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MASS SPECTROMETRY OF METHYLATED METHYL GLYCOSIDES

PRINCIPLES AND ANALYTICAL APPLICATION

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Abstract—A novel approach to the structural analysis and identification of partially methylated monosaccharides involves deuteromethylation and subsequent mass spectrometry to determine the position of the trideuteromethyl groups. The mass spectra of a number of typical partially methylated monosaccharides derived from the hexopyranose, pentopyranose, pentofuranose and 6-deoxyhexose series are discussed in detail.

INTRODUCTION

METHYLATION remains a conventional method for structural analysis of polysaccharides and other natural compounds containing carbohydrate monomer units. The value of this old method has become greater as a result of improvements in the methylation procedure[1,2] and the elaboration of new methods for the separation of partially methylated monosaccharides, involving partition[3,4] and gas-liquid[5] chromatography. The identification of partially methylated monosaccharides, however, requires a set of authentic samples, the problem becoming difficult if these are not available.

The mass spectra of methyl 2,3,4,6-tetra-O-methyl-α,D-glucopyranoside and its deuteroanalogues investigated[6] prompted a novel approach to this problem.[7] The partially methylated monosaccharide under investigation is subjected to deuteromethylation, and the mass spectrum of the compound obtained compared with the calculation on the basis of the fragmentation patterns characteristic of the same type of methylated glycoside. Identity of the calculated and experimental spectra indicates the position of the trideuteromethyl groupings, and consequently, of the free hydroxyl in the starting partially methylated monosaccharide. The validity of the approach has been demonstrated with trideuteromethylated methyl α,D-glucosides, and the calculated data presented as a Table.[7]

The advantages of the new approach are obvious: it uses only readily available permethyl-glycosides as authentic samples, enables identification with minor amounts of substances (about 1 mg) and provides a basis for standardization of the analysis of partially methylated monosaccharides.

The realization of the new approach needed, however, the solution of number of

[1] R. Kuhn, H. Trischmann, I. Löw, *Angew. Chem.* **67**, 32 (1955).
[2] S. Hakomori, *J. Biochem.* **55**, 205 (1964); H. S. Srivastava, S. N. Harshe, Prem Pal Singh, *Tetrahedron Letters* 1869 (1963); H. S. Srivastava, Prem Pal Singh, S. N. Harshe, *Ibid.* 493 (1964).
[3] G. N. Kowkabany, *Adv. Carbohydrate Chem.* **9**, 304 (1954).
[4] W. W. Binkley, *Adv. Carbohydrate Chem.* **10**, 55 (1955).
[5] C. T. Bishop, F. P. Cooper, *Canad. J. Chem.* **38**, 388 (1960); G. O. Aspinall, *J. Chem. Soc.* 1676 (1963).
[6] N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, B. M. Zolotarev, *Tetrahedron* **19**, 2204 (1963).
[7] N. K. Kochetkov, O. S. Chizhov, *Biochem. Biophys. Acta* **83**, 134 (1964).

problems. Firstly, could the data obtained with one member of the series e.g., methyl α,D-glucopyranoside, be applied to other members, e.g., methyl α- or β-,D-galactopyranosides, methyl α,D-mannopyranoside, etc. Consequently the mass spectra of methyl 2,3,4,6-tetra-O-methyl-β,D-galactopyranoside and its deuterium labelled analogues were investigated and the results compared, after appropriate treatment, with those obtained previously for glucose.[7] It was necessary also to obtain information concerning mass spectra of 6-deoxyhexose, pentopyranose and pentofuranose in order to apply the new approach to large number of monosaccharides present in natural carbohydrate polymers. For this purpose, permethyl methyl β,D-fucoside, methyl β,L-arabopyranoside and methyl α,β,L-arabofuranoside were investigated.

GENERAL REMARKS

In order to elucidate the fragmentation patterns and the structure of ions arising from the compounds under investigation, a procedure analogous to that applied earlier to methyl 2,3,4,6-tetra-O-methyl-α,D-glucopyranoside was used.[6] Analogues of the compounds mentioned were prepared with a trideuteromethyl label in various positions. The shift of the peak in the mass spectrum of the analogue with respect to that of the unlabelled compound indicated that the corresponding fragment contained one or more trideuteromethyl groupings and this provides a reasonable basis for suggesting the structure of ion.

As the mass spectra of the compounds investigated usually contained no metastable peaks, it was possible to deduce the patterns by comparing structures of ions; however, the conclusions must in several cases be regarded as preliminary.

Series of ions characterized by similar structures and, presumably, similar origin, will be designated by letters A, B, C, etc. The index below corresponds to the number of stages needed for the transformation of the molecular ion to the given fragment. If several isomeric ions are formed, the index above refers to the ordinal number. For example, designation B_3^2 indicates that the corresponding fragment was formed from the molecular ion by pathway B, by three consecutive transformations, being the second isomer. Analogous series originated from different substances are designated by the same capital, and analogous isomeric ions from different substances have the same ordinal numbers.

The contributions of isomeric ions to the total peak intensity were calculated as described earlier.[6] Knowing the structures of ions and their contributions it appears possible to predict the spectra of labelled analogues with trideuteromethyl groupings at all the possible positions. The corresponding calculations were made starting with data for the substances investigated, and presented in the form of Tables.

The mass spectra were measured with MX-1303 mass spectrometer at ionizing potential 70 ev and temperature in the sample port 175°. With methyl 2,3,5-tri-O-methyl-α,β,L-arabofuranoside and its analogues the ionizing potential was 30 ev and the temperature of sample port 75°. The peak intensities in pyranoside spectra were expressed as a percentage of the intensity of the peak at m/e 88, or the sum of intensities of peaks at m/e 88, 91 and 94 with the labelled analogues. With arabofuranosides, the intensities were expressed in terms of the intensity of peak m/e 101, or the sum of intensities of peaks m/e 101, 104 and 107 with the labelled analogues, assumed arbitrarily 500%.

*Mass spectra of methyl 2,3,4,6-tetra-O-methyl-β,D-galactopyranoside and deutero-methyl analogues**

The mass spectra of methyl 2,3,4,6-tetra-O-methyl-β, D-galactoside (I) and its deuterated analogues—methyl 2,3,4,6-tetra-O-trideuteromethyl-β,D-galactoside (II), methyl 2,4,6-tri-O-methyl-3-O-trideuteromethyl-β,D-galactoside (III), methyl 2,3,4-tri-O-methyl-6-O-trideuteromethyl-β,D-galactoside (IV) and methyl 2,3-di-O-methyl-4,6-di-O-trideuteromethyl-β,D-galactoside (V) are presented in Table 1. The synthesis of the deuterated analogues is described in another paper.[8]

TABLE 1. MASS SPECTRA OF I AND ITS DEUTERATED ANALOGUES*

m/e Values	Relative intensities of peaks in mass spectra of I	II Found	II Sum	III Found	III Sum	IV Found	IV Sum	V Found	V Sum
187	0·4	—		0·3		—		—	
190	—	—	0·3	—	0·3	0·4	0·4	—	0·3
193	—	—		—		—		0·3	
196	—	0·3		—		—		—	
176	1·8	—		—		2·0		—	
179	—	—	1·7	1·7	1·7	—	2·0	1·6	1·6
182	—	—		—		—		—	
185	—	1·7		—		—		—	
159	1·0	—		0·6		—		—	
162	—	—	1·0	0·5	1·1	1·1	1·1	—	0·9
165	—	—		—		—		0·9	
168	—	1·0		—		—		—	
149	11	—		—		—		—	
152	—	—	11	10	10	12	12	10	10
155	—	11		—		—		—	
131	2·5	—		—		2·8		—	
134	—	—	2·2	2·4	2·4	—	2·8	2·3	2·3
137	—	—		—		—		—	
140	—	2·2		—		—		—	
127	3·4	—		2·4		2·2		—	
130	—	—	3·7	0·9	3·3	1·8	4·0	2·6	3·6
133	—	3·7		—		—		1·0	
101	52	—		41		46		9	
104	—	2	55	10	51	8	54	40	57
107	—	53		—		—		8	
88	100	—		5		100		89	
91	—	5	100	95	100	—	100	11	100
94	—	95		—		—		—	
75	58	—		11		44		37	
78	—	41	53	41	52	7	51	13	50
81	—	12		—		—		—	

* The data about intensities several small peaks (m/e) 219, 205, 173, 155, 111) are omitted.

The data presented in Table 1 enable deduction of the structures, corresponding to the major peaks of the spectrum, and calculation of the contributions of the isomeric ions. The results of this calculation are presented in Table 2 and are almost identical to those obtained for methyl 2,3,4,6-tetra-O-methyl-α,D-glucoside.[6] Also

* This part of work was done together with B. M. Zolotarev

[8] N. K. Kochetkov, O. S. Chizhov, B. M. Zolotarev, to be published.

in Table 3 the predicted mass spectra of all the possible trideuteroanalogues of I are very close to that deduced for methyl 2,3,4,6-tetra-O-methyl-α,D-glucoside.[7] Hence, at least in the case of methylated pyranose, the results indicate the possibility of

TABLE 2. STRUCTURES AND CONTRIBUTIONS OF MAJOR IONS ARISING FROM II

m/e	Structure	Designation	Contribution
176	$CH_3O-\dot{C}H-CH(CH_2O)-CH(OCH_3)-CH=\overset{+}{O}CH_3$	B_1	100
159	$CH_3O-CH_2-\overset{+}{C}H-CH(OCH_3)-CH=CH-OCH_3$	C_2^2	55
	$CH_3O-CH_2-CH(CH_2-\overset{+}{C}H-CH-OCH_3)-CH-OCH_3$	C_2^3	45
149	$CH_3O-CH_2-CH=\overset{+}{O}-CH(OCH_3)_2$	D_1	100
101	$CH_3O-CH=CH-CH=\overset{+}{O}CH_3$		
	$C_1\ C_2\ C_3$	$F_1^1(G_1^1)$	2
	$C_2\ C_3\ C_4$	$F_1^2(G_1^2)$	65
	$C_4\ C_5\ C_6$	$F_1^3(G_1^3)$	15
	$CH_3O-CH-CH-OCH_3$ (+/CH)		
	C_1-C_2 / C_3	G_1^4	2
	C_3-C_4 / $C_{1(6)}$	G_1^5	13
	C_5-C_6 / $C_{2(5)}$	G_1^6	3
88	$(CH_3O-CH=CH-OCH_3)^+$		
	$C_1\ C_2$	H_1^1	5
	$C_2\ C_3$	H_1^2	84
	$C_3\ C_4$	H_1^3	11
75	$CH_3O-CH=\overset{+}{O}CH_3$	J_1^1	70
	C_1, CH_3O—groups from C(1) and (C3)		

using Tables to identify other methylated monosaccharides of the series from the data obtained for one methylated glycoside.

The mass spectrum of permethyl-arabofuranoside (see below) added new details to the fragmentation pattern of methylated methyl-pyranosides. The most important being rearrangement involving migration of the methoxy grouping from C(3) to C(1). This phenomenon also noticed recently by Scharmann,[9] made it necessary to reconsider the structure of several ions. First of all, this refers to the structure of the ion

[9] H. Scharmann, *Intern. Symp. über die Chem. der Kohlenhydrate* Münster, Juli (1964).

with m/e 75, which in case I also contains two methoxyls on the same carbon atom, namely at C(1), due to migration of methoxyl from C(3). This is in accordance with the high percentage of incorporation of C(3)-methoxyl, which can not be explained in terms of the structure previously proposed.[6] Futher, the structure of ion with m/e 149 was deduced on the basis of the following considerations. The m/e 149 fragment, containing carbon, oxygen and hydrogen atoms, must have a brutto-formula $C_6H_{13}O_4$. As revealed by the data of Table 1, this fragment must contain the methoxy groupings from C(3) and C(6) only. Obviously, one of the two remaining oxygen atoms must

TABLE 3. MASS SPECTRA OF I AND ITS DEUTERATED ANALOGUES*

	Position of CD_3O—groups				
I	2	3	4	6	2,3
176	179	179	179	176	182
149	149	152	149	152	152
101	101 (20)	101 (82)	101 (17)	101 (85)	101 (15)
	104 (80)	104 (18)	104 (83)	104 (15)	104 (72)
					107 (13)
88	88 (11)	88 (5)	88 (89)	88	
	91 (89)	91 (95)	91 (11)		91 (16)
					94 (84)

	Position of CD_3O—groups				
I	2,4	2,6	3,4	3,6	4,6
176	182	179	182	179	179
149	149	152	152	155	152
101	101 (2)	101 (5)	101 (2)	101 (67)	101 (17)
	104 (33)	104 (95)	104 (95)	104 (33)	104 (68)
	107 (65)		107 (3)		107 (15)
88		88 (11)	88 (5)	88 (5)	88 (89)
	91	91 (89)	91 (84)	91 (95)	91 (11)
			94 (11)		

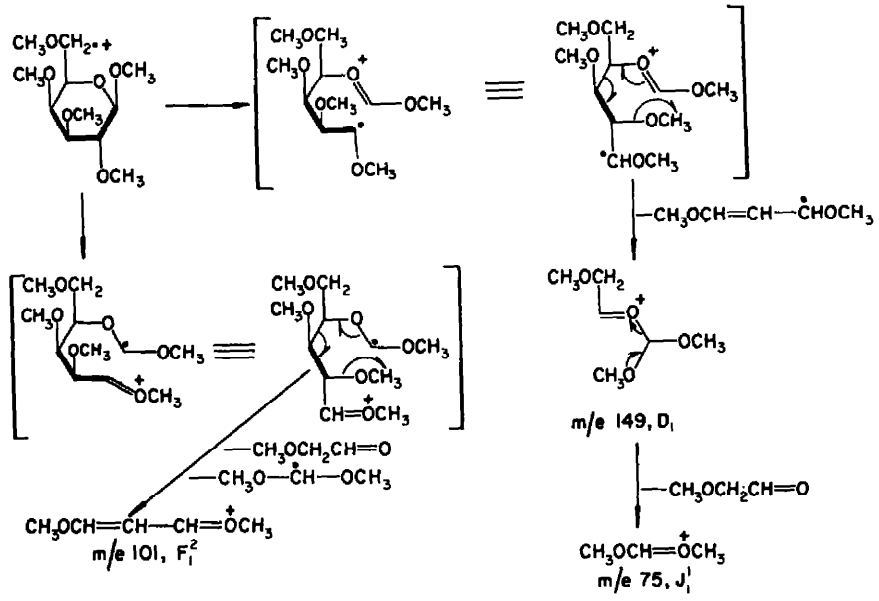
* The data about peaks unnecessary from analytical viewpoint are omitted.

belong to the glycosidic methoxyl, and the other must be the oxygen atom of the cycle. As it is impossible to imagine a structure, containing C(1), C(3), C(6) and the oxygen atom of the cycle, and, at the same time, having a brutto-formula $C_6H_{13}O_4$, the only possibility is that the ion arises by a rearrangement, particularly, by migration of methoxyl from C(3) to C(1).

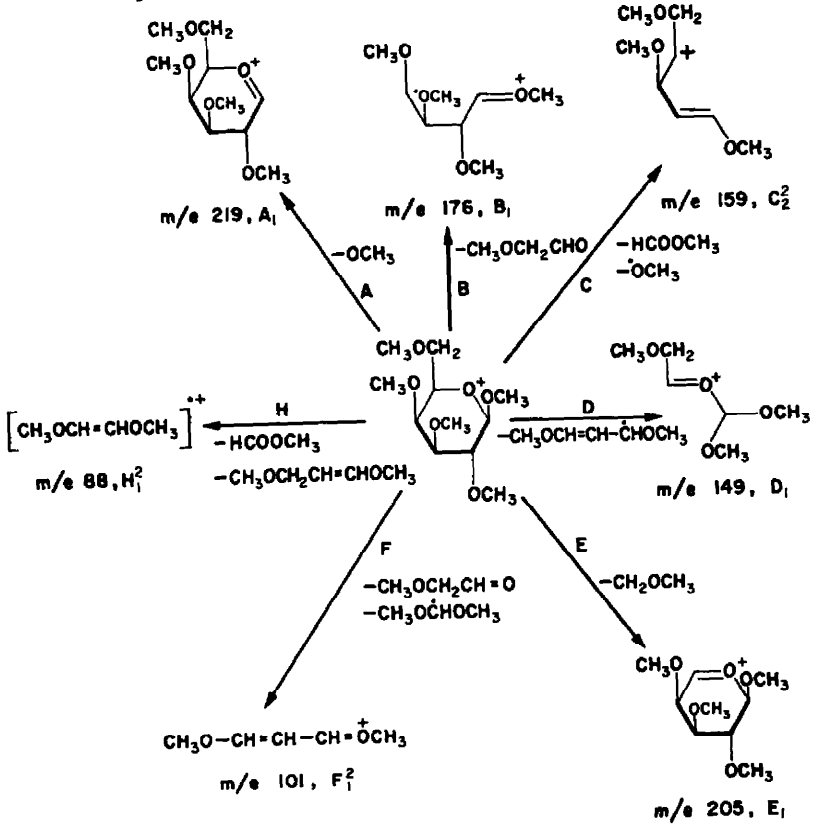
The new pattern also explains the origin of one of the ions with m/e 101, i.e., ion F_1^2, which gives the greatest contribution to the corresponding peak intensity, and also the origin of the ion with m/e 75.

Finally, bearing in mind the analogy in the structures of ions m/e 159 in mass spectra of methylated hexopyranosides and ions with m/e 243 in the mass spectra of acetylated methyl hexopyranosides, studied by Biemann,[10] as well as the absence of any parallelism in changing intensities of these peaks and peak m/e 187 in the mass spectra of different hexopyranosides, it may be assumed, that ions m/e 159 arise not from that at m/e 187, but directly from the molecular ion, although the absence of the corresponding metastable peak precludes a final conclusion.

[10] D. C. De Jongh, K. Biemann, *J. Amer. Chem. Soc.* **85**, 2289 (1963).



Hence, the following partially reconsidered fragmentation pattern can now be proposed for methylated methyl hexopyranosides:



Methyl 2,3,4-*tri*-O-*methyl*-β,D-*fucoside* (VI) *and analogues**

For interpretation of the mass spectrum of VI, the following compounds were prepared and investigated: methyl 2-O-trideuteromethyl-3,4-di-O-methyl-β,D-fucoside (VII), methyl 2,3-di-O-methyl-4-O-trideuteromethyl-β,D-fucoside (VIII), methyl 2-O-methyl-3,4-di-O-trideuteromethyl-β,D-fucoside (IX). The synthesis of these derivatives are described in another paper.[11]

The mass spectra of VI and deuterated analogues VII–IX are presented in Table 4; the structures and contributions of ions deduced on the basis of this evidence are

TABLE 4. MASS SPECTRA OF VI AND ITS DEUTERATED ANALOGUES

m/e	Relative intensities of peaks in mass spectra of						
		VII		VIII		IX	
Values	VI	Found	Sum	Found	Sum	Found	Sum
176	3·6	—		—		—	
179	—	3·1	3·1	2·7	2·7	—	2·5
182	—	—		—		2·5	
131	3·6	—		—		—	
134	—	2·8	2·8	3·0	3·0	—	3·0
137	—	—		—		3·0	
129	7·7	2·8		0·9		—	
132	—	3·7	6·5	6·4	7·3	4·7	7·1
135	—	—		—		2·4	
119	3·9	3·2	3·2	3·8	3·8	—	3·5
122	—	—		—		3·5	
101	24	3·2		6·5		1·5	
104	—	16·7	19·9	14·3	20·8	18·0	21·0
107	—	—		—		1·5	
88	100	16·7		91·1		10·0	
91	—	83·3	100	8·9	100	78·7	100
94	—	—		—		11·3	
75	39	26·8	34·2	26·0	32·8	1·4	32·6
78	—	7·4		6·8		31·2	
73	25	12·5	22·7	19·2	22·7	10·5	21·8
76	—	10·2		3·5		11·3	
72	14·4	12·0	12·0	—	12·2	—	11·7
75	—	—		12·2		11·7	
71	5·3	4·6	4·6	1·6	7·6	—	5·2
74	—	—		6·0		5·2	
45	21·0	6·0	18·0	13·0	16·6	7·7	16·8
48	—	12·0		3·6		9·1	

presented in Table 5. It appears, that methylated fucoside VI decomposes after electron impact according to a mechanism essentially the same as that of permethylated methyl β,D-galactoside I. As with methyl β,D-galactoside, the most intense peaks of the mass spectrum of VI are at m/e 88, 101 and 75; the major contributions to the peak intensity are provided by ions H_1^2 for that at m/e 88, F_1^2—for that at m/e 101, and J_1^1—for that at m/e 75. The peaks corresponding to ions not containing the fifth and sixth carbon atoms occupy the same positions as those in the mass spectra of methylated methyl-hexopyranosides (e.g., fragments with m/e 176 and 131).

* This part of work was done together with B. M. Zolotarev and V. Sh. Sheinker.

[11] N. K. Kochetkov, O. S. Chizhov, B. M. Zolotarev, V. Sh. Sheinker, to be published.

TABLE 5. STRUCTURES AND CONTRIBUTIONS OF MAJOR IONS ARISING FROM VI

m/e	Structure	Designation	Contribution
176	$CH_3O-\dot{C}H-CH(OCH_3)-CH(OCH_3)-CH=\overset{+}{O}CH_3$	B_1	100
129	$CH_3-CH=CH-CH(OCH_3)-CH=\overset{+}{O}CH_3$	C_1^1	13
	$CH_3-\overset{+}{C}H-CH(OCH_3)-CH=CH-OCH_3$	C_1^2	48
	$CH_3-CH-\overset{+}{C}H$ $CH_3O-CH-CH-OCH_3$	C_1^3	39
119	$CH_3-CH=\overset{+}{O}-CH(OCH_3)_2$	D_1	100
101	$CH_3-CH=CH-CH=\overset{+}{O}CH_3$ $C_1\ C_2\ C_3$ $C_2\ C_3\ C_4$ $CH_3O-CH-CH-OCH_3$	F_1^1 (G_1^1) F_1^2 (G_1^2)	9 62
	C_2-C_1 $\diagdown\ \diagup$ C_3	G_1^3	7
	C_2-C_3 $\diagdown\ \diagup$ $C_{1(6)}$	G_1^4	15
	C_3-C_4 $\diagdown\ \diagup$ $C_{2(5)}$	G_1^5	7
88	$(CH_3O-CH=CH-OCH_3)^+$ $C_1\ C_2$ $C_2\ C_3$ $C_3\ C_4$	H_1^1 H_1^2 H_1^3	10 78 12
75	$CH_3O-CH=\overset{+}{O}CH_3$ C_1, CH_3O—groups from C_1 and C_3	J_1^1	60
72	$(CH_3-CH=CH-OCH_3)^+$	K_1	100

The peaks of ions, containing C(5) and C(6), are shifted 30 mass units to lower mass numbers; e.g., peak at m/e 129 is observed instead of that at m/e 159, peak at m/e 119 instead of that at m/e 149, etc.

A characteristic feature of the mass spectrum of fucoside VI, distinguishing it from that of the hexopyranosides discussed, is the complete disappearence of peaks of the E series due to the difficulty of fission of C(6)-methyl as radical; in addition, the

TABLE 6. MASS SPECTRA OF DEUTERATED ANALOGUES OF VI

VI	Position of CD_3O—groups 2	3	4	2,3	2,4	3,4
176	179	179	179	182	182	182
131	134	134	134	137	137	137
129	129 (39)	129 (52)	129 (13)	132 (87)	132 (48)	132 (61)
	132 (61)	132 (48)	132 (87)	135 (13)	135 (52)	135 (39)
119	119	122	119	122	119	122
101	101 (16)	101 (69)	101 (31)	104 (85)	101 (9)	101 (7)
	104 (84)	104 (31)	104 (69)	107 (15)	104 (29)	104 (86)
					107 (62)	107 (7)
88	88 (12)	88 (10)	88 (88)	91 (22)	91	88 (10)
	91 (88)	91 (90)	91 (12)	94 (78)		91 (78)
						94 (12)

TABLE 7. MASS SPECTRA OF X AND ITS DEUTERATED ANALOGUES

m/e Values	X	Relative intensities of peaks in mass spectra of XI Found	XI Sum	XII Found	XII Sum	XIII Found	XIII Sum
176	11	—		—		—	
179	—	9	9	9·5	9·5	—	7
182	—	—		—		7	
175	4	—		—		—	
178	—	4	4	3	3	—	4
181	—	—		—		4	
143	4·5	1		2·5		—	
146	—	5	6	1·5	4	4	5
149	—	—		—		1	
115	11·5	3		6		—	
118	—	8	11	4	10	7·5	10
121	—	—		—		2·5	
101	204	6		151		6	
104	—	171	177	26	177	154	165
107	—	—		—		5	
99	9	8	10·5	5	11	2	10
102	—	2·5		6		8	
88	100	10		10		9	
91	—	90	100	90	100	80	100
94	—	—		—		11	
85	9	4	10	7	9	6	9
88	—	6		2		3	
83	10	4·5	11·5	8	8	7	11·5
86	—	7		—		4·5	
75	84	72	88	12	91	3·5	71·5
78	—	16		79		68	
73	49	27	56	28	54	24	51
76	—	29		26		27	
58	17	24	26	19	19	5	19·5
61	—	2		—		14·5	
45	57	28	54	37	58	21	50
48	—	26		21		29	

compound is characterized by very low intensity of peaks of the A series. On the other hand, the relative intensity of peaks of the C series increases considerably.

Table 6 contains the predicted spectra of the following deuteroanalogues of VI—methyl 2,4-di-O-methyl-3-O-trideuteromethyl-β,D-fucoside, methyl 2,3-di-O-trideuteromethyl-4-O-methyl-β,D-fucoside and methyl 2,4-di-O-trideuteromethyl-3-O-methyl-β,D-fucoside, calculated on the basis of data of Table 5, and also experimental data for mass spectra of VII-IX for comparison . It is seen, that the spectra of all of the isomers differ from each other, so that it appears possible to use Table 6 for identification of partially methylated fucose and presumably other 6-deoxyhexoses.

Methyl 2,3,4-*tri*-O-*methyl*-β,L-*arabopyranoside* (X)*

In order to deduce the structure of ions of the mass spectrum of X, the following labelled analogues were prepared and investigated: methyl 2-O-trideuteromethyl-3,4-di-O-methyl-β,L-arabopyranoside (XI), methyl 3-O-trideuteromethyl-2,4-di-O-methyl-β,D-arabopyranoside (XII), methyl 2-O-methyl-3,4-di-O-trideuteromethyl-β,L-arabopyranoside (XIII). The synthesis of this group of compounds is described in another paper.[12]

The mass spectra of X–XIII are presented as Table 7, and the calculated contributions and deduced structures of ions are given in Table 8.

The mass spectrum of X in some respects resembles the spectra of I and VI. As in the previous cases, the most intense peaks are situated at m/e 88, 101 and 75. The mass spectrum of X differs, however, from those mentioned in that the m/e 101 peak is higher, than that at m/e 88. As for the contribution of ions of different structure or origin, as in the case of I and VI, the major fragment among ions with m/e 101 is F_1^2, among ions with m/e 88 — H_1^2, among ions with m/e 75 — J_1^1. As in the case of VI, the peaks of ions not containing the fifth carbon atom remain at their usual positions (m/e 176, 131), whereas those corresponding to fragments of the A and C series are shifted 44 mass units to lower mass numbers: e.g., peak at m/e 115 appears instead of m/e 159, m/e 175 instead of m/e 219, etc.

The peculiar features of the mass spectrum of X are: (i) the increasing relative intensity of peaks of fragments B_1, C_2^2 and C_2^3, (ii) the decreasing intensity of the peak of fragment D_1 (m/e 105) and (iii) the absence of the E series. The latter must be due to the difficulty of fission of hydrogen at C(5) as H·-radical.

The Table 9 contains predicated mass spectra of the following analogues of X: methyl 2,3-di-O-methyl-4-O-trideuteromethyl-β,L-arabopyranoside, methyl 2,3-di-O-trideuteromethyl-4-O-methyl-β,L-arabopyranoside and methyl 2,4-di-O-trideuteromethyl-3-O-methyl-β,L-arabopyranoside, calculated on the basis of data of Table 8, and also the experimental mass spectra of XI–XIII. Again the spectra of the compounds are characteristic, thus enabling identification of all partially methylated pentopyranoses.

Methyl 2,3,5-*tri*-O-*methyl*-α,β,L-*arabofuranoside* (XIV)†

A mixture of anomers was used for the investigation, as this usually results after hydrolysis of the methylated carbohydrate polymer. The sample of XIV studied

* This part of work was done together with B. M. Zolotarev.

† This part of the work was done in collaboration with N. S. Wulfson and N. F. Madudina.

[12] O. S. Chizhov, B. M. Zolotarev, N. K. Kochetkov, to be published.

TABLE 8. STRUCTURES AND CONTRIBUTIONS OF MAJOR IONS ARISING FROM X

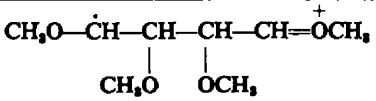
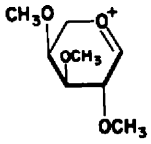
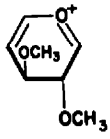
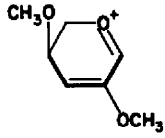
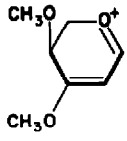
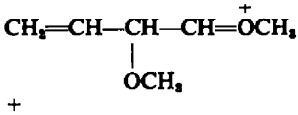
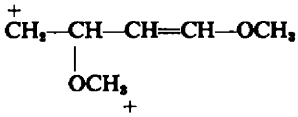
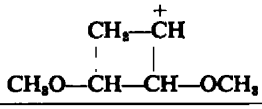
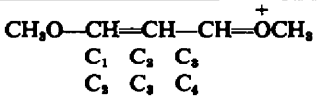
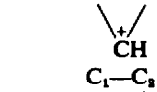
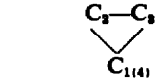
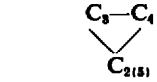
m/e	Structure	Designation	Contribution
176		B_1	100
175		A_1	100
143		A_2^1	20
		A_2^3	60
		A_3^3	20
115		C_2^1	15
		C_2^2	60
		C_2^3	25
101		F_1^1 (G_1^1)	0·3
		F_1^2 (G_1^2)	81·5
		G_1^3	3·6
		G_1^4	11·5
		G_1^5	3·1

TABLE 8. (*Contd.*)

m/e	Structure	Designation	Contribution
88	$(CH_3O—CH=CH—OCH_3)^+$		
	$C_1\ C_2$	H_1^1	10
	$C_2\ C_3$	H_1^2	80
	$C_3\ C_4$	H_1^3	10
75	$CH_3O—CH=\overset{+}{O}CH_3$	J^1_1	88
	C(1), CH_3O—groups from C(1) and C(3)		
73	$CH_3\overset{+}{O}=CH—CH=O$		
	$C_2\ C_3$	H_2^1	50
	$C_3\ C_2$	H_2^2	50
58	$(CH_2=CH—OCH_3)^+$	K_1	100
	$C_5\ C_4$		

TABLE 9. MASS SPECTRA OF DEUTERATED ANALOGUES OF X

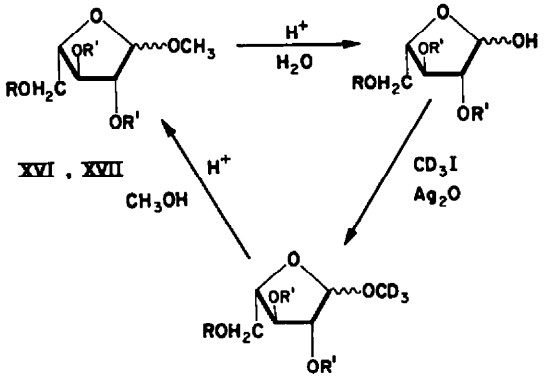
X	Position of CD_3O—groups 2	3	4	2,3	2,4	3,4
176	179	179	179	182	182	182
175	178	178	178	181	181	181
143	146 (80)	146 (40)	146 (80)	149 (20)	149 (60)	149 (20)
	143 (20)	143 (60)	143 (20)	146 (80)	146 (40)	146 (80)
115	118 (75)	118 (40)	118 (85)	121 (15)	121 (60)	121 (25)
	115 (25)	115 (60)	115 (15)	118 (85)	118 (40)	118 (75)
101	104 (96)	104 (15)	104 (85)	107 (11)	107 (82)	107 (3)
	101 (4)	101 (85)	101 (15)	104 (89)	104 (18)	104 (93)
						101 (4)
88	91 (90)	91 (90)	91 (10)	94 (80)		94 (10)
	88 (10)	88 (10)	88 (90)	91 (20)	91	91 (80)
						88 (10)

contained 82·5% α- and 17·5% β- isomer as revealed by gas-liquid chromatographic evidence.* Although the synthesis of labelled analogues of XIV—methyl 2-O-trideuteromethyl-3,5-di-O-methyl-α,β,L-arabofuranoside (XV), methyl 2,3-di-O-methyl-5-O-trideuteromethyl-α,β,L-arabofuranoside (XVI) and methyl 2,3,5-tri-O-trideuteromethyl-α,β,L-arabofuranoside (XVII) are described in another paper,[13] a brief account of the preparation of these substances is given. In order to introduce the trideuteromethyl label into XVI and XVII, the corresponding derivatives were heated in sealed tubes at 100° with trideuteromethyl iodide in the presence of excess silver oxide. Due to unsufficient agitation of the reaction mixture, localized acidic

* The analysis was performed using Argon-Pye instrument; column 200 × 0·4 cm of 5% polyethylene glycol adipate on Celite 535; 160°; 45 ml argone per min; sample 0·1 μl 10% solution of the mixture in methanol.

[13] O. S. Chizhov, N. F. Madudina, to be published.

zones may arise. As water is formed during the methylation and on account of the high sensitivity of furanosides to acidic hydrolysis, the glycosidic methoxyl was substituted to some extent by a trideuteromethyl grouping, as revealed in fact by mass spectral evidence.



XVI α , XVII α

XVI, XVIa R=CD_3, R'=CH_3; XVII, XVIIa R=R'=CD_3

The mass spectra of XIV–XVII are given in Table 10. Although a number of papers[6,11,14,15] point out the specifity of mass spectra of furanose derivatives, no detailed investigation of these compounds has been published. Consequently, the deduction of structures for fragments, corresponding to major peaks of the mass spectrum of XIV, are analysed in detail.

As with pyranose derivatives (I, VI, X) a series of fragments of similar structure and, probably, of common origin, can be followed in the mass spectrum of XIV.

"A"-*Series*. The greatest fragment of this series is A_1 (m/e 175) which is formed, evidently, by fission of methoxyl (31 mass units) from molecular ion M^+. This departing methoxyl can be only the glycosidic one, as corresponding shifts are observed with XV, XVI and XVII. The next important peak of this series is situated at m/e 143. Two fragments (A_2^1 and A_2^2) contribute to its intensity. They are formed from A_1 by fission of methanol (32 mass units) from C(3) and C(5), respectively (Table 10). Calculated contributions are 77% for A_2^1 and 23% for A_2^2.

"C"-*Series*. The greatest fragment of this series (C_1) gives a faint peak at m/e 146. The mass of the C_1 fragment is 60 mass units less than that of the M^+, corresponding to fission of methyl formate. The ion-radical C_1 is stabilized by fission of one of the methoxyls as CH_3O·-radical, leading to three isomeric ions with m/e 115 (C_2^1, C_2^2 and C_2^3) and contributions respectively, 28%, 53% and 19% of the total peak intensity. Fission of methanol from isomeric ions C_2 affords isomeric ions with m/e 83.

"E"-*Series*. Fragments with m/e 161 (E_1) and m/e 129 (E_2^1 and E_2^2) belong to this series. The mass of fragment E_1 is 45 mass units less than that of M^+, corresponding to fission of CH_3OCH_2·-grouping. In accordance with this, the peak of E_1 in the mass spectrum of XV is shifted 3, and in the mass spectrum of XVII—6 mass units. No shift is observed in the mass spectrum of XVI. The mass of fragments E_2^1

[14] K. Biemann, H. K. Schnoes, J. A. M. McCloskey, *Chem. & Ind.* 448 (1963).
[15] K. Biemann, D. C. De Jongh, H. K. Schnoes, *J. Amer. Chem. Soc.* 85, 12 (1963).

TABLE 10. MASS SPECTRA OF XIV AND ITS DEUTERATED ANALOGUES

m/e Values	Relative intensities of peaks in mass spectra of XIV	XV Found	XV Sum	XVI Found	XVI Sum	XVII Found	XVII Sum	XVIIa Found	XVIIa Sum
175	7	6·7		—		—		—	
178	—	—	6·7	5·7	5·7	—	6·3	—	5·6
181	—	—		—		—		—	
184	—	—		—		6·3		5·6	
161	28	—		25		—		—	
164	—	26	26	—	25	—	28	—	25
167	—	—		—		28		8	
170	—	—		—		—		17	
146	0·9	—		—		—		—	
149	—	0·7	0·7	1·1	1·1	—	1·2	—	1·2
152	—	—		—		—		—	
155	—	—		—		1·2		1·2	
143	6·5	—		1·7		—		—	
146	—	6	6	5·3	7	—	9·6	—	6
149	—	—		—		9·6		6	
129	6	1·7		5		—		—	
132	—	5·4	7·1	—	5	6	6	1	6
135	—	—		—		—		5	
115	18	3·7		6·3		—		—	
118	—	16	19·7	16	22·3	—	20	—	16·5
121	—	—		—		20		16·5	
101	500	56		454		—		—	
104	—	444	500	46	500	44	500	20	500
107	—	—		—		456		480	
88	20	—		19·5		—		—	
91	—	26	26	—	19·5	7·3	19·5	—	20
94	—	—		—		12·2		20	
75	126	113		109		—		—	
78	—	14	127	20	129	127	142	30	134
81	—	—		—		15		104	
45	85	58	83	62	96	10	65	—	63
48	—	25		34		45		63	

and E_2^2 is 32 mass units lower than that of E_1, corresponding to fission of methanol. In the mass spectrum of XVII a shift of only 3 mass units is observed. As in the mass spectrum of XV, in place of one peak two peaks arise at m/e 129 and 132, this fission of methanol may occur in one of two ways—from C(3) (ion E_2^1, contribution 76%), or from C(2) (ion E_2^2, contribution 24%).

The most intense peak of the spectrum is situated at m/e 101. This peak in the mass spectrum of XVII being almost completely shifted to m/e 107, the corresponding ions must contain two methoxyls. The remaining 39 mass units must be distributed between 3 carbon atoms and 3 hydrogen atoms. Ions of this composition may have the following two structures[10]: $CH_3O—CH=CH—CH=\overset{+}{O}CH_3$ (F_1) and $CH_3O—\underset{\diagdown\,\diagup}{CH}—CH—OCH_3$ (G_1). Only two ions of the F_1 type can be

TABLE 11. STRUCTURES AND CONTRIBUTIONS OF MAJOR IONS ARISING FROM XIV

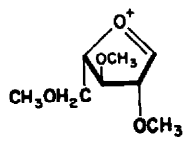
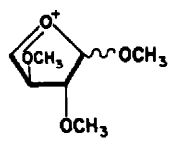
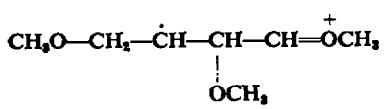
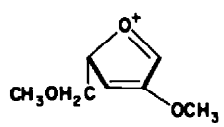
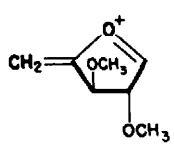
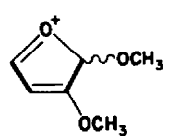
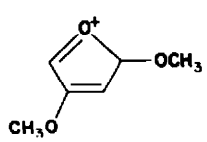
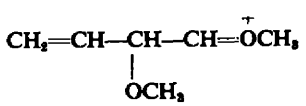
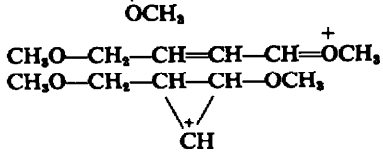
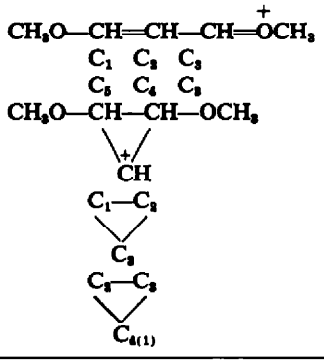
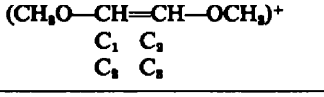
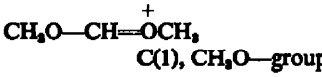
m/e	Structure	Designation	Contribution
175		A_1	100
161		E_1	100
146		C_1	100
143		A_2^1	77
		A_2^2	23
129		E_2^1	76
		E_2^2	24
115		C_2^1	28
		C_2^2 C_2^3	53 19

TABLE 11. (*Contd.*)

m/e	Structure	Designation	Contribution
101			
		F_1^1 (G_1^1)	2
		F_1^3 (G_1^3)	9
		G_1^4	7
		G_1^5	82
88			
		H_1^1	38
		H_1^2	62
75		J_1^1	75
	C(1), CH_3O—groups from C(1) and C(3)		

imagined (if we do not consider possible rearrangements): one containing the glycosidic methoxyl and the methoxyl at C(3) (F_1^1), and the other containing methoxyls at C(3) and C(5) (F_1^3). For ions G_1 except the two possibilities, mentioned for F_1 (G_1^1 and G_1^3) there exist two more: with methoxyls from C(1) and C(2) (G_1^4) and from C(2) and C(3) (G_1^5). Calculation reveals that 82% of the total intensity arises from G_1^5, 9% from G_1^3 (F_1^3), 7% from G_1^4 and 2% from G_1^1 (F_1^1). The mechanism of the formation of ions F_1 and G_1 is still obscure.

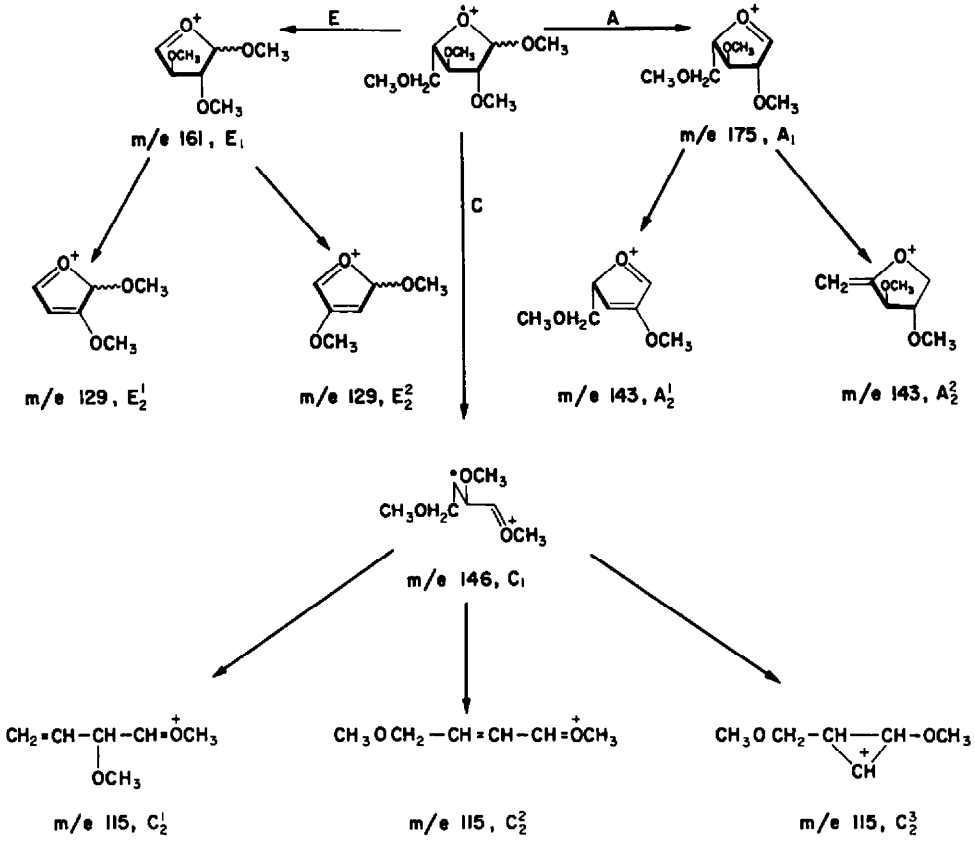
The second intense peak of the spectrum is situated at m/e 75. A considerable peak is situated in the mass spectrum of XVIIa at m/e 81, corresponding to incorporation of at least two methoxyls in the ion m/e 75. The remaining 13 mass units must correspond to a CH-grouping. As no one carbon atom in the starting molecule (XIV) bears more than one methoxyl, the only possibility is migration of a methoxyl. In the mass spectrum of XVII the intensity of peak at m/e 81 becomes lower, whereas that of peak at m/e 78 increases. The compound XVII was obtained from XVIIa by heating with 2% methanolic hydrogen chloride. Under these conditions only the glycosidic trideuteromethoxyl can be exchanged for methoxyl. Hence, the difference of intensities of peaks at m/e 78 and m/e 81 in the spectra of XVII and XVIIa is adequately accounted for by the assumption, that one of the methoxyls of ion m/e 75 is the glycosidic methoxyl. Calculation on the basis of Table 10 reveals that more than 75% of the intensity of the peak at m/e 75 is contributed by ion, including methoxyls of C(1) and C(3). Having elucidated the origin of ion m/e 75 the structure previously proposed was reconsidered.

Peak m/e 88 in the mass spectrum of XIV is of a relatively low intensity. Two ions contribute to it, one including C(1) and C(2) with their methoxyls (H_1^1) and the other containing C(2) and C(3) with their methoxyls (H_1^2). These contributions are 38% and 62%, respectively.

The data concerning structures and contributions of major ions of the mass spectrum of XIV are surveyed in Table 11.

On the basis of this evidence, Table 12 was composed. This contains calculated mass spectra of methyl 3-O-trideuteromethyl-2,5-di-O-methyl-α,β,L-arabofuranoside, methyl 2,5-di-O-trideuteromethyl-3-O-methyl-α,β,L-arabofuranoside, methyl 2,3-di-O-trideuteromethyl-5-O-methyl-α,β,L-arabofuranoside and methyl 2-O-methyl-3,5-di-O-trideuteromethyl-α,β,L-arabofuranoside, and experimental evidence obtained for XV and XVI. As in the case of pyranosides, it is seen, that all the deuteromethyl analogues of XIV have different spectra, and the data of Table 12 can be used for identification of partially methylated pentofuranoses.

The fragmentation pattern of XIV is shown below:



This pattern is essentially similar to that deduced for hexopyranosides: in both cases decomposition starts with cleavage of bonds in the β-position with respect to the oxygen atom of the cycle e.g., by fission of the glycosidic methoxyl (A), by fission of the side chain (E). by fission of the bond between C(1) and C(2) and by subsequent fission of the bond between C(4) and the cyclic oxygen (C). However, in spite of this similarity, the pathways of fragmentation of methylated hexopyranosides and pentofuranosides considerably differ. For example, XIV does not decompose by pathways B and D, as revealed by absence of peaks at m/e 132 and 105. On the other hand,

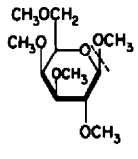
TABLE 12. MASS SPECTRA OF DEUTERATED ANALOGUES OF XIV

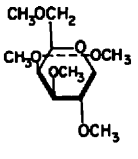
XIV	Position of CD_3O—groups					
	2	3	5	2,3	2,5	3,5
175	178	178	178	181	181	181
161	164	164	161	167	164	164
143	146	143 (77)	143 (23)	146 (77)	146 (23)	146
		146 (23)	146 (77)	149 (23)	149 (77)	
129	129 (25)	129 (75)	129	132	129 (25)	129 (75)
	132 (75)	132 (25)			132 (75)	132 (25)
115	115 (19)	115 (81)	115 (28)	118 (72)	118 (47)	118 (81)
	118 (81)	118 (19)	118 (72)	121 (28)	121 (53)	121 (19)
101	101 (11)	101 (7)	101 (91)	104 (18)	101 (11)	101 (7)
	104 (89)	104 (93)	104 (9)	107 (82)	104 (89)	104 (84)
						107 (9)
88	91	88 (37)	88	91 (37)	91	88 (37)
		91 (63)		94 (63)		91 (63)

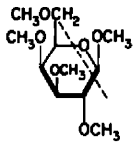
hexopyranosides do not afford ions of the types A_2^2, E_2^2 and C_2^1, characteristic of furanosides. The first step although occurring in a manner similar to that observed with pyranosides, the presence of a furanose cycle adds new specific features to subsequent fragmentation. The specifity of the furanoside fragmentation pattern can be visualized also in other cases, e.g., the difference in origin of ions with m/e 101. For all the pyranosides investigated, the major ion of this peak is of the type F_1, containing methoxyls of C(2) and C(4), whereas in the mass spectrum of arabofuranoside this peak is largely due to the ion, containing methoxyls of C(2) and C(3). Hence, the marked effect of the size of the cycle on the character of mass spectrum[6,11,14,15] appears still more apparent after detailed investigation. At the same time, however, it should be remembered that the principles of fragmentation of the two types of compounds have much in common; this conclusion has not been emphasized in previous publications cited.

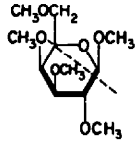
CONCLUSION

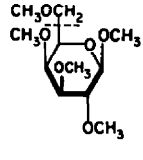
The data presented permits some general conclusions regarding the possibilities of mass spectrometry as a tool for structural analysis of carbohydrates. This first comparative investigation of fragmentation patterns of various types of methylated methyl glycosides demonstrates their close similarity, so that structural peculiarities and stereochemistry are of effect only upon the prelevance of one or other pathway. The major fragmentation pathways (A, B, C, D, E etc.) can be now outlined for pyranosides and furanosides. In all cases, the decomposition of molecules starts with fission of the bond, occupying the β-position with respect to the cyclic oxygen atom, followed sometimes by fission of the α-bonds:

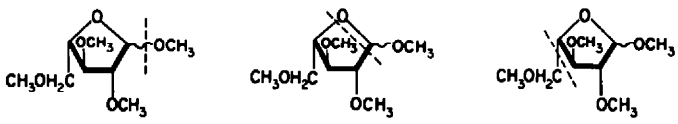

A


B


C


D,F


E



A C E

The prelevance of one or other fragmentation pathway depends on the structure of the starting monosaccharide. For example, pathways B and C are preferred in the case of arabopyranoside X; in case of arabofuranoside XIV the peaks of the E-series are very intense. The accumulation of additional experimental data concerning mass spectrometry of different sugar derivatives will probably lead to more definite conclusions.

At the same time, it appeared obvious, that monosaccharide derivatives of similar structure, e.g., the majority of methylated methyl-hexopyranosides have very similar mass spectral characteristics, so that the data obtained for one epimer can be used for the analysis of other stereoisomers. On the other hand, investigation of a variety of methylated monosaccharides indicated that the nature of substituents and the size of cycle considerably affect the character of spectra, which can be utilized for analysis of the corresponding derivatives, thus providing a new tool for carbohydrate chemistry

The above data presented demonstrates the new approach as valid for the identification of partially methylated monosaccharides—an important step in the structural analysis of polysaccharides. The above Tables (3, 6, 9 and 12) show, that the mass spectra of deuterated derivatives of each of the partially methylated monosaccharides have characteristic features, enabling differentiation between isomers. Moreover, as the mass spectra of the derivatives of closely related monosaccharides are practically identical (e.g., glucose and galactose), the data for one monosaccharide can be applied also to its stereoisomers. The Tables contain data concerning hexopyranoses, 6-deoxyhexopyranoses, pentopyranoses and pentofuranoses, i.e., all the most important neutral monosaccharides present in natural polysaccharides.

The novel approach to the structural analysis of partially methylated monosaccharides thus consists of the following stages. The mixture of partially methylated monosaccharides, in the form of methyl glycosides is subjected to separation by one of the method available. The substances obtained are treated with trideuteromethyl iodide; the constants of the permethyl derivatives obtained enable identification of the parent monosaccharide. Comparing its mass spectrum with the Tables of this paper, it is possible to determine the position of the trideuteromethyl groupings, i.e., of free hydroxyls in the starting partially methylated monosaccharide.

The approach proposed needs only readily available permethyl methyl-glycosides as authentic samples for comparison and only minor amounts of substance (a satisfactory mass spectrum can be obtained with an usual class instrument using ca. 1 mg sample), enabling standardization of partially methylated monosaccharides structural analysis. The advantages for the structural chemistry of carbohydrates are obvious.

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