Journal of Chromatography, 172 (1979) 450–452

CHROM. 11,616

Note

Gas chromatographic procedure for the measurement of bile acids in rat intestine

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(First received September 5th, 1978; revised manuscript received November 16th, 1978)

In rat liver, chenodeoxycholic acid is metabolized to α- and β-muricholic acids, which are secreted in the bile*. In the colon some bile acids are metabolized to hyodeoxycholic acid and ω-muricholic acid[1], both of which are excreted in considerable amounts in rat faeces. In order to study the metabolism of bile acids in the rat it is necessary to identify and estimate these bile acids separately.

The separation of bile acid methyl ester acetates of lithocholic, deoxycholic, chenodeoxycholic, cholic and hyodeoxycholic acid has been described[2]. The bile acids are eluted in that sequence when 2.25% SE-30 silicone rubber gum on Chromosorb W (60–80 mesh) is used, the less polar hyodeoxycholic acid being retained more strongly than cholic acid. Neither OV-17 nor OV-225 separates the muricholic acid methyl ester acetates satisfactorily. When OV-17 is used ω- and β-muricholic acid methyl ester acetates are not adequately separated (peak resolution: 0.4); and when OV-225 is used α- and ω-muricholic acid methyl ester acetates are not adequately separated (peak resolution: 0.5).

Bile acid trimethylsilyl (TMS) ethers have been separated on Hi-eff-8B[3], but α-muricholic and cholic acids are not resolved in this system. The separation of the TMS ethers of cholic and α-, β- and ω-muricholic acids on OV-17 is described[4], but chenodeoxycholic and deoxycholic acids are not resolved in this system. It has been reported[5] that TMS ether derivatives are not stable and that this can lead to multiple peaks from individual trihydroxy bile acids. In this paper a procedure is described which separates the following bile acids in sequence: lithocholic, deoxycholic, chenodeoxycholic, cholic, hyodeoxycholic, hyocholic, α-muricholic, ω-muricholic, and β-muricholic acids.

* Common and systematic names of compounds mentioned in the text are as follows: nordeoxycholic acid, 23-nor-5β-cholanoic acid-3α,12α-diol; lithocholic acid, 5β-cholanoic acid-3α-ol; deoxycholic acid, 5β-cholanoic acid-3α,12α-diol; chenodeoxycholic acid, 5β-cholanoic acid-3α,7α-diol; ursodeoxycholic acid, 5β-cholanoic acid-3α,7β-diol; hyodeoxycholic acid, 5β-cholanoic acid-3α,6α-diol; cholic acid, 5β-cholanoic acid-3α,7α,12α-triol; hyocholic acid, 5β-cholanoic acid-3α,6α,7α-triol; α-muricholic acid, 5β-cholanoic acid-3α,6β,7α-triol; β-muricholic acid, 5β-cholanoic acid-3α,6β,7β-triol; ω-muricholic acid, 5β-cholanoic acid-3α,6α,7β-triol.

MATERIALS AND METHODS

Cholic, chenodeoxycholic, deoxycholic, lithocholic and 23-nordeoxycholic acids were obtained from Maybridge Chemical Co., Tintagel, Great Britain. Hyodeoxycholic, hyocholic, ursodeoxycholic and 3α,6β-dihydroxy-5β-cholanoic acids were obtained from Steraloids, Wilton, N.H., U.S.A. The α- and β-muricholic acid methyl esters were a generous gift from Professor H. Eyssen, Rega Institute for Medical Research, Leuven, Belgium. ω-Muricholic acid methyl ester was prepared from rat colon by thin-layer chromatography (TLC)[6] and identified by gas–liquid chromatography (GLC) as a TMS ether on OV-17[4] and SE-30[6].

The small-intestinal and colonic contents of rat were washed out with distilled water, freeze-dried, and weighed. Bile acids and salts were extracted, hydrolysed, and partially purified according to Evrard and Janssen[7]. Following extraction with diethyl ether, bile acids were methylated using a dimethoxypropane–methanol–conc. HCl mixture (50:20:1). It was necessary to further purify methyl esters of colonic bile acids by TLC. Methyl ester acetates were then formed by a modification of the method of Roovers *et al.*[8]. The methyl esters were acetylated for 2 h at 0° with 1 ml of a mixture of acetic acid–acetic anhydride–70% $HClO_4$. (700:500:1). At the end of this period, 10 ml of a 20% (w/v) solution of NaCl in water was added, and the methyl ester acetates were extracted with three portions of diethyl ether. The pooled extracts were washed with distilled water and then taken to dryness.

Separation of the methyl ester acetate derivatives was carried out on a Pye Unicam Series 104 dual-column chromatograph, equipped with hydrogen flame ionisation detectors. Five-ft. columns were silanized, and packed with 3% SE-30 on 100–120 mesh Supelcoport (Supelco, Bellefonte, Pa., U.S.A.). Nitrogen (oxygen free) was used as carrier gas at a flow-rate of 35 ml/min, and inlet pressure 206 kPa (30 p.s.i.). The operating temperature for column and detector was 262°, and the inlet temperature was 280°. The internal standard was 23-nordeoxycholic acid.

TABLE I

RELATIVE RETENTION TIMES OF BILE ACID METHYL ESTER ACETATES ON 3% SE-30 AT 262°

Peak no.	*5β-Cholanoic acid*	*Retention time relative to 23-nordeoxycholic acid*
1	23-nor-3α,12α-diol	1.00
2	3α-ol	1.06
3	3α,12α-diol	1.34
4	3α,7α-diol	1.55
5	3α,7α,12α-triol	1.69
6	3α,6α-diol	1.89
7	3α,6β-diol	1.97
7	3α,7β-diol	1.97
—	3α,6α,7α-triol	2.28
8	3α,6β,7α-triol	2.41
9	3α,6α,7β-triol	2.82
10	3α,6β,7β-triol	3.11

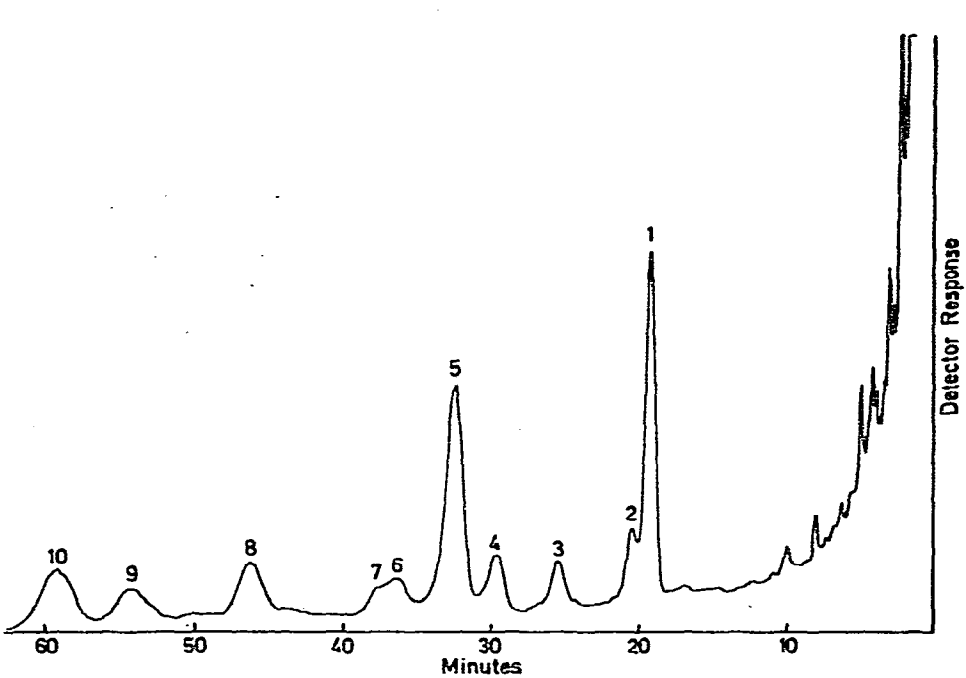

Fig. 1. GLC separation of the bile acid methyl ester acetates in rat intestine. Column, 3% SE-30 on 100–120 mesh Supelcoport; temperature, 262°. For peak identification see Table I.

RESULTS

The retention times of the methyl ester acetate derivatives of bile acids relative to 23-nordeoxycholic acid are shown in Table I.

A gas chromatographic recording of total intestinal bile acid methyl ester acetates is shown in Fig. 1.

ACKNOWLEDGEMENTS

We thank the World Health Organisation for assistance with a Fellowship (K.T.), and the Incorporated National Association of British and Irish Millers Ltd. for financial assistance.

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