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Short Communication

Trifluoroacetylation of muricholic acids and hyocholic acids

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ABSTRACT

Trifluoroacetylation of α-muricholic acid and α- and β-hyocholic acids is incomplete under routine conditions (at room temperature with trifluoroacetic anhydride for 30 min), whereas β-muricholic acid reacts completely. Complete trifluoroacetylation of these bile acids was achieved by reaction at room temperature for 16–24 h. Trifluoroacetylation of α-muricholic acid at room temperature for 48 h or at a higher temperature (50°C) for 0.5–1.0 h led to another unknown peak.

INTRODUCTION[a]

Methyl ester trimethylsilyl ethers of bile acids are popular derivatives for their determination by gas–liquid chromatography. However, it is well known that the derivatives tend to make a flame ionization detector dirty in a short period.

Trifluoroacetylation of a hydroxyl group is also frequently adopted in the analysis of bile acids by gas–liquid chromatography [2–7]. Usually, an excess amount of trifluoroacetic anhydride is added to bile acid methyl esters and left for 15–30 min or more at room temperature [4]. Most of the bile acids are quantitatively trifluoroacetylated by this procedures, but α-muricholic acid and α- and β-hyocholic acids are not, as described by Sjövall [8] and Mott *et al.* [9].

We describe here a method for the trifluoroacetylation of these bile acids.

EXPERIMENTAL

The α- and β-muricholic acids and β-hyocholic acid were kindly supplied by Dr. Isao

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[a] Nomenclature for bile acids was based on a recent proposal by Hofmann *et al.* [1]. In this paper the following trivial names are used: α-muricholic acid, 3α,6β,7α-trihydroxy-5β-cholanoic acid; β-muricholic acid, 3α,6β,7β-trihydroxy-5β-cholanoic acid; β-hyocholic acid, 3α,6α,7β-trihydroxy-5β-cholanoic acid; cholic acid, 3α,7α,12α-trihydroxy-5β-cholanoic acid; 7-epicholic acid, 3α,7β,12α-trihydroxy-5β-cholanoic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid; chenodeoxycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; ursodeoxycholic acid, 3α,7β-dihydroxy-5β-cholanoic acid; hyodeoxycholic acid, 3α,6α-dihydroxy-5β-cholanoic acid; lithocholic acid, 3α-hydroxy-5β-cholanoic acid.

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Horibe (Shionogi Research Laboratories, Osaka, Japan). α-Hyocholic acid was purchased from Calbiochem-Behring (San Diego, CA, USA). Trifluoroacetic anhydride was obtained from Wako (Osaka, Japan).

Bile acids were dissolved in small amounts of methanol and methylated with freshly prepared diazomethane. The reaction mixture was evaporated to dryness under a stream of nitrogen and reduced pressure. Then, 0.3 ml of trifluoroacetic anhydride was added to the bile acid methyl esters. The test-tubes containing the reaction mixtures were capped and left for various periods (0.5–48 h) at room temperature, 0°C or 50°C. The reaction mixture was evaporated to dryness under a stream of nitrogen and reduced pressure.

The bile acid derivatives were dissolved in carbon disulphide–methyl ethyl ketone (1:1, v/v) and analysed by gas–liquid chromatography using a Hewlett-Packard (Avondale, PA, USA) HP 5890A gas chromatograph equipped with a hydrogen flame ionization detector. A 15 m × 0.25 mm I.D. capillary column coated with DB-17 (J & W Scientific, Folsom, CA, USA) was used. The operating conditions were as follows: oven temperature, increased from 200°C (held for 1 min) to 280°C at 7.5°C/min; detector temperature, 300°C; injection port temperature, 280°C; carrier gas, helium at 25 cm/s; splitting ratio, 1:50; data processor, HP 3393A; automatic sampler, HP 7673A.

RESULTS

Fig. 1 shows the chromatograms of methyl α-muricholate (Me-αMC) trifluoroacetylated for 0.5 h and 16 h at room temperature, Me-αMC trifluoroacetylated for 0.5 h gave two major peaks (5.48 and 10.27 min) and one minor peak (10.90 min), while the bile acid trifluoroacetylated for 16 h gave only one peak at 5.48 min.

Table I shows the effects of reaction temperature and reaction time. When Me-αMC was trifluoroacetylated for a shorter duration or at a lower temperature, peaks with longer retention times (10.27 and 10.90 min) appeared, but they disappeared when the trifluoroacetylation reaction was performed for 16–25 h at room temperature. However, when the reaction was performed for 48 h at room temperature or 0.5–1 h at 50°C, another minor peak appeared with a retention time of 5.80 min.

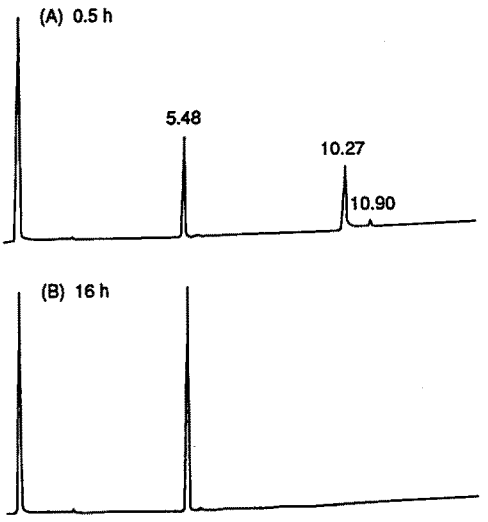

Fig. 1. Gas–liquid chromatograms of methyl α-muricholate after trifluoroacetylation with trifluoroacetic anhydride at room temperature for (A) 0.5 h and (B) 16 h. Numbers at peaks indicate retention times in min.

GC–MS analysis revealed that the peaks with retention times of 5.48, 10.27 and 10.90 min

TABLE I

EFFECTS OF REACTION TEMPERATURE AND REACTION TIME ON TRIFLUOROACETYLATION OF METHYL α-MURICHOLIC ACID

Results given are extent of trifluoroacetylation (%).

Temperature (°C)	Reaction time (h)	Retention time (min)			
		5.48	5.80	10.27	10.90
0	2	23.7	nd[a]	64.3	11.9
Room temp.	0.5	39.7	nd	49.5	10.7
Room temp.	1	58.3	nd	36.5	5.1
Room temp.	16	100.0	nd	nd	nd
Room temp.	24	100.0	nd	nd	nd
Room temp.	48	98.5	1.5	nd	nd
50	0.5	93.8	1.5	4.8	nd
50	1	98.3	1.7	nd	nd

[a] nd = Not detectable.

were tri-, di- and monotrifluoroacetylated Me-αMC, respectively. The peak at 5.80 min could not be identified.

Table II shows the effect of the reaction time on trifluoroacetylation of Me-αMC, Me-βMC, methyl α-hyocholate (Me-αHC) and methyl β-hyocholate (Me-βHC). In this experiment, Me-αMC gave only two peaks after reaction for 0.5 h at room temperature, but it gave one peak (5.48 min) after reaction for 16 h. Me-βMC showed one peak after reaction for both 0.5 and 16 h. Me-αHC gave three peaks and Me-βHC two peaks after a 0.5-h reaction, but both of them gave one peak after a 16-h reaction.

Table II shows the results of the analysis of these bile acids. When 0.1 μg of each bile acid was applied to the column, the peak area was *ca.* 20 000 (arbitrary units) for each bile acid, as shown by Jones *et al.* [10], after reaction for 16 h, suggesting that the reaction had proceeded quantitatively under this condition. In contrast, the peak areas of Me-αMC, Me-αHC and Me-βHC after a 0.5-h reaction were much lower than those after a 16-h reaction, even when the peak areas at 0.5 h were combined for each bile acid.

Although the experiments were carried out at one time in each instance, the data obtained were reasonable among the compared group, hence the data in Tables I and II are thought to be reliable.

TABLE II

PEAK AREAS OF α- AND β-MURICHOLIC ACIDS AND α- AND β-HYOCHOLIC ACID AFTER TRIFLUOROACETYLATION FOR 0.5 AND 16 h AT ROOM TEMPERATURE

Results are peak areas (in arbitrary units) corresponding to 0.1 μg of each bile acid, and in parentheses are the percentages of the values after a 16-h reaction.

Compound[a]	Peak retention times (min)	Reaction time (h)	
		0.5	16
Me-αMC	5.48	9290 (45.6%)	20 356
	10.27	4180 (20.5%)	nd[b]
Me-βMC	7.07	20 472 (105.1%)	19 485
Me-αHC	7.30	4948 (25.2%)	19 671
	9.81	2791 (14.2%)	nd
	11.20	5016 (25.5%)	nd
Me-βHC	7.44	3198 (16.6%)	19 232
	9.83	827 (4.3%)	nd

[a] MCA = Muricholic acid; HCA = hyocholic acid.
[b] nd = Not detectable.

DISCUSSION

The trifluoroacetylation of a hydroxyl group with trifluoroacetic anhydride is considered to proceed quantitatively within 30 min at room temperature [4], and this is true for most of the bile acids such as cholic, 7-epicholic, deoxycholic, chenodeoxycholic, ursodeoxycholic, hyodeoxycholic and lithocholic acids. However, the bile acids possessing hydroxyl groups at the C-3, C-6 and C-7 positions were found to require reaction times of over 16 h at room temperature to complete the reaction quantitatively. Me-αMC, Me-αHC and Me-βHC are the bile acids requiring a longer reaction time, while Me-βMC and methyl cholate and chenodeoxycholate are easily trifluoroacetylated. Therefore, the hydroxyl group at the 6α- or 7α(β)-position is considered to inhibit trifluoroacetylation, probably owing to steric hindrance by the previously formed trifluoroacetyl group at the 6α- or 7α(β)-position.

Mott *et al.* [9] reported the complete trifluoroacetylation of α-HCA at 80°C for 30 min but not at 35°C for 20 min. In our case, when α-MCA was treated with trifluoroacetic anhydride, trifluoroacetylation at 50°C for 30 min or at room temperature for 48 h led to another unknown peak, although it was minor (less than 2%). Therefore, the trifluoroacetylation of bile acids, including α-MCA, with trifluoroacetic anhydride should be performed at room temperature for 16–24 h.

These findings can be explained as follows. We assumed that the rate-determining step of these reactions is not the second trifluoroacetylation but the third. Further, the second trifluoroacetylation of trihydroxy bile acids would occur at the 6-position because 3,6-ditrifluoroacetates are sterically more stable than the corresponding 3,7-ditrifluoroacetates. On trifluoroacetylation, the direction of the lone pair orbitals of the

oxygen atom in the hydroxyl group on the bile acids is closely involved in the reactivity as the reagent attacks the lone pair of electrons in these orbitals. In ditrifluoroacetylates of bile acids, except for that of α-MCA, the carbonyl oxygen in the trifluoroacetyl group introduced would form a hydrogen bond with the proton in the adjacent hydroxyl group, which is the third reaction centre, so that the direction of the lone pair orbitals of the oxygen atom in this group is defined.

Molecular orbital calculation by the PM3 method in MOPAC (version 5.00) (QCPE No. 455) and molecular modelling suggest that the direction of the lone pair orbitals in the ditrifluoroacetylate of β-MCA is the most favourable to the reaction, *i.e.*, no steric hindrance for the approach of the reagent, that of α-HCA is sterically unfavourable as the lone pair orbitals comes under the B-ring, and that of β-HCA is sterically most unfavourable at the orbitals are directed to the C-15 atom so that it is difficult for the reagent to approach the reaction centre. On the other hand, with α-MCA, such hydrogen bonds cannot be formed because the 6- and 7-substituents are in opposite directions. Therefore, the reactivity on trifluoroacetylation of the 3,6,7-trihydroxy bile acids increases in order β-MCA, α-MCA, α-HCA and β-HCA.

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