Synthesis of 5β-Cholestane-3α,6β,7α,25,26-pentol and Identification of a Novel Bile Alcohol, α-Trichechol, Present in the West Indian Manatee Bile

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In order to confirm the structure of α-trichechol, the major bile alcohol of the West Indian manatee, chemical synthesis of 5β-cholestane-3α,6β,7α,25,26-pentol was carried out. The chain of 3α-hydroxy-5β-chol-6-en-24-oic acid was elongated by an Arndt-Eistert reaction to form 3α-hydroxy-26,27-dinor-5β-cholest-6-en-25-oic acid. The unsaturated C_{25} bile acid was converted into 3α,6β,7α-trihydroxy-25-homo-5β-cholan-25-oic acid by 1,2-glycol formation of the Δ^6-double bond. The acetylated derivative of the trihydroxy C_{25} bile acid was then converted into 3α,6β,7α,26-tetraacetoxy-27-nor-5β-cholestan-25-one by successive treatment with thionyl chloride, diazomethane, and acetic acid. A Grignard reaction of the 25-oxo compound with methylmagnesium iodide afforded the desired bile alcohol, 5β-cholestane-3α,6β,7α,25,26-pentol. By direct comparison with the synthetic pentahydroxy bile alcohol, the structure of the naturally occurring α-trichechol was determined to be 5β-cholestane-3α,6β,7α,25,26-pentol.

Keywords 5β-cholestane-3α,6β,7α,25,26-pentol; α-trichechol; bile alcohol; manatee; chemical synthesis

Recently, the West Indian manatee, *Trichechus manatus latirostris*, a herbivorous marine mammal, was found to have bile that was devoid of bile acids and contained instead bile alcohol sulfates of a structure not previously described as its major bile salts.[1] The most abundant bile alcohol of the West Indian manatee was identified provisionally by chromatography, mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy as 5β-cholestane-3α,6β,7α,25,26-pentol (VII) and was named α-trichechol. However, an unequivocal determination of its structure was hampered by the absence of a synthetic standard. In order to confirm the structure of α-trichechol, the chemical synthesis of 5β-cholestane-3α,6β,7α,25,26-pentol was undertaken.

The synthetic route to VII is shown in Fig. 1.

3α-Hydroxy-5β-chol-6-en-24-oic acid (III) was prepared from chenodeoxycholic acid (I) according to the procedure reported previously.[2] The side chain of the cholenic acid (III) was elongated by an Arndt-Eistert reaction, to give 3α-hydroxy-26,27-dinor-5β-cholest-6-en-25-oic acid (IV). The epoxidation of the methyl ester of IV with *m*-chloroperbenzoic acid followed by refluxing with acetic acid and then alkaline hydrolysis afforded 3α,6β,7α-trihydroxy-26,27-dinor-5β-cholestan-25-oic acid (V).[2] The trihydroxy C_{25} acid (V) was acetylated, and then treated with thionyl chloride. The resulting acid chloride was treated with diazomethane to give a diazoketone. The diazoketone was treated with acetic acid to give 3α,6β,7α,26-tetraacetoxy-27-

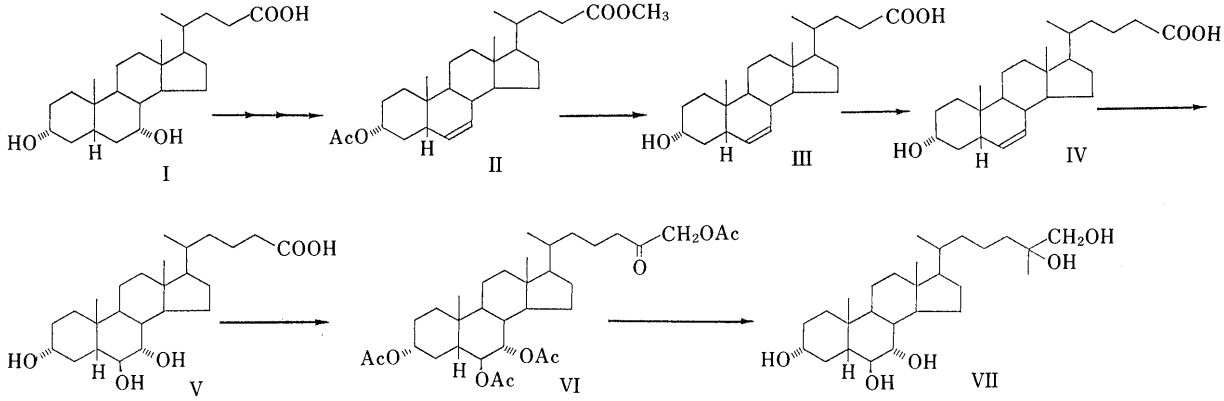

Fig. 1. Synthesis of 5β-Cholestane-3α,6β,7α,25,26-pentol

I, chenodeoxycholic acid; II, methyl 3α-acetoxy-5β-chol-6-en-24-oate; III, 3α-hydroxy-5β-chol-6-en-24-oic acid; IV, 3α-hydroxy-26,27-dinor-5β-cholest-6-en-25-oic acid; V, 3α,6β,7α-trihydroxy-26,27-dinor-5β-cholestan-25-oic acid; VI, 3α.6β.7α,26-tetraacetoxy-27-nor-5β-cholestan-25-one; VII, 5β-cholestane-3α,6β,7α,25,26-pentol.

TABLE I. *Rf* Values on TLC and Relative Retention Times on GLC of the Naturally Occurring α-Trichechol and the Chemically Synthesized 5β-Cholestane-3α,6β,7α,25,26-pentol

	Rf values on TLC		Relative retention times[c] on GLC[d]	
	EA[a]	CE[b]	OV-1	Poly I-110
Naturally occurring α-trichechol	0.18	0.17	2.38	1.14
Chemically synthesized 5β-cholestane-3α,6β,7α,25,26-pentol	0.18	0.17	2.38	1.14

a) EA = ethyl acetate : acetone, 3 : 2 (v/v). *b*) CE = chloroform : ethanol, 4 : 1 (v/v). *c*) Relative to the trimethylsilyl ether of methyl cholate (1.00). *d*) Bile alcohols were chromatographed as their trimethylsilyl ethers.

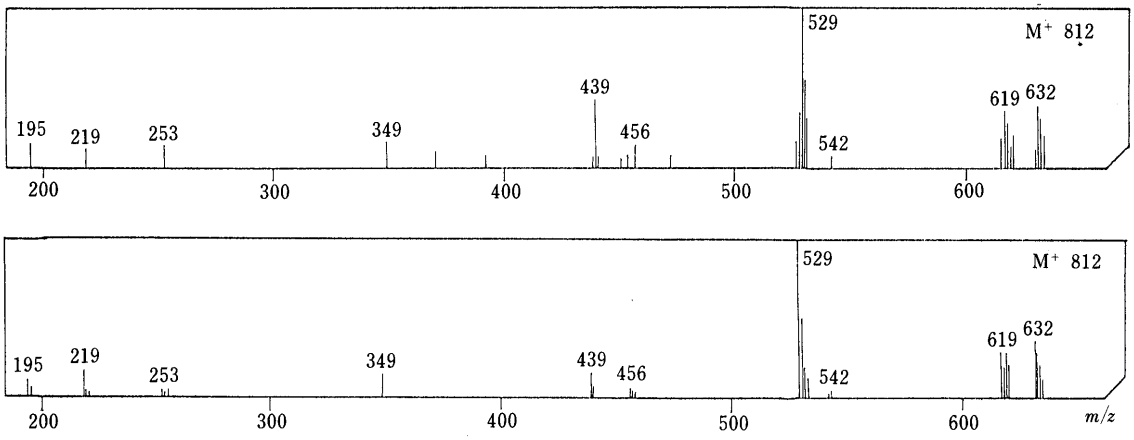

Fig. 2. Mass Spectra of Trimethylsilyl Ether Derivatives of the Naturally Occurring α-Trichechol (Top) and the Chemically Synthesized 5β-Cholestane-3α,6β,7α,25,26-pentol (Bottom)

TABLE II. NMR Data for the Naturally Occurring α-Trichechol and the Chemically Synthesized 5β-Cholestane-3α,6β,7α,25,26-pentol in Pyridine-d_5

	H-18	H-19	H-21	H-27	H-3	H-6	H-7	H-26
Naturally occurring α-trichechol	0.77 (s)	1.55 (s)	1.13 (d)	1.54 (s)	3.89 (m)	4.32 (m)[a]	4.35 (m)[a]	3.92 (s)
Chemically synthesized 5β-cholestane-3α,6β,7α,25,26-pentol	0.77 (s)	1.53 (s)	1.12 (d)	1.53 (s)	3.86 (m)	4.30 (m)	4.30 (m)	3.87 (s)

a) Assignments may be interchanged in each line.

nor-5β-cholest-25-one (VI). A Grignard reaction of the 25-oxo compound (VI) with methylmagnesium iodide gave the desired bile alcohol, 5β-cholestane-3α,6β,7α,25,26-pentol (VII).

By direct comparison with the specimen synthesized in the present work, α-trichechol, the major bile alcohol of the West Indian manatee, was shown to be 5β-cholestane-3α,6β,7α,25,26-pentol, though its absolute configuration at C-25 remains to be established. The naturally occurring α-trichechol had the same chromatographic properties (Table I), mass spectrum (Fig. 2) and NMR spectrum (Table II) as those of the reference compound.

Experimental

General Melting points were determined on a Kofler hot-stage apparatus. Thin-layer chromatography (TLC) was carried out on precoated silica-gel G plates (0.25 mm thickness, Merck). Spots were detected by spraying with phosphomolybdic acid (10% in ethanol) followed by heating at 110 °C for 5 min. Infrared (IR) spectra were obtained with a Shimadzu model IR-408 spectrophotometer as KBr discs. ^{1}H-NMR spectra were recorded with a Hitachi model R-40 spectrometer at 90 MHz, using tetramethylsilane as an internal standard. Gas-liquid chromatography (GLC) was performed with a Shimadzu model GC-14A gas chromatograph equipped with a flame ionization detector. The columns used were a glass capillary column (25 m × 0.32 mm i.d.) coated with OV-1 (Hewlett Packard Co.), and a glass packed column (2 m × 4 mm i.d.) of 2% Poly I-110 (Gasukuro Kogyo Inc.). Gas-liquid chromatography-mass spectrometry (GLC-MS) was carried out on a Shimazdu model QP-1000 gas chromatograph-mass spectrometer under the following conditions: column, a glass open tube column (40 m × 1.2 mm i.d.) coated with G-250 (Chemicals Inspection and Testing Institute, Japan); column temperature, 260—280 °C, increasing at a rate of 2 °C min^{-1}; ionization energy, 70 eV.

'The usual work-up' refers to dilution with a large amount of water, extraction with organic solvent, washing of the extract with water, drying over anhydrous Na_2SO_4, filtration, and evaporation of the filtrate under reduced pressure. The synthetic route is summarized in Fig. 1.

3α-Hydroxy-5β-chol-6-en-24-oic Acid (III) Methyl 3α-acetoxy-5β-chol-6-en-24-oate (II) (4.0 g), prepared from chenodeoxycholic acid (I) according to the procedure reported previously,[2] was refluxed in 5% methanolic KOH (100 ml) for 30 min. After acidification with dilute HCl, the ususal work-up (ether) gave a residue, which was recrystallized from methanol to give III as crystals (3.7 g). mp 207.5—208.0 °C. *Rf* on TLC, 0.23 (ethyl acetate : iso-octane : acetic acid, 10 : 10 : 0.1). IR (cm^{-1}): 1700, 3300. ^{1}H-NMR (pyridine-d_5) δ: 0.64 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3), 0.98 (d, J = 6 Hz, 3H, 21-CH_3), 5.49 (d, J = 9.9 Hz, 1H, 6-H), 5.65 (ddd, J_1 = 9.9 Hz, J_2 = 4.7 Hz, J_3 = 2.6 Hz, 1H, 7-H).

3α-Hydroxy-26,27-dinor-5β-cholest-6-en-25-oic Acid (IV) A solution of III (3.4 g) in formic acid (60 ml) was heated at 55 °C for 4 h. The reaction mixture was poured into ice water (600 ml) and the precipitate was collected by filtration. The precipitate was dissolved in thionyl chloride (14 ml), and the reaction mixture was placed in a dark room for 2 h. The excess of thionyl chloride was evaporated off at room temperature followed by repeated evaporation with dry benzene. The residue was dissolved in dry bezene (30 ml) and the solution was added dropwise to a freshly prepared ethereal diazomethane solution on an ice bath. The reaction mixture was allowed to stand at room temperature overnight. Evaporation of excess diazomethane and the solvent left 3α-formoxy-25-diazo-26,27-dinor-5β-cholestan-24-one as an oily residue. A solution of this diazoketone in benzyl alcohol (9.6 ml) and collidine (9.6 ml) was added to a preheated flask (200 °C), and the temperature was maintained at 180—200 °C for 15 min. The usual-work up (ether) gave an oily residue, which was refluxed in 10% methanolic KOH (100 ml) for 1 h. After acidification with dilute HCl, the usual work-up (ether) gave a residue, which was purified by column chromatography using an octadecylsilyl column (310 mm × 25 mm i.d., Merck) with 85% methanol as an eluting solvent. The column effluents were monitored by TLC. The effluent fractions containing the major product were combined and the solvent was evaporated off. The product (2.8 g) was recrystallized from methanol and water to give IV as white needles (1.6 g). mp 138.5—139.5 °C. *Rf* on TLC, 0.31 (ethyl acetate : iso-octane : acetic acid, 10 : 10 : 0.1). IR (cm^{-1}): 1700, 3300. ^{1}H-NMR (pyridine-d_5) δ: 0.64 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.98 (d, J = 6.6 Hz, 3H, 21-CH_3), 5.50 (d, J = 10.6 Hz, 1H, 6-H), 5.65 (ddd, J_1 = 10.1 Hz, J_2 = 4.6 Hz, J_3 = 2.6 Hz, 1H, 7-H).

3α,6β,7α-Trihydroxy-26,27-dinor-5β-cholestan-25-oic Acid (V) Compound IV (900 mg) was treated with a freshly prepared ethereal diazomethane solution. After 2 h, excess diazomethane and the solvent were driven off by gentle heating. *m*-Chloroperbenzoic acid (600 mg) was added to a solution of the resulting methyl ester in chloroform (40 ml), and the reaction mixture was placed in a dark room at room temperature for 24 h. After treatment of the reaction mixture with 1 N NaOH in order to remove the excess *m*-chloroperbenzoic acid, the usual work-up (ether) gave an oily residue. The residue was refluxed in acetic acid (80 ml) for 3.5 h. The usual work-up (ether) gave an oily residue, which was purified on column chromatography of silica-gel (50 g) with 20% ether in benzene as an eluting solvent. The product was refluxed in 5% methanolic KOH (100 ml) for 1 h.

After the usual work-up (ethyl acetate), the hydrolyzed product was recrystallized from methanol to give V as crystals (376 mg). mp 240.0—241.5 °C. *Rf* on TLC, 0.35 (benzene: iso-propanol: acetic acid, 30: 10: 1). IR (cm^{-1}): 1700, 3400. ^{1}H-NMR (pyridine-d_5) δ: 0.76 (s, 3H, 18-CH_3), 0.99 (d, J=6.6 Hz, 3H, 21-CH_3), 1.44 (s, 3H, 19-CH_3), 3.80 (m, 1H, 3-H), 4.21 (s, 2H, 6- and 7-H).

3α,6β,7α,26-Tetraacetoxy-27-nor-5β-cholestan-25-one (VI) Compound V (213 mg) was heated overnight with acetic anhydride (2 ml) and anhydrous sodium acetate (60 mg) on a steam bath. The reaction mixture was poured into ice water (50 ml), and the precipitate was collected by filtration. The precipitate was dissolved in thionyl chloride (4 ml), and the solution was placed in a dark room at room temperature for 2 h. After removal of excess thionyl chloride using the procedure mentioned above, the residue was added dropwise to a freshly prepared ethereal diazomethane solution at 0 °C, and the solution was allowed to stand at room temperature overnight. The reaction mixture was evaporated down, and the residue was dissolved in acetic acid (14 ml); and this solution was kept at room temperature for 24 h. The usual work-up (ether) gave an oily residue, which was purified by column chromatography on silica gel (40 g) with benzene: ethyl acetate (9: 1) to give VI as a non-crystaline material (75 mg). *Rf* on TLC, 0.40 (benzene: ethyl acetate, 4: 1). IR (cm^{-1}): 1730. ^{1}H-NMR (pyridine-d_5) δ: 0.62 (s, 3H, 18-CH_3), 0.90 (d, J=6.0 Hz, 3H, 21-CH_3), 1.03 (s, 3H, 19-CH_3), 1.97, 2.00, 2.02, 2.06 (s, 3H×4, -$OCOCH_3$×4), 4.56 (m, 1H, 3-H), 4.90 (m, 1H, 6- or 7-H), 5.03 (m, 1H, 6- or 7-H).

5β-Cholestane-3α,6β,7α,25,26-pentol (VII) A solution of VI (56 mg) in dry benzene (5 ml) was added to a ethereal solution of CH_3MgI (5 ml; 0.95 M, 10 eq). The reaction mixture was refluxed for 2.5 h, and then allowed to stand at room temperature overnight. After treatment with 1 N HCl to decompose the Grignard product, the usual work-up (ethyl acetate) gave an oily residue, which was purified by column chromatography of silica-gel (5 g) with a mixture (4: 1) of ethyl acetate and acetone. The effluent fractions containing the major product were combined and the solvent was evaporated off. The residue was recrystallized from methanol to give VII as crystals (5.6 mg). mp 126.0—129.0 °C. *Rf* on TLC, 0.67 (ethyl acetate: acetone, 3: 10). IR (cm^{-1}): 3340. ^{1}H-NMR (pyridine-d_5) δ: 0.77 (s, 3H, 18-CH_3), 1.12 (d, J=6.0 Hz, 3H, 21-CH_3), 1.53 (s, 6H, 19- and 27-CH_3), 3.86 (m, 1H, 3-H), 3.87 (s, 2H, 26-CH_2), 4.30 (m, 2H, 6- and 7-H).

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