Journal of Chromatography, 236 (1982) 361–367
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,390

GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF ALDOSES AS O-METHOXIME, O-2-METHYL-2-PROPOXIME AND O-*n*-BUTOXIME PER-TRIFLUOROACETYL DERIVATIVES ON OV-225 WITH METHYLPROPANE AS IONIZATION AGENT

II. HEXOSES

HORST SCHWEER
Chemical Institute, Veterinary School, Bischofsholer Damm 15, 3000 Hannover (G.F.R.)
(Received September 9th, 1981)

SUMMARY

All aldohexoses as their trifluoroacetylated O-methoximes, O-2-methyl-2-propoximes and O-*n*-butoximes have been separated by gas chromatography on a glass capillary column, wall coated with OV-225, and identified by chemical ionization mass spectrometry with methylpropane. The 2-methyl-2-propoximes have similar retention times to the corresponding methoximes, thus emerging much earlier than isomeric *n*-butoximes.

Fragmentation patterns of the *syn* and *anti* isomers are reported, affording always $m/e = M+1$ as the most intense ion peak, except for the *syn* isomer of the glucose *n*-alkoximes. Glucose gave a different pattern lacking in other hexoses and involving a loss of $F_3C–COO–CH_2$ ($M = 127$) and of $F_3C–COO$ ($M = 113$). All spectra contain few masses and are easily interpreted.

INTRODUCTION

The analysis of carbohydrates by gas chromatography (GC) or high-performance liquid chromatography may difficult with samples containing many different carbohydrates along with other compounds. Mass spectrometric (MS) selected ion monitoring may add an additional dimension in such cases. Thus, carbohydrates with different molecular masses or fragmentation patterns can be detected even where they are not distinguishable from other compounds by conventional detectors.

Chemical ionization proves useful here because it affords clear-cut fragmentation patterns. In the preceding paper I reported the characterization of pentoses[1]. Here data are presented on the GC–MS properties of aldohexoses.

EXPERIMENTAL

Apparatus

A quadrupole gas chromatograph–mass spectrometer MAT 44S (MAT,

0021-9673/82/0000–0000/$02.75

Bremen, G.F.R.) equipped with a 50-m glass capillary column, wall coated with OV-225 (WGA, Griesheim, G.F.R.), was used.

Materials

The hexoses D-allose, D-altrose, D-gulose and D-idose were products of Sigma (St. Louis, MO, U.S.A.), D-glucose, D-mannose and D-galactose of E. Merck (Darmstadt, G.F.R.) and D-talose of Serva (Heidelberg, G.F.R.). Ethyl acetate and sodium acetate p.a. were purchased from E. Merck. O-Methylhydroxylamine hydrochloride was obtained from Merck-Schuchardt (Hohenbrunn, G.F.R.), O-2-methyl-2-propylhydroxylamine hydrochloride from Fluka (Buchs, Switzerland) and O-*n*-butylhydroxylamine hydrochloride from Applied Science Europe (Oud-Beijerland, The Netherlands). Trifluoroacetic anhydride, *ca.* 99%, was a product of Sigma.

Derivatization was performed as described previously[1].

Conditions for GC–MS were as described for pentose O-alkoxime pertrifluoroacetates[1].

RESULTS AND DISCUSSION

Table I shows the retention times, t_R, of the derivatized hexoses. As in the case of pentoses[1], hexose 2-methyl-2-propoximes have approximately the same t_R values as the corresponding methoximes. The first peak of each *syn–anti* isomer pair generally appears somewhat earlier, and the second peak somewhat later, than the corresponding methoxime peak (Fig. 1). The *n*-butoximes exhibit more normal t_R values, emerging about 3 min later than the methoxime and 2-methyl-2-propoxime derivatives. This effect is due to the shape selectivity of OV-225.

TABLE I

RETENTION TIMES OF THE O-ALKOXIME PERTRIFLUOROACETATES OF HEXOSES ON OV-225

Carbohydrate	*Peak*	t_R *(min)*		
		Methoxime	*2-Methyl-2-propoxime*	*n-Butoxime*
D-Allose	1	12.73	12.57	15.15
	2	14.08	14.48	17.38
D-Altrose	1	13.37	13.18	15.82
	2	14.68	15.13	18.33
D-Glucose	1	14.55	14.22	17.35
	2	15.62	15.78	19.55
D-Mannose	1	13.83	13.87	16.58
	2	14.95	15.45	18.63
D-Gulose	1	14.27	14.07	16.77
	2	15.67	15.70	19.50
D-Idose	1	14.85	14.75	17.73
	2	16.60	16.78	20.77
D-Galactose	1	14.30	14.10	17.32
	2	16.50	16.95	20.82
D-Talose	1	14.05	14.18	16.88
	2	15.18	15.88	19.17

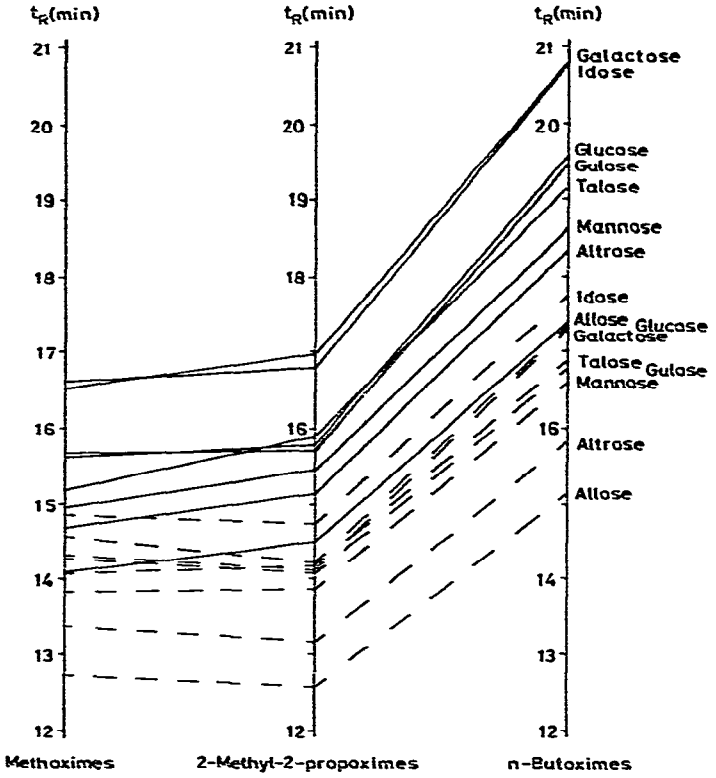

Fig. 1. Retention times of hexose O-*n*-butoxime pertrifluoroacetates: – – –, *syn* isomer; ———, *anti* isomer.

A chromatogram of all the hexoses as their O-*n*-butoxime pertrifluoroacetates obtained using selected ion monitoring (m/e = 732 = M+1) is presented in Fig. 2. The mass spectra (Tables II–IV) show that m/e = M+1 is always the most intense peak except for the first isomer of glucose, derivatized with O-methylhydroxylamine

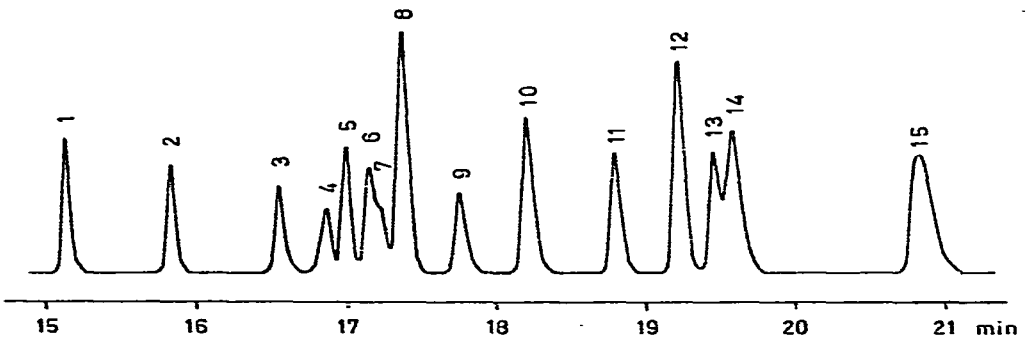

Fig. 2. Chromatogram of O-*n*-butoxime pertrifluoroacetyl derivatives of hexoses using selected ion monitoring (m/e = 732). For this analysis, 5 μl of each of the eight original derivative solutions were mixed by injecting through a septum into a vial containing 40 μl trifluoroacetic anhydride and 20 μl ethyl acetate; 1 μl of this mixture was injected. 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. The components of peak 15 were overlapped.

TABLE II
MASS SPECTRAL DATA OF O-METHOXIME PERTRIFLUOROACETATES OF HEXOSES

m/e	*Assignment*	*Relative intensity (%)*															
		D-*Allose*		D-*Altrose*		D-*Glucose*		D-*Mannose*		D-*Gulose*		D-*Idose*		D-*Galactose*		D-*Talose*	
		1	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*
746	M + 57	3	1	4	1	2	1	3	1	4	1	5	1	5	1	2	1
732	M + 43	11	7	9	9	5	7	10	8	22	13	15	11	10	12	6	6
691	M + 1 (^{13}C)	20	19	19	19	1	20	19	19	20	20	20	20	20	19	20	19
690	M + 1	100	100	100	100	7	100	100	100	100	100	100	100	100	100	100	100
689	M	52	49	48	52	3	47	50	49	60	55	48	48	59	46	58	53
576	M + 1 − 114	5	14	11	22	1	1	5	20	6	16	9	21	2	32	2	11
464	M + 1 − 2 × 113	14	9	18	17	7	16	14	12	20	14	15	15	16	21	5	11
462	M + 1 − 2 × 114									25							
451	M + 1 − 113 − 127 (^{13}C)					14											
450	M + 1 − 113 − 127	7				100											
449	M − 113 − 127					46											
350	M + 1 − 2 × 113 − 114	2	3	4	3	2	3	3	3	7	5	4	5	4	5		3
348	M + 1 − 3 × 114	1	3		6	1		2	2	16	4		5	2	5		
336	M − 2 × 113 − 127					16											
222	M − 2 × 113 − 114 − 127					54											

TABLE III

MASS SPECTRAL DATA OF O-*n*-BUTOXIME PERTRIFLUOROACETATES OF HEXOSES

m/e	*Assignment*	*Relative intensity (%)*															
		D-*Allose*		D-*Altrose*		D-*Glucose*		D-*Mannose*		D-*Gulose*		D-*Idose*		D-*Galactose*		D-*Talose*	
		1	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*
788	M + 57	3	1	3	1	1	1	3	1	3	1	2	1	3	1	2	1
774	M + 43	4	2	3	3	1	2	3	2	4	3	3	3	5	3	3	2
733	M + 1 (^{13}C)	23	24	24	23	5	24	23	23	24	24	24	24	23	23	24	24
732	M + 1	100	100	100	100	20	100	100	100	100	100	100	100	100	100	100	100
731	M	13	14	12	12	20	10	14	13	16	14	15	18	16	13	8	8
618	M + 1 − 114	7	7	12	10	3	8	5	11	7	14	6	9	7	13	7	10
562	M + 1 − 57 − 113	1	1	1	1		1	1	1	1	1	2	1	1	1	1	1
548	M − 56 − 127					8											
506	M + 1 − 2 × 113	16	9	14	12	8	10	18	13	14	10	16	12	14	13	15	12
493	M + 1 − 113 − 127 (^{13}C)					20											
492	M + 1 − 113 − 127					100											
491	M − 113 − 127					8											
449	M + 1 − 57 − 2 × 113	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	2
392	M + 1 − 2 × 113 − 114	4	2	3	3	2	3	5	4	5	4	5	4	4	4	4	4
378	M − 2 × 113 − 127					5											

TABLE IV

MASS SPECTRAL DATA OF O-2-METHYL-2-PROPOXIME PERTRIFLUOROACETATES OF HEXOSES

m/e	*Assignment*	*Relative intensity (%)*															
		D-*Allose*		D-*Altrose*		D-*Glucose*		D-*Mannose*		D-*Gulose*		D-*Idose*		D-*Galactose*		D-*Talose*	
		1	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*	*1*	*2*
733	M + 1 (^{13}C)	24	25	24	25	24	25	24	24	25	25	25	24	25	24	25	25
732	M + 1	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
731	M	18	20	18	21	18	20	17	21	19	21	18	19	21	21	18	19
718	M + 43 − 56	10	5	8	7	7	7	5	7	11	8	9	6	10	6	9	5
677	M + 1 − 56 (^{13}C)	13	3	9	8	7	6	5	12	8	7	6	6	5	6	8	5
676	M + 1 − 56	64	18	50	40	33	33	29	53	39	36	30	34	24	34	42	26
675	M − 56	11	3	29	7	18	5	16	9	21	7	16	6	12	6	7	3
618	M + 1 − 114	13	18	14	40	7	32	6	29	9	31	9	24	6	29	9	19
562	M + 1 − 56 − 114	9	3	11	11	4	8	5	12	5	11	6	6	4	7	7	3
506	M + 1 − 2 × 113	20	11	29	39	15	36	16	48	23	38	18	29	12	34	14	16
450	M + 1 − 56 − 2 × 113	4	2	9	7	4	7	5	12	9	9	4	5	3	6	1	2
433	M + 1 − 73 − 2 × 113	5	5	4	8		8	3	10	2	5	4	6	1	6	5	5
392	M + 1 − 2 × 113 − 114	7	5	8	11	3	12	6	19	8	16	7	11	3	11	7	7
380	M + 1 − 2 × 113 − 126					7											
336	M + 1 − 56 − 2 × 113 − 114	8	5	8	18		20	5	5	3	15	7	15	1	16	9	9
324	M + 1 − 56 − 2 × 113 − 126					3											

hydrochloride or O-*n*-butylhydroxylamine hydrochloride. In these derivatives of glucose, C–C bond clearage occurs with loss of F_3C–COO (M = 113) and F_3C–COO–CH_2 (M = 127).

The O-methoxime and O-*n*-butoxime pertrifluoroacetates of hexoses can add to $C_4H_9^+$ (M = 57), the predominant ion of methylpropane at 10^{-3} bar, and to $C_3H_7^+$ (M = 43), *cf.*, Fig. 3. However, since the corresponding addition products of the O-2-methyl-2-propoxime pertrifluoroacetates are very unstable, only *m/e* = M+1 (M +57−56; C_4H_8, M = 56) and *m/e* = M−13 (M+43−56) are observed. Besides these fragments, loss of trifluoroacetic acid (M = 114) and O–C_4H_9 (M = 73) occurs.

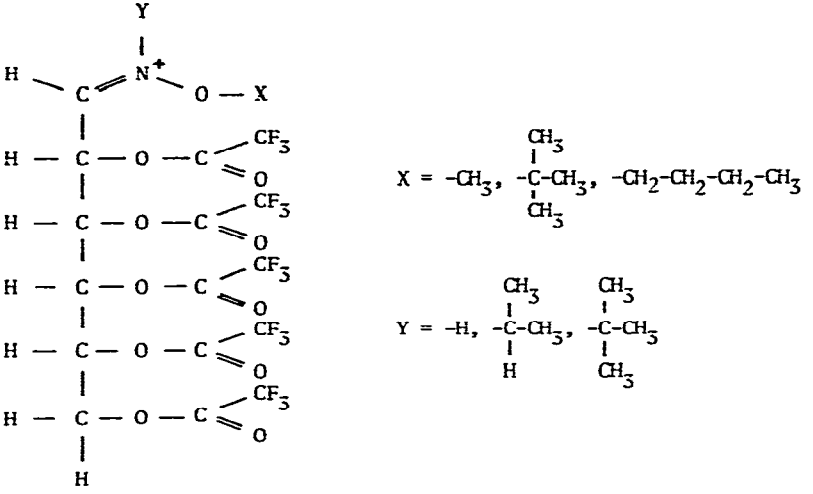

Fig. 3. Ions with masses greater than M.

The unique fragmentation pattern of the *syn* isomers of glucose *n*-alkoximes (*cf.*, Tables II and III) is very significant in terms of the unique biochemical rôle of glucose. It may even allow specific detection of glucose in the presence of all the other hexoses at M + 1 − 113 − 127 since only D-allose affords minute amounts of this glucose-specific fragment.

ACKNOWLEDGEMENTS

The author thanks Professor P. Decker for helpful discussions and Mrs. A. Büthe and Dr. W. Heidmann for technical assistance in the operation of the gas chromatograph–mass spectrometer.

This work was supported by the Stiftung Volkswagenwerk.

REFERENCE

1 H. Schweer, *J. Chromatogr.*, 236 (1982) 355.