Journal of Chromatography, 243 (1982) 149–152
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,844

Note

Gas chromatographic separation of carbohydrate enantiomers as (—)-menthyloxime pertrifluoroacetates on silicone OV-225

HORST SCHWEER
Chemical Institute, Veterinary School, Bischofsholer Damm 15, 3000 Hannover (G.F.R.)
(Received February 22nd, 1982)

Two methods for the separation of enantiomeric carbohydrates are possible. The carbohydrates can be derivatized with achiral reagents and a chiral stationary phase is utilized. The separation of trifluoroacetyl (TFA) and TFA-methylglycoside derivatives of enantiomeric carbohydrates on a capillary column coated with XE-60–L-valine-*S*-α-phenylethylamide has been reported[1,2]. Each carbohydrate enantiomer produces up to four peaks due to the α- and β-pyranosides and furanosides, respectively.

Alternatively derivatives can be prepared with chiral reagents. Oxidation of aldoses to aldonic acids, esterification with a chiral alcohol and acetylation result in diastereomeric derivatives, which allow partial separation[3,4]. Drawbacks of this method are the difficult preparation of derivatives and its limitation to aldoses. Also, trimethylsilyl (—)-2-butanol glycoside derivatives of carbohydrates have been applied in gas chromatography (GC)[5]. In both instances the chiral alcohols used were not optically pure.

In this paper new diastereomeric derivatives of enantiomeric carbohydrates useful for GC are described. Each carbohydrate enantiomer produces two peaks, the *syn*(*Z*) and *anti*(*E*) alkoximes, derivatization is very easy and GC on OV-225 mostly shows good separations of enantiomers.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 5830A gas chromatograph equipped with 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), held on both sides by quartz-wool.

Materials

The carbohydrates and trifluoroacetic anhydride were obtained from Sigma (Munich, G.F.R.) and (—)-menthol, (±)-2-butanol, (—)-2-methyl-1-butanol, sodium acetate and ethyl acetate from Fluka (Buchs, Switzerland). O-(—)-Menthylhydroxylammonium chloride, O-(±)-2-butylhydroxylammonium chloride and O-(—)-2-methyl-1-butylhydroxylammonium chloride were synthesized from the sodium alcoholates and chloramine[6].

0021-9673/82/0000–0000/$02.75

TABLE I

RETENTION TIMES OF THE (±)-2-BUTOXIME PERTRIFLUOROACETYL DERIVATIVES OF CARBOHYDRATES

50-m capillary column; conditions as in Fig. 1. Each *E* and *Z* isomer can give two peaks, the (+)- and the (−)-2-butoxime derivatives.

Carbohydrates	*Retention time (min)*					
	Anti (E) isomer			*Syn (Z) isomer*		
Erythrose		15.11			16.41	
Ribose		17.29		18.76		18.87
Arabinose		17.52			20.03	
Xylose	19.04		19.13		20.59	
Lyxose		18.77		20.21		20.34
Allose		19.02		20.93		21.09
Altrose	19.64		19.76		21.59	
Glucose	20.91		21.01		22.62	
Mannose	20.45		20.57	22.05		22.19
Gulose		20.69		22.55		22.75
Idose	21.47		21.58		23.77	
Galactose		20.79			23.97	
Talose		20.90		22.55		22.68
Fructose		21.21			22.55	
Sorbose		22.23			22.96	
Tagatose		23.64			23.83	
Fucose		15.62			18.25	
Rhamnose	16.41		16.47	17.34		17.47

Derivatization

To about 0.5 mg of a carbohydrate a solution of 4 mg of O-(−)-menthylhydroxylammonium chloride, 2.5 mg of O-(±)-2-butylhydroxylammonium chloride or 2.5 mg of O-(−)-2-methyl-1-butylhydroxylammonium chloride and 3 mg

TABLE II

RETENTION TIMES OF (−)-MENTHYLOXIME PERTRIFLUOROACETYL DERIVATIVES OF ENANTIOMERIC CARBOHYDRATES

50-m capillary column; conditions as in Fig. 2. Each carbohydrate enantiomer gave two peaks, the *E* and the *Z* isomers.

Carbohydrate	*Retention time (min)**			
	D-*Enantiomer*		L-*Enantiomer*	
Glyceraldehyde	11.94	15.24	11.71	15.14
Ribose	18.55	25.01 (A)	17.50	24.84 (A)
Arabinose	18.15	29.53	19.01	29.94
Xylose	22.10 (B)	31.44 (C)	22.18 (B)	31.17 (C)
Lyxose	20.80	30.95	22.03	31.37
Glucose	25.54 (D)	40.30 (E)	25.78 (D)	40.28 (E)
Mannose	25.03	37.05	26.15	37.63
Galactose	27.95	45.60	26.63	44.63
Fucose	15.31	23.36	14.64	22.89

* Pairs of peaks labelled A, B, C, D and E overlap.

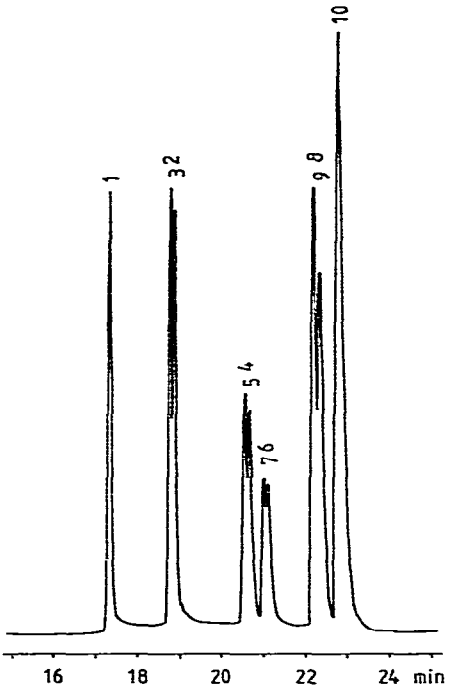

Fig. 1. Gas chromatogram of (±)-2-butoxime pertrifluoroacetyl derivatives of ribose, mannose and glucose. Temperatures: column, 100°C for 2 min then increased from 100 to 180°C at 5°C/min; injection and detector, 250°C. Gas flow-rates: nitrogen carrier gas, 2 ml/min; hydrogen, 20 ml/min; air 200 ml/min. Sample volume: 1 μl. Splitting ratio: 1:12. Peaks: 1,2,3 = ribose; 4,5,8,9 = mannose; 6,7,10 = glucose.

of sodium acetate in 100 μl of water were added. Further preparation was carried out as described previously[7].

RESULTS AND DISCUSSION

First experiments to separate enantiomeric carbohydrates as alkoxime pertrifluoroacetates were made with 2-methyl-1-butoximes and 2-butoximes. The (−)-2-methylbutoxime pertrifluoroacetates of racemic mixtures of carbohydrates were not separated on a 50-m capillary column coated with OV-225, whereas the (±)-2-butoxime pertrifluoroacetates of the investigated aldoses mostly gave three peaks, but hexulose (±)-2-butoxime pertrifluoroacetates showed no splitting (see Table I and Fig. 1). If the aldose had a *threo* configuration at $C_{2,3}$, preferentially the first peak split, whereas an *erythro* configuration at $C_{2,3}$ resulted in splitting of the second peak [except that erythrose, arabinose, galactose and fucose (6-deoxygalactose) gave only two peaks and mannose and rhamnose (6-deoxymannose) gave four peaks].

As the separation of carbohydrate 2-butoxime pertrifluoroacetates was not good and *R*- and *S*-2-butanol are very expensive and moreover not optically pure, experiments with (−)-menthyloxime pertrifluoroacetates were carried out. Of the nine aldoses investigated the enantiomers of two (xylose and glucose, *xylo* configuration at $C_{2,3,4}$) cannot be separated and the others showed separation of the *anti* (*E*)

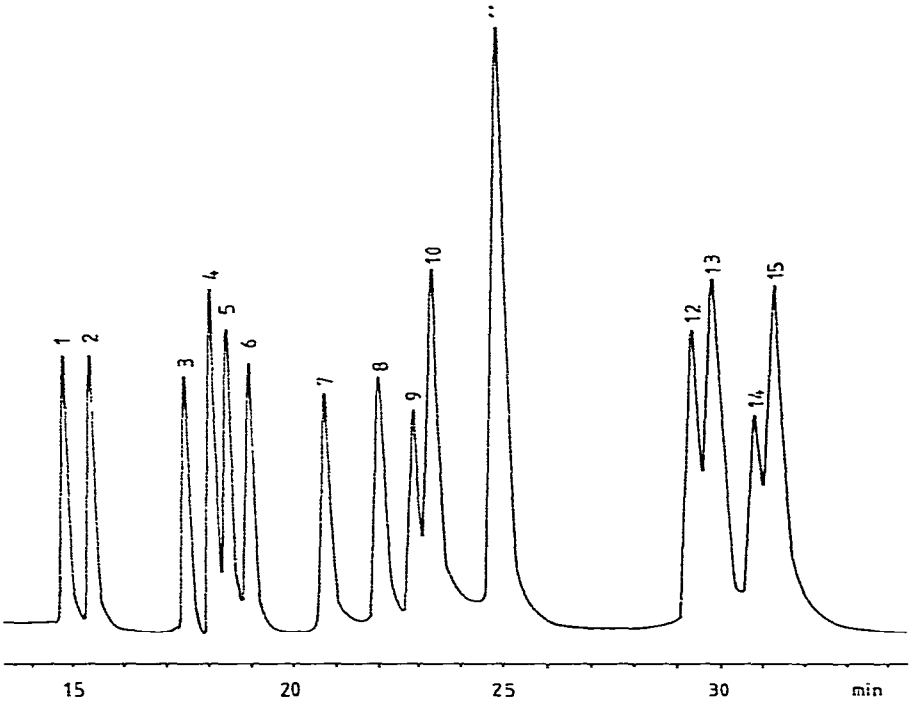

Fig. 2. Gas chromatographic separation of enantiomers of fucose, ribose, arabinose and lyxose as (−)-menthyloxime pertrifluoroacetates. Column temperature: 180°C (isothermal). Other GC conditions as in Fig. 1. Peaks: 1,9 = L-fucose; 2,10 = D-fucose; 3,11* = L-ribose; 4,12 = D-arabinose; 5,11* = D-ribose; 6,13 = L-arabinose; 7,14 = D-lyxose; 8,15 = L-lyxose. * Overlapping peaks.

isomer of the oxime (arabinose) or of the *E* and *Z* isomer (glyceraldehyde, ribose lyxose, mannose, galactose and fucose; see Table II and Fig. 2). It is remarkable that the enantiomer with the hydroxyl function at C_2 on the left-hand side in Fischer's projection always appeared first.

REFERENCES

1 W. A. König, I. Benecke and H. Bretting, *Angew. Chem.*, 93 (1981) 688–690.
2 W. A. König, I. Benecke and S. Sievers, *J. Chromatogr.*, 217 (1981) 71–79.
3 G. E. Pollock and D. A. Jermany, *J. Gas Chromatogr.*, 6 (1968) 412–415.
4 G. E. Pollock and D. A. Jermany, *J. Chromatogr. Sci.*, 8 (1970) 296.
5 G. J. Gerwig, J. P. Kamerling and J. F. G. Vliegenthart, *Carbohydr. Res.*, 62 (1978) 349–357.
6 W. Theilacker and K. Ebke, *Angew. Chem.*, 68 (1956) 303.
7 P. Decker and H. Schweer, *J. Chromatogr.*, 236 (1982) 369–373.