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ANALYTICAL AND PREPARATIVE GAS–LIQUID CHROMATOGRAPHY OF THE FIFTEEN METHYL ETHERS OF METHYL α-D-GALACTOPYRANOSIDE

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SUMMARY

By combining chromatography on silica gel columns with preparative gas–liquid chromatography, the 15 methyl ethers of methyl α-D-galactopyranoside were isolated from the mixture of all derivatives obtained by partial methylation of methyl α-D-galactopyranoside. Methyl ethers were identified by mass spectrometry of their peracetyl glycitol derivatives.

INTRODUCTION

The partial methylation of methyl glycosides is a satisfactory source of monosaccharide methyl ethers and several procedures have been described for preparing methyl derivatives of various monosaccharides which are more rapid and easier than specific organic syntheses. Using this method, some methyl ethers of methyl α-D-mannopyranoside were prepared by Handa and Montgomery[1]. Allen *et al.*[2] described the preparation of all methyl ethers of methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside and their isolation by preparative chromatography on Dowex 1 resin. Using preparative gas–liquid chromatography (GLC) in the manner described by Ovodov and Evtushenko[3,4] for the separation of methyl β-D-xylopyranoside methyl ethers, we were able to isolate in a pure state the 15 methyl ethers of methyl α-D-mannopyranoside[5–8]. In this paper, we describe the preparation and identification of the 15 methyl ethers of methyl α-D-galactopyranoside.

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MATERIALS AND METHODS

Partial methylation of methyl α-D-galactopyranoside

To a cooled (0°), stirred solution of methyl α-D-galactopyranoside (8 g) (K & K Labs., Plainview, N.Y., U.S.A.) in N,N-dimethylformamide (144 ml) (Merck, Darmstadt, G.F.R.) were added, in small portions, methyl iodide (8 ml) (Prolabo, Paris, France) and silver oxide (16 g), freshly prepared according to Whistler and Wolfrom[9]. After 7 h at 20°, the mixture was filtered and the insoluble material was carefully washed on the filter with chloroform. The subsequent experimental procedures followed were the same as previously described[6]. An aqueous phase containing mono-, di- and trimethyl ethers of methyl α-D-galactopyranoside and an organic phase containing tri- and tetramethyl ethers of methyl α-D-galactopyranoside were obtained. The two phases were evaporated to dryness.

Preparative chromatography of the aqueous phase on a silica gel column

The methyl ethers of methyl α-D-galactopyranoside contained in the aqueous phase were first separated into mono-, di- and trimethyl fractions by preparative chromatography on a silica gel column as follows. The concentrated aqueous phase (500 mg) dissolved in 5 ml of chloroform–methanol (9:1) was chromatographed on a 60 × 3 cm column of silica gel (Kieselgel, 0.05–0.2 mm, highest purity, 70–325 mesh; Merck) using chloroform–methanol (9:1) as the solvent. The methylated derivatives were located in the eluate using phenol–sulphuric acid reagent[10]. Three fractions (K-1, K-2 and K-3) were isolated and fractionated further by GLC.

Gas–liquid chromatography

Analytical chromatography. The organic phase, the aqueous phase and fractions K-1, K-2 and K-3 were analysed by GLC under the following conditions: Varian-Aerograph apparatus fitted with a flame-ionization detector; glass column (300 × 0.3 cm) packed with Chromosorb W HMDS (100–120 mesh) containing 3% Carbowax 6000; column temperature, 195°; injector temperature, 210°; detector temperature, 200°; flow-rate of carrier gas (nitrogen), 20 ml/min. Retention times are quoted relative to methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside.

Preparative chromatography. The methyl ethers of methyl α-D-galactopyranoside were isolated from the organic phase, the aqueous phase and fractions K-1, K-2 and K-3 by preparative GLC under the following conditions: Varian-Aerograph Model 705 apparatus (Autoprep type); metal column (600 × 0.92 cm) packed with Chromosorb W (80–100 mesh) containing 3% Carbowax 6000; column temperature, 195°; injector temperature, 210°; detector temperature, 200°; collector temperature, 135°; flow-rate of carrier gas (nitrogen), 220 ml/min (20 ml/min in the direction of the detector and 200 ml/min in the direction of the collector); sensitivity, 10/8; volume injected manually or automatically at each operation, 125 μl of an 8% (w/v) solution of the methyl ethers in methanol.

Under the above conditions, the separation of 2,3,6-, 2,4,6- and 3,4,6-tri-O-methyl, 2,4- and 3,4-di-O-methyl and 2- and 3-mono-O-methyl derivatives was not sharp and the compounds were collected as mixtures. The mixed fractions were separated as the acetate derivatives by the following procedure. Each methylated ether fraction (5 mg), vacuum-dried over phosphorus pentoxide, was added to a solution

containing pyridine and acetic anhydride in equal proportions (1 ml) and kept in the dark for 15 h at room temperature. The acetyl derivatives were fractionated by preparative GLC under the following conditions: Varian-Aerograph Model 705 apparatus (Autoprep type); metal column (600 × 0.92 cm) packed with Chromosorb W HMDS (100–120 mesh) containing 3% butane-1,4-diol succinate polyester (BDSP); column temperature, 185°; injector temperature, 210°; detector temperature, 200°; flow-rate of carrier gas (nitrogen), 220 ml/min (20 ml/min in the direction of the detector and 200 ml/min in the direction of the collector); volume injected, 80 μl of a 4% (w/v) solution of the acetylated derivatives in methanol. The deacetylation of the acetate derivatives (1 mg) was carried out at 20–22° for 60 min in acetone solution (0.5 ml) to which was added 0.1 *N* sodium hydroxide (2 ml)[11]. The solution was neutralized with 0.1 *N* hydrochloric acid and desalted by passing through columns (10 × 1 cm) of cation exchanger (Dowex 50, H^+) and anion exchanger (Duolite A-102 D, $HCOO^-$). The neutral effluent and water washings were evaporated to dryness under reduced pressure.

Mass spectrometry

The methylated derivatives were analysed by mass spectrometry after reduction with sodium borodeuteride followed by peracetylation according to the method described previously[7].

RESULTS AND DISCUSSION

Analytical GLC of the organic and aqueous phases

The yields of the organic and aqueous phases were 1.2 and 7.85 g, respectively, starting from 8 g of methyl galactopyranoside submitted to partial methylation.

The GLC analysis of the organic phase revealed the presence of the permethylated ether and four trimethylated ethers. The methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside, the methyl 2,3,4- and methyl 3,4,6-tri-O-methyl-α-D-galactopyranosides were well separated and the methyl 2,3,6- and methyl 2,4,6-tri-O-methyl-α-D-galactopyranosides were eluted as a single peak (Fig. 1A).

The aqueous phase contained the four tri-O-methyl ethers, the six di-O-methyl ethers and the four mono-O-methyl ethers. Because the methyl 3,4- and 2,4-di-O-methyl-α-D-galactopyranosides and the methyl 2- and 3-mono-O-methyl-α-D-galactopyranosides had almost the same retention times (Fig. 1B), their direct preparative GLC was not possible. To resolve this problem, the aqueous phase was fractionated by chromatography on a silica gel column. Table I indicates the retention time of each ether relative to the methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside.

Preparative chromatography of the aqueous phase on a silica gel column

Preparative chromatography of the aqueous phase on a silica gel column provided three fractions (K-1, K-2 and K-3), which correspond, according to their order of emergence from the column, to the tri-, di- and mono-O-methyl ethers of methyl α-D-galactopyranoside (Fig. 2). Amounts of 87.5 mg of trimethyl ethers (Fig. 3A), 173.8 mg of dimethyl ethers (Fig. 3B) and 107.2 mg of monomethyl ethers (Fig. 3C) were obtained from 500 mg of water-soluble derivatives, corresponding to a final yield of 82%.

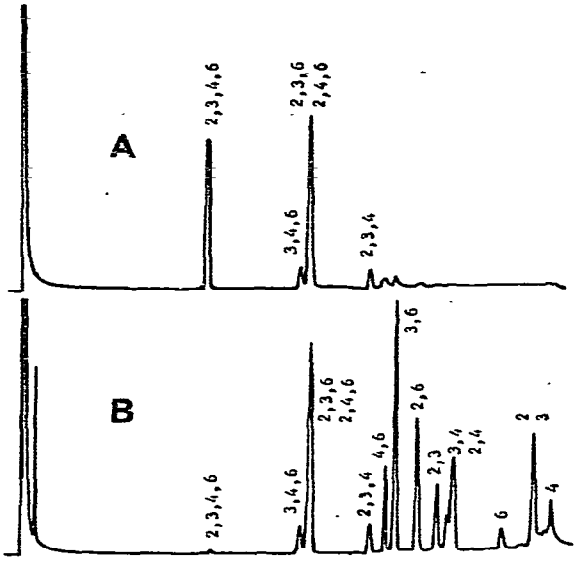

Fig. 1. GLC on a Carbowax 6000 column of the methyl α-D-galactopyranoside methyl ethers present in the organic phase (A) and in the aqueous phase (B) of the mixture obtained by partial methylation.

Preparative GLC of the methyl glycoside methyl ethers

Preparative GLC of the organic phase. Starting from 10 mg of the mixture of tetra- and tri-O-methyl derivatives, we obtained pure methyl 2,3,4,6-tetra-O-methyl- and methyl 2,3,4-tri-O-methyl-α-D-galactopyranosides, and a mixture of methyl 2,3,6-, 2,4,6- and 3,4,6-tri-O-methyl-α-D-galactopyranosides corresponding to a final yield of 45.3% (Table II). The methyl 2,3,6-, 2,4,6- and 3,4,6-tri-O-methyl-α-D-galactopyranosides were separated by GLC in pure form after O-acetylation (Fig. 4A and Table III). Trimethyl ethers were obtained by O-deacetylation in yields varying between 74 and 99% (Table IV).

Preparative GLC of the three silica gel fractions obtained from the aqueous phase. The yields of the preparative GLC of fractions K-1, K-2 and K-3 are given in Table V.

TABLE I

RETENTION TIMES OF THE TRIMETHYL, DIMETHYL AND MONOMETHYL ETHERS OF METHYL α-D-GALACTOPYRANOSIDE RELATIVE TO THE METHYL 2,3,4,6-TETRA-O-METHYL-α-D-GALACTOPYRANOSIDE

Methylated ether	*Retention time*	*Methylated ether*	*Retention time*
2,3,4,6-Tetra-O-methyl	1.00	2,3-Di-O-methyl	2.24
3,4,6-Tri-O-methyl	1.45	3,4-Di-O-methyl	2.28
2,3,6-Tri-O-methyl	1.51	2,4-Di-O-methyl	2.30
2,4,6-Tri-O-methyl	1.51	6-O-Methyl	2.55
2,3,4-Tri-O-methyl	1.84	2-O-Methyl	2.70
4,6-Di-O-methyl	1.96	3-O-Methyl	2.70
3,6-Di-O-methyl	2.02	4-O-Methyl	2.84
2,6-Di-O-methyl	2.15		

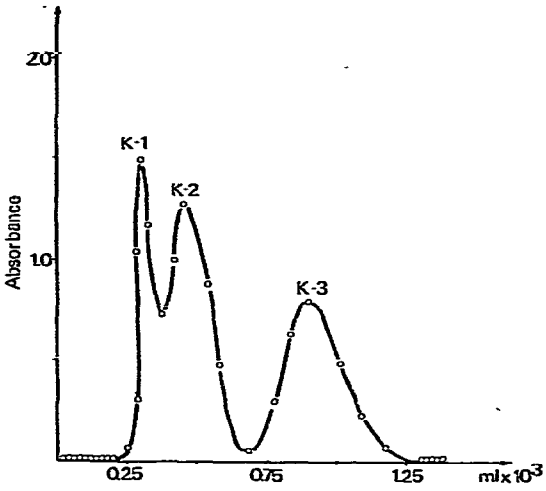

Fig. 2. Adsorption chromatography on a silica gel column of the methyl ethers in the aqueous phase. K-1, K-2 and K-3: mixtures of the tri-, di- and mono-O-methyl ethers, respectively.

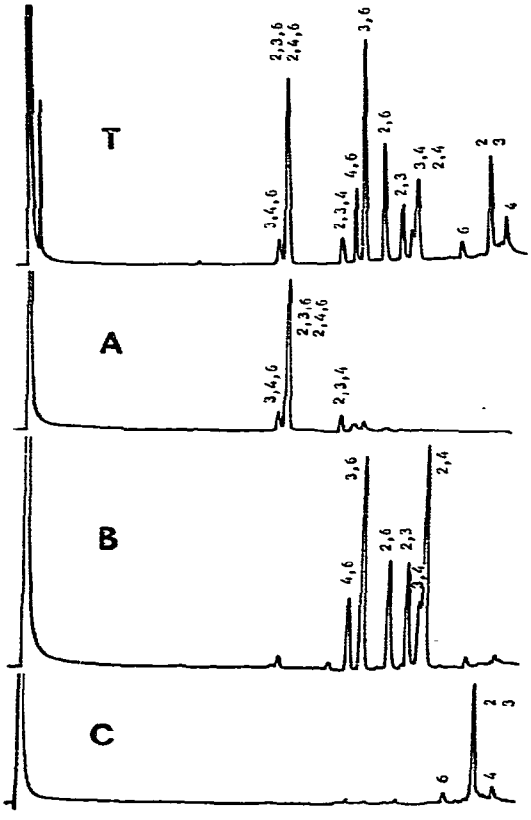

Fig. 3. GLC on a Carbowax 6000 column of the methyl α-D-galactopyranoside methyl ethers contained in the three silica gel fractions. A, B, C: fractions K-1, K-2 and -K3, respectively. T: standard mixture.

TABLE II

AMOUNTS OF METHYLATED ETHERS OBTAINED BY PREPARATIVE GLC FROM 10 mg OF THE ORGANIC PHASE

Compound	*Amount (mg)*
2,3,4,6-Tetra-O-methyl	0.73
2,3,4-Tri-O-methyl	0.18
2,3,6-Tri-O-methyl 3,4,6-Tri-O-methyl 2,4,6-Tri-O-methyl	3.64

TABLE III

AMOUNTS OF MONOACETYL TRIMETHYL ETHERS OBTAINED BY PREPARATIVE GLC FROM 10 mg OF THE ACETYLATED MIXTURE OF THE METHYL 2,3,6-, 3,4,6- AND 2,4,6-TRI-O-METHYL-α-D-GALACTOPYRANOSIDES

Compound	*Amount (mg)*
4-O-Acetyl 2,3,6-tri-O-methyl	2.88
2-O-Acetyl 3,4,6-tri-O-methyl	1.17
3-O-Acetyl 2,4,6-tri-O-methyl	0.43

TABLE IV

AMOUNTS OF TRIMETHYL ETHERS OBTAINED BY O-DEACETYLATION FROM THE AMOUNTS OF THE MONOACETYL TRIMETHYL ETHERS IN TABLE III

Compound	*Amount (mg)*	*Yield (%)*
2,3,6-Tri-O-methyl	1.88	74.2
3,4,6-Tri-O-methyl	0.75	75.7
2,4,6-Tri-O-methyl	0.38	99

TABLE V

AMOUNTS OF METHYLATED ETHERS ISOLATED FROM SILICA GEL FRACTIONS K-1, K-2 AND K-3 OF THE AQUEOUS PHASE BY PREPARATIVE GLC OF 10 mg OF EACH FRACTION

Fraction	*Compound*	*Amount (mg)*	*Yield (%)*
K-1	2,3,4-Tri-O-methyl	0.9	48.4
	2,3,6-Tri-O-methyl 2,4,6-Tri-O-methyl 3,4,6-Tri-O-methyl	3.96	
K-2	4,6-Di-O-methyl	0.87	44.1
	3,6-Di-O-methyl	1.34	
	2,6-Di-O-methyl	0.34	
	2,3-Di-O-methyl	0.29	
	3,4-Di-O-methyl 2,4-Di-O-methyl	1.59	
K-3	6-O-Methyl	0.37	27.2
	2-O-Methyl 3-O-Methyl	2.23	
	4-O-Methyl	0.12	

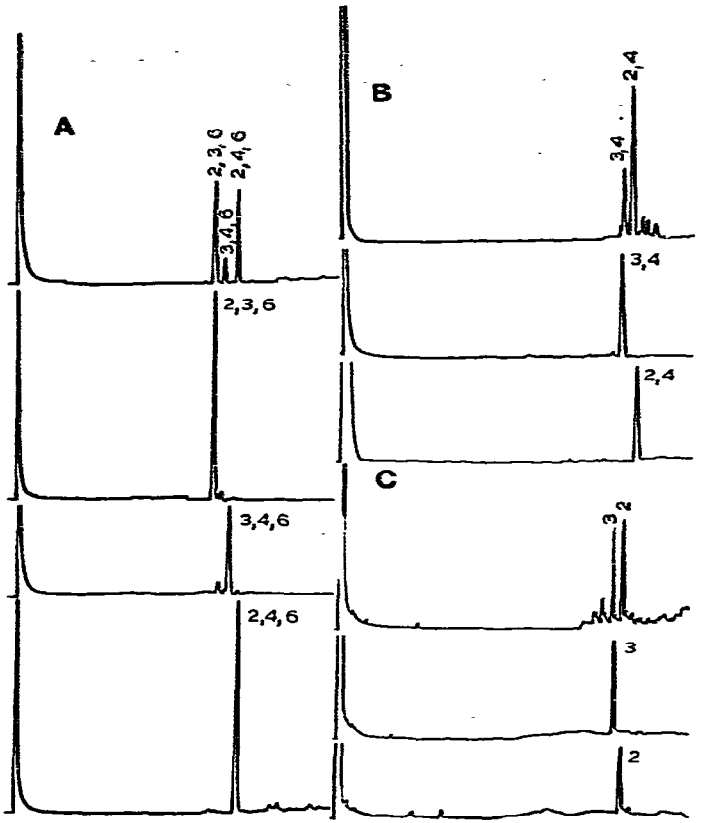

Fig. 4. GLC on a BDSP column of the acetylated methyl ethers of the methyl α-D-galactopyranoside. A, acetylated derivatives of methyl 2,3,6-, 3,4,6- and 2,4,6-tri-O-methyl-α-D-galactopyranosides; B, acetylated derivatives of methyl 3,4- and 2,4-di-O-methyl-α-D-galactopyranosides; C, acetylated derivatives of methyl 2- and 3-mono-O-methyl-α-D-galactopyranosides.

The methyl 2,3,6-, 2,4,6- and 3,4,6-tri-O-methyl-α-D-galactopyranosides were separated as described for the organic phase (see above). The methyl 2,4- and 3,4-di-O-methyl-α-D-galactopyranosides, which were obtained as a mixture, were well separated as their acetate derivatives (Fig. 4B) in 48% yield (Table VI). The dimethyl

TABLE VI

AMOUNTS OF MONOMETHYL AND DIMETHYL ETHERS ISOLATED AFTER ACETYLATION BY PREPARATIVE GLC OF 10 mg OF EACH FRACTION

Compound	*Amount (mg)*	*Yield (%)*
Diacetyl dimethyl ethers		
2,6-Di-O-acetyl 3,4-di-O-methyl	1.19	48.2
3,6-Di-O-acetyl 2,4-di-O-methyl	3.63	
Triacetyl monomethyl ethers		
2,4,6-Tri-O-acetyl 3-O-methyl	2.4	50
3,4,6-Tri-O-acetyl 2-O-methyl	2.6	

TABLE VII

AMOUNTS OF DIMETHYL AND MONOMETHYL ETHERS OBTAINED BY O-DEACETYLATION FROM THE AMOUNTS OF THE DIACETYL DIMETHYL ETHERS AND TRIACETYL MONOMETHYL ETHERS IN TABLE VI

Compound	*Amount (mg)*	*Yield (%)*
Dimethyl ethers		
3,4-Di-O-methyl	0.77	89.5
2,4-Di-O-methyl	1.84	70
Monomethyl ethers		
3-O-Methyl	1.26	84.5
2-O-Methyl	1.12	70

derivatives were obtained after O-deacetylation in yields varying between 70 and 89.5% (Table VII).

The preparative GLC of methyl 2- and 3-mono-O-methyl-tri-O-acetyl-α-D-galactopyranosides gave the pure derivatives in 50% yield (Fig. 4C and Table VI). The O-deacetylation led to preparation of pure methyl α-D-galactopyranoside monomethyl ethers in yields varying between 70 and 84.5% (Table VII).

Identification of the methylated ethers by mass spectrometry

Mass spectrometry of peracetylated galactitol methyl ethers permitted the unambiguous location of methoxy groups. According to Björndal *et al.*[12], the primary cleavages in the carbon chain occur preferentially between the carbon atoms that carry the methoxyl and acetyl groups, the positive charge being stabilized by the methoxyl group. The primary fragments formed from each methyl ether are described in Table VIII.

TABLE VIII

PRIMARY FRAGMENTS OF THE O-ACETYLATED METHYL ETHERS OF GALACTITOL

Acetylated derivative of D-*galactitol methyl ethers*	*m/e*											
	45	*118**	*161*	*162*	*189*	*190*	*205*	*233*	*234*	*261*	*262*	*305*
2,3,4,6-	+	+	+	+			+					
2,3,6-	+	+		+				+				
3,4,6-	+		+			+			+			
2,4,6-	+	+	+									
2,3,4-		+		+	+			+				
4,6-	+		+								+	
3,6-	+					+		+				
2,6-	+	+										
2,3-		+		+						+		+
3,4-					+	+		+	+			
2,4-		+			+				+			+
6-	+											
3-						+				+		
2-		+										
4-					+						+	

* The values in the upper row represent the fragments in which the C-1 position is deuterated.

CONCLUSIONS

The procedures described in our previous papers[5–8] on the preparation of the 15 methyl ethers of methyl α-D-mannopyranoside have been successfully applied to the preparation and isolation of the 15 methyl ethers of the methyl α-D-galactopyranoside. Rather than a separate organic synthesis of each methyl derivatives, the procedure is based on chromatographic fractionations of a mixture of all of the methyl ethers obtained in one step by partial methylation. The methyl derivatives are separated in good yields by silica gel chromatography followed by GLC of the fractions on Carbowax 6000 and butane-1,4-diol succinate polyester. Each ether was identified by mass spectrometry of its peracetylated alditol derivatives.

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