Configuration at C-25 in 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid isolated from human bile

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Summary This report describes the isolation of the natural isomer of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid from human bile by a method that retains the configuration at C-25. The stereochemistry at C-25 in this bile acid was defined as 25R by a direct comparison with standard (25R) 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid.—**Batta, A. K., G. Salen, S. Shefer, B. Dayal, and G. S. Tint.** Configuration at C-25 in 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid isolated from human bile. *J. Lipid Res.* 1983. **24:** 94–96.

Supplementary key words bile acids • *Alligator mississippiensis* bile

5β-Cholestane-3α,7α,12α,26-tetrol is an obligatory precursor in the biosynthesis of cholic acid from cholesterol (1). Inasmuch as the hepatic microsomal and mitochondrial enzymes responsible for the 26-hydroxylation of the cholesterol side-chain are shown to be stereospecific (2–4), this tetrahydroxy bile alcohol is expected to be present in the bile as the 25R or the 25S-diastereoisomer. Oxidation of this tetrol in the liver yields 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA; **Fig. 1**) (1) which may also be present in the bile predominantly as a single isomer. Recently, we have been able to isolate the natural diastereoisomer of THCA in the bile of *Alligator mississippiensis* (5) and from an X-ray crystallographic examination, it has been shown to be the 25R-diastereoisomer (6). THCA is present in human bile in trace quantities (7), but it accumulates in the bile of patients with certain liver abnormalities (8, 9). It has been isolated from human bile after saponification with strong alkali (7) but, since rigorous alkaline hydrolysis causes isomerization at C-25 in THCA (5, 10), this method cannot be used to establish configuration at C-25 in the natural isomer. In the present study, we describe a nondestructive method for the isolation of THCA from human bile and, by direct comparison with standard THCA (5, 6), we have proved that the natural isomer of THCA in human bile is the 25R-diastereoisomer.

Abbreviations: THCA, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; TMS, trimethylsilyl.

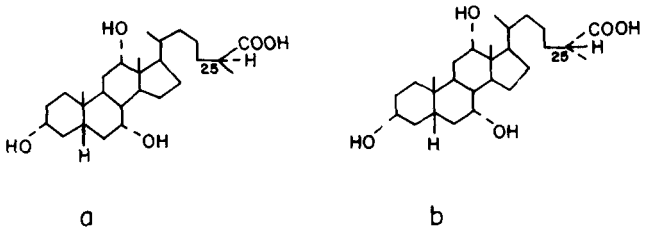

Fig. 1. Structures of (25R) and (25S) 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acids: a, 25R; b, 25S.

METHODS

Melting points were determined on a Thermolyne apparatus, model MP-12600, and are uncorrected.

TLC was done on silica gel G plates (Brinkmann, 0.25 mm thickness). The spots were detected with phosphomolybdic acid (3.5% in isopropanol) and sulfuric acid (20%).

Optical rotations were determined at 25°C in ethanol on a Carey model 60 spectropolarimeter.

GLC. The bile acids, as their methyl ester TMSi ether derivatives were analyzed on a 180 cm × 4 mm column packed with 1% HiEFF-8BP on 80/100 mesh Gas Chrom Q, column temp. 240°C, N_2 flow, 40 ml/min (Hewlett-Packard model 7610 gas chromatograph).

Mass spectra were obtained with a Varian MAT-111 gas chromatograph–mass spectrometer (Varian Associates, Palo Alto, CA).

RESULTS

Isolation of standard (25R) and (25S) diastereoisomers of THCA (Fig. 1 a, b). Bile of *Alligator mississippiensis* (10 ml) was deproteinized and the residue was hydrolyzed with 10 ml of 25% sodium hydroxide in diethylene glycol as described by Batta et al. (6). The (25R) and (25S) diastereoisomers of THCA were separated by preparative TLC of the hydrolyzate (6) and crystallization from ethyl acetate: (25S) diastereoisomer, mp 200–201°C, R_f 0.44 (solvent system, chloroform–acetone–methanol 70:50:10 (v/v/v), developed twice) and (25R) diastereoisomer, mp 180–182°C, R_f 0.41 in the above-mentioned solvent system (6).

Isolation of (25R) diastereoisomer of THCA from human bile (Fig. 1a). THCA was isolated from human gallbladder bile (250 ml; collected from five male normolipidemic subjects, 38–55 years of age, with chronic peptic ulcer disease) by the procedure described in the flow sheet (**Fig. 2**). The pure compound thus obtained (2.5 mg) melted at 180–183°C, $[\alpha]_D^{25} = +30.1°$ (c, 1.60 in ethanol); methyl ester, mp 153–154°C, $[\alpha]_D^{25} = +30.6°$ (c, 1.20, in ethanol); methyl ester-TMSi ether, GLC retention time relative to 5α-cholestane, 2.63 (retention

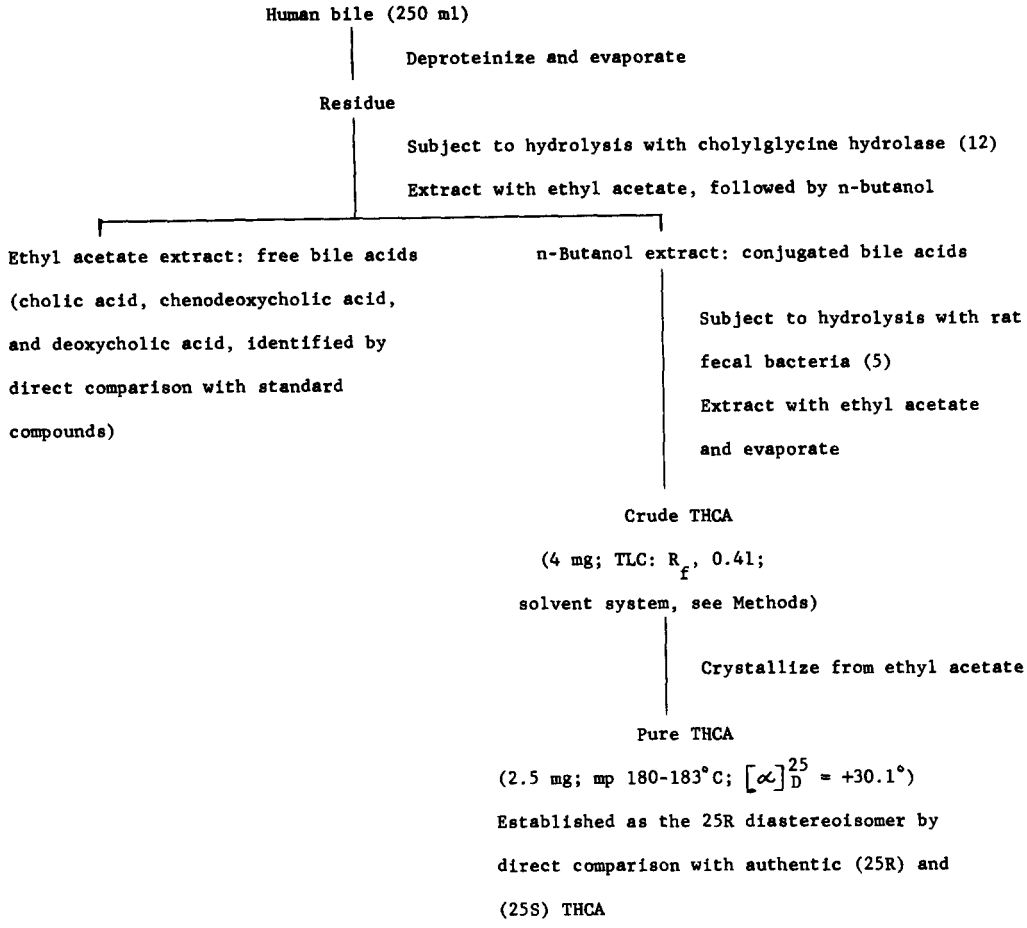

Fig. 2. Isolation of the natural isomer of THCA in human bile.

time of 5α-cholestane, 5.8 min.). The acid showed the following major peaks in the mass spectrum: m/z 450 (M^+; 2%), 432 (M-2 × 18; 35%), 399 [M-(2 × 18 + 15); 9%], 396 (M-3 × 18; 21%), 385 (m/z 399 − 14; 18%), 381 [M-(3 × 18 + 15); 8%], 289 [M-(18 + side chain); 30%], 271 [M-(2 × 18 + side chain); 100%], 253 [M-(3 × 18 + side chain); 82%], 247 [M-(18 + side chain + D ring); 15%], 226 [M-(3 × 18 + side chain + C-16, 17); 26%] and 199 [M-(3 × 18 + side chain + A ring); 18%]. The GLC retention time and mass spectrum were identical with those of the authentic THCA and the mass spectral fragmentation pattern was similar to that reported for THCA (11). On TLC, the THCA obtained from human bile had an R_f of 0.41 (solvent system: see above), which agreed with the R_f value of (25R) diastereoisomer of THCA.

DISCUSSION

Carey and Haslewood (7) isolated THCA from the bile of healthy subjects, but the rigorous alkaline hydrolysis conditions they used would result in isomerization at C-25 in the natural diastereoisomer. We have recently shown that rat fecal bacteria are able to hydrolyze conjugated THCA (5), whereas cholylglycine hydrolase is specific for the C_{24} bile acids (12). We utilized this finding for the separation of the relatively small amounts of THCA from large amounts of cholic, chenodeoxycholic, and deoxycholic acids present in human bile. The configuration at C-25 was established as 25R by direct comparison with standard (25R) THCA (obtained from the bile of *Alligator mississippiensis* (6)).

Microsomal and mitochondrial enzymes are responsible for the oxidation of cholesterol sidechain in man under normal conditions (13) and the isolation of a single (25R) diastereoisomer of THCA in human bile supports the role of mitochondria in ω-hydroxylation. The relative roles of the microsomal and mitochondrial enzyme systems in the ω-hydroxylation of cholesterol sidechain have been studied by Hanson, Leeuwen, and Williams (14) in a patient with bile fistula. After administration of [2-^{14}C]mevalonic acid, the THCA recovered in the bile showed only about 7.5% radioactiv-

ity at C-26. From this they inferred that the ω-hydroxylation of 5β-cholestane-3α,7α,12α-triol involves hydroxylation of the methyl groups derived from C-3 of mevalonate (C-27) by mitochondrial enzymes.

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