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GAS–LIQUID CHROMATOGRAPHY OF TRIMETHYLSILYL DISACCHARIDES

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SUMMARY

The relative retention times of twenty-three trimethylsilyl disaccharides on three liquid phases varying in polarity, *viz.* OV-1, OV-17 and OV-25, are reported. Comparison of these values makes it clear that they are systematically influenced by each of the structural elements of the disaccharide.

INTRODUCTION

Gas–liquid chromatography (GLC) has become an important tool in the analysis of carbohydrates since the introduction of the trimethylsilyl (TMS) group as a protecting group by SWEELEY *et al.*[1]. A large amount of data are available on the GLC of TMS monosaccharides. The application of this technique to TMS ethers of disaccharides is mainly restricted to a few common representatives of this series[1–6], although PERCIVAL[7] investigated some less common ones.

In the course of our studies on the structure determination of carbohydrate-containing polymers we needed a method for the separation and identification of rather complex mixtures of oligosaccharides. With this in mind the GLC of TMS ethers of 23 disaccharides was studied. In this series of model compounds the possible variations in the position of the glycosidic bond are considered. Three liquid phases were tested for their suitability as column coatings *viz.* OV-1 (non-polar), OV-17 (medium-polar) and OV-25 (polar).

MATERIALS AND METHODS

Disaccharides

α,α-D-Trehalose dihydrate, β-D(+)-maltose monohydrate, β-D(+)-cellobiose, α-D(+)-lactose monohydrate, isomaltose, β-gentiobiose, α-D-melibiose monohydrate, D(+)-sucrose and D-lactulose were purchased from J. T. Baker Chemicals N.V.; turanose was purchased from Pierce Chemicals Company and palatinose from EGA-Chemie K.G. The following compounds were gifts: β,β-trehalose, laminaribiose, mannobiose, maniocose, 6-O-α-D-mannopyranosyl-D-glucose, 6-O-β-D-galactopyranosyl-D-galactose, 3-O-β-D-galactopyranosyl-D-arabinose, 2-O-β-D-glucopyranosyl-L-arabinose, α-kojibiose octaacetate, α-sophorose monohydrate, neolactose and prime-

TABLE I

R_s VALUES AND PEAK AREA RATIOS OF THE TMS-ETHERS OF DISACCHARIDES

Relative peak areas of the anomers are given in parentheses.

	Carbohydrate		3% *OV-1*	3% *OV-17*	3% *OV-25*
I	α-D-Glucopyranosyl-(1 → 1)-α-D-glucopyranoside	(α,α-trehalose)	1.34	1.38	1.31
II	β-D-Glucopyranosyl-(1 → 1)-β-D-glucopyranoside	(β,β-trehalose)	1.77	1.90	1.70
III	α-D-Glucopyranosyl-(1 → 2)-D-glucose	(kojibiose)	1.38 (10) 1.69 (7)	1.40 (10) 1.82 (7)	1.31 (10) 1.65 (7)
IV	β-D-Glucopyranosyl-(1 → 2)-D-glucose	(sophorose)	1.59 (3.5) 1.85 (10)	1.66 (3.5) 1.99 (10)	1.57 (3.5) 1.82 (10)
IV*	β-D-Glucopyranosyl-(1 → 2)-α-D-glucose		1.85	1.99	1.82
V	β-D-Glucopyranosyl-(1 → 3)-D-glucose	(laminaribiose)	1.56 (8) 1.76 (10)	1.64 (8) 1.80 (10)	1.53 (8) 1.62 (10)
VI	α-D-Glucopyranosyl-(1 → 4)-D-glucopyranose	(maltose)	1.12 (5) 1.30 (10)	1.19 (5) 1.33 (10)	1.18 (5) 1.26 (10)
VI*	α-D-Glucopyranosyl-(1 → 4)-β-D-glucopyranose		1.30	1.33	1.26
VII	β-D-Glucopyranosyl-(1 → 4)-D-glucopyranose	(cellobiose)	1.15 (7) 1.67 (10)	1.22 (7) 1.70 (10)	1.21 (7) 1.57 (10)
VII*	β-D-Glucopyranosyl-(1 → 4)-β-D-glucopyranose		1.67	1.70	1.57
VIII	β-D-Galactopyranosyl-(1 → 4)-D-glucopyranose	(lactose)	1.00 (7) 1.50 (10)	1.09 (7) 1.54 (10)	1.10 (7) 1.44 (10)
VIII*	β-D-Galactopyranosyl-(1 → 4)-α-D-glucopyranose		1.00	1.09	1.10
IX	β-D-Galactopyranosyl-(1 → 4)-D-altropyranose	(neolactose)	0.85	0.81 (4) 0.87 (10)	0.86 (4) 0.88 (10)

X	β-D-Mannopyranosyl-(1 → 4)-D-mannopyranose	(mannobiose)	1.21 1.59	(10) (4)	1.18 1.52	(10) (4)	1.30 1.40	(10) (4)
XI	α-D-Glucopyranosyl-(1 → 5)-D-glucofuranose	(maniocose)	2.01 2.49	(4.5) (10)	2.40 2.80	(4.5) (10)	2.15 2.42	(4.5) (10)
XII	α-D-Glucopyranosyl-(1 → 6)-D-glucose	(isomaltose)	2.02 2.48	(3) (10)	2.37 2.77	(3) (10)	2.14 2.42	(3) (10)
XIII	β-D-Glucopyranosyl-(1 → 6)-D-glucose	(gentiobiose)	2.02 2.50	(4) (10)	2.36 2.76	(4) (10)	2.14 2.40	(4) (10)
XIV	α-D-Galactopyranosyl-(1 → 6)-D-glucose	(melibiose)	2.02 2.26	(5) (10)	2.16 2.33	(5) (10)	1.98 2.08	(5) (10)
XIV*	α-D-Galactopyranosyl-(1 → 6)-α-D-glucose		2.02		2.16		1.98	
XV	α-D-Mannopyranosyl-(1 → 6)-D-glucose		1.55 1.98	(9) (10)	1.70 2.08	(9) (10)	1.59 1.85	(9) (10)
XVI	β-D-Galactopyranosyl-(1 → 6)-D-galactose		2.26		2.38		2.08	
XVII	β-D-Glucopyranosyl-(1 → 2)-L-arabinose		0.76 0.83	(8) (10)	0.95		0.92	
XVIII	β-D-Galactopyranosyl-(1 → 3)-D-arabinose		0.63 0.74 0.80	(10) (6) (5)	0.73 0.90 1.03	(10) (6) (6)	0.75 0.90	(10) (9)
XIX	β-D-Xylopyranosyl-(1 → 6)-D-glucose	(primeverose)	1.49		1.92 1.96	(7) (10)	1.68 1.78	(10) (7)
XX	α-D-Glucopyranosyl-(1 → 2)-β-D-fructofuranoside	(sucrose)	1.00		1.00		1.00	
XXI	α-D-Glucopyranosyl-(1 → 3)-D-fructose	(turanose)	1.28		1.28 1.56	(10) (4)	1.21 1.47	(10) (4)
XXII	β-D-Galactopyranosyl-(1 → 4)-D-fructose	(lactulose)	1.00		0.94		0.97	
XXIII	α-D-Glucopyranosyl-(1 → 6)-D-fructofuranose	(palatinose)	1.25 1.35 1.43	(1) (2) (10)	1.39 1.48 1.79	(2) (10) (1)	1.34 1.40 1.70	(2) (10) (1)

verose. Sugars were anomerized in water for 48 h at room temperature, and subsequently lyophilized.

Preparation of TMS derivatives

The disaccharides were converted to the TMS derivatives by means of hexamethyldisilazane (Koch Light Ltd.) and trimethylchlorosilane (Schuchardt) in pyridine as described earlier[8].

Gas–liquid chromatography

An F and M gas chromatograph Model 700 equipped with a dual flame ionization detector and coiled stainless steel columns (2.70 m × 3.2 mm O.D.) was used. The packing materials were: 3% OV-1, 3% OV-17 and 3% OV-25 on Chromosorb W (H.P.), 80–100 mesh, and were obtained from Pierce Chemicals Company. The temperature conditions were the following: injection port 270°, detector 310°, column oven 228°. The gas flow rates for H_2 and air were 45 ml/min and 375 ml/min, respectively. The gas flow rate of the carrier gas N_2 was 26 ml/min on 3% OV-1, 18 ml/min on 3% OV-17 and 5 ml/min on 3% OV-25.

RESULTS AND DISCUSSION

The R_S values and peak area ratios for the TMS-derivatives of disaccharides I–XXIII on the three stationary phases are presented in Table I. TMS-sucrose was used as an internal standard (R_S = 1.00); the retention times of this compound were 14.6, 12.1 and 13.1 min on OV-1, OV-17 and OV-25, respectively. In the cases of α-D-G*p*-(1→2)-D-G (III), α-D-Man*p*-(1→6)-D-G (XV) and β-D-Gal*p*-(1→6)-D-Gal (XVI) in addition to the peaks mentioned in Table I, there are some small peaks which probably represent the furanose forms. The gas chromatograms of α-D-G*p*-(1→5)-D-G*f* (XI), α-D-G*p*-(1→6)-D-G (XII) and β-D-G*p*-(1→6)-D-G (XIII) show a great similarity. There are a few minor peaks present besides the main peaks which were attributed to the furanose forms of XI and the pyranose forms of XII and XIII.

Stationary phases

The three liquid phases OV-1 (non-polar), OV-17 (medium-polar) and OV-25 (polar) differ in the ratio of phenyl to methyl groups in the silicone oil. In general OV-17 gives the best separation, although, incidentally, OV-1 has definite advantages, for *e.g.* in the resolution of the following pairs of compounds: α-D-G*p*-(1→4)-D-G*p* (VI) and β-D-Man*p*-(1→4)-D-Man*p* (X); β-D-G*p*-(1→2)-L-Ara (XVII) and β-D-Gal*p*-(1→4)-D-Fru (XXII); and α-D-Man*p*-(1→6)-D-G (XV) and β-D-G*p*-(1→4)-D-G*p* (VII). OV-1 and OV-17 are nearly equivalent in their ability to separate anomeric forms, and can be used in preference to OV-25, except in the case of β-D-Xyl*p*-(1→6)-D-G (XIX). This aspect of fractionating anomeric forms is important because reducing sugars, obtained as breakdown products from oligo- or polymers, will mostly consist of mixtures of anomers. The occurrence of different forms may be an advantage as the combination of R_S and peak area data can be used for the identification of the disaccharides.

The suitability of OV-17 for the separation of TMS disaccharides is illustrated in Fig. 1. In complex mixtures the R_S values of the components remained identical

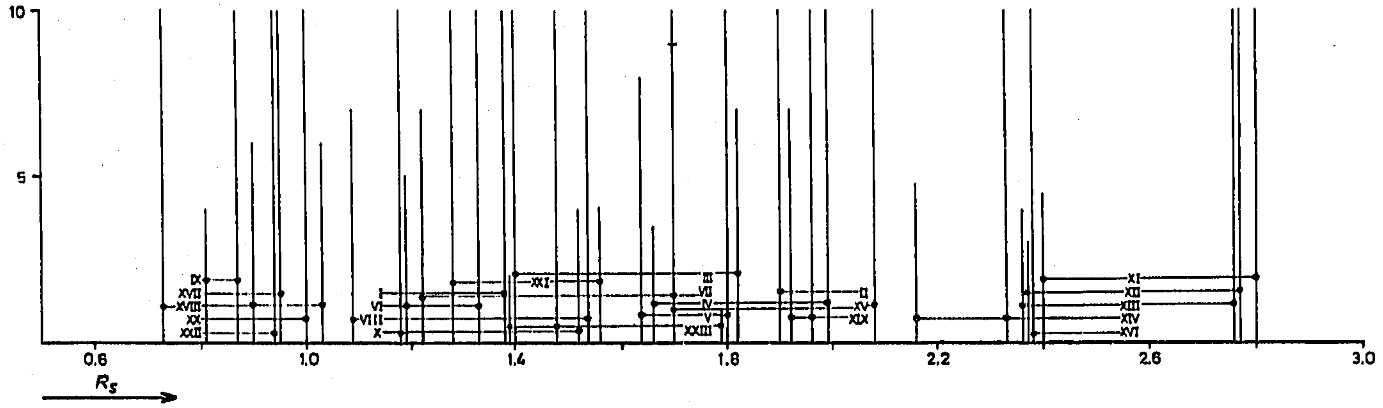

Fig. 1. The data compiled in Table I are graphically presented in this figure. The R_S values are given on the abcissa and the ratio of the peak areas of the anomers of each disaccharide on the ordinate. At R_S = 1.70 two peaks coincide.

to those listed in Table I. The resolution of α-D-G*p*-(1 → 5)-D-G*f* (XI), α-D-G*p*-(1 → 6)-D-G (XII) and β-D-G*p*-(1 → 6)-D-G (XIII) could not be achieved on any of the stationary phases.

Comparison of R_S values

Table I shows that for the (1 → 4) and (1 → 6) aldohexosyl-aldohexoses: α-D-G*p*-(1 → 4)-D-G*p* (VI); β-D-G*p*-(1 → 4)-D-G*p* (VII); β-D-Gal*p*-(1 → 4)-D-G*p* (VIII) and α-D-Gal*p*-(1 → 6)-D-G (XIV), which all contain D-glucose at the reducing end, the β-anomer has a longer retention time than the α-anomer. These results are analogous to the observations of SWEELEY, who found that the monosaccharide form with an equatorial hydroxyl group on C_1 has the longest retention time, provided that the stable conformation is the chair form C1 or 1C. However, β-D-G*p*-(1 → 2)-D-G (IV) shows the reverse sequence of anomers. This anomalous behaviour may be a consequence of the bulky substituent at C_2 of the reducing D-glucose unit. It would be worthwhile investigating whether this feature is specific for (1 → 2)-aldohexosyl-aldohexoses. The reversal of the R_S values of the primeverose anomers (XIX) going from OV-17 to OV-25 indicates, that when the R_S values of the anomers are very close together, the stationary phase may influence the sequence of the peaks.

Comparison of the R_S values of TMS disaccharides, differing only in the con-

TABLE II

COMPARISON OF THE R_S VALUES OF DISACCHARIDES DIFFERING IN THE CONFIGURATION OF THE GLYCOSIDIC BOND

	Carbohydrate	*R_S on OV-1*	*R_S on OV-17*	*R_S on OV-25*
I	α-D-G*p*-(1 → 1)-α-D-G*p*	1.34	1.38	1.31
II	β-D-G*p*-(1 → 1)-β-D-G*p*	1.77	1.90	1.70
III	α-D-G*p*-(1 → 2)-D-G	1.38 and 1.69	1.40 and 1.82	1.31 and 1.65
IV	β-D-G*p*-(1 → 2)-D-G	1.59 and 1.85	1.66 and 1.99	1.57 and 1.82
VI	α-D-G*p*-(1 → 4)-D-G*p*	1.12 and 1.30	1.19 and 1.33	1.18 and 1.26
VII	β-D-G*p*-(1 → 4)-D-G*p*	1.15 and 1.67	1.22 and 1.70	1.21 and 1.57

TABLE III

EFFECT OF CHANGING THE ALDOHEXOSE UNIT ON THE R_S VALUE

	Carbohydrate	*R_S on OV-17*
VII	β-D-G*p*-(1 → 4)-D-G*p*	1.22 and 1.70
VIII	β-D-Gal*p*-(1 → 4)-D-G*p*	1.09 and 1.54
	β-D-G*p*	1.68[a]
	β-D-Gal*p*	1.08[a]
VIII	β-D-Gal*p*-(1 → 4)-D-G*p*	1.09 and 1.54
IX	β-D-Gal*p*-(1 → 4)-D-Alt*p*	0.81 and 0.87
	D-G*p*	(1.00 and 1.57)[b]
	D-Alt*p*	(0.65 and 0.68)[b]
XII	α-D-G*p*-(1 → 6)-D-G	2.37 and 2.77
XIV	α-D-Gal*p*-(1 → 6)-D-G	2.16 and 2.33
XV	α-D-Man*p*-(1 → 6)-D-G	1.70 and 2.08
	α-D-G*p*	1.05[a]
	α-D-Gal*p*	0.87[a]
	α-D-Man*p*	0.63[a]

[a] TMS-sorbitol is taken as an internal standard at 150°.
[b] Measured by SWEELEY *et al.*[1] on SE-52 at 140°, relative to α-D-G*p*.

figuration of the glycosidic bond (Table II) shows, that in general the components with the β-configuration have the longest retention times.

The influence of the constituent monosaccharides on the R_S values of the disaccharides can be demonstrated by comparison of disaccharides which differ in one monosaccharide (Table III). Replacement of an aldohexose unit X in a disaccharide by a stereoisomer Y results in a shift of the R_S value of the TMS disaccharide to lower values, in the case where the R_S value of Y is lower than that of X.

The changes in the R_S values of the disaccharides, observed so far, are restricted to components differing in one structural aspect only.

CONCLUSIONS

The optimal separation of TMS disaccharides is obtained on 3% OV-17 as stationary phase. Nevertheless it seems advisable to use at least two liquid phases with different polarity for the analysis of unknown mixtures.

For the identification of reducing sugars by GLC it may be advantageous to analyse equilibrium mixtures of anomeric forms. The combination of R_S values and peak areas gives more characteristic information about such a component than the single R_S value obtained after reduction to the corresponding alditol. However, for the preparative separation of disaccharides via GLC, reduction to alditols may be preferred as this conversion greatly diminishes the number of components in the mixture.

Comparison of R_S values of disaccharides which differ in one structural element, *viz.* the configuration of the anomeric C atom of the reducing unit, the configuration of the glycosidic bond, or the constituent monosaccharides, shows that at least qualitatively similar changes occur such as are known for monosaccharides.

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