Studies of the synthesis of biomarkers. XI. Synthesis of 4,5-secocholestane and 4-methyl-4,5-secocholestane

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*4,5-Secocholestane (**1a**) and 4-methyl-4,5-secocholestane (**1b**) were synthesized from cholesterol (**2**) in five and seven steps, respectively. The key intermediate, 5-oxo-4,5-secocholestan-4-al (**7**) was reduced by the Clemmensen method to afford **1a**. Meanwhile, **7** underwent selective Wittig reaction, Clemmensen reduction, and hydrogenation to give another target molecule, **1b**. The structure of an unknown biomarker was shown to be different from the proposed **1a** by gas chromatographic and mass spectrometric comparison.* (*Steroids* **57**:551–553, 1992)

Keywords: steroids; secocholestane; synthesis; biomarkers; 4,5-secocholestane; sterols

Introduction

Yang and Yang reported that a novel secosterane was discovered in crude oils from the Kelamayi oilfield in northwestern China.[1] Judging from the mass spectrum, they suggested that the structure was a 13,17-secosterane. Considering that C-14 or C-15 can be preferentially functionalized under some reaction conditions and that 13(17)-diasterenes are biomarkers occurring in crude oils, we synthesized the authentic samples of 5α-(17R,20R)-14,15-secocholestane and 13,17-secodiacholestanes for comparison, but the results indicated that the secocholestane in Kelamayi crude oils have neither a 14,15-secocholestane nor a 13,17-secodiacholestane structure.[2,3] Analyzing the mass spectra of the novel secosterane more carefully, however, Jiang et al.[4] inferred the structure of the novel secosterane to be more consistent with a 4,5-secosterane (**1**, Scheme 1). In order to ascertain the structure of secosterane of Yang and Yang,[1] we decided to synthesize the authentic samples of 4,5-secocholestane (**1a**) and 4-methyl-4,5-secocholestane (**1b**).

Experimental

All melting points (mp) were uncorrected. Infrared spectra were recorded on a Nicolet FT-IR-5DX spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured on Varian FT-80A and Bruker AM-400 spectrometers, using $CDCl_3$ as solvent and tetramethylsilane as internal reference. Mass spectra were obtained on Finnigan-4021C and VG-7070E spectrometers (EI, 70 eV). Gas chromatography (GC) was performed on a Shimadzu GC-9A instrument. A 30 m × 0.25 mm SE-54 quartz glass column was programmed from 100 to 350 C at 4 C/min with helium as carrier gas.

*4β,5-Dihydroxy-5α-cholestane (**4**), 5-hydroxy-5α-cholestan-4-one (**5**), and 4α,5-epoxy-5α-cholestane (**6**)*

To a solution of 200 mg of cholest-4-ene (**3**) in 75 mL of acetic acid and 5 ml of water was added 300 mg of potassium permanganate in 15 ml of water within 10 minutes under stirring at room temperature and the mixture was stirred for an additional 2 hours. Then 350 mg of sodium hydrogen sulfite was added to reduce the excess potassium permanganate and manganese dioxide. The solution was extracted with ether (3 × 30 ml) to provide 185 mg of white solid. The crude product was separated by silica gel column chromatography, and eluted with petroleum ether-ethyl acetate (15 : 1 to 8 : 1, v/v). Compound **6** (35 mg, 17%), **5** (40 mg, 18%), and **4** (89 mg, 41%) were obtained successively. **6**, mp 100–101 C (colorless needles from acetone), literature[5] 101–103 C. The ^{1}H NMR spectral data agree with those reported.[5] **5**, mp 158–160 C (colorless needles from methanol), literature[6] 158–161 C. The mass spectrum of **5** agree with the literature.[7] **4**, mp 171–173 C (colorless needles from diethyl ether-petroleum ether), lit[6] 172–173 C. The mass spectral data and ^{13}C NMR spectral data agree with those reported.[7,8]

Reduction of **5** (50 mg) with lithium aluminum hydride (50 mg) in diethyl ether (10 ml) at room temperature provided **4** (40 mg, 80%).

A mixture of **6** (30 mg), 2% sulfuric acid (0.5 ml), and methanol (10 ml) was stirred at room temperature for 30 minutes to yield **4** (18 mg, 58%).

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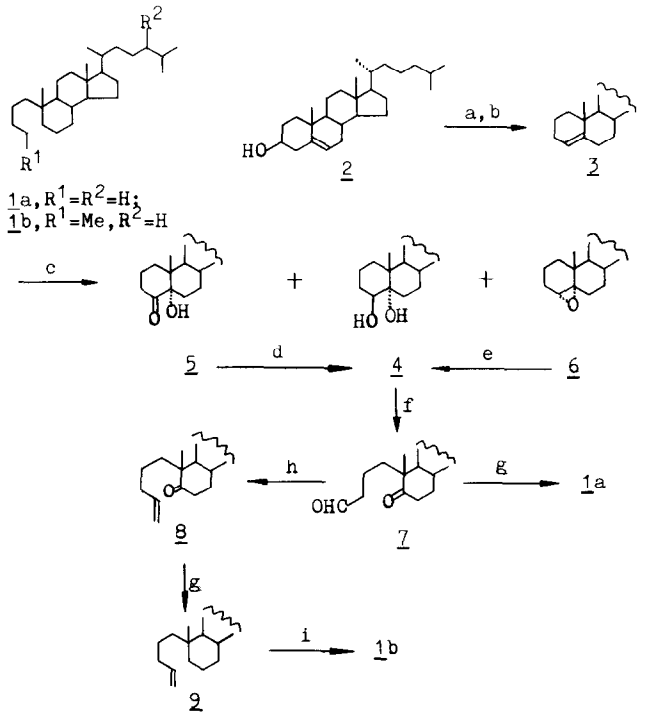

Scheme 1 Reagents: a, $(i\text{-PrO})_3Al$, cyclohexanone; b, $LiAlH_4$, $AlCl_3$; c, $KMnO_4$; d, $LiAlH_4$; e, H^+, H_2O; f, $Pb(OAc)_4$; g, Zn—Hg, HCl; h, $Ph_3P{=}CH_2$; i, Ni, H_2.

5-Oxo-4,5-secocholestan-4-al (7)

A mixture of 200 mg of **4**, 75 ml of dry benzene, 300 mg of lead tetraacetate, and 5 ml of pyridine was stirred under nitrogen at room temperature for 24 hours. The solution was diluted with water. The solid was removed by filtering and the solution was extracted with ether to provide 190 mg of crude **7**. Pure **7** (180 mg, 90%) was obtained by a short column of silica gel: ν_{max} 1,726, 1,705 cm^{-1}; δ_H 0.725(3H, s, 18-CH_3), 0.864, 0.868(6H, 2d, J = 6.6Hz, 26, 27-CH_3), 0.913(3H, d, J = 6.5Hz, 21-CH_3), 1.072(3H, s, 19-CH_3), 9.453(1H, t, J = 2.4Hz, -CHO) ppm; δ_C 202.91(4), 215.06(5), 51.02(10) ppm; m/z 402(4, M^+), 332(100), 317(18), 247(8).

4,5-Secocholestane (1a)

A mixture of 60 mg of **7**, 0.5 g of amalgamated zinc, 20 ml of ethanol, and 6 ml of concentrated hydrochloric acid was refluxed for 6 hours, cooled, diluted with water, and extracted with ether to provide a light yellow oily substance (55 mg). The crude product was separated on 7 g of silica gel containing 7% silver nitrate. Eluting with petroleum ether gave 25 mg of **1a** (yield 45%) as a colorless viscous oil: δ_H 0.649(3H, s, 18-CH_3), 0.821(3H, s, 19-CH_3), 0.860, 0.864(6H, 2d, J = 6.6 Hz, 26, 27-CH_3), 0.889(3H, t, J = 6.6 Hz, 4-CH_3), 0.897(3H, d, J = 6.6 Hz, 21-CH_3)ppm; δ_C 42.46(1), 23.81(2), 25.22(3), 19.82(4), 38.05(5), 21.99(6), 32.13(7), 35.79(8), 42.72(9), 35.10(10), 21.62(11), 40.30(12), 42.68(13), 56.58(14), 24.37(15), 28.32(16), 56.49(17), 12.09(18), 14.23(19), 35.85(20), 18.73(21), 36.26(22), 23.91(23), 39.59(24), 28.05(25), 22.59(26), 22.83(27)ppm; m/z 374(10,M^+), 359(2), 317(100), 220(21), 219(15), 177(34), 109(34) 95(50).

4-Methylene-4,5-secocholestan-5-one (8)

A solution of 60 mg of **7** in 20 ml of diethyl ether was added dropwise to the solution of methylene triphenylphosphorane in 35 ml of diethyl ether (made from 550 mg of triphenyl phosphine methyl bromide and 1.54 mmol phenyl lithium) under nitrogen. The mixture was stirred at 10 C for 1.5 hours. The solution was diluted with water, and the water layer was extracted with ether. The combined etheral liquid was dried and evaporated in vacuum to yield crude **8** (50 mg). After purification through a short column, 45 mg of **8** was obtained (yield 70%): ν_{max} 3,068, 1,707, 1,638 cm^{-1}; δ_H 0.723(3H, s, 18-CH_3), 0.864, 0.868(6H, 2d, J = 6.6 Hz, 26, 27-CH_3), 0.914(3H, d, J = 6.5 Hz, 21-CH_3), 1.058(3H, s, 19-CH_3), 4.929 (1H, dd, J = 8.1 Hz, 2.0 Hz, —CH=CH_2-cis), 5.002(1H, dd, J = 17.1 Hz, 2.0 Hz, —CH=CH_2-trans), 5.796(1H, m, —CH=CH_2)ppm; δ_C 38.23(1), 21.39(2), 34.70(3), 138.99(4), 114.29 (=CH_2), 215.23(5), 34.26(6), 31.14(7), 34.50(8), 47.11(9), 50.90(10), 21.08(11), 39.47(12), 42.51(13), 56.04(14)ppm; m/z 400(22, M^+), 385(17), 359(17), 332(100), 243(21), 182(37), 105(95).

4-Methylene-4,5-secocholestane (9)

Compound **8** (40 mg) underwent Clemmensen reduction to provide **9** (23 mg, yield 60%): ν_{max} 3,076, 1,641 cm^{-1}; δ_H 0.646(3H, s, 18-CH_3), 0.826(3H, s, 19-CH_3), 0.859, 0.863(6H, 2d, J = 6.6 Hz, 26,27-CH_3), 0.894(3H, d, J = 6.6Hz, 21-CH_3), 4.928 (1H, dd, J = 8.1 Hz, 2.0 Hz), 5.002(1H, dd, J = 17.1 Hz, 2.0 Hz), 5.793(1H, m)ppm; δ_C 42.16(1), 19.78(2), 34.79(3), 139.32(4), 114.15(=CH_2), 35.10(5), 21.92(6), 32.08(7), 35.70(8), 49.59(9), 35.10(10), 21.57(11)ppm; m/z 386(27, M^+), 371(6), 317(100), 231(19), 177(25).

4-Methyl-4,5-secocholestane (1b)

Compound **9** (20 mg) was hydrogenated over Raney nickel (0.2 g) in tetrahydrofuran (20 ml) at room temperature for 2 hours. Removal of the catalyst and solvent yielded crude **1b** (20 mg). The crude product was purified by 2 g of silica gel containing 7% silver nitrate, eluted with petroleum ether to give 19 mg of **1b** as a colorless viscous oil: δ_H 0.648(3H, s, 18-CH_3), 0.819(3H, s, 19-CH_3), 0.860, 0.865(6H, 2d, J = 6.6 Hz, 26, 27-CH_3), 0.880(3H, t, J = 7.2 Hz, C-4-CH_3), 0.896 (3H, d, J = 6.5 Hz, 21-CH_3)ppm; δ_C 42.63(1), 22.75(2), 32.99(3), 22.51(4), 19.83(4-CH), 37.97(5), 21.93(6), 32.07(7), 35.71(8), 35.80(9), 35.08(10), 21.56(11), 40.22(12), 42.63(13), 56.51(14), 24.32(15), 28.29(16), 56.38(17), 12.05(18), 14.15(19), 35.80(20), 18.68(21), 36.19(22), 23.84(23), 39.53(24), 28.02(25), 22.58(26), 22.82(27)ppm; m/z 388(7, M^+), 373(6), 317(100), 234(19), 233(17), 177(29), 109(45), 95(74).

Results and discussion

The target molecules, 4,5-secocholestane **(1a)** and 4-methyl-4,5-secocholestane **(1b)**, were synthesized from cholesterol **(2)** as indicated in Scheme 1.

The preparation of cholest-4-ene **(3)** was achieved according to the literature.[3] Compound **3** was oxidized by $KMnO_4$ in HOAc-H_2O at room temperature to give a mixture of 4β,5-dihydroxy-5α-cholestane **(4)**, 5-hydroxy-5α-cholestan-4-one **(5)**, and 4α,5-epoxy-5α-cholestane **(6)**. Separation of the mixture by silica gel column chromatography provided **4, 5,** and **6** in 41%, 18%, and 17% yields, respectively. Potassium permanganate hydroxylation usually give cis-diols; the formation of 4β,5α-diol **4** should come from epoxide **6** with hydrolysis. Actually, hydrolysis of **6** under acidic condition provided **4**. Compound **5** is obviously the oxidation product of **4** under the reaction conditions. Compound **4** can also be obtained by reduction of **5** with lithium aluminum hydride. Treatment of **4** with lead

tetraacetate in the presence of pyridine in benzene at room temperature provided 5-oxo-4,5-secocholestan-4-al (**7**) in 90% yield. The IR and ^{1}H NMR spectra of **7** agree with those reported.[9] The signals at δ_H9.453(1H, t, J = 2.4 Hz, -CHO)ppm and at δ_C202.91 (—CHO) and 215.06 (C=O)ppm established the structure as shown in Scheme 1. Reduction of **7** by the Clemmensen method afforded **1a** in 45% yield. The by-products were olefins, alcohols, and enols. Fieser et al.[10] obtained **1a** in their degradation studies of cholesterol, but they did not give any spectral data. In our synthesis, the ^{1}H NMR spectrum of **1a** showed a triplet at δ_H 0.889(3H, J = 6.6 Hz) ppm assigned to 4-CH_3. The ^{13}C NMR spectrum of **1a** showed six methyl signals and the signal of 4-CH_3 was at δ_C 19.82 ppm.

4-Methylsecosteranes as homologs coexist with secosteranes in the Kelamayi crude oils.[1] To compare more carefully with the oil sample, we also synthesized 4-methyl-4,5-secocholestane (**1b**). At room temperature (10 C), methylene triphenylphosphorane selectively reacted with the aldehyde group in **7** to give 4-methylene-4,5-secocholestan-5-one (**8**). The signals at δ_H 4.929(1H, dd, J = 8.1 Hz, 2.0 Hz), 5.002(1H, dd, J = 17.1 Hz, 2.0 Hz), and 5.796(1H, m) ppm and at δ_C 114.29, 138.99, and 215.23 ppm established the structure of **8** as shown in Scheme 1. Upon Clemmensen reduction, **8** gave 4-methylene-4,5-secocholestane (**9**). The ^{13}C NMR spectrum of **9** showed no carbonyl signal. The alkene **9** was hydrogenated over Raney nickel to provide **1b**, in which the C-4-methyl appeared at δ_H 0.880(3H, t, J = 7.2 Hz) ppm and at δ_C 19.83 ppm.

The synthetic **1a** and **1b** were different from secocholestanes from the Kelamayi crude oils in terms of their retention time on GC and their electron impact mass spectra (EI-MS). The retention time of the secocholestane from the Kelamayi crude oils was 68.2 minutes, but that of **1a** was 70.4 minutes. The EI-MS of the secocholestane from the Kelamayi crude oils showed a base peak at m/z 219, together with m/z 374(22, M^+) and m/z 317(9) as the characteristic peaks. But the EI-MS of **1a** showed m/z 317 as base peak and the peaks, like m/z 374(10) and m/z 219(15), were quite weak. Similarly, the EI-MS of 4-methyl-secocholestane from the Kelamayi crude oils showed m/z 233 as base peak together with m/z 388(20) and m/z 317(9). But the EI-MS of **1b**, just like **1a**, showed m/z 317 as base peak and the peaks, like m/z 388(7) and m/z 233(17), were quite weak.

Contrary to the expectation of Jiang et al.,[4] the structure of secosterane from the Kelamayi crude oils is not 4,5-secosterane. The synthesis of other likely candidates is being actively pursued in our laboratory.

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