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Structural Characterization of Methylenedianiline Regioisomers by Ion Mobility-Mass Spectrometry, Tandem Mass Spectrometry, and Computational Strategies. 2. Electrospray Spectra of 3-Ring and 4-Ring Isomers

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Abstract

Building on results from our previous study of 2-ring methylenedianiline (MDA), a combined mass spectrometry approach utilizing ion mobility-mass spectrometry (IM-MS) and tandem mass spectrometry (MS/MS) coupled with computational methods enables the structural characterization of purified 3-ring and 4-ring MDA regioisomers in this current study. The preferred site of protonation for the 3-ring and 4-ring MDA was determined to be on the amino groups. Additionally, the location of the protonated amine along the MDA multimer was found to influence the gas phase stability of these molecules. Fragmentation mechanisms similar to the 2-ring MDA species were observed for both the 3-ring and 4-ring MDA. The structural characterization of 3-ring and 4-ring MDA isomers using modern MS techniques may aid polyurethane synthesis by the characterization of industrial grade MDA, multimeric MDA species, and methylene diphenyl diisocyanate (MDI) mixtures.

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Supporting Information

NMR spectroscopy of purified MDA isomers, Figures S1 and S2; comparison of theoretical and experimental CCS data for helium and nitrogen data, Figures S3 and S4; mass spectra of doubly charged ions, Figures S5 and S6; and additional computational data, Figures S7–S31. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01084.

Notes

The authors declare no competing financial interest.

Methylenedianiline (MDA) is commonly used as the starting material in the production of methylene diphenylene diisocyanate (MDI), which is a major hard block component in polyurethanes. Industrial grade MDA is composed of 2-ring MDA and larger multimeric species which are formed by the reaction of aniline and formaldehyde and named according to the number of aniline rings present.^{1,2} Structural isomers reflecting attachment of additional anilines at different sites can exist in industrial grade MDA which contributes to heterogeneity in many polyurethane systems.³ Determining preferred protonation sites and location of aniline attachments for larger MDA multimers is of great interest in polymer chemistry, because isomeric characterization can contribute to an enhanced understanding of structure–property relationships of many commercially relevant MDI-based PUR systems.⁴

In our previous work, purified 2-ring MDA regioisomers (4,4'-MDA, 2,4'-MDA, and 2,2'-MDA) were structurally characterized using tandem mass spectrometry (MS/MS), ion mobility-mass spectrometry (IM-MS), and computational modeling.⁵ We observed that protonation can occur both on amine groups and on the aniline ring, which is consistent with previous findings.⁶ Depending on the location of the amine group in the 2-ring MDA species, gas-phase protonation on the amine can lead to low energy fragmentation pathways via a charge-directed process.⁷ In the present study, we aim to characterize purified 3-ring and 4-ring MDA multimeric regioisomers. Possible protonation sites on the 3-ring and 4-ring MDA species are illustrated in Figure 1. In Figure 1a, each aniline ring is attached with its amine group in the para position which represents the primary solution-phase conformation based on NMR studies (Supporting Information, Figures S1 and S2). Due to the location of the amine groups and bridging carbons, protonation in Figure 1a is possible only on the amine group and not the aniline ring. The structures shown in Figure 1b have one terminal aniline ring with its amine group attached in the ortho position (this structure is only representative of <5% of the NMR signals for both the 3-ring and 4-ring MDA). Thus, while it is possible for both 3-ring and 4-ring MDA species to have the last aniline ring attached with an amine group in the ortho position (Figure 1b), this isomer is not preferred. As a result, this manuscript will focus on the structures and protonation sites shown in Figure 1a.

A series of MS techniques are utilized in this study to structurally characterize these larger MDA multimers. MS/MS is used to map fragmentation pathways of the MDA multimers,

while IM-MS provides broad insight into the gas-phase conformations of ionized MDA species.⁸⁻¹¹ Two IM-MS instruments are utilized in this study: a commercial traveling wave instrument which supports multiple stages of fragmentation and a commercial drift tube instrument which supports direct CCS measurements in a variety of drift gases. Computational strategies can then supplement these experimental approaches to provide enhanced structural interpretation. These combined techniques facilitate the separation and characterization of isomeric species that exist for 3-ring and 4-ring MDA. The structural characterization of these multimeric MDA species and their fragments provides a basis for characterization of industrial MDA and MDI mixtures.

EXPERIMENTAL SECTION

Materials

3-ring MDA and 4-ring MDA samples were provided by Dr. Stefan Wershofen, Bayer MaterialScience AG, 47812 Uerdingen, Germany. The authenticity of each sample was determined by ¹H NMR, as provided in the Supporting Information (Figures S1 and S2). Tetraalkylammonium salts and solvents were purchased from Sigma-Aldrich (St. Louis, MO). These included tetraalkylammonium bromides (TAA 1-8) and HPLC grade methanol. Water blended with 0.1% formic acid (optima grade) was obtained from Fisher Scientific.

Instrumentation

Traveling Wave ESI-IM-TOF/MS—Synapt G2 and G2-S (Waters Corporation, Milford, MA) mass spectrometers were used for acquiring MS, traveling wave (TW) IM-MS, and tandem MS/MS data. The MS/MS capabilities of these instruments enable fragmentation experiments to be conducted both before and after the IM region. Although collision cross section (CCS) values cannot be obtained directly from the kinetic theory of gases using TW IM-MS experimental drift time values, a relative CCS method that uses quaternary ammonium salts as CCS calibration standards was utilized to obtain CCS data from these instruments.¹²

Further experimental details can be found from our earlier study,⁵ but, briefly, the TWIM drift cell settings were as follows: TWIM pressure 3 mbar (2.25 Torr), electrodynamic wave height 35 V, wave velocity of 700 m/s, TOF resolution ca. 20,000 (m/ m), 3.00 kV ESI capillary voltage, 80 °C source temperature, 150 °C desolvation temperature, 10 V sampling cone, 2 V (source offset) extraction cone, 20 L/h cone gas flow, 1 mL/min trap gas flow, and 90 mL/min IMS gas flow. Sodium formate clusters were used for TOFMS calibration. Collision-induced dissociation (CID) experiments were performed either prior to or following TWIM mobility separation (MS/IM-MS, and MS-IM/MS, respectively). Lab frame energies were converted to center-of-mass (COM) collision energies for MS/MS experiments to better represent the energy available for rearrangement and fragmentation.¹³ All samples were analyzed as positive ions generated by ESI. MDA 3-ring and 4-ring polymer samples were dissolved at a concentration of 0.1 mg/mL in 9:1 methanol:water, the latter containing 0.1% formic acid (v/v). A direct infusion flow rate of 6.00 μL/min was used for all samples.

Drift-Tube ESI-IM-TOFMS—Drift tube ion mobility (DTIM) measurements using both N₂ and He buffer gases were performed on a commercial ESI-IM-QTOF mass spectrometer (6560, Agilent Technologies, Santa Clara, CA) which was modified to support various drift gases. Details of this instrumentation are provided elsewhere.¹⁴ Briefly, the IM-MS consists of a 78 cm uniform-field drift tube coupled to a QTOF mass spectrometer (m/z ca. 40,000). The buffer gas was maintained at a pressure of ca. 4 Torr, and drift voltages were varied in order to correct for the non-IM flight time of ions through the interfacing ion optics. CCS values were calculated from drift times using the Mason-Schamp equation. Nitrogen-based CCS measurements were obtained for direct comparison with TWIM CCS measurements, while helium-based CCS values were measured for comparison with computational CCS calculations. MDA samples were analyzed at a concentration of 0.095 mg/mL in 9:1 methanol:water, the latter containing 0.1% formic acid (v/v). A direct infusion flow rate of 6.00 μ L/min was used.

Computational Methods

As IM provides a general picture of ion structure in the form of an orientationally averaged surface area (CCS), IM-MS results are often supplemented with computational studies to gain further insight into the gas phase conformations of the ion of interest. A more detailed description of the computational methods used here is included in our previous paper in this series.⁵ A simulated annealing protocol implemented in AMBER¹⁵ was used for conformational sampling with a separate simulation for each of the possible protonation sites on both the 3-ring (3 sites) and 4-ring (4 sites) MDA species. A combination of the projection approximation (He) and the trajectory method (N₂) utilizing MOBCAL¹⁶⁻¹⁸ was then used to calculate theoretical CCS values for the generated conformations. The theoretical conformations were then aligned with the experimental CCS values to provide structural insight into the 3-ring and 4-ring MDA isomers. Alignment of theoretically generated conformations with experimental CCS values is shown in the Supporting Information, Figures S3 and S4.

RESULTS AND DISCUSSION

I. Characterization by MS and Tandem MS

ESI spectra were obtained for the protonated 3-ring ($[M + H]^+ = 304$ Da) and 4-ring ($[M + H]^+ = 409$ Da) MDA species and are shown in Figure 2a. In addition to the protonated precursor ions, significant peaks are observed at 211 and 106 Da for the 3-ring MDA species and peaks at 316, 211, and 106 Da are observed for the 4-ring MDA. These peaks correspond to fragments of the precursor ion and become more intense in the tandem mass spectra as shown in Figure 2b. Even before collision energy is applied to induce fragmentation, the peak at 211 Da for the 3-ring and 316 Da for the 4-ring MDA correspond to the base peaks of the spectra, signifying that the corresponding precursor ions are readily dissociated. It is also interesting to note that the mass difference of 93 Da between the precursor ion and its most abundant fragment present in the spectra is the same mass difference that was observed for the 2-ring MDA species between the 199 Da precursor ion and its major fragment at 106 Da, corresponding to the loss of a terminal neutral aniline.

In addition to the fragment peaks, additional signals are observed at m/z 152.6 and m/z 158.6, respectively, for both the 3-ring and 4-ring spectra. These masses correspond to doubly charged ions for the 3-ring and the 4-ring isomers based on isotope spacing. When these ions were mass selected and fragmented via an MS/MS experiment, singly charged ions at higher m/z values were produced further indicating that these peaks correspond to doubly charged species (Supporting Information, Figures S5 and S6).

The conversion from precursor ion to fragment ions was monitored as a function of applied collision energy for both the 3-ring and 4-ring MDA as shown in Figure 3. The IM-MS/MS structural depletion of the precursor ion is shown in Figure 3a,b, and the breakdown curves for the fragment ions are shown in Figure 3c,d. These breakdown curves show the percentage of precursor ion to fragment conversion observed in the spectra at various collision energies and were obtained by taking the intensity of the indicated fragment ion and dividing by the intensity of its corresponding precursor ion. In Figure 3c, 3-ring MDA has two breakdown curves, representative of the 106 and 211 fragments. The precursor ion breaks down into the 211 fragment at a significantly lower energy threshold than the 106 fragment. Figure 3d corresponds to 4-ring MDA, which has three curves, representative of the 106, 211, and 316 fragments. Again the 316 and 211 fragments form at lower collision energies than the 106 fragment. This observation is further discussed later in the manuscript in the context of IM and computational results.

In our previous study involving 2-ring MDA, the 106 fragment formed readily when external amines were located ortho to the bridging carbon (2,2'-MDA and 2,4'-MDA) but not readily for the 4,4'-MDA. The observation that the 106 fragment requires higher collision energy than the other isomers to form from both the 3-ring and 4-ring MDA species suggests that the external amines are located in para positions to the bridging carbon in a similar manner as the 2-ring 4,4'-MDA. The observation of the 106 fragment ion forming at higher collision energies further indicates that the structures shown in Figure 1a are accurate structural depictions of the 3-ring and 4-ring isomers. However, when comparing Figure 3c,d with the 2-ring MDA breakdown curves from our previous study there is a notable difference between the 3-ring and 4-ring isomers, relative to the 2-ring isomers. Specifically, the 2-ring isomers showed ~30% fragmentation at a center-of-mass (COM) of 4.0 eV, and the 4-ring isomer in this work shows essentially the same behavior in Figure 3d. On the other hand, the 3-ring isomer requires a collision energy of 5.8 eV to reach a similar 30% fragmentation yield. This indicates that there must be a significant structural difference between the 3-ring and 4-ring isomers, making production of 106 Da ions a higher energy pathway in the 3-ring isomer system, *vide infra*.

II. Structural Analysis Using IM-MS and Computational Methods

Collision cross section (CCS) values were measured for the $[M + H]^+$ ions using both TWIM and DTIM instrumentation and are provided in Table 1. The number of measurements for each CCS value is shown in parentheses. Slight differences were observed for the N_2 CCS values between the two instrument platforms most likely reflecting the chemical mismatch of the TAA calibration standards used for TWIM CCS measurements. As discussed in our previous study, these differences likely reflect the exposure of the proton

charge on the MDA molecules, which would result in stronger inelastic interactions with the N_2 buffer gas, as compared to the charge-shielded tetraalkylammonium salts used for calibration. Of particular note is the second conformation observed in the mobility spectrum for 4-ring MDA using the TWIM ($199.5 \text{ \AA}^2$) but not observed in the DTIM analysis. This is reflective of the gas phase stability of these ions; the temperature of the DTIM ion source ($225 \text{ }^\circ\text{C}$) probably causes thermal depletion of the more labile species, which is not depleted in the lower temperature TWIM ion source ($80 \text{ }^\circ\text{C}$). CCS values were also obtained in helium in order to provide better comparison with the theoretical CCS values calculated for the computationally generated conformations. Alignment of theoretical and experimental data in both helium and nitrogen drift gases is available in the Supporting Information, Figures S3 and S4.

The TWIM, IM traces obtained in nitrogen shown in Figure 4a indicate two conformations for both the 3-ring and 4-ring MDA species. The larger CCS conformation observed for the 3-ring MDA species is much less abundant than the smaller CCS conformation, accounting for $\sim 2\%$. For the 4-ring MDA, the larger CCS conformation is more abundant than the smaller CCS conformation, the latter accounting for $\sim 8\%$ of the intensity. The IM-MS/MS structural depletion study in Figure 3 shows the stability of these conformations at varying collision energies. The mobility profiles were obtained at increasing collision energies to compare the depletion rates of the precursor ions. Only the smaller CCS 3-ring MDA conformation appears once collision energy is applied (Figure 3a) compared to the IM trace with no collision energy (Figure 4a). The two conformations for the 4-ring MDA are visible for all collision energies used. Figure 3b also shows that the larger CCS conformation of the 4-ring MDA decreases at a much faster rate (100% to 52% to 6%) than the smaller CCS conformation (100% to 73% to 43%) indicating that the species having the larger CCS conformation is less stable than species represented by the smaller CCS. Although we ascribe the rate of fragmentation to directly depend on the conformational state of the precursor, or directed thermodynamically, we cannot rule out minor influence arising from proton mobility.

In order to facilitate direct comparison between the 3-ring and 4-ring MDA structures, the COM collision energies are considered. Using COM energies, the 1.2 eV in the middle panel for the 3-ring MDA can be compared with the 1.3 eV in the bottom panel for the 4-ring MDA, showing that the 3-ring and smaller 4-ring MDA CCS conformations decrease at similar percentages ($\sim 50\%$) which suggests that the two have similar structural conformations. It is also worth noting that the depletion rates do not mimic those observed for the 4,4', 2,2', or 2,4' 2-ring MDA isomers from our previous paper. This observation suggests two differences between the 2-ring MDA isomers and 3-ring and 4-ring MDA. First, from our earlier paper, the 4,4'-MDA 2-ring isomer has the same $[M + H]^+$ intensity at 0.0 and 1.7 eV and decreased only to 46.2% at 4.2 eV. In this work the 3-ring isomer $[M + H]^+$ intensity in Figure 3a is reduced to 13.2% at 1.7 eV, while the major 4-ring isomer peak shows only 6.1% at 1.3 eV in Figure 3b. This suggests that, although the 4,4'-MDA 2-ring, the 3-ring, and the 4-ring isomers all have terminal para-amino groups, different processes dominate fragmentation for the higher order multimers which are characterized by the m/z 211 and 106 fragments. Second, the behavior of the lower abundance peak in Figure 3b extrapolates to a value of about 25% intensity for the energy of 1.7 eV, a behavior

comparable to that of the 2,2'- and 2,4'-MDA 2-ring isomers from our earlier study. These observations indicate that fragmentation of the 3-ring and 4-ring isomers must be dominated by the internal amine group(s), leading to lower energy pathways, *vide infra*.

Computational modeling generates theoretical structures that can be aligned with the experimental data according to their CCS values. This gives insight into the conformations that were observed experimentally and why a difference in stability is observed. For both 3-ring and 4-ring MDA, the structures shown in Figure 1a were modeled separately with an additional proton for each possible protonation site. This resulted in 3 unique starting structures for the 3-ring MDA and 4 unique starting structures for the 4-ring MDA. The conformational space plots based on the molecular dynamics conformational space sampling simulations are shown in Figure 4b. The overlaid colors on the conformational space plots represent independently modeled protonation sites, with the color scheme following protonation on the amine nitrogen from left to right across the structures shown in Figure 1a. For the 3-ring MDA, the protonation sites are indicated in black, red, and blue, respectively, across the structure, and for the 4-ring MDA the protonation sites are indicated in black, red, cyan, and blue, respectively, across the structure. Figure 4c,d show the results from each independently modeled protonation site that are combined in Figure 4b. Conformation space plots for each protonation site for both helium and nitrogen exhibit similar distributions as seen in the Supporting Information, Figures S3 and S4. The plots in Figure 4b show two distinct conformational clouds indicated with the black circles for both the 3-ring and 4-ring MDA, which agrees with the experimental bimodal IM traces in Figure 4a.

Figure 5 shows selected conformations based on root mean squared deviation (RMSD) clustering and alignment with experimental CCS values. Each conformation is labeled with its theoretical CCS value along with the percentage of conformations that the structure represents based on RMSD clustering analysis. Each conformation shown represents a different protonation site and thus a separately modeled set of conformations. The percentage indicates how representative the conformation shown is of the independently modeled protonation sites and therefore does not show relations between the conformations in Figure 5 since each of these protonated species was modeled separately. A comprehensive set of representative conformations that were generated for each protonation site can be found in the Supporting Information, Figures S7–S31.

Conformations for the 3-ring MDA are shown in Figure 5a–c. The conformations in Figure 5a,b represent the smaller CCS conformational space cluster and are the result obtained when protonation occurs at the amines on the terminal rings. These conformations agree with experimental CCS helium values obtained on the DTIM instrument (Table 1) and are the most representative structures based on RMSD clustering analysis. The smaller CCS, and more stable conformation of the 3-ring MDA, reflects the structure in which the two external amine groups share the additional proton. Likewise, the conformation in Figure 5c is representative of protonation on the internal amine and aligns with the larger CCS conformational space cluster for the 3-ring MDA (Figure 4b). This conformation is a cluster representative structure but due to gas-phase stability does not have a corresponding experimental CCS value from the DTIM instrument. Given that the CCS value we observe experimentally for the 3-ring MDA aligns with the smaller CCS conformational cluster, we

surmise that the larger experimental CCS value corresponds to the larger CCS conformational space cloud. The position of the proton on the internal amine is very similar to that of protonation on both the 2,2' and 2,4' 2-ring MDA species when protonation occurs on the ortho amine. It is also important to note that this additional proton on the amine is aligned in an energetically favorable position with the most basic region of the adjacent aniline ring.

For the 4-ring MDA, two conformational clusters exist in Figure 4b with representative structures shown in Figure 5d–i. The conformations in Figure 5d,e are representative of the smaller CCS cluster, in agreement with Table 1, and represent conformations that are protonated on the external amines. Protonation at the periphery of the molecule form smaller CCS conformations, similar to the 3-ring MDA, that wrap around and share the proton between the two external amine groups. The conformations in Figure 5f–i are representative of the larger CCS cluster, for which there are two possibilities. The first is shown in Figure 5f,g where protonation occurs on the external amines, similar to the conformations in Figure 5d,e, but here the conformation remains in an extended form rather than forming a cyclic species to share the proton. Figure 5h,i shows the other possibility where protonation occurs on one of the two internal amines. Again, this position of the proton on the internal amine is very similar to that of protonation on both the 2,2' and 2,4' 2-ring MDA species when protonated on the ortho amine. This similarity suggests the 3-ring and 4-ring MDA should fragment via a similar mechanism to that of the 2-ring MDA.

There is an interesting difference in the IM intensities shown in Figure 3a,b for both the 3-ring and 4-ring MDA. As indicated above, the 3-ring MDA peak corresponding to the lower CCS structures (Figure 5a,b) is intense, whereas the lower CCS peak for the 4-ring MDA spectra (Figure 5d,e) is much lower in abundance. Also, the onset of the 106 Da fragment peak is accessed at higher collision energies for the 3-ring isomer, as seen in Figure 3c. These observations correlate well with the known behavior of macrocyclic structures. Specifically, there is an optimum size for the formation of large cyclic structures, the most stable in the range of 15 atoms in the ring.¹⁹ The cyclic 3-ring MDA structures in Figure 5a,b have 16 atoms, while the cyclic 4-ring MDA structures in Figure 5d,e are composed of 20 atoms; and thus the former should be the more stable. Compatible with this, the noncyclic structures in Figure 5f,g are prominent for the 4-ring MDA but not for the 3-ring isomer. Because of the shared hydrogen in Figure 5a,b this stability is reflected in the higher fragmentation energy required for dissociation of the 3-ring MDA. A similar effect has been noted previously for hydrogen transfer reactions in the MS/MS fragmentation of aramids.²⁰

III. Mechanism for Protonation and Fragmentation of MDA Isomers

Based on the conformational observations from both IM and theoretical simulations and the results from tandem MS studies, fragmentation mechanisms can be suggested for both the 3-ring and 4-ring MDA species. These mechanisms are shown in Scheme 1. For the 3-ring MDA, there are two fragments in the MS/MS spectra in Figure 2b at 106 and 211 Da compared to the 2-ring MDA isomers in our previous manuscript which only yielded a single fragment at 106 Da. Scheme 1a shows how both of these fragments are formed from the 304 Da precursor ion. Here, the 106 Da fragment forms via an α -cleavage reaction of the

304 Da ion that also results in the loss of a neutral 198 Da fragment that mimics the 2,4' 2-ring MDA. The conformation of the 304 Da precursor ion for this reaction is shown in Figure 5a,b and has a theoretical He CCS of 105.3 Å² which is very similar to the He experimental value in Table 1 of 105.5 Å². As previously mentioned, the 15-member ring present in these conformations is energetically favorable and must therefore be opened before the α -cleavage reaction can occur. This explains why there is not significant formation of the 106 Da fragment in Figure 3c until ca. 4 eV. Figure 3c also shows that at 0 eV the 304 Da fragment is already converted to the 211 Da fragment over 70%, suggesting that this fragmentation pathway is readily accessible. This low energy 1,5 hydrogen shift mechanism was previously observed for the 2,2' and 2,4' 2-ring MDA isomers but not with protonation on an internal amine as shown here in Scheme 1a with the 3-ring MDA. Although the larger 3-ring conformation shown in Figure 5c does not have a corresponding experimental He CCS value, this conformation is assigned to the 304 Da precursor ion for the 1,5 hydrogen shift fragmentation pathway due to the location of the additional proton near the most basic site on the adjacent aniline ring. This 1,5 hydrogen shift fragmentation results in loss of neutral aniline as well as the 211 Da fragment.

For the 4-ring MDA there are three fragments present in the MS/MS spectra in Figure 2b at 106, 211, and 316 Da. Scheme 1b shows how these three fragment ions are formed from the 409 Da precursor ion. Similar to the 3-ring MDA, the 106 Da fragment forms via a high-energy α -cleavage reaction. Also similar to the 3-ring MDA, the 4-ring MDA forms a cyclic conformation as seen in Figure 5d,e with theoretical He CCS values of 133.1 and 132.7 Å², respectively. These conformations exhibit the best agreement with the experimental He CCS value of 132.7 Å² in Table 1. At 20 atoms though, these cyclic structures are no longer as energetically favored as the 3-ring MDA, and therefore the extended conformations shown in Figure 5f,g are also possible. The proposed favorability of this extended conformation is further validated by the observation that significant production of the 106 Da fragment occurs at 2.75 eV in Figure 3d compared to the 4 eV for the 3-ring MDA. Additionally, the larger CCS conformation for the 4-ring MDA is more abundant than the smaller CCS conformation in Figure 4a. Figure 3d also shows that the 409 Da precursor ion has converted to the 316 Da fragment at 94% at 0 eV. Similar to the 3-ring MDA, the low energy 1,5 hydrogen shift fragmentation mechanism is initiated by protonation on an internal amine, which is represented by the conformation in Figure 5h. This fragmentation reaction results in loss of neutral aniline as well as the 316 Da ion fragment. The 211 Da fragment of the 4-ring MDA also occurs via a 1,5 hydrogen shift initiated by protonation on an internal amine, but instead of the protonated amine being located adjacent to an external aniline this internal amine is located directly in the middle of the 4-ring MDA structure with two 2-ring MDA units on each side as shown in the conformation in Figure 5i. In order for the fragmentation mechanism to occur, a larger portion of the structure must be orientated such that the additional proton is in proximity to the most basic site on the adjacent aniline ring. This explains why significant formation of the 211 Da fragment does not occur until 2.25 eV in Figure 3d. These fragmentation mechanisms observed for the 3-ring and 4-ring MDA, which mimic the α -cleavage and 1,5 hydrogen shift observed for the 2-ring MDA isomers, will serve as the basis for characterizing larger MDA sample mixtures possessing additional internal amines which can serve as possible protonation sites.

CONCLUSIONS

In this study, 3-ring and 4-ring MDA species have been characterized using an MS-based analytical approach. We conclude that the protonation sites for the 3-ring and 4-ring MDA regioisomers are only at the amine group. Additionally we determined that protonation on either external or internal amine groups greatly influences the gas-phase stability of these species. The use of MS/MS, IM-MS, and computational modeling allowed for the structural characterization of these gas-phase ions and further promotes the benefit of combining several different MS-based technologies for comprehensive characterization studies.

These larger MDA units are composed primarily of linking anilines with the amine group in the para position to the bridging carbon but do not maintain the gas-phase stability of the 4,4'-MDA due to possible protonation on internal amine groups, leading to charge-directed fragmentation of the 3-ring and 4-ring MDA. A thorough understanding of the fragmentation mechanisms initiated by internally protonated amines for both the 3-ring and 4-ring MDA will greatly facilitate the interpretation of fragmentation spectra of the larger MDA units which are expected to dissociate via similar mechanisms. Further studies on larger MDA units will allow us to observe if this trend continues as chain length increases or if other, more stable attachment patterns form. Understanding that these chains fragment easily when protonated on internal amines will prove helpful when identifying the structures of precursor and fragment ions with MS. Tandem MS studies will provide fragmentation titration curves that can be compared to those for the 2-, 3-, and 4-ring MDA to determine if ions formed from the larger MDA units fragment via similar pathways. IM and theoretical simulations have been demonstrated to separate different protonation sites for the 2-, 3-, and 4-ring isomers and should help to identify different conformers in mixtures of larger MDA species, where the structural heterogeneity is more diverse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Structures of 3-ring and 4-ring MDA (theoretical neutral molecule monoisotopic masses = 303.17 and 408.23 Da, respectively). (a) All para substitution; (b) one ortho substitution. Potential protonation sites are labeled with an asterisk.

Figure 2.

(a) Mass spectra of 3-ring and 4-ring MDA using direct infusion ESI-TOFMS. (b) Tandem mass spectra for 3-ring and 4-ring MDA parent ions ($[M + H]^+ = 304.18$ Da and $[M + H]^+ = 409.24$ Da, respectively).

Figure 3.

Collisionally activated CCS profiles of $[M + H]^+$ ions for (a) 3-ring MDA and (b) 4-ring MDA. Center of mass energies are shown. The inset relative percentages represent signal intensities compared to those without collisional activation. Vertical lines are added for visual alignment. Collision-induced dissociation curves monitoring the transition of precursor ions to fragment ions for (c) 3-ring MDA and for (d) 4-ring MDA. Center of mass collision energies (CE) are shown on the lower axis. (See text for details.)

Figure 4.

(a) CCS profiles for 3-ring MDA (top) and 4-ring MDA (bottom) extracted from TWIM data. (b) Conformational space plots for the possible protonation sites of 3-ring MDA (top) and 4-ring MDA (bottom) generated using computational conformational search methods. The theoretical nitrogen CCS is plotted against the relative energy for each conformation. Theoretical conformations for each protonation site are black, red, and blue, respectively, across the structure depicted in Figure 1 for the 3-ring MDA and black, red, cyan, and blue, respectively, across the structure for the 4-ring MDA. Insets of the conformations resulting from individual protonation species are shown in (c) and (d) to clarify the conformational clusters present on each plot.

Figure 5. RMSD clustering representatives from computational conformational sampling are shown for each of the protonation sites for the 3-ring MDA (a–c) and for the 4-ring MDA (d–i). Labeled bond distances are used to show the proximity of the additional proton to the terminal nitrogen or bridging carbon that would lead to a 1,5-hydrogen shift fragmentation.

Scheme 1.

Proposed Fragmentation Pathways for 3-Ring and 4-Ring MDA Structures

Table 1

Collision Cross Section Values for 3-Ring and 4-Ring MDA Ions Obtained on TWIM and Drift Tube IM-MS Instrumentation^a

MDA species (ion)	TWIM N ₂ (Å ²)	DTIM N ₂ (Å ²)	DTIM He (Å ²)
3-ring [M + H] ⁺	171.3 ± 0.1 (<i>n</i> = 6)	178.7 ± 0.6 (<i>n</i> = 14)	105.5 ± 0.6 (<i>n</i> = 18)
4-ring [M + H] ⁺ (1)	191.2 ± 0.7 (<i>n</i> = 6)	202.1 ± 1.0 (<i>n</i> = 14)	132.7 ± 1.1 (<i>n</i> = 19)
4-ring [M + H] ⁺ (2)	199.5 ± 1.0 (<i>n</i> = 6)	N/A	N/A

^aErrors shown represent the respective standard deviations. The number of measurements on which each CCS value is based are shown in parentheses.