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ISOTHERMAL CAPILLARY COLUMN GAS CHROMATOGRAPHY OF TRIMETHYLSILYL DISACCHARIDES

ZIVKO L. NIKOLOV and PETER J. REILLY*
Department of Chemical Engineering, Iowa State University, Ames, IA 50011 (U.S.A.)
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SUMMARY

The relative retention times of seventeen trimethylsilyl disaccharides (cellobiose, gentiobiose, isomaltose, kojibiose, lactose, lactulose, laminaribiose, maltose, maltulose, melibiose, nigerose, palatinose, sophorose, sucrose, α,α-trehalose, turanose and xylobiose) on a fused-silica capillary column coated with SE-54 are reported. Equilibrium anomeric compositions of fourteen of these disaccharides in pyridine are also given. Isothermal operation at 240°C allowed complete quantitation of the mixture.

INTRODUCTION

In their original publication, Sweeley *et al.*[1] described a simple procedure for preparing trimethylsilyl (TMS) ethers of sugars and showed that TMS derivatives up to tetrasaccharides are sufficiently volatile for separation by gas chromatography. The application of their method to the separation of TMS disaccharides in complex mixtures was reported by several authors[2–6]. However, a general difficulty was the appearance of multiple peaks in the chromatogram due to the presence of tautomeric forms of reducing sugars.

In order to reduce the number of peaks. Toba and Adachi[5] and Adam and Jennings[7] converted the disaccharides into oximes before forming the TMS ethers. Their results indicate that there is no advantage in use of oxime TMS disaccharides, since for reducing sugars usually at least two peaks per disaccharides appeared anyway. Therefore, for clear identification and quantitation of a complex mixture of disaccharides, mutarotation equilibrium data for the component sugars have to be used if overlapping occurs[8].

All prior work on separation by gas chromatography of TMS disaccharide mixtures was conducted with packed columns[2–6]. The retention values of the tautomeric forms of TMS derivatives indicated that techniques yielding higher resolution are required for complete separation.

Therefore, in this work we investigated the separation of seventeen TMS disaccharides on a fused-silica capillary column and determined equilibrium compositions of the reducing disaccharides in pyridine. By choice of the proper separation conditions, all seventeen disaccharides could be identified and quantitated.

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MATERIALS AND METHODS

Disaccharides

α,α-Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside), turanose (3-O-α-D-glucopyranosyl-D-fructose), β-cellobiose (4-O-β-D-glucopyranosyl-β-D-glucose), lactulose (4-O-β-D-galactopyranosyl-D-fructose), isomaltose (6-O-α-D-glucopyranosyl-D-glucose), β-gentiobiose (6-O-β-D-glucopyranosyl-β-D-glucose), α-melibiose (6-O-α-D-galactopyranosyl-α-D-glucose) and palatinose (6-O-α-D-glucopyranosyl-D-fructose) were supplied by Sigma (St. Louis, MO, U.S.A.). Sucrose (α-D-glucopyranosyl-β-D-fructofuranoside), maltose (4-O-α-D-glucopyranosyl-D-glucose) and α-lactose (4-O-β-D-galactopyranosyl-α-D-glucose) were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). α-Kojibiose (2-O-α-D-glucopyranosyl-α-D-glucose) and α-sophorose (2-O-β-D-glucopyranosyl-α-D-glucose) were purchased from Adams (Round Lake, IL, U.S.A.). Xylobiose (4-O-β-D-xylopyranosyl-D-xylose) was prepared in our laboratory by partial acid hydrolysis of xylan followed by charcoal–Celite column chromatography. The following compounds were gifts: nigerose (3-O-α-D-glucopyranosyl-D-glucose) from Professor John F. Robyt of Iowa State University and Dr. Elwyn T. Reese of the U.S. Army Natick Research and Development Laboratories, laminaribiose (3-O-β-D-glucopyranosyl-D-glucose) from Dr. Reese and maltulose (4-O-α-D-glucopyranosyl-D-fructose) from Dr. H. D. Scobell of A. E. Staley Manufacturing Co.

Reagents

Hexamethyldisilazane (specially purified grade), pyridine (silylation grade), trifluoroacetic acid (sequanal grade) and TRI-SIL Z (a 21%, w/v, solution of trimethylsilylimidazole in dry pyridine) were purchased from Pierce (Rockford, IL, U.S.A.). Acetone-d_6 came from Norell (Landisville, NJ, U.S.A.).

Derivatization

For each disaccharide, 2–3 mg were dissolved in 1 ml pyridine containing 0.2 *M* 2-hydroxypyridine catalyst[9] and left for 15 h at 40°C to achieve mutarotation equilibrium. Equilibrated sugars were derivatized with hexamethyldisilazane by the procedure of Brobst and Lott[10]. Disaccharides with known anomeric composition were derivatized directly with TRI-SIL Z in order to obtain retention times for each anomer.

Gas chromatography

Analyses were made with a Perkin-Elmer Model Sigma 1 gas chromatograph controlled by a Sigma 15 chromatography data station. TMS sugars were separated on a 30 m × 0.26 mm I.D. fused-silica capillary column (J & W Scientific, Rancho Cordova, CA, U.S.A.) coated with SE-54.

The column oven was held at 240°C, with injector and flame-ionization detector temperatures of 280 and 300°C, respectively. The carrier gas was helium at an average linear velocity of 0.24 m/sec as measured by methane. The splitting ratio was 1:100.

Proton magnetic resonance ^1H NMR spectroscopy

^{1}H NMR spectroscopy of the TMS derivatives of isomaltose and xylobiose was conducted following the procedure of Kamerling *et al.*[11], using a Bruker WM 300 spectrophotometer locked on deuterium of the solvent acetone-d_6. The chemical shifts are relative to tetramethylsilane. Anomeric configuration of each gas chromatographic peak could be assigned by comparing the ratios of their areas with those of ^{1}H NMR doublets. In turn, anomeric configuration of the latter could be assigned using the findings of Kamerling *et al.*[11] that, for aldoses with the C-2 hydroxyl oriented as it is in glucose, anomeric protons in the α-configuration give lower coupling constants and generally higher chemical shifts (δ) than anomeric protons in the β-configuration. For TMS isomaltose, δ = 5.10 (3.42 Hz) corresponds to $H_G^{\alpha\rightarrow\alpha}$, δ = 5.02 (3.02 Hz) to $H_G^{\alpha\rightarrow\beta}$, δ = 4.97 (3.34 Hz) to H^{α}, and δ = 4.51 (7.47 Hz) to H^{β}, following the nomenclature of Kamerling *et al.*[11]. For TMS xylobiose, δ = 4.95 (3.10 Hz) corresponds to H^{α}, δ = 4.46 (7.10 Hz) to H^{β}, δ = 4.28 (7.45 Hz) to $H_G^{\beta\rightarrow\beta}$, and δ = 4.25 (7.56 Hz) to $H_G^{\beta\rightarrow\alpha}$.

RESULTS AND DISCUSSION

The relative retention values and resolutions of the TMS ethers of the seventeen disaccharides are presented in Table I. Each of the TMS reducing sugars gave rise to two sharp peaks in the chromatogram, due to the pyranose anomers present in pyridine before trimethylsilylation, except for the fructose-containing disaccharides palatinose and turanose, which gave three peaks each, and lactulose and maltulose, which gave one sharp and one broad peak each. When pure anomers or disaccharides with known anomeric composition were available, retention times of their TMS derivatives were compared to those given in Table I and configuration was assigned as appropriate. Relative retention times were calculated from four independent analyses.

Resolutions (R_s) of adjacent peaks, defined as the difference in their retention times (t_i) divided by their average baseline peak width (w_i):

$$R_s = \frac{t_2 - t_1}{(w_1 + w_2)/2}$$

are shown on Table I. As may be seen there and on Fig. 1, there are six cases where no resolution between peaks of different TMS disaccharides is possible, and equilibrium data for the two anomeric forms of the same disaccharide after complete mutarotation must be used to allow composition to be determined. These data, obtained at 40°C in dry pyridine, are presented in Table II for fourteen of the seventeen disaccharides. For comparison, equilibrium compositions of some of these compounds in water are also presented. It may be noted that, where configuration may be assigned, α-pyranose/β-pyranose equilibrium ratios are the same or higher in pyridine than in water except for kojibiose and sophorose, the two (1→2) linked glucopyranosyl–glucoses.

As was observed by Haverkamp *et al.*[4] for (1→4) and (1→6) linked TMS disaccharides which contain D-glucose of the reducing end, the β-anomer has a longer retention time than the α-anomer. In addition, the β-anomer is present in higher concentrations than the α-anomer in solution. These two facts have been explained by the more stable conformation of the β-pyranose anomer in aqueous solution[12]. However, the analogy between elution order and anomeric ratio cannot be completely

TABLE I

RELATIVE RETENTION TIMES AND RESOLUTION OF THE TMS ETHERS OF DISACCHARIDES

Carbohydrate	*Anomer peak*	*Anomer concentration (g/l)*	*Peak width (min)*	*Relative retention time (t_R)**	*Resolution (R_s)*
Xylobiose	1 (α)	0.063	0.25	0.585 ± 0.001	
Lactulose	1	0.40	1.5	~0.927	>7
Xylobiose	2 (β)	2.0	0.40	0.934 ± 0.001	0.19
Lactulose	2	0.60	0.40	0.959 ± 0.001	1.62
Sucrose	1	0.60	0.35	1.000	2.85
Lactose	1 (α)	0.77	0.35	1.000 ± 0.001	0.0
Maltose	1 (α)	0.72	0.45	1.119 ± 0.001	7.72
Cellobiose	1 (α)	0.44	0.40	1.145 ± 0.001	1.60
Maltulose	1	0.56	0.45	1.187 ± 0.001	2.56
Maltulose	2	0.83	0.60	1.205 ± 0.001	0.90
Turanose	1 + 2 + 3	1.1	1.5	1.236** ± 0.001	0.77
Nigerose	1	0.96	0.45	1.245 ± 0.001	0.23
Maltose	2 (β)	0.88	0.45	1.273 ± 0.001	1.62
α,α-Trehalose	1	1.0	0.45	1.330 ± 0.002	3.29
Kojibiose	1 (β)	0.99	0.45	1.330 ± 0.001	0.0
Nigerose	2	0.64	0.50	1.343 ± 0.001	0.72
Palatinose	1 + 2	1.3	0.50	1.383*** ± 0.003	2.08
Lactose	2 (β)	0.83	0.50	1.445 ± 0.001	3.22
Laminaribiose	1	0.90	0.50	1.514 ± 0.001	3.59
Sophorose	1 (β)	0.36	0.50	1.517 ± 0.001	0.15
Cellobiose	2 (β)	0.46	0.50	1.579 ± 0.002	3.22
Palatinose	3	0.12	0.55	1.630 ± 0.002	2.53
Kojibiose	2 (α)	0.50	0.60	1.639 ± 0.001	0.41
Laminaribiose	2	1.1	0.60	1.670 ± 0.002	1.34
Sophorose	2 (α)	0.74	0.60	1.765 ± 0.001	4.12
Melibiose	1 (α)	0.45	0.65	1.905 ± 0.001	5.82
Isomaltose	1 (α)	0.44	0.65	1.977 ± 0.004	2.88
Melibiose	2 (β)	0.65	0.65	2.050 ± 0.001	2.92
Gentiobiose	1 (α)	0.32	0.75	~2.24	7.06
Gentiobiose	2 (β)	0.67	0.75	2.255 ± 0.004	0.52
Isomaltose	2 (β)	0.56	0.75	2.346 ± 0.007	3.16

* TMS–sucrose was used as an internal standard (retention time = 26.00 min), and relative retention times were normalized to it. Standard deviations are based on four samples.

** Based on first incompletely separated peak.

*** Based on second incompletely separated peak.

generalized to other linkages, because while both β-anomers of kojibiose and sophorose elute before their respective α-anomers, opposite to (1 → 4) and (1 → 6) linked TMS disaccharides, their equilibrium anomeric ratios are quite different from each other. More α- than β-sophorose is present in solution[4], as would be expected from the fact that TMS α-sophorose eluted second, but the opposite is true with α- and β-kojibiose. Among disaccharides whose anomeric configuration is not known, the same type of anomolous behavior is exhibited by nigerose.

It is perhaps not too surprising that elution order and anomeric ratio are not always well correlated, as the former may be changed by varying chromatographic conditions. When analyzed isothermally at 240°C, TMS β-gentiobiose eluted after TMS α-gentiobiose. The reverse sequence of anomers resulted when temperature programming was applied. Since better resolution of TMS gentiobiose anomers was obtained with temperature programming, it was employed for equilibrium composition analysis (Table II).

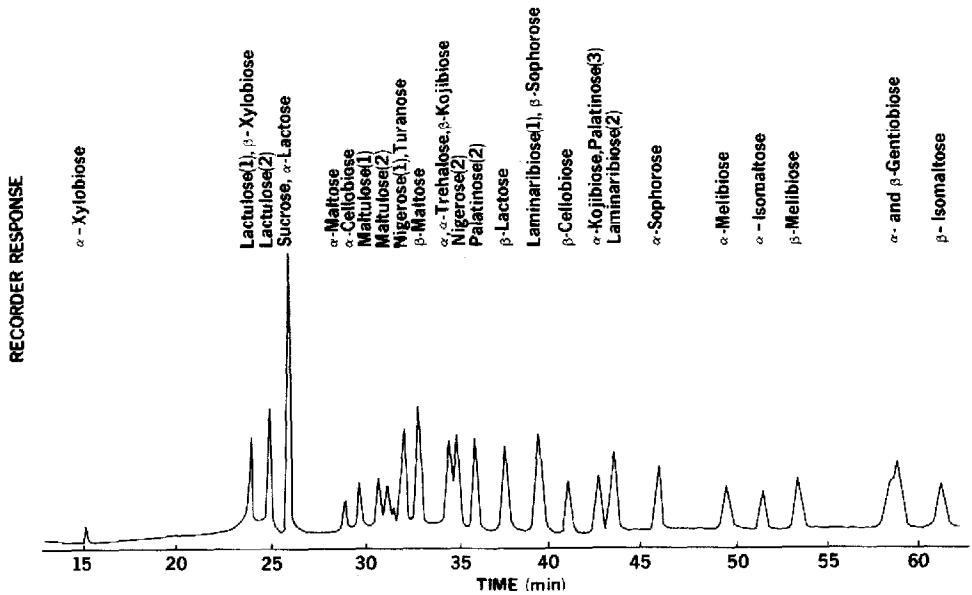

Fig. 1. Gas chromatogram of seventeen TMS disaccharides conducted on a 30 m × 0.26 mm I.D. fused-silica capillary column coated with SE-54 and operated isothermally at 240°C with a flow of 0.24 m/sec helium.

Chromatographic conditions in our case were optimized with respect to the best separation of maltose, nigerose and kojibiose anomers. Neither isothermal operation at higher temperatures nor temperature programming separated these three TMS derivatives as well as did isothermal operation at 240°C.

The total analysis time can be shortened either by using hydrogen as a carrier gas at higher velocities or by applying temperature programming at 8°C/min from 240 to 260°C, starting at 35 min.

In summary, isothermal separation of a complex mixture of TMS disaccharides on a capillary column has been shown to be a reliable and simple analytical method. Increased complexity of the chromatogram due to multiple anomeric peaks is not a problem when high-efficiency capillary columns are used and mutarotation equilibria are known.

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TABLE II

EQUILIBRIUM COMPOSITION OF ANOMERS IN PYRIDINE

Carbohydrate	*Anomer peak*	*Equilibrium composition (%)*		
		*This work**	*Haverkamp et al.***	*Toba and Adachi****
Xylobiose	1 (α)	4	–	–
	2 (β)	96	–	–
Kojibiose	1 (β)	66.21 ± 0.19	59	53
	2 (α)	33.79	41	47
Sophorose	1 (β)	32.86 ± 0.22	26	–
	2 (α)	67.14	74	–
Nigerose	1	60.34 ± 1.83	–	56
	2	39.66	–	44
Laminaribiose	1	44.60 ± 1.94	44	–
	2	55.40	56	–
Maltose	1 (α)	44.78 ± 0.46	33	44
	2 (β)	55.22	67	56
Cellobiose	1 (α)	49.12 ± 0.48	41	41
	2 (β)	50.88	59	59
Isomaltose	1 (α)	44.16 ± 0.51	23	–
	2 (β)	55.84	77	–
Gentiobiose §	1 (α)	32.44 ± 0.97	29	100
	2 (β)	67.56	71	–
Lactose	1 (α)	47.82 ± 1.61	41	41
	2 (β)	52.18	59	59
Melibiose	1 (α)	41.06 ± 0.75	33	44
	2 (β)	58.94	67	56
Maltulose	1	39.50 ± 1.12	–	–
	2	60.50	–	–
Palatinose	1 + 2	91.5	92	–
	3	8.5	8	–
Lactulose	1	40	100	23
	2	60	–	77

* Equilibration in pyridine with 0.2 *M* 2-hydroxypyridine at 40°C for 15 h. Standard deviations are based on three or four samples.

** Equilibration in water at room temperature for 48 h (ref. 4).

*** Equilibration in water at room temperature for 48 h (ref. 5).

§ Temperature programming was used for separation: Initial temperature, 200°C; heating rate, 5°C/min; final temperature, 270°C.

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