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Note

Gas chromatographic separation of silylated derivatives of disaccharide mixtures on open tubular glass capillary columns

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Although several workers have reported the gas chromatographic separations of selected disaccharides on packed columns (*e.g.*, refs. 1–3), the retention characteristics of the disaccharides determined by Haverkamp *et al.*[4] indicate that a clear separation of these substances as components of a mixture would require much higher resolution than is normally attained with packed gas chromatographic columns. Larson *et al.*[2] observed that, because of the temperature limitations and long retention times for disaccharides, liquid phases of higher polarity were generally unsuitable for disaccharide analysis, and the liquid phases of lower polarity did not provide adequate separation. This latter objection might be resolved through the use of columns of higher efficiency and low polarity. Szafranek *et al.*[5] used an open tubular glass capillary column coated with SE-30 admixed with a fumed silicon dioxide (Silanox 101) to achieve the separation of mixtures of monosaccharide derivatives. Dizdaroglu and Von Sonntag[6] reported the separation of silylated derivatives of the radiolysis products of cellobiose (through C_6 compounds), and Schomburg and Husmann[7] separated silylated reaction products of gamma-irradiated glucose on open tubular glass capillary columns. In our work, concerned with the products of the irradiation of saccharides, we have been unable to establish a satisfactory material balance; there are indications that some larger products may arise during this treatment. We have therefore been interested in developing a high-resolution analysis that would include these larger compounds.

EXPERIMENTAL

Reagents

Anhydrous pyridine (reagent grade) and trimethylchlorosilane (TMCS) were obtained from E. Merck, Darmstadt, G.F.R. N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and methoxylamine hydrochloride (95%) were purchased from Serva, Heidelberg, G.F.R. Sucrose, cellobiose, trehalose, maltose and palatinose were obtained from Serva, lactose and gentiobiose from Merck and melibiose from Fluka, Buchs, Switzerland.

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Derivatization

The method used was essentially that described by Laine and Sweeley[8]. A 500-μl volume of an aqueous solution containing 10^{-2} mole/l of each of the eight disaccharides (sucrose, lactose, cellobiose, trehalose, maltose, gentiobiose, palatinose and melibiose) was placed in a desiccator over phosphorus pentoxide and evaporated to dryness. The dried sample (*ca.* 14 mg) was added to a PTFE-capped vial containing 12 mg of methoxylamine hydrochloride dissolved in 500 μl of pyridine and the mixture was heated for 2 h at 80°. Trimethylsilyl (TMS) derivatives were formed by adding 300 μl of BSTFA containing 4% of TMCS and heating for 15 min at 80°. Injections utilized aliquots of this solution.

Gas chromatography

Separations were made with a Carlo Erba Fraktovap Model 2101 GC gas chromatograph, fitted with a simple concentric tube glass inlet splitter and adapted to a glass capillary column, 45 m × 0.25 mm I.D., coated with OV-101 (ref. 9). Deadspace in the detector connection was minimized by carefully straightening *ca.* 15 cm of the outlet end of the column and leading this section through a 6 mm O.D., 1 mm I.D. heavy-walled glass capillary liner that filled the detector column connection; the glass capillary column terminated just below the quartz flame tip.

Analyses were isothermal at 272°, with the inlet and detector at 290°; the carrier gas was helium at an average linear velocity of 24 cm/sec (0.9 ml/min) as measured by propane injection. At this temperature, the difference in retention times between methane and propane is negligible. The splitting ratio was 1:460.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram of the reaction mixture from the eight disaccharides. The early peaks immediately following the solvent peak represent fructose and glucose and appear in every analysis including that of sucrose; they probably result from a small degree of sucrose hydrolysis that occurs during the drying step. Each of the reducing sugars (lactose, cellobiose, maltose, gentiobiose and melibiose but not palatinose) gives rise to two peaks, one large and one small. These peaks probably represent the *syn* and *anti* forms of the oxime. In Fig. 1, the minor derivative of cellobiose, representing approximately 15% of the cellobiose, co-chromatographs with trehalose and is evident as an asymmetric leading edge on the trehalose peak. Similarly, the minor fraction of gentiobiose appears as an asymmetric leading edge on the palatinose peak. We have been unable to achieve improved resolution of these overlapping pairs on the OV-101 column. The separation between a pair of peaks is generally improved by lowering the column temperature[10], but this is not universally true. It has been reported[11], for example, that the ratio of the retention times of isooctane and *n*-heptane has a positive temperature dependence, increasing from 1.13 to 1.25 as the temperature is increased from 0 to 70° in a nylon capillary coated with dinonyl phthalate. Neither isothermal operation at other temperatures (250, 260, 280, 290°) nor temperature programming separated the co-chromatographing disaccharide derivatives as well as did the 272° isothermal operation. This may relate to the observation of Scott[12], who argued that a capillary column operated at or above its optimum gas velocity should exhibit an optimum

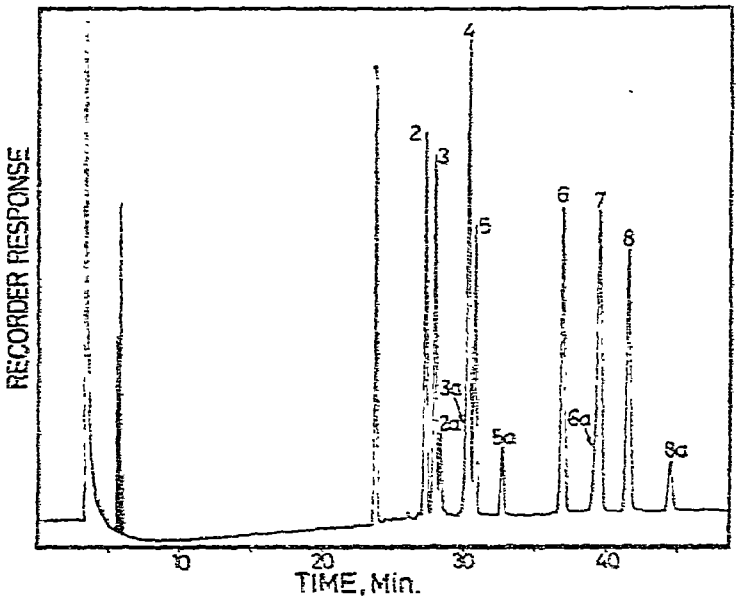

Fig. 1. Chromatogram of 4 μl (splitting ratio 1:460) of TMS and MO-TMS derivatives of a disaccharide mixture on an open tubular glass capillary column, 45 m × 0.25 mm I.D., coated with OV-101 and operated isothermally at 272°. Peaks: 1, sucrose TMS; 2, lactose MO-TMS; 3, cellobiose MO-TMS; 2a, lactose MO-TMS; 3a, cellobiose MO-TMS; 4, trehalose TMS; 5, maltose MO-TMS; 5a, maltose MO-TMS; 6, gentiobiose MO-TMS; 6a, gentiobiose MO-TMS; 7, palatinose MO-TMS; 8, melibiose MO-TMS; 8a, melibiose MO-TMS. In each instance, the "a" following a number denotes the minor MO-TMS derivative.

temperature that would give the maximum resolution for a particular pair of solutes. It is doubtful whether the resolution of these pairs on this column can be further improved.

Table I shows the Kováts retention indices of each of the components separated.

TABLE I

KOVÁTS RETENTION INDICES (*I*) OF TRIMETHYLSILYL DERIVATIVES OF DISACCHARIDES AND OF DISACCHARIDE METHYL OXIMES (MO) ON OV-101 AT 272°

*Peak No.**	*Component*	*I*
1	Sucrose TMS	2750
2	Lactose MO-TMS, major	2790
3	Cellobiose MO-TMS, major	2800
2a	Lactose MO-TMS, minor	2810
3a	Cellobiose MO-TMS, minor	2835
4	Trehalose TMS	2840
5	Maltose MO-TMS, major	2845
5a	Maltose MO-TMS, minor	2865
6	Gentiobiose MO-TMS, major	2925
6a	Gentiobiose MO-TMS, minor	2950
7	Palatinose MO-TMS	2960
8	Melibiose MO-TMS, major	2980
8a	Melibiose MO-TMS, minor	3010

* See Fig. 1.

as determined on the OV-101 open tubular glass capillary column at 272°. The values were determined on the individual disaccharides (which also established the assignments shown in Fig. 1), and re-measured as components of the more complex mixture; interaction between components of the mixture (which would affect the Kováts indices) was not evident.

Because of the relatively low polarity of OV-101, retention (and analysis) times are relatively short, yet the resolution obtained with the open tubular glass capillary column is sufficiently high to afford good separations in most instances. Columns of this type, which are capable of routine use at 300° and occasional exposure to temperatures as high as 340°, are particularly suited to the analysis of these higher boiling compounds.

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