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IDENTIFICATION OF METHYL (METHYL *O*-ACETYL-*O*-METHYLHEXOPYRANOSID)URONATES BY MASS SPECTROMETRY*

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ABSTRACT

Fragmentation of methyl (methyl *O*-acetyl-*O*-methyl-α-D-gluco- and -galactopyranosid)uronates has been studied at 70 and 12 eV. At 12 eV, the production of ions resulting from secondary and further processes is greatly diminished and the spectra are simpler and easier to interpret. The energy required for the elimination of acetic acid and ketene has been calculated from the ion-appearance potentials. The number and location of methyl groups in methyl (methyl *O*-acetyl-*O*-methylhexopyranosid)uronates can be determined. The procedure is particularly suitable for g.l.c.–m.s. of the uronic acid portion of methanolysates of methylated biopolymers and other substances containing uronic acids. From the presence or absence of peaks for molecular ions in the 12-eV spectra, *gluco* and *galacto* isomers which do not contain a methoxyl group at C-4 can be distinguished. The synthesis of methyl (methyl 2,3-di-*O*-methyl-α-D-galactopyranosid)uronate is also described.

INTRODUCTION

Methyl (methyl *O*-methylhexopyranosid)uronates are formed on methanolysis of methylated biopolymers and other uronic acid-containing substances. Compounds of this class can be identified[1–3] by mass spectrometry after exhaustive trideuteriomethylation or after the conversion of the per-*O*-trideuteriomethyl compounds into the corresponding amides. Mass spectrometry can also be applied[4] in the structure determination of methyl derivatives of 4,5-unsaturated 4-deoxyhexopyranuronates resulting from a β-elimination side-reaction during methylation. Acetylation is cheaper and easier to effect than trideuteriomethylation and does not usually give by-products. Moreover, methyl (methyl *O*-acetyl-*O*-methylhexopyranosid)uronates possess good gas-chromatographic properties[5]. The use of g.l.c.–m.s. in the analyses of mixtures containing acetates of partially methylated uronic acids required studies

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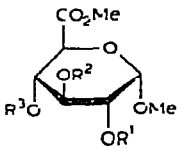
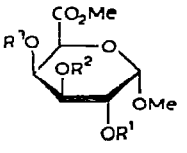
on model compounds We now report on the mass-spectral fragmentation of **1–14** and a simple procedure for identifying acetylated methyl (methyl *O*-methylhexopyranosid)uronates



1 - 7

	R^1	R^2	R^3
1 8	Ac	Ac	Ac
2 9	Me	Ac	Ac
3,10	Ac	Me	Ac
4,11	Ac	Ac	Me



8 - 14

	R^1	R^2	R^3
5 12	Me	Me	Ac
6 13	Me	Ac	Me
7 14	Ac	Me	Me

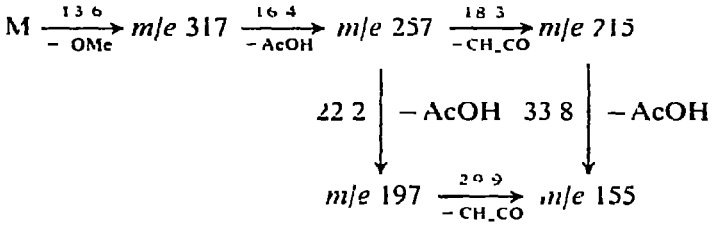
RESULTS AND DISCUSSION

The mass-spectral fragmentation of methyl *O*-acetyl-*O*-methylglycosides at 70 eV, as well as in the energy range (20–23 eV) used in g l c –m s , is a more complicated process[6,7] than that of other sugar derivatives Eliminations of acetic acid and ketene give rise to several types of ions resulting from secondary and further processes, as exemplified by the 70-eV spectrum of methyl (methyl 3-*O*-acetyl-2,4-di-*O*-methyl-α-D-galactopyranosid)uronate (**13**) shown in Table I Interpretation of such spectra is difficult and conditions to give simpler, more-informative spectra were therefore sought

We have previously recommended[1–4] that the mass spectra of methylated uronic acids be obtained at 12 eV In order to find the most suitable ionization-energy, the fragmentation curves of selected ions formed from **1**, **2**, and **5** were studied, two of which are given in Fig 1 The peaks at *m/e* 129 are those of the F_1 (Ac–O–CH=CH–CH=ÕMe) ions[2], and subsequent elimination of ketene gives rise to the ions at *m/e* 87 The curve shows that, below 20 eV, the production of the ions at *m/e* 129 increases with decreasing electron energy, whereas the production of the ions at *m/e* 87 decreases Thus, in order to obtain simpler, interpretable spectra of this class of substance, the use of an ionizing energy of 12 eV is further confirmed In Table I, the comparative 70- and 12-eV spectra of **13** are recorded

The use of low-energy electrons did not simplify the spectra of the acetates as much as those of the simple methyl ethers[1–4] In order to clarify this situation, the ionisation potential for methyl (methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosid)-uronate (**8**) and the appearance potential for the A_1 and further ions of this series formed by the elimination of acetic acid and ketene were measured The results are given in Table II, from which it followed that the individual processes of the A-series

consume the following amounts of energy (in kJ/mol)



The elimination of acetic acid and ketene occurring in the secondary and tertiary processes requires relatively small amounts of energy and, consequently, although the intensity of the relevant ions compared to those of the corresponding ions in the 70-eV spectra is greatly reduced, the ions resulting from non-primary processes have low appearance potentials and appear also in the 12-eV spectra

TABLE I

MASS SPECTRA OF METHYL (METHYL 3-*O* ACETYL 2,4-DI-*O*-METHYL-α-D-GALACTOPYRANOSID)URONATE **13**

m/e	*70 eV*		*12 eV*		m/e	*70 eV*		*12 eV*	
	% Σ	%	% Σ	%		% Σ	%	% Σ	%
345			0 10	0 4	99	1 15	6 9		
292	0 03	0 2	0 22	0 9	98	0 22	1 3		
261	0 38	2 3	0 61	2 5	89	0 82	4 9		
233	1 51	9 2	1 43	5 8	88	6 14	36 7	0 62	2 5
228	0 03	0 2	0 17	0 7	87	1 97	11 8	0 42	1 7
201	1 40	8 4	1 61	6 6	85	8 14	48 7		
200	0 33	2 0	1 37	5 6	75	2 52	15 1	22 50	91 1
187	0 30	1 8	0 11	0 5	74	2 36	14 1	3 59	14 5
173	4 50	26 9	5 81	23 5	73	1 73	10 3		
172	0 41	2 5	0 79	3 2	71	1 21	7 2		
169	0 49	3 0	0 33	1 3	69	0 49	3 0		
159	1 21	7 2	1 69	6 8	61	0 22	1 3		
158	0 47	2 8	0 48	2 0	59	1 81	10 8		
157	0 38	2 3	0 36	1 5	57	0 49	3 0		
155	0 26	1 5			55	0 80	4 8		
144	0 38	2 3	0 51	2 1	47	0 49	3 0		
141	5 62	33 7	3 18	13 2	45	3 48	20 8		
131	1 01	6 1							
130	3 29	19 7	4 53	18 3					
129	1 32	7 9	1 18	4 8					
127	0 80	4 8							
117	7 87	47 1	1 75	7 1	145	11 47	11 6		
116	13 68	81 8	24 70	100 0	103	4 52	4 6		
115	0 77	4 6			60	1 77	1 8	30 51	0 7
113	1 37	8 2			43	82 22	83 4	69 49	1 6
111	1 45	8 7							
101	16 73	100 0	21 92	88 7					

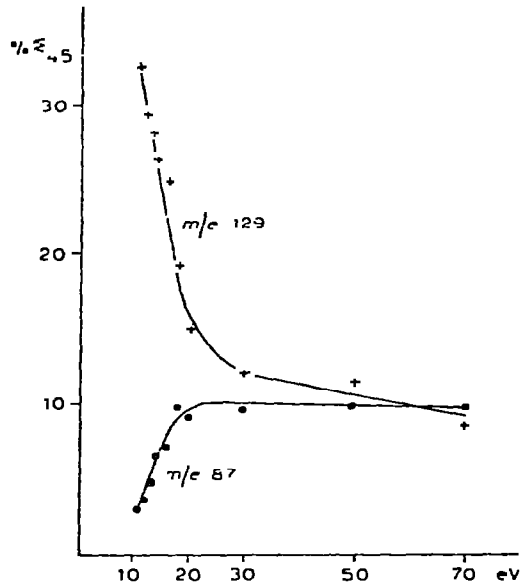


Fig 1 Fragmentation curves of the F_1 ion (AcO–CH=CH–CH=$\overset{+}{O}$Me) at *m/e* 129 and the ($F_1 - CH_2CO$) ion at *m/e* 87 for I

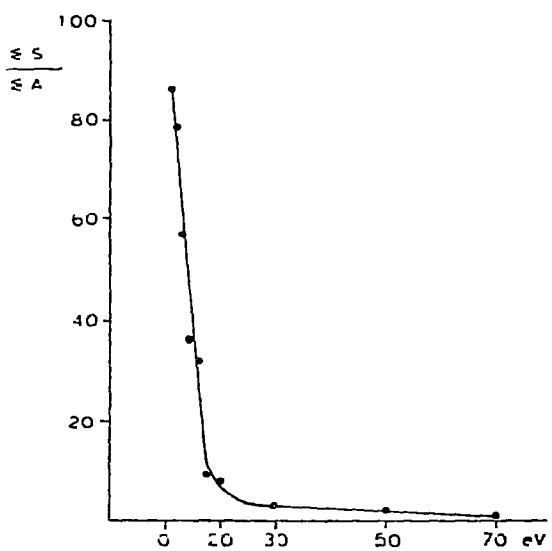


Fig 2 Effect of ionizing energy on the ratio of the summation of ions associated with the sugar skeleton (ΣS) and that of the ions formed from eliminated acetic acid (ΣA)

TABLE II

IONIZATION (I p) AND APPEARANCE POTENTIALS (A p) OF SELECTED IONS

Ions	m/e	*I p , A p (eV)*	*kJ/mol*
M	348	9 96	961 6
A_1	317	10 10	975 2
A_1-60	257	10 27	991 6
$A_1-60-42$	215	10 46	1000 9
$A_1-60-60$	197	10 50	1013 8
$A_1-60-42-60$	155	10 81	1043 7

Another advantage of low-energy mass spectra of acetates is shown by Fig 2, which is a plot of the ratio of the summation of the ions associated with the sugar skeleton (ΣS) and that of the "useless ions" originating from the elimination of acetic acid (ΣA) against the electron energy applied Whereas, for the triacetates, the ratio of intensities of useful and "useless" ions in the 70-eV spectrum is 2, this ratio is 16 in the 12-eV spectrum The convenience of the low-energy spectra is emphasised by the di-*O*- and mono-*O*-acetyl compounds, where these ratios increase from values of 2–4 and 6–8 to 60–80 and 80–100, respectively Hence, in taking the low-energy spectra, a larger sample pressure can be used without risking perturbations due to too high an ion current

The selected, characteristic peaks that allow the number and the location of methyl groups in methyl (methyl *O*-acetyl-*O*-methylhexopyranosid)uronates to be determined are given in Tables III and IV For ion series also given by methylated hexuronic acids[2], capital letters are used Of these, the A_2 ions are those o $[A_1 - AcOH]^+$ or, for derivatives bearing a methoxyl group at C-3, of $[A_1 - MeOH]^+$ A different series characteristic of the fragmentation of acetylated methyl glycosides[6] starts with the elimination of acetic acid, giving rise to weak signals of the $[M - AcOH]^+$ ion radicals From these ions, after retro-Diels-Alder fragmentation, methyl formate is liberated, producing the ions $[M - AcOH - HCOOMe]^+$ For the di- and tri-acetates, the given pathway is followed by the elimination of ketene

A comparison of the 12-eV spectra of the *gluco* and *galacto* derivatives **1–14** revealed differences which allowed some members of these two series of diastereoisomers to be distinguished, according to the presence or absence of peaks for molecular ions (0 2–1 6% of the base peak) appearing together with $[M+43]^+$ ion[6] peaks Whereas only those glucose derivatives having a methoxyl group at C-4 gave peaks for molecular ions, the corresponding peak, together with that of $[M+43]^+$ ions, is present in the 12-eV spectra of all methyl (methyl *O*-acetyl-*O*-methyl-α-D-galactopyranosid)uronates Thus, the presence of an M^+ peak in the 12-eV spectra of the 2,3,4-tri-*O*-acetyl (**8**), 3,4-di-*O*-acetyl-2-*O*-methyl (**9**), 2 4-di-*O*-acetyl-3-*O*-methyl (**10**), and 4-*O*-acetyl-2,3-di-*O*-methyl (**12**) derivatives of methyl (methyl α-D-galactopyranosid)uronate renders it possible to distinguish them from the corresponding *gluco* analogues

The data in Tables III and IV can be used to determine the number and the location of methyl groups in methyl (methyl *O*-acetyl-*O*-methylhexopyranosid)-uronates in the following manner (*1*) The 12-eV mass spectra of each *galacto* derivative, and of those *gluco* derivatives bearing a methoxyl group at C-4, contain a peak for the molecular ion For *gluco* derivatives that do not give peaks for molecular ions in the 12-eV spectra, or from the 70-eV spectra of all methyl (methyl *O*-acetyl-*O*-

TABLE III

CHARACTERISTIC FEATURES OF THE FRAGMENTATION OF METHYL (METHYL *O* ACETYL *O* METHYL-α D GLUCOPYRANOSID)URONATES

Ions	m/e	% Σ_{45}[a]						
		—	*2*[b]	*3*	*4*	*2,3*	*2,4*	*3,4*
M + 43	391							
	363							
	335							
M	348							
	320							
	292							
A_1	317							
	289							
	261							
A_2	257							
(A_1 − 60)	229							
	201							
A_3	257							
(A_1 − 32)	229							
E_1	289							
	261							
	233							
E_2	229	×						
(E_1 − 60)	201		×					
	173							
F_1	157	× ×						
	129		× × ×	×	× × ×	× × ×		× ×
	101						× × ×	
H_1	144	× ×						
	116		× ×				× ×	× ×
	88					× ×		
J_1	75			× × ×		× × ×	×	× × ×
M − 120	228							
	200		×					
	186							
M − 120 − 42	186	× ×						
	158		× × ×					

[a]Peak intensities 0 5> , 1> >0 5, 5> >1, 10> × > 5, 20> × × > 10, × × × > 20% [b]The numbers refer to the positions of methyl groups

methylhexopyranosid)uronates, the molecular weight can be calculated from the equations, $M = A_1 + 31$ and $M = E_1 + 59$. The magnitude of the molecular weight indicates the number of methyl groups present in the molecule (*2*). The location of the methyl groups in the di-*O*-methyl derivatives follows from the *m/e* values of the intense F_1 and H_1 ions. For the 2,3-di-, 3,4-di-, and 2,4-di-*O*-methyl derivatives, the key peaks appear at *m/e* 129 and 88, 129 and 116, and 101 and 116, respectively (*3*). Methyl (methyl *O*-methylhexopyranosid)uronates show the following characteristic features. The base peak in the spectra of the 2- and 4-*O*-methyl derivatives

TABLE IV

CHARACTERISTIC FEATURES OF THE FRAGMENTATION OF METHYL (METHYL *O*-ACETYL *O*-METHYL-2-D GALACTOPYRANOSID)URONATES

Ions	m/e	% Σ_4 [a]						
		—	*2*	*3*	*4*	*2,3*	*2,4*	*3,4*
M + 43	391							
	363							
	335							
M	348							
	320							
	292							
A_1	317							
	289							
	261							
A_2	257							
($A_1 - 60$)	229							
	201							
A_2	257							
($A_1 - 32$)	229							
E_1	289							
	261							
	233							
E_2	229	×						
($E_1 - 60$)	201							
	173						×	
F_1	157	×						
	129		× × ×		× × ×	× × ×		× × ×
	101						× × ×	
H_1	144	×						
	116		× ×		×		× ×	× ×
	88					×		
J_1	75			× × ×		× × ×	× ×	× × ×
M − 120	228							
	200							
M − 120 − 42	186	× ×						
	158		× ×					

[a] See footnotes to Table III

is that of the F_1 ions (*m/e* 129), and the base peak in the spectra of the 3-*O*-methyl derivatives is that of the J_1 ions (*m/e* 75), the latter derivatives are also characterized by the presence of $(A_1\text{-MeOH})^+$ ions The 2- and 4-*O*-methyl derivatives can be distinguished from each other according to the intensities of the $[M-120]^+$ and $[M-120-42]^+$ ions at *m/e* 200 and 158 which are strong for the 2-*O*-methyl compounds, but weak for the 4-*O*-methyl derivatives

These criteria hold for 12-, 20–23-, and 70-eV spectra

EXPERIMENTAL

Compounds **1–14** were prepared by conventional treatment of methyl (*O*-methyl-α-D-gluco- and- galacto-pyranosid)uronates[8–13] (2–5 mg) with acetic anhydride–pyridine Methyl (methyl 2,3-di-*O*-methyl-α-D-galactopyranosid)uronate was synthesised by methylation of methyl (methyl 2-*O*-methyl-4-*O*-*p*-nitrobenzoyl-α-D-galactopyranosid)uronate[13] (400 mg) with diazomethane–boron trifluoride etherate[13], to give methyl (methyl 2,3-di-*O*-methyl-4-*O*-*p*-nitrobenzoyl-α-D-galactopyranosid)uronate (350 mg, 84%), which was isolated by column chromatography as a syrup having $[\alpha]_D^{23}$ +112° (*c* 1, chloroform) (Found C, 50 89, H 5 41, N, 3 29 $C_{17}H_{21}NO_{10}$ calc C, 51 13, H, 5 30 N, 3 51%

The di-*O*-methyl derivative (250 mg) was de-*p*-nitrobenzoylated with aqueous sodium hydroxide[11], and the crude product was purified by chromatography to give syrupy methyl (methyl 2,3-di-*O*-methyl-α-D-galactopyranosid)uronate (133 mg, 85%), $[\alpha]_D^{23}$ +136° (*c* 1, chloroform)* (Found C, 48 08, H, 7 14, OMe, 49 92 $C_{10}H_{18}O_7$ calc C, 48 11, H, 7 25, OMe, 49 61%)

The 70-eV mass spectrum of the substance was qualitatively identical with that of the corresponding D-*gluco* analogue[1]

Mass spectra were obtained at 70 and 12 eV, and an emission of 500 μamp, using an MCh 1306 instrument The temperature at the site of evaporation was 40° and that of the ionizing chamber was 130° The peak intensities (Table I) are expressed as a percentage of the base peak and of total ionisation Σ_{45} The summation does not include the intensities of the peaks at *m/e* 60, 43, 103, and 145 formed by the elimination of acetic acid and ketene[6] The intensities of those peaks which do not contain[6] the carbon atoms of the saccharide skeleton are evaluated separately (Table I) From the spectra of **1**, **2**, and **5**, obtained at 70, 50, 30, 20, 18, 16, 15, 14, 13, 12, and 11 eV, fragmentation curves of the characteristic ions were constructed For the determination of the ionizing potential (i p) of **8** and the ion-appearance potential of selected ions, the semi-logarithmic plot method was used Benzene was employed as a reference substance (i p 9,245 eV) Ionization and appearance potentials were calculated from the initial, linear regions of the ionization efficiency curves, as a mean value of the potential difference in four points (0 05, 0 1, 0 5, and 1% of the maximum ion current at 20 eV)

*The only reference to the physical constants of this substance appears in Ref 14 It follows from the original work[15] that the erroneously cited[14] values (m p 69 $[\alpha]_D$ +172) refer to the tri-*O* methyl derivative

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