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MASS SPECTROMETRY OF CARBOHYDRATE DERIVATIVES

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Abstract—Mass spectra of carbohydrate methyl ethers of different types and other monosaccharide derivatives have been measured. Fragmentation patterns of these compounds, characteristic features of their mass spectra and potential analytical importance of mass spectrometry for structural study in the carbohydrate field are discussed.

THE progress in sugar chemistry is limited by the lack of physical methods for structural analysis of natural carbohydrates. The recent successful application of mass spectrometry to different classes of natural compounds,[1,2] encouraged the present investigation, aimed at elucidating the potentialities and the scope of the method in the carbohydrate field. The literature concerning mass spectrometry of carbohydrates contains only a few short communications by Reed *et al.*[3,4]

The choice of carbohydrate methyl ethers for this investigation was prompted by their importance in carbohydrate structural analysis as well as by their volatility, thus removing the major limitation of mass spectrometry. A few other carbohydrate derivatives of practical or theoretical importance also have been investigated.

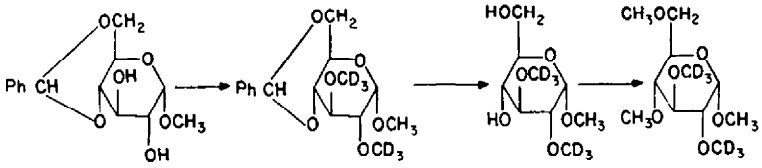
Materials and methods

The mass spectra of the following 26 compounds have been measured: methyl 2,3,4,6-tetra-0-methyl-α,D-glucopyranoside (I), methyl 2-0-trideuteromethyl-3,4,6-tri-0-methyl-α,D-glucopyranoside (Ia), methyl 2,3-di-0-trideuteromethyl-4,6-di-0-methyl-α,D-glucopyranoside (Ib), methyl 2,3,4-tri-0-methyl-6-0-trideuteromethyl-α, D-glucopyranoside (Ic), methyl 2,3-di-0-methyl-4,6-di-0-trideuteromethyl-α,D-glucopyranoside (Id), methyl 2,3,4,6-tetra-0-methyl-β,D-glucopyranoside (II), methyl 2,3,4,6-tetra-0-methyl-α,D-galactopyranoside (III), methyl 2,3,4,6-tetra-0-methyl-β, D-galactopyranoside (IV), methyl 2,3,4,6-tetra-0-methyl-α,D-mannopyranoside (V), methyl 2,3,4,6-tetra-0-methyl-β,D-mannopyranoside (VI), phenyl 2,3,4,6-tetra-0-methyl-α, D-glucopyranoside (VII), phenyl 2,3,4,6-tetra-0-methyl-β,D-glucopyranoside (VIII), methyl 2,3,4,6,2′,3′,6′-heptamethyl-β-cellobioside (IX), methyl 2,3,4,6,2′,3′,6′-heptamethyl-α,β-maltoside (X), 2,3,4,6-tetra-0-methyl-D-glucopyranose (XI), 2,3,4,6-tetra-0-methyl-D-galactopyranose (XII), 2,3,5,6-tetra-0-methyl-D-glucofuranose (XIII),

[1] K. Biemann, *Angew. Chem.* **74**, 102 (1962).
[2] K. Biemann, "*Mass spectrometry*". McGraw-Hill, New York (1962).
[3] P. A. Finan, R. I. Reed, *Nature, Lond.* **184**, 1866 (1959); P. A. Finan, R. I. Reed, W. Snedden, *Chem. & Ind.* 1172 (1958); R. I. Reed, W. K. Ried, J. M. Wilson, *Symp. on Mass Spectrometry.* Oxford, September (1961).
[4] When our preliminary data were already published (N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, B. M. Zolotarev, *Dokl. Acad. Nauk U.S.S.R.* **147**, 1369 (1962)), a short communication of Biemann *et al.* appeared on mass spectrometry of mainly sugar acetates [K. Biemann, H. K. Schnoes, J. A. McCloskey, *Chem. & Ind.* 448 (1963)].

2,3,5-tri-O-methyl-L-arabofuranose (XIV), 2,3,4-tri-O-methyl-L-rhamnopyranose (XV), 2,3,4-tri-O-methyl-D-xylopyranose (XVI), methyl 3,4,6-tri-O-methyl-α,D-glucopyranoside (VXII), methyl 2,3,4-tri-O-methyl-α,D-glucopyranoside (XVIII), methyl 2,3,4,6-tetra-O-acetyl-α,D-glucopyranoside (XIX), methyl 2,3,4,6-tetra-O-acetyl-β,D-glucopyranoside (XX), methyl 2,3,4,6-tetra-O-acetyl-α,D-galactopyranoside (XXI), methyl 2,3,4,6-tetra-O-acetyl-α,D-mannopyranoside (XXII). The compounds I–VIII were obtained by methylating the corresponding methyl- or phenyl-glycosides according to Haworth's procedure and subsequent treatment with methyl iodide by the method of Purdie. The compounds IX and X similarly were prepared by methylating the corresponding disaccharides. The substances XI–XVI were kindly presented by Drs. Yu. S. Ovodov and V. E. Vaskovsky of this laboratory. Compound XVII was obtained by the procedure of Fletcher *et al.*[5]; XVIII was prepared by the method of Robertson and Waters.[6] Acetates XIX–XXII were obtained by acetylating the corresponding methyl glycosides with acetic anhydride in pyridine. All the compounds were homogenous as revealed by thin layer silica gel and paper chromatography. Their constants corresponded to those published in the literature.

Deuterated compounds Ia, Ic and Id were obtained by methylating XVII, XVIII and methyl 2,3-di-O-methyl-α, D-glucopyranoside with trideuteromethyl iodide (CD_3J, 98% deuterium) in the presence of silver oxide; Ib was synthesized according to the following scheme:



XXIII XXIV XXV Ib

Deuterated compounds Ia–d, XXIV and XXV have not been previously described; their constants were close to those of the corresponding unlabelled substances.[7]

The spectra were measured by means of a "MX-1303" mass spectrometer with a modified sample port.[8] All the measurements were performed at 175° and ionizing potential 70 eV as standard conditions with samples of 0·5–1·0 mg. The choice of these particular conditions was made after preliminary experiments. It appeared that decreasing the ionizing potential to 15–20 eV lead to no marked changes of spectra except decrease of all the intensities. Thermal decomposition processes lead to poor reproducibility and considerable decrease of intensities of a number of important peaks at temperatures of 200–225°. The spectra obtained at 140–150° do not practically differ from those measured at 175°; however, less volatile carbohydrate derivatives do not completely evaporate at this lower temperature. These most convenient standard conditions were not changed even when working with

[5] H. B. Wood, R. Allerton, H. W. Diehl, H. G. Fletcher Jr., *J. Org. Chem.* **20**, 875 (1955).
[6] A. Robertson, R. B. Waters, *J. Chem. Soc.* 1709 (1931).
[7] T. Purdie, J. C. Irvine, *J. Chem. Soc.* **85**, 1049 (1904); J. C. Irvine, J. P. Scott, *Ibid.* **103**, 575 (1913).
[8] N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, B. M. Zolotarev, *Dokl. Acad. Nauk U.S.S.R.* **151**, 336 (1963).

volatile derivatives so that all results are comparable. The intensities of the peaks were expressed in relation to the base peak, which was assigned an arbitrary value of 100%.

General features of the mass spectra of carbohydrate derivatives

The mass spectra of different types of derivatives investigated are presented in Tables 1–7. The mass spectra of compounds I and XI, in Fig. 1, show typical patterns.

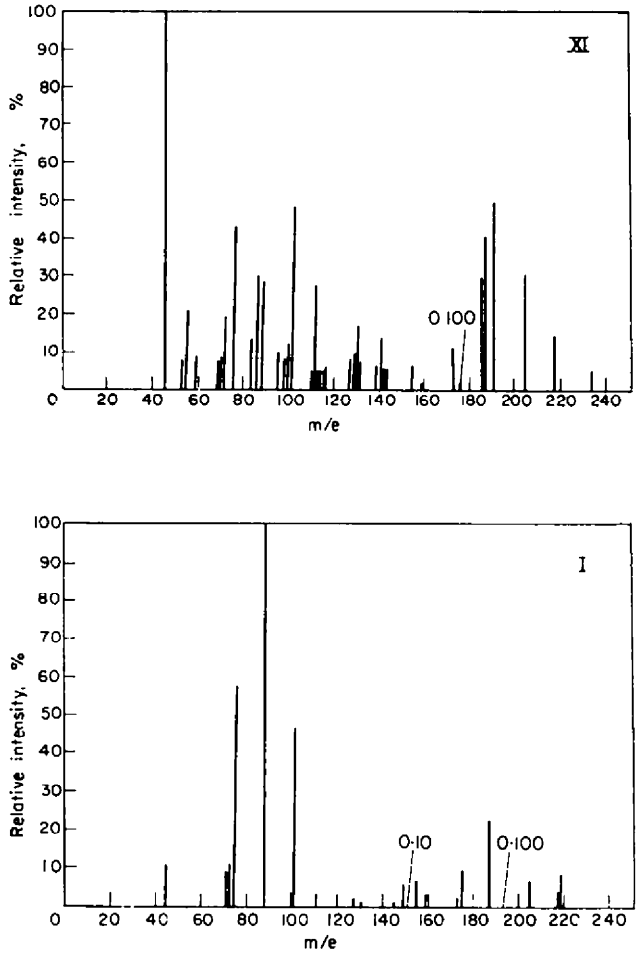


FIG. 1. The mass spectra of 2,3,4,6-tetra-O-methyl-D-glucopyranose (XI) and methyl 2,3,4,6-tetra-O-methyl-α,D-glucopyranoside (I).

The highest peaks are usually those at m/e 101, 88, 75 and 45 or those corresponding to them. Peaks at m/e above 101 are usually rather low. No molecular ion peak was identified in either of the spectra. These data suggest very rapid and extensive fragmentation of carbohydrate molecules proceeding simultaneously along several pathways.

In spite of the absence of peaks, corresponding to molecular ion in all of the spectra investigated, the molecular weights of methylated monosaccharides can be

TABLE 1. MASS SPECTRA OF I AND LABELLED ANALOGUES

m/e	Relative intensity, %								
	I	Ia		Ib		Ic		Id	
		found	total	found	total	found	total	found	total
45	10·7	6·37	8·87	4·80	8·10	3·36	6·48	6·80	13·7
48		2·50		3·30		3·12		6·90	
71	8·90	7·60	7·60	8·25	8·25	5·05	8·85	—	7·90
74		—		—		3·80		7·90	
73	10·5	6·20	11·6	—	9·25	10·6	10·6	8·90	8·90
76		5·40		9·25		—		—	
75	57·5	51·0	60·0	8·00	60·0	59·5	59·5	47·0	55·4
78		9·00		52·0		—		8·40	
88	100	16·5		—		100		84·0	
91		83·5	100	21·0	100	—	100	16·0	100
94		—		79·0		—		—	
101	47·0	4·50		4·30		42·0		14·5	
104		37·0	41·5	30·0	46·1	3·80	45·8	28·2	46·7
107		—		11·8		—		3·96	
111	3·10	1·79	2·84	1·89	3·62	3·46	3·46	0·42	2·17
114		1·05		1·73		—		1·75	
127	2·21	0·97		0·45		1·23		—	
130		1·30	2·27	1·65	2·05	1·15	2·38	1·40	1·93
133		—		—		—		0·53	
131	1·30	—		—		1·30		0·53	
134		1·05	1·05	—	1·10	—	1·30	0·84	1·37
137		—		1·10		—		—	
145	1·30	—		—		1·25		—	
148		0·97	0·97	—	1·00	—	1·25	1·10	1·10
151		—		1·00		—		—	
149	5·70	6·00	6·00	—	5·20	—	5·25	—	4·20
152		—		5·20		5·25		4·20	
155	0·72	0·25	0·73	—	0·72	0·53	0·86	—	0·66
158		0·48		0·72		0·33		0·66	
159	0·35	0·24		—		—		—	
162		0·23	0·47	0·32	0·32	0·33	0·33	—	0·25
165		—		—		—		0·25	
173	0·28	—	0·28	—	0·20	0·30	0·30	—	0·24
176		0·28		0·20		—		0·24	
176	1·15	—		—		1·14		—	
179		1·04	1·04	—	0·86	—	1·14	0·91	0·91
182		—		0·86		—		—	
187	2·31	0·21		—		—		—	
190		2·10	2·31	2·10	2·48	2·38	2·38	0·36	2·19
193		—		0·38		—		1·83	
205	0·072	—		—		0·088		—	
208		0·061	0·061	—	0·066	—	0·088	0·058	0·058
211		—		0·066		—		—	
218	0·041	—		—		—		—	
221		0·037	0·037	—	0·038	0·060	0·060	—	0·028
224		—		0·038		—		0·028	
219	0·087	—		—		—		—	
222		0·095	0·095	—	0·090	0·096	0·096	—	0·076
225		—		0·090		—		0·076	

calculated from the mass number of the largest fragment equal to M-18. For example, the maximum m/e value in spectra of XI and XII (M = 236) was 218, being equal to 188 for XV (M = 206) and 174 for XVI (M = 192). The maximum m/e values for methyl and phenyl glycosides are M-31 and M-93 respectively.

The spectra of the compounds investigated are rather similar, but their fine structure usually makes it possible to distinguish between individual substances. The majority of spectra though containing peaks at the same m/e values, different intensity of these peaks enables identification of compounds or groups of compounds with common structural features. For example (Tables 3–7), the base peak of methylated methyl hexopyranosides I–VI is that at m/e 88, for methylated hexoses (XI, XII) at m/e 45, for pentoses (XVI) and 6-deoxyhexoses (XV) at m/e 101 and for methylated furanose (XIII) at m/e 89. Hence, even the position of the base peak may serve for identification of these groups of compounds. More detailed analysis of characteristic features of mass spectra, distinguishing different types of compounds will be made in the following sections of the paper.

Fragmentation patterns of methyl 2,3,4,6-*tetramethyl-α,*D-*glucopyranoside*

Similarity of the mass spectra of the carbohydrate derivatives investigated suggests a similarity of their fragmentation patterns. Hence, precise knowledge of the origin of at least major peaks for only one compound of the series would enable ready interpretation of mass spectra for analytical applications and provide a firm basis for speculations concerning the general pathways of fragmentation.

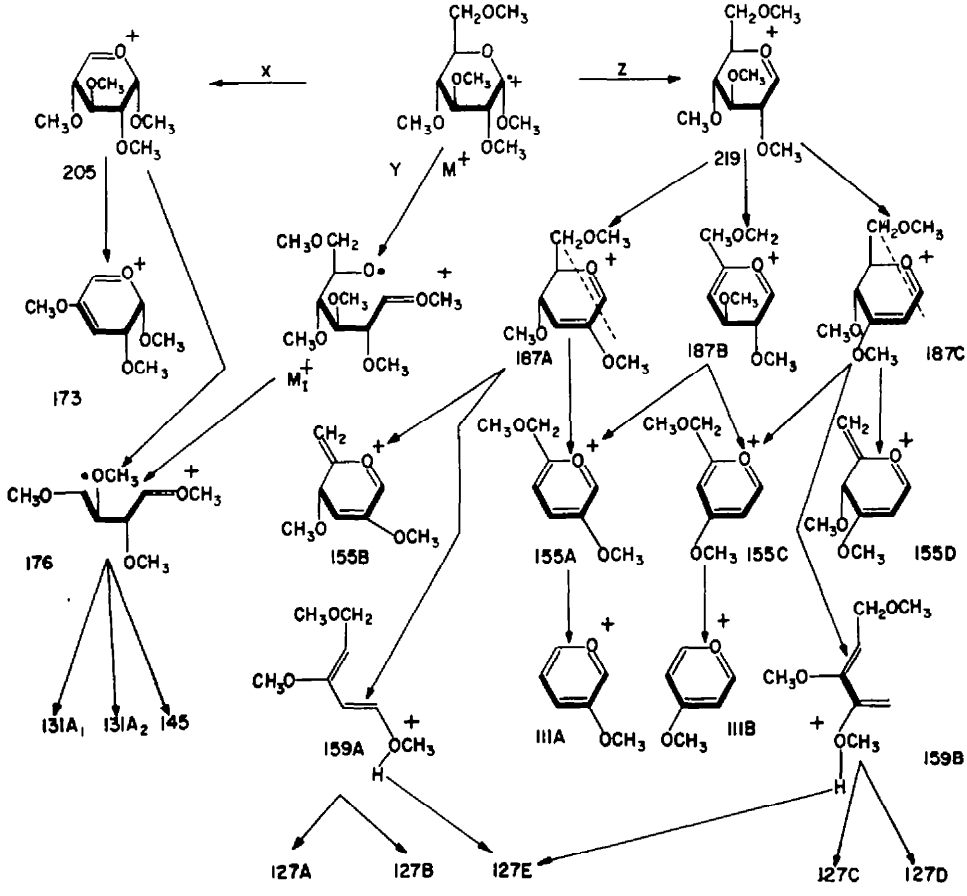
For this purpose, the fragmentation of methyl 2,3,4,6-tetramethyl-α,D-glucopyranoside (I) was investigated as a typical representative of the class of methylated sugars, using partially deuterated analogues of I–Ia, Ib, Ic and Id. The data obtained (Table 1) served as a basis for proposing the structures of major fragments of I arising after electron impact. It is characteristic of the mass spectra of carbohydrate derivatives that almost all the peaks originate from several isomeric ions. Moreover, even ions of the same structure appear sometimes of different origin. For example, the peak at m/e 101 corresponds to two isomeric ions 101A and 101B, each being formed by three independent pathways. The contributions of isomeric ions and ions of different origin to the total intensity of the peak have been calculated.

Deduction of structures and calculation of isomeric ion contributions can be shown by consideration of the isomeric ions 187A, 187B and 187C, corresponding to the m/e 187 peak, which is the highest peak corresponding to relatively large fragments. The peak of the largest mass number is situated at m/e 219. The corresponding fragment can arise from the intact molecule (M=250) only by elimination of the C(1) methoxyl (M = 31), as it contains methoxyls at C(2), C(3), C(4) and C(6). The m/e 187 peak mass number differs from that of the m/e 219 fragment by 32 mass units, corresponding to a CH_3OH molecule. The data presented in Table 1 reveal that this molecule may have eliminated from positions 2, 3 and 4, but not from position 6. Bearing in mind the relatively high intensity of the peak at m/e 187 and the well known stabilization of ions with conjugated bond systems,[1,2] we can conclude, that only structures 187A, 187B and 187C (Table 2) can account for the patterns obtained. In order to calculate the contributions of these structures to the m/e 187 peak intensity it was necessary first to calculate the average total intensity. This was found equal to 2·33. Further, all the intensities were multiplied by ratio

2·33/total intensity of the spectrum. Calculation starting from the reduced values obtained, afforded better superimposability of the results. Using reduced values, we obtain:

$$A + B = 2{\cdot}11; \quad C = 0{\cdot}21 \text{ (from spectrum Ia)}$$
$$A + C = 1{\cdot}97; \quad B = 0{\cdot}36 \text{ (from spectrum Ib)}$$
$$A + C = 1{\cdot}96; \quad B = 0{\cdot}38 \text{ (from spectrum Ic)}$$

Chart 1. The pathways of fragmentation of I after electron impact.



This leads to A = 1·75 (average from the three equations), i.e., A = 75%, B = 16% and C = 9% of the m/e 187 peak total intensity. The structures of other ions were assigned on the basis of analogous considerations. The calculated contributions of isomeric ions to peak intensities are presented in Table 2. The values obtained, although rather approximate, proved very useful for the following speculations.

The data, presented in Table 2, suggest fragmentation of I to proceed according to pathways shown in Chart 1.

The molecular ion M^+ resulting after removal of one electron is subjected to fragmentation in the three directions—X, Y and Z.

Direction X. Elimination of the sixth carbon atom results in the formation of

m/e 205 fragment, the latter giving rise to a stable conjugated ion m/e 173, by elimination of CH_3OH from the position 3, or to a stable ion-radical m/e 176 through ring cleavage.

Direction Y. Cleavage of the bond between the C(1) atom and the ring oxygen atom leads to the formation of an ion-radical M_1^+, eliminating a molecule of methoxyacetic aldehyde to give rise to m/e 176 fragment. The latter may eliminate the methoxyl

TABLE 2. THE STRUCTURES OF MAJOR IONS, PRODUCED BY MOLECULE I, AND THE DATA, CONCERNING THEIR CONTRIBUTION TO THE TOTAL PEAK INTENSITIES

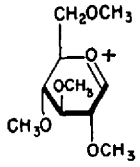
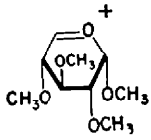
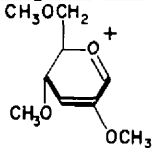
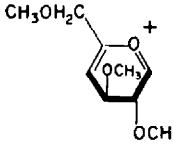
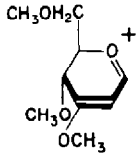
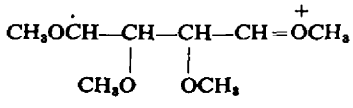
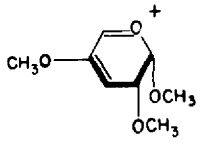
m/e	Structure of ion	Designation	Contribution to peak intensity, %
219		219	100
205		205	100
187		187A	75
		187B	16
		187C	9
176		176	100
173		173	100

TABLE 2 (*continued*)

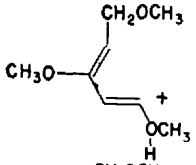
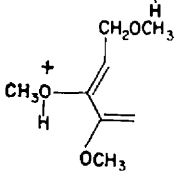
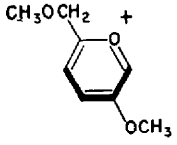
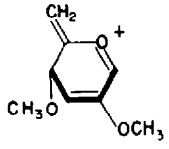
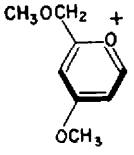
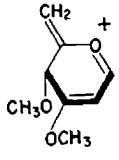
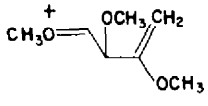
m/e	Structure of ion	Designation	Contribution to peak intensity, %
159		159A	50
		159B	50
155		155A	66
		155B	
		155C	34
		155D	
149	was not established		
145		145	100
131	$CH_3O{=}CH{-}\underset{\displaystyle OCH_3}{\underset{|}{C}}{=}CH{-}OCH_3$ (C2, C3 and C4)	$131A_1$	61
	the same structure (C1, C2 and C3)	$131A_2$	39

TABLE 2 (*continued*)

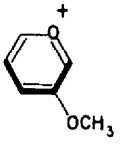
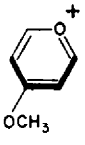
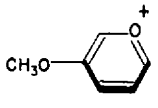
m/e	Structure of ion	Designation	Contribution to peak intensity %
127	$CH_2CH=C(OCH_3)-CH=CH-OCH_3$	127A	
	$CH_3O-CH=CH=CH=CH-CH=OCH_3$	127B	
	$CH_2-CH=C(CH_3O)-C(OCH_3)=CH_2$	127C	
	$CH_3O-CH=CH-CH=C(OCH_3)-CH_2$	127D	
	$CH_3O=CH-CH=C(OCH_3)-CH=CH_2$	127E	
111		111A	37
		111B	11
		111C	52
101	$CH_2O=CH-CH=CH-OCH_3$ (C4, C5 and C6)	$101A_1$	9
	the same structure (C2, C3 and C4)	$101A_2$	60
	the same structure (C1, C2 and C3)	$101A_3$	2
	$CH_2=C(OCH_3)-CH=OCH_3$ (C4, C3 and C2)	$101B_1$	26
	the same structure (C1, C2 and C3)	$101B_2$	
	the same structure (C3, C2 and C1)	$101B_3$	3
88	$CH_3O\dot{C}H-CH=OCH_3$ (C1 and C2)	$88A_1$	5
	the same structure (C2 and C3)	$88A_2$	79
	the same structure (C3 and C4)	$88A_3$	16

TABLE 2 (*continued*)

m/e	Structure of ion	Designation	Contribution to peak intensity %
75	$CH_3OCH=CH-\overset{+}{O}H_2$ (C2 and C1)	$75A_1$	15
	the same structure (C2 and C3)	$75A_2$	
	the same structure (C3 and C2)	$75A_3$	72
	the same structure (C3 and C4)	$75A_4$	
	the same structure (C4 and C3)	$75A_5$	13
73	$CH_3\overset{+}{O}=CH-CH=O$ (C2 and C3)	$73A_1$	47
	the same structure (C3 and C2)	$73A_2$	53
71	$CH_2=CH-CH=\overset{+}{O}CH_3$ (C6, C5 and C4)	$71A_1$	43
	the same structure (C4, C5 and C6)	$71A_2$	57
45	$CH_3\overset{+}{O}=CH_2$ (C6)	$45A_1$	48·5
	the same structure (C4)	$45A_2$	2
	the same structure (C3)	$45A_3$	12·5
	the same structure (C2)	$45A_4$	28
	the same structure (C1)	$45A_5$	9

from position 1 with the formation of m/e 145 fragment, or lose one of the terminal CH_3OCH-groups with the formation of ions $131A_1$ and $131A_2$.

Direction Z. Fission of the C(1) methoxyl results in the formation of the m/e 219 fragment. The latter may eliminate methanol to afford ions 187A, 187B and 187C, these ions eliminating one more methanol molecule with the formation of isomeric ions 155 A–D, or giving rise to isomeric ions 159A and 159B, through ring cleavage, shown by the dotted line. Fragments 159A and 159B eliminate a CH_3OH to give isomeric ions 127A–E.

Speculative mechanisms on the formation of smaller fragments could be discussed on the basis of data, presented in Table 2, but this has not been done as the possible pathways are too numerous to be sure that all of them are taken into consideration.

The influence of the substituent and configuration at C(1) *upon the pathways of fragmentation*

Permethyl methyl hexopyranodises (I–VI). The mass spectra of these compounds are presented in Table 3.

Consideration of Table 3 shows the difference between mass spectra of anomers: peak at m/e 187 in mass the spectra of α-anomers (I, III and V) is several times higher than that at m/e 176, a reversed relationship being observed in the mass spectra of β-anomers (II, IV and VI). This difference can be explained in terms of the above speculations (preceding section). The glycosidic methoxyl in α-anomers in their stable C1 conformation occupies the axial, sterically hindered position and its ready elimination leads to the predominance of the Z direction. The absence of such sterical hindrance in β-anomers leads to the predominance of the Y direction.

Permethyl phenyl glucosides. The mass spectra of these compounds are presented in Table 4.

TABLE 3. MASS SPECTRA OF METHYLATED METHYL HEXOPYRANOSIDES

m/e	Relative intensity, % I	II	III	IV	V	VI
45	11	21	31	27	22	18
71	9	12	9	17	13	4
73	11	11	18	26	18	13
75	58	30	75	82	62	73
88	100	100	100	100	100	100
101	47	46	50	73	51	47
111	3·1	—	4·0	—	3·0	—
127	2·2	9·0	4·0	5·0	2·0	2·1
131	1·3	2·5	2·5	4·0	1·5	1·2
145	1·3	1·8	2·0	2·2	3·2	2·3
149	6·0	7·0	12	3·0	7·0	5·0
155	0·7	—	0·2	—	0·3	0·07
159	0·35	0·3	1·5	1·5	—	0·12
173	0·3	0·3	—	0·5	0·85	0·23
176	1·15	3·1	0·5	1·5	1·3	0·3
187	2·3	0·3	6·1	0·5	3·0	0·1
205	0·07	0·1	0·20	0·1	0·3	0·2
218	0·04	0·02	0·2	0·05	0·25	0·07
219	0·09	0·05	0·5	0·15	0·5	0·15

TABLE 4. MASS SPECTRA OF PERMETHYL PHENYL GLUCOSIDES

m/e	Relative intensity, % VII	VIII	m/e	Relative intensity, % VII	VIII
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			

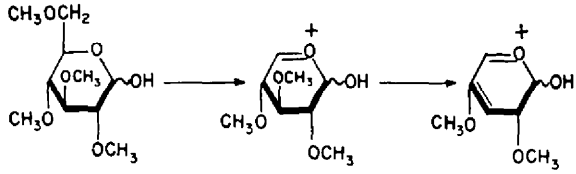
The spectra of anomeric phenyl glucosides VII and VIII are very similar. The peak at m/e 187 is very intense, the peak at m/e 238 (corresponding to the peak at m/e 176 of methylated methyl glycosides) is absent. It is characteristic of these spectra, that the intensities of peaks at m/e 155, 127 and 111, corresponding to fragments which must have originated from 187A ion, is considerably increased if compared with the spectrum of I. The intensities of all the peaks which cannot arise from

ion 187A are at the same time considerably decreased; for example, the peak at m/e 88 becomes very small, as the corresponding fragment can now originate from ion 187B only. It is noteworthy, that VII and VIII do not give ions with the phenyl nucleus bound to sugar residue fragments. This fact is revealed by the following evidence. The mass spectra of VII and VIII do not contain peaks at m/e values, which can be obtained by adding 62 ($M_{C_6H_5} - M_{CH_3} = 77 - 15 = 62$) to those corresponding in spectrum of I to fragments with intact C(1) methoxyl, e.g., peaks at m/e 205, 176 and 173. The instability of these fragments is the consequence of the relatively high electron affinity of the phenyl residue, which enables fragmentation in the direction Z only.

Methylated hexopyranoses (XI, XII). The mass spectra of XI and XII are presented in Table 5.

As is seen from the data presented in Table 5, the mass spectra of methylated hexopyranoses differ from those of the corresponding glycosides by the presence of a greater number of intense peaks. The spectra of XI and XII are very close and, therefore, identification of epimers will be difficult.

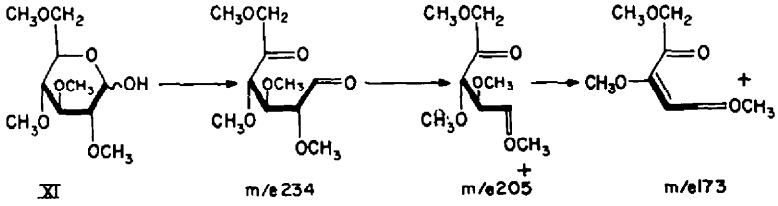
Fragmentation of methylated hexoses follows rather peculiar regularities and details of their mass spectra cannot be interpreted in terms of the above speculations concerning methylated methyl glycosides. There are several parallel pathways of fragmentation for methylated hexopyranoses. One of these pathways, leading to fragments of m/e 191, 159 etc., resembles the direction X of methyl glycosides fragmentation:



The peaks corresponding to this series of fragments are relatively small.

Another series of peaks (at m/e 218, 187 etc.) corresponds to fragments which are formed by reactions of the same type, as those in the direction Z of glycosides fragmentation. These peaks too are of small intensity.

The presence of the third series (peaks at m/e 234, 205, 173, 141) with a number of very intense peaks cannot be explained from the viewpoint of the speculation based on investigation of fragmentation of methylated glycosides. The formation of these fragments is obviously due to the presence of the unprotected glycosidic hydroxyl and ring opening which occurs more readily than in the case of glycosides.



This scheme is supported by spectra of labelled XI analogues which will be published later.

TABLE 5. MASS SPECTRA OF METHYLATED HEXOPYRANOSES

m/e	Relative intensity, % XI	XII	m/e	Relative intensity, % XI	XII
45	100	100	114	5	5
53	8	7	115	6	5
55	21	20	127	8	7
59	9	7	129	9	15
68	8	8	130	17	20
69	9	8	131	7	12
71	19	19	139	6	4
75	43	29	141	14	13
83	14	14	142	6	7
85	33	33	143	5	5
88	28	7	154	6	4
95	10	9	159	2	2
98	9	10	173	11	15
99	12	14	186	0·3	0·4
101	48	56	187	0·4	0·5
110	5	5	191	0·5	0·7
111	28	23	205	0·3	0·2
112	5	5	218	0·15	0·2
113	5	5	234	0·05	0·04

The dependence of the direction of fragmentation and the fragmentation pattern on the ring size

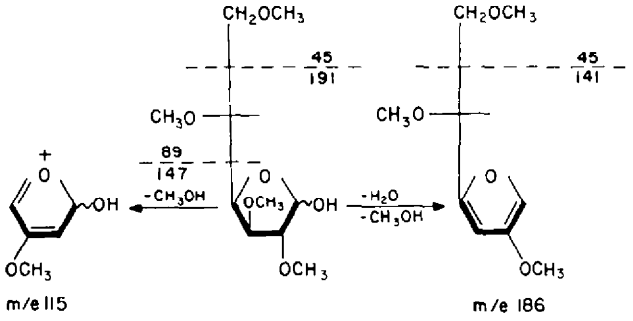
The mass spectra of methylated furanoses (XIII and XIV) are presented in Table 6.

Comparison of the mass spectra of XI (Table 5) and XIII (Table 6) shows that a change of ring size leads to a marked difference in the mass spectra.[4] The latter differs from the former in the relatively high intensity of a number of peaks (m/e 58, 59, 87, 89, 115, 141, 186), which are faint (or absent) in the spectrum of XI.

TABLE 6. MASS SPECTRA OF METHYLATED FURANOSES (XIII AND XIV)

m/e	Relative intensity, % XIII	XIV	m/e	Relative intensity, % XIII	XIV
45	89	100	117	10	—
55	11	15	126	9	—
57	—	8	128	—	4
58	57	—	129	6	2
59	66	10	130	9	—
71	27	16	131	6	4
73	6	13	141	82	4·8
83	15	34	142	19	4·8
85	26	9	147	—	3·5
87	79	91	159	12	—
88	20	27	160	—	1·5
89	100	7	172	6	—
101	85	100	173	—	0·9
114	17	—	175	3	—
115	34	83	186	9	
116	15	—	191	1	
			218	0·3	

The formation of some of the corresponding fragments can be explained by the following scheme:



It is obvious, that this kind of fragment cannot arise from pyranonses. Fragmentation of XIV follows in general the same scheme except that no fragments of m/e 186 and 89 can be formed, a number of other fragments exhibiting consequently different relative intensities.

The dependence of the mass spectra of partially methylated methyl glycosides on the position of the unprotected hydroxyl

Mass spectra of partially methylated methyl glycosides (XVII and XVIII) are presented in Table 7.

There is a very sharp difference between the spectra of XVII and XVIII. The deviation of these spectra from that of I indicate unequivocally the position of the

TABLE 7. MASS SPECTRA OF PARTIALLY METHYLATED METHYL GLYCOSIDES

m/e	Relative intensity, % found	calculated	m/e	Relative intensity, % found	calculated	m/e	Relative intensity, % found	calculated
				XVII				
45	22	7	101	22	5	144	2·2	—
58	9	—	102	14	—	149	2·5	6·0
59	10	6	111	3·4	1·7	159	2·9	0·6
71	90	9	113	3·5	—	161	17	1
74	45	—	115	6·6	—	173	6·3	2·3
75	100	100	117	3·8	1·0	174	0·6	—
87	20	45	127	6·6	1·0	187	0·2	0·2
88	57	18	130	2·6	1·0	191	0·5	0·7
89	15	5	131	2·6	1·0	205	0·2	0·1
99	7	—	141	4·1	0·6			
				XVIII				
45	15	4	101	45	43	141	0·35	0·4
57	2	5	111	1·3	3	143	0·35	0·4
71	4	5	113	1·2	1·2	145	1·0	1·6
73	17	10	127	0·8	1·2	173	2·7	2·6
75	58	60	131	0·9	1·3	176	1·4	1·0
88	100	100	135	1·1	6·5	205	0·15	0·15

unprotected hydroxyl. As anticipated, the peaks of the XVIII mass spectrum corresponding to C(6) containing fragments (m/e 205, 173, 145, 141, 135, 113, 57) are shifted 14 mass units in the direction of less m/e values if compared with the corresponding peaks of the mass spectrum of I (m/e 219, 187, 159, 155, 149, 127, 71). The intensities calculated from data presented in Tables 1 and 2 on the basis of the very approximate assumption of little influence of substituting methoxyl for hydroxyl on the stability of the fragment are cited for comparison in Table 7. Deviations beyond experimental error of the observed spectrum from the predicted pattern are found for only four peaks (m/e 135, 111, 73, 45).

Peaks, corresponding to the C(2) containing fragments, in the spectrum of XVII are again shifted 14 mass units in the direction of less m/e values if compared with that of I. Calculation of intensities in this case is complicated by the ready conversion of one of the base fragments of m/e 74, corresponding to the $88A_2$ fragment of I, into ion of m/e 75, which proceeds through capture of one hydrogen atom:

$$\underset{\text{m/e 74}}{CH_3O\dot{C}H-CH=\overset{+}{O}H} \xrightarrow{+H} \underset{\text{m/e 75}}{CH_3OCH=CH-\overset{+}{O}H_2}$$

This complication leads inevitably to considerable error in evaluating the intensity of the base peak and, consequently, of all the intensities. However, even in this case there exists qualitative correspondence between the predicted and the observed spectra. Calculated spectra of the two other possible trimethyl methyl glucosides as well reveal the usefulness of mass spectrometry in assigning the position of the unprotected hydroxyl. The above examples illustrate the potentialities of mass spectrometry in the analysis of partially methylated monosaccharides. Further experimental verification of this approach is now in progress.

Other derivatives

Methylated disaccharides (IX, X). The spectra of disaccharides are very complicated because of fragmentation proceeding simultaneously with the two moieties of the molecule to give many isomeric ions. Spectra of disaccharides consist of two regions. The region between m/e 45 and 219 resembles spectra of methylated methyl glycosides, and differs from them in the absence of only a few fragments (m/e 149, 176). The region between m/e 219 and 380 contains peaks, corresponding to fragments composed of both halves of the molecule (m/e 273, 279, 280, 289, 304, 379). The presence of these peaks in the second region shows the mass spectra to be of potential value in assigning the position of the glycosidic bond. However, the minor deviations between the spectra of IX and X cannot at present be related to the change of the glycosidic bond configuration.

Methyl glycopyranoside acetates (XIX–XXII). The spectra of methyl glycoside acetates are very close to those reported recently by Biemann *et al.*[4] for monosaccharide penta-acetates. The type of fragmentation of these compounds is the same as that of methylated glycosides. However, the spectra of acetates are complicated due to the instability of the acetate grouping as indicated by the absence of peaks at m/e values above 230. On the other hand, the ready fission of the acetate grouping leads to the formation of a series of fragments in place of single ions in the patterns of methylated glycosides. The mass numbers of fragments within such a series differ by 60 (CH_3COOH), or 42 (—CH_2CO—). This complication leading to much closer

similarity of patterns of differing compounds together with the absence of peaks at high m/e values will, in our opinion, considerably limit the use of sugar acetates in mass spectrometry.

Conclusions

The above discussed data reveal potentialities of mass spectrometry as a useful tool which can help in solution of structural problems like determination of ring size and the position of an unprotected hydroxyl in partially methylated monosaccharides, molecular weight determination and assignment of configuration at C(1).

The preliminary data obtained in experiments now in progress suggest that mass spectrometry will become useful in assigning the position of glycosidic bonds in disaccharides, of aminogroups in aminosugars and CH_2-groups in deoxysugars.

It is believed, that further investigation of different types of carbohydrate derivatives will open new applications of mass spectrometry in the specific field of carbohydrate chemistry, but it is at present quite obvious that the new approach can at least partially compensate for the shortcomings of the existing physical methods.