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Collision-induced loss of AgH from Ag^+ adducts of alkylamines, aminocarboxylic acids and alkyl benzyl ethers leads exclusively to thermodynamically favored product ions

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The loss of AgH from $[M + Ag]^+$ precursor ions of tertiary amines, aminocarboxylic acids and aryl alkyl ethers is examined by deuterium labeling combined with collision activation (CA) dissociation experiments. It was possible to demonstrate that the AgH loss process is highly selective toward the hydride abstraction. For tertiary amines and aminocarboxylic acids, hydrogen originates from the α-methylene group carrying the nitrogen function (formation of an immonium ion). In all cases examined, the most stable, i.e. the thermodynamically favored product ion is formed. In the AgH loss process, a large isotope effect operates discriminating against the loss of D. The $[M + Ag]^+$ ion of benzyl methyl ether loses a hydride ion exclusively from the benzylic methylene group supporting the experimental finding that the AgH loss reaction selectively cleaves the weakest C–H bond available.

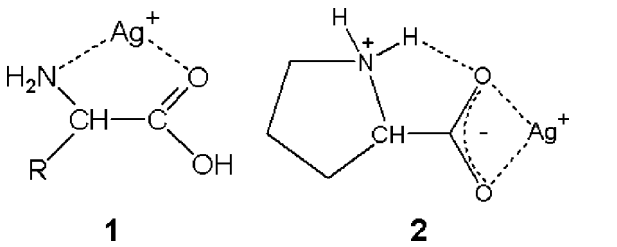
Keywords: Ag^+ adducts; tertiary amines; MS/MS-fragmentation; primary isotope effect; AgH loss

Introduction

During our investigations on the gas-phase structure of solution-phase zwitterions[1,2] we also tested the influence of Ag^+ on the fragmentation behavior of derivatized amino acids. In this context, we observed the loss of AgH from $[M + Ag]^+$ precursor ions upon collision activation (CA). The fragmentation reactions of Ag^+ molecular adduct ions of primary amines, α-amino acids and peptides have been studied extensively *inter alia* by *Shoeib, Siu* and *Hopkinson*.[3–10] Additional to the tandem MS experiments, the studies included computational modeling calculations to elucidate the energetics of the AgH loss reaction mechanism.[3–10] It turned out that for all $[M + Ag]^+$ precursor ions possessing a hydrogen atom in the α-position $[M - H]^+$ product ions are obtained upon CA by loss of AgH.[3,11] Additionally, *O'Hair et al.* demonstrated that electrospray-MS^n (ESI-MS^n) of other silver amino acid clusters $[(M + Ag - H)_x + Ag]^+$ with M = glycine or *N*,*N*-dimethylglycine, deliver Ag_3^+, Ag_5^+, Ag_7^+ and silver hydride cluster ions Ag_2H^+, Ag_4H^+ and Ag_6H^+ in the gas phase.[12]

For $[M + Ag]^+$ precursor ions of α,ω-diamino alkanes, a competition occurs between the loss of AgH and H_2.[4] However, similar reaction schemes were observed for a number of $[M + X]^+$ molecular ions of alkyl amines with singly charged group 8–10 transition metal ions X^+ as well as for Cu^+ a member of transition metal group I (electron configuration s^0d^{10}).[13,14]

For α-amino acids with alkyl side chains, the most stable conformation was calculated to be a bidentate complex with Ag^+ coordinated to the nitrogen of the NH_2-group and the carbonyl oxygen (*charge-solvation* structure **1** in Scheme 1). Owing to the higher gas-phase basicity of the NH group in proline, a zwitterionic structure is found to be more stable than an alternative *charge-solvation* structure.[3,10] In that zwitterionic structure of proline, the Ag^+ ion is bound to the negatively charged carboxylate group, which in turn is stabilized via a salt bridge to the protonated nitrogen (structure **2** in Scheme 1). For amino acids with functionalized side chains, theory predicts the formation of tridentate complexes where a side chain heteroatom or an aromatic residue provides a third binding site for the Ag^+ ion (e.g. lysine, histidine, phenylalanine etc.).[5,8,9,15,16] The rather limited D-labeling studies reported in literature show that the hydrogen lost in AgH neither originates from the exchangeable hydrogen atoms bound to nitrogen or oxygen[3,8,9,17] nor from the benzylic ones in the case of phenylalanine.[15]



Scheme 1. Structures of $[M + Ag]^+$ molecular ions of α-amino acids: *charge-solvation* **1**, *salt bridge* structure **2** of proline.

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Experimental

Chemicals

6-Dimethylaminohexanoic acid[18] was derived from the respective ε-amino acid in an Eschweiler-Clarke reaction. 6-Aminohexanoic acid was therefore refluxed in HCHO/HCOOH/H_2O for 16 h. After evaporation of the reaction mixture, the resulting white solid was suspended in toluene. The solvent was removed under reduced pressure and the crystalline crude product was purified by crystallization from acetonitrile (yield 74%).

N-Methylproline[19] was produced by methylation of proline by treatment with an aqueous formaldehyde solution (37%) for 16 h at 20 °C under reductive conditions (Pd/C + H_2 at 1 bar). After filtration over celite, the yellow liquid was concentrated under reduced pressure. The resulting oil was taken up in methanol. After removal of the solvent under reduced pressure, the desired product was obtained as a yellow solid in 98% yield.

[1,1-D_2]-1-Dimethylaminohexane[20] was synthesized from hexanoic acid dimethylamide by reduction with $LiAlD_4$ for 20 h at 20 °C in diethyl ether. After quenching with saturated Na/K-tartrate solution, the resulting mixture was extracted with *tert*-butyl methyl ether four times. The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure to give the product in 89% yield as slightly yellowish oil.

[1-D]-1-Dimethylaminohexane[20–22] was produced in three steps, starting with the reduction of hexanal with $LiAlD_4$ for 92 h at 20 °C. After the addition of saturated Na/K-tartrate solution, the reaction mixture was extracted three times with ethyl acetate. The combined organic layers were dried over magnesium sulfate and the solvent was removed under reduced pressure. [1-D]-hexanol was isolated as colorless oil in 86% yield. [1-D]-Hexanol was dissolved in dichloromethane and subsequently treated with 0.5 equ. PBr_3 for 2 h at 0 °C and for 63 h at 20 °C. After the addition of saturated $NaHCO_3$-solution, the organic layer was separated and the aqueous layer was extracted three times with *tert*-butyl methyl ether. The organic layers were combined, dried over magnesium sulfate and the solvent was removed under reduced pressure to give [1-D]-1-bromohexane as colorless oil in 65% yield. [1-D]-1-bromohexane was taken up in methanol and treated with $(CH_3)_2NH$ (40% in H_2O) for 1 h at 0 °C and for 16 h at 20 °C. After addition of sodium hydroxide (10% in H_2O), the mixture was extracted with *tert*-butyl methyl ether four times and the combined organic layers were dried over magnesium sulfate. After removal of the solvent under reduced pressure [1-D]-1-dimethylaminohexane was isolated as yellowish oil in 64% yield.

Benzyl trideuteromethyl ether[23] was synthesized by reacting benzyl bromide for 1 h at 20 °C with CD_3ONa in 2-methyltetrahydrofuran. After the addition of water, the aqueous layer was extracted with ethyl acetate two times. The combined organic layers were dried over magnesium sulfate and the solvent was removed under reduced pressure to give the product as yellowish liquid in 80% yield.

[α,α-D_2]-Benzyl methyl ether[20,21,23] was obtained in a three step synthesis, beginning with the reduction of benzoic acid ethyl ester with $LiAlD_4$ in diethyl ether for 1 h at 0 °C and for 2 h at 35 °C. After acidification with sulfuric acid (25%) the mixture was extracted with ethyl acetate four times. The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure to give the product as liquid in 91% yield. The resulting [α,α-D_2]-benzyl alcohol was treated with 0.5 equ. PBr_3 in CH_2Cl_2 for 1 h at 0 °C and for 15 h at 20 °C. After the addition of water, the organic layer was washed with saturated $NaHCO_3$-solution and dried over magnesium sulfate. The solvent was evaporated to give [α,α-D_2]-benzyl bromide as yellowish oil in 80% yield. This oil was reacted with CH_3ONa in methanol for 3 h at 0 °C and for 16 h at 20 °C. The resulting mixture was taken up in water and extracted four times with *tert*-butyl methyl ether. The organic layers were combined and dried over magnesium sulfate. After removal of the solvent under reduced pressure [α,α-D_2]-benzyl methyl ether was obtained as yellowish oil in 77% yield.

All chemicals were used as purchased without further purification (Acros Organics, Geel, Belgium and ABCR, Karlsruhe, Germany). Toluene and diethyl ether were dried by distillation from benzophenone and sodium, dichloromethane by distillation from calcium hydride. Methanol was distilled from magnesia and all other solvents were distilled over a column prior to use.

Mass spectrometry

Mass spectral data were obtained with a MAT 900 ST instrument providing an EB-quadrupole ion trap (QIT) geometry and equipped with an ESI II ion source (Thermo Fisher Scientific, formerly Finnigan MAT, Bremen, Germany). Analyte solutions were pumped through a fused silica capillary (inner diameter 100 µm; Mascom, Bremen, Germany) by a syringe pump (Harvard Apparatus, Holliston, MA, USA) safeguarding for a constant flow rate of 3 µl/min. The fused silica capillary was fed through a stainless steel capillary to which the ESI spray voltage of 3.6 kV was applied. No additional sheath gas was used to support the spraying process. The front end of the ESI II ion source was a heated transfer capillary which was held at 230 °C to provide complete desolvation. ESI-ion source conditions (adjustable voltages on the tube, ESI-capillary and the skimmer) were optimized with regard to maximum abundance of the desired $[M + Ag]^+$ ion species selected as precursor ions for the CA-MS/MS experiments. The resolution of the double focusing two sector field analyzer system was adjusted to be >5000 (10% valley). The samples were dissolved in methanol or water to give stock solutions with approximately 0.01 mM concentration. Ag^+ adduct ions of the compounds of interest were generated by adding 10 µl of a 0.01 mM solution of CF_3COOAg in CH_3CN to the respective analyte solution prior to ESI-MS experiments. The ESI-MS spectra of the analytes containing nitrogen exhibited predominantly protonated molecular ions $[M + H]^+$ along with $[M + Ag]^+$ adduct ions of moderate abundance. In the ESI-MS spectrum of benzyl methyl ether the sodium adduct ion $[M + Na]^+$ exceeded the respective $[M + Ag]^+$ ion in abundance. The ESI-MS spectrum of hexyl methyl ether exclusively exhibited the $[M + Na]^+$ molecular ion. All ion source optimization efforts failed for that analyte to generate $[M + Ag]^+$ species of notable abundance. Fragmentation reactions of the $[M + Ag]^+$ precursor ions were induced by low energy CA either in an octapole located in front of the QIT or in the QIT itself (helium was used in the QIT as bath gas with $\sim 2 \times 10^{-3}$ Pa; helium diffusing in the octapole was utilized as collision gas therein, collision gas pressure in the octapole was not measured separately). For all product ion experiments monoisotopic precursor ion selection was adjusted. The center of mass collision energy E_{cm} and the laboratory collision energy E_{lab} of the tandem MS measurements in either the QIT or the octapole were not explicitly determined.[24] Appropriate experimental conditions were adjusted independently for each individual experiment to yield significant analytical information.

The AgH loss reaction was investigated toward a primary kinetic isotope effect (KIE) by tandem MS with the $[M + Ag]^+$ precursor ion

of [1-D]-1-dimethylaminohexane. General understanding of KIEs distinguishes certain cases.[25] For the examined fragmentation reaction, the precursor ion decomposes to give identical but isotopically distinct product ions, i.e. $[M + Ag - AgH]^+$ or $[M + Ag - AgD]^+$. In this case, it is justified to approximate a primary KIE (i.e. the ratio of kinetic constants of the competing fragmentation channels as functions of internal energy of the precursor species) by the ratio of the signal intensities of the respective product ions. Furthermore, it is reasonable to assume that a given KIE of that kind might not vary much over a narrow range of energy.[25] The experiments with [1-D]-1-dimethylaminohexane were executed in the QIT and the octapole of the Finnigan MAT 900 instrument under respective CA conditions. Both experiments yielded a ratio of the product ion abundances related to the AgH-to-AgD loss of about 2 : 1. Hence, the distribution of internal energy of the precursor ions after CA in the QIT or in the octapole seems to be at least comparable.

The measurement of the exact ion masses of the $[M + {}^{107}Ag]^+$ and $[M + {}^{109}Ag]^+$ precursor ions of 6-dimethylaminohexanoic acid and of the shared $[M - H]^+$ product ion, i.e. generated after the ${}^{107/109}AgH$ loss, were conducted on LTQ-Orbitrap XL instrument (Thermo Fisher Scientific, Bremen, Germany) at a resolution of 100 000 fwhh by Dr E. Damoc. The measurement of the exact ion masses were first carried out with external calibration and repeated referring to ${}^{107}Ag^+$ and ${}^{109}Ag^+$ as internal standard ions. The absolute experimental error of the exact ion mass measurements ranged between 0.3 and 0.4 mmu, the relative error was 1.1 – 1.9 ppm. The contribution of Dr E. Damoc (Thermo Fisher, Bremen Germany) is explicitly acknowledged.

Results and Discussion

For our systematic study of the AgH loss reaction, we selected tertiary amino acid compounds with the intention to block the competing H_2 loss fragmentation channel that is observed for $[M + Ag]^+$ molecular ions of primary and secondary amines upon CA.[4,10–13] Furthermore, we decided to study deuterated tertiary amines with an unsymmetrical substitution pattern to enable a differentiation of the $[M + Ag - AgH]^+$ immonium ions observed as product ions of the AgH loss.

Hence, the $[M + Ag]^+$ molecular ion of 6-dimethylaminohexanoic acid was selected for CA experiments (in all investigations, parallel experiments were performed with ions containing the isotopes ${}^{107}Ag$ and ${}^{109}Ag$). CA of the $[M + Ag]^+$ molecular ion of 6-dimethylaminohexanoic acid delivered exclusively the respective $[M + Ag - AgH]^+$ product ion (Fig. 1). A control experiment with the COOD analog of 6-dimethylaminohexanoic acid showed that the deuterium atom is completely retained in the $[M + Ag - AgH]^+$ product ion, i.e. an exclusive AgH loss was observed. With that we assume that the tertiary nitrogen plays a more important role for the complexation of the silver ion in the $[M + Ag]^+$ ion than the oxygens of the acid moiety. However, the experimental findings are in good agreement with the published data on the AgH loss reactions of amines.[3,8,9,17] Nevertheless, the exact structure of the $[M + Ag - AgH]^+$ product ion cannot be deduced.

To yield structural information on the particular immonium ion and to study the selectivity of this reaction [1,1-D_2]-1-dimethylaminohexane was chosen as a model compound for the analog amino acid. The $[M + Ag]^+$ precursor ion of [1,1-D_2]-1-dimethylaminohexane exhibits an exclusive AgD loss upon CA in the QIT (Fig. 2), thus proving evidence for the exclusive formation of the thermodynamically favored immonium ion (structure **3** in Fig. 2). To see to what extent an isotope effect[25] may operate, [1-D]-1-dimethylaminohexane was synthesized and investigated. CA experiments of the respective mono-deuterated $[M + Ag]^+$ precursor ion in the octapole and in the QIT exhibited a ratio of the AgH-to-AgD loss of about 2 : 1 (Fig. 3). It is worth noting that notwithstanding the substantial isotope effect and despite of the statistical ratio of 1 : 3 for $CD_2/2CH_3$ for [1,1-D_2]-1-dimethylaminohexane elimination occurs only from the CD_2 methylene group. In this context, it is interesting to note that an isotope effect of similar extend (1.6 and 1.7) discriminating against deuterium was found for the abstraction of benzylic hydride ions

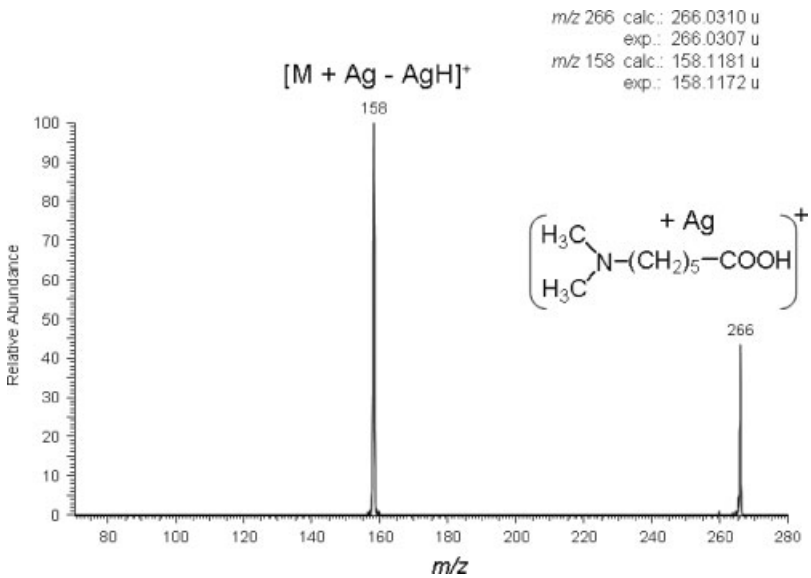


Figure 1. QIT-MS^2 product ion spectrum of the $[M + {}^{107}Ag]^+$ ion of 6-dimethylaminohexanoic acid at *m/z* 266. The neutral loss of $\Delta m = 108$ u was determined by exact mass measurements of the precursor and product ion to be the loss of ${}^{107}AgH$. Analogous results were obtained for the loss of ${}^{109}Ag$ from $[M + {}^{109}Ag]^+$.

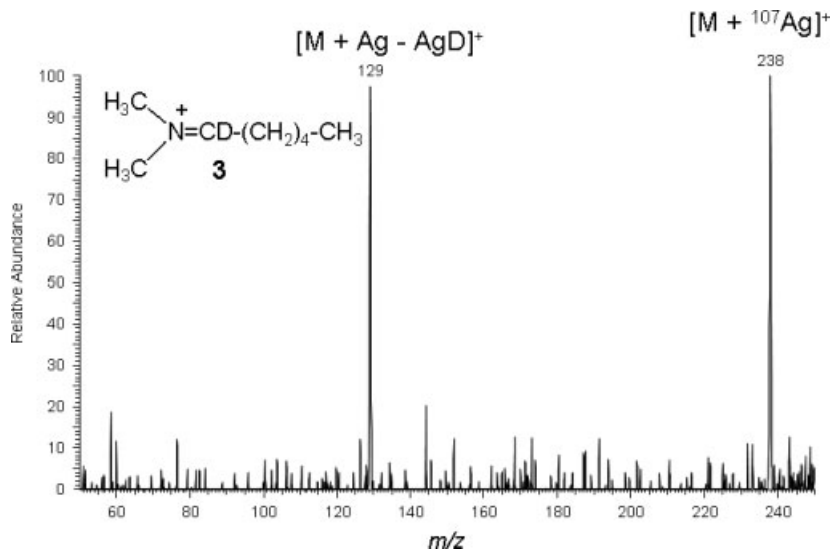


Figure 2. QIT-MS^2 product ion spectrum of the $^{107}Ag^+$ molecular adduct ion of [1,1-D_2]-1-dimethylaminohexane at *m/z* 238. The exclusive neutral loss of $\Delta m = 109$ u shows the selective loss of ^{107}AgD and the formation of the respective $[M + Ag - AgD]^+$ immonium ion **3** at *m/z* 129.

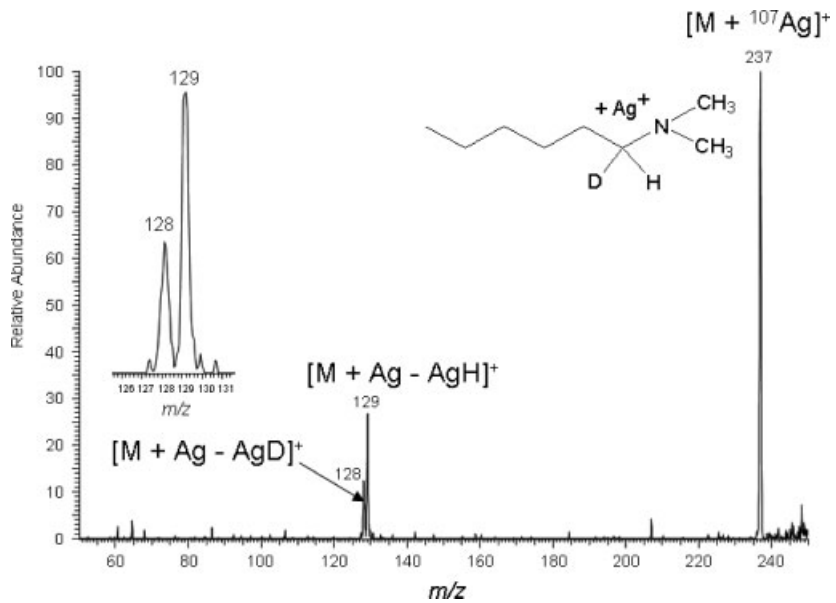


Figure 3. Octapole-MS/MS product ion spectrum of the $[M + {}^{107}Ag]^+$ ion of [1-D]-1-dimethylaminohexane at *m/z* 237. An isotope effect was documented by the ratio of the signal intensities of the product ions at *m/z* 128: 129 (i.e. the loss of AgD *vs* loss of AgH) which was found to be ≈1:2.

by *tert*-butyl cations (CH_2 *vs* CD_2).[26,27] Analog KIEs were found for the highly regioselective CA decomposition FeO^+ adduct ions of methoxybenzenes and related analytes, in which the C–H bond of the methoxy group was cleaved.[28] Finally, the gas-phase reaction of *N*,*N*-dimethylanilines with the oxo iron(IV) porphyrin radical cation exhibits a formal hydride transfer which is consistent with the operation of a substantial KIE.[29]

However, the results presented above show with convincing clearness that the AgH loss strictly accounts for the stability of the product ion as it exclusively leads to the formation of the higher substituted immonium ion regardless to the hydrogen isotope present.

After the examination of the tertiary amine with different *n*-alkyl substituents and analogous *tert*-amino acid compounds we chose a cyclic analog for further testing. Obviously, *N*-methylproline is an adequate candidate for this purpose as the AgH loss behavior of the natural amino acid analog was closely examined before.

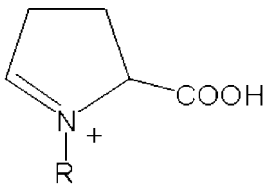
In *N*-methylproline, the proton affinity[30] of the nitrogen atom is further increased as a result of the additional alkyl substituent making a "*salt bridge*" structure of the $[M + Ag]^+$ ion in the gas phase energetically even more favored than in the case of proline (structure **2** in Scheme 1). The loss of AgH is the main fragmentation process upon CA of the $[M + Ag]^+$ ion of *N*-methylproline. The hydrogen atom of the acid moiety is retained in the $[M + Ag - AgH]^+$ product ion of *N*-methylproline as a D_2O exchange experiment shows. In that experiment, an interesting loss of 3 u is observed with minimal abundance (data not shown). In analogy to published data of proline, we conclude that a HD elimination is detected, which requires an internal protonation of the nitrogen atom prior to the fragmentation

reaction.[10] Accordingly, the presence of a gas-phase zwitterion of *N*-methylproline in the $[M + Ag]^+$ ion can be proposed.

These considerations offer another aspect to the discussion of the experimental results achieved with 6-dimethylaminohexanoic acid. As presented above in the product ion experiment, the respective $[M + Ag]^+$ ion loses exclusively AgH. Consequently, the absence of H_2 loss in the case of 6-dimethylaminohexanoic acid points toward the presence of a charge solvation structure in the $[M + Ag]^+$ ion. With that, the criteria that are determining the formation of gas-phase zwitterions can be evaluated anew. Clearly, the amphoteric character of the analyte has to be sufficiently pronounced (i.e. a high gas-phase basicity and acidity), but, the molecule structure is crucial as the formation of efficient salt bridge interactions has to be arranged to substantially stabilize a zwitterion in the gas phase. According to this interpretation, the latter criterion permits the formation of a zwitterion structure of *N*-methylproline but prevents the same for 6-dimethylaminohexanoic acid in the respective $[M + Ag]^+$ ions. However, it must be emphasized, that the nature and the physicochemical character of the counter ion, i.e. the ion radius, charge state and polarizability play a major role in the formation and stabilization of zwitterions as well.[31,32]

For the silver and the alkali metal adduct ions of proline $[M+X]^+$ with X = Li, Na, K, Ag theory predicts a gas-phase structure including an internal salt bridge, i.e. a zwitterionic structure in which the negatively charged carboxylate group coordinates the metal ion and the nitrogen function is protonated (*salt bridge* structure **2** in Scheme 1).[5,10,33,34] This structural assignment is supported by the results of the CA experiments: a loss of H_2 (2u) is observed for the $[M + Ag]^+$ precursor ion of proline and a loss of HD (3u) is found in an analogous CA experiment with D_2O exchange.[10] Although this unique fragmentation channel is of low abundance, it is a strong hint toward the presence of zwitterionic structure of proline and its *N*-methylated analog in their gas-phase $[M + Ag]^+$ ions.

The structure of the $[M + Ag - AgH]^+$ immonium ion of proline was elucidated by CA experiments. Such an experiment with the $[M + Ag]^+$ ion of [2-D]-proline showed retention of the D-atom, i.e. a loss of AgH and of H_2 was detected.[10] Additionally, the exchangeable H-atoms at the carboxyl and the amine functionality are retained during the loss of AgH. In agreement with the structures for the respective immonium ions discussed above the $[M + Ag - AgH]^+$ ion of proline was assigned to be structure **4a** (Scheme 2). Owing to the very similar fragmentation behavior of *N*-methylproline and proline regarding the loss of AgH and H_2 we assume that both immonium ions formed are closely related (structure **4a** and **4b** in Scheme 2).



4a, R=H **4b**, R=CH_3

Scheme 2. Structures of $[M+Ag-AgH]^+$ immonium product ions formed by the AgH loss from $[M + Ag]^+$ precursor ions of proline **4a** and its *N*-methyl derivative **4b**.

According to Pearson,[35] Ag^+ is a soft Lewis acid and should therefore bind preferentially to soft bases like N- or S-functionalities rather to the harder rated oxygen if available at the same time. This assumption is convincingly supported by the results of the series of CA experiments with the $[M + Ag]^+$ ions of the amino acid compounds as the exchangeable hydrogens are retained in the $[M + Ag - AgH]^+$ product ions.

In that context it seemed of interest to test the behavior of Ag^+ toward isolated oxygen functionalities. The only pertinent observation in this respect reported in literature is that several methyl glycosides[36] form $[M + Ag]^+$ ions under Fast Atom Bombardment (FAB) conditions. The silver adduct ions of these molecules lose AgH when the methoxy group and the C-2 bound hydroxyl group are in *trans* position (observed in mass-analyzed ion kinetic energy, MIKE, experiments). Specific D-labeling at C-1, C-2 and C-6 and exchange of the four OH-groups to OD showed that only the anomeric D (C-1) is lost as AgD. Coordination of Ag^+ with the hydroxyl group at C-2 and elimination of AgH in a five-membered transition state was suggested.

To examine $[M + Ag]^+$ of compounds containing an oxygen we chose compounds with an ether functionality, instead of the tertiary amino nitrogen. In a first attempt *n*-hexyl methyl ether was tested. However, we failed to generate $[M + Ag]^+$ ions of notable abundance of that dialkyl ether. The obviously lacking stability of the $[M + Ag]^+$ ions of this compound is very likely due to the absence of a second coordination site for the Ag^+ ion. This prerequisite is however fulfilled in the case of benzyl methyl ether where the twofold coordination of Ag^+ to the oxygen and to the π-system of the phenyl ring is responsible for the observation of an abundant $[M + Ag]^+$ ion of obviously sufficient stability (*cf* phenylalanine).[5,15,16,30] Specific D-labeling of the methyl and the benzylic methylene group showed exclusive AgH loss from the former and AgD loss from the latter (Fig. 4(a) and (b)). These results are in complete accordance to the experimental findings of the tertiary amines and respective amino acids. In all the cases investigated exclusively the most stable fragment ion is formed by loss of AgH.

In addition, to further the ESI-tandem-MS investigations of silver adduct ions of hydrocarbons,[37] the examination of respective gas-phase $[M + Ag]^+$ species by extended density functional theory calculations are projected.

Conclusions

The study of the AgH loss reaction observed upon CA dissociation from $[M + Ag]^+$ precursor ions yielded detailed information on the structure of the product ions and indirect evidence on the structure of the precursor ions as well. By selective labeling experiments it was possible to demonstrate that the AgH loss process is highly selective toward the hydride abstraction. Tertiary amines and aminocarboxylic acids lose a hydride from the α-methylene group carrying the nitrogen function (formation of an immonium ion). In all cases examined, the thermodynamically favored product ion, i.e. the most effectively stabilized immonium ion, is formed. The $[M + Ag]^+$ ion of benzyl methyl ether loses a hydride H- exclusively from the benzylic position, again forming the most stable oxonium product ion $[M + Ag - AgH]^+$. The formation of the most stable product ion seems to be a universal feature of the AgH loss reaction independent from the nature of the binding partners that form

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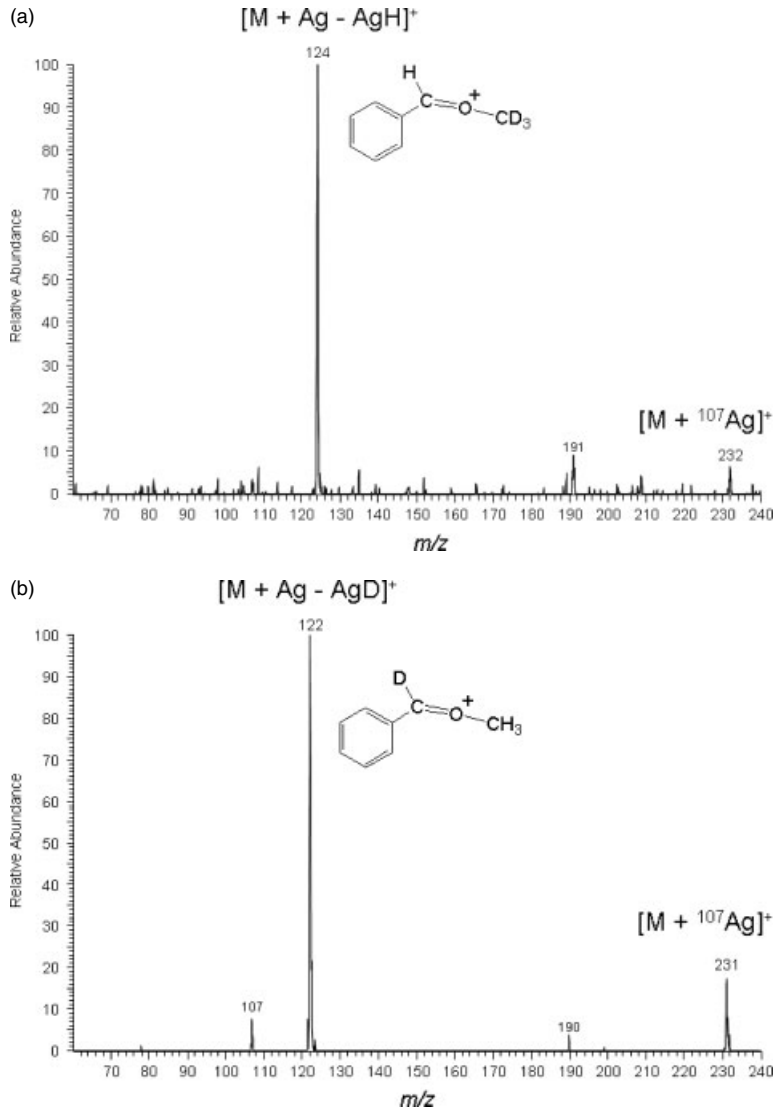

Figure 4. (a) QIT-MS^2-product ion spectrum of the $[M + {}^{107}Ag]^+$ ion of benzyl trideuteromethyl ether at *m/z* 232, (b) QIT-MS^2-product ion spectrum of the $[M + {}^{107}Ag]^+$ ion of [α,α-D_2]-benzyl methyl ether at *m/z* 231. The formation of the low abundant product ions at *m/z* 191 in (a) and 190 in (b) cannot be reasonably explained by CA of the benzyl methyl ether. We assume that the detected neutral loss of 41 u points toward the unintended CA dissociation of an isobaric species at *m/z* 232 and 231, respectively.

the complex with the silver ion (e.g. nitrogen, oxygen, aromatic π-system).

The examination of the $[M + Ag]^+$ ion of proline and its *N*-methylated derivative gave rise to an additional product ion species formed by the loss of H_2. Labeling experiments clearly hint toward the presence of zwitterionic structures for those amino acid compounds that show this characteristic neutral loss in the gas-phase. However, the hydrogen loss reaction was only found with minimal abundance and is therefore error-prone. In that respect more evidence has to be collected.

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