IDENTIFICATION OF METHYL *O*-METHYL-D-XYLOFURANOSIDES BY MASS SPECTROMETRY

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(Received December 6th, 1971, accepted for publication, February 21st, 1972)

ABSTRACT

The fragmentation of methyl *O*-methyl-D-xylofuranosides under conditions of electron-impact mass spectrometry has been studied and compared with that of fully methylated methyl pentofuranosides Characteristic differences in fragmentation of the differently substituted methyl *O*-methyl-D-xylofuranosides have been found which permit unambiguous assignment of both the number and the location of the methyl groups Thus, compounds in this series can be identified without derivatization prior to the analysis The g l c–m s technique can be conveniently used to identify the products of methanolysis of methylated polysaccharides

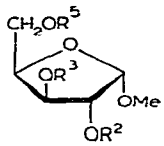
INTRODUCTION

Methanolysis of a fully methylated polysaccharide containing D-xylopyranose as a structural unit results in the formation of methyl D-xylofuranosides in addition to methyl *O*-methyl-D-xylopyranosides G l c [1–4] is valuable in studying such complicated mixtures of carbohydrate derivatives Many of the possible products of methanolysis of methylated polysaccharides are directly amenable to g l c and the identification of individual components is usually based on retention times Identification is more reliable if the mass spectra of the substances are also obtained by using combined g l c–m s [5,6] We now report on the mass spectrometry of all the theoretically possible methyl *O*-methyl-D-xylofuranosides without derivatization

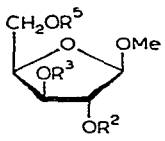
The fragmentation of fully methylated methyl pentofuranosides has been described[5,7,8] By using trideuteriomethyl derivatives, the fragmentation mechanisms of methyl per-*O*-methylpentofuranosides have been found to be different from those of the pyranose analogues[7–9] The presence of free hydroxyl groups in partially methylated hexopyranosides alters[10] the mode of fragmentation, both qualitatively and quantitatively. The characteristic fragmentation of partially methylated methyl *O*-methyl-D-xylofuranosides has now been investigated

EXPERIMENTAL

Methyl *O*-methyl-α-D-xylofuranosides (**1–7**) and methyl *O*-methyl-β-D-xylofuranosides (**9–15**) were prepared as described previously[11–13] Methyl α- and β-D-xylofuranosides (**8** and **16**) were made according to the method of Augestad and Berner[14]



1—8



9—16

	R²	R³	R⁵		R²	R³	R⁵
1,9	Me	Me	Me	**5a**	Me	D	D
2,10	H	Me	Me	**6,14**	H	Me	H
2a	D	Me	Me	**6a**	D	Me	D
3,11	Me	H	Me	**7,15**	H	H	Me
3a	Me	D	Me	**7a**	D	D	Me
4,12	Me	Me	H	**8,16**	H	H	H
4a	Me	Me	D	**8a**	D	D	D
5,13	Me	H	H				

The *O*-deuterated compounds **2a–8a** were prepared by evaporation of solutions of **2–8** in D_2O directly in the mass spectrometer The overall degrees of deuteration were **2**, 92%, **3**, 95%, **4**, 95%, **5**, 65%, **7**, 87%, **8**, 71% The mass spectra were obtained with a MCh 1306 mass spectrometer (U S S R) at an ionizing potential of 70 eV The inlet temperature was 20–30° and that in the ionizing chamber 110–120°

TABLE I

THE MASS-SPECTRAL DATA FOR THE METHYL *O*-METHYL-α-D-XYLOFURANOSIDES

m/e	**1** (*2,3,5*)[a]	**2** (*3,5*)	**3** (*2,5*)	**4** (*2 3*)	**5** (*2*)	**6** (*3*)	**7** (*5*)	**8**
175	0 084							
161	2 500	0 500	0 673	3 328				
147		2 555	0 734		2 147	2 590	0 587	
146	0 046						0 129	
143	0 941		1 751					
133							1 671	2 356
132		0 266	0 499	0 532				0 327
131	0 328							
129	0 673	0 500	0 547	0 732	0 897	0 153	1 223	
118					1 196	1 122	0 836	
117	0 237	0 532	0 273	0 233	0 224	0 388	0 597	0 369
115	0 857	1 370	0 379	0 166	0 299	1 165	0 477	0 959
111	0 336		0 450	0 133				
105							0 358	0 753
104								0 906
103							0 567	0 390
102	2 142	0 484		2 063				
101	37 634	6 094	2 056	34 875	1 121	1 908	0 328	0 516
100		0 582	2 410		2 018	1 765	0 686	
99	0 964			1 531		0 360		
89	0 964		1 409				1 164	
88	2 753	1 193	4 429	3 228	5 605	3 136	1 477	
87	0 551	29 262	45 111	3 527	37 369	26 984	5 073	3 403

TABLE I (*Continued*)

m/e	1 (2,3,5)[a]	2 (3,5)	3 (2,5)	4 (2,3)	5 (2)	6 (3)	7 (5)	8
86					1 232	0 729	2 984	2 908
85	1 285	1 290	1 727	1 098	1 868	2 110	1 980	1 802
75	12 392	9 956	2 033	11 381	1 570	8 800	2 874	4 899
74	1 147	2 426	2 909		2 691	3 031	4 377	3 814
73	3 366	3 079	1 752	3 561	3 587	2 652	28 348	25 498
71	2 371	5 369	4 439	3 460	4 933	6 162	2 686	2 002
69				3 128	1 794	1 846		0 801
68					1 495	1 750		1 897
61			1 255	1 123	2 466	2 738	3 680	6 111
60						0 324	2 089	3 340
59	1 071	2 942	3 345	2 296	4 036	4 057	3 084	2 360
58		1 298	1 046		1 570	2 925	3 581	2 360
57	0 765	2 596	3 450	1 730	4 709	5 103	8 057	11 506
55	1 071		1 324	1 530	5 830	1 655	1 142	2 455
45	24 325	22 249	12 934	19 634	10 762	12 373	18 352	14 414

[a]The figures in brackets refer to the location of the methoxyl groups

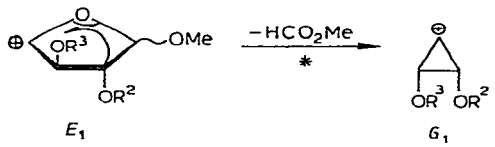
RESULTS AND DISCUSSION

The mass spectra of the methyl *O*-methyl-α-D-xylofuranosides are summarized in Table I To facilitate a comparison of individual peak intensities, the respective data are given as percentages of the total ionisation (% Σ_{45}) The mass spectra of the methyl *O*-methyl-β-D-xylofuranosides were generally very similar to those of the corresponding α-D anomers, the differences being within the range of the experimental error The only factor of difference was the presence of low-intensity peaks $(M-MeOH)^+$ at *m/e* 160, 146, or 132 in the spectra of the β-D anomers Thus, the anomeric configuration cannot be assigned solely on the basis of mass spectrometry data However, in view of the fact that pairs of α-and β- anomers of methyl *O*-methyl-D-xylofuranosides are readily separated by g l c [3,4], it is possible, without prior derivatization, to identify any of the theoretically possible methyl *O*-methyl-D-xylofuranosides by g l c –m s

The fragmentation of the methyl di-*O*-methyl-D-xylofuranosides **2–4** and **10–12** compares well with the scheme proposed by Kochetkov and Chizhov[7] for methyl 2,3,4-tri-*O*-methylpentofuranosides In Series A, the A_1 ions appear at *m/e* 161, which, after dehydration, give rise to the A_2 ions appearing at *m/e* 143 or, after elimination of a molecule of methanol, at *m/e* 129 In the case of **2**, **3**, **10**, and **11**, the E_1 ions appear at *m/e* 147 whereas, for **4** and **12**, these appear, together with the A_1 ions, at *m/e* 161 In the spectrum of the *O*-deuterated analogue **4a**, both types of ion are recognizable and appear at *m/e* 161 (E_1) and 162 (A_1) By subsequent elimination of water from positions 2 or 3, the E_2 ions at *m/e* 129 and 115 are produced The transitions $A_1 \rightarrow A_2$ and $E_1 \rightarrow E_2$ were proved by the presence of metastable peaks (Table II) The C_1 ions at *m/e* 132, formed by the elimination of $HCOOCH_3$ from the molecular

ions, disintegrate to give rise to the C_2 ions at *m/e* 115 and 101 (m*) after splitting off of the hydroxyl or methoxyl radical

A new mode of disintegration of the C_1 ions was observed in the case of **2, 3, 10,** and **11** which contain a free hydroxyl group at C-2 or C-3 (and also in the case of **5–8** and **13–16** having two or three hydroxyl groups) By the elimination of methanol from the C_1 ions, the ions at *m/e* 100 are formed It could be proved by deuteration analysis that there is an interaction of the methoxyl group with the hydrogen of the hydroxyl group The most prominent are the peaks of the ions G_1 (F_1) at *m/e* 101 and 87 which contain methoxyl or hydroxyl groups mainly at C-2 and C-3 Several hypotheses for the formation of these ions, although not proved, have been expressed previously[5 10] From the relevant metastable peaks, it is proved now that the G_1



E_1 G_1

3,4,9,12	$R^2 = OMe$	$R^3 = OMe$	*m/e* 161	*m/e* 101
2,6,10,14	$R^2 = H$	$R^3 = OMe$		
3,5,11,13	$R^2 = OMe$	$R^3 = H$	*m/e* 147	*m/e* 87
2a	$R^2 = D$	$R^3 = OMe$		
3a	$R^2 = OMe$	$R^3 = D$	*m/e* 148	*m/e* 88
7,8,15,16	$R^2 = H$	$R^3 = H$	*m/e* 133	*m/e* 73
7a,8a	$R^2 = D$	$R^3 = D$	*m/e* 135	*m/e* 75

ions are produced from the E_1 ions, and we assume that this is the main source of the G_1 ions The ions at *m/e* 87, and probably also those at *m/e* 73, partially disintegrate by splitting off of CH_2O (m*) The less-prominent peaks corresponding to the H_1 ions present in the spectra of furanosides appear at *m/e* 88 and 74 The J_1 ions at *m/e* 75 are weak in the case of methyl 2,5-di-*O*-methyl-D-xylofuranosides **3** and **11**; corresponding ions at *m/e* 61 easily disintegrate after elimination of methanol, giving[10] the ions at m/*e* 29

TABLE II

METASTABLE TRANSITIONS IN THE SPECTRA OF METHYL *O*-METHYL-D-XYLOFURANOSIDES

*m**	*Transition*	*Compound*[a]	*m**	*Transition*	*Compound*[a]
127 0	161→143	**3**	63 4	161→101	**12**
113 2	147→129	**7,11,13**	62 7	118→ 86	**5,15**
103 4	161→129	**9,12**	60 6	88→ 73	**1,12**
99 4	133→115	**7,15 16**	51 5	147→ 87	**6,11,13**
90 0	147→115	**6**	47 0	74→ 59	**6**
77 3	132→101	**12**	37 3	87→ 57	**3,6,11**
64 1	118→ 87	**6,13**			**12,13,15**

[a]Only those compounds showing stable transitions are listed

Compared to the methyl di-*O*-methyl-D-xylofuranosides, the fragmentation of methyl mono-*O*-methyl-D-xylofuranosides **5–7** and **13–15** yields ions that are 14 mass units smaller The A_1 ions are at *m/e* 147, and A_2 at *m/e* 129 or 115 The E_1 ions for **5**, **6**, **13**, and **14**, which have a free hydroxyl group at C-5, appear together with the A_1 ions, whereas in the case of **7** and **15** the E_1 ions appear at *m/e* 133. In the spectra of the *O*-deuterio-analogues **5a** and **6a**, the A_1 and E_1 ions again form a pair of peaks with *m/e* 149 and 148 The elimination of water or methanol from the E_1 ions gives rise to ions at *m/e* 129 and 115 which are isomeric with the A_2 ions The peaks corresponding to the C_1 and C_2 ions are at *m/e* 118 and 101 or 87, respectively The liberation of methanol from the C_1 ions produces ions at *m/e* 86 (m*), and the loss of water gives rise to the ions at *m/e* 100 The G_1 (F_1) ions for 2-*O*- and 3-*O*-methyl derivatives are at *m/e* 87, and at *m/e* 73 for the 5-*O*-methyl derivatives For the 2-*O*-methyl derivatives **5** and **13**, the series H_1 comprises the ions at *m/e* 74 and 88 (the latter increases the intensity of the peaks isotopic to those appearing at *m/e* 87) For the 3-*O*-methyl derivatives **6** and **14**, the A_1 ions appear at *m/e* 74, and for the 5-*O*-methyl derivatives **7** and **15** at *m/e* 74 and 60 The J_1 ions at *m/e* 75 are intense only in the

TABLE III

CHARACTERISTIC FEATURES[a] OF THE FRAGMENTATION PATTERNS OF METHYL *O*-METHYL-D-XYLOFURANOSIDES

m/e	**1** (*2,3,5*)	**2** (*3,5*)	**3** (*2,5*)	**4** (*2,3*)	**5** (*2*)	**6** (*3*)	**7** (*5*)	**8**
175								
161								
147								
146	·							
143			·					
132								
133								
129					·			
118					·			
115								
104								
101	× × ×	×		× × ×				·
100								
88[b]								
87		× × ×	× × ×	·	× × ×	× × ×		
86						·	· ·	· ·
75	× ×	× ×	·	× ×		×		
74								· · ·
73	· · ·		·			×	× × ×	× × ×
61			·	·	·			×
60								
45	× × ×	× × ×	× ×	× ×	× ×	× ×	× ×	× ×

[a]Peak intensities ·, <0 5%; · ·, 0 5–1%, · , 1–5%; ×, 5–10%, × ×, 10–20%, × × ×, 20–50%
[b]Values obtained by subtracting the intensities of the peaks isotopic to those at *m/e* 87 from those at *m/e* 88

spectra of the methyl 3-*O*-methyl-D-xylofuranosides **6** and **14**, only the peaks at *m/e* 87 and 45 are stronger in the spectra of these compounds

The mass spectra of the methyl D-xylofuranosides **8** and **16** contain peaks for the A_1+E_1 ions at *m/e* 133, A_2+E_2 at *m/e* 115, C_1 at *m/e* 104, and C_2 at *m/e* 87 The peaks $(C_1-H_2O)^+$ appear at *m/e* 86, G_1 at *m/e* 73, H_1 at *m/e* 74 and 60, and J_1 at *m/e* 61

It follows from the foregoing considerations, as well as from the data summarized in Table I, that there are characteristic differences in the fragmentation patterns of the differently substituted methyl *O*-methyl-D-xylofuranosides The differences are such that it is possible from the mass-spectrometric data alone not only to determine the degree of substitution but also to assign the location of the methyl groups As the anomeric configuration is given by the gas-chromatographic elution pattern[3,4], the g l c–m s technique provides a convenient means of unambigous identification of these compounds The characteristic features of the fragmentation of methyl *O*-methyl-D-xylofuranosides are presented in Table III

As can be seen from Table III, the degree of methylation follows from the values of the peaks A_1 (*m/e* 175, 161, 147, 133), E_1 (*m/e* 161, 147, 133), and C_1 (*m/e* 146, 132, 118, 104), whereas, from the *m/e* values and the relative intensities (% Σ_{45}) of the peaks G_1 (*m/e* 101, 87, 73) and J_1 (*m/e* 75, 61), the location of the individual methyl groups can be assigned

ACKNOWLEDGMENT

The authors are grateful to Mr S Markovič for skilful technical assistance

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