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Determination of Linkage Position in Per-*O*-methylated Xylooligosaccharides by MIKE and CID Mass Spectra of Ammonia and Methylamine Cluster Ions

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The linkage types 1→2, 1→3, and 1→4 of D-xylobioses can be distinguished based on the unimolecular decomposition spectra (MIKE) of the cluster $[M + NH_4]^+$ ions or by collision-induced dissociation (CID) mass spectra of $[M + CH_3NH_3]^+$ ions of per-*O*-methylated compounds. The MIKE spectra of $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ adducts allow the determination of linkage positions between the xylose residues in per-*O*-methylated D-xylotrioses. The branching point of the branched trisaccharides can be deduced from these mass spectra, too.

D-Xylans and (4-*O*-methyl-D-glucurono)xylans are components of industrially important plants [1]. With the aim to use mass spectrometric technique for structure elucidation of oligosaccharides related to D-xylans, we have studied per-*O*-methylated oligosaccharides [2, 3]. As a result, the sequential analysis of oligosaccharides by electron impact (EI) mass spectrometry has been described [3]. In continuation of our studies directed to the mass spectrometric analysis of xylooligosaccharides we have examined the gas-phase reactions of per-*O*-methylated D-xylotriose with several protonated reagents by chemical ionization (CI) mass spectrometry [4]. Under these conditions the ammonium ions produced from ammonia and amines yield abundant cluster ions with per-*O*-methylated oligosaccharides. Nowadays, CID and fast atom bombardment tandem mass spectrometry (FAB MS/MS) have been used successfully at the study of per-*O*-methylated oligosaccharides [5—7]. Common fragmentation process in low energy CID FAB MS/MS cleaves the internal glycosidic bonds to possess oxonium ions [5]. The high energy CID FAB MS/MS has been used for the study of alkali metal cationized and per-*O*-methylated higher oligosaccharides by *Fournet et al.* [6, 7]. As a result, many linkage positions in one compound may be determined by the presence or absence of specific fragment ions that arise from the cleavage of two ring bonds. Here we discuss the results of an investigation by CI of per-*O*-methylated D-xylan type oligosaccharides *I—VIII,* using ammonia and methylamine as a reaction gas. MIKE and CID mass spectrometry were used to study the fragmentation of these cluster ions.

Compounds investigated:

I Methyl per-*O*-methyl-(*O*-β-D-xylopyranosyl-(1→2)-β-D-xylopyranoside)
(Symbol a→2b)

II Methyl per-*O*-methyl-(*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranoside)
(Symbol a→3b)

III Methyl per-*O*-methyl-(*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside)
(Symbol a→4b)

IV Methyl per-*O*-methyl-(*O*-β-D-xylopyranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside)
(Symbol a→2b→4c)

V Methyl per-*O*-methyl-(*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(2←1)-β-D-xylopyranoside)
(Symbol a→4b2←c)

VI Methyl per-*O*-methyl-(*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside)
(Symbol a→3b→4c)

VII Methyl per-*O*-methyl-(*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(3←1)-β-D-xylopyranoside)
(Symbol a→4b3←c)

VIII Methyl per-*O*-methyl-(*O*-β-D-xylopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside)
(Symbol a→4b→4c)

EXPERIMENTAL

The synthesis of methyl glycosides of xylooligosaccharides under study has been described previously [8—12]. The methyl glycosides were per-*O*-methylated by the *Hakomori* method [13] to give chromatographically pure (TLC, GC) compounds *I—VIII*. The CI mass spectra were obtained with VG ZAB-2F mass spectrometer, using the direct inlet system, 70 eV energy, and ion source temperature of approx. 180 °C. Ammonia or methylamine was introduced into the ion source until the pressure reading at the ion source gauge was 10^{-4}—10^{-3} Pa. To obtain the MIKE and CID spectra, the ions under study were focussed magnetically in the second field-free region (2nd FFR) between the magnetic and the electrostatic analyzer, and the spectra were recorded by scanning the deflection voltage of the electrostatic analyzer. To perform CID in the collision cell of the 2nd FFR, He was introduced until the main ion beam was settled to approx. 50 % of the original intensity.

RESULTS AND DISCUSSION

As expected the CI(NH_3) and CI(CH_3NH_2) spectra of *I—VIII* exhibit abundant adduct ions $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$, respectively, permitting an easy determination of the relative molecular masses of the per-*O*-methylated oligosaccharides. The MIKE spectral data of $[M + NH_4]^+$ adducts of xylobiosides *I—III* are given in Table 1. The spontaneous decay of clusters follows two routes. The liberation of ammonia gives rise to the $[M + H]^+$ quasimolecular ions at *m/z* = 367. The production of these ions is exceptionally intense in the case of compounds with 1→3 and 1→4 linkages. In the case of 1→2 linkage (Table 1) the production of baA_1 oxonium ions (*m/z* = 335) dominates. The notation of fragments formed from *I—VIII* is in accordance with the commonly used one at EI fragmentation of per-*O*-methylated oligosaccharides [2, 3]. The baA_1 ions are produced from $[M + H]^+$ ions by the elimination of methanol. The splitting of the interglycosidic linkage, leading to one-unit oxonium ions aA_1 (*m/z* = 175) is less intense. The secondary and tertiary A-type ions (*m/z* = 303, 143, and 111) are also produced. In addition, the A_1-type ions react with the molecule of ammonia to give $[A_1 + NH_3]^+$ ions [14, 15]. In the reversed reaction, the $[A + NH_3]^+$ adduct eliminates the molecule of ammonia giving the ions A_1 at *m/z* = 175 under these conditions. This was proved by MIKE measurement of ions with *m/z* = 192.

Table 1. MIKE Spectra of $[M + NH_4]^+$ Adducts (*m/z* = 384) of D-Xylobiosides *I—III*

m/z	Relative intensity/%			Symbol
	I	*II*	*III*	
367	10	100	100	$[M + H]^+$
352	2	1	1	$[baA_1 + NH_3]^+$
335	100	6	27	baA_1
303	38	14	14	baA_2
192	9	5	1	$[aA_1 + NH_3]^+$
175	1	1	1	aA_1
143	11	18	8	aA_2
111	10	5	1	aA_3

The spontaneous decay of $[M + CH_3NH_3]^+$ cluster ions under the MIKE conditions (Table 2) follows the same route, as when ammonia is used as a reaction gas. Thus the quasimolecular $[M + H]^+$ ions, A-type ions, as well as $[aA_1 + CH_3NH_2]^+$ adducts are produced.

Table 2. MIKE Spectra of $[M + CH_3NH_3]^+$ Ions (*m/z* = 398) of D-Xylobiosides *I—III*

m/z	Relative intensity/%			Symbol
	I	*II*	*III*	
367	19	33	41	$[M + H]^+$
335	77	12	83	baA_1
303	100	97	65	baA_2
271	10	2	5	baA_3
206	41	38	45	$[aA_1 + CH_3NH_2]^+$
175	13	18	100	aA_1
143	17	31	76	aA_2
111	30	100	27	aA_3

A comparison of the MIKE spectra of various linked per-*O*-methyl-D-xylobioses reveals noticeable differences in relative intensities of ion peaks. The determination of the linkage position can simply be based on the *m/z* value of the base peak in the MIKE spectra of cluster ions, using methylamine as reaction gas. Thus the base peak in the case of 1→2 , 1→3,

and 1→4 linkage is at *m/z*: 303, 111, and 175, respectively.

The CID spectra of $[M + NH_4]^+$ clusters, obtained with helium as a collision gas, together with the interpretation of fragments are given in Table 3. In addition to the routes deduced from the MIKE spectra interpretation (Tables 1 and 2), the splitting of pyranoid cycles, similar to that occurring during the EI fragmentation [2, 3] takes place (*e.g.* F, H, and J pathways). The H-type fragmentation, involving the conjugated rupture of the pyranoid ring, dominates at the high energy CID FAB MS/MS methodology [6, 7] and serves to linkage position analysis of metal-cationized per-*O*-methylated oligosaccharides. By comparing the CID spectra of $[M + NH_4]^+$ ions (*m/z* = 384, Table 3), the characteristic differences in the base peak position could be found: *m/z* = 303 for the 1→2, 367 for the 1→3, and 175 for the 1→4 linkage. These constitute an alternative means for the determination of linkage mode of methylated D-xylobioses.

Table 3. CID Spectra of $[M + NH_4]^+$ Adducts (*m/z* = 384) of D-Xylobiosides *I—III*

m/z	Relative intensity/%			Symbol
	I	*II*	*III*	
367	5	100	28	$[M + H]^+$
352	6	3	3	$[baA_1 + NH_3]^+$
335	90	10	15	baA_1
303	100	32	14	baA_2
271	4	2	1	baA_3
207	1	1	6	
192	23	11	8	$[aA_1 + NH_3]^+$
175	79	32	100	aA_1
161	4	1	4	
143	64	44	60	aA_2
129	4	1	3	C_2
111	43	40	17	aA_3
101	39	40	17	F_1
88	12	5	13	H_1
75	13	6	10	J_1
71	13	6	11	K_2
45	7	4	5	$CH_2O^+CH_3$

The complete series of positionally isomeric D-xylotrioses *IV—VIII* have been also studied by MIKE and CID measurements of $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ adduct ions. The MIKE spectra of $[M + NH_4]^+$ species are presented in Table 4. Compound *IV* containing the a→2b→4c linkage fragments almost exclusively by splitting the 1→4 interglycosidic linkage (baA_1 *m/z* = 335). The baA_2 and baA_3 fragment species, as well as adduct ions $[baA_1 + NH_3]^+$ at *m/z*: 303, 271, and 352, accompany the baA_1 ions production. The production of one-unit aA_1 ions with *m/z* = 175 is low in intensity. This splitting is dominant with the compound *V* (a→4b2←c) representing a model for branching point of D-xylan type oligo- and polysaccharides.

Table 4. MIKE Spectra of $[M + NH_4]^+$ Adducts (*m/z* = 544) of D-Xylotriosides *IV—VIII*

m/z	Relative intensity/%					Symbol
	IV	*V*	*VI*	*VII*	*VIII*	
527			87	39	100	$[M + H]^+$
512			12			$[cbaA_1 + NH_3]^+$
495			8	19	30	$cbaA_1$
463			15	100	20	$cbaA_2$
431			4	13	1	$cbaA_3$
352	15		61		63	$[baA_1 + NH_3]^+$
335	100		16		13	baA_1
303	50		100		98	
271	10					
192		14	4	6	20	$[aA_1 + NH_3]^+$
175	5	33	28	1	20	aA_1
143		100	37	12	23	aA_2
111		10	85	30	5	aA_3

The MIKE spectra of $[M + NH_4]^+$ adducts of linear trisaccharides, possessing the a→3b→4c and a→4b→4c linkages are qualitatively the same. All fragment routes discussed earlier (*e.g.* the production of $[M + H]^+$, all A-type and $[A_1 + NH_3]^+$ ions) are reflected in the spectra. In contrast, the model compounds *V* and *VII* with the a→4b2←c and a→4b3←c arrangement do not produce two-ring baA_1 ions (Table 4).

The $[M + CH_3NH_3]^+$ clusters disintegrate under the MIKE conditions by the same routes (Table 5) as their ammonia analogues. The higher stability of cluster quasimolecular ions is probably the reason for the presence of the peak of $[M + H]^+$ ions formed also in the case of the branched trimer *V* with the a→4b2←c linkage arrangement. In the case of compound *VII* the presence of baA_1 (*m/z* = 335) has been observed, too. We are not able to explain the production of these ions in the case of branched trimer. The origin of these ions is probably combined with the rearrangement of $[M + CH_3NH_3]^+$ ions. The small intensity baA_1 peak should be omitted at branching point deduction.

Table 5. MIKE Spectra of $[M + CH_3NH_3]^+$ Cluster Ions (*m/z* = 588) of D-Xylotriosides *IV—VIII*

m/z	Relative intensity/%					Symbol
	IV	*V*	*VI*	*VII*	*VIII*	
527		25	37	17		$[M + H]^+$
495		10	23	10		$cbaA_1$
463				30		$cbaA_2$
431				40		$cbaA_3$
367		100	10	10	66	$[baA_1 + CH_3NH_2]^+$
335	30		17	5	72	baA_1
303	100		20	10	66	baA_2
206	10	20	5	20	26	$[aA_1 + CH_3NH_2]^+$
175	30	100	93	24	93	aA_1
143	10	50	71	43	100	aA_2
111	5	20	61	100	33	aA_3

Table 6. Characteristic Features of MIKE Spectra of Cluster $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ Ions of D-Xylotriosides *IV—VIII*

Symbol	Presence of peaks in the MIKE spectra				
	a→2b→4c	a→4b2←c	a→3b→4c	a→4b3←c	a→4b→4c
$[M + H]^+$	O	O[a]	+	+	+
$cbaA_1$	O	O	+	+	+
baA_1	+	O	+	O	+
aA_1	+	+	+	+	+
Base peak (*m/z*):					
$Cl(NH_3)$	335	143	303	463	527
$Cl(CH_3NH_2)$	303	175	367	111	143

a) In the case of CH_3NH_2: +.

Characteristic features of MIKE spectra of $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ adducts, giving characteristic information about the structure of per-*O*-methylated D-xylan type trimers, are summarized in Table 6. The presence or absence of the characteristic fragments, together with the *m/z* value of base peak of the spectra, give the unambiguous evidence for the linkage positions assignment in the trisaccharide concerned.

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