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Note

Gas–liquid chromatography of hexoses as O-methoxime and O-*n*-butoxime pertrifluoroacetyl derivatives on silicone OV-225

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Different derivatization methods have been applied in the gas–liquid chromatography of carbohydrates, such as permethyl, trimethylsilyl (TMS), peracetyl, isopropylidene and pertrifluoroacetyl (TFA) derivatives. Of these, pertrifluoroacetylation appears to be the most convenient, because the formation of derivatives occurs at low temperature and is much faster than with acetic anhydride. Moreover, TFA derivatives require lower separation temperatures than TMS derivatives and no silicon deposits can be formed in the detector. On the other hand, TFA derivatives decompose on metal surfaces and require all-glass equipment.

In a previous paper[1] we reported on the separation of tetroses and pentoses. Here we discuss the use of silicone OV-225 as a suitable stationary phase for the separation of O-methoxime and O-*n*-butoxime TFA derivatives of hexoses on a capillary column, allowing the rapid and effective separation of all hexoses and hex-2-uloses.

EXPERIMENTAL

Apparatus

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

Materials and derivatization

D-Allose, D-altrose, D-gulose and D-idose were obtained from Sigma (Munich, G.F.R.), D-glucose, D-mannose and D-galactose from Merck (Darmstadt, G.F.R.) and D-talose from Serva (Heidelberg, G.F.R.). Ethyl acetate and sodium acetate were products of Merck. O-Methylhydroxylammonium chloride was obtained from Merck-Schuchardt (Hohenbrunn, G.F.R.), O-*n*-butylhydroxylammonium chloride from Applied Science Europe (Oud-Beijerland, The Netherlands) and trifluoroacetic anhydride (TFAA) (*ca.* 99%) from Sigma.

Derivatization was performed as described previously[1].

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TABLE I

RETENTION TIMES OF THE O-METHOXIME AND O-*n*-BUTOXIME PERTRIFLUOROACETATES OF HEXOSES AND HEX-2-ULOSES

50-m capillary column; conditions as in Fig. 1. Each carbohydrate gave two peaks, the *syn(Z)* and *anti(E)* alkoximes

Carbohydrate	*Retention time (min)**			
	O-Methoxime pertrifluoroacetates		*O-n-Butoxime pertrifluoroacetates*	
Allose	25.17	26.81[1]	28.61	31.91[5]
Altrose	25.88	27.61	29.59	33.12[6]
Mannose	26.51	28.00	30.65	33.96
Talose	26.81[1]	28.42	31.31	34.59[7]
Gulose	27.05[2]	28.90[3]	31.03	34.93
Galactose	27.05[2]	30.21[4]	31.47	37.09[8]
Glucose	27.46	28.90[3]	31.60	35.13
Idose	27.77	30.21[4]	32.37	37.09[8]
Psicose			32.08[5]	33.08[6]
Fructose			31.96[5]	34.55[7]
Sorbose			33.66	35.52
Tagatose			35.68	36.76

* [1–8] = Overlapping peaks according to the corresponding numbers.

RESULTS AND DISCUSSION

The retention times of the hexose derivatives are given in Table I. The separations of a mixture of the pertrifluoroacetylated O-methoximes and O-*n*-butoximes are shown in Figs. 1 and 2, respectively.

The Hewlett-Packard 5830A gas chromatograph affords excellent reproducibility of retention times to at least 0.05 min and of the distances between the peaks of a series to ± 0.01 min. Nevertheless among the O-methoxime derivatives some peak pairs were not separated. Therefore, only with altrose and mannose was direct quantitation by adding the peak areas of the two isomers feasible. However, as the other hexoses, except gulose and galactose, afford at least one well separated peak, they can be identified qualitatively and may even be quantitated indirectly using the fairly reproducible individual peak-area factors for the calculation of each second peak area from the area of the first one:

$$\text{area I}_{(x)} \cdot f_{(x)} = \text{area II}_{(x)}$$

Only gulose and galactose cannot be distinguished and at best sum of both can be obtained by adding the area of the peak containing both sugars (27.05 min) and those of the two peaks (28.90 and 30.21 min) which contain the isomers of each, together with one peak of idose and glucose, respectively, and subtracting from this sum the peak area of idose and glucose contained therein, as calculated from the other isomer thereof by means of specific peak-area factors.

O-*n*-Butoxime derivatives are better separated, only the second peaks of galac-

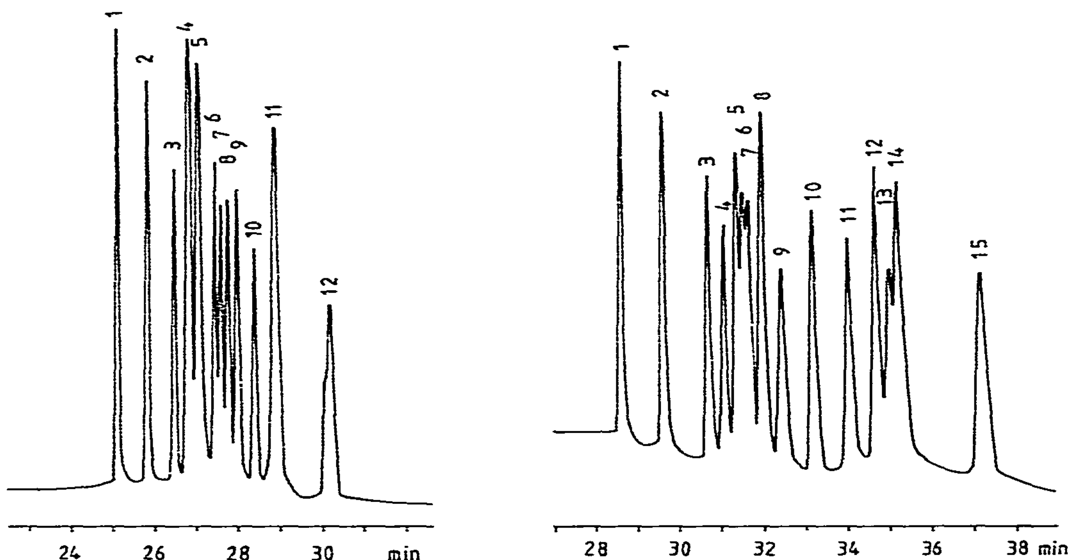

Fig. 1. Gas chromatogram of O-methoxime TFA derivatives of aldohexoses. Temperatures: column, 70°C for 2 min, then increased from 70 to 180°C at 5°C/min and held at 180°C; injector and detector, 250°C. Gas flow-rates: nitrogen carrier gas, 1.5 ml/min; hydrogen, 20 ml/min; air, 200 ml/min. Sample volume: 1 μl. Splitting ratio: 1/15. For this analysis, 5 μl of each of the eight original derivative solutions were mixed by injecting through a septum into a vial containing 40 μl of TFAA and 20 μl of ethyl acetate. Peaks: 1, 4^1 = allose; 2, 7 = altrose; 3, 9 = mannose; 4^1, 10 = talose; 5^2, 11^3 = gulose; 5^2, 12^4 = galactose; 6, 11^3 = glucose; 8, 12^4 = idose. $^{1-4}$ = Overlapping peaks according to the corresponding numbers.

Fig. 2. Gas chromatogram of O-*n*-butoxime TFA derivatives of aldohexoses. Preparation of derivatives and GC conditions as in Fig. 1. Peaks: 1, 8 = allose; 2, 10 = altrose; 3, 11 = mannose; 4, 13 = gulose; 5, 12 = talose; 6, 15* = galactose; 7, 14 = glucose; 9, 15* = idose. * Overlapping peaks.

tose and idose having the same retention time. Thus all hexoses can be identified immediately. For quantitation the partial overlapping of galactose I (I = first appearing isomer) with glucose I and of gulose II with glucose II can be circumvented by using peak-area factors as for galactose and idose. Thus, a quantitative (or semiquantitative) determination of all hexoses in one chromatogram appears feasible.

The redundancy introduced by the presence of the *syn*- and *anti*-forms of O-*n*-butoximes allows even the separation of all hexoses and hex-2-uloses. The retention times (which will be discussed in a forthcoming paper on hex-2- and -3-uloses, recently found in formose[2], and hex-2,5-diuloses[3]) have been included in Table I from independent chromatograms. Psicose I and fructose I should overlap with allose II, psicose II with altrose II and fructose II with talose II. The peaks of sorbose and tagatose should appear separated from the others. Thus small amounts of fructose can be identified only in the absence of both allose and psicose, or of talose, and small amounts of psicose can only be seen in the absence of altrose, or of both fructose and allose.

Thus all hexoses, sorbose, tagatose and, in the absence of altrose and talose, also fructose and psicose, are easily revealed because at least one of their twin peaks remains undisturbed. Altrose and talose very often might be absent; moreover, even

in their absence greater amounts of fructose and/or psicose are easily revealed by considering the peak-area factors and comparing the sizes of the two altrose and talose peaks.

Therefore, the gas chromatographic separation of trifluoroacetylated O-*n*-but-oximes may prove a useful "one pot" method, allowing the separation of all hexoses including the rarer ones (and also the tetroses, pentoses[1] and probably some hep-toses).

OV-225 exhibits good specifity towards structural differences in isomeric sugars, the distance between the slowest and fastest hexose amounting to 60 peak half-widths on the capillary column used.

REFERENCES

1 P. Decker and H. Schweer, *J. Chromatogr.*, 236 (1982) 369–373.
2 P. Decker, H. Schweer and R. Pohlmann, *J. Chromatogr.*, in press.
3 P. Decker and H. Schweer, *Carbohydr. Res.*, in press.