GLC of Methylated and Acetylated Hexitols Prepared from Some *O*-Methyl Ethers of D-Fructose

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During work with the constitution of naturally occurring oligo- and polyfructosides of the kestose series in this laboratory, it was considered desirable to develop existing methods for the characterisation of methylated fructose derivatives by means of GLC.

Various *O*-methyl-D-fructoses have been separated by GLC as their methyl glycosides.[1,2] Each methyl ether gives at least two, but often three to five, peaks in the chromatogram, and the analysis therefore may be complicated by overlapping peaks.

By reduction of a methylated D-fructose a mixture of the corresponding methylated D-glucitol and D-mannitol is obtained.[3,4] This mixture of only two components may give less complex chromatograms by GLC. Further, the identification of the parent monosaccharide may be corroborated by the formation of the two alditols, since only fructose among all the naturally occurring monosaccharides might give rise to this mixture. Another advantage is that some of the methylated D-glucitols and D-mannitols produced from partly methylated D-fructoses by reduction may be prepared, for comparison by GLC, from well known methylated derivatives of D-glucose and D-mannose.

Partially methylated D-glucitol and D-mannitol acetates are separated by GLC.[5,6] They are not reported to originate from the corresponding *O*-methyl-D-fructoses and the retention times seem very close for quantitative estimation.

Anderle and Kováč [7] reported good GLC separation of mono-*O*-methyl-D-glucoses as their D-glucitol trifluoroacetates on the polar liquid phase XE-60.

The present paper reports GLC separation of some reduced *O*-methyl-D-fructoses and -D-glucoses as their acetates and trifluoroacetates.

Under the conditions used, GLC of alditol acetates on the liquid phase ECNSS-M resulted in one peak for each *O*-methyl-D-fructose, whereas the TFA derivatives were resolved when chromatographed on liquid phase XE-60, giving two peaks from each *O*-methyl-D-fructose.

Table 1. Relative retention times of alditol acetates (T^A) and alditol trifluoroacetates (T^{TFA}).

Alditol	T^A	T^{TFA}
1,3,4,6-Tetra-*O*-methyl-D-mannitol	0.73	0.30
1,3,4,6-Tetra-*O*-methyl-D-glucitol	0.73	0.38
1,3,4,6-Tetra-*O*-methyl-L-iditol	–	0.40
2,3,4,6-Tetra-*O*-methyl-D-glucitol	1.00	0.46
1,3,4-Tri-*O*-methyl-D-mannitol	1.88	0.50
3,4,6-Tri-*O*-methyl-D-glucitol	1.88	0.57
1,3,4-Tri-*O*-methyl-D-glucitol	1.88	0.64
2,3,4-Tri-*O*-methyl-D-glucitol	2.39	0.71
3,4-Di-*O*-methyl-D-mannitol	5.04	1.25
3,4-Di-*O*-methyl-D-glucitol	5.04	1.55
D-Ribitol	–	1.00

The identity of the two peaks resulting from GLC of TFA derivatives of each *O*-methyl-D-fructose reduced, was confirmed by co-chromatography. Reduced 1,3,4,6-tetra-*O*-methyl-D-fructose gave one peak in common with those arising from reduced 1,3,4,6-tetra-*O*-methyl-L-sorbose. This must be the D-glucitol derivative. TFA derivative of reduced 3,4-di-*O*-methyl-D-fructose gave one peak with retention time identical to that of the peak arising from TFA derivative of reduced 3,4-di-*O*-methyl-D-glucose.

Because of symmetry 1,3,4- and 3,4,6-tri-*O*-methyl-D-fructose give two different methylated glucitols but the same methylated mannitol after reduction. A co-chromatogram of the TFA derivatives gave three peaks, the one in common being the mannitol derivative.

GLC of TFA derivatives showed that the reduction of *O*-methyl-D-fructoses produced *O*-methyl-D-mannitol and -D-glucitol in a fairly constant ratio of 1 to 2. This is in agreement with previous observations.[4] For both 1,3,4- and 3,4,6-tri-*O*-methyl-D-fructose the ratio was 0.51. Standard deviations

were 0.044 for both, the number of independently reduced samples being 13 and 6, respectively.

The trifluoroacetates were preferred for quantitative GLC. To obtain calibration curves samples were prepared by varying the amount of each sugar relative to the amount of a reference sugar, 1,3,4,6-tetra-*O*-methyl-D-fructose. The ratio of the peak areas in each sample was plotted against the respective molar ratio. The slope of the curves, the relative molar response indexes, were constant for the ratios of interest.

Table 2. Relative molar response indexes (R) for *O*-methyl-D-fructoses and -D-glucoses analysed as trifluoroacetates of the corresponding alditols.

Sugar	R	Molar ratios
1,3,4-Tri-*O*-methyl-D-fructose	0.90	0.2–10
3,4,6-Tri-*O*-methyl-D-fructose	0.90	0.2–10
3,4-Di-*O*-methyl-D-fructose	0.66	0.3–3
2,3,4-Tri-*O*-methyl-D-glucose	0.87	0.3–3
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.17	0.3–3
1,3,4,6-Tetra-*O*-methyl-D-fructose	1.00	–

As the ratio between the D-mannitol and D-glucitol derivatives and the relative molar response indexes are identical for 1,3,4- and 3,4,6-tri-*O*-methyl-D-fructose, the relative amount of the two derivatives in a mixture may simply be determined by measuring the relative amount of the tri-*O*-methyl-D-glucitol trifluoroacetates in the chromatogram.

Experimental. Chromatographically pure *O*-methyl ethers of D-fructose,[8] D-glucose,[9] and L-sorbose [10] synthesized according to known procedures, were used.

The sugars were reduced using $NaBH_4$. Under the actual conditions [5] 2 h were found sufficient to complete the reaction; an aliquot of the sample showed no reducing power when treated with spray reagent for reducing sugars.[11]

Acetylation was performed according to Björndal *et al.*[5] The acetylation mixture was diluted with water, concentrated under reduced pressure to dryness and dissolved in chloroform before injection into the gas chromatograph.

TFA derivatives were prepared by dissolving the reduced sample (1–2 mg) in 50 µl acetonitrile. After adding an equal amount of trifluoroacetic anhydride (TFAA) the solution was kept at 40°C for 2 h, which was found sufficient to complete the reaction. About 1 µl of the reaction mixture was injected into the gas chromatograph.

A Perkin-Elmer F 11 Gas Chromatograph equipped with a hydrogen flame detector was used. The stainless steel columns were 2.1 m × 1/8″. Alditol acetates were separated at a gas flow of 27 ml nitrogen per min on a column containing 3 % (w/w) of ECNSS-M on Gas Chrom Q (80/100 mesh) at 180°C. Injection block temperature 220°C.

Gas flow rate for separation of alditol trifluoroacetates was 20 ml nitrogen per min. The column was packed with 3 % (w/w) XE-60 on Gas Chrom Q (100/120 mesh) and operated at 125°C. Injection block temperature was 170°C.

For quantitative analysis peak areas were determined by measuring height × width at half height.

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