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Gas-Liquid Chromatography of Partially Methylated Alditols as their Acetates

HÅKAN BJÖRNDAL, BENGT LINDBERG and SIGFRID SVENSSON

Institutionen för organisk kemi, Stockholms Universitet, Stockholm, Sweden

A number of partially methylated alditols have been separated as their fully acetylated derivatives by GLC on an ECNSS-M column. The method seems to be of value in methylation analysis of polysaccharides as a complement to analyses of methyl glycosides by GLC.

GLC has become an important method in methylation analysis of polysaccharides, both as an aid in identifying individual methylated sugars and for their quantitative measurement.[1,2] Generally a methanolysate of a fully methylated polysaccharide is investigated. In most cases a methylated sugar gives a mixture of two to four glycosides in fairly constant proportions which sometimes facilitates identification, but more often overlapping of peaks complicates the results.

The same problem is encountered in quantitative analysis of mixtures of sugars, as their trimethylsilyl ethers.[3] Early attempts to analyse sugars as their fully acetylated alditols, when each sugar gives a single peak, were not entirely successful, as the resolution of isomeric substances was often unsatisfactory. Recently, however, Sawardeker *et al.*[4] found that improved separation of acetylated alditols could be obtained using a copolymer of ethylene glycol succinate polyester and a nitrile silicone polymer (ECNSS-M) as the stationary phase. Similar results have been obtained using mixtures of related polymers as the stationary phase.[5]

The present paper reports GLC separations of some partially methylated alditols as their acetates on an ECNSS-M column. As is evident from Table 1, which gives relative retention times at 180°, replacement of a methoxyl group by an acetoxyl group results in a considerable increase in the retention time. Thus tetra-, tri- and di-*O*-methyl hexitol acetates as well as pentitol acetates are well separated. For fully acetylated hexitols and mono-*O*-methyl hexitol acetates, temperatures of 220° and 200°, respectively, give practical retention times.

Table 1. Relative retention times (T) of alditol acetates.

Alditol	T^a
2,3,5-Tri-*O*-methyl-L-arabinitol	0.48
2,4-Di-*O*-methyl-L-arabinitol	1.40
2,3,4-Tri-*O*-methyl-D-xylitol	0.68
3,5-Di-*O*-methyl-D-xylitol	1.08
2,3-Di-*O*-methyl-D-xylitol	1.54
2,3,4,6-Tetra-*O*-methyl-D-glucitol	**1.00**
2,4,6-Tri-*O*-methyl-D-glucitol	1.95
2,3,4-Tri-*O*-methyl-D-glucitol	2.49
2,3,6-Tri-*O*-methyl-D-glucitol	2.50
2,6-Di-*O*-methyl-D-glucitol	3.83
4,6-Di-*O*-methyl-D-glucitol	4.02
3,6-Di-*O*-methyl-D-glucitol	4.40
2,4-Di-*O*-methyl-D-glucitol	5.10
2,3-Di-*O*-methyl-D-glucitol	5.39
3-Mono-*O*-methyl-D-glucitol	9.6
2,3,5,6-Tetra-*O*-methyl-D-galactitol	1.15
2,3,4,6-Tetra-*O*-methyl-D-galactitol	1.25
2,4,6-Tri-*O*-methyl-D-galactitol	2.28
2,3,6-Tri-*O*-methyl-D-galactitol	2.42
2,3,4-Tri-*O*-methyl-D-galactitol	3.41
2,6-Di-*O*-methyl-D-galctitol	3.65
2,4-Di-*O*-methyl-D-galactitol	6.35
3,4-Di-*O*-methyl-D-galactitol	6.93
2,3,4,6-Tetra-*O*-methyl-D-mannitol	1.00
3,4,6-Tri-*O*-methyl-D-mannitol	1.95
2,4,6-Tri-*O*-methyl-D-mannitol	2.09
2,3,4-Tri-*O*-methyl-D-mannitol	2.48
3,4-Di-*O*-methyl-D-mannitol	5.37

[a] Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

It is believed that transforming methylated sugars obtained on hydrolysis of a fully methylated polysaccharide, into the acetylated alditol derivatives and analysing the mixture by GLC offers the following advantages as a complement to analysis of methyl glycosides by GLC. Firstly, by using a temperature gradient, alditols with all degrees of methylation, even nil, could be detected in a single experiment, and thereby undermethylation and/or demethylation during hydrolysis would easily be detected. Secondly, improved separation of some sugars, which are not well separated as their methyl glycosides, is obtained. Thirdly, as each sugar gives a single peak, quantitative evaluation of the chromatogram is considerably facilitated. The objection that information is lost, either because different sugars may give the same alditol or because some alditols have elements of symmetry not present in the parent sugars, should seldom be of practical significance. Probably more important, is the possible loss of volatile alditol methyl ethers during concentration preceding acetylation.

As the concentration of the stationary phase is low, the columns are sensitive to overloading, resulting in unsymmetrical peaks. Reproducibility, even on different columns, is good, generally 1 % or better. To obtain this reproducibility, it is necessary to use two internal standards, which differ considerably in retention times, and estimate the T-values by interpolation. In the present study we have used 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol ($T = 0.48$) and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methyl-D-galactitol ($T = 6.35$).

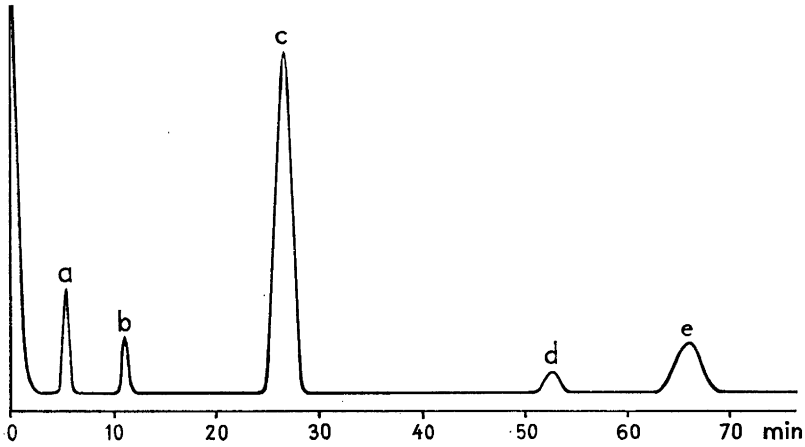

Fig. 1. Analysis of a dextran, containing α-(1→3)-branching points. a: 1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol = 1,4-OAc-2,3,5-OMe-Arab. *b*: 1,5-OAc-2,3,4,6-OMe-Gluc. c: 1,5,6-OAc-2,3,4-OMe-Gluc. d: 1,3,5,6-OAc-2,4-OMe-Gluc. e: 1,3,5,6-OAc-2,4-OMe-Gal.

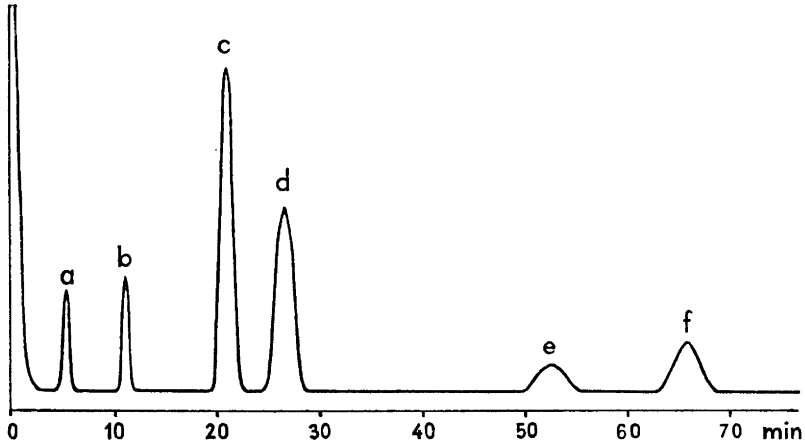

Fig. 2. Analysis of a fungal β-glucan, containing (1→3)- and (1→6)-linkages. a: 1,4-OAc-2,3,5-OMe-Arab. b: 1,5-OAc-2,3,4,6-OMe-Gluc. c: 1,3,5-OAc-2,4,6-OMe-Gluc. d: 1,5,6-OAc-2,3,4-OMe-Gluc. e: 1,3,5,6-OAc-2,4-OMe-Gluc. f: 1,3,5,6-OAc-2,4-OMe-Gal.

The application of the method to methylated polysaccharides is illustrated with two examples, a dextran containing α-(1→3)-branching points (Fig. 1) and a fungal β-glucan, containing (1→3)- and (1→6)-linkages (Fig. 2).[6]

EXPERIMENTAL

Gas-liquid chromatography was carried out by using a Perkin-Elmer 881 Gas Chromatograph. Separations were made at a gas flow rate of 40 ml nitrogen per min on a column (200 × 0.3 cm) containing 3 % (w/w) of ECNSS-M on Gas Chrom Q (100–120 mesh) at 180°.

Methylated sugars (1 mg) were reduced in water (5 ml) with sodium borohydride (10 mg) for 2 h. After treatment with Dowex 50 (H^+) and concentration, boric acid was removed by codestillation with methanol and the product was treated with acetic anhydride-pyridine, 1:1, (2 ml) at 100° for 10 min. The acetylation mixture was either injected into the column or was first diluted with water, concentrated to dryness and dissolved in chloroform.

Methylated polysaccharides were hydrolysed, neutralized and the resulting mixture of methylated sugars was reduced and acetylated following the above procedure.

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