Thin-layer chromatographic separation of conjugates of ursodeoxycholic acid from those of litho-, chenodeoxy-, deoxy-, and cholic acids

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Summary Separation of the glycine and taurine conjugates of ursodeoxycholic acid from those of lithocholic acid, chenodeoxycholic acid, deoxycholic acid, and cholic acid by thin-layer chromatography is described. Thus, on running a silica gel G plate first in a solvent system of n-butanol–water 20:3 and then in a second solvent system of chloroform–isopropanol–acetic acid–water 30:30:4:1, all the above-mentioned conjugated bile acids are separated from one another. The application of this method to study the change in the biliary bile acid conjugation pattern in ursodeoxycholic acid-fed gallstone patients is described. —**Batta, A. K., S. Shefer, and G. Salen.** Thin-layer chromatographic separation of conjugates of ursodeoxycholic acid from those of litho-, chenodeoxy-, deoxy-, and cholic acids. *J. Lipid Res.* 1981. **22:** 712–714.

Supplementary key words conjugated bile acids

Ursodeoxycholic acid has gained considerable importance in recent years as a gallstone dissolving agent (1) and it has been found that when patients with gallstones are fed ursodeoxycholic acid they have large amounts of this acid in their bile (2). Inasmuch as the bile acids in the gallbladder bile are present in the form of conjugates with glycine and taurine, it is important to know the change in the conjugation pattern of these bile acids produced in the bile of gallstone patients who are fed ursodeoxycholic acid. Various solvent systems have been proposed for the partial or complete separation of conjugated bile acids by thin-layer chromatography (TLC) (3–6, and references therein) but there has been no report on the separation of the glycine and taurine conjugates of ursodeoxycholic acid from those of its isomeric dihydroxy bile acids, chenodeoxycholic acid and deoxycholic acid. We now describe a solvent system that separates conjugates of these three bile acids from one another. By making use of the difference in the relative mobilities of the various glycine- and taurine-conjugated bile acids due to changing the pH of the solvent system, we have been able to separate the glycine and taurine conjugates of lithocholic, chenodeoxycholic, deoxycholic, ursodeoxycholic, and cholic acids from one another on the same TLC plate.

Abbreviation: TLC, thin-layer chromatography.

MATERIALS AND METHODS

The glycine and taurine conjugates of lithocholic, chenodeoxycholic, deoxycholic, and cholic acids were purchased from Calbiochem, Los Angeles, CA. Ursodeoxycholic acid was purchased from Tokyo Tanabe Co., Tokyo, Japan and was purified by preparative TLC (benzene–dioxane 70:30 (v/v)) (7); the plate was developed twice in the solvent system. The glycine and taurine conjugates of ursodeoxycholic acid were prepared according to Tserng, Hachey, and Klein (8) as described for the preparation of the corresponding conjugates of chenodeoxycholic acid. The conjugated bile acids were dissolved in methanol and 2–4 μg was applied on a 0.25 mm thick silica gel G plate (Brinkmann, Westbury, NY). The plate was developed in the appropriate solvent system and the solvent was allowed to rise 5 cm from the starting line when the neutral solvent system containing butanol and water was used and 17–18 cm from the starting line when an acidic solvent system was used. Before a second development, the plate was dried with hot air. After the final development, the plate was sprayed with 20% sulfuric acid and phosphomolybdic acid (3.5% in isopropanol) (EM Laboratories, Westbury, NY) and heated at 110°C for 2 min. The spots thus obtained generally had a maximum diameter of 1 cm.

RESULTS AND DISCUSSION

Conjugates of the epimeric ursodeoxycholic and chenodeoxycholic acids can be completely separated from each other by developing the plate once in chloroform–isopropanol–acetic acid–water 30:30:4:1 (solvent system A_1 of Batta, Salen, and Shefer (3)). However, a second development in the solvent system is necessary when conjugated deoxycholic acid is also present. Since the mobilities of the glycine conjugates of these bile acids are quite high in this solvent system, the second development resulted in only a slight enhancement in the resolution of these conjugates. We subsequently found that, on reducing the polarity of the solvent system, the resolution was improved. **Fig. 1** shows the TLC separation of the glycine and taurine conjugates of these three bile acids by two developments in chloroform–isopropanol–acetic acid–water 60:60:3:0.75.

Although the latter solvent system is suitable for the separation of the conjugates of ursodeoxycholic, chenodeoxycholic, and deoxycholic acids, difficulty arises when other bile acid conjugates are present. Thus, the mobility of the biologically important glycocholic acid is very close to that of tauroursodeoxycholic

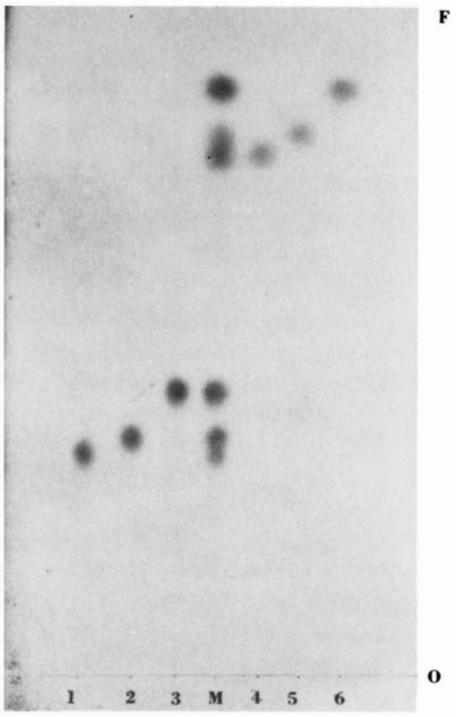

Fig. 1. TLC separation of glycine and taurine conjugates of ursodeoxycholic acid, chenodeoxycholic acid, and deoxycholic acid. Solvent system, chloroform–isopropanol–acetic acid–water 60:60:3:0.75, two developments. 1, Taurodeoxycholic acid; 2, taurochenodeoxycholic acid; 3, tauroursodeoxycholic acids; M, mixture of conjugated dihydroxy bile acids; 4, glycodeoxycholic acid; 5, glycochenodeoxycholic acid; 6, glycoursodeoxycholic acid; 0, origin; and F, solvent front.

acid, and on increasing the amount of acetic acid in the solvent system, it is incompletely separated from taurolithocholic acid. We have previously shown that the mobilities of the glycine conjugates are reduced markedly as compared to those of the corresponding taurine conjugates on increasing the pH of the solvent system from acidic to neutral (3) and this property of the two types of conjugates has now been used for the separation of glycocholic acid from tauroursodeoxycholic acid and taurolithocholic acid. Thus, on developing the plate in the neutral solvent system of n-butanol–water 20:3, and allowing the solvents to rise 5 cm from the starting line, glycocholic acid moved much slower (1 cm) compared to taurolithocholic acid (2.4 cm) and tauroursodeoxycholic acid (2.2 cm). When the plate was then developed twice in chloroform–isopropanol–acetic acid–water 30:30:4:1, glycocholic acid had a mobility intermediate between that of tauroursodeoxycholic acid and taurolithocholic acid. In this way, the glycine and the taurine conjugates of cholic, deoxycholic, chenodeoxycholic, ursodeoxycholic and lithocholic acids could be separated from one another; **Table 1** shows the R_f values. While developing the plate in n-butanol–water

TABLE 1. R_f values of conjugated bile acids

Compound	R_f	Compound	R_f
Taurocholic acid	0.18	Glycocholic acid	0.47
Taurodeoxycholic acid	0.34	Glycodeoxycholic acid	0.73
Taurochenodeoxycholic acid	0.37	Glycochenodeoxycholic acid	0.76
Tauroursodeoxycholic acid	0.43	Glycoursodeoxycholic acid	0.81
Taurolithocholic acid	0.53	Glycolithocholic acid	0.87

Solvent system: First development, n-butanol–water 20:3 (solvent front, 5 cm); second and third developments, chloroform–isopropanol–acetic acid–water 30:30:4:1 (solvent front, 17 cm).

system, it was noticed that if the solvents were allowed to rise higher than 5 cm, tauroursodeoxycholic acid moved close to glycocholic acid after the plate was developed in the acidic system; if the solvents were allowed to rise less than 5 cm, taurolithocholic acid did not move appreciably enough compared to glycocholic acid to allow an effective separation between the two. The application of this method to resolve and compare the bile acid components in the bile of patients with gallstones, before and after ursodeoxy-

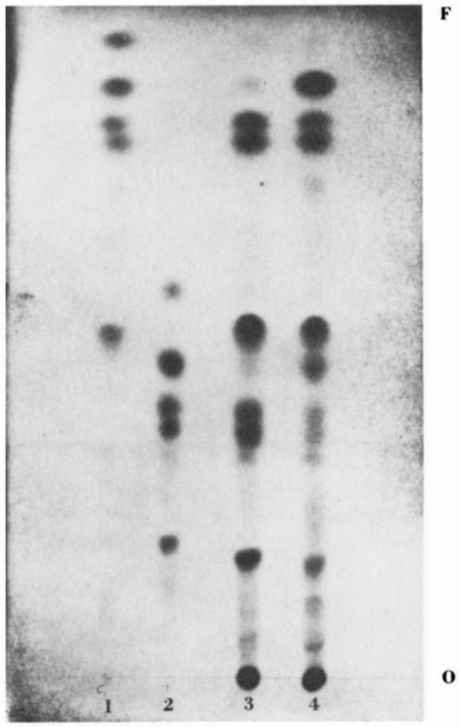

Fig. 2. TLC of the bile of a gallstone patient before and after feeding ursodeoxycholic acid. Solvent system: first development, n-butanol–water 20:3 (solvent front, 5 cm); second development, chloroform–isopropanol–acetic acid–water 30:30:4:1, two developments (solvent front, 17 cm). 1, Mixture of glycine conjugates of cholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, and lithocholic acid; 2, mixture of the corresponding taurine conjugated bile acids; 3, bile of gallstone patient; 4, bile of gallstone patient after feeding ursodeoxycholic acid.

cholic acid feeding is illustrated in **Fig. 2.** It is clear that before ursodeoxycholic acid feeding, only a trace amount of conjugated ursodeoxycholic acid is present in the bile, while after feeding this acid, glyco- and tauroursodeoxycholic acids appear in appreciable amounts in the bile.

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