Note

Separation by gas–liquid chromatography of tetra-*O*-methylaldohexoses and other sugars as acetates

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Gas–liquid chromatography was first applied in the carbohydrate field to methyl glycosides of methylated sugars[1]. Since that time, data on the retention times of many such compounds have been published[2]. The use of methyl glycosides means that, in most cases, one compound will produce several peaks; these complicate the chromatogram even though the pattern of the peaks for a particular sugar may be a useful diagnostic feature. The most common way of avoiding this problem is to reduce the methylated sugars and to separate them as methylated alditol acetates[3].

In situations where neither of these methods give satisfactory resolution of certain components of a mixture it has been possible, in many cases, to achieve separations of methyl glycosides[4,5] and free sugars[6–8] as their *O*-trimethylsilyl derivatives or as acetylated methyl glycosides[9,10].

Unfortunately none of these combinations gives satisfactory resolution of a mixture containing 2,3,4,6-tetra-*O*-methyl-D-glucose, -D-galactose, and -D-mannose. The necessity for achieving this separation arises in studies on galactoglucomannans, such as occur in wood[11,13], where each of the tetra-*O*-methylaldohexoses in a mixture must be determined or, alternatively, the absence of one in the presence of the other two needs to be confirmed positively. Table I shows that these three ethers

TABLE I

RETENTION TIMES OF METHYLATED ALDOHEXOSE ACETATES

Compound (as peracetate)	*Retention time (min)* *peak 1*	*peak 2*	*Approximate ratio peak 1/peak 2*
(a) Isothermal at 170°			
2,3,4,6-Tetra-*O*-methylglucose	9.4	11.5	0.85
2,3,4,6-Tetra-*O*-methylgalactose	13.4	20.2	1.4
2,3,5,6-Tetra-*O*-methylgalactose	15.1		
2,3,4,6-Tetra-*O*-methylmannose	17.9	23.2	20
(b) Isothermal at 185°			
2,3,4,6-Tetra-*O*-methylglucose	4.8	5.2	0.85
2,3,6-Tri-*O*-methylglucose	14.8	17.0	1.3
2,3,6-Tri-*O*-methylmannose	31.2		
2,3-Di-*O*-methylglucose	34.8	39.7	0.3

may be separated readily in the form of their acetates. Although this method causes each compound to give two peaks, the retention times of these peaks serve for characterization.

Table I also shows that, at a slightly higher column-temperature, di-*O*-methyl-aldohexoses are sufficiently volatile to be separated as their acetates; particularly noteworthy is the marked difference in retention times between 2,3,6-tri-*O*-methyl-D-glucose and the corresponding D-mannose analog. This is particularly useful since these two compounds as alditol acetates[12,13] and as methyl glycosides[2] have similar retention times.

These results demonstrate that, for the examples cited, separation of methylated sugars as their acetates is the preferred method. This system is thus a useful complement to those previously described, and has the additional merit that the free sugar may be readily regenerated for further characterisation. The only other report of the use of acetates of methylated sugars appears to be that of H. G. Jones, J. K. N. Jones, and Perry[14], who used this method in a study on the separation of mono-, di-, tri-, and tetra-*O*-methyl-D-glucose derivatives.

EXPERIMENTAL

2,3,4,6-Tetra-*O*-methyl-D-glucose, -D-galactose, and -D-mannose were prepared from the corresponding sugars by a standard procedure[15]. 2,3,6-Tri-*O*-methyl-D-glucose was obtained by hydrolysis of a commercial methylated cellulose[16]. 2,3,6-Tri-*O*-methyl-D-mannose[17] and 2,3-di-*O*-methyl-D-glucose[18] were available from previous studies. All compounds and/or their derivatives had physical properties in accord with literature values[19,20] .

Sugars were acetylated by dissolving them in acetic anhydride–pyridine (1:1) and heating the mixtures under anhydrous conditions (conveniently in a sealed tube) for 15 min on a steam bath. The acetates were extracted with chloroform and the solution was washed twice with M hydrochloric acid, followed by aqueous sodium hydrogen carbonate, and water. The dried ($CaCl_2$) extract was concentrated to small volume for injection. Omission of the washing procedure gave extraneous peaks.

Gas–liquid chromatography was carried out on a F and M 720 instrument using dual column (8 ft × 3/16 in.) of 3% ECNSS-M on Gas-Chrom Q with a helium flow-rate of 86 ml/min.

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Note added in proof (11 April 1972). 2,3,4,6-Tetra-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-L-rhamnose, a pair of compounds difficult to separate as methyl glycosides or alditol acetates, may be resolved conveniently as acetates. Using a column (4 ft × 1/4 in.) containing 5% butanediol succinate on Diatoport S (80–100 mesh) and a temperature program from 110 to 210° at 2°/min, the acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose were eluted at 27.9 and 30.2 min, and those of 2,3-di-*O*-methyl-L-rhamnose at 37.0 and 40.7 min. In addition, two unidentified peaks (*ca* 7% of total peak area) were eluted at 16.1 and 18.5 min.