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GAS–LIQUID CHROMATOGRAPHY ON OV-225 OF TETROSES AND ALDOPENTOSES AS THEIR O-METHOXIME AND O-*n*-BUTOXIME PERTRIFLUOROACETYL DERIVATIVES AND OF C_3–C_6 ALDITOL PERTRIFLUOROACETATES

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SUMMARY

The separation of tetroses and aldopentoses as the pertrifluoroacetates of acyclic O-methoximes or O-*n*-butoximes on silicon OV-225 using packed or capillary columns is reported. The trifluoroacetates are formed at lower temperatures than acetates, require lower separation temperatures and do not cause silicon deposits in the flame-ionization detector. OV-225 gives a relatively large response to structural differences of isomeric sugars. Besides the pentoses, OV-225 allows a fast separation of C_3–C_6 alditols as pertrifluoroacetates.

INTRODUCTION

Monosaccharides have been separated by gas chromatography (GC) as volatile permethyl[1,2], trimethylsilyl[3–5], peracetyl[6,7], isopropylidene[8] and pertrifluoroacetyl[9–12] derivatives. Since direct derivatization produces mixtures of α- and β-pyranose and furanose derivatives respectively, acyclic derivatives have been introduced. The simplest method, reduction to alditols, has been widely used but implies a loss of information, since a given alditol can originate from different ketoses and aldoses. Derivatization to oximes and alkoximes avoids this drawback. Each aldose gives two peaks, the *syn (Z)* and *anti (E)* isomers. Whereas this redundancy increases the risk of overlapping, it may also save information where one isomer peak is convoluted.

We propose a new combination, the separation of alkoxime pertrifluoroacetates on OV-225. This method combines the use of well defined derivatives, the redundancy introduced by *cis-trans* isomers, the ease of trifluoroacylation and the special selectivity of OV-225 towards differences in the shapes of isomeric carbohydrate derivatives. It proved useful in the separation of the complex monosaccharide mixture arising from the autocatalytic formaldehyde condensation[13,14]. Here we report the separation of all tetroses and aldopentoses and of C_3–C_6 alditol pertrifluoroacetates.

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EXPERIMENTAL

Apparatus

For column gas chromatography a Becker-Packard gas chromatograph was equipped with on-column injection, FID and glass columns (2 and 4 m × 3 mm I.D.) filled with 1% OV-225 on Chromosorb W HP (80–100 mesh).

For capillary gas chromatography, a Hewlett-Packard 5830 A gas chromatograph was equipped with a flame-ionization detector and a 50-m capillary column wall-coated with OV-225 (WGA, Griesheim, G.F.R.). The split liner was filled about 2 cm with 3% OV-225 on Chromosorb W HP (80–100 mesh), fixed on both sides by quartz wool.

Materials

The carbohydrates glycolic aldehyde, D-erythrose, D-erythrulose and D-arabinose were obtained from Serva (Heidelberg, G.F.R.), D-ribose from C. Roth (Karlsruhe, G.F.R.), and D-lyxose and D-xylose from Fluka (Buchs, Switzerland). Threose was a gift of Dr. Morgenlie, Agricultural University, Department of Chemistry, N-1432 Åas-NLH, Norway. Ethyl acetate and sodium acetate *pro analysi* were obtained from E. Merck (Darmstadt, G.F.R.), O-methylhydroxylamine hydrochloride from Merck-Schuchardt (Hohenbrunn, G.F.R.), O-*n*-butylhydroxylamine hydrochloride from Applied Science Europe (Oud-Beijerland, The Netherlands) and trifluoroacetic anhydride, *ca.* 99% from Sigma (München, G.F.R.). A mixture of erythrose, threose and erythrulose was obtained in 10 min by incubation of a solution of glycolic aldehyde at pH 11 at room temperature.

Derivatization

To about 1 mg of the carbohydrate in an 1-ml vial were added a solution of 3 mg O-methylhydroxylamine hydrochloride resp. 5 mg O-*n*-butylhydroxylamine hydrochloride and 6 mg sodium acetate in 0.1 ml water. The mixture was held at 60°C for 1 h. Water was then removed by evaporation in an air flow at 60°C. A 0.1-ml volume of methanol was added and evaporated as above, producing a crystalline precipitate. The last traces of water were removed as an azeotrope by adding 0.1 ml of benzene and again evaporating to dryness. The vial was closed immediately with a PTFE-coated spetum. To the dry oximes (or 1 mg alditol) were added 0.03 ml trifluoroacetic anhydride (TFAA) and 0.015 ml ethyl acetate using a 50-μl syringe. After 12 h in a refrigerator or 2 h at room temperature, the derivatives were ready for injection.

Condensation of formaldehyde and reduction of carbohydrates

0.016 *M* Formaldehyde, $1.6 \cdot 10^{-3}$ *M* glycolic aldehyde and 0.036 *M* calcium acetate in 0.087 *M* NaOH were held for 1 min at 70°C. The reaction was started by adding the NaOH and stopped by neutralization. The carbohydrates were reduced by $NaBH_4$[9] and the alditols derivatized as above.

RESULTS AND DISCUSSION

The retention times of the derivatives of the tetroses and the aldopentoses,

TABLE I

RETENTION TIMES (t_R IN min) OF THE O-METHOXIME AND O-*n*-BUTOXIME PERTRIFLUOROACETATES OF TETROSES AND ALDOPENTOSES

50-m Capillary column; conditions as in Fig. 1.

Carbohydrate	*O-Methoxime pertrifluoroacetates*		*O-n-Butoxime pertrifluoroacetates*	
D-Erythrose	20.28	21.13	23.38	25.61
Threose	20.74	22.42	23.79	26.98
D-Erythrulose	21.84	22.66	25.34	26.88*
D-Ribose	22.83	24.22	26.48	28.82
D-Arabinose	23.23	25.48	26.85*	30.61
D-Lyxose	24.71	25.67	28.52	30.95
D-Xylose	25.03	26.41	29.01	31.67

* Overlapping of peaks.

separated by capillary gas chromatography, are presented in Table I. A capillary gas chromatogram of O-methoxime petrifluoroacetyl derivatives of aldopentoses is shown in Fig. 1; one of the O-*n*-butoxime pertrifluoroacetates is in Fig. 2. In both series the sequence of pentoses is ribose, arabinose, lyxose and xylose for both the *syn* and the *anti* isomers. The pentose derivative with all hydroxyl groups on one side in Fischer's projection, ribose, emerges first, that with alternating hydroxyl groups on the right and on the left side, xylose, appearing last.

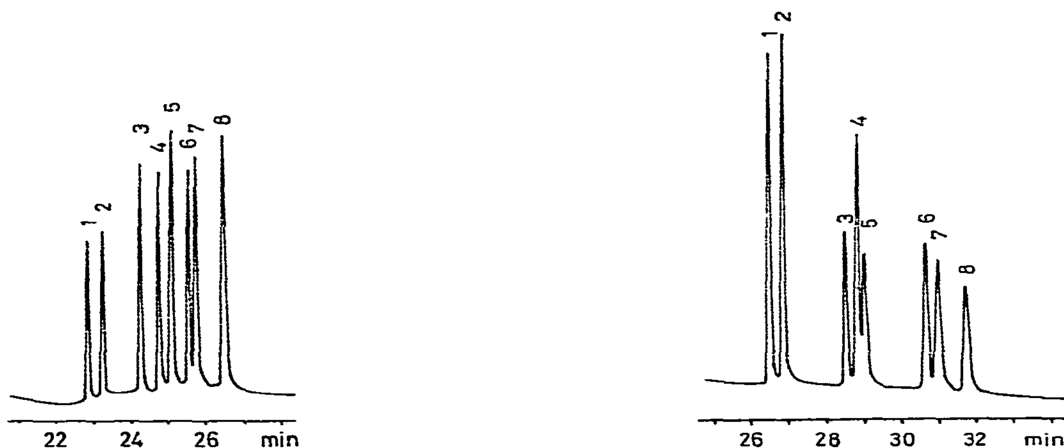

Fig. 1. Gas chromatogram of O-methoxime pertrifluoroacetyl derivatives of aldopentoses. Temperatures: column, 70°C for 2 min, then 70–180°C at 5°/min, finally 180°C; injection and detector, 250°C. Gas flow-rates: N_2 carrier gas, 1.5 ml/min; H_2, 20 ml/min; air, 200 ml/min. Sample volume; 1 µl. Splitting ratio: 1/15. For this analysis, 5 µl of each of the four original derivative solutions were mixed by injecting through a septum into a vial containing 55 µl TFAA and 25 µl ethyl acetate; 1 µl of this mixture was injected. Peaks: 1,3 = ribose; 2,6 = arabinose; 4,7 = lyxose; 5,8 = xylose.

Fig. 2. Gas chromatogram of O-*n*-butoxime pertrifluoroacetyl derivatives of aldopentoses. Preparation of derivatives and GC conditions as in Fig. 1. Peaks: 1,4 = ribose; 2,6 = arabinose; 3,7 = lyxose; 5,8 = xylose.

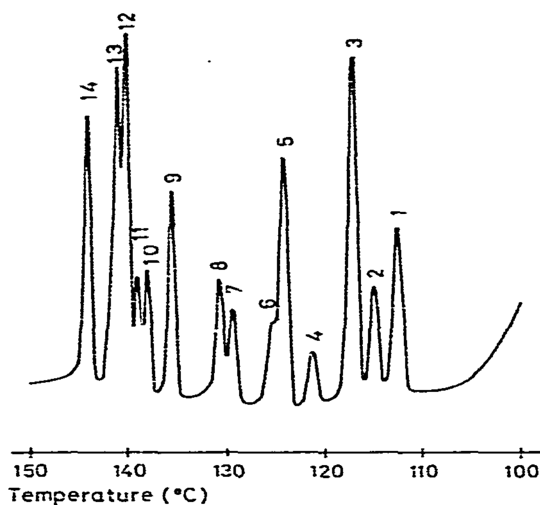

Fig. 3. Gas chromatographic separation of O-methoxime pertrifluoroacetates of tetroses and aldopentoses on a 2-m packed column. Temperature program: 2 min at 100°C, then increased at 10°/min. Peaks: 1,3 = erythrose; 2,5 = threose; 4,6 = erythrulose; 7,9 = ribose; 8,12 = arabinose; 10,13 = lyxose; 11,14 = xylose.

Owing to the redundancy of isomers, also in chromatograms obtained on 2-m and 4-m packed columns all pentoses can easily be identified using 5°/min or 10°/min temperature programs, in spite of partial overlapping (Fig. 3). 4-m columns are also sufficient for the separation of pertrifluoroacetates of alditols. All alditols from C_3 to C_6 are separated, with the exception of iditol and galactitol, which remain at least partially overlapped with the glucitol peak (Fig. 4).

We prefer sodium acetate and ethyl acetate[10] for oximation, thus avoiding a pyridine solvent peak which would interfere with the low-molecular-weight sugars.

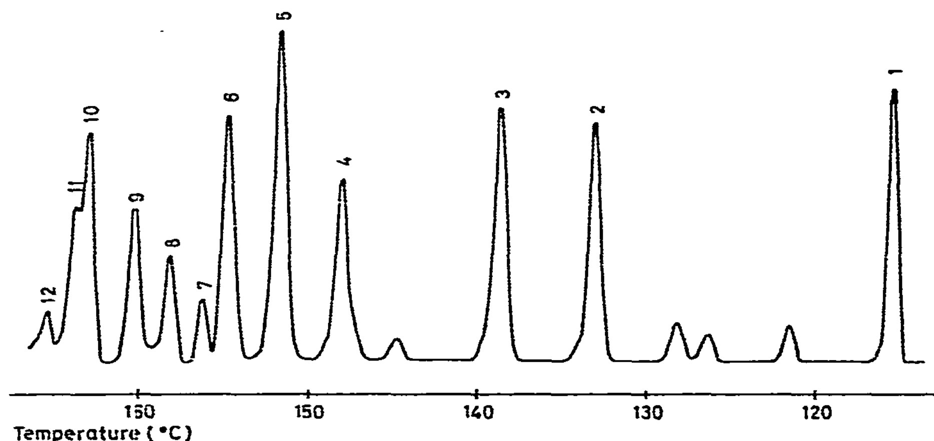

Fig. 4. Gas chromatogram of alditol pertrifluoroacetates from a formaldehyde condensation on a 4-m packed column. Temperature program: 3 min at 100°C, then increased at 5°/min to 180°C. Peaks: 1 = glycerol; 2 = erythritol; 3 = threitol; 4 = ribitol; 5 = arabinitol; 6 = xylitol; 7 = allitol; 8 = mannitol; 9 = altritol; 10 = glucitol; 11 = iditol; 12 = galactitol. Other peaks belong to unknown products.

This allows us to apply trifluoroacylation which occurs at room temperature and much faster than the reaction with acetic anhydride. Trifluoroacetyl (TFA) derivatives separate at much lower temperatures than acetates: pentose O-methoximes emerge at < 145°C from a 2-m column and the C_3–C_6 alditol derivatives appear between 120 and 170°C, allowing one to perform consecutive runs (including recooling) each in about 15 min.

So far, OV-225 has been applied only casually in GC of sugars[15]. It exhibits an excellent selectivity towards the shape of isomeric sugar derivatives. Even on a 2-m column at 10°/min a distance of 5.5 peak half-widths (of 6 sec) between erythritol and threitol, and 13 half-widths between the slowest pentitol (xylitol) and the hexitol (galactitol) respectively are obtained. On the capillary column the region of pentose derivatives extends over as much as 50 half-widths.

In the case of O-*n*-butoximes we observe even an overlapping of subsequent isomer series (see Table I): the second peak of erythrulose has nearly the same retention time as the first peak of arabinose, followed by the second peak of threose.

There are interesting differences between the retention times of the O-methoxime and the corresponding O-*n*-butoxime derivatives, a field that deserves further exploration since in temperature-programmed GC, retention times are linear functions of the functional groups (and interactions thereof) of both the stationary phase and the solute[16,17]. Therefore a variation in substitution of the derivatizing agent theoretically should entail variations of retention times similar to those resulting from variations in the constitution of the stationary phase. Such a strategy would have the advantage of being much easier to apply than the use of different columns.

There are minor drawbacks in our method. TFA derivatives may decompose on metal surfaces and therefore require all-glass equipment, and the maximum temperature of OV-225 makes it unsuitable for the separation of oligosaccharides.

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