Reaction of arylium ions with the collision gas N₂ in electrospray ionization mass spectrometry

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A number of studies revealed that certain product ions undergo ion-molecule reactions with neutral molecules inside mass spectrometers to form unexpected adduct ions which cannot arise from the precursor ion. Such peaks were found to be due to reaction of arylium ions with N₂ in the collision cell. Therefore, this reaction was studied in detail with representative compounds.

**METHODS:** Various classes of compounds were dissolved in acetonitrile/water/formic acid and studied by electrospray ionization mass spectrometry to record their MS² and pseudo-MS³ spectra in a QqQ mass spectrometer and their accurate m/z values in an Orbitrap Elite instrument. Arylium ions were found to react with N₂ in the collision cell. The reaction was confirmed by pseudo-MS³ studies, by comparison with authentic diazonium ions, and by the pressure dependence of the product ion survival yield.

**RESULTS:** Reactions of arylium ions with N₂ were observed with p-toluenesulfonic acid, o-toluenesulfonamide, phenylphosphonic acid, phenol, aniline, aminonaphthalenes, benzoic acid, benzophenone, and other compounds. By using a QqQ mass spectrometer, we observed that the protonated compounds produce arylium ions, which then react with N₂ to form diazonium ions. The diazonium ion was produced with N₂ but not with Ar in the collision cell, and its abundance increased with increasing N₂ pressure.

**CONCLUSIONS:** Arylium ions generated from a wide variety of compounds in electrospray ionization tandem mass spectrometry may react with N₂ to form diazonium ions. The abundance of the diazonium ions is affected by the collision energy and N₂ pressure. This reaction should be considered when annotating peaks in MS/MS libraries. Published in 2015. This article is a U.S. Government work and is in the public domain in the USA.
The compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in acetonitrile/water/formic acid (50:50:0.1) at a concentration of about 0.1 mg/mL. N₂ (99.999%), Ar (99.999%) and CO₂ (99.5%) were utilized as collision gases for mass spectrometers. HCD (Higher-energy Collision Dissociation) spectra were acquired with an Orbitrap Elite instrument (Thermo Fisher Scientific, Waltham, MA, USA) with 3.75 mTorr (0.5 Pa) N₂ pressure. Spectra were collected using various collision energies and the resolution for MS² was set at 30,000. For experiments with different collision gases and different collision cell pressures, which were monitored by a Baratron capacitance manometer, we used a Micromass Quattro Micro triple quadrupole instrument (QqQ) (Waters Corp., Milford, MA, USA). First the mass spectra were recorded at different cone voltages to optimize the abundances of the precursor ions. Then, the precursor ion at the optimal cone voltage was selected for fragmentation in the collision cell [usually with 0.21 Pa (1.6 mTorr) pressure of Ar as the collision gas] and the MS/MS spectrum was recorded at 20 different collision voltages. The range of collision voltage spanned from near zero V up to a value where no precursor ion remained. We also used a high cone voltage to produce in-source fragments from the precursor ion, which were then selected into the collision cell for MS/MS study of their dissociation reactions; for this type of experiment we use the term ‘pseudo-MS³’ for sake of abbreviation, as used before. Spectra were acquired in ‘centroid’ mode, whereby signals within each individual time interval in a given spectrum were centered and integrated by the instrument data system. Typically, m/z values were within 0.2 of the theoretical m/z values throughout the m/z range of interest. When the collision gas was changed, a rapid flow of the new gas through the collision cell for about an hour was generally sufficient to fully replace the old gas; this was confirmed by recording the spectra at several time intervals after the gas change. N₂ produces less fragmentation than Ar because of its lower mass, but we used both gases at the same pressure. A few experiments were also carried out using a quadrupole time-of-flight (QTOF) instrument (model 6530; Agilent Technologies, Santa Clara, CA, USA) which uses N₂ as a collision gas, but at a much lower pressure, only 0.02 mTorr (reported as collision pressure in the collision chamber).

Synthesis of diazonium compounds

1-Aminonaphthalene (1 mg) was dissolved in dilute sulfuric acid (0.1 mL) and kept in an ice bath. Then sodium nitrite (1.5 mg) in water (0.1 mL) was added dropwise. The resulting mixture was stirred in the ice bath for 10 min. This naphthalenediazonium sulfate solution was then diluted 10 times with the electrospray solvent (acetonitrile/water/formic acid, 50:50:0.1) and infused into the mass spectrometer for MS/MS analysis. The same procedure was used to synthesize benzenediazonium sulfate from aniline.

RESULTS AND DISCUSSION

High-resolution MS/MS spectra were acquired with the Orbitrap Elite instrument for inclusion in the NIST MS/MS library. As part of the quality control procedures, we attempted to assign all the product ion peaks to a reasonable chemical formula derived from the original compound. In many cases we noted product ion peaks that could arise from the precursor ion by any combination of the elemental compositions. Some of these peaks were found to arise from the reaction of product ions with H₂O, which were reported in our recent publication. However, occasionally additional peaks were found at a mass 10 m/z units higher than that. Exact mass measurements suggested that these extra peaks might be due to the addition of N₂, the collision gas, to a product ion. Therefore, we performed a more detailed study with some representative compounds to confirm this reaction.

Production of aryl cations and their reaction with N₂

p-Toluene sulfonic acid was the first compound that was examined in detail. The HCD MS/MS spectra of its [M+H]⁺ ion were acquired by the Orbitrap Elite at various collision energies. Figure 1(a) shows the spectrum acquired at intermediate collision energy (collision voltage, 10 V). The [M+H]⁺ precursor ion (m/z 173.0267) undergoes loss of water to form [M+H-H₂O]⁺ (m/z 155.0161), loss of SO₂ to form

Figure 1. MS/MS spectra of protonated p-toluene sulfonic acid. (a) HCD spectrum acquired with the Orbitrap Elite mass spectrometer, using 3.75 mTorr N₂ in the collision cell, at collision voltage 10 V. The m/z values of the peaks are given with the high accuracy provided by the Orbitrap. (b) Spectrum obtained with the Micromass QqQ, using 1.6 mTorr N₂ as collision gas, at collision voltage 16 V. (c) Spectrum obtained with the Micromass QqQ, using 1.6 mTorr Ar as collision gas, at collision voltage 12 V. The precursor ion peak is marked p.
Reactions of product ions with N2

[M+H–SO3]⁺ (m/z 109.0647), and loss of both water and SO₂ to form [M+H–SO₂–H₂O]+ (m/z 91.0541). However, a peak at m/z 119.0663 is also observed that cannot arise from the precursor ion by any combination of the elemental compositions. The abundance of this ion varies with the collision energy. The ion appears concurrently with the ion [M+H–SO₂–H₂O]+, but disappears earlier when the collision energy is raised. The exact mass difference between these two ions is 28.0062 m/z units, which corresponds to the mass of N₂. Since the product ion [M+H–SO₂–H₂O]+ is probably the p-methylphenylium ion (CH₃C₆H₄) it is likely that this ion reacts with N₂ in the collision cell to yield the diazonium ion [CH₃C₆H₄N=N]+, which is sufficiently stable to be detected as a product ion.

To confirm that this product ion is formed only when N₂ is used as the collision gas, we carried out experiments with the Micromass QqQ mass spectrometer. In contrast to the Orbitrap instrument, which is run with N₂, the collision gas in the Micromass can be changed from the commonly used Ar to N₂ and this permits us to examine the effect of this change on the peak at m/z 119 ascribed to the diazonium ion. Our Micromass instrument, however, does not provide high-resolution spectra so that it cannot distinguish between the mass of N₂ (28.00615) and that of CO (27.99491). That distinction is clearly made with the Orbitrap.

Figure 1(b) shows the MS/MS spectrum of protonated p-toluenesulfonic acid at a collision voltage of 16 V under N₂, and Fig. 1(c) shows the spectrum at a collision voltage of 12 V under Ar. Clearly, the peak at m/z 119 appears only under N₂. Figure 2(a) shows the dependence of the relative intensities of the main peaks in the spectra upon the collision voltage in the QqQ mass spectrometer. The first peak to appear at low collision voltage is that at m/z 155 ([M+H–H₂O]+). At slightly higher voltage, the peaks at m/z 109 and 91 begin to appear concurrently. The peak at m/z 65 appears at much higher voltage and is clearly due to fragmentation of the benzene ring. Figure 2(b) shows similar results obtained by replacing Ar with a similar pressure of N₂ as collision gas, and in this case a peak at m/z 119 is clearly observed along with those at m/z 91 and 109. Furthermore, when the pressure of N₂ was 1.6 mTorr the abundance of the m/z 119 peak, at its highest value, which was achieved at a collision voltage of 22 V, was 11% of the total ion current (Fig. 2(b)). When the pressure was increased by a factor of two, the maximum intensity of the m/z 119 peak increased to 40% (data not shown). Similar results were observed in the QTOF instrument. A more detailed study of the N₂ pressure was carried out with aniline and is discussed below.

Figure 2 demonstrates another difference between Ar and N₂, i.e. their effectiveness as collision gases. To decrease the intensity of the precursor ion (m/z 173) by a certain fraction requires a higher voltage under N₂ than under Ar, when both are present at the same pressure, and this is mainly due to the lower mass of N₂ than Ar.[23,24] Another observation in Fig. 2 should be noted because it appears to be a common behavior of sulfonic acids. The curve for the precursor ion at m/z 173 does not begin at 100% when the collision voltage is near zero. It begins at a lower value and may increase before it begins to decrease with increasing voltage. This is because some of the precursor ions remain hydrated at low collision voltage and are observed at m/z 191 instead of m/z 173. The sum of the abundances of these two ions reaches the 100% level. Electrospray ionization of many sulfonic acids yields such mixtures of [M+H]+ and [M+H+H₂O]+ ions because of the strong bonding of water to these acids.

The above findings suggest that many other arylium ions, formed by loss of some neutral molecules from protonated precursors, may react with the collision gas N₂ to form diazonium ions.

We confirmed this hypothesis by investigating other classes of compounds, where X = SO₃H, SO₂NH₂, PO₃H₂, CO₂H, OH, NH₂, CO₂H₅S, and R represents one or more alkyl groups or the benzene ring replaced with a naphthalene. The results are summarized in Table 1.
o-Toluenesulfonamide was found to exhibit the same behavior as p-toluenesulfonic acid except that the first neutral loss involves NH₃ instead of H₂O. The MS/MS spectra show the precursor ion [M+H]+ at m/z 172.0426, and the major product ions [M+H–NH₃]+ (m/z 155.0161) and [M+H–NH₃–SO₂]+ (m/z 91.0540), and the N₂ addition product ion [CH₃C₆H₅N≡N]+ (m/z 119.0604). The spectra at different collision energies are included in the 2014 version of the NIST tandem mass spectral library.

Protonated phenylphosphonic acid (m/z 159.0206) (Fig. 3(a)) undergoes loss of water to form the m/z 141.0101 ion, loss of HPO₂ to form the m/z 95.0491 ion, and loss of H₃PO₃ to form the phenylium ion (m/z 77.0384). In addition, a peak at m/z 79.0541, assigned to C₆H₅H⁺, and another at m/z 98.9841, assigned to H₄PO₄⁺, are observed. The most abundant product ion, however, is at m/z 105.0447, which is ascribed to the product of the reaction of the phenylium ion with N₂ to form the diazonium ion. The HCD spectrum of the [M+H–H₂O]+ ion (m/z 141.0101) (Fig. 3(b)) also shows formation of the ions at m/z 77.0384, 95.0491, and 105.0447, but not those at m/z 79.0541 and 98.9841. The latter two ions cannot be formed from the [M+H–H₂O]+ ion, and this is in line with their structures assigned above.

<table>
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<tr>
<th>Compound</th>
<th>Structure</th>
<th>m/z [M+H]+</th>
<th>Product ion [Ar]+</th>
<th>m/z [Ar]+</th>
<th>m/z [Ar+N₂]+</th>
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<td>H₃CC₆H₄⁺</td>
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<td>H₃CC₆H₄⁺</td>
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<td>119</td>
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<tr>
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<td>C₆H₅⁺</td>
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<td>C₆H₅⁺</td>
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<td>C₁₀H₁⁷⁺</td>
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<td>127</td>
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<td>172</td>
<td>C₁₀H₁⁷⁺</td>
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<td>155</td>
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The phenylium ion (m/z 77) reacts with N₂ to form the benzenediazonium ion (m/z 105). To confirm this reaction, we produced the m/z 77 ion in the ion source of the QqQ instrument by raising the cone voltage to 80 V and then selected it into the collision cell to acquire pseudo-MS³ spectra at different collision voltages. When the collision voltage was very low we observed the m/z 77 ion as a precursor ion and also the ions formed by addition of neutral molecules. Under Ar (Fig. 3(c)) we observe the water adduct at m/z 95 (as we reported previously[14]) and under N₂ (Fig. 3(d)) we observe both the m/z 95 ion and the m/z 105 ion of the diazonium. Protonated phenol (m/z 95) undergoes loss of water to form the phenylium ion (m/z 77), which reacts with H₂O, under Ar as collision gas, and with both H₂O and N₂, under N₂ as collision gas. The products of these reactions, at m/z 95 and 105, are clearly observed in Fig. 4(b). Pseudo-MS³ measurements gave similar results to those in Figs. 3(c) and 3(d).

Protonated aniline behaves in a similar manner. Its MS/MS spectrum under N₂ as collision gas (Fig. 4(b)) is similar to that of phenol (Fig. 4(a)) except that the initial dissociation involves loss of ammonia instead of water. This initial loss produces the phenylium ion (m/z 77), which reacts with H₂O, under Ar as collision gas, and with both H₂O and N₂, under N₂ as collision gas. The products of these reactions, at m/z 95 and 105, are clearly observed in Fig. 4(b). Pseudo-MS³ measurements gave similar results to those in Figs. 3(c) and 3(d).

The competing reactions of phenylium (m/z 77) with water and with nitrogen were studied as a function of gas pressure in the collision cell. Figure 5 shows that when the pressure of N₂ is raised from near zero to about 3 Pa, the abundances of the products of the reaction with H₂O (m/z 95) and with N₂ (m/z 105) are somewhat similar, but when the...
N₂ pressure is raised further, up to 13 Pa, the reaction with N₂ becomes more dominant. This finding provides further support for the involvement of N₂ in the production of the m/z 105 ion. It also indicates that the abundance of such diazonium ions in the MS/MS spectra measured with different instruments may differ if the collision gas or its pressure is different, or if the relative concentrations of water and nitrogen in the collision cell are different. A plot similar to that shown in Fig. 5 is also recorded with 1-naphthylamine (data not shown).

Benzoic acid and benzophenone present examples of spectra where high mass resolution is critical to demonstrate the stepwise reactions. Protonated benzoic acid [M+H]⁺ (m/z 123) undergoes loss of water to form the m/z 105 ion [M+H-H₂O]⁺, and further loss of CO to form the phenylium ion [M+H-H₂O-CO]⁺ at m/z 77 which reacts with N₂ to generate the m/z 105 ion [M+H-H₂O-CO+N₂]⁺. A low accuracy mass spectrometer cannot distinguish between the m/z values of [M+H-H₂O]⁺ and [M+H-H₂O-CO+N₂]⁺. As a result, the peak at m/z 105 is generally assigned as [M+H-H₂O]⁺ only, but in fact it is a mixture composed of [M+H-H₂O]⁺ and [M+H-H₂O-CO+N₂]⁺. In parallel experiments performed with the high mass accuracy Orbitrap Elite instrument, the HCD spectra of protonated benzoic acid show clearly two peaks, [M+H-H₂O]⁺ at m/z 105.0334, dominant at low collision energies, and [M+H-H₂O-CO+N₂]⁺ at m/z 105.0443, dominant at high energies. Similarly, the HCD spectra of protonated benzophenone show the separation between the [M+H-C₆H₆]⁺ (m/z 105.0334) and [M+H-C₆H₆-CO+N₂]⁺ (m/z 105.0443) ions.

To compare phenylm with larger arylium ions, we examined the MS/MS spectra of protonated 1-amino- and 2-aminonaphthalene. The [M+H]⁺ ions of both these compounds (m/z 144) undergo loss of NH₃ to produce the 1- and 2-arylium ions (m/z 127). These ions react with N₂ to form the naphthalenediazonium ions (m/z 155). A representative spectrum acquired with 2-aminonaphthalene is shown in Fig. 4(c). To confirm that the m/z 155 ion originates from the naphthylum ion (m/z 127), pseudo-MS³ experiments were carried out with the QqQ mass spectrometer, as described above. The results (Fig. 4(d)) show that the naphthylum ion reacts with both H₂O and N₂ to form the ions at m/z 145 and 155, respectively.

Experiments were also carried out with 1-amino- and 2-aminanthracene, 2-aminofluorene, 4-aminobiphenyl, and 6-aminochrysene. Tandem mass spectra of the protonated compounds show loss of NH₃ as the most significant initial dissociation in all cases. However, pseudo-MS³ spectra of the [M+H-NH₃]⁺ ions under N₂ as collision gas show low abundance of the diazonium ions, <1.5% of the base peak in all cases. This might be due to the lower stability of these diazonium ions or other competing reactions in the collision cell of the mass spectrometer.

Comparison of the N₂ addition product ions and authentic diazonium ions

To prove the identity of the N₂ addition product ions, diazonium compounds were synthesized from the corresponding amino compounds by the well-known diazotization method. 1-Aminonaphthalene was first examined due to its relatively larger backbone that can result in more product ions in the MS/MS spectra. 1-Naphthalenediazonium sulfate was synthesized from 1-aminonaphthalene with high yield, as observed in the full mass spectrum. MS/MS spectra of 1-naphthalenediazonium ion are found to be very similar to the pseudo-MS³ spectra of the [M+H-NH₃+N₂]⁺ ion (Fig. 5). The diazonium ion undergoes loss of N₂ to form the m/z 127 ion, which adds a water molecule, to produce the m/z 145 ion, or further fragments to generate the phenylum ion at m/z 77. Figure 6 shows that the relative intensities of the main peaks as a function of collision voltage are very similar in the pseudo-MS³ spectra of the [M+H-NH₃+N₂]⁺ ion and the MS² spectra of the authentic diazonium ion. Experiments with aniline gave similar results, although the number of peaks available for comparison was smaller.
Reaction of aryl ions with carbon dioxide

Although CO₂ is not often used as collision gas, we decided to examine its behavior by comparison with that of N₂ because it is a generally more reactive gas. We used o-methylbenzoic acid to study both the positive and negative ions. The MS/MS spectra of the [M+H]^+ ion at m/z 137 shows loss of CO₂ to produce the m/z 93 ion and loss of formic acid to produce the m/z 91 ion. Neither the MS/MS spectra nor the pseudo-MS³ spectra of the o-methylphenylion ion (m/z 91) show any reaction with CO₂. This is expected since the product ion o-CH₃C₆H₄COO⁺ is unstable and is known to undergo rapid decarboxylation. On the other hand, the MS/MS spectra of the [M–H]⁻ precursor ion at m/z 135 show loss of CO₂ to produce the o-methylphenyl anion at m/z 91, and the pseudo-MS³ spectra of this product show that it reacts with CO₂ to reconstitute the precursor o-methylbenzoate anion, in line with previous reports. This latter reaction did not take place when CO₂ was replaced with Ar as collision gas.

CONCLUSIONS

A wide variety of compounds which contain an aromatic ring undergo collision-induced dissociation to produce arylium ions. Electrospray ionization tandem mass spectra show that the arylium ions can react with ‘inert’ N₂ in the collision cell to yield diazonium ions. High accuracy HCD spectra in the ppm range confirm that N₂ (exact mass 28.0061) is added to the arylium ions. Replacing N₂ with Ar in the collision cell eliminates formation of the diazonium ions. The abundance of diazonium increases with increasing N₂ pressure. Pseudo-MS³ spectra of the [M+H–N₂H+H₂]⁺ ion, produced in the mass spectrometer from 1-aminonaphthalene, are identical to the MS² spectra of authentic 1-naphthalenediazonium synthesized from 1-aminonaphthalene. This reaction of arylium ions with N₂ occurs in parallel with their reaction with H₂O, which has been reported previously. Both these reactions should be taken into account during quality control procedures to achieve a more complete annotation of peaks in MS/MS libraries. Although the reaction with water is more common, since it occurs with many types of ions and when using any collision gas, the reaction with N₂ should also be considered with any compound that contains an aromatic ring. Simple compounds, such as those examined in this study, undergo losses of small neutral molecules and then exhibit the reaction with N₂. More complex compounds, which contain an aromatic ring, may undergo successive neutral losses until a substituted phenylion remains, which then reacts with N₂. Although such reactions are observed mainly at high collision energies, we found many examples where the abundance of the diazonium ion in the HCD spectra is substantial. A few examples are: benzocaine, doxepin, oleyl anilide, guanabenz, resveratrol glucuronide, methylyhippuric acid, carminic acid, dextromethorphan, vitamin K₁, theaflavin, dichloroquinoline, estriol and its derivatives, primisulfuron-methyl, and various chalcones and flavones. Because the widely used Orbitrap mass spectrometers utilize N₂ as the collision gas, it is important to take into account the formation of diazonium ions.

REFERENCES


