Synthesis and Metabolism of Coprostane-7α-ol-3-one

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Bergström and Lindstedt [1,2] have shown that both 7α-hydroxycholesterol and coprostane-3α,7α-diol are converted to cholic and chenodeoxycholic acid in the bile-fistula rat. Yamasaki and coworkers [3] have found that 7α-hydroxycholesterol is oxidized in rat liver homogenates to an α,β-unsaturated ketone which was suggested to be Δ^4-cholestene-7α-ol-3-one. This compound was proposed as an intermediate in the postulated formation of coprostane-3α,7α-diol from 7α-hydroxycholesterol. The identification of Δ^4-cholestene-7α-ol-3-one as a metabolite of 7α-hydroxycholesterol in mouse liver homogenates has recently been described [4]. This compound was found to be metabolized in the bile-fistula rat to cholic and chenodeoxycholic acid, but several unidentified acids were also formed [5]. The possibility that this compound might be an intermediate in the conversion of cholesterol to bile acids can, however, not be excluded. If Δ^4-cholestene-7α-ol-3-one were an intermediate one possible metabolite of this compound would be coprostane-7α-ol-3-one, *i.e.* the saturated compound with the rings A and B in *cis*-junction as in the normal bile acids.

In a communication published while this work was in progress, Yamasaki *et al.*[6] reported the synthesis of coprostane-7α-ol-3-one and suggested that this compound was an intermediate in the proposed conversion of 7α-hydroxycholesterol to coprostane-3α,7α-diol. In the present report an alternative and more simple synthesis of coprostane-7α-ol-3-one is described as well as the metabolism of tritium labeled coprostane-7α-ol-3-one in the bile-fistula rat.

Experimental. Coprostane-3α,7α-diol was synthesized according to Bergström and Krabisch[7] and had m.p. 78–79°, reported 84–86°. The infrared spectrum of the material of m.p. 78–79° was identical with that of m.p. 84–86°. Tritium-labeled coprostane-3α,7α-diol was prepared by exposure of part of above-mentioned material to tritium gas according to the method of Wilzbach[8] in the apparatus described by Bergström and Lindstedt[9] and purified by repeated chromatography [10]. Labeled coprostane-3α,7α-diol was also prepared by electrolytic coupling of *iso*valeric acid with chenodeoxycholic acid that had been labeled with tritium by the Wilzbach procedure. After purification by chromatography this labeled coprostane-diol was diluted with inactive material of m.p. 78–79°.

The method of synthesis of coprostane-7α-ol-3-one was based on the finding of Jones *et al.*[11] that cholic acid can be selectively oxidized at the 3-position by Oppenauer oxidation.

Synthesis I. 150 mg coprostane-3α,7α-diol randomly labeled with tritium, spec. act. 33 000 c.p.m./mg, were dissolved in 5 ml dry benzene and heated under reflux for 6 h with 200 mg aluminium *tert.*-butoxide in 3 ml acetone. The reaction mixture was acidified with 2 N sulfuric acid and the benzene layer washed with sodium carbonate and water and evaporated to dryness. The residue was chromatographed on a 15 g column of aluminum oxide (Woelm, Eschwege, Germany, grade III). After elution of coprostane-3,7-dione (42 mg) with benzene, coprostane-7α-ol-3-one (59 mg, spec.act. 19 800 c.p.m./mg) was eluted with 5 % ethyl acetate in benzene. Repeated crystallization from petroleum ether and methanol/water afforded 32 mg, m.p. 120–121°, reported m.p. 121–22°.

Synthesis II. 140 mg coprostane-3α,7α-diol labeled with tritium in the "chenodeoxycholic acid moiety" of the molecule, spec. act. 32 000 c.p.m./mg, were oxidized with same amounts of reagents as above, but only for 3 h. The reaction mixture was extracted and chromatographed as above. In this case little of the coprostane-dione was formed (8 mg) and the main product was coprostane-7α-ol-3-one (79 mg, spec.act. 23 000 c.p.m./mg). Crystallization as above afforded 47 mg, m.p. 121°.

Animal experiments. 2.6 mg of coprostane-7α-ol-3-one from synthesis I and 3.5 mg of material from synthesis II were injected intraperitoneally as emulsions with bovine serum albumin solution into two bile-fistula rats (RI and RII resp.). After saponification the first 24 h portions of bile were chromatographed with phase system F 1 [12] to separate the cholic and the chenodeoxycholic acid fractions. The cholic acid fractions were rechromatographed in phase system C 1 [12] and the chenodeoxycholic acid fractions in phase system F 1.

Results. When administered to bile-fistula rats coprostane-7α-ol-3-one was rapidly excreted in bile as bile acids (52 % of injected dose in R I and 25 % in R II during the first 24 h). The radioactivity

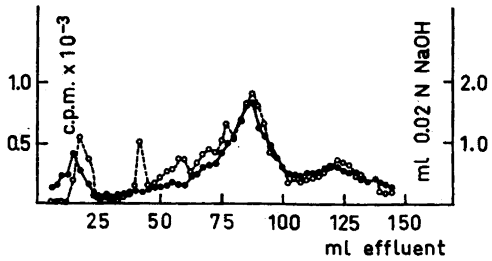

Fig. 1. Chromatography of the cholic acid fraction of hydrolyzed bile from Rat I. Column: 4.5 g hydrophobic Hyflo Supercel. Phase system C 1. Solid line: titration values. Broken line: radioactivity.

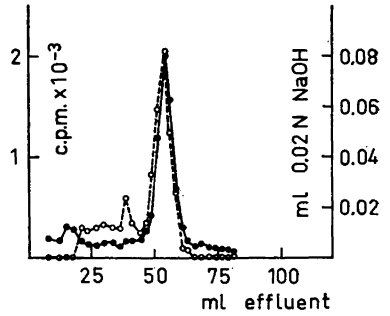

Fig. 2. Chromatography of the chenodeoxycholic acid fraction of hydrolyzed bile from Rat I. Column: 4.5 g hydrophobic Hyflo Supercel. Phase system F 1.

was distributed between the cholic and the chenodeoxycholic fractions in the proportion 6.5:3.5 (R I) and 6:4 (R II), resp. Rechromatography of the cholic acid fraction showed that a substantial part of the radioactivity coincided with the titration peak of cholic acid (Fig. 1) and the identity of the radioactive material with cholic acid was established by isotope dilution. The main part of the radioactivity in the chenodeoxycholic acid fraction was eluted as chenodeoxycholic acid (Fig. 2) and the identity was confirmed by isotope dilution.

Thus, coprostane-7α-ol-3-one is efficiently converted in the bile-fistula rat to both cholic and chenodeoxycholic acid. However, evidence for the formation of this compound from cholesterol, 7α-hydroxycholesterol or Δ^4-cholestene-7α-ol-3-one is lacking and it appears that discussions of coprostane-7α-ol-3-one as a possible intermediate in the formation of bile acids from cholesterol should await further experimentation.

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