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Note

Separation of monosaccharides as trimethylsilylated alditols on fused-silica capillary columns

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The preparation of suitable volatile derivatives of the monosaccharides formed by acid hydrolysis and their separation by gas–liquid chromatography (GLC) is a standard procedure for the characterization of carbohydrate polymers.

Each monosaccharide formed by hydrolysis will be in at least two forms in aqueous solution due to the anomeric center at the glycosidic position. Some monosaccharides, *e.g.* arabinose, exist as four isomers in solution, *i.e.* as the α- and β-anomers of the furanose and pyranose forms. GLC analysis is facilitated if the anomeric center is removed by reduction to give the straight-chain alditol. Usually the alditols obtained from the neutral aldoses in native polysaccharide hydrolyzates are acetylated[1]. Good separation of these derivatives can be obtained on columns packed with support coated with polar phases such as ECNSS-M[2] or SP2330 and SP2340[3]. However, there are some disadvantages to this type of analysis. The polar stationary phases are relatively volatile and tend to bleed, leading to short column lifetimes and an unstable baseline particularly if temperature programming is used. Because of the high bleed rate, these polar phases are not suitable for capillary column use where low loadings are required. A recent report claims that a polar chiral siloxane stationary phase offers improved thermal stability and its application on glass capillary columns to separate alditol acetates was described[4].

This report describes how trimethylsilylation of alditols can offer a convenient alternative to acetylation for derivatization and quantification of the acid hydrolyzates of polysaccharides. The separation of monosaccharides as trimethylsilyl (TMS) derivatives was first reported by Sweeley *et al.*[5] and GLC–mass spectrometry (MS) has been used to characterize the individual TMS derivatives of alditols[6] and aldonic acids[7]. The reduced, trimethylsilylated irradiation products of low-molecular-weight carbohydrates have been estimated by GLC and GLC–MS using glass capillary columns[8]. The TMS derivatives of alditols have not been used for the analysis of acid hydrolyzates of polysaccharides, however, because they are not separated on packed columns[1,9]. On the other hand, there are several advantages to using TMS derivatives. The reaction of alditols with the silylating agent used in this study is rapid and quantitative within a few minutes at room temperature. These conditions are less rigorous than those used for acetylation where a long reaction time, *e.g.* overnight at room temperature[10] or the action of heat, *e.g.* 100°C for 10 min[2], in acetic anhydride–pyridine mixtures is required. In addition, because of tail-

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ing problems in GLC analysis, some workers have found it necessary to evaporate this reaction mixture and dissolve the residual alditol acetates in a small volume of solvent, *e.g.* chloroform[10] prior to injection.

The TMS derivatives are readily hydrolyzed to the original alditols but they are stable for several months if stored with excess silylating agent in a sealed tube in a freezer. Most efficient separation of the TMS derivatives is obtained on stable, low-polarity silicone gum stationary phases, which are readily adaptable to capillary columns. Størset *et al.*[11] recently have shown that resolution of the TMS derivatives of alditols present in human seminal fluid could be improved using SE-30 coated glass capillary columns. This report describes the use of recently developed fused-silica columns[12] for the separation of the TMS derivative of the alditols from a polysaccharide hydrolyzate.

MATERIALS AND METHODS

A Perkin-Elmer 900 Series instrument adapted to accommodate the fused-silica capillary columns was used for GLC separations. The columns were 25 and 50 m × 0.23 mm I.D., OV-101 coated fused-silica capillary columns supplied by Perkin-Elmer. The carrier gas head pressure was set at 14 p.s.i. and for an injected sample size of 1 μl a sample splitter (150:1) was used. Hydrogen "make up" gas was added to the carrier jar at the end of the column. The column was operated isothermally at 190°C or was temperature programmed from 175 to 220°C at 2°C/min. Standard alditols were obtained commercially and were silylated at room temperature using Tri-Sil "Z" (Pierce, Rockford, IL, U.S.A.). The silylation was considered complete after stirring for 5 min.

Corn bran hemicellulose was isolated from destarched corn bran according to the method of Wolf *et al.*[13] and was purified by dialysis. The hemicellulose (10 mg) was hydrolyzed in a sealed vial with trifluoroacetic acid (2 *N*, 2 h, 121°C).

For quantitative analysis a 1-ml aliquot of erythritol solution (0.8 mg/ml) was added before hydrolysis. The acid was removed on a rotary evaporator and the hydrolyzates reduced with sodium borohydride (5 mg) in 5 ml of water at room temperature (1 h) with stirring. Dowex 50W-X8 (H^+) resin was added to remove sodium ions, the solution filtered and taken to dryness while a few milliliters of methanol were added five times to remove boric acid as volatile methyl borate. The alditols were silylated with Tri-Sil "Z" (1 ml). The identity of the hydrolysis products was confirmed by co-chromatography and GLC–electron impact MS using a Finnegan 3300 system.

RESULTS AND DISCUSSION

The separation of nine TMS-alditols on a 25-m column is shown in Fig. 1. By using a 50-m column (Fig. 2), resolution can be improved but analysis times are longer and we find that by analyzing mixtures of known composition, quantitation of polysaccharide hydrolyzates can be satisfactorily carried out using the 25-m column. Response factors and retention times for the alditols are given in Table I.

The gas chromatogram of TMS derivatives of the alditols, prepared from the corn bran hemicellulose hydrolyzate is shown in Fig. 3. By using an internal standard,

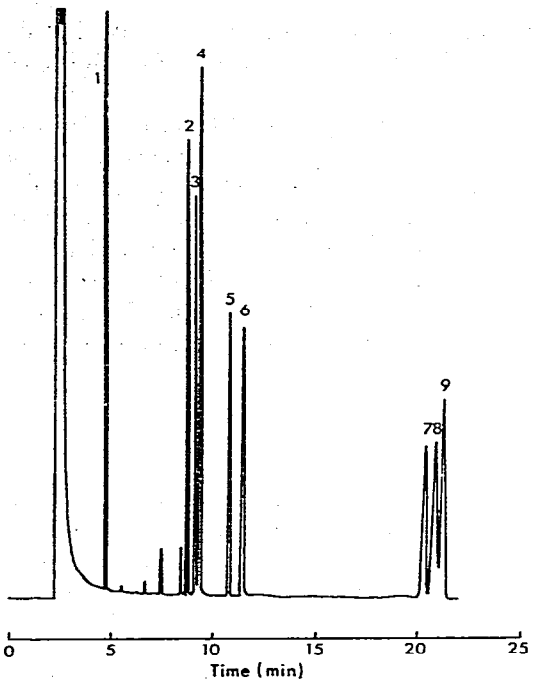

Fig. 1. Gas chromatogram of TMS derivatives of alditols on a 25-m OV-101 coated fused-silica capillary column. Isothermal 190°C. Peaks: 1 = erythritol; 2 = xylitol; 3 = arabinitol; 4 = ribitol; 5 = fucitol; 6 = rhamnitol; 7 = mannitol; 8 = glucitol; 9 = galactitol.

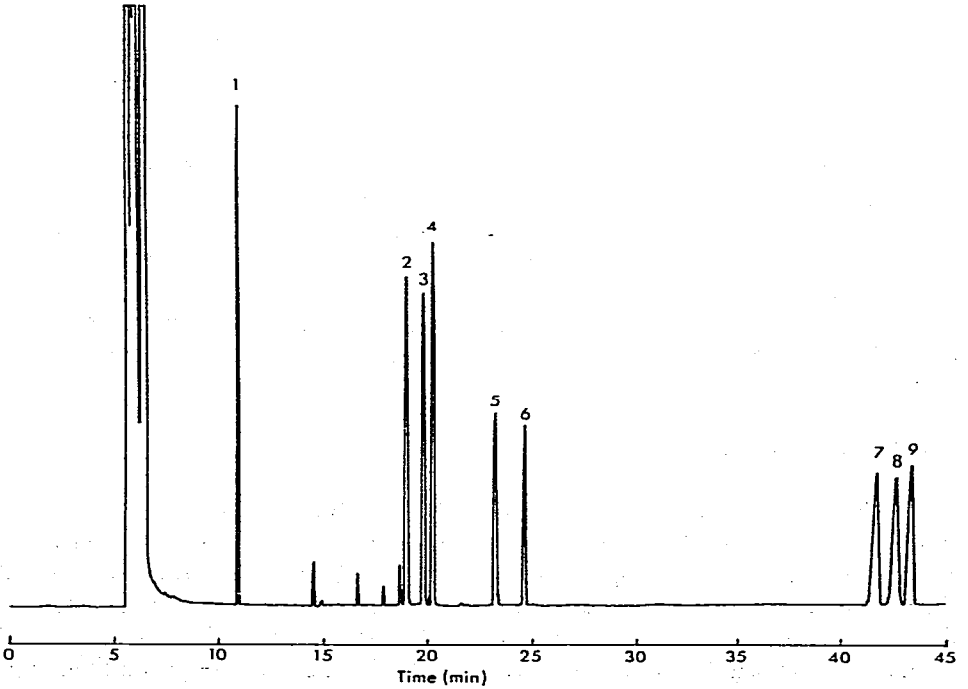

Fig. 2. Gas chromatogram of TMS derivatives of alditols on a 50-m OV-101 coated fused-silica capillary column. Isothermal 190°C. Peaks as in Fig. 1.

TABLE I

RESPONSE FACTORS AND RETENTION TIMES FOR TMS-ALDITOLS SEPARATED ON A 25-m OV-101 FUSED-SILICA CAPILLARY COLUMN

Column run isothermally, 190°C.

TMS derivative	*Response factor**	*Retention time (min)*
Erythritol	1.00	4.78
Xylitol	1.33	8.79
Arabinitol	1.33	9.22
Ribitol	1.33	9.46
Fucitol	1.27	10.89
Rhamnitol	1.27	11.58
Mannitol	1.69	20.39
Glucitol	1.69	20.93
Galactitol	1.69	21.34

* Relative to erythritol = 1. Peak area/response factor = relative moles.

the weight percentages of the monosaccharides in the corn bran hemicellulose, xylose, arabinose, galactose and mannose (trace) could be calculated. Uronic acid analysis and GLC–MS labelling experiments indicated that at least some of the glucitol derivative was formed from glucuronic acid. The estimated values show good agreement

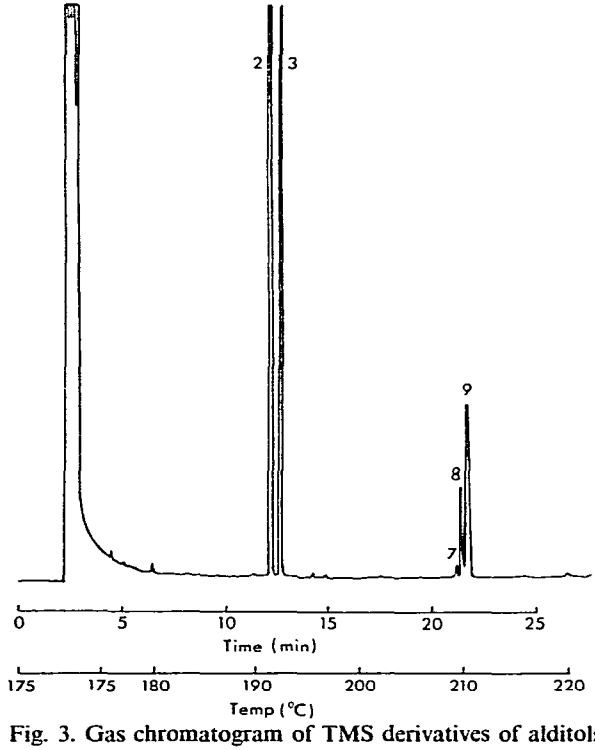

Fig. 3. Gas chromatogram of TMS derivatives of alditols prepared from the hydrolyzate of corn bran hemicellulose. Temperature program: 175°C for 4 min, then 175–220°C at 2°C/min. Peaks as in Fig. 1.

with those determined for corn bran hemicellulose using other analytical methods[14]. The steady baseline and scarcity of superfluous peaks indicate good column stability and the absence of degradation during derivatization. Earlier reports[10,15] had indicated that quantitation of carbohydrates by GLC was difficult to achieve with trimethylsilylation. This was attributed to interference by small quantities of water[15]. This was not apparent in our analyses as repeat samples gave good reproducibility. This may be due to the choice of silylating agent, Tri-Sil "Z", which is specified as being able to remove traces of water from carbohydrate samples without affecting quantitation[16].

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