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Dear Sir

Mass-analysed Ion Kinetic Energy and Collisional Activation Spectra of Clusters of Per-*O*-methylated β-D-Xylotriose with Protonated Reagents Formed Under the Conditions of Chemical Ionization

In continuation of our systematic study of mass spectrometry of oligosaccharides related to D-xylans[1,2,3] we have examined the gas-phase reactions of model oligosaccharides of this type with several protonated reagents by chemical ionization mass spectrometry. It is well known that saccharides and oligosaccharides form adduct ions or cluster ions with ammonium ions[4] and protonated reagents carrying hydroxy groups[3] which are useful for a mass spectrometric analysis of carbohydrates. In a preliminary study we prepared adducts of a series of protonated reagents **1**–**12**, (Table 1) to oligosaccharides and obtained the mass-analysed ion kinetic energy (MIKE) and collisional activation (CA) spectra of these cluster ions.

As a model substance per-*O*-methylated *β*-D-xylopyranosyl-(1 → 4)-*β*-D-xylopyranosyl-(1 → 4)-*β*-D-xylopyranose (xylotriose, **X**) was chosen representing the main chain of D-xylans and D-*β*-glucurono-D-xylans. Reagent gases were introduced into a modified chemical ionization (CI) ion source of a VG ZAB-2F mass spectrometer by the normal CI-gas inlet line and liquid reagents by a heated inlet system with a leak valve until the ion source housing pressure was 10^{-6}–10^{-5} mbar. In the case of methanol, ethylene diol and glycerol some isobutane was admitted additionally into the ion source to ensure protonation of the alcohols. The energy of the ionizing electron was 70 eV and the ion source temperature was ~180 °C. The model oligosaccharide **X** was introduced by a solid sample probe by gentle heating. The cluster ions under study were focused magnetically into the second field-free region of the ZAB-2F instrument, and the MIKE and CA spectra were obtained by the usual technique, using helium as the collision gas.

Table 1. Reagents used for the cluster ion formation

Reagent (R)	$[X+RH]^+$ cluster	PA(R)[5] kJ mol⁻¹
methane (**1**)	—	552
isobutane (**2**)	—	683
methanol (**3**)	—	761
ethylene glycol (**4**)	—	
glycerol (**5**)	—	
1,2-dimethoxyethane (**6**)	—	857
ammonia (**7**)	+	853
methylamine (**8**)	+	896
dimethylamine (**9**)	+	923
pyridine (**10**)	+	924
2-aminoethanol (**11**)	+	926
1,2-diaminoethane (**12**)	+	945

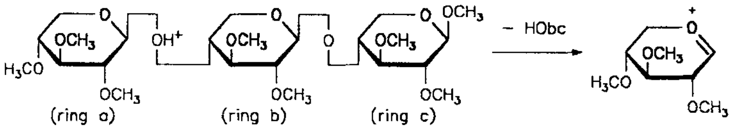
As expected the protonated species of the hydrocarbon reactants methane (**1**) and isobutane (**2**) do not form any adduct ions with **X** nor can the $[M+H]^+$ ion be observed. Instead the exothermic proton transfer from $[CH_5]^+$ and $[t\text{-}C_4H_{11}]^+$ to **X** results in a protiolytic cleavage of one of the glycosidic bonds and the formation[1] of oxonium ions of type **A** (Scheme 1).

Similarly, the cleavage of the glycosidic bond between the rings '**b**' and '**c**' of **X** gives rise to **baA**$_1$, *m/z* 335. A subsequent elimination of methanol from the ions of type **A** gives rise to the fragment ions *m/z* 303 (**baA**$_2$), *m/z* 143 (**aA**$_2$), and *m/z* 111 (**aA**$_3$), respectively.[1]

The protonated species $[CH_5O]^+$, $[C_2H_7O_2]^+$ and $[C_3H_9O_3]^+$ formed from methanol (**3**), ethylene glycol (**4**) and glycerol (**5**), respectively, also do not form adduct ions, and their reaction with **X** under CI conditions produces similar mass spectra as the hydrocarbon reagents **1** and **2**. Dimethoxyethane gives rise to abundant ions *m/z* 91 under CI conditions after addition of isobutane. However, no adduct ions of this protonated species with the oligosaccharide **X** are formed, and the mass spectrum obtained corresponds qualitatively to the EI mass spectrum of **X**.[1]

In contrast to the protonated species generated from the reagents **1**–**6** the ammonium ions produced from NH_3 (**7**) and the amines (**R**) **8**–**12** under CI conditions yield abundant cluster ions $[X+RH]^+$ appearing at *m/z* 544 (**7**), *m/z* 558 (**8**), *m/z* 572 (**9**), *m/z* 606 (**10**), *m/z* 588 (**11**) and *m/z* 587 (**12**), respectively. No systematic study of the ease of a cluster ion formation by the different ammonium ions has been performed. However, the tendency to yield cluster ions with **X** depends obviously very much on the structure of the ammonium ions without a clear correlation to the proton affinity of the amine. Especially abundant (and stable) clusters are given by protonated **8**, **9** and **12**. For example, a mixture NH_3/CH_3NH_2 with only a few per cent of **8** gives rise predominantly to clusters $[X+\mathbf{8}+H]^+$, and the cluster ions $[X+\mathbf{9}+H]^+$ and $[X+\mathbf{12}+H]^+$ do not give any fragment ions in the MIKE spectra. Metastable cluster ions $[X+RH]^+$ with **R** = **7**, **8**, **10** or **11** decompose mainly by loss of **R** to yield $[X+H]^+$, *m/z* 527, and by cleavage of a glycosidic bond generating the sequence ions **cbaA**$_1$, *m/z* 495, **baA**$_1$, *m/z* 335, and **aA**$_1$, *m/z* 175 (Scheme 2 and Table 2). Interestingly, the cluster ions $[\mathbf{baA}_1+\mathbf{R}]$ and $[\mathbf{aA}_1+\mathbf{R}]$ also are observed in the MIKE spectra with a relative intensity depending on **R** (Table 2).

$$\text{e.g. } [CH_5]^+ + X \longrightarrow [X+H]^+ + CH_4$$



$[X+H]^+$

aA$_1$, *m/z* 175

Scheme 1

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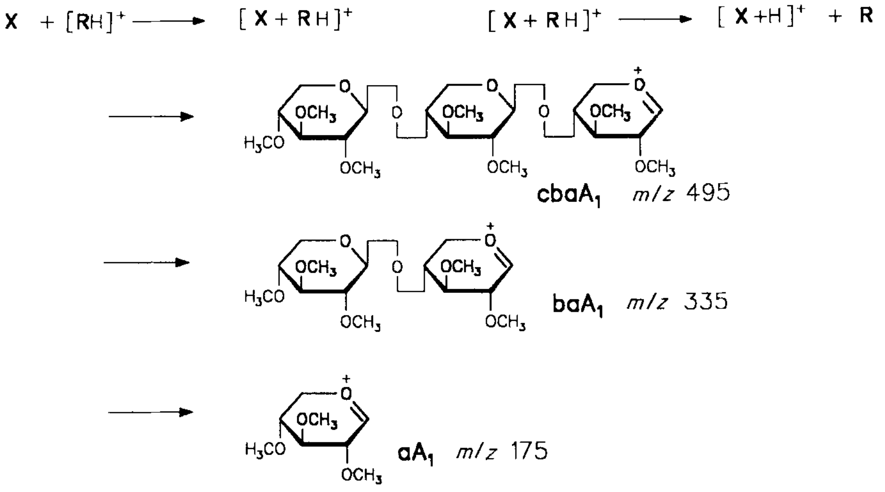

Scheme 2

Obviously $[\mathbf{RH}]^+$ is attached at the different monosaccharide units of **X** and catalyse a protiolytic cleavage of the glycosidic bond accompanied by loss of the saccharide units c and bc, respectively. The CA spectra of all cluster ions, including those of **9** and **12**, exhibit abundant sequence ions $\mathbf{cbaA_1}$, $\mathbf{baA_1}$, and $\mathbf{aA_1}$ and $[\mathbf{RH}]^+$. In addition, fragment ions by loss of CH_3OH from the sequence ions are observed. The cluster ions of the fragment ions $[\mathbf{baA_1} + \mathbf{R}]$ and $[\mathbf{aA_1} + \mathbf{R}]$ exhibit a reduced relative intensity, however (Table 2).

In conclusion, the present study shows that ammonium ions and protonated amines generate abundant adduct ions allowing a straightforward determination of the relative molecular mass of per-*O*-methylated oligosaccharides. In particular, protonated methylamine, dimethylamine and 1,2-diaminoethane form very stable adducts. The MIKE and CA spectra of these cluster ions contain large peaks of the sequence ions allowing a determination of the units of the oligosaccharides. Thus, the gas-phase reactions of protonated amines with per-*O*-methylated oligosaccharides yields valuable information for the analysis of oligosaccharides.[5]

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Yours

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Table 2. Sequence ions A and cluster ions $[\mathbf{A} + \mathbf{R}]^+$ in the MIKE and CA spectra of the parent cluster ions $[\mathbf{X} + \mathbf{RH}]^+$

Reagent	$[aA_1 + R]/aA_1$ MIKE	CA	$[baA_1 + R]/baA_1$ MIKE	CA
ammonia (**7**)	0.10	0.03	5.00	0.70
methylamine (**8**)	0.12	0.06	0.90	0.60
dimethylamine (**9**)	—	[a]	—	[a]
pyridine (**10**)	0.50	0.33	0.38	0.20
2-aminoethanol (**11**)	0.25	0.06	0.48	0.38
1,2-diaminoethane (**12**)	—	[a]	—	[a]

[a] Only ions $\mathbf{aA_1}$ and $\mathbf{baA_1}$ are observed.

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