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Short Communication

Capillary gas chromatography of partially methylated alditol acetates on a high-polarity, cross-linked, fused-silica BPX70 column

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ABSTRACT

A new high-polarity, cross-linked, fused-silica BPX70 capillary column was used to separate complex mixtures of partially methylated alditol acetates derived from the monosaccarides: arabinose, xylose, fucose, rhamnose, galactose, glucose and mannose. These partially methylated alditol acetates are separated by gas chromatography and identified by mass spectrometry by comparison with standard spectra as well as with their retention times relative to *myo*-inositol hexacetate, an internal standard.

INTRODUCTION

Glycosyl linkage positions between monosaccharides in glycoconjugates and polysaccharides are established using methylation analysis [1–6]. A quick and easy method for preparing such partially methylated alditol acetates is described by Doares *et al.* [7]. The separation of the resultant partially methylated alditol acetates can be achieved using capillary gas chromatography (GC) and the derivatives identified by both retention times relative to an internal standard and mass spectrometry (MS).

Partially methylated alditol acetates have been chromatographed on capillary columns coated with high-polarity cyanoalkyl silicone phases. They are CP-Sil88 [8], BP-75 [9], SP-2330 [7] and SP-2340 [10]. The CP-Sil88, SP-2330 and SP-2340 are all non-bonded phase columns whereas the BP-75 is bonded phase. Bonded phase columns are of benefit during GC–MS analysis due to their inherent low bleed characteristics as well as displaying greater stability. The BP-75 column is no longer available. The separation of some partially methylated alditol acetates which would otherwise co-elute can be achieved by chromatography on columns coated with phases of varying polarity. This paper describes the separation of partially methylated alditol acetates using a new, high-polarity, cross-linked, fused-silica BPX70 column with a phase equivalent to 70% cyanopropyl siloxane, recently released by SGE, Melbourne, Australia.

EXPERIMENTAL

Materials

Methyl-α-D-glucopyranoside, methyl-α-D-galactopyranoside, methyl-α-D-mannopyranoside,

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myo-inositol, L-fucose, L-rhamnose and sodium borodeuteride (98 atom% ^2H) were obtained from Sigma (St Louis, MO, USA); methyl-α-D-xylopyranoside was obtained from Pfanstiehl Labs. (Waukegan, IL, USA); L(+)-arabinose was obtained from BDH (Poole, UK). Methanolic HCl (1 *M*) was prepared by diluting 3 *M* methanolic HCl obtained from Supelco (Bellefonte, PA, USA); potassium methylsulphinyl methanide was prepared from potassium hydride (20% in oil) and dimethylsulphoxide as described by Harris *et al.* [11]; *myo*-inositol hexaacetate was prepared as described by Doares *et al.* [7]; methyl iodide and 1-methylimidazole were obtained from Fluka (Buchs, Switzerland). All other reagents were of the highest purity commercially avaliable.

Synthesis of methyl glycosides

The synthesis of methyl glycosides of L-fucose, L-rhamnose and L-arabinose was according to the method by Doares *et al.* [7]

Preparation of partially methylated alditol acetate standards

The methyl glycosides of the sugar were partially methylated, hydrolysed, reduced and acetylated by the procedure outlined by Doares *et al.* [7] using potassium methylsulphinyl methanide carbanion and methyl iodide. The partially methylated alditol acetates were extracted into dichloromethane, dried and reconstituted into an appropriate volume of dichloromethane. *Myo*-inositol hexa-acetate was added as an internal standard.

Gas chromatography–mass spectrometry

Partially methylated alditol acetates were separated and identified by GC–MS on a fully automated Finnigan MAT 1020B GC–MS (Sunnyvale, CA, USA).

A 25 m × 0.22 mm I.D., film thickness 0.25 μm, BPX70 (equivalent to 70% cyanopropyl siloxane), cross-linked, fused-silica capillary column (SGE) was used for GC. The column was interfaced with the ion source via a separator oven held at 260°C. The injector port was held at 240°C. The oven was programmed from 185 to 260°C, ramping at a rate of 3°C/min and held at final temperature for 10 min.

The partially methylated alditol acetates were introduced into the column via a split/splitless injector operating in the split mode. The carrier gas used was helium (ultra-high purity, C.I.G., Melbourne, Australia) at a flow-rate of 0.78 ml/min.

Electron impact ionisation at an ionisation potential of 70 eV was used for MS. Using the reconstructed ion chromatogram (RIC) obtained by scanning from 100 to 350 *m/z* in 0.3 s, derivatives eluting from the gas chromatograph were detected and identified by comparison of their mass spectra with standard spectra.

RESULTS AND DISCUSSION

The retention times of the partially methylated sugars relative to the internal standard, *myo*-inositol hexa-acetate, separated on a high-polarity, bonded phase, fused-silica BPX70 capillary column, are listed in Table I. *myo*-Inositol hexa-acetate eluted at 24.28 min. Peaks were identified by comparison of their mass spectra with standard spectra.

Fig. 1 shows the reconstructed ion chromatogram for partially methylated galactitol acetates separated on a BPX70 capillary column. The peaks which have not been assigned represent contaminants, such as plasticisers, introduced during the preparation of partially methylated alditol acetates. The improved resolution provides easy identification of the derivatives on the basis of both their mass spectra and relative retention times. The BPX70 being a bonded phase column also has the advantage of minimal bleed during chromatography.

Other high-polarity columns such as the CP-Sil88 [8], BP-75 [9], SP-2330 [7] and SP-2340 [10] have also been used to separate partially methylated alditol acetates. The order of polarity for the columns from lowest polarity to highest polarity based on average McReynolds constants [12] is the BPX70, BP-75, SP-2330, CP-Sil88, and SP-2340. The latter two being equivalent in polarity.

The BPX70 exhibited the ability to separate several derivatives which would co-elute on the

TABLE I

RETENTION TIMES OF PARTIALLY METHYLATED ALDITOL ACETATES RELATIVE TO *myo*-INOSITOL ON A BPX70 CAPILLARY COLUMN

Position of O-methyl group[a]	Parent monosaccharide[b]						
	Ara	Xyl	Fuc	Rha	Gal	Glc	Man
None	0.624	0.726	0.512	0.491	0.906	0.949	0.868
2-	0.529	0.586*	0.445	0.430	0.813	0.827	0.779
3-	0.553	0.586*	0.501	0.478	0.897*	0.864	0.855*
4-	0.546	0.586*	0.494	0.455	0.897*	0.909	0.855*
5-	0.440	–	–	–	–	–	–
6-	–	–	–	–	0.699	0.742	0.667
2,3-	0.404*	0.436+	0.384	0.344*	0.742	0.723	0.677
2,4-	0.406*	0.400	0.365	0.344*	0.751	0.701	0.710+
2,5-	0.357	–	–	–	–	–	–
2,6-	–	–	–	–	0.617	0.636	0.595
3,4-	0.413	0.436+	0.395	0.334	0.780	0.727	0.710+
3,5-	0.329	–	–	–	–	–	–
3,6-	–	–	–	–	0.667	0.663	0.654
4,6-	–	–	–	–	0.622	0.648	0.587
2,3,4-	0.270	0.277	0.266	0.219+	0.605	0.533	0.530
2,3,5-	0.228	–	–	0.219+	–	–	–
2,3,6-	–	–	–	–	0.533	0.544	0.501
2,4,6-	–	–	–	–	0.499	0.470	0.478°
2,5,6-	–	–	–	–	–	–	–
3,4,6-	–	–	–	–	0.526	0.482	0.478°
2,3,4,6-	–	–	–	–	0.381	0.342	0.338
2,3,5,6-	–	–	–	–	–	–	–

[a] Denotes 2-O-methyl galactitol = 1,3,4,5-tetra-O-acetyl-2-O-methyl galactitol, etc.

[b] Ara = Arabinitol; Xyl = xylitol; Fuc = fucitol; Rha = rhamnitol; Gal = galactitol; Glc = glucitol and Man = mannitol. *, + and ° denote co-eluting derivatives.

other columns [7–10]. These derivatives being 4,6-OCH_3/2,6-OCH_3-galactitol which co-eluted on the CP-Sil88 [8], SP-2330 [7] and the SP-2340 [10] and 2,3-OCH_3/4,6-OCH_3-glucitol which co-eluted on the SP-2330 [7] and the SP-2340 [10]. The elution order of fucitol derivatives on all four columns was identical. For derivatives of arabinose, 3,4-OCH_3/5-OCH_3 exhibited a reversed order of elution on the CP-Sil88 [8]. It is also worth noting that while some derivatives were not separated on the BPX70, they were on one or more of the other high-polarity columns. These include: (a) Arabinitol derivatives; 2,3-OCH_3/2,4-OCH_3 which were separated on the BP-75 [9] and CP-Sil88 [8]. (b) Xylitol derivatives; 3-OCH_3 which were separated from 2-OCH_3/4-OCH_3 on both the BP-75 [9] and CP-Sil88 [8] but all three derivatives co-eluted on the BPX70. (c) Rhamnitol derivatives; 2,3,4-OCH_3/2,3,5-OCH_3 which were separated on the BP-75 [9] and 2,3-OCH_3/2,4-OCH_3 were separated on the CP-Sil88 [8]. (d) Mannitol derivatives; 2,4,6-OCH_3/3,4,6-OCH_3-mannitol which were separated on the BP-75 [9] and CP-Sil88 [8]; and 3,4-OCH_3/2,4-OCH_3 which were separated on the SP-2330 [7] and SP-2340 [10].

Although there are some differences in the order of elution of some derivatives on the different high-polarity columns, more significant changes can be achieved by using a capillary column of low polarity such as the SP-2100 [13] and the CP-Sil5 [10]. Both the SP-2100 and CP-Sil5 are dimethyl polysiloxane phase. Thus by using two columns, one of high polarity and one of low polarity, laboratories without access to MS facilities are provided with a greater

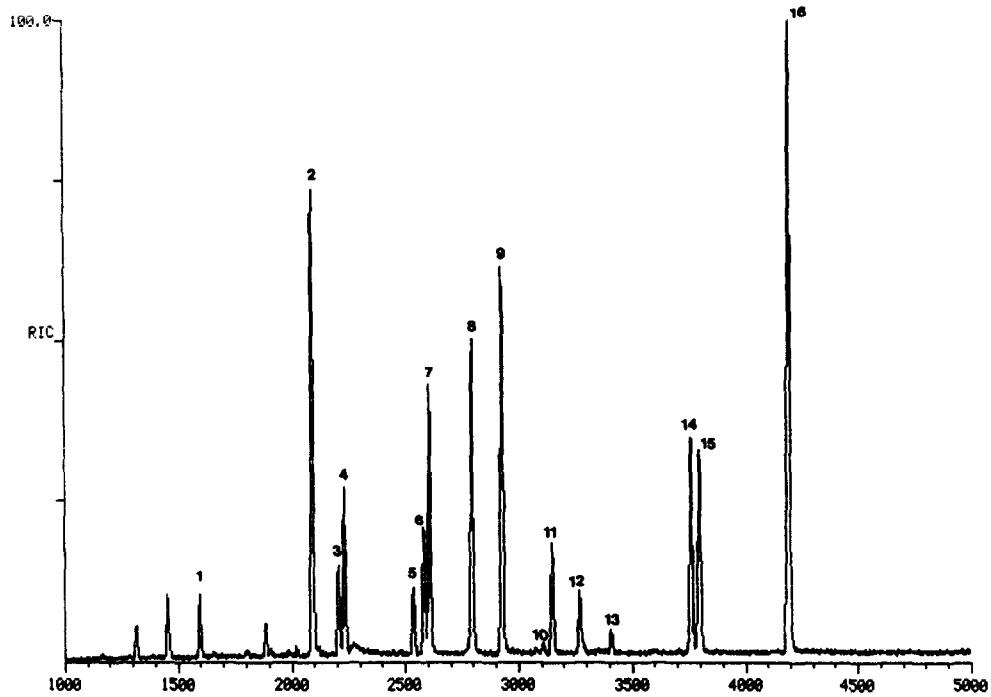

Fig. 1. Reconstructed ion chromatogram of partially methylated galactitol acetates. 1 = 2,3,4,6-OCH_3; 2 = 2,4,6-OCH_3; 3 = 3,4,6-OCH_3; 4 = 2,3,6-OCH_3; 5 = 2,3,4-OCH_3; 6 = 2,6-OCH_3; 7 = 4,6-OCH_3; 8 = 3,6-OCH_3; 9 = 6-OCH_3; 10 = 2,3-OCH_3; 11 = 2,4-OCH_3; 12 = 3,4-OCH_3; 13 = 2-OCH_3; 14 = 3-OCH_3/4-OCH_3; 15 = galactitol hexa-acetate; 16 = *myo*-inositol hexa-acetate (internal standard).

degree of certainty in peak identification based solely on retention times. Routine separation of partially methylated alditol acetates in this manner also provides the added advantage of quantification of the individual components in a mixture.

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