CHROM. 4707

GAS CHROMATOGRAPHY OF PARTIALLY METHYLATED ALDITOLS AS TRIFLUOROACETYL DERIVATIVES

I. SEPARATION OF MONO-O-METHYL-PER-O-TFA-D-GLUCITOLS

D. ANDERLE AND P. KOVÁČ
Institute of Chemistry, Slovak Academy of Sciences, Bratislava (Czechoslovakia)

(Received March 11th, 1970)

SUMMARY

The reduction of mono-O-methyl-D-glucoses to their corresponding mono-O-methyl-D-glucitols and gas chromatography of the trifluoroacetyl derivatives of the latter resulted in an excellent separation of the compounds under investigation. Trimethylsilyl derivatives of the same compounds were not satisfactorily separated. The retention times of the mono-O-methyl-per-O-trifluoroacetyl-D-glucitols were comparable with those of trimethylsilyl derivatives. Trifluoroacetylation of mono-O-methyl-D-glucitols was found to be sufficiently fast for the procedure to be used for routine analyses.

INTRODUCTION

Gas chromatography is a useful tool for both the qualitative and quantitative evaluation of the analysis of polysaccharides by methylation[1,2]. Methanolysis of polysaccharides, and even of monosaccharides, usually results in the formation of complicated mixtures of glycosides, amongst which the methylglycosides of mono-O-methylhexoses are poorly volatile, and when gas chromatographed are almost completely retained in the column.

The problem of poor volatility of the methyl-mono-O-methyl-hexosides was solved by the development of the silylation technique and its application to carbohydrate chemistry[3]. However, the problem of overlapping peaks corresponding to individual anomers and stereoisomers remained. Reduction of monosaccharides to their corresponding sugar alcohols and subsequent gas chromatography as their trimethylsilyl ethers has met with little success[3]. Alditols have been separated by SAWARDEKER *et al.*[4] as their more polar acetates on a copolymerized stationary phase (ECNSS-M). Similar results have also been obtained by SJÖSTRÖM *et al.*[5] using mixed stationary phases. After reduction to the corresponding alditols, BJÖRNDAL *et al.*[6] successfully separated partially methylated aldoses as partially methylated alditol acetates. Gas chromatography of the acetates of some other D-glucose and D-glucitol methyl ethers was recently reported by JONES AND JONES[7]. The main difficulty encountered in gas chromatography of alditolacetates is the high temperature necessary to obtain reasonable retention times, as usually the temperature is very close to that of the stability of the stationary phase. The possibility of the stationary phase bleeding, which results in an increased noise level when using a flame ionization detector (FID), then becomes an additional hazard.

Only a few workers[8-10] have investigated the use of trifluoroacetates for the gas chromatography of carbohydrates. The trifluoroacetates of some common alditols have been successfully separated by SHAPIRA[11] at a reasonable temperature and with a short analysis time.

The present paper describes the separation of mono-O-methyl-D-glucoses after reduction to their corresponding D-glucitols as per-O-trifluoroacetyl derivatives.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Research Chromatograph, Model 5750 G, with a dual column system and flame ionization detectors was employed. The composition of equilibrium mixtures of monosaccharides was determined quantitatively using Hewlett-Packard Integrator 3370 A.

Operating conditions

The gas chromatography of TMS derivatives was carried out on various columns: (A) 6 ft. × 1/8 in. (O.D.) Al, packed with 10% Apiezon L on Gas-Chrom Z 80–100 mesh; (B) 6 ft. × 1/8 in. (O.D.) Al, packed with 3% ECNSS-M on Chromatone N AW HMDS 70–80 mesh (Lachema, Brno); (C) 10 ft. × 1/8 in. (O.D.) stainless steel, packed with 1% XE 60 on Gas-Chrom Z 80–100 mesh.

Helium was used as a carrier gas. The flow rate was adjusted so that the columns showed the maximum efficiency.

Derivatives

Chromatographically pure mono-O-methyl-D-glucoses, synthesized according to known procedures[12], were used. Before derivatization, the free monosaccharides (3–5 mg) were equilibrated in 1 N H_2SO_4 (3–5 ml) at 100° for 2 h which was found to be sufficient time for the ratio of the α- and β-forms of the sugars to become

TABLE I

COMPOSITION OF EQUILIBRIUM MIXTURES OF MONO-O-METHYL-D-GLUCOSES AS FOUND BY GLC OF TMS ETHERS

Compound	α- (%)	β- (%)
2-O-Me[a]	54.8	45.2
3-O-Me	41.2	58.8
4-O-Me	47.0	53.0
6-O-Me	45.7	54.3

[a] 2-O-Me-D-glucopyranose etc.

constant. Sulfuric acid was removed by an anion-exchange resin (Ionenaustauscher I OH^-, Merck A.G.) and water was removed under reduced pressure below 40°. The composition of the equilibrium mixtures is shown in Table I.

The TMS derivatives were prepared by dissolving the dry residue, after equilibration or reduction to sugar alcohols, in 0.3 ml of pyridine and adding 0.1 ml of Tri-Sil Concentrate (Pierce Chemical Co.). The mixture was shaken vigorously for about 30 sec and then left at room temperature for 1 h. The samples on storage in a desiccator over P_2O_5 at room temperature showed no change in composition over a period of three months.

The reduction of the mono-O-methyl-D-glucoses into the corresponding D-glucitols and the preparation of the TFA derivatives was carried out in the following manner: 5 mg of each component was dissolved in 2 ml of water, 50 mg of sodium borohydride was added and the mixture was left at room temperature for 4 h. Dilution with water was followed by demineralization with a cation-exchange resin (Dowex 50 W H^+) and evaporation of the boric acid with methanol. The residue was left in a desiccator over P_2O_5 overnight and trifluoroacetylated with 0.3 ml of trifluoroacetic anhydride in the presence of 3 μl of pyridine. The reaction mixture was shaken vigorously for about 30 sec and left at room temperature for 1 h which was found to be sufficient time for the complete conversion of mono-O-methyl-D-glucitols into per-O-trifluoroacetates.

Both TMS and TFA derivatives were prepared in 10 ml drop-shaped vials equipped with polyethylene caps.

RESULTS AND DISCUSSION

Since, up to the present, a satisfactory method for the gas chromatographic separation of a mixture of mono-O-methyl-D-glucoses had not been found we first tried to solve the problem by using the known silylation technique. We employed various stationary phases and several combinations of operating conditions. As shown in Table II the separation achieved was not satisfactory for quantitative work. Our next attempt was to try to separate a mixture of mono-O-methyl-D-glucoses as their TMS ethers after reduction to their respective mono-O-methyl-D-glucitols. Although the reduction to sugar alcohols reduces the number of possible components by half,

TABLE II

RELATIVE RETENTION TIMES OF EQUILIBRATED MONO-O-METHYL-D-GLUCOSES AS TMS ETHERS

Compound	*Column A (210°)*	*Column B (120°)*	*Column C (145°)*
α-2-O-Me	0.82	0.80	0.56
β-2-O-Me	1.17	1.17	0.79
α-3-O-Me	0.65	0.57	0.41
β-3-O-Me	1.03	0.88	0.67
α-4-O-Me	0.78	0.66	0.47
β-4-O-Me	0.89	1.17	0.82
α-6-O-Me	1.04	1.00	0.79
β-6-O-Me	—	1.37	1.00
α-Glucose	1.00 (7.25 min)	1.00 (2.97 min)	1.00 (6.97 min)
Inositol	—	2.86 (8.5 min)	1.64 (11.42 min)

TABLE III

RELATIVE RETENTION TIMES OF EQUILIBRATED MONO-O-METHYL-D-GLUCOSES AS PER-O-TRIFLUORO-ACETATES

Compound	*Column C temp. programmed 130–150° (1°/min)*	*Column C temp. programmed 120° (4 min)–150° (2°/min)*
α-2-O-Me	0.44	0.56
β-2-O-Me	0.50	0.63
α-3-O-Me	0.33	0.44
β-3-O-Me	0.55	0.66
α-4-O-Me	0.37	0.48
β-4-O-Me	0.50	0.62
α-6-O-Me	0.18	0.24
β-6-O-Me	0.26	0.34
Inositol	1.00 (13.8 min)	1.00 (17.7 min)

the separation achieved was still not satisfactory on either of the columns used. It was thus obvious that in order to achieve a satisfactory separation the TMS group had to be replaced by a more polar group. Since BJÖRNDAL *et al.*[6] had previously shown that the acetyl group did not give rise to alditol derivatives having retention times comparable with those of TMS derivatives, we selected the trifluoroacetyl group, which was known to give satisfactory results with common alditols[11] and amino acids[13].

In our experience, trifluoroacetic anhydride fulfills all the conditions necessary as a derivatization agent in the gas chromatography of carbohydrates. Trifluoroacetylation was found to be a fast reaction, the derivatives formed were sufficiently stable and gave retention times comparable with those of TMS ethers. Thus, the present paper demonstrates the possibility of separating a mixture of mono-O-methyl-D-glucoses as the TFA derivatives of the corresponding mono-O-methyl-D-glucitols.

Tables III and IV show the retention times of mono-O-methyl-per-O-trifluoroacetyl-D-glucoses and mono-O-methyl-per-O-trifluoroacetyl-D-glucitols relative to that of inositol which we chose as an internal standard because it never occurres in polysaccharide hydrolyzates.

For comparison we show the separation of a mixture of the equilibrated mixture of mono-O-methyl-D-glucoses as per-O-TMS ethers in Fig. 1; Fig. 2 shows the sepa-

TABLE IV

RELATIVE RETENTION TIMES OF MONO-O-METHYL-PER-O-TRIFLUOROACETYL-D-GLUCITOLS

Compound	*Column C temp. programmed 130–150° (1°/min)*
2-O-Me	0.63
3-O-Me	0.79
4-O-Me	0.88
6-O-Me	0.29
Inositol	1.00 (15.7 min)

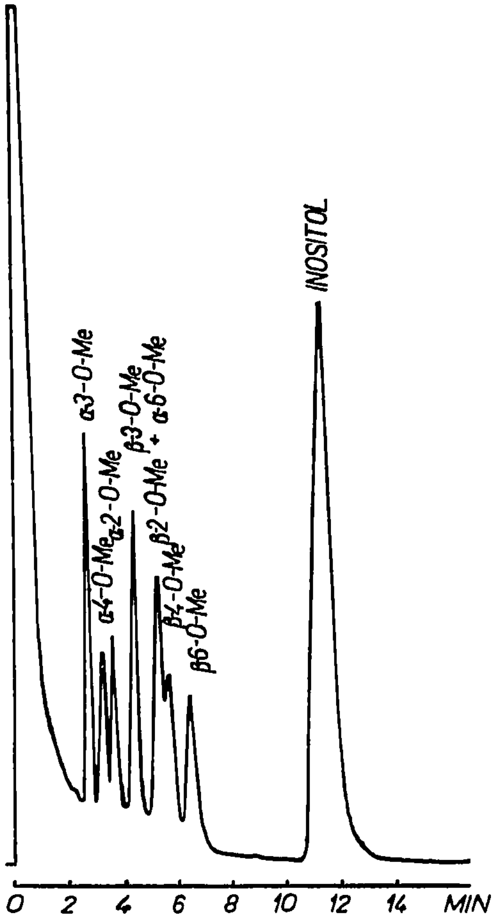

Fig. 1

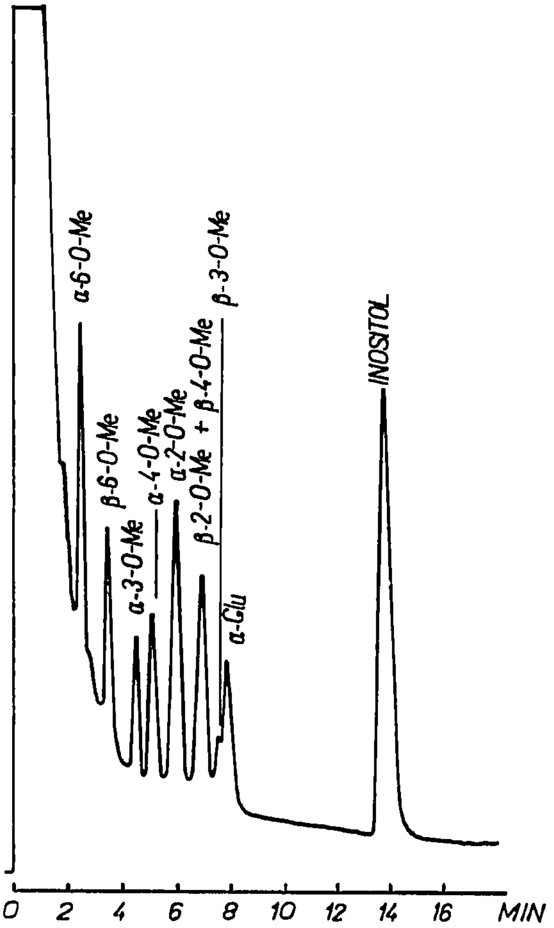

Fig 2

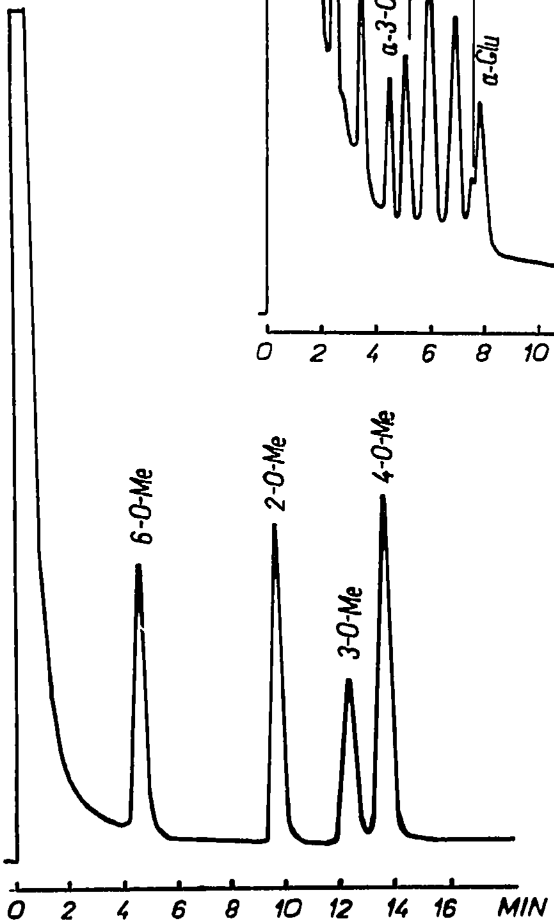

Fig 3

Fig 1 GLC of an equilibrated mixture of mono-O-methyl-D-glucoses as their per-O-TMS ethers Column C, 145°. Carrier gas flow rate 20 ml/min.

Fig. 2. GLC of an equilibrated mixture of mono-O-methyl-D-glucoses as their per-O-TFA derivatives. Column C; 130–150° (1°/min). Carrier gas flow rate 30 ml/min

Fig 3. GLC of mono-O-methyl-per-O-TFA-D-glucitols. Column C, 130–150° (1°/min). Carrier gas flow rate 25 ml/min.

ration of the same compounds as their per-O-TFA derivatives, and Fig. 3 the separation of a mixture of mono-O-methyl-D-glucoses after reduction to mono-O-methyl-D-glucitols and GC as the per-O-TFA derivatives.

For quantitative work it is necessary that the resolution of consecutive peaks is of the highest possible value (possibly higher than 1). It can be seen from the presented data that the resolution of the TFA mono-O-methyl-D-glucitols was better than 1.5 which corresponds to a separation better than 99.7%.

It is clear that, for both the qualitative and quantitative investigation of mixtures of mono-O-methyl-D-glucoses, the per-O-TFA derivatives are more advantageous than the per-O-TMS-ethers. It is even better to reduce mono-O-methyl-D-glucoses in the mixture to the corresponding D-glucitols and gas chromatograph these as per-O-TFA derivatives. The separation is excellent and the gas chromatography needs less time and a shorter column than in the case of the acetates. The procedure described is suitable for routine analyses. The work on separation of other carbohydrate derivatives as trifluoroacetates is in progress and will be published later.

REFERENCES

1 G. O. ASPINALL, *J. Chem. Soc.*, (1963) 1676.
2 C. T. BISHOP, *Advan. Carbohydrate Chem.*, 19 (1964) 95
3 C. C. SWEELEY, R. BENTLEY, M MAKITA AND W. W. WELLS, *J. Am. Chem. Soc.*, 85 (1963) 2497.
4 J. S. SAWARDEKER, J. H. SLONEKER AND A. JEANES, *Anal. Chem.*, 12 (1965) 1602.
5 E. SJÖSTRÖM, P. HÅGLUND AND J. JANSON, *Svensk. Papperstid.*, 69 (1966) 381.
6 H BJÖRNDAL, B. LINDBERG AND S SVENSSON, *Acta Chem. Scand.*, 21 (1967) 1801.
7 H. G. JONES AND J. K. N. JONES, *Can J. Chem.*, 47 (1969) 3269
8 M. VILKAS, HIN-I-JAN, M. BOUSSAC AND M. BONNARD, *Tetrahedron Letters*, 14 (1966) 1441.
9 Z. TAMURA AND T. IMANARI, *Chem. Pharm. Bull. (Tokyo)*, 15 (1967) 246.
10 K. YOSHIDA, N. HONDA AND N. TINO, *Carbohydrate Res.*, 10 (1969) 333.
11 J. SHAPIRA, *Nature*, 222 (1969) 729.
12 E. J. BOURNE AND S. PEAT, *Advan. Carbohydrate Chem.*, 5 (1950) 145.
13 C V. GEHRKE, D. ROACH, R. W. ZUMWALT, D. L. STALLING AND L L. WALL, *Quantitative Gas-Liquid Chromatography of Amino Acids in Proteins and Biological Substances*, Analytical Biochemistry Laboratories, Inc., Columbia, 1968