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Fragmentation pathways of organoarsenical compounds by electrospray ion trap multiple mass spectrometry (MS^6)

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Abstract

With its detection limit well below 30 pg μl^{-1} LC–MS–MS has become a sensitive and thus popular analytical technique for organoarsenical compounds. Collision induced dissociation (CID) is a valuable tool for speciation and facilitates a positive identification of the species detected. However, it is not straightforward to understand the fragmentation pathways of organoarsenical compounds when only CID-MS–MS data is available. In the present paper we have investigated multiple mass spectrometry (MS^n, $n=1, 2, 3, 4, 5, 6$) with electrospray CID fragmentation for a number of organoarsenical compounds likely to occur in the environment. The investigated compounds were tetramethylarsonium, trimethylarsinoxide, monomethylarsonic acid, dimethylarsinic acid, arsenobetaine, arsenocholine, and dimethylarsinoylethanol. By CID of (protonated) organoarsenical cations mostly even-electron fragments are produced after neutral loss processes such as elimination of H_2, H_2O, CH_4, C_2H_2, C_2H_4, C_2H_6, HCHO, CH_3OH, C_2H_5OH, C_2H_4O, and CH_2CO. However, abundant odd-electron fragments are also formed after elimination of radical species. Evidence for reduction of As(V) to As(III) as a driving force in the odd-electron ion formation is obtained.

Keywords: Mass spectrometry; Interfaces, LC–MS; Organoarsenic compounds

1. Introduction

The biochemistry and environmental fate of organometallic compounds are of great importance, primarily due to their high toxicity. Elemental speciation of arsenic has received considerable attention. Until recently the applied analytical techniques often did not assure a complete speciation scheme or did not allow for a positive identification of the species detected and eventually quantified. With the development of atmospheric pressure ionisation (API) sources APCI (atmospheric pressure chemical ionisation) and ESI (electrospray ionisation) it has become technically feasible to analyse polar and non-volatile compounds with liquid chromatography–mass spectrometry (LC–MS) in a similar way to gas chromatography (GC)–MS of non-polar volatile compounds. By coupling LC to a triple quadrupole mass spectrometer structural information on an analyte can be obtained by collision induced dissociation (CID) MS–MS. The first example of this technique for organoarsenical compounds was presented by Siu et al. as early as 1988 [1]. These authors presented APCI-MS–MS spectra of (protonated) dimethylarsinic acid (DMAA), monomethylarsonic acid (MMAA), arsenobetaine (AB), and arsenocholine (AC). However, not until the development of robust and affordable ESI sources in

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the mid-1990s has the use of LC–MS for analysis of organoarsenical compounds in real samples been demonstrated [2–6]. Interpretation of CID mass spectra has been offered by Siu et al. [1] and Florencio et al. [7] for (protonated) DMMA, MMAA, AB, AC as well as tetramethylarsonium (TMA) [7] obtained with LC–ESI-MS–MS and by Pergantis et al. for protonated and deprotonated arsenosugars obtained by fast atom bombardment LC–MS–MS [8].

It is not straightforward to propose fragmentation pathways when only CID-MS–MS data is available. With the development of ion trap MS it has become possible to repeat the MS–MS process a number of times by CID directly in the trap through resonant excitation followed by collision with helium buffer gas atoms and thus obtain further information on the observed CID fragments. With API sources ion-trap mass spectrometers can easily be coupled to liquid chromatography, a technique referred to as LC–MS^n. ESI and APCI produce even-electron (EE) molecular ions for which decomposition processes and pathways are much better understood today than just a few years ago. They include simple bond cleavages, cleavages with hydrogen transfer rearrangement and skeletal rearrangements. In a typical LC–MS^n experiment, full scan LC–MS spectra are first recorded and the (de)protonated molecule(s) are identified. Next, LC–MS–MS spectra are recorded by isolating the (de)protonated molecule(s) in the ion-trap followed by CID. The energy required in this process typically varies between 10 and 40% of the total available resonant excitation collision energy and can be optimised to preserve a signal of the precursor ion in the order of 5–10%. This process can be repeated a number of times (MS^n) by successive isolation of one of the generated ions (product ions) as long as the CID process yields products ions larger than the instrumental lower mass range limit.

In the present investigation we have applied LC–MS^n to the study of CID fragmentation of seven organoarsenical compounds likely to occur in the environment, i.e., (protonated) TMA, DMAA, MMAA, AB, AC together with trimethylarsinoxide (TMOA) and dimethylarsinoylethanol (DMAOE). While MS–MS spectral interpretations have been proposed before in the literature for TMA, DMAA, MMAA, AB, AC, and TMOA nothing has been done to confirm these interpretations. In the present paper we investigate consistency of the proposed interpretations by multiple mass spectrometry (MS^n, $n=1, 2, 3, 4, 5, 6$) and present CID data on DMAOE for the first time.

2. Experimental

The ESI-MS analysis was performed with a Finnigan MAT LCQ ion trap mass spectrometer equipped with a 250-μl diffusion pump and an ESI interface. The ion source was operated in the positive ion mode. The sheath and auxiliary gases were N_2 (80 arbitrary units) and He (10 arbitrary units), respectively. The capillary temperature was 265°C, and the spray voltage 4.1 kV. Preliminary experiments with APCI showed that this ionisation technique is less specific and sensitive than ESI for the investigated compounds.

CID multiple MS spectra (MS^n, $n=1, 2, 3, 4, 5, 6$) were acquired with standard solutions of the pure compounds (10 ng ml^{-1}) injected with the infusion pump (30 μl min^{-1}). Full scan LC–MS spectra were first recorded and the (protonated) molecule(s) were identified. Next, LC–MS–MS spectra were recorded by isolating the (protonated) molecule(s) in the ion trap followed by (wide-band) activation energy CID. The energy required in this process varied between 10 and 90% of the total available collision energy and was selected to preserve a signal of the precursor ion in the order of 5–10%. This process was repeated up to six times by successive isolation of one of the generated ions (product ions). The obtained information served as basis for proposing fragmentation pathways. It is important to notice that the pathways still await confirmation, e.g., by extensive isotope labelling studies and are drawn mainly to rationalise the observed fragments.

All organoarsenical reference compounds were obtained from Tri Chemical Laboratory as neat crystals of a purity greater than 98% except for dimethylarsinoylethanol, which was synthesised and kindly provided by Rob Ritsema.

3. Results and discussion

Until now the organoarsenical compounds have been analysed by LC–MS with the use of a triple

quadrupole spectrometer operated in the tandem MS mode (MS–MS), which in the scan mode provides mass spectra for compound identification and which in combination with selected reaction monitoring (SRM) can provide excellent selectivity and sensitivity [1,8]. The ESI-MS–MS spectra obtained in the present study by resonant excitation ion trap mass spectrometry are shown in Fig. 1 for each compound after CID of the (protonated) molecular ions. By varying the applied collision energy different intensities of the product ions could be observed, but additional fragments could not be obtained. The amount of CID energy was optimised for each compound to produce the most intensive signal for a major fragment. The following values were obtained: AC (m/z 165→m/z 121, 50% wide-band activation), TMA (m/z 135→m/z 120, 35% narrow-band activation), TMAO (m/z 137→m/z 119, 35% wide-band activation), DMAOE (m/z 167→m/z 105, 45% wide-band activation), AB (m/z 179→m/z 161, 35% wide-band activation), DMAA (m/z 139→m/z 121, 90% narrow-band activation), MMAA (m/z 141→m/z

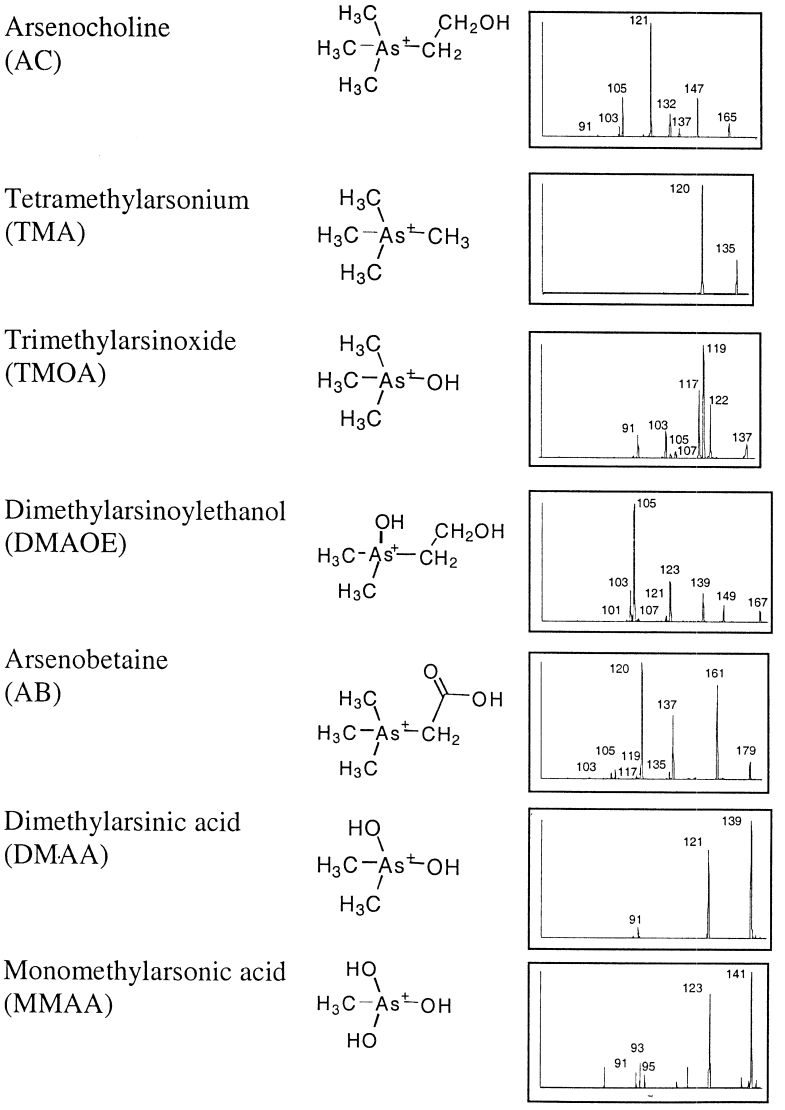

Fig. 1. Chemical structures and ESI-MS–MS-CID ion trap mass spectra of protonated organoarsenical compounds.

123, 90% wide-band activation). By coupling the ion trap to a HPLC system (data not shown) very good sensitivities could be reached with this SRM acquisition table for the investigated compounds. The obtained detection limits ($S/N=3$) were well below 30 pg μl^{-1}, which is comparable though slightly inferior to the detection limits reported for LC–MS–MS using micro-LC and a triple quadrupole spectrometer [3].

3.1. Multiple mass spectrometry (MS^n) fragmentation

The spectral interpretation of organoarsenical compounds is not trivial and assignments of plausible structures of observed fragments require additional experimental data than those obtainable with CID-MS–MS. In the following fragmentation pathways after CID multiple mass spectrometry are proposed and discussed for each of the investigated compounds.

3.1.1. Tetramethylarsonium

The ESI-MS–MS spectrum of TMA (m/z 135) is very simple and contains only one fragment ion at m/z 120, which obviously must derive from the loss of a methyl radical (Fig. 1). The most common decomposition processes and pathways after CID of EE molecular ions involve formation of new EE ions after elimination of stable, neutral molecules. Yet, the elimination of radical species to produce odd-electron (OE) molecular ions can be observed in organic compounds when the resulting radical ions are structurally stabilised [13]. During the investigation of organoarsenical compounds the formation of OE molecular ions was frequently observed and can be attributed to a change in the oxidation step in the central As atom of the organometallic complex. The reduction of As(V) in the tetramethylarsonium to As(III) is fulfilled in the MS^3 step by the elimination of a second methyl radical to form $(CH_3)_2As^+$ as shown in Fig. 2. Additional CID steps (MS^4 and MS^5) lead to consecutive elimination of molecular hydrogen by formation of cyclic As(III) complexes (Fig. 2). The fragment ion at m/z 103 has previously been observed as a major product ion in the triple quadrupole MS–MS spectrum of TMA [7] and assigned the non-cyclic structure $(CH_2)_2As^+$, in

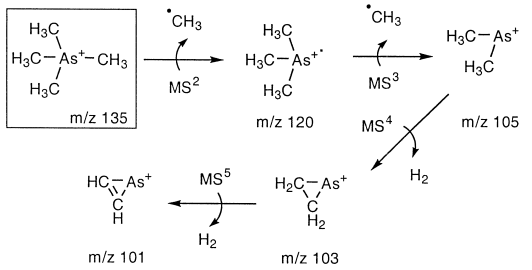

Fig. 2. CID (MS^n) fragmentation scheme for tetramethylarsonium (TMA).

which arsenic occurs as As(V). From the multiple MS sequence (m/z 105→m/z 103→m/z 101→none) observed in the present ion trap study a cyclic structure with arsenic preserved as As(III) is more likely.

The product ions obtained from TMA and all other investigated organoarsenic compounds by MS–MS and subsequent MS^n are in overall agreement (different intensities) with those obtained in a previous study by LC–ESI triple quadrupole MS–MS [7] with one important exception. High-energy CID (65 V) by triple quadrupole MS–MS produce fragment ions by reduction of arsenic all the way to As(I). This was not the case for in-the-trap CID and highlights the intrinsic differences of the two techniques.

3.1.2. Trimethylarsinoxide

The ESI-MS–MS spectrum of protonated TMAO (m/z 137) is very complex and contain EE as well as OE product ions (Fig. 1). The favoured decomposition process involves the elimination of a water molecule to produce a fragment ion at m/z 119 (Fig. 3). Additional CID displays three multiple MS sequences (m/z 119→m/z 117→m/z 115→none), (m/z 119→m/z 91→m/z 89→none) and (m/z 119→m/z 103→none). The former sequence is favoured and points to the cyclic structures for the fragment ions at m/z 117 and m/z 115 assigned in Fig. 3. The second sequence excludes a cyclic structure for the fragment ion at m/z 103, which would have been continued by a further elimination of molecular hydrogen. The third and least favoured sequence proceeds via the elimination of molecular ethene and involves the disruption of two C–As bonds.

Another significant decomposition process of pro-

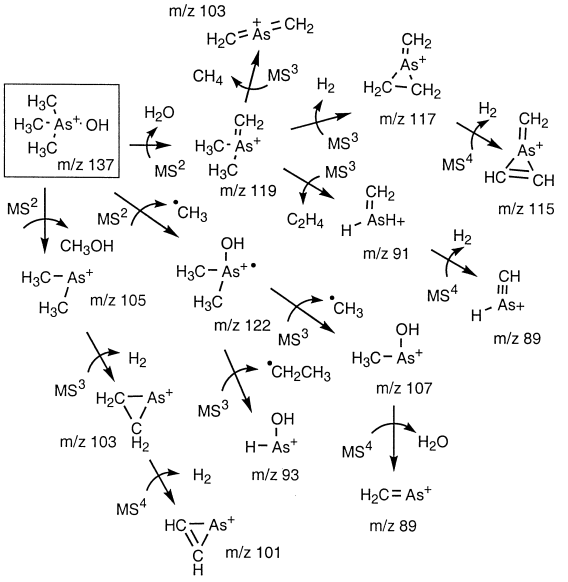

Fig. 3. CID (MS^n) fragmentation scheme for protonated trimethylarsinoxide (TMAO).

tonated TMAO involves the elimination of a methyl radical to produce an intensive fragment ion at m/z 122 with only one possible structure. Just as for the OE ion in the spectrum of TMA the MS^3 of this OE ion fulfills the reduction of As(V) to As(III) by the elimination of a second alkyl radical in the form of $^{\cdot}CH_3$ to form $CH_3–As^+–OH$ (preferred) and in the form of $^{\cdot}CH_2CH_3$ to form $H–As^+–OH$. The proposed structure of the former ion is supported by its ability to eliminate molecular water in the next CID step (MS^4) to form $CH_2=As^+$.

A minor yet detectable decomposition process involves the elimination of methanol to produce a multiple MS sequence (m/z 105→m/z 103→m/z 101→none) already discussed above for TMA.

3.1.3. Dimethylarsinic acid

High energy (90%) can be applied to protonated DMAA without significantly reducing the base peak at m/z 139 (Fig. 1). The elimination of a water molecule produces a major fragment at m/z 121, which can be assigned the structure $(CH_3)_2As^+=O$ on the basis of the MS^n analysis shown in Fig. 4. The three multiple MS sequences: (m/z 121→m/z 91→none) after elimination of ethane, (m/z 121→m/z 93→m/z 91→none) after elimination of ethene and

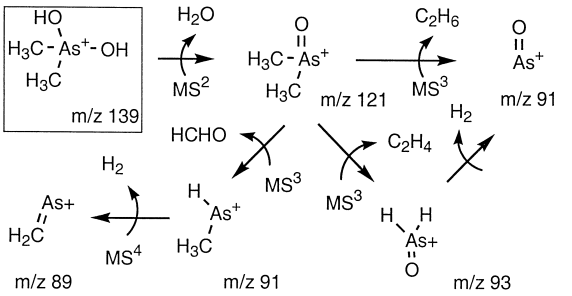

Fig. 4. CID (MS^n) fragmentation scheme for protonated dimethylarsinic acid (DMAA).

subsequently molecular hydrogen, and (m/z 121→m/z 91→m/z 89→none) after the elimination of formaldehyde and subsequently molecular hydrogen all involve simultaneous disruption of more than one C–As bond and are therefore not very evident in the MS–MS spectrum of DMAA.

Fragment ions at m/z 124 (elimination of methyl radical), m/z 109 (elimination of ethane), and m/z 108 (elimination of ethyl radical) have previously been observed by triple quadrupole MS–MS using APCI [1] and ESI [3,7]. These ions were not detected in the present MS^n study after CID in the ion trap.

3.1.4. Monomethylarsonic acid

Protonated MMAA can also be supplied at very high energy (90%) in the ESI-MS–MS analysis without significantly reducing the base peak at m/z 141 (Fig. 1). Elimination of a water molecule produces the major fragment ion at m/z 123 with the obvious structure of protonated methylarsinic acid as depicted in Fig. 5. MS^3 analysis produces three fragment ions, namely $HO–As^+–H$ after elimination

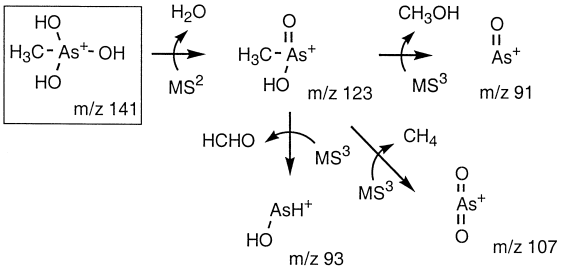

Fig. 5. CID (MS^n) fragmentation scheme for protonated monomethylarsonic acid (MMAA).

of formaldehyde (favoured), $O{=}As^+$ after elimination of methanol and $^+AsO_2$ after elimination of methane (minor). Fragment ions at *m/z* 109, *m/z* 77 and *m/z* 75, which have previously been observed by LC–ESI triple quadrupole MS–MS [3,7] were not detected in the present MS^n study.

3.1.5. Arsenobetaine

The ESI-MS–MS spectrum of AB (*m/z* 179) is very complex and contain EE as well as OE product ions (Fig. 1). Multiple MS data obtained with TMA and protonated TMAO facilitated the assignment of structures to the obtained product ions from AB. The favoured decomposition process involves the elimination of a water molecule to produce a fragment ion at *m/z* 161 (Fig. 6). Additional CID produces OE product ions at *m/z* 120 (elimination of ketenyl radical, favoured) and at *m/z* 146 (elimination of methyl radical, minor). The former exhibits a multiple MS sequence identical to the fragment ion at *m/z* 120 obtained by MS–MS of TMA (*m/z* 120→*m/z* 105→*m/z* 103→*m/z* 101→none). The latter proceeds by a second elimination of a methyl radical to form a methylketenyl–As(III)$^+$ ion at *m/z* 131, which structure is supported by the MS^5 data that demonstrate elimination of CO and ketene.

The second important decomposition process of AB is the elimination of ketene to produce protonated TMAO at *m/z* 137. Further CID of this product ion confirmed its structure by its identical multiple MS sequence to that of the reference compounds. We have often observed the formation of the ketene

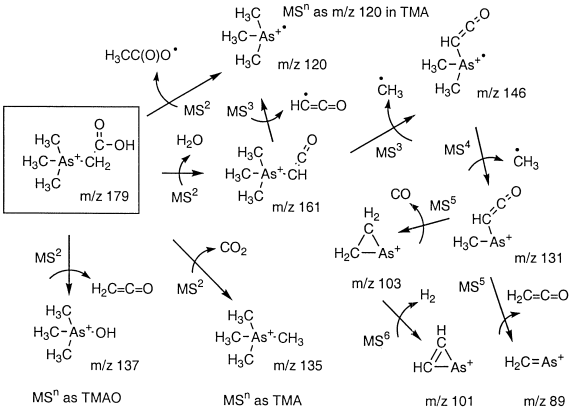

Fig. 6. CID (MS^n) fragmentation scheme for arsenobetaine (AB).

structure by CID of compounds containing an ethanoic acid segment in their molecular structure [9–12]. However, for deprotonated carboxylic acids the elimination of CO_2 is favoured. For AB elimination of CO_2 to produce TMA is insignificant but detectable and confirmed by MS^n of the product ion at *m/z* 135. Previous triple quadrupole MS–MS experiments have also shown the insignificance of CO_2 elimination from AB [1,3,7].

A third important decomposition process of AB is initiated by the disruption of the As–CH_2 bond to produce an OE product ion at *m/z* 120, which is identified by MS^n analysis to be identical to the OE product ion at *m/z* 120 in the mass spectrum of TMA.

One interesting point to note is the low intensity of the product ion at *m/z* 105 in the MS–MS spectrum of AB, which has been detected previously as a major product ion by triple quadrupole MS–MS [1,7]. This fragment derives from multiple elimination steps and requires more energy than that available from resonant excitation collision with He in the ion trap. By increasing the wide-band activation energy it was possible to observe enhanced signals at *m/z* 105.

3.1.6. Arsenocholine

The ESI-MS–MS spectrum of AC (*m/z* 165) is also very complex and contain EE as well as OE product ions (Fig. 1). Nevertheless, multiple MS data obtained with protonated TMAO facilitated the assignment of structures to the obtained product ions from AC. The favoured decomposition process involves the elimination of a neutral molecule with the molecular mass 44 u to produce an intense product ion at *m/z* 121 (Fig. 7). The proposed structure for the eliminated compound is the cyclic ethylene oxide, in agreement with an electron cascade mechanism initiated by migration rearrangement of the hydrogen atom from the alcohol group to As. Additional CID produces $(CH_3)_2As^+$ (elimination of methane) and further MS^n follows the previously described sequence for this fragment ion. The formation of the product ion at *m/z* 121 has been observed by triple quadrupole ESI-MS–MS [3] but is in contrast with triple quadrupole APCI-MS–MS data, which does not report this ion at all [1] but produces a product ion at *m/z* 120 as the main peak in the

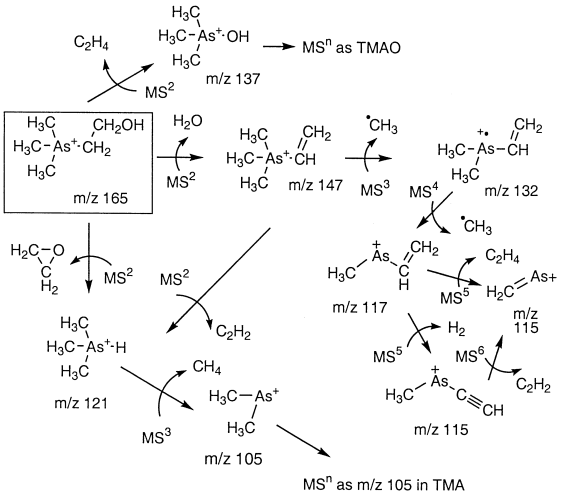

Fig. 7. CID (MS^n) fragmentation scheme for arsenocholine (AC).

spectrum. As before, the differences may be explained by the different energy available for CID in the two methods.

Another favoured decomposition process involves the elimination of a water molecule to produce a fragment ion at m/z 147, which by subsequent CID proceeds via an OE process to yield the fragment ion at m/z 132 (loss of methyl radical). In the same way as for the OE ions in the spectrum of TMA, protonated TMAO, and AB the MS^3 of this OE ion fulfills the reduction of As(V) to As(III) by the elimination of a second alkyl radical to produce $CH_3{-}As^+{-}CH{=}CH_2$ at m/z 117, the structure of which is supported by the MS^5 and MS^6 analysis.

A less important decomposition process of AC is the elimination of ethene to produce a fragment ion at m/z 137. Further CID identified the structure of this ion to be protonated TMAO by comparison of its multiple MS sequence to that of the reference compounds. The mechanism for the elimination of ethene from the ethanolic segment of AC may be explained by an electron cascade reaction initiated by the shift of the electrons from the C–O bond to As.

3.1.7. Dimethylarsinoylethanol

CID fragmentation of protonated DMAOE has not been reported in the literature before. The ESI-MS–MS spectrum is complex but contains only EE product ions (Fig. 1). By analogy to the fragmentation pathways described above for the other investigated organoarsenical compounds – in particular the similar compound AC – we were able to propose a fragmentation scheme for DMAOE and assign structures to the obtained product ions. The base peak in the MS–MS spectrum is the product ion $(CH_3)_2As^+$ at m/z 105. This ion can be formed by three different fragmentation sequences (Fig. 8). First, by initial elimination of ethylene oxide (as also seen for AC) to form the fragment ion at m/z 123 followed by elimination of a water molecule. Second, by initial elimination of a water molecule (analogue to AC) to form the fragment ion at m/z 149 followed by the elimination of ethenol. The third pathway is barely detectable and initiates with the elimination of formaldehyde to form protonated TMAO.

Another favoured decomposition process involves the elimination of ethene (mechanism explained for AC) to produce a fragment ion at m/z 139. Further MS^n analysis identifies the structure of this ion to be protonated DMAA by comparison of its multiple MS sequence to that of the reference compounds.

A minor decomposition process involves the elimination of ethanol to produce a fragment ion at m/z 121. This ion can also be obtained by sequential elimination of H_2O and ethene and is identified to be $(CH_3)_2As^+{=}O$ after MS^n analysis as described for DMAA.

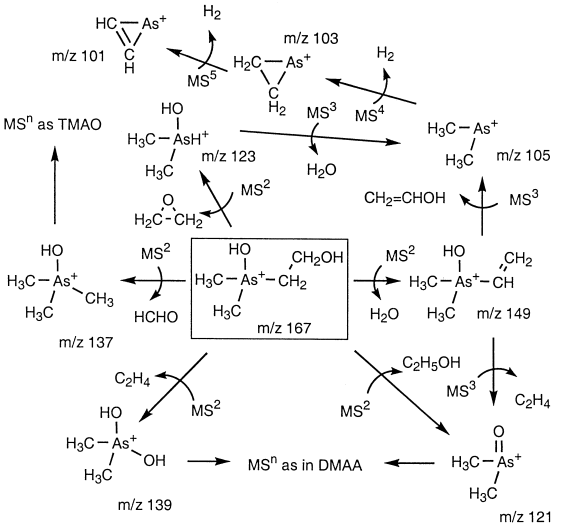

Fig. 8. CID (MS^n) fragmentation scheme for protonated dimethylarsinoylethanol (DMAOE).

4. Conclusion

The CID fragmentation pathways for organoarsenical compounds have been mapped by ESI multiple mass spectrometry coupled with LC–ESI using an ion trap mass spectrometer. The obtained data are in overall agreement with data obtained by triple quadrupole MS–MS analysis. However, the energy available from resonant excitation collision with helium atoms in the ion trap is significantly lower than that available from collision with argon in a triple quadrupole mass spectrometer. This alters the relative intensities of the observed fragments in the CID mass spectra and does not lead to the formation of reduced fragment ions containing As(I). Organoarsenical compounds are mainly fragmented into EE product ions after elimination of H_2, H_2O, CH_4, C_2H_2, C_2H_4, C_2H_6, HCHO, CH_3OH, C_2H_5OH, C_2H_4O, and CH_2CO. Nonetheless, some abundant OE fragments are also formed after elimination of radicals species possibly driven by redox processes in which As(V) is reduced to As(III).

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