3H, s, CH_3CO—), 5.73 (1H, t, J = 1 Hz, 15-H) ppm.

Address reprint requests to Dr. Yu-Lin Li, Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou 730000, People's Republic of China.
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3β-Acetoxy-5α14,15-secocholesta-14,15-dione **(6)**

A mixture of **5a** and **5b** (50 mg) was reduced by dimethyl sulfide (0.5 ml) in dichloromethane (3 ml) at room temperature for 10 hours. The crude product was purified by silica gel column chromatography to give 40 mg of **6** (yield 74%). ν_{max}: 1,731, 1,700, 1,030 cm^{-1}. δ_H: 0.87 (6H, d, J = 6.4 Hz, 26,27-CH_3), 0.89 (3H, s, 19-CH_3), 0.92 (3H, d, J = 6.8 Hz, 21-CH_3), 1.10 (3H, s, 18-CH_3), 2.03 (3H, s, CH_3CO—), 2.52 (1H, td, J = 11.2 Hz, 3.0 Hz, 8β-H), 4.68 (1H, m, 3α-H), 9.77 (1H, t, J = 2.4 Hz, —CHO) ppm.

5α-14,15-Secocholesta-3,14,15-trione **(8)**

A mixture of 35 mg of **6,** 7 ml of methanol, 0.5 ml of water, and 38 mg of potassium carbonate was stirred at room temperature for 4 hours, diluted with water, and extracted with ether to provide 30 mg of **7,** which was confirmed by its IR spectrum (3,340 cm^{-1}) and ^1H NMR spectrum (3.60 ppm, m, 3α-H). Compound **7** (25 mg) was then oxidized by pyridinium dichromate (0.2 g) in dimethylformamide (2 ml) at room temperature for 12 hours to yield 22 mg of **8** (overall yield from **6,** 83%). δ_H: 0.85 (6H, d, J = 6.8 Hz, 26, 27-CH_3), 0.92 (3H, d, J = 6.8 Hz, 21-CH_3), 1.08 (3H, s, 19-CH_3), 1.13 (3H, s, 18-CH_3), 2.57 (1H, td, J = 12.0 Hz, 3.6 Hz, 8β-H), 9.77 (1H, t, J = 2.4 Hz, —CHO) ppm.

Clemmensen reduction of **8**

The preparation, isolation, and identification of compounds 9, 10, 11, and 12. A mixture of 40 mg of **8,** 0.5 g of amalgamated zinc, 12 ml of ethanol, and 4 ml of concentrated hydrochloric acid was refluxed for 3.5 hours, cooled, diluted with water, and extracted with ether to provide a light yellow oily substance (about 40 mg). The crude product was separated on 5 g of silica gel containing 10% silver nitrate. Eluting with petroleum ether gave about 20 mg of hydrocarbons which contained 50% of **12,** as shown by gas chromatography. The column was then eluted with mixtures of petroleum ether and diethyl ether in the ratios 8 : 1, 5 : 1, and 2 : 1. Compounds **10** (3 mg), **11** (8 mg), and **9** (4 mg) were obtained successively. Compound **10** was a white solid. ν_{max}: 3,482, 1,682, 1,056 cm^{-1}. δ_H: 0.65 (3H, s, 19-CH_3), 0.83, 0.85 (6H, 2d, J = 6.6 Hz, 26, 27-CH_3), 0.87 (3H, d, J = 6.7 Hz, 21-CH_3), 1.02 (3H, s, 18-CH_3), 3.57 (1H, dd, J = 11.8 Hz, 7.5 Hz, 15β-H) ppm. δ_C (numbering of carbon): 38.9 (1), 21.1 (2), 26.5 (3), 28.6 (4), 52.7 (5), 30.9 (6), 31.9 (7), 55.0 (8), 51.1 (9), 38.6 (10), 21.9 (11), 45.8 (12), 48.2 (13), 221.6 (14), 78.4 (15), 26.5 (16), 45.7 (17), 22.6 (18), 14.4 (19), 34.3 (20), 19.8 (21), 31.1 (22), 25.8 (23), 40.1 (24), 28.0 (25), 22.7 (26), 22.8 (27) ppm. m/z: 402 (10, M^+), 384 (3, M^+-H_2O), 233 (100, M^+ − side chain − ring D), 125 (52), 109 (42). Compound **11** was a colorless oily substance. ν_{max}: 3,341, 1,048 cm^{-1}. δ_H: 0.75 (3H, s, 18-CH_3), 0.84 (3H, s, 19-CH_3), 0.87 (6H, d, J = 6.6 Hz, 26,27-CH_3), 0.90 (3H, d, J = 7.0 Hz, 21-CH_3), 3.63 (2H, m, 15-CH_2) ppm. δ_C: 38.5 (1), 22.1 (2), 26.9 (3), 29.1 (4), 46.9 (5), 29.0 (6), 33.5 (7), 32.3 (8), 54.3 (9), 35.3 (10), 20.7 (11), 29.7 (12), 37.5 (13), 35.3 (14), 64.9 (15), 45.3 (16), 53.0 (17), 19.9 (18), 12.2 (19), 31.8 (20), 22.1 (21), 37.0 (22), 26.4 (23), 39.4 (24), 27.9 (25), 22.6 (26), 22.7 (27) ppm. m/z: 372 (0.05, M^+ − H_2O), 219 (100, M^+ − side chain), 151 (1), 149 (13), 137 (27), 123 (67). Compound **9** was a white solid ν_{max}: 3,480, 1,707, 1,703, 1,054 cm^{-1}. δ_H: 0.84, 0.86 (6H, 2d, J = 6.6 Hz, 26,27-CH_3), 0.88 (3H, s, 19-CH_3), 0.88 (3H, d, J = 6.7 Hz, 21-CH_3), 1.06 (3H, s, 18-CH_3), 3.63 (1H, dd, J = 11.8 Hz, 7.5 Hz, 15β-H) ppm. δ_C: 38.9 (1), 37.8 (2), 211.5 (3), 45.6 (4), 52.7 (5), 30.6 (6), 32.0 (7), 54.6 (8), 50.1 (9), 37.8 (10), 21.8 (11), 44.2 (12), 48.3 (13), 221.4 (14), 77.9 (15), 26.3 (16), 45.3 (17), 22.5 (18), 13.5 (19), 34.3 (20), 19.7 (21), 31.2 (22), 25.8 (23), 39.7 (24), 28.0 (25), 22.6 (26), 22.7 (27) ppm. m/z: 416 (10, M^+), 398 (8, M^+ − H_2O), 247 (100, M^+ − side chain − ring D), 123 (32).

5α-(17R,20R)-14,15-Secocholestane **(12)**

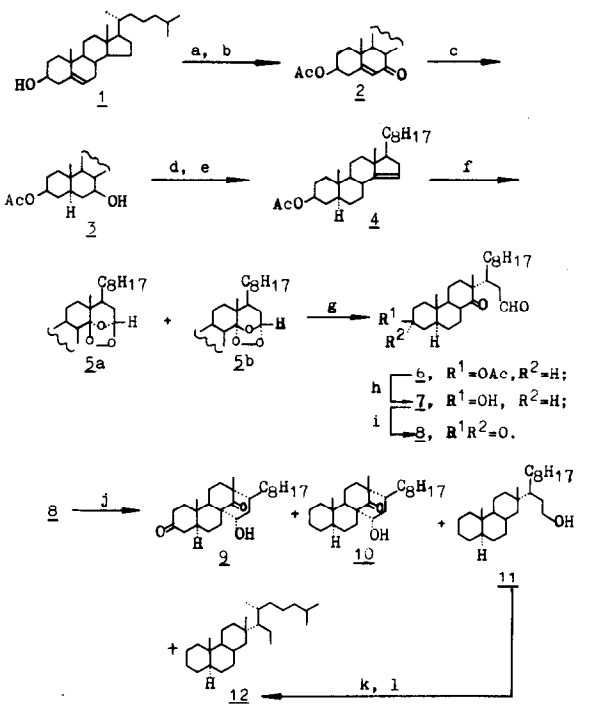
Compound **11** (5 mg) was treated with tosyl chloride (10 mg) in pyridine (0.2 ml) at room temperature for 16 hours to provide the crude tosylate. After purification by column chromatography, 6 mg of 5α-(17R,20R)-14,15-secocholest-15-yl tosylate was obtained. This was reduced by lithium aluminum hydride (4 mg) in tetrahydrofuran (1 ml) at refluxing temperature for 3 hours. The usual work-up gave a crude product, which was purified by 1 g of 10% silver nitrate-silica gel column chromatography (petroleum ether as eluant) to give 3 mg of **12** (overall yield from **11,** 63%). δ_H: 0.75 (3H, s, 18-CH_3), 0.81 (3H, s, 19-CH_3), 0.86 (6H, d, J = 6.6 Hz, 26,27-CH_3), 0.93 (3H, d, J = 7.1 Hz, 21-CH_3), 0.94 (3H, t, J = 7.5 Hz, 15-CH_3) ppm. δ_C: 38.5 (1), 22.1 (2), 26.9 (3), 29.2 (4), 46.9 (5), 29.7 (6), 33.3 (7), 31.8 (8), 54.4 (9), 36.2 (10), 20.7 (11), 29.0 (12), 37.8 (13), 45.4 (14), 22.4 (15), 35.4 (16), 59.2 (17), 17.2 (18), 12.2 (19), 32.6 (20), 29.9 (21), 37.0 (22), 26.6 (23), 39.5 (24), 28.0 (25), 22.7 (26), 22.7 (27) ppm. m/z (EI): 261 (0.34), 219 (100), 151 (1.5), 149 (12), 123 (59), 109 (63). m/z (CI): 373 (5, M^+ − 1), 219 (100), 205 (16).

Results and discussion

The target molecule, 5α-(17R,20R)-14,15-secocholestane **(12),** has been synthesized from cholesterol **(1)** as indicated in Scheme 1.

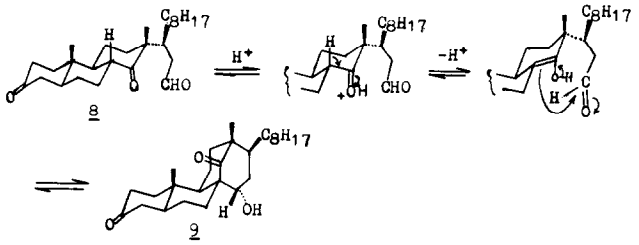
The preparation of 3β-acetoxycholest-5-en-7-one **(2)** from cholesterol **(1)** has already been reported by Fieser et al.[5] Hydrogenation of **2** over W-2 Raney nickel in absolute ethanol at room temperature provided **3** in quantitative yield. The configurations of C5 and C7 were confirmed by the ^1H NMR spectrum.

The synthesis of 3β-acetoxy-5α-cholest-14-ene **(4)** was achieved in 75% yield, according to the methods described in the literature.[6,7] Compound **4** was ozonized in dichloromethane at −20° C to provide a mixture of **5a** and **5b** (4 : 1). The ratio of **5a** and **5b** was determined by ^1H NMR spectrum (400 MHz).[2,3] The ^1H NMR data of **5a** and **5b** agree well with those of Wife et al.[2]



Reagents: a, Ac_2O, Py; b, CrO_3; c, Ni-H_2; d, $CuSO_4$; e, HCl, $CHCl_3$; f, O_3, CH_2Cl_2; g, Me_2S; h, K_2CO_3-MeOH-H_2O; i, PDC; j, Zn-Hg, HCl; k, TsCl, Py; l, $LiAlH_4$.

Scheme 1



Scheme 2

The mixture of **5a** and **5b** was reduced by dimethyl sulfide in dichloromethane to give compound **6**. The signal of 15-CHO was a triplet (J = 2.4 Hz) at 9.77 ppm, and the signal of 8β-H was at 2.52 ppm (td, J = 11.2 Hz, 3.0 Hz). Hydrolysis of **6** with potassium carbonate in methanol gave **7**; which was then oxidized by pyridinium dichromate to yield a somewhat unstable product **8**.

Compound **8** could not be reduced cleanly to our target molecule **12** by Clemmensen reduction. Instead, the reaction provided a complex mixture from which four compounds (**9, 10, 11,** and **12**) could be isolated and characterized. Compound **9** was isomeric with **8**, the genesis of a secondary alcohol (δ_H 3.63, dd; δ_C 77.9, d) at the expense of an aldehydic group being the result of a typical intramolecular aldol condensation, as shown in Scheme 2. Reductive removal of the 3-keto group from **9** produced **10.** These two compounds belong to modified steroids with an unusual skeleton.[8] The third polar product, **11,** was spotted between **9** and **10** on thin-layer chromatography. The IR spectrum indicated that there was a hydroxyl (3,341, 1,048 cm^{-1}) in the molecule. The signals at δ_H 3.63 ppm (2H, m) and at δ_C 64.9 ppm (t) established the structure as shown in Scheme 1.

The nonpolar part of the Clemmensen reduction products of **8** was still a complex mixture, as indicated by gas chromatography/mass spectrometry. The major component of the mixture was **12.** It constituted 50% of the mixture, and its presence was confirmed by coinjection with the pure synthetic standard of **12** from **11.**

The alcohol **11** was treated by tosyl chloride in pyridine at room temperature, and the tosylate was reduced by lithium aluminum hydride in tetrahydrofuran to give the target molecule **12.** The purity was more than 98%, as determined by gas chromatography. Its ^1H NMR spectrum showed a triplet (3H, J = 7.5 Hz) at 0.94 ppm assigned to 15-CH_3, together with angular methyl signals at 0.75 (18-CH_3) and 0.81 (19-CH_3) ppm. The electron impact mass spectrum showed no molecular ion, but did show a major fragment (base peak) at m/z 219 (M^+-side chain), presumably because of the facile cleavage of the C13-C17 bond on electron impact. The chemical ionization mass spectrum showed a molecular ion at m/z 373 (M^+-1).

It is obvious that the structure of secocholestane in Kalamayi crude oils[1] is incompatible with **12.** The synthesis of other likely candidates is being actively pursued in our laboratory.

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