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

I. PENTOSES

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SUMMARY

Pentoses have been identified by gas chromatography–mass spectrometry using chemical ionization by methylpropane of the O-methoxime, O-2-methyl-2-propoxime and O-*n*-butoxime pertrifluoroacetyl derivatives, separated on a glass capillary column wall coated with OV-225. Each pentose gave two peaks, the *syn* (*Z*) and the *anti* (*E*) alkoxime. The *syn* derivatives of O-2-methyl-2-propoximes give shorter retention times than the corresponding derivatives of O-methoximes. The fragmentation patterns are discussed. M + 1 gives the most intense peak.

INTRODUCTION

Several volatile and thermally stable carbohydrate derivatives have been proposed for gas chromatography and gas chromatography–mass spectrometry (GC–MS), *e.g.*, permethyl[1,2], trimethylsilyl[3–7], peracetyl[8,9], pertrifluoroacetyl[10–12] and isopropylidene[13,14]. Carbonyl groups can be left underivatized, reduced to a hydroxyl group or derivatized to an oxime. The last appears preferable since reduction entails a loss of information and underivatized carbohydrates can produce up to four peaks each. Oximes always gave two peaks, the *syn*(*Z*) and *anti*(*E*) isomers (with the exception of symmetrical dihydroxyacetone and 3-pentuloses).

Chemical ionization (CI) MS has been preferred since CI spectra are more readily interpreted, giving fewer fragment ions and more diagnostic hints for the elucidation of molecular structure. It avoids certain drawbacks of conventional electron impact MS as low intensity of high mass ions, absence of the molecular ion and multiplicity of fragmentation pathways.

EXPERIMENTAL

Apparatus

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

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

Materials

D-Arabinose, p.a. was obtained from Serva (Heidelberg, G.F.R.), D-ribose, purum from C. Roth (Karlsruhe, G.F.R.), D-lyxose and D-xylose, puriss. from Fluka (Buchs, Switzerland), ethyl acetate and sodium acetate, p.a. from E. Merck (Darmstadt, G.F.R.), O-methylhydroxylamine hydrochloride from Merck-Schuchardt (Hohenbrunn, G.F.R.), O-2-methyl-2-propylhydroxylamine hydrochloride from Fluka, O-*n*-butylhydroxylamine hydrochloride from Applied Science Europe (Oud-Beijerland, The Netherlands) and trifluoroacetic anhydride (TFAA), *ca.* 99% from Sigma (München, G.F.R.).

Derivatization

In a 1-ml vial, a solution of 3 mg O-methylhydroxylamine hydrochloride, 5 mg O-2-methyl-2-propylhydroxylamine hydrochloride or 5 mg O-*n*-butylhydroxylamine hydrochloride respectively and 6 mg sodium acetate in 0.1 ml water was added to about 1 mg of a pentose. The mixture was heated at 60°C for 1 h. Water was then evaporated in an air flow at 60°C, 0.1 ml methanol was added and then evaporated. This procedure affords a crystalline precipitate. The last traces of water were removed as an azeotrope by adding 0.1 ml benzene and again evaporating to dryness. The vial was closed immediately with a PTFE-coated septum and 0.03 ml TFAA and 0.015 ml ethyl acetate were injected. After 12 h in a refrigerator or after 2 h at room temperature, the derivatives were ready for injection. The samples are stable at 0°C for several months.

Conditions for GC–MS

Temperature program: 120°C for 2 min, increased at 5°/min, to 180°C then held for 10 min. Carrier gas (helium) flow-rate: 1.5 ml/min. Splitting ratio: 1/10. Sample volume: 1 μl. Pressures: in CI box, 390 μbar; of the forepump, 37 μbar. Electron energy: 150 eV. Emission current: 0.7 mA. Voltage of the secondary electron multiplier: 1800 V. Mass spectrum scanned from *m/e* 200 to 800; scan duration 2 sec. Temperatures: injection port, 250°C; GC separator, 220°C; GC line of sight, 220°C; source, 220°C.

RESULTS AND DISCUSSION

Table I shows the retention times of the O-methoxime, O-2-methyl-2-propoxime and O-*n*-butoxime pertrifluoroacetyl derivatives of the pentoses. As expected, the methoximes show the lowest and the butoximes the highest retention times. It is expected that 2-methyl-2-propoximes always should have longer retention times than the corresponding methoximes. However, three of the four first (*Z*) isomer peaks of the methoximes emerge after the corresponding 2-methyl-2-propoxime analogue. This extreme effect of the bulkiness of a substituent on volatility (outweighing that of the three CH_2 groups) is interpreted as a result of the special selectivity of the OV-225 stationary phase towards shape differences of isomeric sugars.

The predominant ion at 10^{-3} bar of methylpropane is $C_4H_9^+$ (M = 57)[15]. For

TABLE I

RETENTION TIMES OF O-ALKOXIME PERTRIFLUOROACETATES OF PENTOSES ON OV-225

Carbohydrate	*Peak*	t_R *(min)*		
		Methoxime	*2-Methyl-2-propoxime*	*Butoxime*
D-Ribose	1	10.88	11.15	13.63
	2	12.07	12.85	15.37
D-Arabinose	1	11.28	11.27	13.95
	2	13.10	13.83	16.82
D-Xylose	1	12.75	12.60	15.45
	2	13.92	14.18	17.33
D-Lyxose	1	12.50	12.35	15.12
	2	13.32	13.98	16.90

all the O-alkoxime pertrifluoroacetyl pentose derivatives, the ion m/e = M + 1 (M + 57 − 56) has the highest intensity (Tables II-IV). The O-methoxime and O-*n*-butoxime derivatives produce fragment ions with higher masses, resulting from the addition of $C_3H_7^+$ (M+43) and $C_4H_9^+$ (M+57). The O-2-methyl-2-propoxime derivatives also add to these voluminous substituents (Fig. 1), but the ions are unstable and always lose C_4H_8, giving the masses M+1 (M+57−56) and M−13 (M+43−56). Another feature of the O-2-methyl-2-propoxime derivatives is the appearance of the masses M+1−56 and M−56. The masses M−113 (F_3C–COO·) and M−114 (F_3C–COOH) are also observed.

Fig. 2 shows a chromatogram of all the pentoses as their O-*n*-butoxime pertrifluoroacetyl derivatives obtained by using selected ion monitoring (m/e = 606 = M + 1). Thus, especially in complex mixtures of natural products, selected ion monitoring represents an useful detection method for the diverse carbohydrate species.

TABLE II

MASS SPECTRAL DATA OF O-METHOXIME PERTRIFLUOROACETYL DERIVATIVES OF PENTOSES

m/e	*Assignment*	*Relative intensity (%)*							
		D-*Ribose*		D-*Arabinose*		D-*Xylose*		D-*Lyxose*	
		1	*2*	*1*	*2*	*1*	*2*	*1*	*2*
620	M + 57	5	1	5	1	5	2	5	1
606	M + 43	8	8	8	10	10	12	10	8
565	M + 1 (^{13}C)	16	16	16	17	17	16	16	17
564	M + 1	100	100	100	100	100	100	100	100
563	M	15	50	15	51	53	49	50	48
450	M + 1 − 114	2	16	5	22	3	31	5	22
338	M + 1 − 2 × 113	15	11	16	19	17	17	17	13
222	M + 1 − 3 × 113	1	3	2	2	2	5	2	3

TABLE III

MASS SPECTRAL DATA OF O-2-METHYL-2-PROPOXIME PERTRIFLUOROACETYL DERIVATIVES OF PENTOSES

m/e	*Assignment*	*Relative intensity (%)*							
		D-*Ribose*		D-*Arabinose*		D-*Xylose*		D-*Lyxose*	
		1	*2*	*1*	*2*	*1*	*2*	*1*	*2*
607	M + 1 (^{13}C)	20	20	19	20	20	20	19	19
606	M + 1	100	100	100	100	100	100	100	100
605	M	21	21	20	22	21	20	19	24
592	M + 43 − 56	11	7	10	5	8	5	7	5
551	M + 1 − 56 (^{13}C)	7	3	2	3	2	3	3	3
550	M + 1 − 56	52	20	15	22	15	23	14	21
549	M − 56	9	3	2	3	3	4	3	3
492	M + 1 − 114	9	15	5	24	8	25	7	22
436	M + 1 − 56 − 114	7	5	3	8	8	8	5	7
380	M + 1 − 2 × 113	16	18	10	27	15	28	16	29
324	M + 1 − 56 − 2 × 113	2	2	1	2	2	2	2	2
266	M + 1 − 2 × 113 − 114	2	2	1	2	2	2	1	2
210	M + 1 − 56 − 2 × 113 − 114	5	8	2	10	3	11	3	14

TABLE IV

MASS SPECTRAL DATA OF O-*n*-BUTOXIME PERTRIFLUOROACETYL DERIVATIVES OF PENTOSES

m/e	*Assignment*	*Relative intensity (%)*							
		D-*Ribose*		D-*Arabinose*		D-*Xylose*		D-*Lyxose*	
		1	*2*	*1*	*2*	*1*	*2*	*1*	*2*
662	M + 57	3	1	5	1	3	1	3	1
648	M + 43	3	3	6	3	5	3	4	3
607	M + 1 (^{13}C)	20	20	19	20	19	19	20	20
606	M + 1	100	100	100	100	100	100	100	100
605	M	9	10	13	13	9	9	8	8
492	M + 1 − 114	27	8	8	11	11	11	5	8
380	M + 1 − 2 × 113	16	15	18	13	16	12	23	20
322	M + 1 − 56 − 2 × 114	1	1	1	1	1	1	1	1
266	M + 1 − 2 × 113 − 114	2	2	2	2	2	2	2	2
210	M + 1 − 56 − 2 × 113 − 114		1	1	1		1	1	1

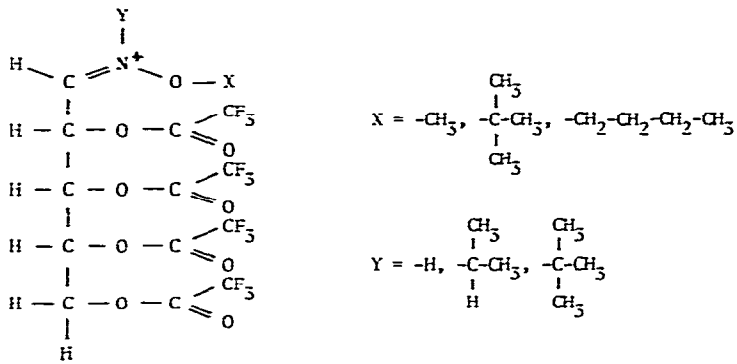

Fig. 1. Ions with masses greater than M.

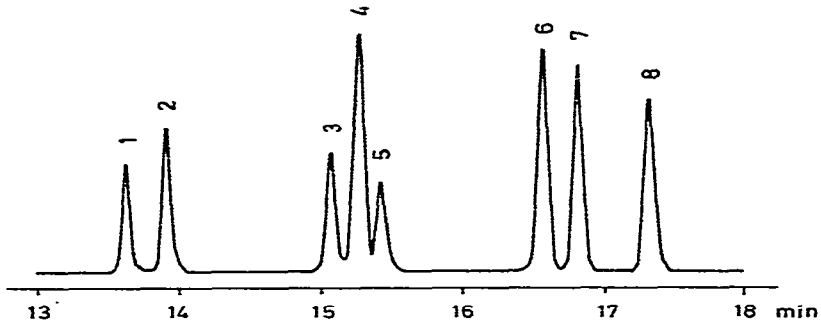

Fig. 2. Chromatogram of O-*n*-butoxime pertrifluoroacetates of pentoses using selected ion monitoring (*m/e* = 606). For this analysis, 5 μl of each of the four original derivative solutions were mixed by injecting through a septum into a vial containing 55 μl TFAA and 25 μl ethyl acetate; 1 μl of this mixture was used. Peaks: 1.4 = ribose; 2.6 = arabinose; 3.7 = lyxose; 5.8 = xylose.

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