permitted thorough mixing on vigorous shaking. The solutions were shaken vigorously intermittently over a period of 30 min.

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Tetracyanoethylated pentaerythritol: an efficient polar liquid phase for analysis of trimethylsilyl derivatives of sugars and sugar alcohols

In recent years application of gas–liquid chromatography (GLC) for analysis of carbohydrates and other polyhydroxy compounds has been accomplished by preparing their volatile derivatives. Trimethylsilyl (TMS) ethers are some of the most suitable derivatives for routine determination of these compounds. Identification of closely related compounds by GLC generally requires that analysis be made on both polar and nonpolar liquid phases. If only one phase is used, resolution may be incomplete. The nonpolar phases such as silicone gums SE-52[1], SE-30[5], and XE-60[1] have been used to separate TMS derivatives. By linear temperature programming these nonpolar phases can be used for separating a mixture of compounds with a wide range of boiling points. Polar phases are usually more selective than nonpolar liquid phases. Polar phases which have been used with partial success for analyses of TMS derivatives include polyethylene glycolsuccinate (EGS)[1], Carbowax 1540* (ref. 1) and Carbowax 20M[2,3].

This report describes the use of dimethylsilicone gum OV-1 as a nonpolar phase and tetracyanoethylated pentaerythritol (TCEPE) as a polar phase for the separation of TMS derivatives of various sugars and sugar alcohols.

* Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

Experimental

Materials. All sugars and sugar alcohols were obtained from Sigma Chemical Co., St. Louis, Mo. Hexamethyldisilazane and trimethylchlorosilane were obtained from Applied Science Labs, Inc., State College, Pa.

Preparations of TMS derivatives. TMS derivatives were prepared from pure anhydrous carbohydrates according to SWEELEY *et al.*[1] except that in the final stage of preparation the pyridine was evaporated and TMS derivatives were redissolved in hexane.

Erythrose was a mixture of "α" and "β" forms; deoxyribose, arabinose, and ribose were in "β" form; rhamnose, xylose, mannose, fructose and galactose were all in "α" form.

Gas chromatography. A Barber-Colman model 5000 gas chromatograph equipped with a hydrogen flame ionization detector was used throughout the study. Nitrogen was the carrier gas.

The columns and chromatographic conditions for separation were as follows:

(i) A U-shaped glass column 6 ft. × 5 mm I.D. was packed with 15% Carbowax 20M coated on 80–100 mesh Chromosorb W (Applied Science Labs, Inc.). Temperatures were: injector, 265°; detector, 225°; and column, 145°. Nitrogen inlet pressure was adjusted to 20 p.s.i.

(ii) A U-shaped glass column 6 ft. × 5 mm I.D. was packed with 3% TCEPE on 70–80 mesh Aeropack 30 (Varian Aerograph, Walnut Creek, Calif.). Temperatures were: detector, 225°, and injector, 265°. Column temperature was programmed from 100° to 140° at the rate of 2°/min. Nitrogen inlet pressure was maintained at 15 p.s.i.

(iii) A U-shaped glass column 6 ft. × 5 mm I.D. was packed with 3% OV-1 on 60–80 mesh Gas-Chrom Q (Applied Science Labs, Inc.). Temperatures were: detector, 290°, and injector, 350°. Column temperature was programmed from 120° to 190° at the rate of 3°/min. Nitrogen inlet pressure was adjusted to 20 p.s.i.

Results and discussion

Fig. 1 illustrates the degree of separation of the TMS derivatives of a mixture containing ten standard sugars and sugar alcohols on the 3% OV-1 column. This nonpolar liquid phase is comparable to SE-30 and SE-52 in separating characteristics; it has the advantage of having a more stable base line when it is temperature programmed. In addition, this column can be used at higher temperatures with less base line drift than that which occurs with SE-30 or SE-52.

TCEPE as a liquid phase previously has been used for separating methyl esters of unsaturated and satured fatty acids[4]. Fig. 2 shows the separation of the ten TMS derivatives on the 3% TCEPE column. Most of the individual sugars and sugar alcohols are well separated on this column under the conditions employed. The time required for analysis of the ten TMS derivatives was less than 21 min. Moreover, the base line drift during temperature programming conditions (100–140° at 2°/min) was negligible.

The retention times of TMS derivatives of 14 sugars or sugar alcohols on the three liquid phases used in this study are shown in Table I. Carbowax 20M was used as a polar phase to compare with TCEPE since it was previously shown to be superior to EGS for analysis of TMS derivatives[2]. Comparison of retention times on the two polar columns indicates that TCEPE is superior to Carbowax 20M. Erythrose and

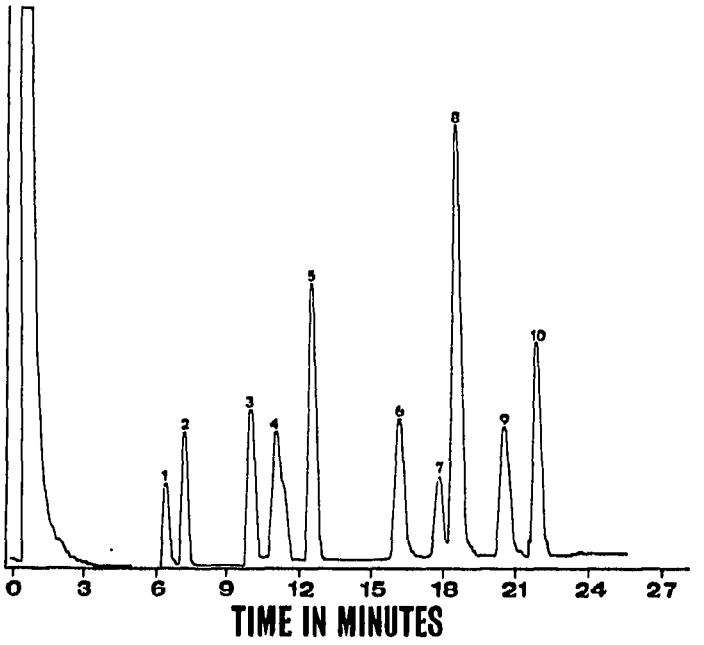

Fig. 1. Gas–liquid chromatogram of TMS derivatives of ten sugars and sugar alcohols obtained from 3% OV-1 column: 1, deoxyribose ; 2, erythritol; 3, arabinose; 4, ribose; 5, xylose; 6, mannose; 7, galactose; 8, α-glucose; 9, sorbitol; and 10, β-glucose.

erythritol had identical retention times on Carbowax 20M (2 min), but they were well separated on TCEPE. The difference in retention time of arabinose and rhamnose was 0.5 min on TCEPE compared to only 0.2 min on Carbowax 20M. Fructose and mannose were also well separated on the TCEPE column. In addition to poorer separation, the Carbowax 20M exhibited excessive column bleeding and base line drift when the temperature was programmed.

TABLE I

RETENTION TIMES OF TMS DERIVATIVES OF SUGARS AND SUGAR ALCOHOLS IN MINUTES

No.	*Compound*	*3% OV-1*	*15% Carbowax 20 M*	*3% TCEPE*
1	Erythrose	3.5	2	3.3
2	Deoxyribose	6.3	2.7	4.5
3	Erythritol	7.1	2	4.0
4	Arabinose	9.9	3.5	7.1
5	Rhamnose	10.0	3.3	6.6
6	Ribose	11.0	4.2	7.8
7	Xylose	12.4	5.7	8.7
8	Xylitol	12.7	5.0	9.7
9	Mannose	16.1	7.7	12.8
10	Fructose	16.2	7.1	11.7
11	Galactose	17.7	11.0	15.7
12	α-Glucose	18.5	11.7	14.9
13	Sorbitol	20.4	14.9	18.0
14	β-Glucose	21.8	21.9	19.1

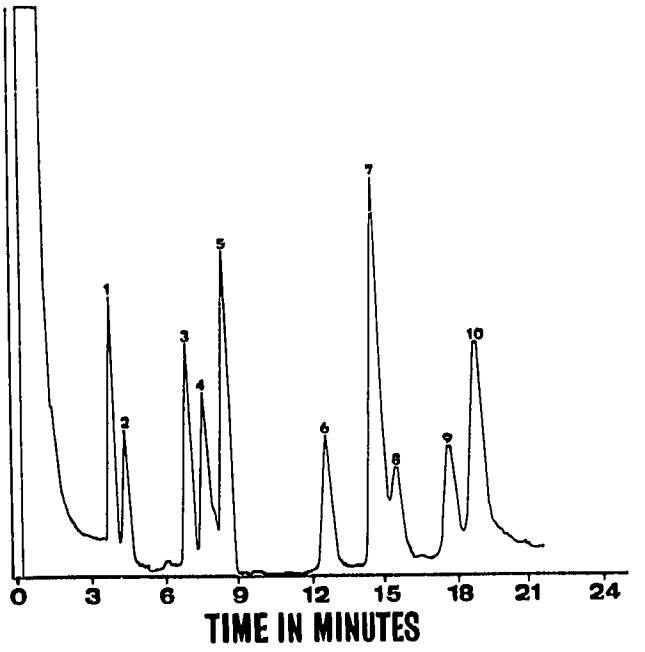

Fig. 2. Gas–liquid chromatogram of TMS derivatives of ten sugars and sugar alcohols obtained from 3% TCEPE column: 1, erythritol; 2, deoxyribose; 3, arabinose; 4, ribose; 5, xylose; 6, mannose; 7, α-glucose; 8, galactose; 9, sorbitol; and 10, β-glucose.

It is interesting to note that on the OV-1 and Carbowax 20M column glucose had a higher retention time than did galactose; the opposite elution pattern for these two sugars was observed on the TCEPE column (compare peaks 7 and 8 in Figs. 1 and 2). This characteristic of the TCEPE column is very useful in analyzing biological materials, such as plasma, which contain glucose and galactose. Similar effects were observed for deoxyribose and erythritol (peaks 1 and 2 in Figs. 1 and 2). Thus it appears that using OV-1 and TCEPE as nonpolar and polar phases for analysis of TMS derivatives is extremely valuable. In addition to sugars and sugar alcohols examined in this study, TCEPE and OV-1 may also prove useful in the analysis of other classes of compounds which form TMS derivatives.

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