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On the Synthesis and Metabolism of Cholest-4-en-7α-ol-3-one

Bile Acids and Steroids 156

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A new method of synthesis of cholest-4-en-7α-ol-3-one is described. This compound was prepared from chenodeoxycholic acid in a yield of about 5 % by electrolytic coupling of chenodeoxycholic acid with isovaleric acid, oxidation of the resulting 5β-cholestane-3α,7α-diol to 5β-cholestan-7α-ol-3-one, and dehydrogenation of this compound with selenium dioxide.

Tritium-labeled cholest-4-en-7α-ol-3-one, prepared from randomly tritium-labeled chenodeoxycholic acid, was converted, when administered in trace amounts, mainly into cholic and chenodeoxycholic acids in the bile fistula rat.

A previous communication [1] described the synthesis of cholest-4-en-7α-ol-3-one, which was prepared from cholesteryl benzoate in an over-all yield of about 0.2 %. The biological formation of cholest-4-en-7α-ol-3-one from cholest-5-ene-3β,7α-diol was demonstrated in an earlier report [2] and the formation of cholic and chenodeoxycholic acids from cholest-4-en-7α-ol-3-one in the bile fistula rat was also established.[1] In addition to cholic and chenodeoxycholic acids, several unidentified bile acids were formed in these experiments. The tritium-labeled cholest-4-en-7α-ol-3-one used had been prepared from randomly tritium-labeled cholesterol of low specific activity.

Further studies on the possible role of cholest-4-en-7α-ol-3-one as an intermediate in the biogenesis of cholic and chenodeoxycholic acids required the use of material of considerably higher specific acitivity. The present communication describes an improved method of synthesis of cholest-4-en-7α-ol-3-one and studies of the *in vivo* metabolism of tritium-labeled cholest-4-en-7α-ol-3-one, prepared from tritium-labeled chenodeoxycholic acid.

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EXPERIMENTAL

5β-Cholestane-3α,7α-diol. 5β-Cholestane-3α,7α-diol was prepared by electrolytic coupling of chenodeoxycholic acid, 2 g, and isovaleric acid, 12 g, as described by Bergström and Krabisch.[3] The reaction mixture was diluted with water and extracted with ether. The ether extract was washed with a 5 % solution of sodium carbonate and then with water until neutral. The residue of the ether extract was chromatographed on a column of 100 g of aluminum oxide, grade III (Woelm, Eschwege, W. Germany). The column was eluted with increasing concentrations of ethyl acetate in benzene. Ethyl acetate, 20 % in benzene, eluted 720 mg of 5β-cholestane-3α,7α-diol. Crystallization from a methanol-water mixture afforded 615 mg of 5β-cholestane-3α,7α-diol, m.p. 83–85°, reported [3] m.p. 84–86.°

5β-Cholestan-7α-ol-3-one. 5β-Cholestane-3α,7α-diol, 400 mg, was dissolved in 14.3 ml dry benzene and 8.6 ml acetone, 570 mg of aluminum *tert.*-butoxide were added and the mixture was refluxed for 3 h.[4] The reaction mixture was acidified with 1 M sulfuric acid. The benzene layer was washed with a 5 % solution of sodium carbonate and then with water until neutral. The benzene extract was evaporated to dryness and the residue was chromatographed on a column of 50 g of aluminum oxide, grade III, eluting with increasing concentrations of ethyl acetate in benzene. Ethyl acetate, 5 % in benzene, eluted 280 mg of 5β-cholestan-7α-ol-3-one. Crystallization from a methanol-water mixture yielded 242 mg of 5β-cholestan-7α-ol-3-one, m.p. 120°, reported [4] m.p. 121–122°.

Cholest-4-en-7α-ol-3-one. 5β-Cholestan-7α-ol-3-one, 200 mg, was dissolved in 50 ml 96 % ethanol and treated with 150 mg of selenium dioxide at 70° for 4 h.[5] The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was chromatographed on a column of 27 g of hydrophobic Hyflo Super-Cel using phase system III.[6] The effluent was analyzed by reading the ultraviolet absorbance of the fractions at 243 mμ. Cholest-4-en-7α-ol-3-one was eluted between 480 and 840 ml of effluent. These fractions were combined and the solvent was evaporated. Crystallization of the residue from a methanol-water mixture afforded 61 mg of cholest-4-en-7α-ol-3-one, m.p. 181–182°, reported [1] m.p. 183–184°. The material obtained had the same properties as that prepared previously [1] in thin layer and gas chromatography and there was no depression of melting point upon mixing the two materials.

Tritium-labeled cholest-4-en-7α-ol-3-one. Tritium-labeled 5β-cholestane-3α,7α-diol was prepared by electrolytic coupling of isovaleric acid and tritium-labeled chenodeoxycholic acid, labeled by exposure for 4 weeks to 2 C of tritium gas (Radiochemical Centre, Amersham, England). Tritium-labeled 5β-cholestane-3α,7α-diol, 52 mg, specific activity 30 μC per mg, was oxidized with aluminum *tert.*-butoxide as above yielding 32 mg of 5β-cholestan-7α-ol-3-one, specific activity 23 μC per mg. This material was treated with selenium dioxide as above. The reaction mixture was chromatographed on a column of 4.5 g of hydrophobic Hyflo Super-Cel using phase system III. The fractions collected between 80 and 165 ml of effluent were combined and the solvent was evaporated. The residue, 14 mg, was crystallized from a methanol-water mixture yielding 9.3 mg of cholest-4-en-7α-ol-3-one, m.p. 179–181°, specific activity 11 μC per mg.

Animal experiments. Male rats of the Sprague-Dawley strain weighing about 200 g were used. Bile fistulas were prepared in the usual manner. The labeled compound was administered intraperitoneally as an emulsion stabilized with serum albumin. Bile was collected in 24 h portions. Hydrolysis of bile was carried out with 1 M sodium hydroxide in 50 % aqueous ethanol in sealed steel tubes at 110° for 11 h. The hydrolysate was acidified with hydrochloric acid and was extracted with ether. The ether extract was washed with water until neutral and the solvent was evaporated. The residue was chromatographed with phase system F 1.[7] The trihydroxycholanoic acid fraction was rechromatographed with phase system C 1 [7] and the dihydroxycholanoic acid fraction with phase system F 1.

RESULTS AND DISCUSSION

Tritium-labeled cholest-4-en-7α-ol-3-one, in doses of 1–2 μC, was administered to three bile fistula rats. The first 24 h portions of bile contained 26, 27, and 30 % of the administered isotope. Chromatography of hydrolyzed bile

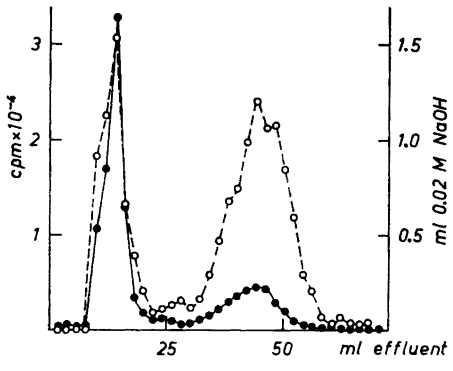

Fig. 1. Chromatogram of first 24 h portion of hydrolyzed bile from bile fistula rat treated with injection of tritium-labeled cholest-4-en-7α-ol-3-one. Column, 9 g of hydrophobic Hyflo Super-Cel; phase system F 1. Solid line, titration values; broken line, radioactivity.

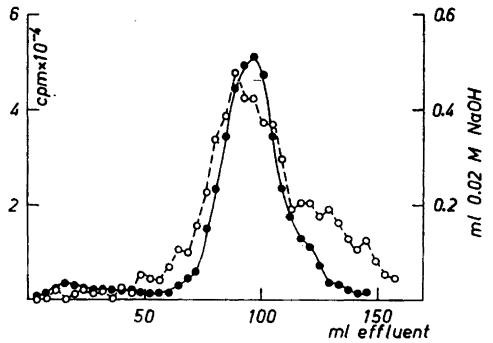

Fig. 2. Rechromatography of cholic acid fraction (10—25 ml of effluent) in the chromatogram shown in Fig. 1. Column, 4.5 g of hydrophobic Hyflo Super-Cel. phase system C 1. Symbols as in Fig. 1.

with phase system F 1 showed that 70—80 % of the labeled material was eluted together with cholic and chenodeoxycholic acids (*cf.* Fig. 1). The cholic acid fraction (between 10 and 25 ml of effluent, Fig. 1) was rechromatographed with phase system C 1 (Fig. 2) and the main part of the radioactivity was eluted together with cholic acid. The identity of this labeled material with cholic acid was established by crystallization to constant specific activity after addition of unlabeled cholic acid. The labeled material eluted after the cholic acid peak had the elution volume characteristic of β-muricholic acid (3α,6β,7β-trihydroxy-5β-cholanoic acid), a metabolite of chenodeoxycholic acid. The chenodeoxycholic acid fraction (between 35 and 60 ml of effluent, Fig. 1) was rechromatographed with phase system F 1 and it was shown that the labeled material in this fraction consisted predominantly of chenodeoxycholic acid. The ratio between the amount of labeled cholic acid and the amount of labeled chenodeoxycholic acid formed was 1:0.3, 1:0.9, and 1:0.8.

The results of the present investigation differ in some respects from those obtained earlier.[1] In the previous investigation unidentified acids were formed in addition to cholic and chenodeoxycholic acids. This finding can be explained, at least in part, by the difference in the specific activities of the labeled cholest-4-en-7α-ol-3-one used. In fact, when labeled cholest-4-en-7α-ol-3-one, prepared as described in the present investigation, but diluted ten-fold with unlabeled material, was administered to bile fistula rats the pattern of labeled products in bile differed from that observed when undiluted cholest-4-en-7α-ol-3-one had been administered. Thus, in addition to cholic and chenodeoxycholic acids varying amounts of unidentified bile acids, the same as or similar to those observed previously,[1] were present.

The method of synthesis of cholest-4-en-7α-ol-3-one described has the advantage of enabling the preparation of material of high specific activity, as chenodeoxycholic acid is easily labeled with tritium by exposure to tritium gas.

Recent work [8] on the metabolism of tritium-labeled cholest-4-en-7α-ol-3-one *in vitro* has shown that this compound is hydroxylated in the 12α-position to yield cholest-4-ene-7α,12α-diol-3-one, a probable intermediate in the biogenesis of cholic acid.[5] The availability of cholest-4-en-7α-ol-3-one of high specific activity may facilitate further studies on the role of this compound in the biosynthesis of cholic and chenodeoxycholic acids from cholesterol.

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