Synthesis of 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one, an intermediate in the 25-hydroxylation pathway of cholic acid biosynthesis from cholesterol

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Summary This paper describes the chemical synthesis of 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one via selective oxidation of 5β-cholestane-3α,7α,12α,24ξ,25-pentol with silver carbonate on celite. The structure of this 24-keto bile alcohol was confirmed by gas–liquid chromatography and mass spectrometry. Synthesis of this compound via pyridinium chlorochromate oxidation of the triacetoxy derivative of 5β-cholestane-3α,7α,12α,24ξ,25-pentol followed by saponification further established its structure. 3α,7α,12α,25-Tetrahydroxy-5β-cholestan-24-one was required for the in vivo and in vitro studies of side-chain oxidation and cleavage in the 25-hydroxylation pathway of cholic acid biosynthesis.—**Dayal, B., G. S. Tint, A. K. Batta, S. Shefer, G. Salen, A. K. Bose, and B. N. Pramanik.** Synthesis of 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one, an intermediate in the 25-hydroxylation pathway of cholic acid biosynthesis from cholesterol. *J. Lipid Res.* 1983. **24:** 208–210.

Supplementary key words bile alcohols • keto-bile alcohol intermediate • side-chain oxidation

A 25-hydroxylation pathway of cholic acid biosynthesis has been recently demonstrated in patients with cerebrotendinous xanthomatosis (CTX), in normal subjects and in the rat (1, 2). This pathway proceeds via 5β-cholestane-3α,7α,12α,25-tetrol and 5β-cholestane-3α,7α,12α,24S,25-pentol (1). It was postulated that a 24-keto intermediate might be involved and that the final cleavage of the side-chain is catalyzed by a hydrolase whose action leads to the breaking of the carbon-carbon bond between C-24 and C-25, resulting in the formation of cholic acid and acetone.

The present study describes the synthesis of the putative keto intermediate, 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one, via selective oxidation of 5β-cholestane-3α,7α,12α,24ξ,25-pentol. The location of the keto group at C-24 was ascertained via a two-step procedure that employs pyridinium chlorochromate oxidation of 3α,7α,12α-triacetoxy-5β-cholestane-24ξ,25-diol in which the only secondary hydroxyl group at C-24 was oxidized.

METHODS

Physical measurements

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

NMR spectrum, in Hertz, was obtained in deuterated chloroform ($CDCl_3$) solution using a XL-200 MHz NMR spectrometer.

GLC. The bile alcohols, as the TMSi derivatives, were analyzed on a 180 cm × 4 mm column packed with 1% Hi-EFF-8BP on 80/100 mesh Gas Chrom Q; column temp. 240°C; N_2 flow, 40 ml/min. (Hewlett-Packard model 7610 gas chromatograph) (Hewlett-Packard, Palo Alto, CA).

EI–MS of the TMSi derivatives of the bile alcohols was obtained with a Varian MAT-111 gas chromatograph–mass spectrometer (Finnigan MAT, Sunnyvale, CA) at an ion source pressure of $2–3 \times 10^{-6}$ Torr and an electron energy of 70 eV, as described previously (3).

CI–MS of 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one was recorded by the direct probe technique using a Biospect spectrometer (Scientific Research Instruments Corporation, Baltimore, MD) (4).

TLC. The bile alcohols were separated on silica gel G plates (Brinkman Instruments, Westbury, NJ, 0.25 mm thickness) and the spots were made visible either with iodine or with water.

RESULTS

Preparation of 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one (Fig. 1, II) Scheme 1. Ag_2CO_3/celite oxidation of 5β-cholestane-3α,7α,12α,24ξ,25-pentol

Seven mg of 5β-cholestane-3α,7α,12α,24ξ,25-pentol [I; (5)] was placed in a 15-ml round-bottom flask and dissolved in about 5 ml of dry benzene; 160 mg (40

Abbreviations: CTX, cerebrotendinous xanthomatosis; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMSi, trimethylsilyl; NMR, nuclear magnetic resonance; PMR, proton magnetic resonance; RRT, relative retention time; EI–MS, electron ionization–mass spectrometry; CI–MS, chemical ionization–mass spectrometry.

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mmol) of oxidant (Ag_2CO_3/celite) was added and the mixture was refluxed azeotropically (to remove any H_2O from the reaction mixture) while stirring (6, 7). Progress of the reaction was monitored by TLC, using the solvent system chloroform–acetone–methanol 70:20:7.5 (v/v). After 30–40 min of refluxing, the solid material was filtered off and washed with benzene. The solvent was evaporated on a rotary evaporator and the residue (5 mg) was separated by analytical and preparative TLC on silica gel G as described above. The following R_f values were observed: 0.12, starting material, I (0.5 mg); R_f 0.33, 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one, II, [4.0 mg (57% yield)]; GLC, RRT 2.47 (RT of 5α-cholestane, 6.02 min); CI–MS, m/z 468, [$(M + NH_4)^+$, pseudo molecular ion (PM)] (80%), 454, ($M\text{-}CH_2 + NH_4$) (100%), 366 (PM^+-102) (60%), 330, (366-2H_2O) (41%); and 312 (366-3H_2O) (55%); R_f 0.25, 3α,7α,12α-trihydroxy-5β-cholan-24-al, III (1.0 mg); GLC, RRT 1.57; EI–MS, m/z 518, M-90 (1%), 428, M-2 × 90 (19%), 385, M-2 × 90 + 43 (2%) (McLafferty rearrangement), 338, M-3 × 90 (32%), 295, M-3 × 90 + 43 (7%) (McLafferty rearrangement), 253, M-3 × 90 + side-chain (100%), 243, C_5-C_7 (9%), 227, 226, 211, M-3 × 90 + D ring scission (16%, 19%, and 17%, respectively).

Scheme 2. Preparation of 3α,7α,12α-triacetoxy-5β-cholestane-24ξ,25-diol

Acetylation followed by oxidation with osmium tetroxide of 5β-cholest-24-ene-3α,7α,12α-triol (5) gave 3α,7α,12α-triacetoxy-5β-cholestane-24ξ,25-diol in 65% yield; mp, 102–104°C; R_f, 0.1, solvent system, benzene–ethyl acetate 90:10 (v/v). The 200 MHz PMR spectrum in $CDCl_3$ showed the following signals: 0.74 (s, 18-CH_3), 0.92 (s, 19-CH_3), 0.83 (d, 21-CH_3, 6Hz), 1.16 (s, 26-CH_3), 1.21 (s, 27-CH_3), 2.05, 2.10, 2.15, (3-OAc, 9H). The following major peaks were observed by mass spectrometry: m/z 500, [0.2%, M^+-(HOAc + H_2O)]; 482, (1.1%, 500-H_2O); 458, (13.2%, M^+-2 × HOAc); 443, [3.2%, M^+-(2 × HOAc + CH_3)]; 440, [3.2%, M^+-(2 × HOAc + H_2O)]; 422, [2.1%, M^+-(2 × HOAc + 2H_2O)]; 400, [35.3%, M^+-{2 × HOAc + $(CH_3)_2$ C = O}; 382, [11.6%, M^+-{2HOAc + $(CH_3)_2$ C = O + H_2O}]; 340, [44.2%, M^+-{3 × HOAc + $(CH_3)_2$ C = O}]; 322, [14.7%, M^+-{3 × HOAc + $(CH_3)_2$ C = O + H_2O}]; 313, [47.4%, M^+-(2 × HOAc + side chain)]; 253, [62.1%, M^+-(3 × HOAc + side chain)] and 43, (100%, CH_3CO^+).

Pyridinium chlorochromate oxidation of 3α,7α,12α-triacetoxy-5β-cholestane-24ξ,25-diol

To a stirred solution of pyridinium chlorochromate (50 mg) in dichloromethane (15 ml) was added 3α,7α,12α-triacetoxy-5β-cholestane-24ξ,25-diol (12 mg).

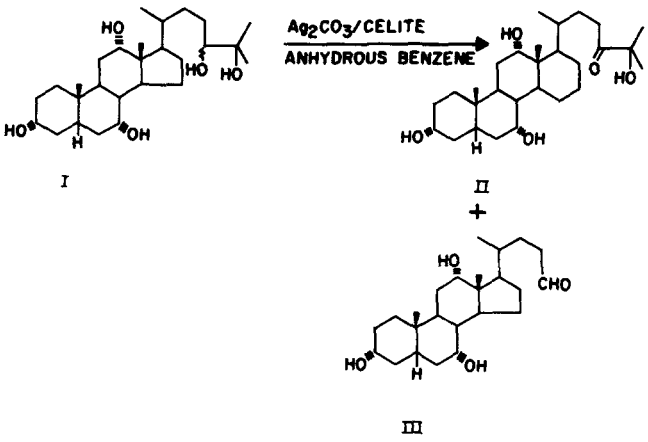
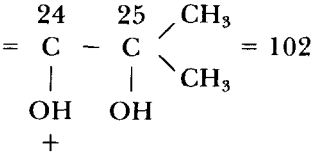


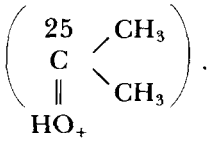
Fig. 1. Synthesis of 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one. (Scheme 1). I, 5β-cholestane-3α,7α,12α,24ξ,25-pentol; II, 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one; III, 3α,7α,12α-trihydroxy-5β-cholan-24-al.

Stirring was continued for 30 min at ambient temperature and the reaction was worked up as described before (8). The resulting material (7 mg) was purified by preparative TLC [silica gel G, benzene–ethyl acetate 90:10 (v/v)]. The band corresponding to R_f 0.74 (3α,7α,12α-triacetoxy-25-hydroxy-5β-cholestan-24-one), when analyzed by mass spectrometry (EI–MS, direct injection underivatized) gave the following major peaks m/z 456, (M-2 × 60, 5%); 413, [M-(60 + 102 + H), 22%],



= 102

due to McLafferty rearrangement; 397, [M-(2 × 60 + 59), 6%]; 396, [M-(3 × 60), 7%]; 370, [M-(2 × 60 + 87-H), 20%]; 353, [M-(2 × 60 + 102 + H), 61%]; 337, [M-(3 × 60 + 59), 11%]; 310, [M-(3 × 60 + 87-H), 70%]; 295, [M-(3 × 60 + 102 − H), 28%]; 253, (M-(3 × 60 + 143), 100%]; 227, [M-(3 × 60 + 143 + C − 16, C − 17, − H), 14%]; 59, loss of C_{25} to C_{27} moiety



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Saponification of 3α,7α,12α-triacetoxy-25-hydroxy-5β-cholestan-24-one

Saponification of the above acetoxy-keto-ol (6 mg) in 1 ml of 3% ethanolic KOH at ambient temperature for 15 min yielded a product that was resolved by preparative TLC [silica gel G; solvent system, chloroform–

acetone–methanol 70:20:7.5 (v/v)] and the band corresponding to R_f 0.33 was eluted with ethyl acetate–methanol 90:10. The resulting compound (0.7 mg) had similar mass spectral (CI–MS) characteristics as 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one (Fig. 1, II) synthesized by the Ag_2CO_3/celite oxidation.

DISCUSSION

We have demonstrated (6) the high selectivity of silver carbonate adsorbed to celite in the oxidation of the 3α-OH group to a 3-keto group in the bile acid series. Recently, we have found in preliminary experiments that 5β-cholestane-3α,24ξ,25-triol (9), when subjected to Ag_2CO_3/celite oxidation, resulted mainly in the formation of the corresponding 24-keto compound. In this case the 3β-hydrogen cannot approach the surface of the oxidizing reagent (Ag_2CO_3/celite) and consequently the oxidation at C-3 does not take place (10). Taking advantage of the high selectivity for the C-24 hydroxyl group in bile alcohols with 1,2-glycol system in the side-chain, we have oxidized 5β-cholestane-3α,7α,12α,24ξ,25-pentol with Ag_2CO_3/celite which yielded 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one as the major product (57%). The 24-keto, 25-ol system in the side-chain was confirmed by comparison to the product obtained via the two-step procedure that employs pyridinium chlorochromate oxidation. These polyhydroxy compounds proved to be too nonvolatile and thermolabile for recording meaningful spectra in an electron impact mass spectrometer. The CI–MS of this keto-compound using ammonia and argon as the reagent gas gave several fragment ions, one of which (m/z 366) corresponded to cleavage due to a McLafferty rearrangement of a 24-keto-25-hydroxycholestane derivative (4).

The synthetic 3α,7α,12α,25-tetrahydroxy-5β-cholestan-24-one showed the same TLC (R_f = 0.33) and GLC characteristics and had identical mass-spectral fragmentation pattern as the biosynthetic 24-keto bile alcohol isolated from the incubation of 5β-cholestane-3α,7α,12α,24S,25-pentol with the cytosolic fraction of rat liver homogenate in the presence of NAD.

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