GC Behavior of Disaccharide Trimethylsilyl Oximes

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Abstract

Twenty-five disaccharides are converted to trimethylsilyl oximes and injected on two capillary columns of different polarities (methyl and phenyl–methyl silicone). Their Kovats retention indices are determined at three temperatures. Multivariate data analysis is used in order to obtain relations between retention and some structural features. Phenylmethyl silicone have better chromatographic characteristics for the assayed compounds. The two columns can provide useful chromatographic information when a very complex disaccharide mixture has to be analyzed.

Introduction

Carbohydrates are frequently analysed by gas chromatography (GC) as their trimethylsilyl (TMS) derivatives. Because tautomeric forms of reducing sugars can produce multiple peaks, some approaches have been taken in order to suppress the anomeric center before silylation, the most popular being the formation of oximes from the carbonyl group. The *syn* (*E*) and *anti* (*Z*) isomers produced in the reaction can be separated by GC. This method has been used to analyze different monosaccharides (1,2). Although several disaccharides have been analyzed as their TMS oximes, GC retention data for these derivatives are relatively scarce. Previous works report the separation data of up to 11 disaccharides using apolar columns such as DB-5 (3,4), DB-1 (5), and OV-101 (6).

Disaccharides with glucose, galactose, mannose, and fructose as monosaccharide moieties occur in nature, but most are difficult to isolate and synthesize. An exhaustive study of their chromatographic properties cannot be carried out because, among all the disaccharide structures differing in their monosaccharide constituents and in the possible positions and configuration of the glycosidic linkage, only a few of them are commercially available as standard compounds.

In this work, we have determined the retention indices of TMS oximes of 25 disaccharides (many of them are reported for first time) on two stationary phases. The effect of temperature operation and stationary phase polarity on the retention indices have been evaluated.

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Experimental

Standard substances

Analytical standards of cellobiose (4-*O*-β-D-glucopyranosyl-D-glucose), epilactose (4-*O*-β-D-galactopyranosyl-α-D-mannose), 3α-galactobiose (3-*O*-α-D-galactopyranosyl-D-galactose), 4α-galactobiose (4-*O*-α-D-galactopyranosyl-D-galactose), 6β-galactobiose (6-*O*-β-D-galactopyranosyl-D-galactose), 3-*O*-α-D-galactopyranosyl-D-arabinose, β-gentiobiose (6-*O*-β-D-glucopyranosyl-β-D-glucose), isomaltose (6-*O*-α-D-glucopyranosyl-D-glucose), kojibiose (2-*O*-α-D-glucopyranosyl-D-glucose), lactose (4-*O*-β-D-galactopyranosyl-D-glucose), lactulose (4-*O*-β-D-galactopyranosyl-D-fructose), laminaribiose (3-*O*-β-D-glucopyranosyl-β-D-glucose), maltose (4-*O*-α-D-glucopyranosyl-D-glucose), 2α-mannobiose (2-*O*-α-D-mannopyranosyl-D-mannose), 3α-mannobiose (3-*O*-α-D-mannopyranosyl-D-mannopyranose), nigerose (3-*O*-α-D-glucopyranosyl-D-glucopyranose), and sucrose (2-*O*-α-D-glucopyranosyl-β-D-fructofuranoside) were obtained from Sigma Chemical Co. (St. Louis, MO). Leucrose (5-*O*-α-D-glucopyranosyl-D-fructose); melibiose (6-*O*-α-D-galactopyranosyl-D-glucose); palatinose (6-*O*-α-D-glucopyranosyl-D-fructose); α,α-trehalose (1-*O*-α-D-glucopyranosyl-α-D-glucopyranoside); and turanose (3-*O*-α-D-glucopyranosyl-D-fructose) were from Fluka (Buchs, Switzerland). Maltulose (4-*O*-α-D-glucopyranosyl-D-fructose) was from Aldrich Chemical Co. (Milwaukee, WI), and sophorose (2-*O*-β-D-glucopyranosyl-D-glucose) was from Sarsynthèse (Merignac, France).

Carbohydrate analysis

Pure disaccharides were equilibrated in water, evaporated under vacuum at 30ºC, and then treated with 2% hydroxylamine chloride in pyridine and heated to 75ºC for 30 min in order to form sugar oximes. Persilylation was carried out with hexamethyldisilazane (HMDS) and trifluoroacetic acid at 45ºC for 30 min (7). After reaction, the samples were centrifuged at 7000 g for 5 min at 5ºC (8).

GC analysis of TMS oximes was carried out using two fused-silica columns. Column A (25-m × 0.25-mm i.d. × 0.1-µm d_f) was an Rtx-65 TG from Restek (Bellefonte, PA) and was coated with 35% dimethyl–65% diphenyl polysiloxane. Column B (30-m × 0.25-mm i.d. × 0.25-µm d_f) was an SPB-1 from Supelco (Bellefonte, PA) was coated with methyl polysiloxane.

Column A was installed in a Hewlett-Packard (HP) 5890 gas

chromatograph (Palo Alto, CA), and helium was the carrier gas. Column B, installed in a GC-8000 (Fisons Instruments, Milan, Italy) used nitrogen as carrier gas; column flow was adjusted in both cases to the Van Deemter optimum. Injections were made in the split mode, with a split flow rate of 40 mL/min. Chromatographic peaks were measured using an HP ChemStation and a Chromcard acquisition system, respectively.

Derivatives were carefully examined by GC–mass spectrometry in order to check for the absence of artifacts or partially derivatized compounds because oximation of monosaccharides at temperatures higher than 60°C can lead to the formation of dicarbonyl species (1). Therefore, both columns were installed in an HP-5890 GC with a HP-5971 quadrupole mass detector operated at 70 eV (Hewlett-Packard). Results (yield and lack of artifacts) were considered satisfactory.

Kovats retention indices (*I*) were calculated from the retention times of TMS oximes of disaccharides and the suitable *n*-alkanes. Hold-up time was measured by injecting *n*-pentane.

Statistical analysis

Statistical analysis was carried out with the statistical package BMDP (9), using the BMDP 2R program (stepwise multiple regression).

Results and Discussion

TMS oximes of 25 disaccharides were injected on two capillary columns coated with methyl silicone and phenyl–methyl silicone. Nonreducing disaccharides (sucrose and α,α-trehalose) gave only a peak corresponding to the *octakis*-TMS derivative. Disaccharides having glucose, galactose, or mannose as the reducing moiety gave two well-resolved peaks whose ratio varied within 3:1 and 10:1; the major peak, which always eluted first, was assigned to *syn* (*E*) isomer and the minor to the *anti* (*Z*) isomer according to the results found by Funcke & von Sonntag (10) for aldohexoses. Disaccharides having fructose as the reducing moiety gave two peaks whose resolution was significantly worse (except palatinose), and with an approximate 1:1 area ratio, which was similar to that found by the same authors. In this case, no assignation of *syn* and *anti* isomers could be performed.

Table I. Kovats Retention Indices (I) for Each TMS Oxime Disaccharide on Two Different Columns and Their Increments per 10°C

				Column A				Column B			
				E (1)*		Z (2)*		E (1)*		Z (2)*	
Compound*	Monosaccharide constituents	Union linkage	Glycosidic bond	*I* at 230°C	DI/10°C†	*I* at 230°C	DI/10°C†	*I* at 250°C	DI/10°C‡	*I* at 250°C	DI/10°C‡
Trehalose	Glu–Glu	1,1	α	2630	–9			2841	0		
Kojibiose	Glu–Glu	1,2	α	2706	–11.75	2746	–9.75	2979	–0.25	3022	1.5
Sophorose §	Glu–Glu	1,2	β	2694	–20.25	2733	–17.5	2956	–5.5	3001	–3.5
Nigerose	Glu–Glu	1,3	α	2684	–15.5	2719	–13.5	2963	–3.25	3000	–1.5
Laminaribiose §	Glu–Glu	1,3	β	2664	–19.5	2696	–16.75	2942	–6.5	2975	–3.75
Maltose	Glu–Glu	1,4	α	2684	–12.25	2696	–9.25	2968	–0.25	2984	1.5
Cellobiose	Glu–Glu	1,4	β	2632	–17.25	2659	–17.25	2910	–3.5	2937	–3.25
Isomaltose	Glu–Glu	1,6	α	2836	–13.5	2867	–11.25	3080	–3.25	3120	–0.75
Gentiobiose	Glu–Glu	1,6	β	2785	–20	2811	–17.75	3032	–7	3064	–4.5
Mannobiose§	Man–Man	1,2	α	2619	–15	2656	–12.25	2914	–7.25	2968	0.25
Mannobiose§	Man–Man	1,3	α	2568	–14.25	2624	–11.75	2878	–1	2940	0.75
Mannobiose§	Man–Man	1,4	α	2598	–13.75	2624	–9.25	2902	–1	2946	1.75
Galactobiose§	Gal–Gal	1,3	α	2732	–13.75	2776	–11	2998	–2	3040	0.5
Galactobiose§	Gal–Gal	1,4	α	2679	–13.25	2722	–11.5	2954	–1.25	3000	0
Galactobiose§	Gal–Gal	1,6	β	2771	–14.5	2814	–12.25	3054	–3.25	3101	–1.25
Sucrose	Glu–Fru	1,2	α	2515	–13			2733	–0.75		
Turanose	Glu–Fru	1,3	α	2681	–13.75	2690	–12.75	2964	–1.25	2973	–1
Maltulose	Glu–Fru	1,4	α	2635	–12.25	2649	–12.25	2935	–0.5	2948	–0.25
Leucrose§	Glu–Fru	1,5	α	2676	–10.75	2683	–10.5	2968	2	2976	2.25
Palatinose	Glu–Fru	1,6	α	2756	–12.75	2778	–13.25	3023	–1.5	3041	–2
Lactose§	Gal–Glu	1,4	β	2597	–11	2603	–10	2894	–0.25	2905	1
Melibiose	Gal–Glu	1,6	α	2815	–16.25	2851	–13.5	3073	–4	3118	–1.75
Lactulose§	Gal–Fru	1,4	β	2581	–13.5	2592	–13.75	2884	–0.5	2894	–1.5
Epilactose§	Gal–Man	1,4	β	2627	–14.25	2659	–12	2919	–1.75	2959	0.25
Galactosyl arabinose§	Gal–Ara	1,3	α	2435	–14.25	2443	–16.25	2663	–4.75	2669	–2.75

* Peaks were named 1 and 2 when E and Z forms could not be assigned.
† Mean value, –13.55 ± 2.75.

Kovats retention indices (*I*) were calculated at 190ºC, 210ºC, and 230ºC for column A and 250ºC, 270ºC, and 290ºC for column B. Table I shows *I* values for each compound at 230ºC (column A) and at 250ºC (column B) and also its increment per 10ºC.

Retention indices of disaccharide TMS oximes were noticeably lower in the polar column (A), as was also found for disaccharide TMS ethers (11). The retention factor (*k*), which was calculated for 6β-galactobiose and corrected by taking into account the phase ratio of both columns, was 2.8 times higher for column B at 250ºC than for column A at 230ºC, which indicated that disaccharide absolute retention was also lower in column A. The behavior of both columns is compared in Figure 1, which shows as an example the separation of the TMS oximes of eight disaccharides. As a result of the different selectivity of the two columns, the elution pattern presents important changes, and some peaks that overlap in one column can be resolved on the other one.

Resolution, which depends on column efficiency and stationary phase selectivity, is difficult to quantitate objectively in complex mixtures of variable composition. A useful parameter for column resolution is the peak capacity, defined as the maximum number of nonoverlapping peaks in a given interval (12). If we were considering that the first disaccharide to elute was sucrose and the last disaccharide was isomaltose, peak capacity for disaccharides was approximately 100 for column B and 200 for column A. Because column A efficiency is only slightly higher than that of column B, methylphenyl polysiloxane seems to present a better general selectivity for TMS oximes of carbohydrates than dimethyl polysiloxane. Although the number of compounds that can be correctly resolved in real samples is of course lower, peak capacity values indicate that these columns can be used in mixtures of disaccarides of medium–high complexity, such as those occurring in samples from natural sources.

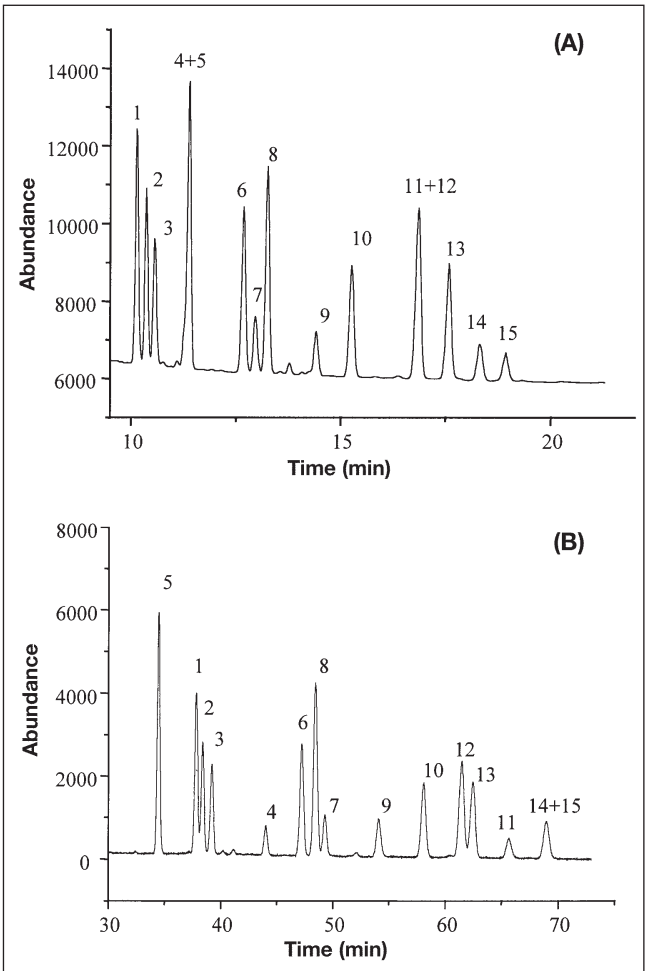

Figure 1. GC profiles of a standard mixture of 8 disaccharide TMS-oximes on a dimethyldiphenyl polysiloxane column (A) at 230°C and on a dimethylpolysiloxane column (B) at 250°C. The peaks represent the following: (1) 3α-mannobiose (E); (2) lactulose 1; (3) lactulose 2; (4) α-mannobiose (Z); (5) α,α-trehalose; (6) maltose (E); (7) maltose (Z); (8) kojibiose (E); (9) kojibiose (Z); (10) 6β-galactobiose (E); (11) 6β-galactobiose (Z); (12) melibiose (E); (13) isomaltose (E); (14) melibiose (Z); and (15) isomaltose (Z).

The variation of *I* values with temperature depended clearly on the stationary phase. Values of Δ*I*/10ºC (listed in Table I) for both stationary phases were obtained from a linear fitting of *I* values measured at three different temperatures. Mean values were –13.55 for column A and –1.59 for column B. The dispersion for the Δ*I*/10ºC values was also higher for column A, and differences in the elution pattern when using different column temperature conditions are more probable in this column.

Structural aspects

For simplicity, the monosaccharide with the glycosidic linkage unit was abbreviated as M1 and the monosaccharide with the free reducing end unit as M2. Some features relating retention and structure are easily observed in Table I: nonreducing disaccharides (trehalose and sucrose) did not form oximes and were eluted first, which can be explained by their lower molecular weight. Compounds with a β-glycosidic link were eluted before those with an α-linkage. All assayed 1–6 disaccharides were eluted last. A similar behavior, which has been found for disaccharide TMS ethers (13), is explained by considering that the more planar molecules were more retained (11,14). General rules for other structural units cannot be deduced because the number of disaccharide-standard compounds was too low.

Raso et al. (11) indicated an additive contribution of monosaccharides to the TMS disaccharide retention, which was significatively dependent on its position (M1 or M2). A statistical study was carried out in order to evaluate the possible influence of some structural features on the retention, although the scarce number of available compounds limited its significance. In a stepwise regression analysis, experimental values in Table I were taken as independent variables, and dependent values were considered in turn to be different structural descriptors, such as monosaccharide type in M1 and M2 and position of the glycosidic link.

The values of the partial correlation and of the F-statistic for each independent variable were used to compare the influence of structural characteristics on the retention. As expected, the 1–6 link presented the highest relationship with retention. This link had F-values that were 69.8 for *I* in column A and 55.1 for *I* in column B, and the partial correlations (*r*) were 0.79 for *I* in column A and 0.75 for *I* in column B. Their positive values indicated that the presence of an 1–6 linkage contributed positively to the retention index. The 1–4 linkage F-values were 19.7 for *I* in column A and 15.8 for *I* in column B. This link had *r* values that were –0.57 and –0.52, which showed a negative effect on the retention index. The rest of structural the parameters showed less

significant values: the existence of a glucose moiety in M2 was negatively (r = –0.38 for column A and r = –0.35 for column B) related with the ΔI/10ºC values (F = 7.3 for column A and F = 5.9 for column B). The use of several retention variables only slightly improved the mentioned relations.

The described chromatographic behavior indicates that dimethyl–diphenyl polysiloxane is a useful phase for disaccharide separation. If compounds with different characteristics are present in the analyzed samples, the high values of ΔI/10ºC can be an advantage when selecting the most adequate operating temperature. Both operation temperature and phase selectivity indicate that methylphenyl polysiloxane seems to be a better choice than dimethyl polysiloxane for preparing columns for oligosaccharides separation. Carbohydrate samples from natural sources, however, can be very complex, and dimethyl polysiloxane columns can also be used in order to provide additional information in difficult separation problems.

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