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## GAS-LIQUID CHROMATOGRAPHY OF SOME PARTIALLY METHYLATED ALDITOLS AS THEIR TRIMETHYLSILYL ETHERS

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## SUMMARY

The relative retention times and molar response factors of trimethylsilyl ethers of some partially methylated D-galactitols, L-arabinitols and L-rhamnitol on the stationary phases SE-52, SE-30, OV-101, OV-17, OV-25, QF-1 and XE-60 have been determined and the relative efficiencies of these systems compared.

## INTRODUCTION

The gas-liquid chromatography (GLC) of partially methylated sugars, either as their methyl glycoside<sup>1,2</sup> or alditol acetate<sup>3,4</sup> derivatives, has been extensively studied<sup>5</sup>. Both methods, however, have some disadvantages.

The polar phases generally used for the separation of the methyl glycosides suffer from insufficient thermal stability. The operational temperature range of these packings is very limited owing to the relatively high concentration (5-20%) of stationary phase that is required to prevent significant changes in packing composition and consequent variation of retention characteristics. At high temperatures excessive column bleed is experienced, whereas at lower temperatures the retention times of the methyl glycosides become too long. Methyl di-O-methylhexosides have retention times of up to 2 h at 160° and the glycosides of monomethylhexoses and of hexoses themselves are completely retained<sup>2</sup>. The multiple peaks obtained, owing to anomers of the methyl glycosides, lead to poor resolution and difficulties in peak assignment. This imposes further restrictions on the method.

The use of the alditol acetate derivatives of the partially methylated sugars eliminates most of these disadvantages. Retention times are relatively shorter, monomethylhexoses and hexoses are eluted and only one peak is obtained for each sugar<sup>3,4</sup>. However, the thermal stability of the organosilicone polyester phase, consisting of ethylene glycol succinate chemically combined with a silicone of the cyanoethyl type, is still not ideal and column deterioration is rapid<sup>6-8</sup>.

The GLC of the trimethylsilyl (TMS) ethers of carbohydrates has been investigated in some detail<sup>5,9</sup> and has, in fact, been successfully applied to partially methylated glucitols<sup>10</sup>. As the formation of the TMS derivatives is rapid, quantitative<sup>11</sup>

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TABLE I  
RETENTION TIMES OF PARTIALLY METHYLATED ALDITOLS ON VARIOUS STATIONARY PHASES

TMS ethers of methylated alditols	Stationary phase and concentration	OV-101 (3%)		SE-30 (3.8%)		SE-52 (3%)		OV-17 (3%)		QF-1 (5%)		OV-25 (3%)		XE-60 (3%)		Relative molar response factor, $R_s$		
		60-80 mesh Chrom. W	60-80 mesh Supelcoport Chrom. W	80-100 mesh Chrom. W	60-80 mesh Chrom. W	80-100 mesh Chrom. W	60-80 mesh Chrom. W	80-100 mesh Chrom. W	60-80 mesh Chrom. W	80-100 mesh Chrom. W	60-80 mesh Chrom. W	60-80 mesh Chrom. W	Found	Determined from graph				
Galactitols 2,3,4,6G <sup>b</sup> 2,4,6G 2,3,6G 3,4,6G 2,3,4G 2,6G 2,4G 2,3G 3,4G 2G (3)4G 6G G	Solid support Rohr-schneider const. (2)	0.50	0.50	—	1.79	3.55	2.15	3.62	1.00 (9)	1.00 (11)	1.00 (5)	1.00 (8)	1.00 (2)	1.00 (4)	1.00 (3)	1.00	1.00	
		1.25	1.25	1.19	0.92	—	—	—	—	—	—	—	—	—	—	—	1.06	1.06
		1.34	1.35	1.28	1.00	—	—	—	—	—	—	—	—	—	—	—	1.09	1.09
		1.39	1.38	1.32	1.22	—	—	—	—	—	—	—	—	—	—	—	1.10	1.10
		1.57	1.57	1.50	1.20	—	—	—	—	—	—	—	—	—	—	—	1.16	1.16
		1.79	1.79	1.64	1.00	—	—	—	—	—	—	—	—	—	—	—	1.21	1.21
		1.99	1.99	1.84	1.14	—	—	—	—	—	—	—	—	—	—	—	1.28	1.28
		2.08	2.09	1.95	1.27	—	—	—	—	—	—	—	—	—	—	—	1.31	1.31
		2.18	2.19	2.00	1.26	—	—	—	—	—	—	—	—	—	—	—	1.33	1.33
		2.89	2.88	2.62	1.35	—	—	—	—	—	—	—	—	—	—	—	1.53	1.53
2.94	2.92	2.64	1.35	—	—	—	—	—	—	—	—	—	—	—	1.55	1.55		
2.99	2.98	2.66	1.39	—	—	—	—	—	—	—	—	—	—	—	1.56	1.56		
4.63	4.61	4.13	1.83	—	—	—	—	—	—	—	—	—	—	—	2.04	2.04		
Arabinitols 2,3,5A 2,5 + 3,5A 2,3 + 3,4A A		0.42	0.42	0.41	0.37	—	—	—	—	—	—	—	—	—	—	0.73	0.73	
		0.62	0.62	0.58	0.41	—	—	—	—	—	—	—	—	—	—	1.05	1.05	
		0.73	0.73	0.69	0.50	—	—	—	—	—	—	—	—	—	—	1.10	1.10	
		1.55	1.54	1.38	0.68	—	—	—	—	—	—	—	—	—	—	1.50	1.50	
Rhamnitol 2,3,4R 3R R		0.58	0.58	0.57	0.49	—	—	—	—	—	—	—	—	—	—	0.73	0.73	
		1.26	1.25	1.14	0.61	—	—	—	—	—	—	—	—	—	—	1.59	1.59	
		1.93	1.92	1.68	0.77	—	—	—	—	—	—	—	—	—	—	1.66	1.66	
		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

<sup>a</sup> Relative molar response factors determined from the graph,  $R_s$  (Found), vs. average retention time,  $T_R$ , between the OV-101 and SE-52 columns (Fig. 2).

<sup>b</sup> Values in parentheses give actual retention time of standard in minutes.

and reversible, it was thought that the separation by GLC of the TMS ethers of partially methylated alditols, on the thermally more stable silicone phases, would be a suitable analytical procedure.

## EXPERIMENTAL

### *Materials*

The methylated sugars used had either been isolated from the hydrolysis products of methylated polysaccharides or had been synthesized in connection with other work. The methyl sugars were reduced in water with sodium borohydride, as described earlier<sup>3,8</sup>, extracted into chloroform and dried *in vacuo*.

### *Preparation of TMS derivatives*

The partially methylated alditols were converted to their TMS derivatives by the method of SWEELEY *et al.*<sup>11</sup>. The mixtures, stored in sealed vials (Clinbritic vials, with septa), were allowed to stand for 1 h before injection.

### *Gas-liquid chromatography*

A Beckman GC-4 chromatograph with a flame ionization detector and glass columns (1.80 m  $\times$  6.25 mm O.D.) was used. The chromatograph was operated at 140°, the flow-rate of the carrier gas, helium, being 60 ml/min. Stationary phases were deposited on the solid supports by the "slurry" method. The various packings used are shown in Table I.

Relative molar response factors,  $R_s$ , were calculated by injection of the TMS derivatives of weighed samples of the partially methylated alditols. A minimum of three injections per standard mixture was used. In all instances the TMS derivative of 2,3,4,6-tetra-O-methyl-D-galactitol was used as the internal standard.

## RESULTS AND DISCUSSION

The results obtained (Table I) indicate that the non-polar stationary phases OV-101, SE-30 and SE-52 are most suitable for the separation of complex mixtures of TMS derivatives of partially methylated alditols, as obtained, for example, during methylation analyses of polysaccharides. The mildly polar phase OV-17 would be of value with simple mixtures, as a complete analysis can be performed in less than 15 min under the conditions specified below. The moderately polar phase OV-25 and the highly polar phase XE-60 are totally unsuitable, giving very poor resolution. Surprisingly, the highly polar phase QF-1 gives slight separation, probably as a result of complex interactions between the sample and this phase.

The Rohrschneider constants ( $\alpha$ )<sup>12,13</sup>, a measure of column polarity, plotted against the retention times, illustrate the relative efficiencies of the various stationary phases. The plots of the TMS derivatives of galactitol, 2-O-methyl-D-galactitol, 2,4-di-O-methyl-D-galactitol and 2,3,4-tri-O-methyl-D-galactitol are shown in Fig. 1. It can be seen that as the Rohrschneider constant ( $\alpha$ ) increases, the relative separation between these partially methylated alditols decreases, the only exception being the phase QF-1.

Complex mixtures of TMS derivatives of partially methylated alditols are best

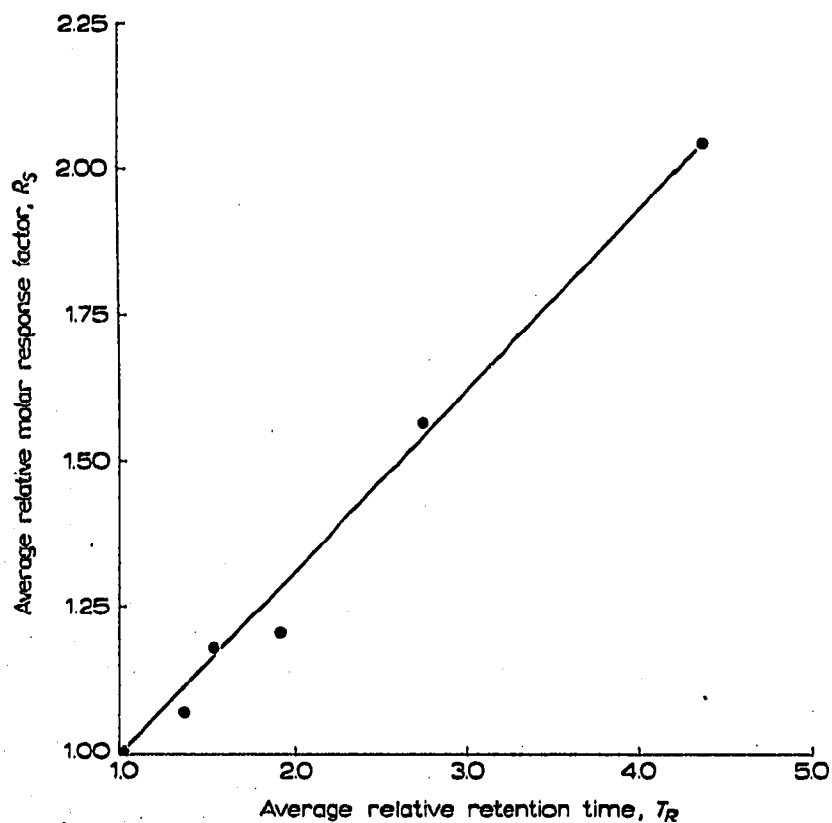
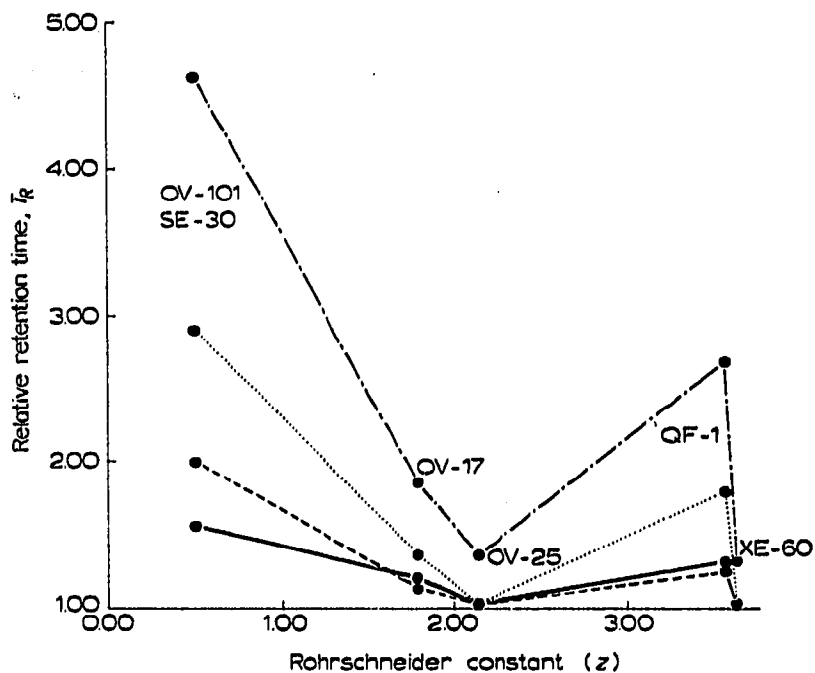


Fig. 1. Relative retention time,  $T_R$ , vs. Rohrschneider constant,  $z$ , for TMS derivatives of some partially methylated galactitols on various stationary phases: ●---●, galactitol; ●····●, 2-O-methyl-D-galactitol; ●---●, 2,4-di-O-methyl-D-galactitol; ●—●, 2,3,4-tri-O-methyl-D-galactitol.

Fig. 2. Average relative molar response factor,  $R_S$ , vs. average relative retention time,  $T_R$ , for TMS derivatives of partially methylated galactitols on OV-101 and SE-52.

separated on non-polar stationary phases having Rohrschneider constants ( $z$ ) less than 1. These stationary phases resolve the various TMS derivatives largely according to their boiling-points. Relative suitabilities of the stationary phases used are as follows: OV-101 ( $\equiv$  SE-30)  $>$  SE-52  $>$  OV-17  $\sim$  QF-1  $>$  OV-25  $>$  XE-60. Stationary phases having values of  $z > 1$  may prove useful for simple mixtures when time is the critical factor.

The relative molar response factors,  $R_s$ , found for the various partially methylated galactitols when plotted against their relative retention times,  $T_R$ , gave a linear relationship (Fig. 2). This relationship was used to obtain unknown  $R_s$  values and to improve on those found experimentally.

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