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Gas chromatographic retention of carbohydrate trimethylsilyl ethers

IV. Disaccharides

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ABSTRACT

Trimethylsilyl ethers of seventeen disaccharides were injected on two stationary phases and their retention indices were calculated. Multiple linear regression was used to discuss relationships between retention indices and structural features of disaccharides.

INTRODUCTION

In spite of the large number of papers dealing with the gas chromatographic (GC) analysis of disaccharides as their trimethylsilyl (TMS) derivatives [1,2], most of them are restricted to only a few compounds (sucrose, lactose and maltose being the most representative). Sweeley *et al.* [3] gave the first retention data for eight disaccharides. The most complete reports are those of Haverkamp *et al.* [4] and Nikolov and Reilly [5]. The former lists relative retentions (with respect to sucrose at 228°C) for 23 dissacharides on three stationary phases, and the latter gives retention times relative to sucrose at 240°C for seventeen disaccharides on SE-54. In both instances the elution orders were related to some structural characteristics of the constituent monosaccharides, but the comparisons were restricted to components differing in one structural aspect only. Percival [6] measured relative retentions of fifteen unusual disaccharides on Apiezon and SE-30.

Unfortunately, it is impossible to collect a complete series of disaccharides, *i.e.*, those having the same rings and glycosidic linkages in all possible positions (twelve isomers) or those differing only in the monosaccharide having a free reducting group (eight possible isomers). Most such compounds have not yet been described, and others are very difficult to prepare.

We have previously studied the chromatographic behaviour of pentoses [7], aldohexoses [8] and ketohexoses [9] as TMS ethers. Some general features were found: an unusual retention when they were compared with other ethers, a decrease in their retention indices with increasing temperature and sta-

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tionary phase polarity and several relationships between some structural descriptors and retention.

In this work we determined the retention indices of seventeen disaccharides on two stationary phases, SE-54 and OV-17. The quantitative relationships between the retention of disaccharides and those of their constituent monosaccharides were evaluated.

EXPERIMENTAL

Samples

Lactose (4-O-β-D-galactopyranosyl-D-glucose) and sucrose (2-O-α-D-glucopyranosyl-β-D-fructofuranoside) were purchased from Ferosa (Spain); lactulose (4-O-β-D-galactopyranosyl-D-fructose), melibiose (6-O-α-D-galactopyranosyl-D-glucose), palatinose (6-O-α-D-glucopyranosyl-D-fructofuranose) and turanose (3-O-α-D-glucopyranosyl-D-fructose) were from Fluka (Buchs, Switzerland); cellobiose (4-O-β-D-glucopyranosyl-D-glucose) and gentiobiose (6-O-β-D-glucopyranosyl-D-glucose) from Merck (Darmstadt, Germany); maltose (4-O-α-D-glucopyranosyl-D-glucose) from Difco; and isomaltose (6-O-α-D-glucopyranosyl-D-glucose), galactobiose (6-O-α-D-galactopyranosyl-D-galactose), laminaribiose (3-O-β-D-glucopyranosyl-D-glucose), nigerose (3-O-α-D-glucopyranosyl-D-glucose), α,α-trehalose (1-O-α-D-glucopyranosyl-α-D-glucopyranoside) from Sigma (Eisenhofen, Germany). Epilactose (4-O-β-D-galactopyranosyl-D-mannose), neolactose (4-O-β-D-galactopyranosyl-D-altrose) and maltulose (4-O-α-D-glucopyranosyl-D-fructose) were prepared by Professor A. Olano (Instituto de Fermentaciones Industriales, CSIC, Madrid, Spain).

Samples were dissolved in pyridine or water and left to stand until equilibrated. Aliquots containing about 1 mg of carbohydrate were silylated with trimethylsilylimidazole [7].

Gas chromatographic analysis

GC analyses were carried out on two columns of different polarity. Their characteristics and operating conditions are summarized in Table I. Nitrogen was used as the carrier gas.

Kováts retention indices were calculated from the retention times of disaccharide TMS ethers and those of suitable *n*-alkanes. The dead time was determined by linear regression [10].

Assignments of peaks were made according to previous references [3–5]. In some instances, peaks were assigned by comparison with NMR equilibrium data.

Calculations

Calculations of retention indices and multiple linear regressions were carried out on a personal microcomputer using a multiple linear regression program written in QuickBasic.

RESULTS AND DISCUSSION

Retention indices on SE-54 and OV-17 of the examined disaccharides are given in Table II and their structures are depicted in the Fig. 1.

Separation and identification

Although the studied disaccharides covered a wide range of retention (367 index units on SE-54 and 431 index units on OV-17), the separation of anomeric forms was worse than that found previously for monosaccharides [7–9], which covered a narrower range (192 and 244 i.u., respectively, for aldohexoses). Most of disaccharides gave only one or two peaks. As expected, sucrose and trehalose showed only one component. All the examined disaccharides having a 1,4-glycosidic bond showed a maximum of two peaks, although in one of them (neolactose) the free monosaccharide corresponding to the reducing unit (altrose) had noticeable

TABLE I

CHARACTERISTICS AND OPERATING CONDITIONS OF THE CAPILLARY COLUMNS USED FOR THE GC ANALYSIS OF DISACCHARIDE TMS ETHERS

Column	Stationary phase	Origin	Temperature (°C)
Pyrex glass (40 m × 0.18 mm I.D.)	SE-54	Laboratory-made	280
Fused silica (25 m × 0.20 mm I.D.)	OV-17	Chrompack	240

TABLE II

RETENTION INDICES OF DISACCHARIDE OTMS ETHERS

Compound	Retention index	
	SE-54	OV-17
α,α-Trehalose	2820	2697
Sucrose	2710	2596
α-Laminaribiose	2844	2757
β-Laminaribiose	2876	2783
α-Nigerose	2780	2678
β-Nigerosa	2806	2714
Turanose	2783	2670
α-Cellobiose	2754	2666
β-Cellobiose	2861	2771
α-Maltose	2744	2652
β-Maltose	2785	2658
α-Lactose	2707	2626
β-Lactose	2833	2738
α-Epilactose	2627	2498
β-Epilactose	2723	2577
α-Neolactose	2671	2544
β-Neolactose	2650	2519
Maltulose-1[a]	2773	2659
Maltulose-2[a]	2774	2667
Lactulose-1[a]	2684	2558
Lactulose-2[a]	2695	2578
α-Melibiose	2924	2848
β-Melibiose	2947	2869
α-Palatinose	2828	2682
β-Palatinose	2828	2726
α-Isomaltose	2946	2879
β-Isomaltose	2994	2929
α-Galactobiose	2831	2741
β-Galactobiose	2901	2814
α-Gentiobiose	2971	2912
β-Gentiobiose	2978	2921

[a] Peaks 1 and 2 from maltulose and lactulose were assigned as β-furanose and β-pyranose, respectively.

proportions of furanose forms [8,11]. It seems that the bulky substituent at C-4 avoids the formation of furanose forms in such compounds.

Furanose forms are possible in 1,6-glycosyl aldohexoses; in fact, galactobiose showed four components. The two main peaks were assigned to α- and β-pyranoses, these being the most abundant forms in the disaccharide [12] and also in the free galactose [13].

Three disaccharides containing fructose as the reducing end (palatinose, turanose and lactulose) showed a main peak with a tail or two partially overlapping peaks on SE-54; the resolution was improved on OV-17. Similar results have been reported by other workers [3–5]. A more detailed study of lactulose revealed that up to three tautomeric forms (β-furanose, β-pyranose and α-furanose) could be separated on a more polar column (SP-3240, with retention indices I_x of 2668, 2700 and 2733) [14]. It is reasonable to suppose a similar overlapping of peaks in turanose, which has the same tautomers as shown by NMR [15]. Palatinose only has furanose forms [16], which could be separated on OV-17. We found four peaks for maltulose, and two of them were assigned as β-furanose and β-pyranose, which have been included in Table II.

The change in column polarity increased the overall resolution, and decreased slightly the I_x values, but it did not produce noticeable changes in the elution order of α- and β-anomers, as was previously observed [4].

Structure–retention relationships

In previous studies of quantitative structure–retention relationships [7–9] we calculated the contributions to the retention index of several structural descriptors of monosaccharides. As the large number of parameters necessary to describe the structure of disaccharides avoids the use of this approach, we have tried a simpler retention model:

$$I_{xp} = a1_p I_{x1p} + a2_p I_{x2p} + c_{ip} + b_0 \quad (1)$$

which assumes that the retention index of a disaccharide x in a stationary phase p depends on the retention indices of the two constituent monosaccharides in the same phase (I_{x1p} and I_{x2p}) and on the type of glycosidic bond i. The intercept b_0 and the coefficients $a1_p$, $a2_p$ and c_{ip} are calculated by multiple linear regression.

The quality of fit obtained using this model is lower than that with monosaccharides because the data/variables ratio is higher. The low fit limits the use of the model in the prediction of retention indices of other disaccharides; however, the values found for a_1, a_2 and c_i can be used to assess quantitatively the influence of the related structural features on the chromatographic retention. These values, the intercept and the regression coefficient are listed in Table III.

When we consider a group of disaccharides dif-

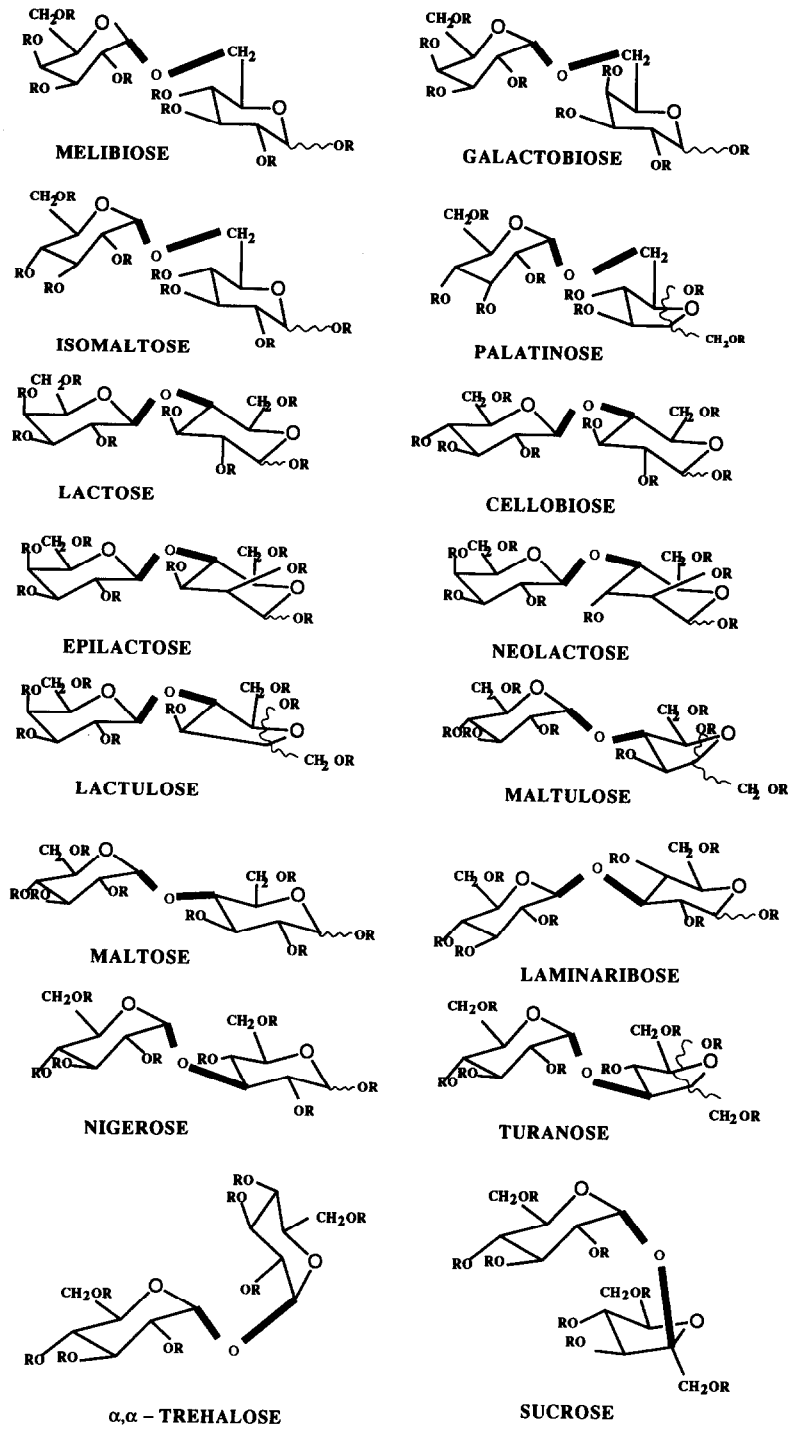

Fig. 1. Structural formulae of the disaccharides investigated.

TABLE III

VALUES OF COEFFICIENTS $a1$, $a2$, c_{ip} AND b_0 CALCULATED BY LINEAR REGRESSION

Phase	$a1$	$a2$	$c_{1,2}$	$c_{1,3}$	$c_{1,4}$	$c_{1,6}$	b_0	r
OV-17	0.455	0.694	10.3	44.4	0	181.2	497.9	0.95
SE-54	0.330	0.375	30.4	60.1	0	168.3	1384.7	0.91

fering in the reducing unit (*e.g.*, 4-O-β-galactosyl derivatives of fructose, altrose, mannose and glucose), their elution order is very similar to that of corresponding monosaccharides.For this reason, the retention model in eqn. 1 includes the experimental retention index of the monosaccharide constituents taken from ref. 8; however the coefficients depend on the position of the monosaccharide unit in the molecule. We have called the monosaccharide with the glycosidic linkage A and the monosaccharide with the free reducing end B.

The contributions associated with the monosaccharide retentions ($a1$ for unit A and $a2$ for unit B) have significant positive values, higher for the OV-17 phase and for unit B. Similar results are found when the relative retentions listed in ref. 4 are converted into retention indices using the experimental data available; the contributions for units A and B are always positive, the latter being higher.

The data of Haverkamp *et al.* [4] show also that glucosyl glucoses were eluted forming three groups: first those with a 1,4-link, second those with 1,1-, 1,2- and 1,3-links and finally those with 1,5- and 1,6-links. Similarly, the 1,4-galactosyl glucoses were eluted before their 1,6-isomers. The contributions c_{ip} calculated for these structural features are similar to those found from our retention indices which appear in Table I. Glycosidic bond contributions seem to be related with the overall molecular shape of the disaccharide. The higher retention of the 1,6-disaccharides can be attributed to the three atoms linking the two rings, which afford a greater flexibility of conformation. Previous data on monosaccharides indicate that the more planar forms present the highest I_x values.

The quality of fit improves when other variables are added to the model in eqn. 1. For instance, when the α- or β-character of A and B monosaccharide units is also considered, the correlation coefficient is 0.96 for OV-17 and 0.94 for SE-54. The contribution of an α-link is positive for the A unit and negative for the B unit; however, the significance of the values is low.

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REFERENCES

1 K. Robards and M. Whitelow, *J. Chromatogr.*, 373 (1986) 81.
2 M. F. Laker, *J. Chromatogr.*, 163 (1979) 9.
3 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Am. Chem. Soc.*, 85 (1963) 2497.
4 J. Haverkamp, J. P. Kamerling and J. F. G. Vliegentart, *J. Chromatogr.*, 59 (1971) 281.
5 Z. L. Nikolov and P. J. Reilly, *J. Chromatogr.*, 254 (1983) 157.
6 E. Percival, *Carbohydr. Res.*, 4 (1967) 441.
7 A. García-Raso, I. Martínez-Castro, M. I. Páez, J. Sanz, J. García-Raso and F. Saura-Calixto, *J. Chromatogr.*, 398 (1987) 9.
8 I. Martínez-Castro, M. I. Páez, J. Sanz and A. García-Raso, *J. Chromatogr.*, 462 (1989) 49.
9 A. García-Raso, M. Fernández-Díaz, M. I. Páez, J. Sanz and I. Martínez-Castro, *J. Chromatogr.*, 471 (1989) 205.
10 R. J. Smith, J. K. Haken and M. S. Wainwright, *J. Chromatogr.*, 334 (1985) 95.
11 S. J. Angyal, *Adv. Carbohydr. Chem. Biochem.*, 42 (1984) 15.
12 J. H. Pazur, F. J. Hiskiel and B. Liu, *Anal. Biochem.*, 174 (1988) 46.
13 T. E. Acree, R. S. Shallenberger and L. R. Mattick, *Carbohydr. Res.*, 6 (1968) 498.
14 I. Martínez-Castro, M. M. Calvo and A. Olano, *Chromatographia*, 23 (1987) 132.
15 P. E. Pfeffer and K. B. Hicks, *Carbohydr. Res.*, 102 (1982) 11.
16 J. Reuben, *J. Am. Chem. Soc.*, 107 (1985) 1747.