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Abstract

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MASS-SPECTROMETRIC STUDY OF CARBOHYDRATES

METHYL ETHERS AND ACETATES OF GLYCOSIDES

We have recently published ([1]) the first data on the application of mass-spectrometric analysis to carbohydrates and have shown that there are real prospects for its use in the study of this class of natural compounds.

When the method was extended to partially methylated monosaccharides, their insufficient volatility could prove to be a substantial methodological difficulty. In this connection we studied the mass spectra of several methylated methyl glycosides, in order to compare the general picture of their fragmentation with the fragmentation of methylated monosaccharides having a free glycosidic hydroxyl, and to assess the possibility of using glycosides for analytical purposes. The study of glycosides was also of independent interest, since it made it possible to judge the influence of the configuration of the glycosidic center on the direction and character of decomposition.

Table 1

Mass spectra of methylated methyl glycosides

m/e	I	II	III	IV	V	VI
45	19	21	31	27	22	18
71	12	12	9	17	13	4
73	11	11	18	26	18	13
75	45	30	75	82	62	73
88	100	100	100	100	100	100
101	47	46	50	73	51	47
127	3	9	4	5	2	2
149	7	7	12	8	7	5
155	1.1	–	0.2	–	0.3	0.07
159	0.35	0.3	1.5	1.5	–	0.12
173	–	0.3	–	0.50	0.85	0.23
176	1.5	3.1	0.50	1.52	1.3	0.29
187	3.65	0.30	6.1	0.5	3.0	0.10

Fig. 1. Device for introducing the sample into the inlet balloon

Figure 1: Fig. 1. Device for introducing the sample into the inlet balloon

m/e	I	II	III	IV	V	VI
205	0.15	0.10	0.20	0.10	0.30	0.20
219	0.20	0.05	0.50	0.15	0.50	0.15

Intensity relative to the peak m/e 88*, %

* In the range m/e 45–150, peaks with intensity less than 5%, and in the range m/e 151–220 with intensity less than 0.05%, as a rule, are not given.

For this purpose we recorded the mass spectra of 2,3,4,6-tetramethyl-α-methylglucoside (I), 2,3,4,6-tetramethyl-β-methylglucoside (II), 2,3,4,6-tetramethyl-α-methylgalactoside (III), 2,3,4,6-tetramethyl-β-methylgalactoside (IV), 2,3,4,6-tetramethyl-α-methylmannoside (V), and 2,3,4,6-tetramethyl-β-methylmannoside (VI).

Compounds (I–VI) were prepared by methylation of the corresponding glycosides by Haworth' s method and then additionally by Purdie' s method. All the substances were chromatographically homogeneous and their constants were in agreement with literature data ([2]). The mass spectra were recorded under the same conditions and on the same instrument as previously ([1]), but the sample-inlet system was somewhat modified (Fig. 1). To the inlet bulb, made of stainless steel, a glass tube was attached through a Kovar seal; into this tube was placed an unsealed ampoule with the sample under investigation, fixed in a small boat with an iron rod sealed in throughout, for moving it along the tube by means of a magnet. The glass tube was sealed off and evacuated to low and high vacuum. After the pumping line had been closed, a heater was placed on the tube and the sample evaporated completely.

A characteristic pair of mass spectra (spectra I and II) is shown in Fig. 2, and the data for the remaining spectra are given in Table 1. Examination of these data shows that the spectra of all glycosides differ substantially from the spectra of the corresponding monosaccharides with an unsubstituted glycosidic hydroxyl: the main peak in the spectra of all methyl glycosides is the peak corresponding to m/e 88, whereas for monosaccharides with a free glycosidic hydroxyl it is m/e 101 ([1]); there is also a substantial difference in the relative intensities of the other peaks.

Another very important conclusion following from consideration of the data obtained is that the mass spectra change substantially depending on the configuration of the glycosidic center.

Substance

To the inlet balloon

Fig. 1. Device for introducing the sample into the inlet balloon

Fig. 2. Mass spectra of 2,3,4,6-tetramethyl-α-methylglucoside (I) and 2,3,4,6-tetramethyl-β-methylglucoside (II)

Thus, in the spectra of all α-glycosides (I, III, V) the intensity of the peak corresponding to m/e 187 is considerably higher than the intensity of the peak 176; in the spectra of β-glycosides (II, IV, VI) the reverse relationship is observed. Further, in the spectra of the β-glycosides there is a small but clearly expressed peak corresponding to m/e 173, which is practically absent in spectra I and III and is considerably inferior in intensity to peaks 187 and 176 in spectrum V. In addition, a number of other regularities can also be traced.

Such a difference in the spectra of α- and β-methyl glycosides can depend only on the difference in the spatial arrangement of the methoxyl groups at C_1. As is known, for glucose, galactose, and mannose the most favorable conformation is the C1 conformation. Thus, in all α-glycosides the anomeric methoxyl group is in the axial position, and in β-glycosides in the equatorial position. For this reason the molecular ions, which in the case of glycosides also could not be fixed (cf. ([1])), may decompose differently for the two types of glycosides.

$$\text{(VII)} \longrightarrow \text{CH}_3\text{O}^\bullet + \text{(VIII)} \longrightarrow \text{(IX)}$$

Apparently, in the case of α-anomers, from the molecular ion ((VIII) in the case of glucose) the axial methoxyl group at C_1 is split off first, and ion (VIII) with m/e 219 is formed, after which β-elimination of methanol occurs, leading to ion (IX) with m/e 187, strongly stabilized by conjugation.

$$\text{(X)} \longrightarrow \text{(XI)} \longrightarrow m/e\ 176$$

For the molecular ions obtained from β-anomers ((X) in the case of glucose), which have an equatorial methoxyl at C_1, rupture of the C_1 bond with the oxygen in the ring is probably more favorable, after which fragmentation of the resulting ion-radical (XI) proceeds further by an entirely different route. It should be borne in mind that the above considerations on the fragmentation pathways of methylated monosaccharides are still, of course, purely hypothetical in character and require experimental verification. The results of this investigation will be reported by us later.

At the same time, the data presented quite convincingly show that anomeric methyl glycosides can be clearly distinguished from one another by their mass spectra, which thus can serve as a means for determining the configuration of the anomeric center in these compounds. Although there is a considerable amount of data on the dependence of mass spectra on configuration in rigid polycyclic systems ([3,4]), such differences for conformationally mobile monocyclic systems,

as far as we know, have been observed for the first time and are of substantial interest. Differences in the spectra of α- and β-methyl glycosides also have their disadvantage, since they complicate the analytical use of the method, because methanolysis of a polysaccharide chain, as is known, always leads to a mixture of both anomeric glycosides.

Table 2

Mass spectra of methylated phenyl glycosides

m/e	Intensity, % XII	Intensity, % XIII	m/e	Intensity, % XII	Intensity, % XIII
45	64	80	101	83	86
55	9	8	102	6	6
57	11	5	111	100	100
59	13	16	112	8	8
69	7	4	113	6	5
71	47	45	115	6	6
73	19	23	116	6	8
74	5	7	127	24	24
75	40	40	129	6	5
77	4	8	131	6	5
81	6	4	143	5	5
83	7.5	5	145	7	8
85	12	9	155	23	23
88	11	25	187	96	93
89	25	27	188	11	10
94	6	12	218	21	19
95	18	16	219	10	9
99	17	15			

In connection with the propositions set forth, it was of interest to establish how the nature of the aglycone affects the appearance of the spectrum. For a first assessment of such an influence we recorded the mass spectra of 2,3,4,6-tetramethyl-α-phenyl-D-glucoside (XII) and 2,3,4,6-tetramethyl-β-phenyl-D-glucoside (XIII) ([5]), in which case the general pattern of decomposition should not have been complicated by fragmentation of the aglycone, in view of the well-known stability of aromatic nuclei. This could also prove useful for determining the configuration of natural glycosides with aromatic aglycones.

The spectra of XII and XIII (Table 2) differed sharply from the spectra of methyl glycosides, but were very close to one another.

The principal peak in both spectra was the peak corresponding to m/e 111, while the peak m/e 88 did not exceed 25%. In both spectra the peak m/e 187 was very intense, and the peak m/e 238 (corresponding to m/e 176 for β-methyl

glycosides) was completely absent in the spectrum of XIII. Thus, in the case of phenyl-

of glycosides, the direction of decomposition depends little on the configuration of the glycosidic carbon atom. The difference in the directions of fragmentation between methyl and phenyl glycosides can be attributed either to the greater electron affinity of phenyl, or to the larger size of phenyl, or, finally, to the combined influence of these two factors. Since preliminary experiments with anomalous methylated disaccharides showed the existence of differences in their spectra, preference should be given to the first explanation.

In addition to studying methylated glycosides, we also attempted to elucidate another possibility for increasing the volatility of carbohydrates —their acetylation—and recorded the mass spectra of several complete acetates of methyl glycosides. As model compounds we selected 2,3,4,6-tetraacetyl-α-methylglucoside (XIV), 2,3,4,6-tetraacetyl-β-methylglucoside (XV), 2,3,4,6-tetraacetyl-α-methylgalactoside (XVI), and 2,3,4,6-tetraacetyl-α-methylmannoside (XVII). The most intense peak in the spectra of all these compounds is the peak corresponding to m/e 43 ($CH_3C^+ = O$); because of the instability of the acetyl group under ionization conditions, most other peaks do not exceed 10% of its intensity. It is especially significant that the spectra of all the compounds tested coincide with one another within experimental error ($±$6%). Thus, mass spectrometry of carbohydrate acetates is unlikely to have great analytical significance. As for the pathways of fragmentation of glycoside acetate molecules, in general outline they repeat the principal fragmentation of methylated glycosides, with the sole difference that peaks corresponding to m/e greater than 230 are practically absent, and instead of one peak in the spectrum of a methylated glycoside there appears a series of peaks corresponding to ions with different numbers of acetyl groups (the difference is 42). Thus, for example, instead of the peak corresponding to the ion with m/e 101, the spectra of acetates show peaks at 157, 115, and 73, and instead of the peak m/e 88—peaks with m/e 144, 102, and 60. Because of the absence of ions with large m/e values, it was not possible to trace the influence of the stereochemistry of the glycosidic center on the character of the mass spectrum of acetates.

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Note: Figure translations are in progress. See original paper for figures.