Synthesis of 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24ξ,25-pentol

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Abstract This paper describes syntheses of 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24ξ,25-pentol which give higher yields than previously published methods. In addition, 5β-cholestane-3α,7α,12α,24ξ,25-pentol was synthesized by a different procedure, namely via performic acid oxidation of the corresponding unsaturated triol, which gave a lower yield but avoided the formation of 5β-cholestane-3α,7α,12α,25,26-pentol, which normally tends to contaminate the final product. Structures were confirmed by gas-liquid chromatography, infrared-, proton magnetic resonance- and mass spectrometry. 5β-Cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24ξ,25-pentol were required for in vivo and in vitro studies of the (hypothetical) 25-hydroxylation pathway of cholic acid biosynthesis.

Patients with the rare familial sterol storage disease, cerebrotendinous xanthomatosis (CTX) (1), secrete a number of C_{27} bile alcohols in bile and feces (2). One of the main components of the bile alcohol fraction was a tetrahydroxy bile alcohol, 5β-cholestane-3α,7α,12α,25-tetrol. A pentahydroxy bile alcohol fraction consisted of two major and several minor components (3). One of the pentahydroxy bile alcohols was 5β-cholestane-3α,7α,12α,24α,25-pentol indicating that in CTX subjects there existed a pathway of bile acid synthesis involving 25-hydroxylated intermediates, rather than 26-hydroxylated compounds, as previously reported for normal subjects.

In order to investigate individual biochemical steps of the 25-hydroxylation pathway of cholic acid biosynthesis, it was necessary to prepare C_{27} bile alcohol derivatives, substituted in the 25-position. This paper describes the synthesis of 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,-24ξ,25-pentol from cholic acid. Certain modifications of the published procedures were required to improve the yield and purity of these substances.

METHODS

Physical measurements

Melting points were determined on a Thermolyne apparatus (Thermolyne Corp., Dubuque, Ia.), model MP-12600 and are uncorrected.

Infrared spectra were recorded on a Perkin-Elmer (Norwalk, Conn.) model 421 grating spectrophotometer as KBr discs. Absorption frequencies are quoted in reciprocal centimeters.

NMR spectra, in parts per million (ppm), were obtained in deuterated methanol ($C^2H_3O^2H$) solution using a Varian XL-100 spectrometer (Varian Associates, Palo Alto, Cal.) equipped with Fourier transform capability.

Optical rotations were determined in methanol on a Carey model 60 spectropolarimeter (Carey Instruments, Monrovia, Cal.)

GLC: The bile alcohols, as the TMSi-derivatives, were analyzed on a 180 cm × 4 mm column packed with 3% QF-1 on 80/100 mesh Gas-Chrom Q (Applied Science Laboratories, State College, Pa.); column temp. 230° C (Hewlett-Packard model 7610 gas chromatograph, Palo Alto, Cal.).

Mass spectra of the bile alcohols were obtained with a Varian MAT-111 gas chromatograph–mass spectrometer, as described previously (3).

TLC: The bile alcohols were separated on silica gel G plates (Brinkmann, Westbury, N.J., 0.25 mm thickness), with the solvent system, chloroform–acetone–methanol 35:25:7.5 (v/v). The spots were visualized either with iodine or phosphomolybdic acid (3.5% in isopropanol).

RESULTS

3α,7α,12α-Triformoxy-24-oxo-25-diazo-25-homo-5β-cholane (III) (Fig. 1)

Compound (III), m.p. 126–128° C [reported m.p. 128–129° C (5)] was prepared according to the procedure of

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Abbreviations: CTX, cerebrotendinous xanthomatosis; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl; NMR, nuclear-magnetic resonance; PMR, proton-magnetic resonance.

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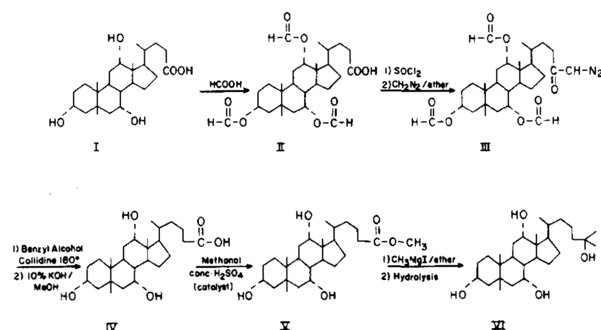

Fig. 1. Synthesis of 5β-cholestane-3α,7α,12α,25-tetrol. I, cholic acid; II, 3α,7α,12α-triformoxy-cholanoic acid; III, 3α,7α,-12α-triformoxy-24-oxo-25-diazo-25-homo-5β-cholane; IV, homocholic acid; V, methyl homocholate; VI, 5β-cholestane-3α,7α,-12α,25-tetrol.

Ruzicka, Plattner, and Heusser (4). The mass spectrum of the diazoketone showed a major fragment at m/e 84 and other prominent peaks at 442, M-(46 + 28); 432, M-84 (scission at C-22–C-23); 414, M-(46 + 41 + 15); 396, M-(2 × 46 + 28); 381, M-(2 × 46 + 28 + 15); 368, M-(2 × 46 + 41 + 15); 350, M-(3 × 46 + 28); 327, M-189 (scission at C-20–C-22); 299, M-(2 × 46 + 125, side chain); 281, M-(3 × 46 + 97); 253, M-(3 × 46 + 125) **(Fig. 2).**

25-Homocholic acid (IV) (Fig. 1)

Compound (IV), m.p. 216–218° C [reported m.p. 219.5–220° C (5)] was prepared from 3α,7α,12α-triformoxy-24-oxo-25-diazo-25-homo-5β-cholane (III) by a modified Arndt–Eistert synthesis as described by Svoboda, Thompson, and Robbins (6).

Methyl ester of 25-homocholic acid (V) (Fig. 1)

A solution of 1.75 g of 25-homocholic acid in 110 ml of anhydrous methanol with 3.3 ml of concentrated sulfuric acid was left overnight at room temperature (7). The next day the reaction mixture was poured in small portions into a beaker with crushed ice and the precipitate was collected (1.57 g). Further purification either by repeated crystallization from acetone or by column chromatography on neutral alumina IV (elution with 2–4% methanol in ethyl acetate) yielded 1.31 g of material, m.p. 149–150° C [reported m.p. 150–151° C; another form melts at 166–167° C (5)]. The mass spectrum of the trimethylsilyl ether of methyl homocholate exhibited a molecular ion at m/e 652, and prominent peaks at 637, M-(15); 562, M-(90); 547, M-(90 + 15); 472, M-(12 × 90); 457, M-(2 × 90 + 15); 441, M-(2 × 90 + 31); 382, M-(3 × 90); 367, M-(3 × 90 + 15); 351, M-(3 × 90 + 31); 343, M-(2 × 90 + 129, side chain) with other peaks at 281, M-371 (scission at C-20–C-22); 253, M-(3 × 90 + 129, side chain); 243, 226 and 211. The mass spectrum of the underivatized methyl homocholate exhibited peaks at 418, M-(18); 400, M-(2 × 18); 385, M-(2 × 18 + 15); 382, M-(3 × 18); 369, M-(2 × 18 + 31); 367, M-(3 × 18 + 15); 281, M-155 (scission at C-20–C-22); 271, M-(2 × 18 + 129, side chain); 253, M-(3 × 18 + 129).

The crude (noncrystalline) form of homocholic acid could be directly treated with methanol/sulfuric acid to yield methyl homocholate.

5β-Cholestane-3α,7α,12α,25-tetrol (VI) (Fig. 1)

Compound VI was prepared according to a procedure described by Pearlman (5), with the exception that the methyl homocholate (930 mg) in dry benzene was added to the Grignard reagent yielding 744 mg of compound VI (Fig. 1). This compound was further purified on a column of neutral alumina V. The fractions eluted with 5–6% methanol in ethyl acetate yielded pure tetrol. The final product was crystallized from ethyl acetate yielding needles of m.p. 189–190° C [reported m.p. 188–189° C (5)]. NMR data were identical to those reported (3) as were the $[\alpha]_D$ (3) and mass spectral data (2). IR results: (KBr disc) 3320 cm^{-1} (OH) **(Fig. 3).**

5β-Cholest-24-ene-3α,7α,12α-triol (VII) and 5β-cholest-25-ene-3α,7α,12α-triol (VIII) (Fig. 4a)

The procedure used was similar to that of Hoshita (8) with modifications. A solution of 110 mg of 5β-cholestane-

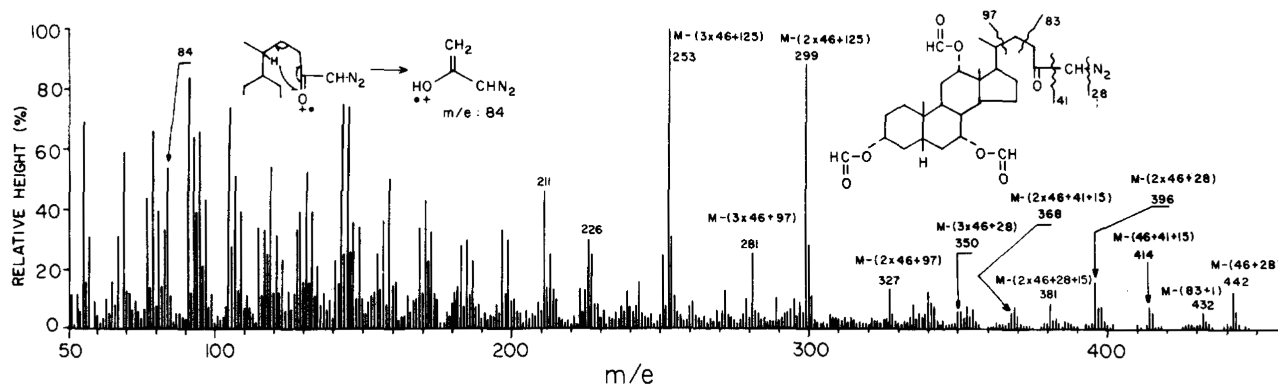

Fig. 2. Mass spectrum of 3α,7α,12α-triformoxy-24-oxo-25-diazo-25-homo-5β-cholane.

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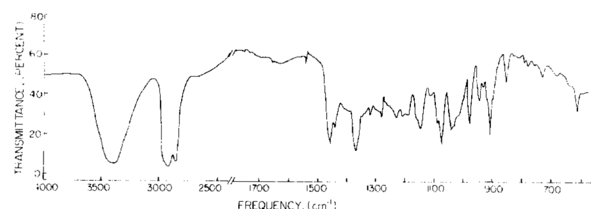

Fig. 3. Infrared spectrum of 5β-cholestane-3α,7α,12α,25-tetrol (VI, KBr disc).

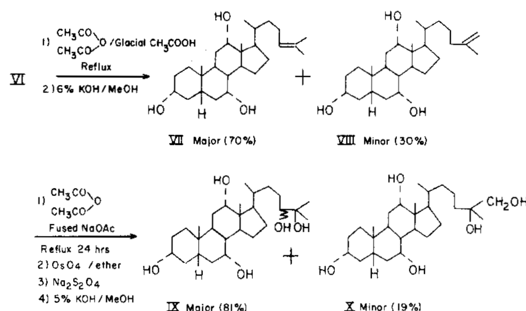

Fig. 4a. Synthesis of 5β-cholestane-3α,7α,12α,24ξ,25-pentol. VI, 5β-cholestane-3α,7α,12α,25-tetrol; VII, 5β-cholest-24-ene-3α,7α,12α-triol; VIII, 5β-cholest-25-ene-3α,7α,12α-triol; IX, 5β-cholestane-3α,7α,12α,24ξ,25-pentol; X, 5β-cholestane-3α,7α,12α,-25,26-pentol.

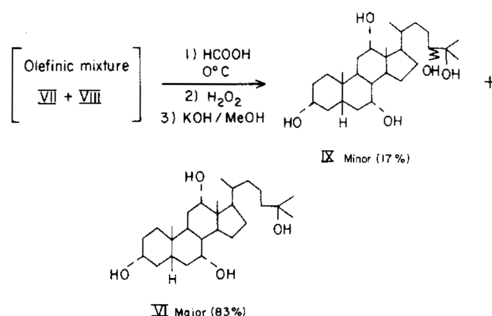

Fig. 4b. Synthesis of 5β-cholestane-3α,7α,12α,24ξ,25-pentol. VII, 5β-cholest-24-ene-3α,7α,12α-triol; VIII, 5β-cholest-25-ene-3α,7α,12α-triol; IX, 5β-cholestane-3α,7α,12α,24ξ,25-pentol; X, 5β-cholestane-3α,7α,12α,25-tetrol.

3α,7α,12α,25-tetrol (VI) (Fig. 1) in 3 ml of glacial acetic acid was refluxed for 3 hr. Two ml of acetic anhydride was added and the reaction mixture was further refluxed for 12 hr. The reaction mixture was evaporated to dryness in vacuo. The residue was hydrolyzed by refluxing with 10.5 ml of 6% methanolic potassium hydroxide for 1.5 hr. The hydrolyzate was poured into a beaker with crushed ice, stirred and scratched. The white precipitate obtained was purified on a column of neutral alumina IV. The fractions eluted with 3–5% methanol in ethyl acetate contained a mixture of Δ^{24}-Δ^{25}-isomers (VII and VIII), which could be separated on silica gel G plates impregnated with silver nitrate [0.25 mm; solvent system: chloroform–acetone–methanol 35:25:7.5 (v/v); R_f, 5β-cholest-24-ene-3α,7α,12α-triol (VII), 0.49 and 5β-cholest-25-ene-3α,7α,12α-triol (VIII), 0.38.

5β-Cholestane-3α,7α,12α,24ξ,25-pentol (IX)

Procedure A (*Fig. 4a*). Compound IX was prepared from 180 mg of the olefinic mixture (VII and VIII) as described by Hoshita (8), except that the osmium complex was decomposed with sodium hydrosulfite (9). The reaction product was purified by column chromatography on neutral alumina V with increasing amounts of methanol in ethyl acetate (3). The fractions eluted with 7.5–12.5% methanol in ethyl acetate contained 94 mg of a mixture of 5β-cholestane-3α,7α,-12α,24α,25-pentol and 5β-cholestane-3α,7α,12α,24β,25-pentol (IX) in a ratio of 62.4:37.6 (3). Further elution with 15% methanol in ethyl acetate yielded 22 mg of 5β-cholestane-3α,7α,12α,25,26-pentol (X). Crystallization of 5β-cholestane-3α,7α,12α,24ξ,25-pentol from ethyl acetate gave a white crystalline material, m.p. 199–200° C; $[\alpha]_D^{25} = +38.8°$ (3).

Procedure B (*Fig. 4b*). Δ^{24}- and Δ^{25}-3α,7α,12α-trihydroxy-5β-cholestene mixture (VII + VIII, 80 mg) was treated with formic acid (0.8 ml; 88%) for 45 min at room temperature. The mixture was cooled to 5° C, then hydrogen peroxide (0.2 ml; 30%) was added dropwise, the temperature being kept below 5° C. The reaction mixture was left with stirring at 0–5° C for 6–8 hr; after addition of ice-cold water, a precipitate was collected. The precipitate was heated to 50° C for 30 min with potassium hydroxide (83 mg) in methanol (1.0 ml) and water (0.2 ml). Evaporation under reduced pressure, addition of water and filtration yielded 50 mg of a mixture of 5β-cholestane-3α,7α,12α,25-tetrol (VI) (Fig. 4b), and the two epimeric pentols, 5β-cholestane-3α,7α,12α,24α,-25-pentol and 5β-cholestane-3α,7α,12α,24β,25-pentol (IX) (Fig. 4b). The different compounds were separated either by thin-layer or column chromatography. On thin-layer plates of silica gel G [solvent system, chloroform–acetone–methanol 35:25:7.5 (v/v)], 5β-cholestane-3α,7α,12α,25-tetrol had an R_f of 0.57 and 5β-cholestane-3α,7α,12α,24ξ,25-pentol had an R_f of 0.30–0.34. On columns of neutral alumina V, the tetrahydroxy bile alcohol (33.2 mg) was eluted with 5% (v/v) methanol in ethyl acetate, and the pentahydroxy bile alcohols (6.8 mg) with 7.5–15% methanol in ethyl acetate. The physical and spectral properties were identical with those described in Procedure A.

DISCUSSION

Degradation of the cholesterol side chain to form bile acids may involve 25-hydroxylated intermediates which constitute an alternative pathway in cholic acid biosynthesis (2). In order to study the possibility that 25-hydroxylated intermediates participate in the biosynthesis of bile acids, 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,-24ξ,25-pentol were synthesized from cholic acid as illustrated in Figs. 1 and 4.

25-Homocholic acid IV (Fig. 1) (m.p. 216–218° C) was prepared from cholic acid by the Arndt-Eistert method (6); the intermediary 3α,7α,12α-triformoxy-24-oxo-25-diazo-25-homo-

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5β-cholane (III) had an interesting mass spectrum fragmentation pattern (Fig. 2). It showed a major fragment of m/e 432 ($M^+ - C_3H_4ON_2$) due to McLafferty-style cleavage of the side chain between C-22 and C-23 suggesting the presence of a ketonic group at C-24. The methyl ester of 25-homocholic acid (V) (Fig. 1) (m.p. 149–150° C) on treatment with methyl magnesium iodide yielded 5β-cholestane-3α,7α,12α,-25-tetrol (VI) (Fig. 1) in 85% yield (m.p. 189–190° C) as compared to a yield of less than 50% when the ethereal solution of methyl magnesium iodide was added to the methyl homocholate (5).

5β-Cholestane-3α,7α,12α,25-tetrol was refluxed for 12 hr with a mixture of glacial acetic acid and acetic anhydride. The reaction was monitored by TLC to ensure complete disappearance of the tetrol. This prolonged boiling yielded a higher proportion of the 5β-cholest-24-ene-3α,7α,12α-triol (VII) (Fig. 4a) than reported by Hoshita (8). It appears that the Δ^{25}-isomer (VIII) rearranges to the thermodynamically more stable Δ^{24}-isomer. The mixture of the Δ^{24}- and Δ^{25}-isomers was purified on an alumina IV column. These isomers could not be separated either by TLC on silica gel G or by GLC on 3% QF-1 column, as TMSi derivatives. However, silica gel G impregnated with silver nitrate separated the two isomers, the Δ^{24} compound having the higher R_f value. Attempts to separate the two isomers by crystallization (8) were not successful.

5β-Cholestane-3α,7α,12α,24ξ,25-pentol was obtained in approximately 65% yield from the olefinic mixture (VII + VIII) (Fig. 4a) by a modification of Hoshita's method (8). Compounds VII + VIII were treated with osmium tetroxide followed by separation of the resulting pentol mixture (IX and X) by column chromatography on alumina V. Careful column chromatography also separated the pentol mixture epimeric at C-24 into 5β-cholestane-3α,7α,12α,24α,25-pentol and 5β-cholestane-3α,7α,12α,24β,25-pentol (3).

An alternative method for hydroxylating the double bond of Δ^{25}- and Δ^{24}-triols (VII and VIII) with hydrogen peroxide and formic acid yielded a mixture of 83% 5β-cholestane-3α,-7α,12α,25-tetrol and 17% 5β-cholestane-3α,7α,12α,24ξ,25-pentol (Fig. 4b). Under these conditions no 5β-cholestane-3α,7α,12α,25,26-pentol was formed. (In the presence of formic acid isomerization of Δ^{25}-triol to the Δ^{24}-triol—the thermodynamically more stable isomer—is taking place and subsequent addition of formic acid to the Δ^{24}-double bond yielded 5β-cholestane-3α,7α,12α,25-tetrol on saponification). JLR

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REFERENCES

1. Salen, G. 1971. Cholestanol deposition in cerebrotendinous xanthomatosis: a possible mechanism. *Ann. Intern. Med.* **75:** 843–851.
2. Setoguchi, T., G. Salen, G. S. Tint, and E. H. Mosbach. 1974. A biological abnormality in cerebrotendinous xanthomatosis. *J. Clin. Invest.* **53:** 1393–1401.
3. Shefer, S., B. Dayal, G. S. Tint, G. Salen, and E. H. Mosbach. 1975. Identification of pentahydroxy bile alcohols in cerebrotendinous xanthomatosis: characterization of 5β-cholestane-3α,7α,12α,24ξ,25-pentol and 5β-cholestane-3α,7α,12α,23ξ,25-pentol. *J. Lipid Res.* **16:** 280–286.
4. Ruzicka, L., P. A. Plattner, and H. Heusser. 1944. Steroide und sexual hormone. Über β′-[3α,7α,12α,Trioxy-norcholanyl-(23)]-$\Delta^{\alpha'\beta'}$ butenolid, ein Homologes der digitaloiden Aglucone. *Helv. Chim. Acta.* **27:** 186–194.
5. Pearlman, W. H. 1947. The preparation of C_{27} steroids from bile acids. I. Coprostanetetrol-3(α),7(α),12(α),-25. *J. Amer. Chem. Soc.* **69:** 1475–1476.
6. Svoboda, J. A., M. J. Thompson, and W. E. Robbins. 1968. 3β-Hydroxy-24-norchol-5-en-23-oic acid—A new inhibitor of the Δ^{24} -sterol reductase enzyme system(s) in the tobacco hornworm, *Manduca sexta* (Johannson). *Steroids.* **12:** 559–570.
7. Hofmann, A. F., and E. H. Mosbach. 1964. Identification of allodeoxycholic acid as the major component of gallstones induced in the rabbit by 5β-cholestan-3β-ol. *J. Biol. Chem.* **239:** 2813–2821.
8. Hoshita, T. 1962. Stero-bile acids and bile sterols XLVII. Syntheses of 3α,7α,12α,25ξ,26- and 3α,7α,12α,24ξ,25-pentahydroxycoprostanes. *J. Biochem.* (Tokyo) **52:** 176–179.
9. Kagan, H. B., and J. Jacques. 1960. Bile acids with hydroxyl groups at positions 6 and 7. *Bull. Soc. Chim. Fr.* 871–878.

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