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Mass-analysed Ion Kinetic Energy and Collisionally Induced Dissociation Mass Spectra of Clusters of Acetylated Xylo-oligosaccharides with Protonated Ammonia and Methylamine

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Per-*O*-acetylated methyl glycosides of D-xylan-type di- and trisaccharides were studied by mass-analysed ion kinetic energy (MIKE) and collisionally induced dissociation (CID) mass spectrometry using protonated ammonia and methylamine, respectively, as reaction gases in chemical ionization (CI). The oligosaccharides form abundant cluster ions, $[M + NH_4]^+$ or $[M + CH_3NH_3]^+$, and the main fragmentation of these ions in the MIKE and CID spectra is the cleavage of interglycosidic linkages. Thus, CI (NH_3) or CI (CH_3NH_2) spectra in combination with the MIKE or CID spectra allow the molecular masses, the masses of monosaccharide units and the branching point in oligosaccharides to be established. In the case of disaccharides, it is possible to distinguish the (1 → 2) linkage from the other types of linkages.

INTRODUCTION

D-Xylans and (4-*O*-methyl-D-glucurono)xylans are components of industrially important woods and other plants.[1] With the aim of using mass spectrometric methods for the structure elucidation of oligosaccharides related to D-xylans, we and others have made systematic studies of per-*O*-methylated oligosaccharides.[2,3] The sequential analysis of oligosaccharides by electron impact (EI) mass spectrometry has also been described.[4] Other important results in this regard have been achieved by the fast atom bombardment (FAB) mass spectrometry of underivatized methyl glycosides with subsequent analysis of the $[M + \text{glycerol} + H]^+$ cluster ions by mass analysed ion kinetic energy (MIKE) and collisionally induced dissociation (CID) mass spectrometry.[5] In continuation of our studies directed at the mass spectrometric analysis of xylo-oligosaccharides, we examined the gas-phase reactions of per-*O*-methylated D-xylotriose with several protonated reagents by chemical ionization (CI) mass spectrometry.[6] The ammonium ions produced from ammonia and methylamine yield abundant cluster ions under these conditions with the per-*O*-methylated oligosaccharides studied. In this contribution, the results of an investigation of the per-*O*-acetylated methyl glycosides of D-xylan-type oligosaccharides (**1–10**) by CI, using ammonia or methylamine as reaction gas, are discussed. MIKE and CID mass spectrometry were used to study the fragmentation of these cluster ions.

1 = α-D-Xylp-(1 → 2)-β-D-Xylp

2 = β-D-Xylp-(1 → 2)-β-D-Xylp

3 = α-D-Xylp-(1 → 3)-β-D-Xylp

4 = β-D-Xylp-(1 → 3)-β-D-Xylp

5 = α-D-Xylp-(1 → 4)-β-D-Xylp

6 = β-D-Xylp-(1 → 4)-β-D-Xylp

7 = β-D-Xylp-(1 → 2)-β-D-Xylp-(1 → 4)-β-D-Xylp

8 = β-D-Xylp-(1 → 3)-β-D-Xylp-(1 → 4)-β-D-Xylp

9 = β-D-Xylp-(1 → 4)-β-D-Xylp-(1 → 4)-β-D-Xylp

10 = β-D-Xylp-(1 → 4)-β-D-Xylp-(2 ← 1)-β-D-Xylp

EXPERIMENTAL

The synthesis of the methyl glycosides of oligosaccharides **1–10** has been described previously[7–11] and the methyl glycosides were per-*O*-acetylated by conventional techniques using acetic anhydride–pyridine.

The CI mass spectra were obtained with a VG ZAB-2F mass spectrometer using the direct inlet system for the sample, 70 eV energy and an ion source temperature of ~180 °C. Ammonia or methylamine was introduced into the ion source as reaction gas until the pressure reading at the ion source gauge was 10^{-5}–

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10^{-6} mbar (1 bar = 10^5 Pa). In the case of disaccharides **1–6** deuterated ammonia was also used as a reaction gas. To obtain the MIKE and CID spectra, the ions under study were focused magnetically into the second field-free region (2nd FFR) between the magnet and the electrostatic analyser, and the spectra were recorded by scanning the deflection voltage of the electrostatic analyser. To perform CID in the collision cell of the 2nd FFR, helium was introduced until the main ion beam was attenuated by ~50%.

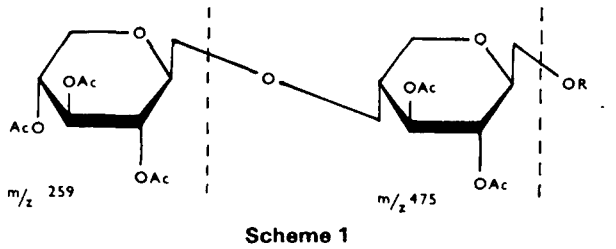
RESULTS AND DISCUSSION

The CI (NH_3) spectra and CI (CH_3NH_2) spectra of **1–10** exhibit abundant cluster ions $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$, respectively, as in the case of the per-*O*-methylated sugars.[6] In particular, the peak of the cluster ions $[M + CH_3NH_3]^+$ is dominant in the spectrum, permitting an easy determination of the relative molecular masses of the per-*O*-acetylated methyl glycosides of the oligosaccharides. However, no fragmentation of these ions is observed in their MIKE spectra. The MIKE spectra of $[M + NH_4]^+$ adduct ions of per-*O*-acetylated disaccharides **1–6** are presented in Table 1. The only fragmentation process observed is the cleavage of glycosidic linkages, as shown in Scheme 1. Interestingly, in the case of a (1 → 2) linkage the cleavage of this interglycosidic bond takes place much easier than in the case of (1 → 3) and (1 → 4) linkages [see the much smaller intensity ratio baA_1/aA_1 (*m/z* 475/259) for **1** and **2**].

More extensive fragmentation is observed in the CID spectra of both types of cluster ions of **1–6** shown in Tables 2 and 3. As in the case of the MIKE spectra, a cleavage of adduct ions $[M + NH_4]^+$ (Table 2) gives rise to the ions $[M - {}^{\bullet}OCH_3]^+$ (baA_1, *m/z* 475) and ions aA_1 (*m/z* 259), representing the non-reducing unit of the disaccharide. Again the cleavage of a (1 → 2) interglycosidic linkage occurs with special ease, as indicated by small baA_1/aA_1 intensity ratio (*m/z* 475/259) for **1** and **2**. Further fragmentations of these ions proceed by elimination of CH_3COOH, CH_2CO and CO molecules as usual, giving rise to the ions A_2 at *m/z* 199, A_3 at *m/z* 157 and 139, A_4 at *m/z* 97 and CH_3CO^+

Table 1. MIKE mass spectra of $[M + NH_4]^+$ ions (*m/z* 524) of disaccharide derivatives 1–6

	Relative intensity (%)					
m/z	1	2	3	4	5	6
475	10	12	100	100	100	100
259	100	100	80	80	30	71



Scheme 1

Table 2. CID mass spectra of $[M + NH_4]^+$ ions (*m/z* 524) of disaccharide derivatives 1–6

	Relative intensity (%)					
m/z	1	2	3	4	5	6
475	7	4	33	25	75	37
259	100	100	100	100	100	100
229	10	12	10	9	8	7
199	52	61	61	50	83	57
169	12	12	8	8	8	5
157	75	71	61	50	75	75
139	50	71	43	50	60	45
109	4	4	4	4	4	4
97	68	71	51	40	44	31
43	60	51	26	30	28	25

at *m/z* 43. The peaks of the other series of ions at *m/z* 229, 169 and 109 are less intense. These fragmentation routes were confirmed by measuring the MIKE and CID spectra of $[M + ND_4]^+$ adduct ions in the CI (ND_3) spectra of **1–6**. Only the masses of the cluster ions have been shifted by 4 u to higher values.

The CID spectra of the cluster ion $[M + CH_3NH_3]^+$ (Table 3) again show distinct signals of fragments baA_1 and a A_1 at *m/z* 475 and 259 due to the cleavages of glycosidic bonds of **1–6**. However, the intensity ratios do not correlate with the type of glycosidic bonds. Furthermore, the degradation of these ions by losses of CH_3COOH and CH_2CO is more intense, compared with the CID spectra of the corresponding $[M + NH_4]^+$ cluster ions, and ions arising from the fragmentation of the pyranoid rings (e.g. at *m/z* 245, 229, 169, 127 and 109) are more pronounced. Finally, a large portion of the fragment ion current is carried away by $CH_3NH_3^+$ ions (*m/z* 32), characteristic of the reactant gas used.

The information obtained from the CI (NH_3) or CI (CH_3NH_2) spectra and from the MIKE or CID spectra of the cluster ions $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$, respectively, can easily be used for the structure elucidation of the per-*O*-acetylated methyl glycosides of disaccharides. The determination of relative molecular mass is unambiguously possible from the

Table 3. CID mass spectra of $[M + CH_3NH_3]^+$ adducts (*m/z* 538) of disaccharide derivatives 1–6

	Relative intensity (%)					
m/z	1	2	3	4	5	6
475	6	31	19	18	55	53
415	9	50	30	25	33	36
259	30	63	52	56	88	100
245	15	31	15	22	48	45
229	13	13	28	16	33	57
199	26	52	59	50	48	45
169	39	57	30	26	22	30
157	93	76	94	87	93	98
139	71	87	94	90	96	84
127	15	21	15	18	15	10
109	19	13	10	10	15	20
97	100	100	100	100	100	77
43	85	68	30	35	77	86
32	278	210	220	215	220	165

masses of the respective cluster ions. The determination of the masses of monosaccharide units uses the large peaks in the MIKE and CID spectra of the cluster ions $[M + NH_4]^+$, and it is also possible to differentiate a $(1 \rightarrow 2)$ interglycosidic linkage from the other types of glycosidic junctions. However, it is not possible to distinguish between α- and β-anomers. Better information can be obtained only by the method of determining interglycosidic linkages in disaccharides using fast atom bombardment high-performance tandem mass spectrometry and high-energy CID measurements of A_1 C(1) carbenium ions derived from trideuteroacetylated dihexopyranosides.[12]

The per-*O*-acetylated trisaccharides **7–10** also give rise to abundant cluster ions $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ in their CI (NH_3) and CI (CH_3NH_2) spectra, respectively. The $[M + CH_3NH_3]^+$ cluster ions are again very stable and do not give MIKE spectra. The MIKE spectra of the $[M + NH_4]^+$ adduct ions are shown in Table 4, cleavages of the glycosidic bonds again being the only processes observed. Again the cleavage of the $(1 \rightarrow 2)$ interglycosidic linkage of **7** is strongly preferred. A branched trisaccharide de- ative (**10**) can in principle not form ions baA_1 at *m/z* 475, so this branching is easily detected.

The CID spectra of the $[M + NH_4]^+$ cluster ions of trisaccharides **7–10** are presented in Table 5. The ions baA_1 formed by splitting of the glycosidic linkages appear at *m/z* 475 and 259, and further fragmentation of these ions gives rise to ions aA_2 at *m/z* 199, aA_3 at *m/z* 157 and 139 and aA_4 at *m/z* 97. The CID spectra of the adduct ions $[M + CH_3NH_3]^+$ of the trisaccharides **7–10** are given in Table 6. Similarly to the MIKE spectra of the $[M + NH_4]^+$ adduct ions, the fragmentation occurs predominantly at the interglycosidic linkages. The high-mass range of the spectra exhibits peaks at *m/z* 694 and 634 due to ions formed by the loss of one or two CH_3COOH molecules from the adduct ions.

Table 4. MIKE mass spectra of $[M + NH_4]^+$ ions (*m/z* 740) of trisaccharide derivatives 7–10

	Relative intensity (%)			
m/z	7	8	9	10
475	10	100	100	
259	100	66	60	100

Table 5. CID mass spectra of $[M + NH_4]^+$ ions (*m/z* 740) of trisaccharide derivatives 7–10

	Relative intensity (%)			
m/z	7	8	9	10
475	20	78	38	
259	100	84	100	100
199	50	92	53	50
157	65	100	57	40
139	35	96	63	30
97	65	78	37	50
43	30	46	20	42

Similarly to the CI (NH_3) and CI (CH_3NH_2) spectra of disaccharides, the spectra of the trisaccharides can be used to determine relative molecular masses. The MIKE and CID spectra of the respective adduct ions, in particular of $[M + NH_4]^+$, give additional structural information concerning the masses of per-*O*-acetylated monosaccharide units. The absence of the oxonium ions baA_1 at *m/z* 475 (and its fragmentation product baA_2 at *m/z* 415) containing two monosaccharide units in the MIKE and CID spectra of the cluster ions of **10** confirms the presence of branching in the trisaccharide. Finally, the preferred cleavage of a $(1 \rightarrow 2)$ interglycosidic linkage is also observed in the MIKE and CID spectra of adduct ions of the trisaccharide.

Table 6. CID mass spectra of $[M + CH_3NH_3]^+$ ions (*m/z* 754) of trisaccharide derivatives 7–10

	Relative intensity (%)			
m/z	7	8	9	10
694	80	78	80	30
634	90	60	94	45
475	10	30	9	
415	10	31	10	
259	100	50	100	81
245	20	26	44	
199	40	74	70	67
169	10	22		
157	50	100	80	100
139	60	85	72	50
97	60	70	65	80
43	20	38	60	42
32	60	72	110	74

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