GAS – LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY OF THE ACETATES OF PARTIALLY METHYLATED METHYL GLYCOSIDES

I. PENTOSES

Yu. N. El'kin, B. V. Rozynov,
A. I. Kalinovskii, A. F. Pavlenko,
N. I. Shul'ga, and A. K. Dzizenko

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For the analysis of partially methylated carbohydrates by mass spectrometry (MS) in the study of the structure of poly- and oligosaccharides a number of cyclic [1-4] and acyclic [5, 6] derivatives have been proposed. In all cases the positions of the OMe groups were established reliably. However, the mass spectra of the stereoisomers were not distinguished.

Methyl glycosides partially methylated with CD_3I [1] have not found wide use for the separation of mixtures because of the impossibility of using gas–liquid chromatography (GLC). Some partially methylated methyl glucosides have been studied by chromato-mass spectrometry [2]. However, this work was not continued.

Silylated partially methylated sugars [3] have not been studied sufficiently systematically for them to be used for GLC-MS analysis. In the methodological respect, these derivatives are unsuitable because of their decomposition on storage, while for the GLC-MS system the existence of a standard collection of samples is necessary.

The absence of systematic GLC-MS characteristics for the acetates of partially methylated sugars [4] has been the reason why these derivatives are not used.

The good GLC separation [5] and a systematic MS study [6] of polyol acetates has led to their predominant use for GLC-MS analysis [7]. The aldononitrile acetates recently proposed [8] are now being studied [9, 10]. With similar aims, we have begun the investigation of acetates of partially methylated methyl glycosides as the most easily obtained sugar derivatives. Below we give the relative retention times* of the acetates of methyl ethers of methyl pentopyranosides (T values).

Initial methyl ethers of methyl glycosides	T	Initial methyl ethers of methyl glycosides	T
Me 2,3,4-OMe_3-β-Xyl	0	Me-2-OMe-β-Xyl	3.34
Me 2,3,4-OMe_3-α-Lyx	0	Me-3-OMe-α-Lyx	3.35
Me 2,3,4-OMe_3-β-Xyl	0.40	Me-3-OMe-β-Ara	3.51
Me 2,3,4-OMe_3-β-Ara	1.20	Me-3-OMe-α-Xyl	3.55
Me 3,4-OMe_2-α-Lyx	1.34	Me-4-OMe-α-Lyx	3.61
Me 3,4-OMe_2-α-Xyl	1.49	Me-4-OMe-β-Xyl	3.79
Me 2,3-OMe_2-β-Xyl	1.69	Me-2-OMe-α-Lyx	4.03
Me 2,3-OMe_2-α-Xyl	2.19	Me-2-OMe-α-Xyl	4.05
Me 2,3-OMe_2-β-Ara	2.22	Me-3-OMe-β-Xyl	4.14
Me 2,4-OMe_2-α-Lyx	2.35	Me-2-OMe-β-Ara	4.21
Me 2,4-OMe_2-β-Xyl	2.36	Me-β-Ara	4.72
Me 2,4-OMe_2-β-Ara	2.79	Me-α-Xyl	4.84
Me 2,4-OMe_2-α-Xyl	3.06	Me-β-Xyl	4.98

* T = 0 is the retention time of the full acetate of hydroxylamine (3.80 min), T = 10 is the retention time of the full acetate of the aldononitrile of D-Gal (27.50 min). Carrier gas – nitrogen, 30 ml/min.

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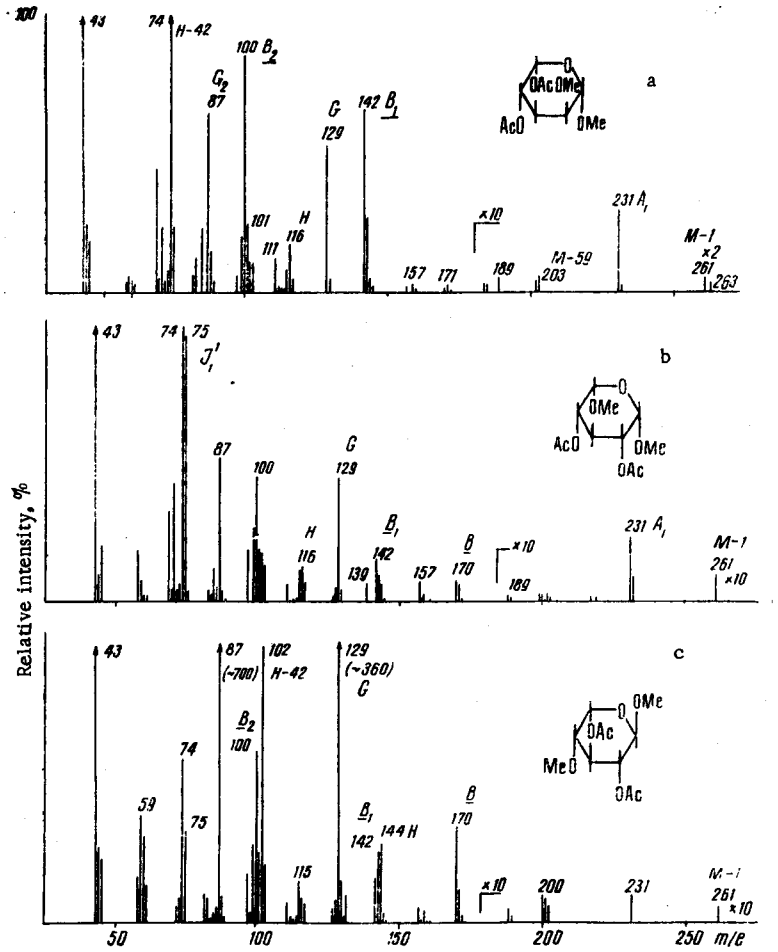

Fig. 1. Mass spectra of the acetates of methyl α-2-O-methyl-D-lyxoside (a), methyl β-4-O-methyl-D-xyloside (b), and methyl α-3-O-methyl-D-xyloside (c).

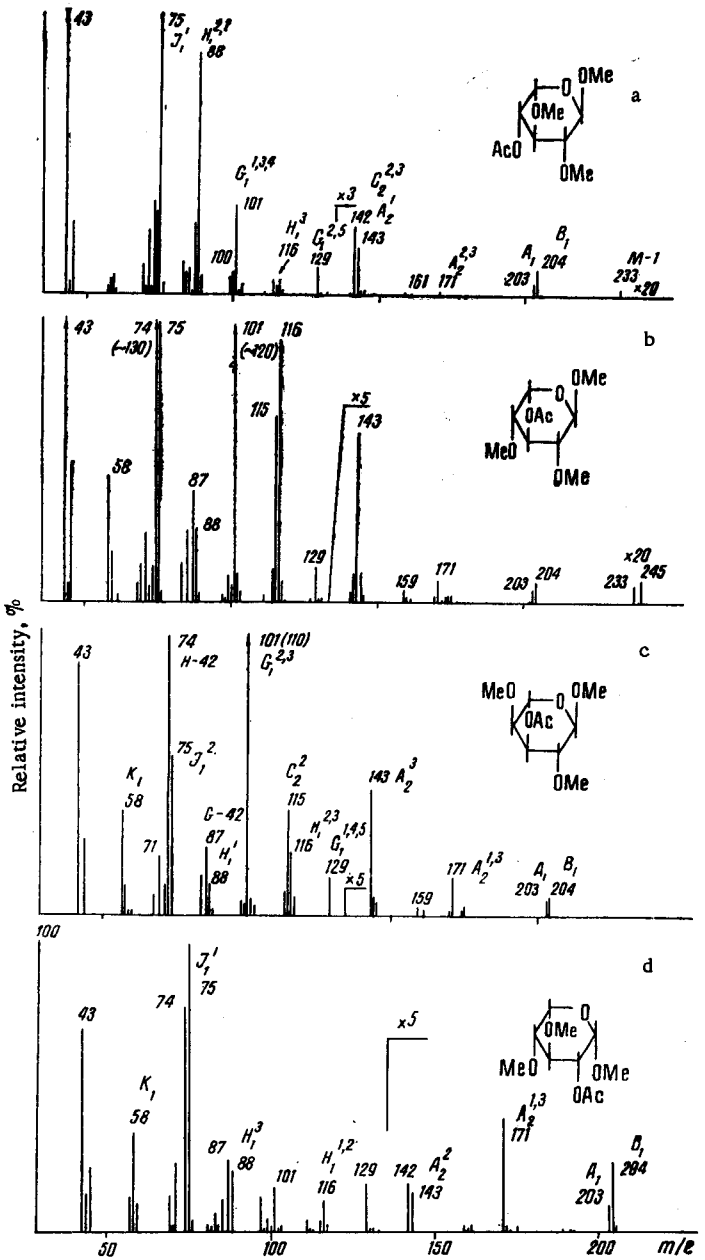

Fig. 2. Mass spectra of the acetates of methyl β-2,3-di-O-methyl-D-xyloside (a), methyl β-2,4-di-O-methyl-D-xyloside (b), methyl β-2,4-di-O-methyl-L-arabinoside (c), and methyl α-3,4-di-O-methyl-D-xyloside (d).

TABLE 1. Relative Contributions of the Ions G Containing an OAc Group

Position of the OAc group	$\frac{RI_{129}+RI_{87}}{RI_{101}}$	
	found	calc.*
4-	1	6
3-	0,25	1,7
2-	1,7	2,7

* According to Table 8 [1].

The mass spectra of the acetates of the partially methylated methyl pentopyranosides with the complete set of positions of the methoxy groups are shown in Figs. 1 and 2. The general laws of the change in the mass spectra of the pentose acetates with the introduction of OMe groups at C_1, C_2, and C_3 of the D-xylose molecule have already been discussed by K. Biemann [4]. The results of the comparison of the spectra in Fig. 1b [4] and 1a show that the replacement of an acetyl aglycone by a methyl aglycone and a change in the configuration at C_2 do not normally change the type of fragmentation of the acetate of 2-O-methyl-D-xylose. Peaks of the ions H, G, and J characteristic of the permethylglycosides [11] appear additionally. The relative intensities (RIs) and mass numbers of these peaks also give information on the position of the OMe groups.

A feature of the mass spectra of the acetates of the 4-monomethylpentosides is the formation of peaks of fragments H containing OAc groups with m/e 144 and H − 42 with m/e 102. The appearance in the spectrum of a considerable peak of ions B with m/e 170 throws doubt on the specificity of the loss of the C_3 substituent in the formation of this ion [4].

The mass spectra of the acetates of the methyl glycosides of the dimethylpentoses are also characteristic (Fig. 2, a, b, c, and d). Thus, characteristic for the mass spectra of the 2,4-dimethylpentoside acetates is a peak with m/e 143, and for the 2,3- and 3,4-dimethylpentoside acetates a peak with m/e 142. At the same time, these isomers differ in the RIs of the peaks with m/e 88, 116, and 74.

The assignment of the main peaks to the ions of the H, G, J, C, B, A, and K series was made on the basis of the assumption that one OAc group in the molecule of the dimethyl ethers does not qualitatively affect the known laws of the decomposition of the permethylated methyl glycosides [1]. However, with the introduction of a OAc group the contributions of the isomeric ions change substantially, and they depend on the position of this group in the molecule, as can be seen in the case of the ions G (Table 1).

As in the case of the permethylated methyl glycosides [1], the anomers of the acetates of the dimethyl ethers are distinguished by the ratio of the intensities of the peaks of the ions A_1 and B_1 with m/e 203 and 204, respectively.

Although stereoisomers can be separated according to their T values (see below), the mass spectra can also be used for these purposes. Thus, the spectra of acetylated methyl β-2,4-di-O-methylxyloside and of acetylated methyl β-2,4-di-O-methylarabinoside have clear differences in the RIs of the peaks with m/e 115, 116, and 129 (see Fig. 2, b and c). These differences can probably be used to identify the stereoisomers, since they are retained in the five spectra of each isomer taken at different times with the use of different GLC columns (BDS and NPGS). Differences have also been observed in the spectra of other pairs of compounds, but they are not given because there are no detailed statistics available. Furthermore, differences were found in the mass spectra of the silylated aldohexopyranoses [12].

Thus, it may be assumed that the statistical treatment of the mass spectra of the acetates of partially methylated methyl glycosides (cyclic sugar derivatives) will permit the determination of the stereoisomers by the use of a GLC-MS computer system. This would fundamentally supplement information obtained from the T values, the use of which in any case requires the calibration of the gas–liquid chromatograph and a collection of standard samples.

It must be noted that when ion sources with electron impact are used, acyclic derivatives of the stereoisomers do not usually give characteristic mass spectra.

EXPERIMENTAL

Samples of mixtures of partially methylated methyl pentosides were obtained according to a known procedure [13] by choosing the time of methylation of α-Me-D-Xyl_p, β-Me-D-Xyl_p, α-Me-L-Lyx_p, β-Me-L-Ara_p.

The mass spectra were taken on an LKB-9000 instrument (column 1.5 m × 3.4 mm, 3% of NPGS on Aeropak 30, 60-80 mesh), and the T values were measured on a Pye-Unicam series 104 chromatograph (glass columns 1.5 m × 6 mm, NPGS 3% on Aeropak 30, 60-80 mesh, at 125-223°C, 5°C/min).

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SUMMARY

For acetates of partially methylated methyl pentosides GLC-MS results have been obtained which permit the positions of the OMe groups to be determined unambiguously and, from the T values, assignments to definite stereoisomers to be made.

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