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Note

Gas chromatography of ether glucuronides as methyl-trifluoroacetyl derivatives

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Glucuronic acid conjugation is a major metabolic route for hydroxyl-containing compounds[1]. Gas chromatography (GC) of glucuronides has in most cases been performed indirectly after enzymatic or chemical hydrolysis. GC of intact glucuronides has been reported after conversion to methyl-trimethylsilyl[2–4], methyl-acetyl[2,5,6], pertrimethylsilyl[7] and permethyl[8] derivatives. Structure confirmation of some of these derivatives was carried out by mass spectrometry (MS)[4,6–8].

Perfluoroacylation has been used for the derivatization of a variety of functional groups for GC analysis (*e.g.*, refs. 9–14). The perfluoroacyl derivatives have high volatility and hydrophobic character, making them eminently suitable for GC work.

The purpose of this investigation was to study the GC and MS properties of ether-type glucuronides after methylation of the carboxylic and trifluoroacetylation of the alcoholic groups. The β-D-glucuronic acid conjugates of 1-naphthol and androsterone were used as model compounds.

EXPERIMENTAL

Gas chromatography

The gas chromatograph was a Varian 1440 equipped with a flame ionization detector. The columns were made of glass (6 ft. × 2 mm I.D.) and packed with 2% OV-17 on 80–100 mesh Chromosorb W HP and 1% OV-1 on silanized 60–80 mesh Chromosorb W. The nitrogen flow-rate was 60–80 ml/min.

Gas chromatography–mass spectrometry

The MS analysis was carried out on an LKB 9000S instrument using an ionizing electron energy of 20 eV. The GC column was made of glass (3 ft. × 1.5 mm I.D.) and packed with 1% OV-1 on silanized 60–80 mesh Chromosorb W. The helium flow-rate was 10 ml/min.

Chemicals

Ethyl acetate, methanol and acetone (analytical grade) were obtained from Fisher Scientific, Pittsburgh, Pa., U.S.A. Diazomethane in diethyl ether was prepared

from N-methyl-N'-nitro-N-nitrosoguanidine (Aldrich, Milwaukee, Wisc., U.S.A.)[15]. Trifluoroacetic anhydride (99% purity) was obtained from Pierce, Rockford, Ill., U.S.A. 5α-Androstane-3α-ol-17-one-β-D-glucuronic acid (androsterone glucuronide) and 1-naphthol-β-D-glucuronic acid (1-naphthol glucuronide) were purchased from Sigma, St. Louis, Mo., U.S.A. An equimolar amount of hydrochloric acid was added to the 1-naphthol glucuronide (sodium salt) before methylation.

Derivatization procedure

Diazomethane in diethyl ether was added to 100 μl of the glucuronide in methanol (1 mg/ml) until a permanent color was obtained. After a reaction time of 15 min at 20°, the solution was evaporated to dryness under nitrogen at 50° and the residue mixed with 200 μl of ethyl acetate and 25 μl of trifluoroacetic anhydride. The mixture was shaken vigorously using a Vortex mixer for 15 min at 20°.

RESULTS AND DISCUSSION

Reaction conditions

The methylation was performed in methanol with diazomethane in diethyl ether as reagent. The trifluoroacetylation was studied in ethyl acetate and acetone and was complete in both solvents within 15 min (20°). The derivatives were stable for at least 24 h in the final reaction mixture (20°). Removal of the trifluoroacetic anhydride by evaporation or solvent extraction resulted in rapid decomposition of the derivatives.

Gas chromatographic properties

The relative retention of the methyl-trifluoroacetyl derivative of 1-naphthol glucuronide to underivatized 1-naphthol on OV-1 and OV-17 is given in Table I. The derivative has a high volatility with little difference in retention compared with the parent compound, especially on OV-17. The derivatives give symmetrical peaks even on a column with a low percentage of a non-polar stationary phase (Fig. 1).

TABLE I

RELATIVE RETENTION OF THE METHYL-TRIFLUOROACETYL DERIVATIVE OF 1-NAPHTHOL GLUCURONIDE ON OV-1 AND OV-17

Compound	$t_{rel.}$ *(OV-1)**	$t_{rel.}$ *(OV-17)***
1-Naphthol	1.0	1.0
Methyl-trifluoroacetyl derivative of 1-naphthol glucuronide	20.5	6.0

* Column temperature, 170°; nitrogen flow-rate, 60 ml/min; retention time for 1-naphthol, 0.3 min.

** Column temperature, 200°; nitrogen flow-rate, 60 ml/min; retention time for 1-naphthol, 0.4 min.

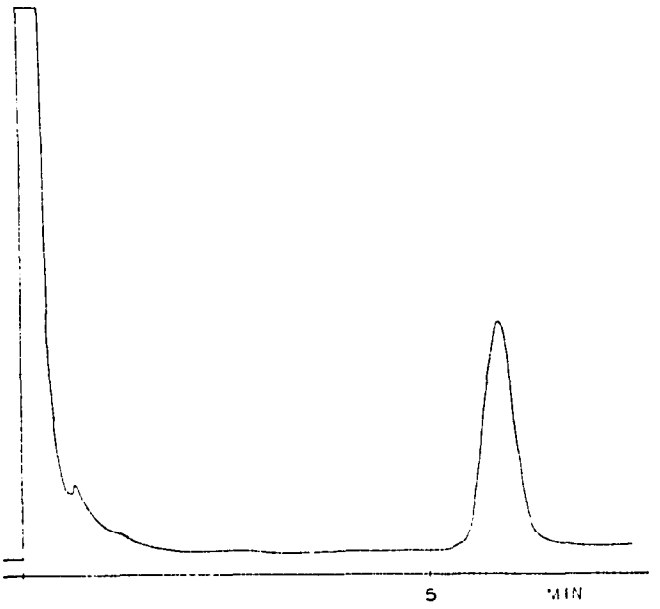

Fig. 1. Gas chromatography of the methyl-trifluoroacetyl derivative of androsterone glucuronide. Column, 1% OV-1. Column temperature, 250°. Injector temperature, 280°. Detector temperature, 250°. Nitrogen flow-rate, 80 ml/min. Amount of derivative injected, 1 μg.

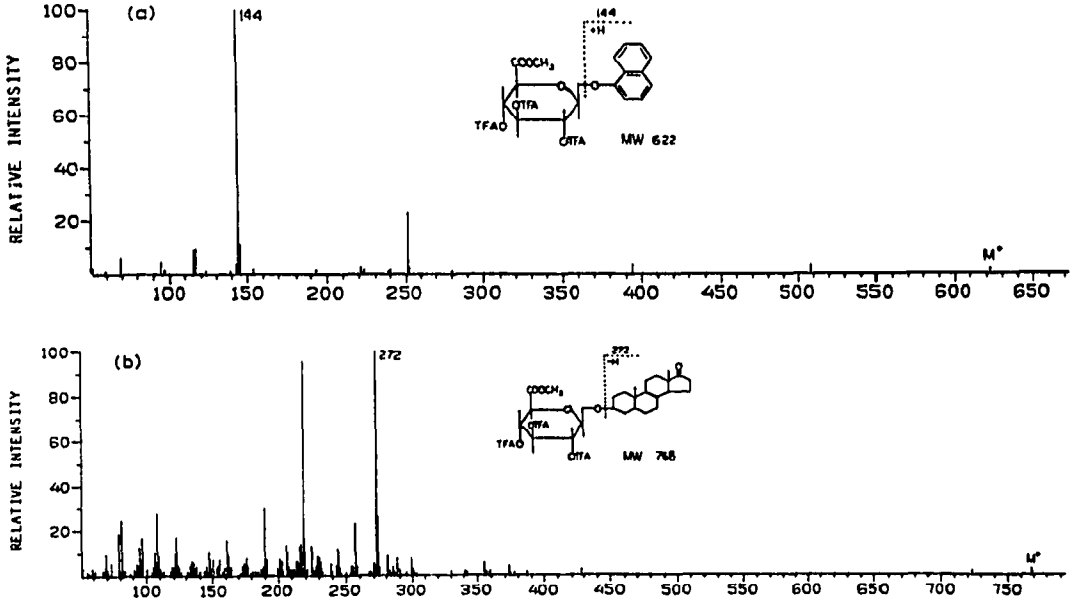

Fig. 2. Mass spectra of the methyl-trifluoroacetyl derivatives of (a) 1-naphthol glucuronide and (b) androsterone glucuronide. Ions with relative intensity <1% of the base peak are excluded.

Mass spectrometry

The mass spectra of the methyl-trifluoroacetyl derivatives of 1-naphthol and androsterone glucuronides are given in Fig. 2. The derivatives exhibit rather intense molecular ions (1.7 and 2.7% of the base peaks). Both spectra contain major fragments formed by cleavage of the exocyclic acetal bond with charge retention on the aglycone moiety. The origin of the base peaks is indicated in Fig. 2 and is similar to findings for methyl-trimethylsilyl[4] and methyl-acetyl[6] derivatives of glucuronides and trifluoroacetyl[16] derivatives of glycosides.

The principle outlined above for GC–MS of glucuronides has been applied successfully to the identification of glucuronide metabolites of propanolol, a beta-adrenoceptor-blocking drug. These results will be published elsewhere.

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