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Note

Capillary gas chromatography of partially methylated alditol acetates on a SP-2100 wall-coated open-tubular column

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The positions of the glycosyl linkages between monosaccharide residues in oligosaccharides, polysaccharides and glycoconjugates are commonly determined by methylation analysis[1–6]. The resulting partially methylated alditol acetates are separated by gas chromatography (GC) and can be identified by mass spectrometry (MS) and by their retention times relative to an internal standard. Recently we reported the retention times of a range of partially methylated alditol acetates on a high-polarity wall-coated open-tubular (WCOT) column, BP-75, produced by bonding the highly polar phase, OV-275, on vitreous silica[7]. Although this column gives excellent resolution of many derivatives, co-elution of some derivatives leads to difficulties in their identification and quantification. Previous reports[8–11] indicate that the order of elution of some partially methylated alditol acetates can be different on phases of lower polarity. In this paper, we report the retention times of a range of partially methylated alditol acetates on a vitreous silica column coated with the low-polarity phase, SP-2100. The partially methylated alditol acetates include those that commonly result from the methylation analysis of plant polysaccharides[12].

EXPERIMENTAL

Partially methylated alditol acetates, prepared as previously described[7], were separated and identified by capillary GC–MS using a fully automated Finnigan MAT 1020B GC–MS instrument (Sunnyvale, CA, U.S.A.).

For GC, a 15 m × 0.24 mm I.D. fused-silica WCOT column coated with SP-2100 (film thickness 0.25 μm) (Supelco, Bellefonte, Pa, U.S.A.) was used. The column was interfaced with the ion source via a separator oven which was maintained at 250°C. The injection port was held at 240°C and the oven temperature was programmed from 120 to 200°C at 2°C/min. Samples, in dichloromethane, were introduced via a Finnigan MAT split/splitless injector using the split mode. Helium (ultra-high purity, C.I.G., Melbourne, Australia) was used as the carrier gas at a flow-rate of 0.78 ml/min. MS conditions were as previously described[7].

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RESULTS AND DISCUSSION

The retention times, relative to *myo*-inositol hexaacetate, of a range of partially methylated alditol acetates separated on a fused-silica WCOT column coated with SP-2100 are shown in Table I.

Geyer *et al.*[8] chromatographed a range of partially methylated alditol acetates on SE-30 and OV-101 WCOT glass capillary columns. OV-101 is a low-polarity dimethyl polysiloxane phase similar to SP-2100[13]. SE-30 and BP-1 differ in that they have a higher molecular weight and, in addition, BP-1 is cross-linked. The order of elution of the partially methylated alditol acetates is the same on SP-2100 as that reported for OV-101[8], SE-30[8] and BP-1[11], but there are differences on phases of higher polarity, such as the highly polar phase, BP-75[7].

TABLE I

RETENTION TIMES OF PARTIALLY METHYLATED ALDITOL ACETATES ON AN SP-2100 WCOT CAPILLARY COLUMN

Retention times are relative to *myo*-inositol hexaacetate = 1.000. Ara = Arabinose; Xyl = xylose; Fuc = fucose; Rha = rhamnose; Gal = galactose; Glc = glucose; Man = mannose.

Position of O-methyl group*	Retention time						
	Parent monosaccharide						
	Ara	*Xyl*	*Fuc*	*Rha*	*Gal*	*Glc*	*Man*
-	0.573	0.583**,***	0.566	0.565	0.936	0.927	0.912
-	0.591	0.587***	0.604	0.599	0.986**	0.955	0.960**
-	0.578	0.583**,***	0.607	0.588	0.986**	0.971	0.960**
-	0.511	–	–	–	–	–	–
-	–	–	–	–	0.857	0.855	0.842§
,3-	0.466	0.468§	0.489	0.479**	0.845	0.832	0.821
,4-	0.474**	0.457	0.504	0.489	0.883***	0.840**	0.856
,5-	0.424	–	–	–	–	–	–
,6-	–	–	–	–	0.761	0.765	0.754
,4-	0.474**	0.468§	0.508	0.479**	0.880***	0.840**	0.842§
,5-	0.420	–	–	–	–	–	–
,6-	–	–	–	–	0.792	0.788	0.790
,6-	–	–	–	–	0.787	0.782	0.768
,3,4-	0.357	0.348	0.394	0.365	0.748	0.698	0.700
,3,5-	0.314	–	–	–	–	–	–
,3,6-	–	–	–	–	0.661	0.673	0.661
,4,6-	–	–	–	–	0.685§	0.655	0.671
,5,6-	–	–	–	–	–	–	–
,4,6-	–	–	–	–	0.685§	0.647	0.655
,3,4,6-	–	–	–	–	0.558	0.526	0.531
,3,5,6-	–	–	–	–	0.537	–	–
None	0.662	0.687	0.656	0.646	1.036	1.023	1.007

* 2-O-Methyl arabinitol = 1,3,4,5-tetra-O-acetyl-2-O-methyl arabinitol; etc.

**,§ Co-eluting derivatives from the same parent monosaccharide.

*** Incompletely resolved derivatives from the same parent monosaccharide.

The order of elution of partially methylated arabinitol acetates on SP-2100 was the same as that on BP-75, except that the 2,4- and 3,4-O-methyl derivatives were not separated on SP-2100. For partially methylated xylitol acetates the order was again the same except that on SP-2100 the 3-O-methyl derivative eluted slightly after, rather than slightly before, the co-eluting 2- and 4-O-methyl derivatives.

For partially methylated fucitol acetates the order of elution of the 2,3- and 2,4-O-methyl derivatives and the 3- and 4-O-methyl derivatives on SP-2100 was reversed compared to BP-75. However, for partially methylated rhamnitol acetates the 2,3- and 3,4-O-methyl derivatives co-eluted before the 2,4-O-methyl derivative on SP-2100, whereas on BP-75 the 2,3- and 2,4-O-methyl derivatives co-eluted after the 3,4-O-methyl derivative.

The order of elution of a number of partially methylated hexitol acetates on SP-2100 and BP-75 was also reversed. These were:

(a) for galactitol derivatives: 2,3,6- and 2,4,6-O-methyl, 2,3- and 6-O-methyl, 3,4- and 2,4-O-methyl;

(b) for glucitol derivatives: 3,4,6- and 2,4,6-O-methyl, 2,3,6- and 2,3,4-O-methyl, 2,3- and 2,4-O-methyl;

(c) for mannitol derivatives: 2,3,6- and 2,4,6-O-methyl, 2,6- and 4,6-O-methyl, 2,3- and 6-O-methyl.

In addition, 3,4,6-O-methyl galactitol acetate co-eluted with 2,4,6-O-methyl galactitol acetate on SP-2100, whereas it eluted after it on BP-75, but before 2,3,6-O-methyl galactitol acetate. Also in contrast to BP-75, 2,3- and 3,4-O-methyl glucitol acetates were resolved on SP-2100, but the latter co-eluted with 2,4-O-methyl glucitol acetate. Similarly, 2,4-O-methyl mannitol acetate and 3,4-O-methyl mannitol acetates were resolved on SP-2100, but the latter co-eluted with 6-O-methyl mannitol acetate.

SP-2100 is thus an excellent second phase that can be used in conjunction with a high-polarity phase, such as BP-75, for the separation of partially methylated alditol acetates. The use of two such columns will be especially useful for those without access to MS facilities and who rely solely on retention times to assign peak identity.

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