Thin-layer chromatographic separation of sulfated and nonsulfated lithocholic acids and their glycine and taurine conjugates

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Summary A method for superior thin-layer chromatographic separation of lithocholic acid and its *N*-glycine and *N*-taurine conjugates, as well as their respective 3α-sulfates, is described. A solvent system of chloroform–methanol–acetic acid–water 65:24:15:9 (v/v) is used with air-dried plates of silicic acid containing calcium sulfate (10% by weight) under conditions of chamber saturation.

Supplementary key words bile acid sulfates · bile acids · conjugated bile acids

Lithocholic acid is the major bacterial metabolite of chenodeoxycholic acid in man, but little is known of its metabolism. Palmer (1) and Palmer and Bolt (2) have reported that lithocholic acid occurs in bile as glycine and taurine conjugates that are sulfated, in part, at the 3-hydroxy group. For our studies concerned with characterizing conjugation and sulfation of lithocholic acid during a single hepatic passage in man, we required a simple method of separating free lithocholic acid and its glycine and taurine conjugates as well as the respective ester sulfates of each of these species. We report here a thin-layer chromatographic system capable of separating these six compounds.

Method. Silica gel H for thin-layer chromatography (E. Merck, Darmstadt, Germany) was mixed with calcium sulfate for thin-layer chromatography (Mallinckrodt Chemical Works, St. Louis, Mo.) 9 parts to 1 (w/w). Thin-layer plates were prepared from an aqueous slurry using a commercial spreader. Plates were dried in air in a 37°C room for 24 hr and were not heat activated. Chromatography tanks were lined with filter paper to ensure vapor saturation before chromatograms were developed. For qualitative detection, phosphomolybdic acid, 10% (v/v) in ethanol, was used.

Lithocholic acid (Mann Research Laboratories, New York) was conjugated with glycine or taurine as described by Norman (3). Lithocholylglycine was purified by adsorption chromatography of the methyl ester on silicic acid as described earlier (4), and lithocholyltaurine was purified by recrystallization from ethanol–ethyl acetate. The pure conjugates were sulfated, as described by Palmer and Bolt (2), and purified.

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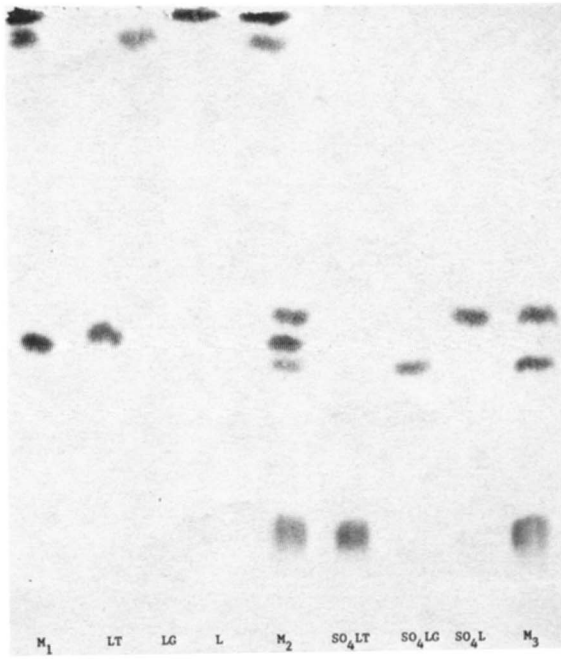

Fig. 1. Thin-layer chromatographic separation of lithocholic acid derivatives (10 μg applied; development time, 1 hr; detection with phosphomolybdic acid). From left to right: M_1, mixture of unsulfated derivatives (from bottom to top, lithocholyltaurine, lithocholylglycine, and lithocholic acid); LT, lithocholyltaurine; LG, lithocholylglycine; L, lithocholic acid; M_2, mixture of entire series (from bottom to top, sulfolithocholyltaurine, sulfolithocholylglycine, lithocholyltaurine, sulfolithocholic acid, lithocholylglycine, and lithocholic acid); SO_4LT, sulfolithocholyltaurine; SO_4LG, sulfolithocholylglycine; SO_4L, sulfolithocholic acid; and M_3, mixture of sulfate esters (from bottom to top, SO_4LT, SO_4LG, and SO_4L).

Results and discussion. This solvent system resolves unconjugated lithocholic acid, its glycine and taurine conjugates, and their respective sulfate esters (**Fig. 1** and **Table 1**). In addition, it offers a clean separation of the

TABLE 1. R_f values of lithocholic acid, its derivatives, and other major bile acids of human bile

	R_f
Lithocholic acid series	
Lithocholic acid	0.99
Lithocholylglycine	0.94
Sulfolithocholic acid	0.48
Lithocholyltaurine	0.44
Sulfolithocholylglycine	0.41
Sulfolithocholyltaurine	0.17
Others	
Deoxycholylglycine	0.84
Chenodeoxycholylglycine	0.83
Cholylglycine	0.65
Deoxycholyltaurine	0.38
Chenodeoxycholyltaurine	0.37
Cholyltaurine	0.31

Solvent system, chloroform–methanol–acetic acid–water 65:24:15:9 (v/v); adsorbent, silica gel H containing 10% calcium sulfate by weight.

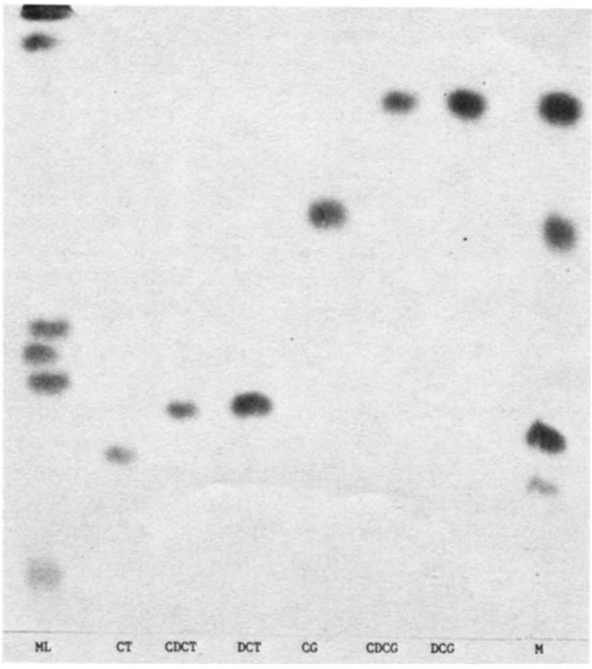

Fig. 2. Thin-layer chromatographic separation of major conjugated bile acids occurring in human bile, as well as lithocholic acid derivatives. Chromatographic conditions as in Fig. 1. ML, mixture of lithocholic acid series (same as M_2 in Fig. 1); CT, cholyltaurine; CDCT, chenodeoxycholyltaurine; DCT, deoxycholyltaurine; CG, cholylglycine; CDCG, chenodeoxycholylglycine; DCG, deoxycholylglycine; and M, mixture (from bottom to top, CT, CDCT and DCT, CG, and CDCG and DCG).

major bile acid classes of human bile from each other and from these lithocholic acid derivatives (**Fig. 2** and Table 1). In our hands, it provides superior resolution of the three lithocholic acid derivatives with intermediate mobility (sulfolithocholic acid, sulfolithocholylglycine, and lithocholyltaurine) compared with the butanol 1 system of Palmer and Bolt (2) and Gänshirt, Koss, and Morianz (5).

The system has been useful for the separation of isotopically labeled metabolites in bile after the administration of labeled lithocholic acid or its derivatives (6). The pattern of separation differs from that observed for the butanol 3 system of Palmer and Bolt (2), so that the system described here provides complementary information when chromatographic behavior is used to infer radiopurity (**Fig. 3**).

A disadvantage of the system is its limited capacity; for preparative isolation, either nonaqueous gel permeation chromatography (7) or high-speed adsorption liquid chromatography is likely to be preferable.

Dr. Robert H. Palmer and Dr. Paul J. Thomas made helpful suggestions.

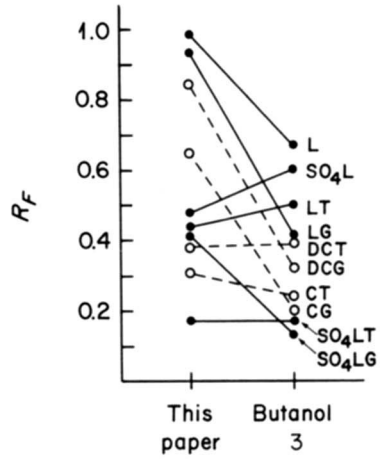

Fig. 3. Chromatographic mobility of lithocholic acid derivatives in the solvent system described in this paper (*left*) compared with that using the butanol 3 system of Palmer and Bolt (2) (*right*). Abbreviations as in Fig. 1.

This investigation was supported in part by Research Grant AM-16770 from the National Institutes of Health, U.S. Public Health Service, and grants-in-aid from the Share Foundation, Mead Johnson & Company, and Eli Lilly and Company.

Manuscript received 20 August 1974; accepted 12 November 1974.

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