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MASS-SPECTRAL FRAGMENTATION OF METHYL 2,3,4-TRI-*O*-METHYL-α-D-GLUCOPYRANOSIDURONAMIDE AND ITS ANALYTICAL APPLICATION*

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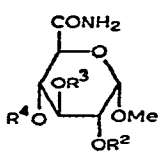
ABSTRACT

Fragmentation pathways for methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosiduronamide are proposed, based on 70- and 12-eV spectra of the compound specifically labelled with CD_3 and ND_2 groups. The presence of the NH_2 group in the molecule gives rise to new fragmentation series. The number and positions of CD_3 groups can be unequivocally determined from the mass spectra. Partially methylated derivatives of hexuronic acids, obtained by methylation analysis of hexuronic acid-containing substances, can be identified by exhaustive trideuteriomethylation and conversion into readily obtainable crystalline amides.

INTRODUCTION

Previously[1], we described the fragmentation patterns of methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate. The published spectra, together with the theoretical conclusions, allow the identification of some of the products of methanolysis of acidic polysaccharides and other hexuronic acid-containing, synthetic and natural products. Uronamides are usually easy to obtain, possess good crystallizing properties, and are often used for characterization and identification of uronic acid derivatives[2]. In order to determine the effect on fragmentation of replacing a methoxyl group by an amido group, we have studied the title compound **1**. For this purpose, analogs of **1** specifically labelled with trideuteriomethyl groups (**2**–**7**) were prepared



	R^2	R^3	R^4
1	Me	Me	Me
2	CD_3	Me	Me
3	Me	CD_3	Me
4	Me	Me	CD_3
5	CD_3	CD_3	Me
6	CD_3	Me	CD_3
7	Me	CD_3	CD_3

*Mass spectrometry of uronic acid derivatives: Part VII[1].

and their behaviour upon impact with electrons of 70 and 12 eV studied. The results are discussed in relation to the assignment of the number and the location of methyl groups in partially methylated hexuronic acid derivatives. The method of identification of a methyl hexosiduronic acid methyl ether involves small-scale per-*O*-trideuteriomethylation, conversion of the fully methylated derivative into an amide, and mass spectrometry.

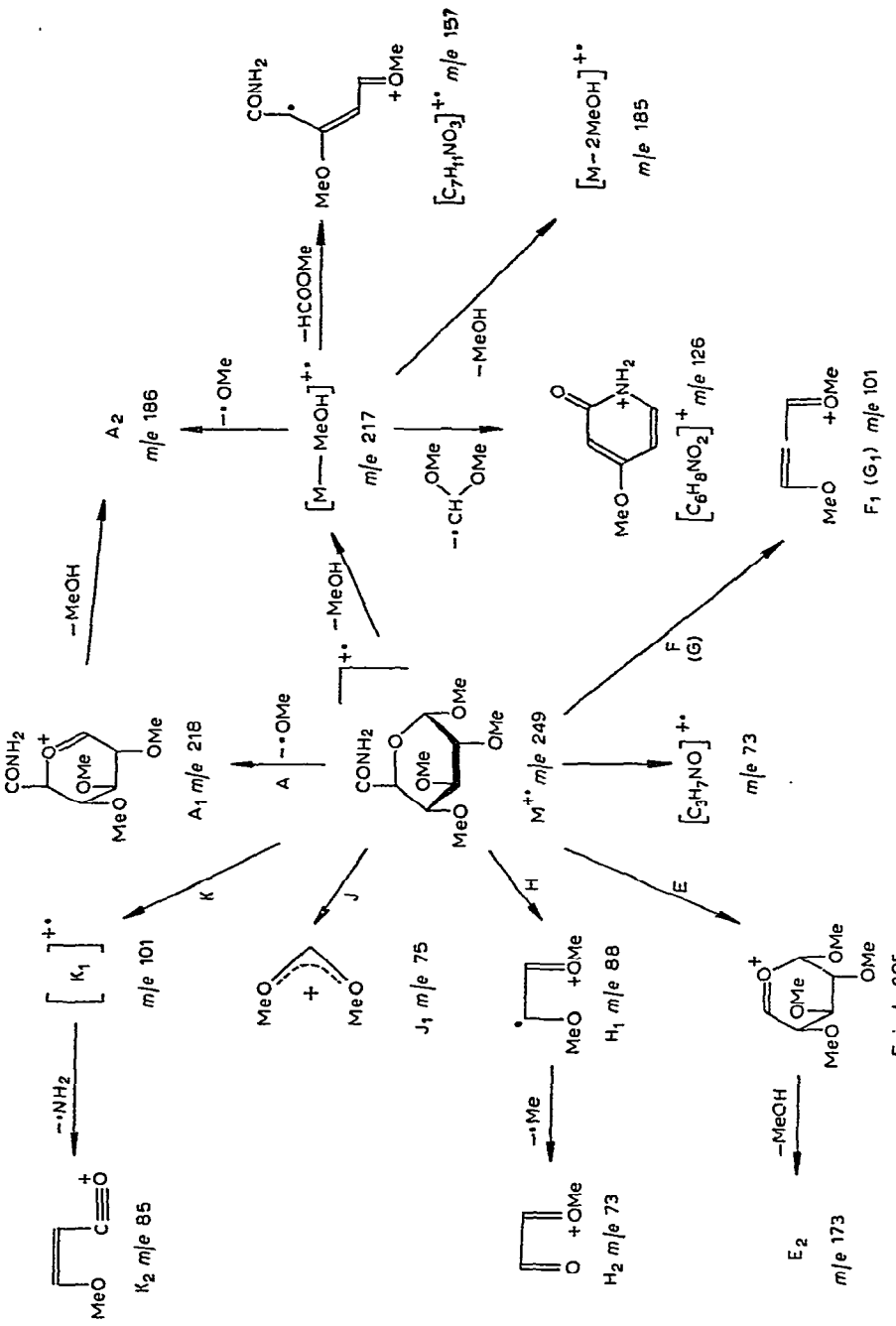
RESULTS AND DISCUSSION

There are only a few points in which the fragmentation of methyl (methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosid)uronate[1] differs qualitatively from that of methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside[3] and other permethylated monosaccharides[4–6]. The most probable centre of ionization is the hemiacetal ring oxygen. This is in agreement with the lower ionization potential (i.p.) of ethers compared with that of methyl esters[7]. The lower electronegativity of nitrogen compared with that of oxygen is responsible for the lower i.p. of amides[7] compared with that of esters. The i.p. of amides is close[7] to that of ethers, two, statistically most-probable, centres of ionization in **1**, and, thus, the occurrence of new fragmentation series can be assumed.

The fragmentation of **1** is only partly governed by the rules developed for methylated methyl glycosides of saccharides. For the fragmentation series common to both types of compound, the symbols introduced by Kochetkov[3] will be used throughout this paper. New fragmentation series will be characterized by the elemental composition of ions or by [M−R], where R is the leaving particle. Of the known series[3–6], A, E, F (G), H, J, and K are also formed from **1**–**7**, but the series B, C, and D do not occur. A complete fragmentation pattern of **1** is shown in Scheme 1.

The spectra of **1**–**7** contain weak peaks for the molecular ions. In addition to series A, started by loss of the glycosidic methoxyl group, the competitive elimination of a molecule of methanol from the molecular ions is also characteristic of the fragmentation of the uronamides under investigation. The ions $[M-MeOH]^{+\cdot}$ are formed neither in the fragmentation of fully methylated glucopyranosyluronic acid[1], nor in that of neutral saccharides[3–6]. These ions occur, however, in the fragmentation of methylated 5-thiopyranosides and 4-thiofuranosides[8], where the centre of ionization is the less-electronegative sulfur atom. Table I shows the *m/e* values, the intensities of A_1 and $[M-MeOH]^{+\cdot}$ ions, and the disintegration products thereof, expressed as a percentage of the total ionization Σ_{45} as found in the spectra of **1**–**7**.

Owing to the formation of A_1 and $[M-MeOH]^{+\cdot}$ ions, the ions A_2 are composed of more isomers than in the fragmentation of permethylated esters[1] or neutral saccharides[3–6]. This is attributed to the fact that the ions A_2 may be formed after loss of an ·OMe radical also from the ions $[M-MeOH]^{+\cdot}$. Thus, five isomeric ions, the relative proportions of which could be calculated from the 70 eV-data (Table I), are formed (Scheme 1). The calculations showed that, at 70 eV, 83, 13, and 2% of the ions $[M-MeOH]^{+\cdot}$ do not contain the methoxyl group at C-3, C-2, and C-4, respectively. The respective values for C-3 and C-2 at 12 eV are 85 and 15%. The



Scheme 1. Fragmentation pattern of methyl 2,3,4-tri-*O*-methyl-α-D-glucopyranosiduronamide.

TABLE I

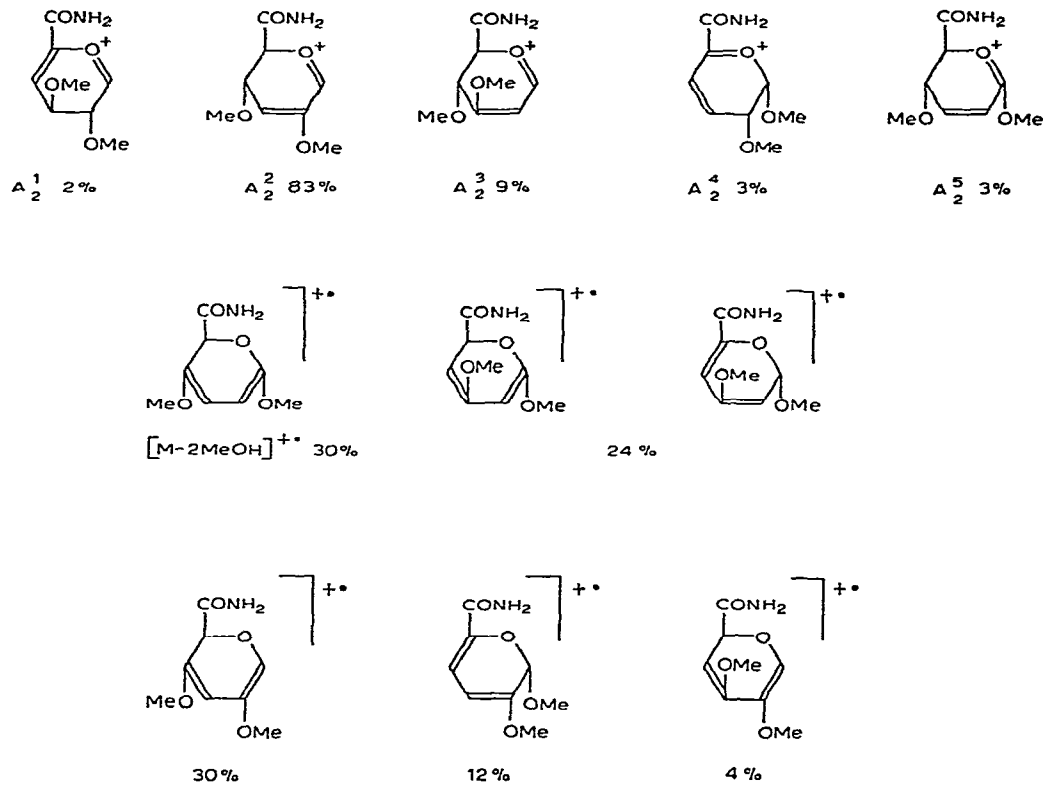
INTENSITIES OF THE FRAGMENT IONS OF THE A AND M−MeOH SERIES

Electron energy (eV)	*Ion series*	m/e	% $\Sigma_{45} \times 100$						
			—	*2*[a]	*3*	*4*	*2,3*	*2,4*	*3,4*
70	A_1	224					6	16	5
		221		16	6	18			
		218	15						
	$[M-MeOH]^{+\cdot}$	223					1	36	5
		220		30	6	45	47	9	23
		217	38	6	38	1			
	A_2	192					4	84	7
		189		95	11	100	127	23	83
		186	103	16	104	10	4		3
	$[M-2MeOH]^{+\cdot}$	191					1		
		188		10	6	18	15	14	16
		185	18	15	16	8	9	5	2
12	A_1	224					32	47	20
		221		93	44	31			
		218	24						
	$[M-MeOH]^{+\cdot}$	223						210	32
		220		202		281	273	47	193
		217	113	32	131				
	A_2	192						58	20
		189		140		94	129	23	107
		186	145	47	87	31	16		
	$[M-2MeOH]^{+\cdot}$	191						23	
		188				94	64	47	97
		185	64	93	87	31	64		

[a]The numbers refer to the positions of CD_3 groups.

ions $[M-MeOH]^{+\cdot}$ consist of six isomers, the relative amounts of which are shown in Scheme 1. Since the intensity of $[M-MeOH]^{+\cdot}$ ions is markedly decreased, the least-intense peaks could not be measured and, consequently, the relative proportion of the isomers formed at 12 eV could not be calculated exactly.

It can be seen from the relative amounts of the ions $[M-MeOH]^{+\cdot}$ and $[M-2MeOH]^{+\cdot}$ (Scheme 2) that MeO-3 is most easily eliminated as methanol, resulting in the formation of a 3,4-double bond. The ions $[M-MeOH]^{+\cdot}$ thus produced disintegrate by means of retro-Diels–Adler fragmentation to give the ions $[M-MeOH-HCOOMe]^{+\cdot}$ at *m/e* 157. These ions, *i.e.*, $[C_7H_{11}NO_3]^{+\cdot}$, contain an NH_2 group (on deuteration, they are shifted by 2 units to *m/e* 159) and MeO-2 and MeO-4 (Table II). Other ions, possibly also formed from $[M-MeOH]^{+\cdot}$ ions, are



Scheme 2. Structures and contributions of isomeric A_2 and $[M-2MeOH]^{+\cdot}$ ions.

TABLE II

INTENSITIES OF THE $[C_7H_{11}NO_3]^{+\cdot}$ AND $[C_6H_8NO_2]^+$ FRAGMENT IONS

Eelectron energy (eV)	*Ions*	m/e	% $\Sigma_{45} \times 100$						
			—	*2*[a]	*3*	*4*	*2,3*	*2,4*	*3,4*
70	$[C_7H_{11}NO_3]^{+\cdot}$	163						94	
		160		*97*		*73*	*129*		*65*
		157	69		76				
	$[C_6H_8NO_2]^+$	129				143		162	162
		126	163	171	167		172		
12	$[C_7H_{11}NO_3]^{+\cdot}$	163						419	
		160		374		437	514		373
		157	250		306				
	$[C_6H_8NO_2]^+$	129				344		303	279
		126	330	280	350		370		

[a]The numbers refer to the positions of CD_3 groups.

those at *m/e* 126, *i.e.*, $[C_6H_8NO_2]^+$. These contain an NH_2 group and MeO-4 (Table II). The ions $[C_7H_{11}NO_3]^{+\cdot}$ and $[C_6H_8NO_2]^+$ are formed at low energies (they appear also in 12-eV spectra).

The intensities and *m/e* values of $F_1(G_1)$ ions are given in Table III. The peak of the ions $[C_5H_9O_2]^+$ was found by high-resolution measurements to be a singlet; a peak for the ions K_1, *i.e.*, $[C_4H_7NO_2]^{+\cdot}$, was not present. From the data in Table III, the relative contributions of the individual F and G isomers were calculated (Table IV).

TABLE III

INTENSITIES OF THE FRAGMENT IONS OF THE F(G) SERIES

Electron energy (eV)	*Ion series*	m/e	% $E_{45} \times 100$						
			—	*2*[a]	*3*	*4*	*2,3*	*2,4*	*3,4*
70	$F_1(G_1)$	107					253	2068	58
		104		2332	476	2060	2113	476	2544
		101	2626	303	2312	507	127	302	127
12	$F_1(G_1)$	107					164	6147	64
		104		6355	131	6281	6125	536	6631
		101	6039	280	6698	313		210	86

[a]The numbers refer to the positions of CD_3 groups.

TABLE IV

STRUCTURES AND RELATIVE CONTRIBUTIONS OF THE F AND G ISOMERIC IONS.

m/e	*Structure*	*Symbol*	*Contribution (%)*	
			70 eV	*12 eV*
101	MeO–CH=CH–CH=$\overset{+}{O}$Me			
	C(1)–C(2)–C(3)	F_1^1	10	3
	C(2)–C(3)–C(4)	F_1^2	74	93
	MeO–CH–CH–OMe \ / CH +			
	C(1)–C(2) \ / C(3)	G_1^1	4	1
	C(2)–C(3) \ / C(4 or 1)	G_1^2	10	2
	C(3)–C(4) \ / C(2 or 5)	G_1^3	2	1

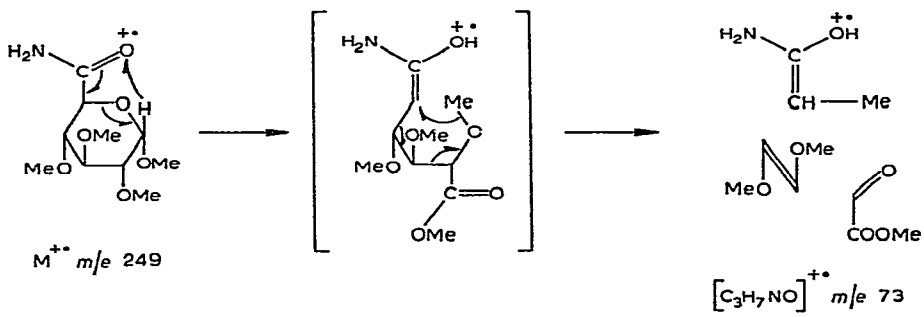
TABLE V

INTENSITIES OF THE FRAGMENT IONS OF THE H AND K SERIES

Electron energy (eV)	*Ion series*	m/e	% $\Sigma_{45} \times 100$						
			—	*2*[a]	*3*	*4*	*2,3*	*2,4*	*3,4*
70	H_1	94					1546		387
	($+K_2$)	91		1960	1927	482	1018	2440	1606
		88	2513	522	691	2348		305	803
	$H_2+[C_3H_7NO]^{+\cdot}$	76		341	211	74	614	389	276
		73	701	382	486	634	167	292	424
	K_2	85	356	285	309		288		
12	H_1	94					514		129
		91		654	613	94	241	698	515
		88	1208	140	66	469			64
	$[C_3H_7NO]^{+\cdot}$	76		140			177	163	
		73	209		131	156			118

[a]The numbers refer to the positions of CD_3 groups.

By conjugated transfer of electrons along the pyranoid ring, the ions of the H and K species are formed, and the intensities are given in Table V. Although the peak of the K_1 ions was not detected by high-resolution measurements, the K_2 ions, *i.e.*, $[C_4H_5O_2]^+$, at *m/e* 85 are strong in the 70-eV spectra. High-resolution measurements showed that, at 70 eV, only 80% of the ions appearing as the peak at *m/e* 73 belong to the H_2 (*i.e.*, $[C_3H_5O_2]^+$) ions and that the rest of the peak intensity belongs to the $[C_3H_7NO]^{+\cdot}$ ions. This can be seen also in the 12-eV spectra, from which it is clear that $[C_3H_7NO]^{+\cdot}$ ions contain the methyl group from C-2. Ions $[C_3H_7NO]^{+\cdot}$ formed from **2**, **5**, and **6**, at 70 eV, contribute to the intensity of H_2 ions at *m/e* 76, and, for the other compounds, to those at *m/e* 73. It can be seen from the spectrum of the ND_2 analog of **1** that the ions $[C_3H_7NO]^{+\cdot}$ contain both hydrogens of the amido group and,



Scheme 3. Mechanism of the formation of $[C_3H_7NO]^{+\cdot}$ ions.

therefore, for the formation of these ions, a two-step mechanism, consisting of a McLafferty rearrangement of the anomeric hydrogen followed by rearrangement of the methyl radical of MeO-2, is proposed (Scheme 3). The first step of this mechanism is supported also by the fact that, in the fragmentation of long-chain aliphatic acid amides (having more than three carbon atoms in sequence), the main phenomenon

TABLE VI

STRUCTURES AND RELATIVE CONTRIBUTIONS OF THE H AND K ISOMERIC IONS

m/e	*Structure*	*Symbol*	*Contribution (%)*	
			70 eV	*12 eV*
88	$MeO-\dot{C}H-CH=\dot{O}Me$			
	C(1)–C(2)	H_1^1	22	9
	C(2)–C(3)	H_1^2	60	74
	C(3)–C(4)	H_1^3	18	17
85	$MeO-CH=CH-C\equiv\overset{+}{O}$			
	C(4)–C(5)	K_2	100	—
73	$Me\overset{+}{O}=CH-CH=O$			
	C(1)	H_2^1	11	—
	C(2)	H_2^2	41	—
	C(3)	H_2^3	39	—
	C(4)	H_2^4	9	—

TABLE VII

INTENSITIES OF THE J_1 AND $CH_2=\overset{+}{O}Me$ FRAGMENT IONS

Electron energy (eV)	*Ion series*	m/e	% $\Sigma_{45} \times 100$						
			—	*2*[a]	*3*	*4*	*2,3*	*2,4*	*3,4*
70	J_1	81					45	115	40
		78		180	416	218	545	177	585
		75	753	638	259	486	116	522	57
	$CH_2=\overset{+}{O}Me$	48		206	199	132	530	282	369
		45	761	437	495	700	271	302	436
12	J_1	81						70	10
		78		93	525	125	756	93	644
		75	966	841	174	563	80	605	43

[a]The numbers refer to the positions of CD_3 groups.

is the McLafferty rearrangement, which takes place[9] even more easily at 12 than at 70 eV. Taking into account the addition of the intensity caused by overlapping of different ion species, the relative contribution of individual isomeric ions of the H and K series (Table VI) was calculated from the data in Table V. The intensities of the J ions, *m/e* values, and structurally similar ions $CH_2{=}\overset{+}{O}Me$ are summarized in Table VII. The methoxyl groups present in the molecule, in all possible combinations, contribute to the formation of the ions of the J series. The statistical distribution of the isomers, calculated from the data in Table VII, is listed in Table VIII.

TABLE VIII

STRUCTURES AND RELATIVE CONTRIBUTIONS OF THE J_1 AND $CH_2{=}\overset{+}{O}Me$ ISOMERIC IONS

m/e	*Structure*	*Symbol*	*Contribution* (%)	
			70 eV	*12 eV*
75	MeO–CH=OMe			
	1 2	J_1^1	7	4
	1 3	J_1^2	57	79
	1 4	J_1^3	16	8
	2 3	J_1^4	5	—
	2 4	J_1^5	12	8
	3 4	J_1^6	5	1
45	$CH_2{=}\overset{+}{O}Me$			
	1		23	—
	2		32	—
	3		29	—
	4		16	—

TABLE IX

SIGNIFICANT PEAKS (*m/e*) IN THE SPECTRA OF METHYL *O*-METHYL-*O*-TRIDEUTERIOMETHYL-HEXOPYRANOSIDURONAMIDES

Ion series	m/e							
	—	*2*[a]	*3*	*4*	*2,3*	*2,4*	*3,4*	*2,3,4*
$[C_7H_{11}NO_3]^{+\cdot}$	157	160	157	160	160	163	160	163
$[C_6H_8NO_2]^+$	126	129	126	126	129	129	126	129
$F_1(G_1)^b$	101	104	101	104	104	107	104	107
$H_1{}^b$	88	91	91	88	94	91	91	94
$J_1{}^b$	75	75	78	75	78	75	78	78

[a]The numbers refer to the positions of CD_3 groups. [b]The given peaks of $F_1(G_1)$, H_1, and J_1 are by far the most-intense of the triads at *m/e* 101, 104, 107; 88, 91, 94, or the pair at *m/e* 75 and 78 of peaks which may be present in the spectra.

The resulting, theoretical conclusions find application in the assignment of the number and the position of CD_3 groups in amides of permethylated *O*-methyl-*O*-trideuteriomethyl derivatives of hexuronic acids, which is done simply by comparing the observed *m/e* values of the significant peaks with the data in Table IX. It consequently permits, after exhaustive trideuteriomethylation and conversion of the fully substituted uronate into the crystalline amide, the assignment of the location of free hydroxyl groups originally present in a molecule of partially methylated methyl (methyl hexopyranosid)uronates found among the products of methylation analysis of hexuronic acid-containing substances.

EXPERIMENTAL

Compounds **1–7** were prepared by ammonolysis[2] of methyl (methyl *O*-methyl-*O*-trideuteriomethyl-α-D-glucopyranosid)uronates[1]. Mass spectra were obtained at 70 and 12 eV, using a MCh 1306 spectrometer (U.S.S.R.). The site of evaporation was kept at room temperature, and the temperature in the ionizing chamber was 90°. Evaporation (thrice) of the solution of **1** in D_2O (99.7% of D_2O) or CH_3OD (99% of CH_3OD) gave the ND_2 analog. Although the degree of deuteration (55%) was independent of the solvent used, the shifts of the *m/e* values of the ND_2-containing peaks were sufficient to indicate the presence of ND_2 groups in the ions. Exact-mass measurements were done with an MS-902S spectrometer (resolving power, 20,000).

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REFERENCES

1 V. Kováčik and P. Kováč, *Org. Mass Spectrom.*, 9 (1974) 172.
2 P. Kováč, *Carbohyd. Res.*, 31 (1973) 377.
3 N. K. Kochetkov, N. S. Wulfson, O. S. Chizhov, and B. M. Zolotarev, *Tetrahedron*, 19 (1963) 2209.
4 N. K. Kochetkov and O. S. Chizhov, *Tetrahedron*, 21 (1965) 2029.
5 K. Heyns and D. Müller, *Tetrahedron*, 21 (1965) 55.
6 K. Heyns and H. Scharmann, *Tetrahedron*, 21 (1965) 507.
7 P. Kiser, *Introduction to Mass Spectrometry and Its Applications*, Prentice–Hall, New York, 1965, p. 308.
8 V. Kováčik, P. Kováč, and R. L. Whistler, *Carbohyd. Res.*, 31 (1973) 377.
9 H. Budzikiewicz, C. Djerassi, and D. H. Williams, *Mass Spectrometry of Organic Compounds*, Holden–Day, San Francisco, 1967, p. 336.