

Fourier Transform Ion Cyclotron Resonance Study of Ion-Molecule Reactions of $[M - OCH_3]^+$ Ions of Methyl 2,3,4,6-Tetra-*O*-methyl- β -D-hexopyranosides with Ammonia

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Dedicated to Professor Kurt Heyns to thank him for the excellent training in carbohydrate chemistry and mass spectrometry

Glycosidic oxocarbenium ions A_1^+ were formed by isobutane chemical ionization from methyl 2,3,4,6-tetra-*O*-methyl- β -D-mannopyranoside, methyl 2,3,4,6-tetra-*O*-methyl- β -D-galactopyranoside and methyl 2,3,4,6-tetra-*O*-methyl- β -D-glucopyranoside (the ring $-O-$ being converted into $-\dot{O}=\$), and their reaction with ammonia was studied by Fourier transform ion cyclotron resonance spectrometry. Very slow formation (reaction efficiency 0.6–1.4%) of the adduct ion $[A_1 + NH_3]^+$ was observed as the main process for carefully thermalized ions A_1^+ . Interestingly, the efficiency of the adduct ion formation depends on the stereochemistry of ions A_1^+ .

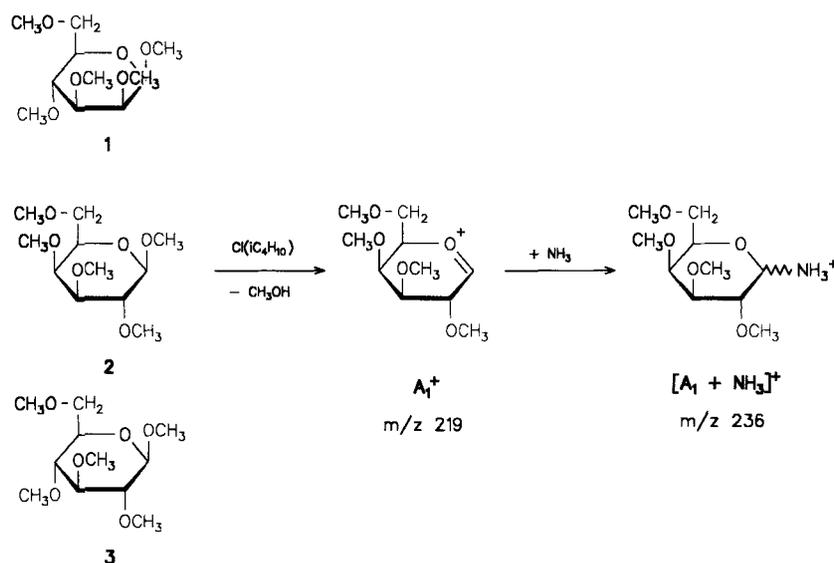
INTRODUCTION

The electron impact (EI) mass spectra of carbohydrate derivatives usually do not show a signal for the molecular ions but a peak of oxocarbenium ions which are formed by the loss of the glycosidic group, and many of the other fragment ions observed are derived from these oxocarbenium ions by further degradation.¹ Similarly, the methane and isobutane chemical ionization (CI) mass spectra of most carbohydrate derivatives contain large signals due to these oxocarbenium ions and no or only small peaks of the protonated molecular species.² A noticeable exception are the ammonia CI mass spectra, which usually display distinct peaks of cluster ions formed by the addition of NH_4^+ to the sugar molecule.^{2,3} Recently, we have shown that per-*O*-methylated oligosaccharides not only form cluster ions with NH_4^+ but also intense cluster ions $[M + RH]^+$ with amine reagents (R) such as pyridine, 2-aminoethanol, methylamine and particularly dimethylamine and 1,2-diaminoethane under CI conditions.⁴ The mass-analysed ion kinetic energy spectra of the cluster ions $[M + NH_4]^+$ and $[M + CH_3NH_3]^+$ contain not only the expected signals for the loss of NH_3 and CH_3NH_2 , respectively, but also peaks for ions $[M + RH - CH_3OH]^+$. Further, this type of ion is also observed in the collisional activation spectra of all cluster ions of the per-*O*-methylated oligosaccharides with protonated amines together with fragment ions A^+ arising from cleavage of the glycosidic bond and analogous ions incorporating the amine R. These latter fragment ions $[A + R]^+$ can be pictured as glycosidic ammonium ions arising from an aminolysis of a protonated glyco-

sidyl group or from an addition of a glycosidic oxocarbenium ion to the amine (Scheme 1). Therefore, we investigated the reaction of the glycosidic oxocarbenium ions of type A^+ with ammonia by Fourier transform ion cyclotron resonance (FT-ICR) spectrometry.

RESULTS AND DISCUSSION

Methyl 2,3,4,6-tetra-*O*-methyl- β -D-mannopyranoside (1), methyl 2,3,4,6-tetra-*O*-methyl- β -D-galactopyranoside (2) and methyl 2,3,4,6-tetra-*O*-methyl- β -D-glucopyranoside (3) were used as model compounds yielding epimeric oxocarbenium ions $1A_1^+$, $2A_1^+$ and $3A_1^+$, respectively, of m/z 219 by loss of methanol from the protonated molecules. These A_1^+ ions were generated from the parent compounds by isobutane CI in the external ion source of a Bruker Spectrospin CMS 47X ICR spectrometer and transferred into the ICR cell. The complete isobutane CI-FT-ICR mass spectra of 1–3 are listed in Table 1. As known from the isobutane CI mass spectra of per-*O*-methylated methyl glycosides,⁵ the peak intensity of the quasi-molecular ions $[M + H]^+$ varies strongly with the temperature of the CI ion source. The main process is the formation of ions A_1^+ followed by the loss of methanol yielding ions A_2^+ , m/z 187. Small peaks are observed for the elimination of a second molecule of methanol (ion A_3^+ , m/z 155) and for the eventual loss of the CH_2OCH_3 substituent at C(5) as a fragment of 44 u yielding ions A_4^+ , m/z 111. The relative abundances of the ions A_2^+ – A_4^+ differ in the isobutane CI-FT-ICR mass spectra of 1–3. In fact, the variation of the A_2^+/A_1^+ abundance ratio with the configuration at C(2) and C(4) is quite large and compares



favourably with the usual ion beam mass spectra¹ with respect to analytical applications. The reason for these increased differences in the FT-ICR mass spectra of stereoisomers may be the longer reaction times for the fragmentation within the FT-ICR instrument, usually milliseconds, compared with microseconds in an ion beam instrument. Hence, also the slow and more sterically controlled fragmentations of only slightly excited quasi-molecular ions are sampled in the FT-ICR instrument.

The ion-molecule reactions of the ions A_1^+ , m/z 219, from 1–3 were explored by isolation of these ions in the ICR cell, carefully ejecting all other ions, and ‘cooling’ the ion of m/z 219 using a 15 ms pulse of argon to remove most of the excess kinetic energy. Following this procedure, the ions A_1^+ were reacted with ammonia at a constant pressure of about 5×10^{-7} mbar (1 mbar = 100 Pa) in the ICR cell for reaction times up to 20 s. The curves obtained for the time dependence of the intensities of ions A_1^+ and the product ions of the ion-molecule reactions are illustrated in Fig. 1 for the case of the D-mannopyranoside 1.

It is seen that NH_4^+ is formed by a fast reaction with ions A_1^+ , but only at the beginning of the reaction period, and that the adduct ion $[A_1 + NH_3]^+$, m/z 236,

and a secondary ion, m/z 204, arise from a slow reaction with ammonia (Scheme 2). The ion of m/z 204 corresponds to $[A_1 + NH_3 - CH_3OH]^+$. Separate experiments using ions A_2^+ ($= [A_1 - CH_3OH]^+$) and ammonia show that ions of m/z 204 are not formed from A_2^+ by addition of NH_3 , but exclusively by elimination of methanol from adduct ions $[A_1 + NH_3]^+$ having excess energy. It is not known, however, which of the four methoxy groups of A_1^+ is lost. Additional product ions are observed at m/z 172 ($[A_1 + NH_3 - 2-CH_3OH]^+$), m/z 187 (A_2^+) and m/z 155 (A_3^+), but their relative intensities are always below 5%.

The pseudo-first-order rate constant k_{exp} of the reaction of ions A_1^+ with ammonia was determined from a semi-logarithmic plot of the data for the decay of A_1^+ . Figure 2 shows the result for the reaction of ions $1A_1^+$ from the mannopyranoside 1. After an initial fast decay of the ions due to the formation of NH_4^+ , good pseudo-first-order kinetics are observed which are attributed to the formation of the adduct ion $[A_1 + NH_3]^+$. The bimolecular rate constant k_{bi} was calculated by taking into account the (constant) concentration of ammonia in the ICR cell. The k_{exp} and k_{bi} values for the reaction of ions $1A_1^+$ – $3A_1^+$ are listed in Table 2 together with the corresponding reaction efficiencies obtained from

Table 1. Isobutane CI-FT-ICR spectra of methyl 2,3,4,6-tetra-O-methyl-D-hexopyranosides

m/z	Intensity (%)		
	1 (D-manno)	2 (D-galacto)	3 (D-gluco)
251	2.2	7.9	1.4
219	100	73.1	11.6
187	66.2	100	100
155	1.2	3.5	0.6
149	5.0	1.7	0.4
127	1.0	1.3	—
111	3.4	14.4	15.5
101	1.4	4.0	0.5
88	1.0	2.8	0.7
75	2.7	10.1	8.6
[187]/[219]	0.7	1.4	8.6

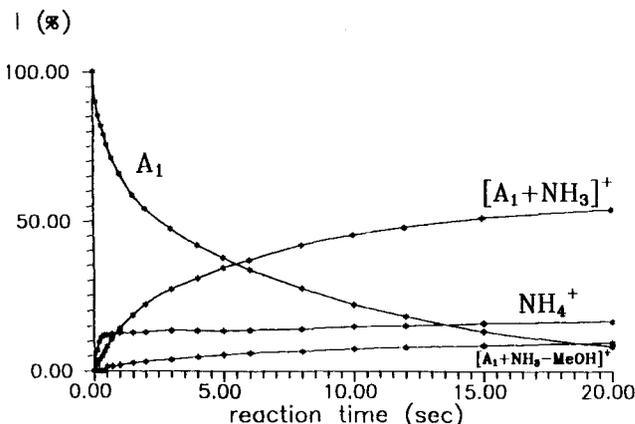
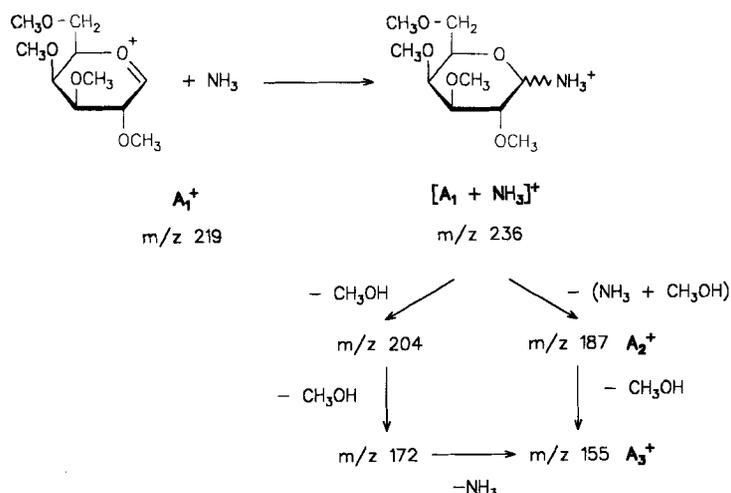


Figure 1. Kinetic curves for the reaction of ions $2A_1^+$ with NH_3 .



Scheme 2

k_{bi}/k_{ADO} , k_{ADO} corresponding to the collision rate between ions A_1^+ and NH_3 as calculated by the ADO theory⁶ using $\mu(NH_3) = 1.47$ D and $\alpha(NH_3) = 2.26 \text{ \AA}^3$.⁷

It is difficult to decide whether the formation of the NH_4^+ ions occurs by an exothermic proton transfer from the ions A_1^+ to NH_3 , since the heats of formation of these ions are not known. However, the formation of NH_4^+ only at the beginning of the reaction period and the non-linear decay of ions A_1^+ in the semi-logarithmic plot in Fig. 1 during this first period indicate an endothermic reaction of A_1^+ with NH_3 to form NH_4^+ . This is also suggested by the observation that the amount of NH_4^+ produced depends very much on the collisional deactivation of the ions A_1^+ after the transfer from the external ion source into the ICR cell. Hence, the NH_4^+ ions probably originate from an endothermic charge exchange of A_1^+ with NH_3 followed by the fast reaction $NH_3^+ + NH_3 \rightarrow NH_4^+ + NH_2^+$.

The addition of NH_3 to the ions A_1^+ is very slow and only one out of 70–170 collisions gives rise to the adduct ion $[A_1 + NH_3]^+$. This is to be expected because the collision complex is highly excited by the electrostatic binding energy which is released during the complex formation. Hence, the complex dissociates back into the components if it is not deactivated by physical or chemical processes. At the low pressures in the ICR cell during the experiment a collisional deacti-

vation is not very likely, and obviously deactivation by radiation is also slow.⁸ Surprisingly, the excess energy present in the collision complex of A_1^+ and NH_3 is not used for an excessive fragmentation. The main fragmentation observed is the loss of one CH_3OH . At a reaction time of 20 s about 90% of the initial ions A_1^+ have reacted, but the main product in the case of 1 and 3 is still the adduct ion $[A_1 + NH_3]^+$, and only in the case of the galactopyranoside 2 about two thirds of the product ions have been converted into the addition-elimination product $[A_2 + NH_3]^+$ and further fragmentation products. It is of interest that the efficiency of galactopyranosyl cations $2A_1^+$ for the reaction with NH_3 is about twice as large as in the case of the epimeric ions $1A_1^+$ and $3A_1^+$. Obviously, this increase in the total reaction rate is due to a faster decomposition of the adduct ion $[2A_1 + NH_3]^+$ by elimination of methanol, so that a smaller portion of these ions revert to the educts. This means that either the collision complex of ions A_1^+ and NH_3 and the resulting adduct ion $[A_1 + NH_3]^+$ is more chemically activated in the case of the galactopyranoside than in the case of the epimeric monosaccharides, or that the elimination of methanol from the NH_3 adduct ion of the galactopyranosyl cation $2A_1^+$ needs less critical energy than for the epimers. It has been observed that the stability of the NH_4^+ adduct ion of cyclic polyol derivatives⁹ and of monosaccharides¹⁰ depends on the stereochemistry, and this may be also true for the $[A_1 + NH_3]^+$ ions studied here. However, for the moment we prefer the second

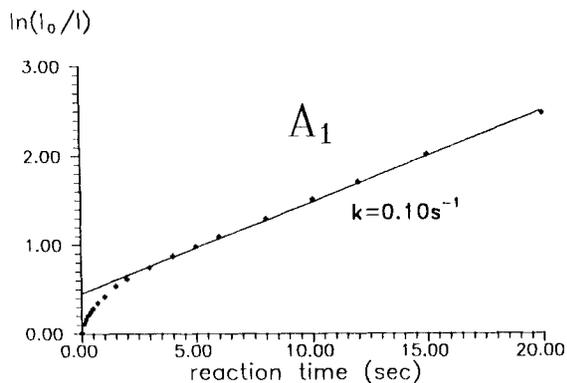


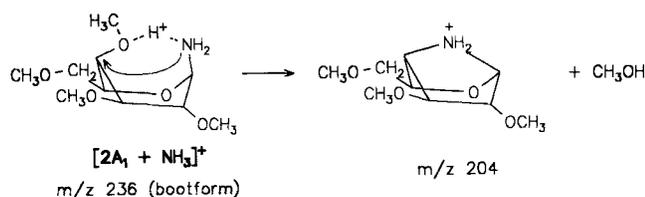
Figure 2. Semi-logarithmic plot for the decay of ions $2A_1^+$ by reaction with NH_3 .

Table 2. Bimolecular rate constants, k_{bi} , for the reaction of stereoisomeric oxonium ions A_1^+ derived from methyl 2,3,4,6-tetra-*O*-methyl-D-hexopyranosides 1–3 with NH_3 .^a

Compound	k_{exp} (s^{-1})	k_{bi} ($10^{-11} \text{ cm}^3 \text{ s}^{-1}$ molecule $^{-1}$)	Efficiency (%)	$[A_1 + NH_3]$ (%) ^b	$\sum f$ (%) ^b
1	0.103	0.93	0.57	72	28
2	0.243	2.19	1.35	40	60
3	0.116	1.04	0.64	64	36

^a $p(NH_3)_{corr} = 4.6 \times 10^{-7}$ mbar.

^b Relative intensity of adduct ions $[A_1 + NH_3]^+$, and of the sum of their fragment ions after a reaction time of 20 s.



Scheme 3

explanation, i.e. an increase in the rate of the methanol elimination from ions $[2A_1 + NH_3]^+$. An interesting, but admittedly at present still speculative, explanation for this effect is depicted in Scheme 3 and includes a methanol elimination induced by a proton transfer from the NH_3^+ group to the axial methoxy group at C(4). In any case, it is an interesting observation that the bimolecular reaction of pyranosyl cations with the nucleophile NH_3 depends on the stereochemistry of the sugar, and further studies of these effects are important for a better understanding of the gas-phase reactions of ionized saccharide derivatives and for the analytical application of mass spectrometry to this class of natural compounds.

EXPERIMENTAL

The methyl tetra-*O*-methylhexopyranosides 1–3 were prepared by standard techniques of carbohydrate chemistry; ammonia was of 99.8% purity (Merck) and used as received.

The FT-ICR measurements were performed with a Bruker Spectrospin CMS 47X FT-ICR spectrometer equipped with a 4.7 T superconducting magnet, a 24 bit/128k-words Aspect 3000 computer, a cylindrical ICR cell of 6 cm diameter and 6 cm length and an external CI ion source. The samples of 1–3 were introduced into the external ion source by a heated inlet system and ionized by isobutane CI. Each CI mass spectrum was recorded with 32k-words data, and under these conditions the base peak in the CI mass spectra of 1–3 corresponds to the $[MH^+ - nCH_3OH]$ ions A_1 and A_2 .

For the study of the ion–molecule reactions of ions A_1^+ with ammonia, these ions were isolated by a pulse sequence consisting of broad-band ejection and of 'single shots' to eliminate ions adjacent in mass to ions A_1^+ . The excess energy of the ions was minimized by the introduction of a 15 ms pulse of argon by a pulsed valve.¹¹ Ammonia was introduced continuously into the ICR cell at a pressure of about 10^{-7} mbar, and the pressure reading was corrected by measuring the rate of the reaction $NH_3^+ + NH_3 \rightarrow NH_4^+ + NH_2^+$ ($k_{bi} = 2.2 \times 10^{-9} \text{ cm}^3 \text{ s}^{-1} \text{ molecule}^{-1}$).¹² The temperature of the ICR cell was 300 K.

Acknowledgements

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