Mass spectrometry of uronic acid derivatives. X.* Identification of methyl (methyl *O*-methylhexopyranosid)uronates by mass spectrometry

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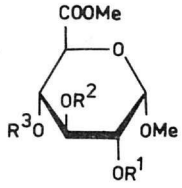
Based on mass spectral fragmentation of methyl (methyl *O*-methyl-α-D-gluco- and galactopyranosid)uronates a simple procedure is proposed for the determination of the number and location of methyl groups in methyl (methyl *O*-methylhexopyranosid)uronates, the products of methanolysis of methylated acidic polysaccharides and other uronic acid-containing substances. The advantage of the use of low-energy electrons (12 eV) for this purpose is demonstrated. The comparison of the 12 eV mass spectra of *gluco* and *galacto* derivatives provided data from which criteria were deduced enabling, except for the fully methylated derivatives, to distinguish between these two diastereoisomeric forms. The most significant factor for the stereochemical assignment is the presence or absence of the molecular, *m/e* 169 and *m/e* 155 ion peaks in the spectra.

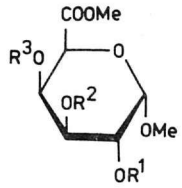
По изучении масс-спектрометрической фрагментации метил(метил-*O*-метил-α-D-глюко- и галактопиранозид)уронатов был предложен простой способ определения количества и положения метиловых групп в метил(метил-*O*-метил гексапиранозид)уронатах как продуктах метанолиза метилированных кислых полисахаридов и других, уроновые кислоты содержащих веществ. Продемонстрировано преимущество применения низких энергий (12 эВ) электронов при измерении масс-спектров. Сравнением 12 эВ масс-спектров *глюко-* и *галакто-*производных были найдены критерии для различения обоих диастереоизомеров в присутствии или отсутствии пиков молекулярных ионов и ионов с *m/e* 169 или 155, за исключением полностью метилированных соединений.

* For Part IX see Ref. [1].

Methyl (methyl *O*-methylhexopyranosid)uronates can be found among the products of methanolysis of methylated acidic polysaccharides and other uronic acid-containing substances. We have previously described methods for the identification of this class of substances after exhaustive trideuteriomethylation [2], trideuteriomethylation, and conversion of the fully methylated derivatives to the corresponding amides [3], or acetylation [1]. The disadvantage of these procedures lies in the fact that derivatization of partially methylated substances must be done prior to mass spectrometry. To eliminate the drawback encountered in the existing methods complete series of methyl (methyl *O*-methyl-α-D-gluco- and galactopyranosid)uronates (*I*—*XVI*) have been studied after impact with 70 and 12 eV electrons



I-*VIII*



IX-*XVI*

No.	R^1	R^2	R^3
I *IX*	H	H	H
II *X*	Me	H	H
III *XI*	H	Me	H
IV *XII*	H	H	Me

No.	R^1	R^2	R^3
V *XIII*	Me	Me	H
VI *XIV*	Me	H	Me
VII *XV*	H	Me	Me
VIII *XVI*	Me	Me	Me

Although this type of substances, owing to the presence of free hydroxyl groups, are less volatile than the fully methylated derivatives they can be separated by gas—liquid chromatography [4] and, therefore, are amenable to the identification by GC—MS technique.

Experimental

Compounds *I*—*XVI* were prepared as described [1, 2, 5—10]. Mass spectra (70 and 12 eV) were obtained with an MCh 1306 instrument modified for direct introduction of samples. The temperature in the site of evaporation was, according to the volatility of the substances, 25—45°C, and that of the ionizing chamber was 120—130°C. Exact mass-measurements were done with an MS 902 S instrument (resolution 20 000).

Results and discussion

A comparison of the 12 eV mass spectra with those obtained with 70 eV electrons showed that the low-energy spectra were much simpler and easier to interpret, since they contained mainly primary, structurally significant ion peaks. The 70 eV spectra, because of the presence of peaks of ions resulting from secondary and further processes, were more complex. The features characteristic of the fragmentation of methyl (methyl *O*-methyl-α-D-glucopyranosid)uronates (*I*—*VIII*) and methyl (methyl *O*-methyl-α-D-galactopyranosid)uronates (*IX*—*XVI*) deduced from the 12 eV spectra are presented in Tables 1 and 2, respectively. The peak intensities are expressed in per cent of the total ionization % Σ_{45}.

Table 1

Features characteristic of the fragmentation of methyl (methyl *O*-methyl-α-D-glucopyranosid)uronates

Ion	*m/e*	% Σ_{45}							
		–	2	3	4	2,3	2,4	3,4	2,3,4
A_1	233								.
	219					.	.	...	
	205		.	.	..				
	191	..							
A_2	201						..		...
	187		..		...	...		..	
	173	...		...					
B_1	176								.
D_1	163								...
E_1	205								.
	191					.	.	.	
	177		.	.	.				
	163	.							
$C_6H_{12}O_5$	164			...		...		...	
$C_5H_8O_4$	132	x	xx	x		xx			

Table 1 (Continued)

Ion	*m/e*	% Σ_{45}							
		—	2	3	4	2,3	2,4	3,4	2,3,4
$C_7H_{13}O_4$	161					x		x	
$C_6H_{11}O_4$	147		.	...	.				
$C_5H_9O_4$	133	...							
$C_5H_9O_3$	117			...	xx	..	x	x	
$C_4H_7O_3$	103	xx	...	...					
F_1	101					...	xxx	x	xxx
	87		xxx	...	xxx	...		...	
	73	xxx							
$C_3H_6O_3$	90	xxx	...		...				
H_1	88					x	...	...	x
	74		xx	xx	..	..	...	...	
	60	...			.				
J_1	75	..		xxx		xxx	..	xxx	x
	61	..			.				

Peak intensities: . < 0.5; 0.5 ≤ .. < 1.0; 1.0 ≤ ... < 5.0; 5.0 ≤ x < 10.0; 10.0 ≤ xx < 20.0; 20.0 ≤ xxx

Table 2

Features characteristic of the fragmentation of methyl (methyl *O*-methyl-α-D-galactopyranosid)uronates (12 eV)

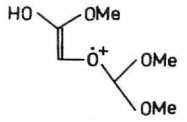
Ion	*m/e*	% Σ_{45} (12 eV)							
		–	2	3	4	2,3	2,4	3,4	2,3,4
M	264								
	250						.	.	
	236		.	.	..				
	222	.							
A_1	233								..
	219					.	..	..	
	205		...	...	...				
	191	...							

Table 2 (Continued)

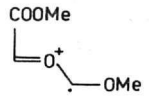
Ion	*m/e*	% Σ_{45} (12 eV)							
		–	2	3	4	2,3	2,4	3,4	2,3,4
A_2	201						.		..
	187		.		.	.		..	
	173	...		..					
A_3	169		..			.			
	155	...		..	..			.	
B_1	176								.
D_1	163								...
E_1	205								.
	191					.	.	.	
	177		.	.	.				
	163	.							
$C_6H_{12}O_5$	164			...		...		...	
$C_5H_8O_4$	132	...	...	...		x		..	
$C_7H_{13}O_4$	161					x		x	
$C_6H_{11}O_4$	147		...	...	.				
$C_5H_9O_4$	133	...							
$C_5H_{11}O_3$	117			...	xxx	...	x	xx	
$C_4H_7O_3$	103	xxx	x	...					
F_1	101					x	xxx	x	xxx
	87		xxx	x	xxx	..		x	
	73	xxx							
$C_3H_6O_3$	90	xxx	...		...				
H_1	88					xx	..	..	xx
	74		xxx	xx	...	..	...	...	
	60	...			...				
J_1	75	...		xxx	x	xxx	..	xxx	xx
	61	..			.				

The presence of free hydroxyl groups in *I—VII* and *IX—XV* alters drastically the mode of fragmentation compared with the fully methylated substances [2] *VIII* and *XVI*. In addition to ions A_1, A_2, E_1, F_1, and J_1 found also in the fragmentation

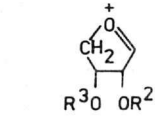
of per-*O*-methyl compounds [2] new fragments are formed and they are marked in Tables 1 and 2 by their elemental compositions. Of these only ions $[C_7H_{13}O_4]^+$ and $[C_6H_9O_4]^+$ have analogy with the fragmentation of partially methylated methyl glucopyranosides [11]. The mechanism of the formation of the fragments resulting from the new fragmentation processes formulated below has been elucidated by labelling experiments [12]



$[C_6H_{12}O_5]^{+\cdot}$ *m/e* 164

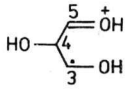


$[C_5H_8O_4]^{+\cdot}$ *m/e* 132



$[C_5H_9O_3]^+$ *m/e* 117*

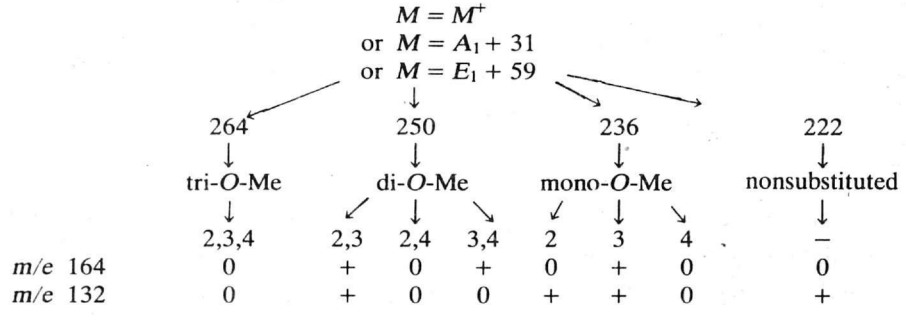
or $[C_4H_7O_3]^+$ *m/e* 103*



$[C_3H_6O_3]^{+\cdot}$ *m/e* 90

* Previously [12] the elemental composition of ions at *m/e* 103 and 117 was erroneously given as $C_5H_9O_3$ and $C_6H_{11}O_3$, respectively.

Based on the found features characteristic of the fragmentation of *I—XVI* a simple procedure is proposed permitting the determination of both the number and the location of methyl groups in partially methylated methyl (methylhexopyranosid)uronates without derivatization (Scheme 1)



Scheme 1

The molecular weight of the substance concerned is given by the *m/e* value of the molecular ion peak. When there is no molecular ion peak present in the spectrum the molecular weight is calculated from the *m/e* values of A_1 and E_1 ion peaks (the peaks having the highest *m/e* values) according to the equations in Scheme 1. The found molecular weight shows immediately degree of substitution and the location of the methyl groups can be simply assigned as follows: The spectra of 2,3-di-*O*-methyl derivatives contain peaks at *m/e* 164 and 132, those of 3,4-dimethyl ethers contain only the peak at *m/e* 164 and 2,4-di-*O*-methyl derivatives do not give rise to either of these two peaks. Of the spectra of mono-*O*-methyl derivatives only that of the 3-*O*-methyl compound contains the peak at *m/e* 164 and 132. The spectra of 2-methyl ether contain only the peak at *m/e* 132 and none of these two peaks is present in the spectra of the 4-methyl analogues. This procedure can be used for the determination of the number and the location of the methoxyl groups also from the 70 eV spectra of methyl (methyl *O*-methylhexopyranosid)uronates, in which case the slight peak at *m/e* 132 present in the spectrum of 3,4-di-*O*-methyl derivatives is neglected. These criteria hold for *gluco* compounds with no exception. An anomaly has been observed in the case of 3,4-di-*O*-methyl *galacto* substance the spectra of which (both 70 and 12 eV) contained the peak at *m/e* 132. For unambiguous distinction between 2,3-di-*O*-methyl and 3,4-di-*O*-methyl isomers the intensities of the peaks at *m/e* 164 and 132, or even better their ratios, should be taken into account (Table 3). The differences are again more pronounced when the data obtained from the 12 eV spectra are considered.

Table 3

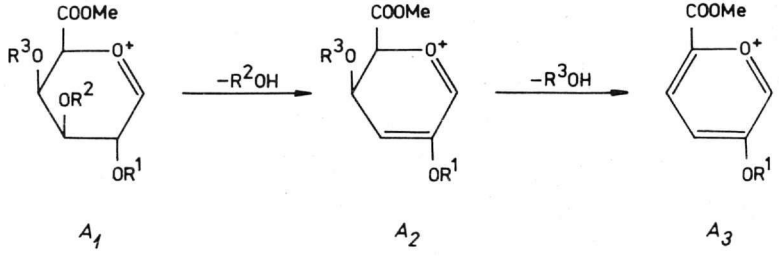
Intensities (% Σ_{45}) of ion peaks at *m/e* 164 and 132 in the mass spectra of methyl (methyl 2,3- and 3,4-di-*O*-methyl-α-D-galactopyranosid)uronates

m/e	70 eV		12 eV	
	2,3-di-*O*-Me	3,4-di-*O*-Me	2,3-di-*O*-Me	3,4-di-*O*-Me
164	0.59	0.59	1.48	2.89
132	2.3	0.31	5.35	0.97
164/132	0.3	1.9	0.3	3.0

During the present study of the fragmentation of C-4-epimeric methyl (methyl *O*-methylhexopyranosid)uronates significant differences (Tables 1 and 2) have been found which permit their distinction. With the exception of those of

2,4-di-*O*-methyl and 2,3,4-tri-*O*-methyl derivatives the 12 eV spectra of *galacto* isomers (Table 2) contain A_3 ions at m/e 169 or 155, not present in the spectra of their *gluco* counterparts (Table 1). The A_3 ions are given rise to [2] after the elimination of methanol or water from C-4 of the substances (Scheme 2).

Hence, based on the presence or absence of the A_3 ions in the 12 eV spectra glucuronic and galacturonic acid derivatives of the studied class can be distinguished from each other in the case of their methyl ester methyl glycosides and their 2-, 3-, 2,3-di-, and 3,4-dimethyl ethers. Still another significant fact follows



Scheme 2

from the 12 eV spectra of the two series of substances under investigation. The spectra of *galacto* isomers *IX—XVI* contain sufficiently intense (0.3—1.2% of the base peak) molecular ion peaks (Table 2). These, except for 2,3-di-*O*-methyl and 2,3,4-tri-*O*-methyl derivatives, are shown in Table 2. Of *gluco* isomers, the molecular ion peak was found only in the case of 4-*O*-methyl derivative, but in this case its intensity was only a fifth of that of the *galacto* analogue. Thus, the presence of molecular ion peak in the spectra of *galacto* derivatives, showing the higher stability of these isomers compared to *gluco* compounds, can also be used as a criterium for distinguishing between these two series of C-4 epimers. Aided by these two noticeable differences in the spectra of partially methylated derivatives of glucuronic and galacturonic acid the stereochemistry can be distinguished in all pairs of compounds except for the fully methylated compounds.

References

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