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Preparation of Ursodeoxycholic Acid and 3α,7β,12α-Trihydroxycholanic Acid

Bile Acids and Steroids 94

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The compounds have been prepared through reduction of the corresponding 7-ketoacids with sodium in *n*-propanol. Reversed phase partition chromatography of the reduction products revealed that the epimeric 7β- and 7α-hydroxycompounds are formed in the proportion of about 3:1.

Ursodeoxycholic acid (3 α,7β-dihydroxycholanic acid) is found in the bile of certain bear species [1] and in the coypu [1]. Traces of this acid has recently also been reported to occur in the rat [2] and man [3]. Urxodeoxycholic acid has previously been prepared synthetically through reduction of 7-ketolithocholic acid (3α-hydroxy-7-ketocholanic acid) with sodium ethoxide and through catalytic hydrogenation in acidic medium. The yield of the 7β-epimer was only about 10 % [4]. Sodium borohydride reduction of 7-ketocholanic acids and derivatives produces specifically the corresponding 7α-hydroxycompounds [5-8]. The 7-ketone in the bile acid series has been reported to be almost resistent to reduction with aluminium *iso*propoxide [8,9]. In one case, however, a successful reduction, which gave predominately the axial epimer (7α), has been described [10]. These authors also reported the preparation of ursodeoxycholic acid in 95 % yield by reduction of 7-ketolithocholic acid with sodium in *n*-propanol. When this method was used in the present investigation and the reaction product separated by reversed phase partition chromatography, it appeared that the reduction gives a mixture consisting of the equatorial 7β- and axial 7α-hydroxyepimers in the approximate proportion of 3:1.

3α,7β,12α-trihydroxycholanic acid has never been synthesized or isolated before. Recently, however, an acid with identical chromatographic properties and sulfuric acid spectrum has been isolated as a metabolite of cholic acid-24-^{14}C in the rat [11] and has been detected in samples of human bile [3].

3α,7β,12α-trihydroxycholanic acid was obtained by reduction of 7-ketodeoxycholic acid (3α,12α-dihydroxy-7-ketocholanic acid) by the same method

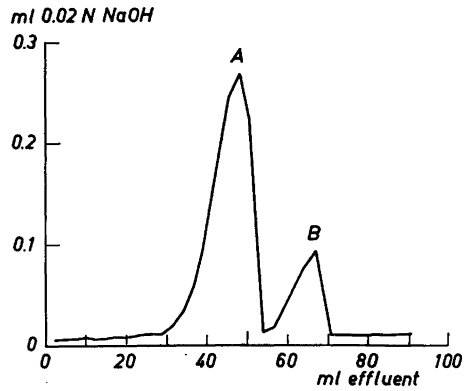

Fig. 1. Chromatography of an aliquot of the reaction product obtained by reduction of 7-ketolithocholic acid with sodium in *n*-propanol. Column: 4.5 g of hydrophobic SuperCel. Moving phase: 55 % (v/v) aqueous methanol. Stationary phase: 10 % (v/v)-heptane in chloroform. A: Ursodeoxycholic acid. B: Chenodeoxycholic acid.

as above. The two epimers, separated chromatographically, were found in the same proportion as in the reduction of 7-ketolithocholic acid. The methyl ester of 3α,7β,12α-trihydroxycholanic acid yielded a tri-*p*-nitrobenzoate on acylation with *p*-nitrobenzoyl chloride in pyridine. Under similar conditions methyl cholate only gives the 3α-mono-*p*-nitrobenzoate [12].

EXPERIMENTAL

Ursodeoxycholic acid. 7-Ketolithocholic acid (m. p. 200—202°) was prepared through sodium dichromate oxidation of methyl 3α-cathyloxy-7α-hydroxycholanate followed by hydrolysis [13,14]. A solution of 0.5 g of the ketoacid in 10 ml of anhydrous *n*-propanol was refluxed with 1 g of sodium for 3 h. The solution was diluted with water, acidified with hydrochloric acid and extracted with ethylacetate. After evaporation to dryness *in vacuo* the residue was chromatographed on hydrophobic Super Cel with 55 % (v/v) methanol as moving phase and 10 % (v/v) heptane in chloroform as stationary phase [15] (Fig. 1).

No unchanged ketoacid could be detected and of the two peaks which appeared, the first one (77 %) was crystallized from ethylacetate/light petroleum. Yield: 0.32 g, m. p. 201—202°. There was no depression of the m. p. on admixture with authentic ursodeoxycholic acid that was kindly supplied by Prof. G. A. D. Haslewood. $[\alpha]_D^{25} = +57 \pm 2°$ (*c* 1.02, dioxane). (Found: C 73.1; H 10.3. Calc. for $C_{24}H_{40}O_4$: C 73.4; H 10.3).

The material in the second peak (23 %) with an elution volume characteristic of chenodeoxycholic acid was crystallized from ethylacetate/light petroleum. M. p. 141—142°, undepressed by authentic chenodeoxycholic acid.

3α, 7β, 12α-trihydroxycholanic acid. Methylcholate was oxidized with N-bromosuccinimide [16] to methyl-7-ketodeoxycholate, purified *via* the diacetate [17] (m. p. 118—118.5°) and saponified. 7-Ketodeoxycholic acid was crystallized according to the directions given by Hoehn and Linsk [18]; m. p. 198—199°. The ketoacid was reduced as described above and the reaction product was separated by reversed phase partition chromatography on hydrophobic Super Cel. Aqueous methanol (50 % (v/v)) was used as the moving phase and *iso*octanol-chloroform (1:1) as stationary phase [19]. (Fig. 2.) The more hydrophilic compound (72 %), 3α,7β,12α-trihydroxycholanic acid (0.2 g), was dissolved in 3 ml methanol, neutralized with 0.1 N NaOH and added slowly to 100 ml of cold

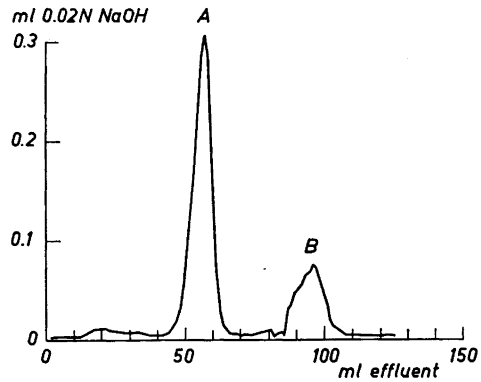

Fig. 2. Chromatography of an aliquot of the reaction product obtained by reduction of 7-ketodeoxycholic acid with sodium in *n*-propanol. Column: 4.5 g of hydrophobic Super Cel. Moving phase: 50 % (v/v) aqueous methanol. Stationary phase: *iso*octanol / chloroform (1:1). A: 3α,7β,12α-trihydroxycholanic acid. B: Cholic acid.

0.05 N HCl, with vigorous stirring. The precipitated acid crystallized after standing 12 h at +4°. 0.155 g (56 % yield calculated on 7-ketodeoxycholic acid) was obtained after filtration, washing with water and drying; m. p. 127—129°. The m. p. was not changed after repeated recrystallizations by this method. $[\alpha]_D^{25} = +62 \pm 2°$ (*c* 1.13, dioxane). (Found C 70.3; H 10.0. Calc. for $C_{24}H_{40}O_5$: C 70.5; H 9.9.)

A sample of this acid was crystallized from ethylacetate/heptane and dried at 130° at 0.001 mm Hg for 24 h; m. p. 157—158° (decomp.). $[\alpha]_D^{25} = +57 \pm 2°$ (*c* 1.06, dioxane). The elemental analysis indicated that the acid had formed a complex with heptane. (Found C 72.7; H 10.4. Calc. for $C_{24}H_{40}O_5 + C_7H_{16}$: C 73.2; H 11.1). Several other organic solvents were tried for recrystallization without success.

Methyl 3α,7β,12α-trihydroxycholanate-tri-p-nitrobenzoate. The method of Borsche [12] was followed. A solution of 75 mg of methyl 3α,7β,12α-trihydroxycholanate, prepared with diazomethane, was dissolved in 2 ml of dry pyridine and cooled to 0°. 180 mg of *p*-nitrobenzoylchloride in 2 ml of pyridine was added. After standing at room temperature for 12 h the reaction mixture was transferred to a mixture of ether, ethylacetate, ice and 2 N HCl. The ether layer was washed successively with 2 N HCl, 5 % aqueous sodium carbonate and water. After evaporation to dryness *in vacuo* the residue was crystallized from ethylacetate/ethanol to yield 121 mg of fine needles, m. p. 188—189°. The concentrated mother liquor gave an additional 24 mg of crystals with the same m. p. Total yield: 94 % $[\alpha]_D^{25} = +140 \pm 3°$ (*c* 1.19, dioxane). (Found C 63.5; H 6.0; N 4.8. Calc. for $C_{46}H_{51}O_{14}N_3$: C 63.5; H 5.9; N 4.8).

The second compound (B in Fig. 2) comprising about 28 % of the two epimers, was eluted at the place of cholic acid. It was crystallized from ethylacetate, m. p. 197—198°, undepressed by authentic cholic acid.

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