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G.L.C. OF THE OXIDATION PRODUCTS OF PENTITOLS AND HEXITOLS AS TRIFLUOROACETYLATED *O*-METHYLOXIME AND *O*-BUTYLOXIME DERIVATIVES

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ABSTRACT

Oxidation of pentitols and hexitols with bromine in the presence of calcium carbonate gave 2- and 3-uloses and 2,5-hexodiuloses, which were separated by capillary g.l.c. on silicone OV-225 as the trifluoroacetylated *O*-methyloxime and *O*-butyloxime derivatives.

INTRODUCTION

Pentuloses and hexuloses can be obtained from aldoses by boiling with pyridine or by treatment with an isomerase, or by oxidation of alditols with a dehydrogenase, mercury(II) acetate, chlorine, or bromine. D-*threo*-2,5-hexodiulose is formed from D-fructose by *Acetobacter suboxydans* or *Gluconobacter cerinus*.

Monosaccharides have been separated by g.l.c. as volatile methyl[1,2], trimethylsilyl[3–6], acetyl[7], isopropylidene[8,9], and trifluoroacetyl[10–12] derivatives. Since direct derivatisation produces α,β-mixtures of pyranoid and furanoid derivatives, acyclic derivatives are preferred. Conversion into alditols involves loss of information, since a given alditol can originate from different ketoses and aldoses. Derivatisation to oximes or alkoximes[3–5] avoids this drawback. Ketoses can give two peaks corresponding to the *syn* (*Z*) and *anti* (*E*) isomers, but 3-pentuloses give only one peak. 2,5-Hexodiuloses give three peaks corresponding to *syn-syn*, *syn-anti*, and *anti-anti* oximes. Whereas this multiplicity of peaks increases the risk of overlapping, it may also give useful information.

We have studied the behaviour of trifluoroacetylated sugar alkoximes on OV-225, a stationary phase that gives good results with other sugar derivatives[8,9]. This method involves well-defined oxime derivatives and easy trifluoroacetylation, and takes advantage of the special selectivity of OV-225 for isomeric carbohydrate derivatives.

The analysis of pentuloses is of interest because of their role as intermediates of the Calvin–Horecker cycle and in photosynthesis. Also, pentuloses and hexuloses are involved in formaldehyde polycondensation.

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EXPERIMENTAL

Apparatus. — A Hewlett–Packard 5830A gas chromatograph fitted with flame-ionisation detectors was used together with a 50-m capillary column, wall-coated with OV-225. A 2-cm length of the split liner was filled with 3% of OV-225 on Chrom W H.P. (80–100 mesh) held on both sides by quartz wool.

Materials. — Alditols were either commercial products or were obtained by conventional borohydride reduction of commercial samples of D-allose, D-talose, or D-idose.

Oxidation of alditols. — Calcium carbonate (~100 mg) and a solution of bromine (100 mg) in water (4 mL) were added to a solution of the alditol (10 mg) in water (1 mL). The mixture was stored in a closed vial for ~3 h at room temperature. Excess of bromine was reduced by Na_2SO_3 and the suspension was filtered. Organic acids and inorganic ions were removed from the filtrate by using a mixed-bed column (200 × 10 mm) of Dowex 50W-X8 and Amberlite IRA-68 resins and washing with distilled water (~150 mL). The deionised solution was concentrated at 30°/15 mmHg, and a solution of the residue in 50% aqueous ethanol (5 mL) was stored in a closed vial in a refrigerator. The products are stable for several months.

Derivatisation and g.l.c. — The aqueous ethanolic solution (1 mL) of each oxidation product was dried in an air stream at 60°. A solution of *O*-methylhydroxylamine hydrochloride (3 mg) or *O*-butylhydroxylamine hydrochloride (5 mg) and sodium acetate (6 mg) in water (0.1 mL) was added, and the mixture was heated at 60° for 1 h and then concentrated as described above. Methanol (0.1 mL) was evaporated from the residue, to give a crystalline precipitate of sodium acetate. The last traces of water were removed as an azeotrope by evaporation of benzene (0.1 mL). Then the vial was closed immediately with a PTFE-coated septum, and ethyl acetate (0.015 mL) and trifluoroacetic anhydride (0.03 mL) were added by using a 50-μL syringe. After 1–2 h at room temperature or after 12 h at 0°, the samples were ready for injection. In a refrigerator, the derivatives were stable for several months.

G.l.c. conditions: 70° for 2 min, →180° at 5°/min, and 180° for 15 min; injection and detector temperature, 250°; flow rates (mL/min), nitrogen 1.5, hydrogen 20, air 200; split ratio, 1/15; sample volume, 2 μL.

RESULTS AND DISCUSSION

Methyl[1,2], trimethylsilyl[3–6], acetyl[7], isopropylidene[8,9], and trifluoroacetyl[10–12] derivatives have been used for g.l.c. of carbohydrates. Of these, the trifluoroacetyl derivatives appear to be the most convenient, because they can be formed at low temperatures and much faster than acetates. Moreover, trifluoroacetates require lower temperatures for separation than trimethylsilyl derivatives, and no silicon deposits are formed in the detector. On the other hand, trifluoroacetates decompose on metal surfaces and require all-glass equipment.

The use of OV-225 as stationary phase and trifluoroacetylated alkoxime

TABLE I

OXIDATION PRODUCTS OF PENTITOLS AND THEIR R_T VALUES AS THE TRIFLUOROACETYLATED *O*-METHYLOXIME AND *O*-BUTYLOXIME DERIVATIVES

Pentitol	*Products*	O-*Methyloxime* R_T *(min)*	O-*Butyloxime* R_T *(min)*
Ribitol	DL-*erythro*-2-Pentulose	25.85	29.87
		25.98	30.42
	erythro-3-Pentulose	26.82	30.89
	Ribitol	29.06	29.12
D-Arabinitol	D-*threo*-2-Pentulose	25.59	29.21
		27.11	32.00
	D-*erythro*-2-Pentulose	25.79	29.84
		25.91	30.38
	D-*threo*-3-Pentulose	27.11	31.35
	D-Arabinitol	30.69	30.78
Xylitol	DL-*threo*-2-Pentulose	25.60	29.23
		27.07	32.00
	erythro-3-Pentulose	26.81	30.88
	Xylitol	32.04	32.17

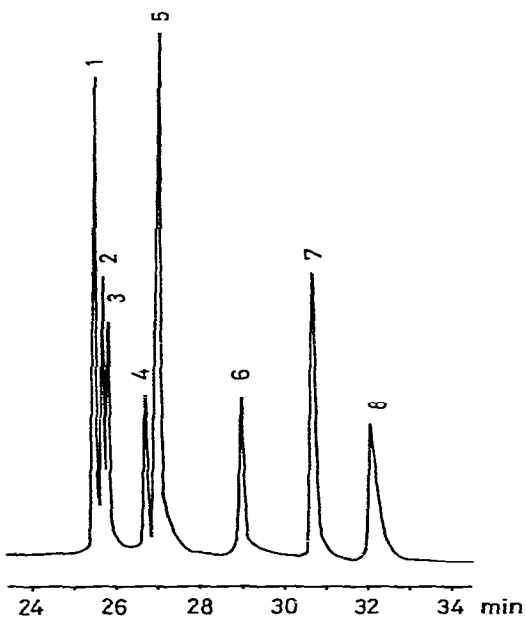

Fig. 1. Gas chromatogram of trifluoroacetylated *O*-methyloxime derivatives of pentuloses and trifluoroacetylated pentitols; 0.5 μL of each solution of derivatised oxidation products from ribitol, D-arabinitol, and xylitol was injected. Peak identities: 1 and 5, DL-*threo*-2-pentulose; 2 and 3, DL-*erythro*-2-pentulose; 4, *erythro*-3-pentulose; 5, D-*threo*-3-pentulose; 6, ribitol; 7, D-arabinitol; 8, xylitol.

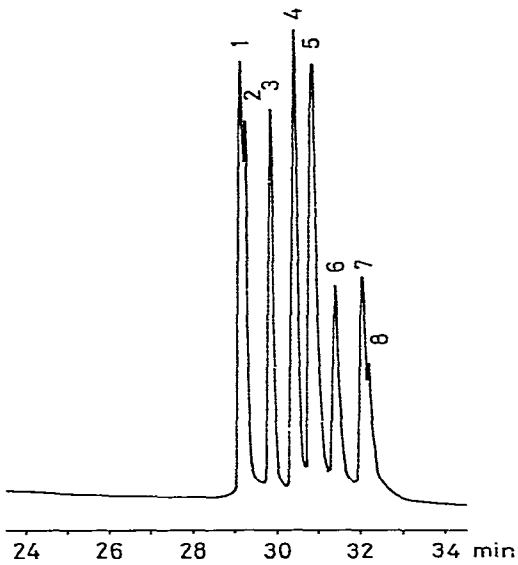

Fig. 2. Gas chromatogram of trifluoroacetylated *O*-butyloxime derivatives of pentuloses and trifluoroacetylated pentitols; 0.5 μL of each solution of derivatised oxidation products from ribitol, D-arabinitol, and xylitol was injected. Peak identities: 1, ribitol; 2 and 7, DL-*threo*-2-pentulose; 3 and 4, DL-*erythro*-2-pentulose; 5, *erythro*-3-pentulose; 5, D-arabinitol; 6, D-*threo*-3-pentulose; 8, xylitol.

derivatives facilitates effective separation of isomeric sugars; indeed, the slowest and fastest peak of pentoses emerge at a distance of 50 peak half-widths[13], and among the hexoses a maximal distance of 60 peak half-widths has been observed[14]. Moreover, the possible variation of the alkyl group of alkoximes affords additional flexibility.

Pentuloses but no (or only small amounts of) pentodiuloses are formed from pentitols by oxidation with bromine; the acids, which were removed by ion exchange, are not considered here. Some pentitols remained when oxidation was terminated. The g.l.c. data are shown in Table I; all of the theoretically possible pentuloses were formed.

The separation of the products was good. Among the trifluoroacetylated *O*-methyloximes, only the second peak for DL-*threo*-2-pentulose has the same retention time as that for D-*threo*-3-pentulose (Fig. 1). Among the trifluoroacetylated *O*-butyloximes, that of *erythro*-3-pentulose could not be separated from the trifluoroacetylated D-arabinitol (Fig. 2). Using both trifluoroacetylated *O*-methyloxime and *O*-butyloxime derivatives, all of the pentuloses and pentitols can be identified.

Since pentodiuloses were not found, they possibly undergo further oxidation or escape detection by our procedure.

All of the theoretically possible 2- and 3-hexuloses were obtained on oxidation of hexitols. Additionally, three peaks were observed in each chromatogram. The oxidation products of D-glucitol, D-mannitol, and D-iditol gave the same three additional peaks with retention times of ~39.35, ~41.25, and ~44.00 min, whereas

TABLE II

OXIDATION PRODUCTS OF HEXITOLS AND THEIR R_T VALUES (MIN) AS THE TRIFLUOROACETYLATED *O*-BUTYLOXIME DERIVATIVES

Products	*Allitol*	D-*Altritol*	D-*Glucitol*	D-*Mannitol*	D-*Iditol*	*Galactitol*
DL-Psicose	32.06	32.09				
	33.07	33.09				
D-Fructose			31.98	31.95		
			34.54	34.56		
DL-Sorbose			33.68		33.63	
			35.66		35.52	
DL-Tagatose		35.69				35.67
		36.79				36.73
DL-*ribo*-3-	35.13		35.18			
Hexulose	35.59		35.66			
D-*arabino*-3-		34.55		34.56		
Hexulose		36.79		36.90		
D-*lyxo*-3-		36.38			36.36	
Hexulose		37.08			37.09	
DL-*xylo*-3-			35.66			35.67
Hexulose			37.48			37.43
erythro-2,5-	38.45	38.45				38.40
Hexodiulose	39.89	39.85				39.80
	42.69	42.65				42.65
DL-*threo*-2,5-			39.33	39.34	39.35	
Hexodiulose			41.24	41.25	41.25	
			44.01	44.01	43.97	
Hexitol[a]			36.35	33.20		37.90

[a]Unchanged starting-material.

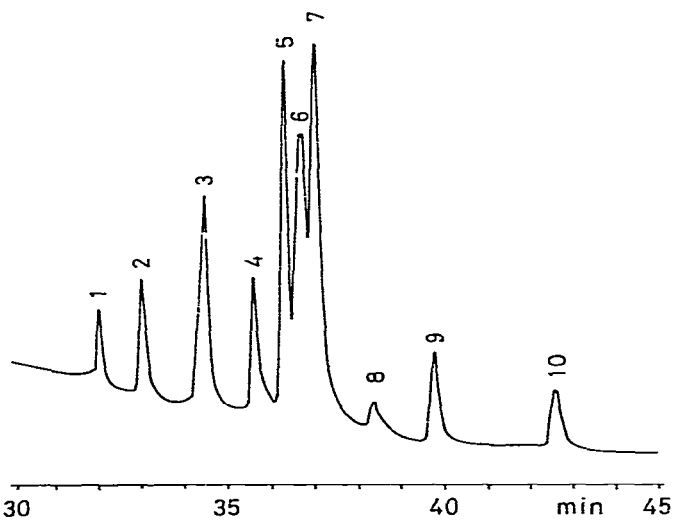

Fig. 3. Gas chromatogram of the trifluoroacetylated *O*-butyloxime derivatives of the oxidation products of D-altritol. Peak identities: 1 and 2, D-psicose; 4 and 6*, D-tagatose; 3 and 6*, D-*arabino*-3-hexulose; 5 and 7, D-*lyxo*-3-hexulose; 8–10, *erythro*-2,5-hexodiulose; *overlapping peaks.

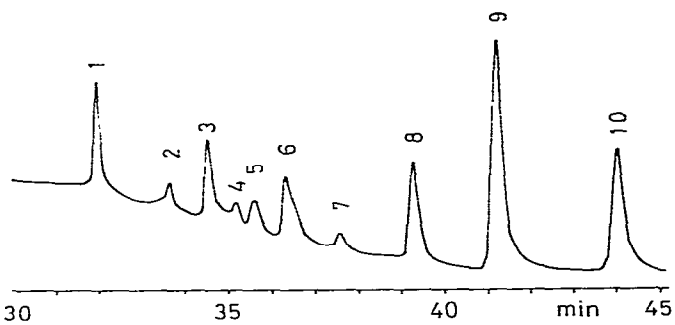

Fig. 4. Gas chromatogram of the trifluoroacetylated *O*-butyloxime derivatives of the oxidation products of D-glucitol. Peak identities: 1 and 3, D-fructose; 2 and 5*, L-sorbose; 4 and 5*, D-*ribo*-3-hexulose; 5* and 7, L-*xylo*-3-hexulose; 8–10, D-*threo*-2,5-hexodiulose; 6, D-glucitol; *overlapping peaks.

the chromatograms of oxidised allitol, D-altritol, and galactitol afforded another set of peaks with retention times of ~38.45, ~39.85, and ~42.65 min (Table II). Since only D-glucitol, D-mannitol, and D-iditol have the *threo* configuration at C-3,4, and allitol, D-altritol, and galactitol have the *erythro* configuration at C-3,4, it is concluded that the additional peaks are due to the symmetrical 2,5-hexodiuloses, which must give three isomeric dioximes.

It is not clear why 2,3-, 2,4-, and 3,4-hexodiuloses were not found. Additional work should show whether such compounds, if formed, undergo further reactions in the oxidation mixture or are lost during work-up and/or derivatisation.

Where the oxidation of hexitols was not complete, the original hexitol was also detected in the chromatograms. Figs. 3 and 4 show the chromatograms of oxidised D-altritol and D-glucitol, respectively. These hexitols yield all theoretically possible 2- and 3-hexuloses, and 2,5-hexodiuloses, and their oxidation products may prove useful as test mixtures in other chromatographic systems.

REFERENCES

1 E. BAYER AND R. WIDDER, *Justus Liebigs Ann. Chem.*, 686 (1965) 181–197.
2 H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG, AND S. SVENSSON, *Angew. Chem.*, 82 (1970) 643–652.
3 R. A. LAINE AND C. C. SWEELEY, *Anal. Biochem.*, 43 (1971) 533–538.
4 R. A. LAINE AND C. C. SWEELEY, *Carbohydr. Res.*, 27 (1973) 199–213.
5 G. PETERSSON, *Carbohydr. Res.*, 33 (1974) 47–61.
6 P. STORSET, O. STOKKE, AND E. JELLUM, *J. Chromatogr.*, 145 (1978) 351–357.
7 W. J. A. VANDENHEUVEL AND E. C. HORNING, *Biochem. Biophys. Res. Commun.*, 4 (1961) 399–403.
8 S. MORGENLIE, *Carbohydr. Res.*, 41 (1975) 285–289.
9 S. MORGENLIE, *Carbohydr. Res.*, 80 (1980) 215–222.
10 J. SHAPIRA, *Nature (London)*, 222 (1969) 792–793.
11 T. IMANARI, Y. ARAKAWA, AND Z. TAMURA, *Chem. Pharm. Bull.*, 17 (1969) 1967–1969.
12 W. A. KÖNIG, H. BAUER, W. VOELTER, AND E. BAYER, *Chem. Ber.*, 106 (1973) 1905–1919.
13 P. DECKER AND H. SCHWEER, *J. Chromatogr.*, 236 (1982) 369–373.
14 P. DECKER AND H. SCHWEER, *J. Chromatogr.*, 243 (1982) 372–375.