Journal of Chromatography, 92 (1974) 185–190
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CHROM. 7299

Note

Analytical and preparative gas–liquid chromatography of methyl α-D-mannoside monomethyl ethers

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(Received November 21st, 1973)

In a previous note[1] we described a simple and rapid method of identifying and isolating all the di-, tri- and tetramethylated derivatives of methyl α-D-mannoside. In this paper we report a solution to the problem of identification and isolation of the monomethyl derivatives of methyl α-D-mannoside, which are obtained by careful methylation of methyl α-D-mannoside followed by fractionation on silica gel columns and preparative gas–liquid chromatography (GLC).

MATERIALS AND METHODS

Partial methylation of methyl α-D-mannoside

To a solution of 8 g methyl α-D-mannoside (K & K Labs., Plainview, N.Y., U.S.A.), vacuum-dried over P_2O_5 for 24 h, in 140 ml of Merck N,N-dimethylformamide are added 16 ml of Prolabo (Paris, France) methyl iodide and 16 g of silver oxide (freshly prepared according to Whistler and Wolfrom[2]) in small fractions and under constant stirring. The mixture is stirred for 1.5 h at 20–22 °. The subsequent experimental procedure is identical with that previously described[1]. This results in an aqueous phase which contains the mono-, di- and trimethyl ethers of methyl α-D-mannoside, and which is evaporated to dryness.

Preparative chromatography on silica gel column

The monomethyl ethers of methyl α-D-mannoside are isolated from the aqueous phase by preparative chromatography on a silica gel column under the following experimental conditions: 1 g of the aqueous phase dissolved in 5 ml of chloroform–methanol (9:1) is chromatographed on a 60×3 cm column of silica gel (Kieselgel, 0.05–0.2 mm, highest purity, 70–325 mesh; Merck, Darmstadt, G.F.R.) in a chloroform–methanol (9:1) solvent system. The methylated compounds are located with a phenol–sulphuric acid reagent[3] and the monomethyl ethers, which appear in the tail fraction, are subjected to GLC under the conditions described below.

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Gas–liquid chromatography

Analytical and preparative GLC of the four methyl α-D-mannoside monomethyl ethers is carried out on compounds peracetylated by treating 100 mg of the monomethyl ether fraction with 24 ml of a mixture of equal parts of pyridine and acetic anhydride for 15 h at 20–22° and then evaporating the resulting solution to dryness under reduced pressure.

Analytical chromatography. The peracetylated monomethylethers of methyl α-D-mannoside are easily separated by GLC under the following experimental conditions: Varian-Aerograph apparatus fitted with a flame ionization detector; glass column (0.3 × 180 cm) packed with Chromosorb W (60–80 mesh) containing 3% ECNSS-M; column temperature, 170°; injector temperature, 200°; detector temperature, 210°; flow-rate of carrier gas (nitrogen), 20 ml/min. The retention times are calculated relative to the methyl 6-O-methyl-2,3,4-tri-O-acetyl-α-D-mannoside.

Preparative chromatography. The peracetylated monomethyl ethers of methyl α-D-mannoside are isolated by GLC under the following experimental conditions: Varian-Aerograph Model 705 apparatus (Autoprep type); metal column (0.92 × 600 cm) packed with Chromosorb W (80–100 mesh) containing 5% butane-1,4-diol succinic polyester (BDSP); temperature of the column 195°, of the injector 210°, of the detector 200° and of the collector 100°; flow-rate of carrier gas (nitrogen), 220 ml/min; decomposition of effluent in the direction of the detector, 20 ml/min and in the direction of the collector, 200 ml/min; sensitivity, 10/8; and volume injected at each operation, manually or automatically, 80 μl of a 4% (w/v) solution of the acetylated derivatives in methanol. The deacetylation of the compounds is carried out in 0.5 ml of an acetone solution containing 1 mg of acetylated compound, to which had been added 2 ml of 0.1 *N* sodium hydroxide (Kunz and Hudson[4]), at 20–22° for 60 min. The solution is then neutralized with 0.1 *N* hydrochloric acid and demineralized by passing it through columns (1 × 10 cm) of cation exchanger (Dowex 50, H^+) and anion exchanger (Duolite A-102 D, $HCOO^-$). The neutral effluent, to which is added the wash water from the columns, is finally evaporated to dryness under reduced pressure.

Identification of the methyl α-D-mannoside monomethyl ethers

The methyl α-D-mannoside monomethyl ethers are identified by means of mass spectrometry after reduction by sodium borodeuteride followed by peracetylation according to the following experimental method. A 5-mg amount of methyl α-D-mannoside monomethyl derivatives is hydrolysed by 500 μl of 1.5 *N* hydrochloric acid at 100° for 1.5 h. The hydrolysate is neutralized by passing it through a column of anion exchanger (Duolite A-102 D, 25–50 mesh, $HCOO^-$). The effluent fraction, to which is added the wash water from the column, is evaporated to dryness under reduced pressure. The residue is taken up in 5 ml of heavy water (C.E.A., Saclay, France) and the solution is again evaporated to dryness. The monomethyl ethers are taken up by 5 ml of heavy water and reduced by 50 mg of sodium borodeuteride (Merck Sharp & Dohme, Montreal, Canada) for 15 h at 20–22°. After treatment with Dowex 50-X8 (25–50 mesh, H^+) and evaporation to dryness, the boric acid is eliminated in the form of methyl borate by means of three co-distillations in the presence of methanol. The D-mannitol monomethyl ethers are then acetylated by 1 ml of a mixture of equal parts of pyridine and acetic anhydride at 20–22° for 15 h.

The monomethyl-pentaacetyl-D-mannitols are dissolved in chloroform and identified by GLC using a column of 3% ECNSS-M directly connected to a Perkin-Elmer Model 270B mass spectrometer and fitted with a Honeywell visicorder.

RESULTS AND DISCUSSION

Preparative chromatography on a silica gel column

Preparative chromatography of the aqueous phase on a silica gel column provides three well-defined fractions, which correspond, according to the order of emergence from the columns, to the tri-, di- and monomethyl ethers of methyl α-D-mannoside, respectively, with a yield of 300 mg of monomethyl ether fraction from 1 g of aqueous phase.

Analytical gas–liquid chromatography

The results of the separation of the monomethyl ethers of methyl tri-O-acetyl-α-D-mannosides are given in Table I and illustrated in Fig. 1. It can be observed that GLC on a column of Chromosorb W (60–80 mesh) with 3% ECNSS-M produces perfect separation of the four isomers. Chromatography on columns of BDSP gives identical results.

TABLE I

RETENTION TIMES OF TRIACETYL MONOMETHYL ETHERS OF METHYL α-D-MANNOSIDE RELATIVE TO THE METHYL 6-MONO-O-METHYL-2,3,4-TRI-O-ACETYL-α-D-MANNOSIDE

Compound	*Relative retention time*
6-Mono-O-methyl-2,3,4-tri-O-acetyl	1
3-Mono-O-methyl-2,4,6-tri-O-acetyl	1.37
4-Mono-O-methyl-2,3,6-tri-O-acetyl	1.65
2-Mono-O-methyl-3,4,6-tri-O-acetyl	1.81

Preparative gas–liquid chromatography

Preparative GLC of the fraction of previously peracetylated monomethyl ethers on a column of BDSP gives the following results. (1) Each injection of 9 mg of a mixture of the monomethyl ethers of methyl tri-O-acetyl-α-D-mannosides gives quantities of pure methyl ethers (Fig. 1) equivalent to a final yield of 62% (Table II). (2) The O-deacetylation gives methyl α-D-mannoside monomethyl ethers in yields varying between 65 snd 95% (Table III).

Identification of methyl α-D-mannoside monomethyl ethers by means of mass spectrometry

Study of the mass spectra of reduced and peracetylated ethers permits unambiguous identification of the position of the methoxy group. As has been shown

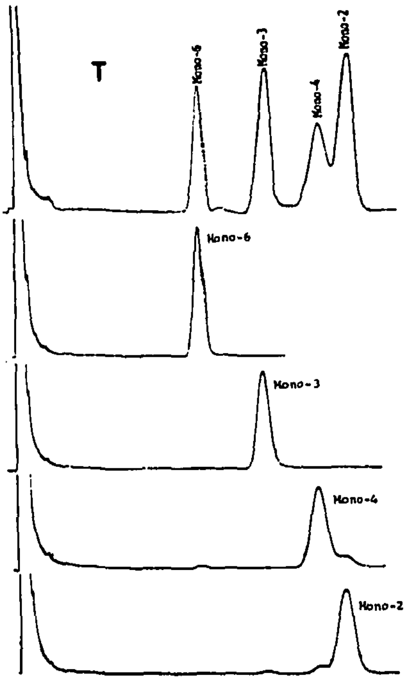

Fig. 1. GLC on an ECNSS-M column of the four monomethyl ethers of methyl tri-O-acetyl-α-D-mannosides obtained by preparative GLC. T = standard mixture.

TABLE II

AMOUNTS OF TRIACETYL MONOMETHYL ETHERS OBTAINED BY PREPARATIVE GLC FROM 9 mg OF A MONOMETHYL ETHER FRACTION ISOLATED BY CHROMATOGRAPHY ON A SILICA GEL COLUMN AND SUBSEQUENTLY O-ACETYLATED

Compound	*Amount* (*mg*)
2-Mono-O-methyl-3,4,6-tri-O-acetyl	1.72
3-Mono-O-methyl-2,4,6-tri-O-acetyl	1
4-Mono-O-methyl-2,3,6-tri-O-acetyl	1.03
6-Mono-O-methyl-2,3,4-tri-O-acetyl	2.14

TABLE III

AMOUNTS OF MONOMETHYL ETHERS OBTAINED BY O-DEACETYLATION FROM QUANTITIES OF THE TRIACETYL MONOMETHYL ETHERS OF TABLE II

Compound	*Amount* (*mg*)
2-Mono-O-methyl-	1.29
3-Mono-O-methyl-	0.6
4-Mono-O-methyl-	0.61
6-Mono-O-methyl-	0.90

by Björndal *et al.*[5], the primary cleavages in the carbon chain occur preferentially between the carbons carrying the methyl and acetyl groups, the positive charge being stabilized by the methoxy group. Thus, we found (Fig. 2) for the 2-mono-O-methyl-1,3,4,5,6-penta-O-acetylmannitol primary fragments of *m/e* 118 and 333, for the 3-mono-O-methyl-1,2,4,5,6-penta-O-acetylmannitol primary fragments of *m/e* 190 and 261, for the 4-mono-O-methyl-1,2,3,5,6-penta-O-acetylmannitol primary fragments of *m/e* 189 and 262, and for the 6-mono-O-methyl-1,2,3,4,5-penta-O-acetylmannitol primary fragments of *m/e* 45 and secondary fragments derived from the primary fragments 362 and 189. Fragment 260 arises from the former by elimination of acetic acid (60) and of ketene (42), whereas fragments 129 and 87 arise from the latter by elimination of acetic acid and a subsequent elimination of ketene.

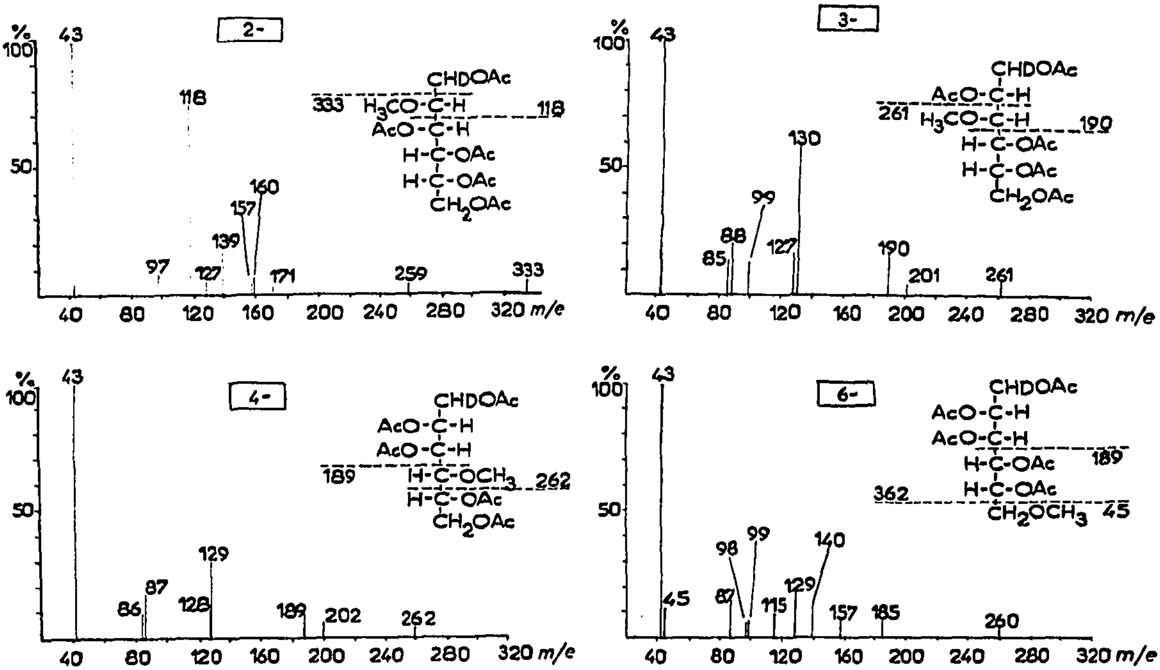

Fig. 2. Simplified mass spectra of the O-acetylated methyl ethers of mannitol. Ordinate: percentage mass intensity relative to mass number 43 as 100%. Conditions of mass spectrometry: electron energy, 70 eV; ionization current, 80 μA; ionization chamber temperature, 170°.

CONCLUSIONS

GLC on a column of ECNSS-M, BDSP or Carbowax 6000 permits identification of the four monomethyl ethers of methyl tri-O-acetyl-α-D-mannosides. These compounds are isolated in the pure state with good yields by means of preparative GLC on a column of Chromosorb W containing 5% BDSP. Saponification of the O-acetylated derivatives leads to the pure mono-O-methyl derivatives of methyl α-D-mannoside, which are identified by mass spectrometry.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the Centre National de la Recherche Scientifique (E.R.A. No. 320: Structure and metabolism of glycoproteins) and from the Commissariat à l'Énergie Atomique.

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