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Formation of Protonated ortho-Quinonimide from ortho-lodoaniline in the Gas Phase by a Molecular-Oxygen-Mediated, ortho-Isomer-Specific Fragmentation Mechanism

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ABSTRACT: Upon collisional activation under mass spectrometric conditions, protonated 2-, 3-, and 4-iodoanilines lose an iodine radical to generate primarily dehydroanilinium radical cations (m/z 93), which are the distonic counterparts of the conventional molecular ion of aniline. When briefly accumulated in the Trap region of a Triwave cell in a SYNAPT G2 instrument, before being released for ion-mobility separation, these dehydroanilinium cations react readily with traces of oxygen present in the mobility gas to form peroxyl radical cations. Although all three isomeric dehydroanilinium ions showed avid affinity for O₂, their reactivities were distinctly different. For example, the product-ion spectra recorded from mass-selected m/z 93 ion from 3- and 4iodoanilines showed a peak at m/z 125 for the respective peroxylbenzenaminium ion. In contrast, an analogous peak at m/z 125 was absent in the spectrum of the 2-dehydroanilinium ion generated from 2-iodoaniline.



Evidently, the 2-peroxylbenzenaminium ion generated from the 2-dehydroanilinium ion immediately loses a 'OH to form protonated *ortho*-quinonimide $(m/z \ 108)$.

KEYWORDS: ortho effect, distonic ions, dehydroanilinium radical cations, dioxygen, quinonimide

INTRODUCTION

Reactions between gaseous ions and neutral molecules have been investigated from the early stages of mass spectrometry.¹⁻³ Many examples have been documented of adventitious or deliberate ion-molecule reactions (IMR) that occur between residual neutral gas molecules and nontargeted or mass-selected ions.⁴⁻¹⁶ Such ion-molecule reactions are often useful as probes for molecular structure and functional group determinations.¹⁷⁻²¹ Some of these reactions have been particularly useful for the differentiation of isomeric radical ions.²²⁻²⁴ Ion-molecule reactions are usually investigated using ion-storing mass analyzers such as ion traps and Fouriertransform ion cyclotron resonance instruments.²⁴ Occasionally, the formation of ion-molecule reaction adducts have also been observed on tandem-in-space instruments as well.^{16,25,20}

One of the fundamentally interesting ion-molecule reactions that has been investigated is the deliberate, or sometimes undesired, addition of dioxygen to radical sites of distonic radical cations.^{16,27-31} For example, both pyridineand aniline-derived distonic radical cations are known to form peroxyl radical cations with molecular oxygen.^{23,32} Similar O₂ addition reactions have been reported also for several distonic radical anions.^{33–37} Moreover, molecular oxygen is known to add to radical ions formed by the cleavage of N–C α bonds of the amide backbone in peptides under electron-capture

dissociation (ECD) or electron-transfer dissociation (ETD) conditions,³⁸ to radical anions generated from iodotyrosine residues,³⁹ to organometallic cobalt(III) and aluminum(III) complexes^{40,41} and other inorganic ions.^{42,43}

The term distonic ion was originally coined by Radom and co-workers to describe radical cations originating (formally) by ionization of zwitterions.⁴⁴ However, the term distonic ion is more commonly used simply to denote ions with formally separated charge and radical centers.44-53 Such ions are of fundamental interest because of the different chemical activities that can take place at two distinct loci within the same species. Kenttämaa and her co-workers⁵⁴ showed that 2-, 3-, and 4-dehydroanilinium ions, which are distonic isomers of the aniline radical cation (the molecular ion), can be formed in the gas phase from the respective iodoanilines. These isomers are kinetically stable and sufficiently long-lived to be investigated separately. They can be distinguished from the

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Figure 1. A schematic diagram of Waters Synapt-G2 HDMS instrument.

isomeric nondistonic radical cation by an ion-molecule reaction because only the three dehydroanilinium ions react by abstracting a thiomethyl radical from dimethyl disulfide.^{54,55}

In a recent study, Hossain et al.⁵⁶ reported the formation of deprotonated quinonoidal imides under electrospray ionization conditions. In the present work, we report the formation of *ortho*-quinonimide from protonated *ortho*-iodoaniline in the gas phase by a molecular-oxygen-mediated, *ortho*-isomerspecific fragmentation mechanism.

EXPERIMENTAL SECTION

Materials. Acetonitrile (ACN) was purchased from Pharmco-Aaper Co. (Brook field, CT). Other chemicals, including o-iodoaniline, m-iodoaniline, p-iodoaniline, and deuteriated water (D_2O) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and used without further purification. Deionized water was obtained from a Milli-Q purification system (Millipore Corporation, U.S.A.). Solutions of all samples (100 ppm) were prepared in a mixture of 50:50 acetonitrile:water. p-Iodo¹⁵N^{aniline} was prepared by adding iodine (10.0 mg, 0.078 mmol) to a solution of [¹⁵N]aniline (8.0 mg, 0.08 mmol) and sodium bicarbonate (11.0 mg, 0.13 mmol) in water (0.5 mL) at 0 $^{\circ}$ C.⁵⁷ The mixture was stirred at room temperature for 1 h, and the black precipitate was filtered and washed with water to obtain the desired product [¹H NMR spectroscopy confirmed 1,4 substitution (400 MHz, $CDCl_3$) δ ; 6.47 (d, 2H, J = 8.0 Hz), 7.41 (d, 2H, J = 8.0 Hz); Isomeric purity 96% (based on GC-MS); 70 eV EI-MS, 220 $(M^{+\bullet}, 100), 127 (15), 93 (75)].$

Mass Spectrometry. All experiments were conducted on a Synapt G2 HDMS (Waters, MA) mass spectrometer equipped with an ESI source (Figure 1). House nitrogen generated by a Parker-Balston model 75-A74 nitrogen generation system (Haverhill, MA, U.S.A.) was used as the nebulizer, desolvation, and cone gases. Mass calibration $(m/z \ 20-1500)$ was performed using a solution of sodium formate (100 ppm). Mass spectra were acquired in the positive-ion mode over a range of $m/z \ 20-1500$. For ion-mobility experiments, the ions of interest were mass selected by the quadrupole analyzer (Q) and transferred to the Triwave cell. Mass-selected ions were briefly accumulated in the *Trap* region of the Triwave cell and released to the N₂-filled traveling wave ion-mobility separation, the ions passed the *Transfer* cell and underwent acceleration by the

pusher before they were mass-separated by the time-of-flight (TOF) analyzer.

The m/z 220 ion generated under ESI conditions, from 100ppm o-, m-, or p-iodoaniline in acetonitrile:water (50:50 v/v; flow rate 20 μ L/min), was mass selected and subjected to ionmobility separation under the following instrumental conditions: Trap collision energy, 4–28 eV; Transfer collision energy, 2–28 eV; IMS wave velocity, 1500 m/s; IMS wave height, 40.0 V; IMS (N₂) gas flow 140 mL/min; scroll pump pressure, 4.34 mbar; source pressure, 3.43 × 10⁻³ mbar; helium cell pressure, 1.40 × 10³ mbar; IMS cell pressure, 3.98 mbar (N₂ supplied from a nitrogen generator); TOF analyzer pressure, 8.36 × 10⁻⁷ mbar; Trap-cell pressure, 3.10 × 10⁻² mbar (Ar); Transfer-cell pressure, 3.38 × 10⁻² mbar (Ar).

Unless otherwise stated, the general ESI source conditions for most experiments were as follows: capillary voltage, 2.85 kV; sampling cone, 30.0 V; extraction cone, 3.0 V; desolvation-gas flow rate, 138 L/h; sample infusion flow rate, 20 μ L/min; Vernier-probe adjuster position, 5.92 mm. The source and desolvation-gas heater temperatures were held at 80 and 100 °C, respectively.

For specificity experiments, *o-*, *m-*, or *p*-iodoaniline solutions (100-ppm in 50:50 acetonitrile:water) were electrosprayed at a capillary voltage of 2.85 kV and a cone voltage of 65 V. The insource-generated m/z 93 ions were mass selected by the quadrupole and subjected to ion mobility separation under low collision-energy settings (*Trap* 4 eV and *Transfer* 2 eV).

Similarly, chronograms were recorded using a dual-spray ESI system (with the baffel plate removed), and the intensities of m/z 93 and 125 ions were monitored on two separate channels. The mass selection by the quadrupole was switched from m/z 93 to m/z 125 every 1.024 s. The ToF analyzer was set to monitor ions between m/z 20–1500. Initially a 100-ppm solution of aniline in 50:50 acetonitrile:water was nebulized continuously via the main electrospray probe at a rate of 20 μ L/min (Figure 1). A high sampling-cone voltage (70 V) was used to generate m/z 93 radical cations from the aniline solution. At 2.0 min, a $10-\mu$ L aliquot of a *p*-iodoaniline solution (100 ppm in 50:50 acetonitrile:water) was introduced via the lock-spray probe at a rate of 20 μ L/min. The baffle plate, which mechanically blocks the signal from one sprayer while the instrument is recording the signal from the other, was removed for this experiment. Other instrumental parameters were: sampling cone voltage 70 V; main probe capillary voltage

3.85 kV; lock-spray probe capillary voltage 2.50 kV; source and desolvation heater temperatures 80 and 100 °C, respectively; cone and desolvation gas flows 34 and 138 L/h, respectively.

Density Functional Theory (DFT) Calculations. DFT calculations were carried out using the Gaussian 09W program at the B3LYP level of theory with a 6-311++G (2d,2p) basis set for C, H, N, O, and LanL2DZ basis set for iodine. Complete geometry optimizations were conducted for all species with subsequent frequency calculations for species at ambient pressure (1 atm) and room temperature (298.15 K) to verify the nature of each stationary state on the potential energy surface. In brief, reactants and products were associated with all positive vibrational frequencies, whereas transition states (TS) were associated with only one imaginary frequency, for which the normal vibrational mode corresponds to the expected bond formation/breaking movements in a specific reaction pathway. Atom coordinates and absolute energies of intermediates and transition states obtained in this way are provided as Supporting Information.

RESULTS AND DISCUSSION

Under ESI-MS conditions *ortho-*, *meta-*, and *para-*iodoanilines generate intense signals at m/z 220 for their respective protonated species.⁵⁸ The m/z 220 ions generated in the ion source by ESI from each isomer were mass selected by the quadrupole filter on a Synapt G2 HDMS instrument and subjected to ion-mobility separation using nitrogen as the mobility gas. The recorded arrival-time profiles showed that the ion generated from the *ortho* isomer manifests a slightly faster mobility than the other two species (Figure 2). When the ion-activation conditions in the *Trap* and *Transfer* collision regions of the Triwave cell were relatively low (4 and 2 eV,



Figure 2. Ion mobility arrival-time distributions of m/z 220 ions of protonated *ortho*- (a), *meta*- (b), and *para*-iodoaniline (c). Insets show the m/z 20 to 250 region of product-ion mass spectra (m/z 20 to 1500) recorded at *Trap* and *Transfer* collision-energy settings of 4 and 2 eV, respectively. The m/z 220 ions were generated by ESI from 100-ppm solutions in acetonitrile:water (50:50) of each iodoaniline. Other conditions: sampling cone voltage 30 V, capillary voltage 2.85 kV; source and desolvation temperatures 80 and 100 °C, respectively; cone and desolvation gas flows 38 and 138 L/h, respectively. Ionmobility separations were carried out at a wave velocity of 1500 m/s and a wave height of 40.0 V and using N₂ as the buffer gas at a flow rate of 140 mL/min.

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respectively), the arrival-time distributions (ATDs) and mass spectra recorded for each species showed only one peak in both arrival-time and mass spectral profiles, establishing that under such low ion-activation conditions, the m/z 220 ions from none of the three isomers undergo any noticeable fragmentation (Figure 2 insets).

However, the product-ion spectra of the three mobilityseparated isomers, recorded after subjecting them to 25 eV collisional activation in the *Transfer*-collision cell, were dominated by a peak at m/z 93 (Figure 3). In fact, these



Figure 3. m/z 20–250 region of product-ion spectra (m/z 20 to 1500) recorded from mass-selected and ion-mobility separated m/z 220 ions generated from *ortho*- (a), *meta*- (b), and *para*-iodoaniline (c) activated at 4 eV *Trap* and 25 eV *Transfer* collision-energy settings. The mobilograms corresponding to each isomer are shown in insets. All other experimental conditions were identical to those given in Figure 2.

spectra were indistinguishable from the product-ion spectra recorded from mass-selected m/z 220 ions by subjecting them to 10 eV activation in the *transfer*-collision cell but without subjecting them to ion-mobility separation (i.e., in the absence of helium or nitrogen in the mobility cell) (Figure 4).

The presence of the base peak at m/z 93 confirmed that the major fragmentation of all three isomers represents the loss of an iodine radical due to the homolytic fragmentation of the C–I bond (Scheme 1). The C–I bond fragmentation generates the respective dehydroanilinium ions as noted previously by Chyall and Kenttämaa.⁵⁴

The homolytic fragmentation of the C–I bond in iodoanilinium ions can easily be attained also in the *Trap*collision cell of the Triwave system. From such an experiment, we expected to see two well-separated peaks in the ionmobility arrival-time profiles: one peak for the precursor m/z220 ion and another for the m/z 93, the major product-ion generated by collision-induced fragmentation in the *Trap* region. However, to our surprise, in the mobilograms recorded from *meta* and *para*-iodoaniline at a *Trap*-collision energy setting of 28 eV, a very significant, but entirely unexpected, additional peak was observed in the 4.80–4.95 ms region (Figure 5b,c).

From the mass spectra corresponding to the peaks at 5.89 and 3.53 ms peaks in the mobility profile of the *para* isomer (and 5.68 and 3.53 ms peaks in the profile of the *meta* isomer; Figure 5b), it was evident that these two peaks represent the m/z 220 precursor ion and the m/z 93 product ion, generated

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Figure 4. Transfer-fragmentation product-ion spectra recorded under nonmobility conditions (no helium or nitrogen in the mobility cell, and no ion accumulation in the *Trap* region) from mass-selected m/z220 ions generated under ESI conditions from protonated *ortho*- (a), *meta*- (b), and *para*-iodoaniline (c). Samples were infused as 100-ppm solutions in acetonitrile:water (50:50), and data were acquired for 0.5 min. The spectra displayed were generated by coadding and averaging for the whole acquisition period. The sampling cone voltage was 30 V; capillary voltage was 2.85 kV; source and desolvation heater temperatures were 80 and 100 °C; and cone- and desolvation-gas flows were 38 L/h and 138 L/h, respectively. *Trap* and *Transfer* collision energies were 4 and 10 eV, respectively.

Scheme 1. Homolytic Fragmentations Manifested by Protonated Iodoanilines upon Collision-Induced Dissociation (CID)



in the *Trap* (Figure 5c). In contrast, the product-ion spectrum corresponding to the 4.92 ms peak in Figure 5c showed three peaks at m/z 125, 109, and 93. The accurate mass of the m/z 125 ion confirmed its molecular formula to be $C_6H_7NO_2$ (Supporting Information Table S1). It was evident that this peak represented an ion produced by the addition of molecular oxygen to the distonic m/z 93 radical cation that was generated in the *Trap* by a homolytic removal of iodine from the m/z 220



Figure 5. Arrival-time distributions (ATDs) recorded from the massselected *m/z* 220 ion of *o*-iodoaniline (a), *m*-iodoaniline (b), and *p*iodoaniline (c) at a *Trap*-collision energy setting of **28 eV**. Insets show spectra that represent ions delivered to the ToF analyzer at specific arrival times. Ions were generated by electrospraying 100-ppm solutions in 50:50 acetonitrile:water at a flow rate of 20 μ L/min. Other experimental conditions were as follows: sampling-cone voltage 30 V; capillary voltage 2.85 kV; source and desolvation temperatures 80 and 100 °C, respectively; cone and desolvation gas flow rates were 38 and 138 L/h, respectively; *Transfer*-collision energy of **2.0 eV**. IM separations were carried out at a wave velocity of 1500 m/s, a wave height 40.0 V, and a mobility gas (N₂) flow rate of 140 mL/min.

precursor ion. The coadded spectra generated from the massselected m/z 221 (M+H)⁺ ion from protonated p-iodo^{[15}N]aniline showed the corresponding O₂-adduct peak at m/z 126 (Supporting Information Figure S1A). Moreover, the averaged spectrum obtained from deuteronated p-iodoaniline [I- $C_6H_4-ND_3^+$ showed the corresponding peak at m/z 128 (Supporting Information Figure S1B). An analogous adduct peak at m/z 125 for the oxygen adduct was observed also in the spectrum recorded from the adduct of meta-iodoaniline (Figure 5b). All these results confirmed that the dehydroanilinium radical ions from meta and para isomers form adducts with dioxygen under the experimental conditions used. Apparently, the addition reaction takes place at the radical site of the distonic ion, with minimal direct involvement of the charge-bearing amino group. The formation of such an adduct was not surprising because the addition of dioxygen to phenylradical distonic ions has been well documented.^{31,36,58-60} For example, a small peak for an oxygen adduct has been seen in the product-ion spectrum recorded from 4-iodoanilinium ion subjected to photodissociation at 260 nm.⁵⁹ In the present work, we observed the formation of such adducts under certain tandem mass spectrometric conditions. Although dehydroanilinium ions show a very high reactivity toward molecular oxygen, no peaks corresponding to such adducts were observed when the mobility cell was not engulfed with nitrogen (Supporting Information Figure S2). Presumably, nitrogen from the relatively high-pressure mobility cell compartment (about 3-4 mbar) leaks out to the low-pressure ion-trapping region (about 10⁻² mbar) (Figure 1). Oxygen present in trace amounts in the nitrogen then reacts with the Trapaccumulated dehydroanilinium ions. If the addition took place in the mobility cell, the arrival-time peaks were expected be broader and diffused in shape. However, the peaks observed

for adducts were symmetrical and nearly Gaussian (Figure 5b,c). Furthermore, product-ion spectra recorded from *Trap*fragmented mass-isolated m/z 220 ions, but without nitrogen in the mobility cell, did not show a peak at m/z 125 or at m/z108 (Supporting Information Figure S2). This result was not surprising because ions are not accumulated in the *Trap* region, but transmitted continuously, when the instrument is not operated under mobility conditions. In contrast, ion packets are accumulated for about 200 μ s in the *Trap* region before they are released for separation under mobility conditions. Thus, there is a sufficient amount of time for ion-molecule reactions to take place in the Trap region when mobility experiments are conducted.

Compared to the arrival-time profiles recorded from protonated meta- and para-aniline that acquired from protonated o-iodoaniline was very different (Figure 5). At first glance, it was surprising that an m/z 125 peak in the 4.6-5.0 ms region for a dioxygen adduct was not visible in the arrival-time profile recorded from the mass-selected m/z 220 ion from o-iodoaniline (Figure 5a). It appeared as if the dehydroanilinium radical cation generated from the ortho isomer does not form a dioxygen adduct. On the other hand, we noticed that the shape of the 3.60 ms peak was now rather skewed, and the spectra corresponding to the tailing end of the peak showed a signal at m/z 108, in addition to that at m/z 93 (Figure 5a). A rigorous scrutiny conducted by extracting ionintensity profiles for m/z 108 and 93 ions deconvoluted the 3.60 ms profile to two peaks with respective maxima at 3.81 and 3.53 ms (Figure 6). Thus, the 3.60 ms peak is a composite representing an m/z 108 ion in addition to the expected m/z93 species (Figure 6c).

Evidently, the addition of dioxygen to the ortho-dehydroanilinium ion follows a different route compared with those taken by meta- and para-dehydroanilinium ions. To rationalize the formation of the ortho-isomer-specific m/z 108 ion, we propose the fragmentation mechanism illustrated in Scheme 2. Calculated electronic energies predicted that the 2-peroxylbenzenaminium ion formed by the initial interaction of dioxygen with the ortho-dehydroanilinium ion is a short-lived species. The energy released (176.4 kJ/mol) in the exergonic formation of the initial adduct far exceeds that required to surmount the small 2.8 kJ/mol energy barrier separating the 2peroxylbenzenaminium ion (7) from the final products (9)(Figure 7). Consequently, the adduct 7 $(m/z \ 125)$ immediately eliminates a hydroxyl radical via a six-membered transition state (TS_{7-8}) and forms protonated orthoquinonimide $(m/z \ 108)$ as the final product. Thus, neither a peak at m/z 125 nor m/z 109 (for a 16-Da oxygen atom loss from the m/z 125 ion) is visible in the spectrum of orthodehydroanilinium ion: the hypothetical cleavage of the peroxyl bond in adduct 7 to form the m/z 109 ion (11) requires 139.8 kJ/mol (Figure 7; $7 \rightarrow 11$), whereas the ejection of HO[•] needs only to surmount a 2.8 kJ/mol energy barrier to form the oquinonimide cation (9, m/z 108) (Figure 7; 7 \rightarrow 9). A loss of HO[•] from oxygen adducts of cation radicals formed from protonated peptides has been noted previously by Xia et al.³⁸ The proposed mechanism of a HO[•] removal by an orthoisomer specific mechanism (Scheme 2) resembles the chargeremote pathway proposed by Moore et al. for the formation of an ortho-quinone derivative from the peroxyl radical generated by the tyrosinyl radical cation combining with oxygen.³⁹ Prendergast et al.⁶¹ reported a similar HO[•] loss by a chargeremote mechanism from the oxygen adduct of 5-(N,N,N-



Figure 6. Arrival-time distributions (ATDs) recorded from the massselected m/z 220 ion of *o*- iodoaniline (c) and from extracted ion profiles of m/z 93 (a) and m/z 108 (b). Profiles were recorded at a *Trap*-collision energy setting of 28 eV. Insets show spectra representing ions delivered to the ToF analyzer at specific arrival times. Ions were generated by electrospraying 100-ppm solutions in 50:50 acetonitrile:water at a flow rate of 20 μ L/min. Other experimental conditions were as follows: sampling-cone voltage 30 V; capillary voltage 2.85 kV; source and desolvation temperatures 80 and 100 °C, respectively; cone and desolvation gas flow 38 and 138 L/h, respectively; *Transfer*-collision energy 2.0 eV. IM separations were carried out at a wave velocity of 1500 m/s, wave height of 40.0 V, and a mobility gas (N₂) flow rate of 140 mL/min.

Scheme 2. Proposed Fragmentation Pathway for the Formation of Protonated *ortho*-Quinonimide (9; m/z 108) from *ortho*-Dehydroanilinium Ion (6)



trimethylammonium)-2-methylphenyl radical cation, forming a charged quinone methide derivative. The same researchers described the formation of an ammonium tagged *o*-benzoquinone derivative from 5-ammonium-2-hydroxyphenyl



Figure 7. Combined relative electronic and zero-point energies [in kJ/mol computed for 0 K by the density functional theory method B3LYP using a 6-311+ + G(2d,2p) basis set] and molecular structures of energy-optimized ions and transition states associated with the formation of m/z 108 (9) and 109 (11) ions upon O₂ addition to *o*-dehydroanilinium radical anion (6).

radical.⁶² In contrast to the aforementioned charge-remote mechanisms, the pathway described in Scheme 2 denotes a transfer from a charge-bearing functionality resulting in the formation of protonated *ortho*-quinonimide (9). This is supported by the observation that the adduct of the deuteronated 2-dehydro[N²H₂]anilinium radical cation (for which a peak was not observed), loses a DO[•] to generate an ion of m/z 110 (Supporting Information Figure S3).

Quinonimides are structurally interesting molecules. Previously, the *o*-quinonimide anion has been generated from *o*azidophenol and examined by photoelectron spectroscopy.⁵⁶ In this study, we have demonstrated the formation of protonated *o*-quinonimide under positive-ion-generating electrospray ionization and Triwave ion-mobility tandem mass spectrometric conditions.

The addition reactions of O2 to meta- and paradehydroanilinium radical cations are also exergonic (Supporting Information Figure S4 and S5). Thus, the formation of the m/z 125 ion is expected to be spontaneous. However, the dissociation of 3- and 4-peroxylbenzenaminium ions (m/z)125) to form m/z 109 ions by losing a 16-Da oxygen atom are highly endergonic reactions (Supporting Information Figures S1, S3, S4, and S5). Thus, it is not surprising that the intensities of the peaks at m/z 109 ions for the oxygen-atom loss are very low. Furthermore, this results is congruent with the observation that in the potential energy surfaces (PES) that describe the energies of the interaction of molecular oxygen with meta- and para-dehydroanilinium ions, the 3- and 4peroxylbenzenaminium ions occupy minima (Supporting Information S4 and S5). In contrast, the 2-peroxylbenzenaminium ion formed from the ortho-dehydroanilinium ion has a more favorable alternative reaction channel available (Figure 7). Thus, the m/z 125 ion for the oxygen adduct of the 2dehydroanilinium cation continues to undergo fragmentation

via the low-energy saddle point TS_{7-8} by a HO[•] elimination (instead of losing an O[•] as that followed by its *meta* and *para* counterparts) and forms the *ortho*-quinonimide (m/z 108) (Figure 7, 9).

To demonstrate the specificity of the O₂-addition reaction, we generated the m/z 93 ions by subjecting protonated o-, m-, and *p*-iodoaniline to collision-induced dissociation in the lowvacuum region of the ion source (Figure 1), by increasing the sampling-cone voltage to 65 V (Supporting Information Figure S6). The m/z 93 ions generated in the ion source in this way were mass-selected by the quadrupole analyzer and subjected to ion mobility separation under low collision activation conditions (Trap 4 eV and Transfer 2 eV). The arrival-time profiles recorded from meta- and para-dehydroanilinium radical cations showed peaks for the respective 3-peroxylbenzenaminium and 4-peroxylbenzenaminium ions $(m/z \ 125)$ in addition to those for the precursor m/z 93 ions (Supporting Figure S6b,c). However, the peaks for adduct ions were very small compared to those obtained from trap fragmentation of m/z 220 ions (Figure 5b,c). Apparently, the 3 and 4dehydroanilinium ions generated in the source are mostly quenched in the source by atmospheric oxygen. Thus, the peaks at 4.97 and 5.04 ms in Supporting Figure S6b,c represent the adducts formed from small amounts 3 and 4dehydroanilinium ions that had survived the onslaught of oxygen in the source. In other words, we believe that the peaks depicting m/z 93 ions in Figure 5 represent the conventional radical cation of aniline (formed by isomerization), which is nonreactive toward oxygen. This proposition is supported by the observation that the product-ion spectra of m/z 93 ions recorded at high transfer-collision energy (20 eV) showed a product ion peak at m/z 66 for a 27-Da HCN loss (Supporting Information Figure S7a and S7b). On the other hand, dehydroanilinium ions are expected to give a product ion

peak at m/z 76 for a 17-Da NH₃ loss. Furthermore, a peak for the m/z 125 adduct was absent in the arrival-time profile recorded from 2-dehydroanilinium ion (Supporting Information Figure S6a). It appears that the 2-dehydroanilinium ion is so reactive that it undergoes complete annihilation in the ion source. The higher reactivity of the 2-dehydroanilinium ion has been noted recently by Kelly et al.²⁴ Thus, the m/z 93 ion delivered from the ion source to the mobility cell is the nonreactive molecular radical-cation of aniline (Supporting Information Figure S6a).

Further support for the above supposition was obtained from the data recorded using a dual-sprayer ionization source hyphenated to the mass spectrometer. The results confirmed that the oxygen addition reaction in fact can take place in the ion source under ambient conditions. To demonstrate this, an experiment was set up to specifically monitor the intensities of the m/z 93 and 125 ions on two channels (Supporting Information Figure S8a,b). Initially, the m/z 93 ion was generated continuously in the ion source by electrospraying a 100-ppm solution of aniline at a high sampling-cone voltage. Under such high cone-voltage conditions, in addition to the m/z 94 ion for protonated aniline, a small abundance of the conventional m/z 93 ion is also generated in the ion source.⁶³ The m/z 93 ion generated in this way is deemed to be a composite consisting primarily of the molecular ion of aniline, which known to be inert to oxygen addition (Supporting Information Figures S7a,b). Thus, intensity of the m/z 125 ion in the ion source was initially low. While continuously spraying the solution of aniline from the main spray probe, a sample of p-iodoaniline was introduced at 2.0 min to the source via the secondary sprayer for 15 s. Immediately after the p-iodoaniline was introduced, the intensity of the m/z 125 signal soared up, confirming the formation the O2-adduct from the in-source generated 4-dehydroanilinium ion generated by the collisioninduced fragmentation of protonated 4-iodoaniline in the ion source (Supporting Information Figure S8b).

CONCLUSIONS

Undesired ion-molecule reactions lead to the formation of ions that cannot be formed directly from the precursor ions. Such reactions complicate mass spectra and not only distort quantification results obtained by multiple reaction monitoring (MRM) procedures⁶⁴ but also mislead artificial intelligence (AI) programs designed to identify compounds either by interpretation or database search of mass spectra.⁶⁵⁻⁶⁷ When the chemistry is properly comprehended, however, the complications due to undesired ion-molecule reactions can sometime become beneficial, and AI can be programed to provide more robust identifications. Herein, we have described the specificity of the undesired oxygen-addition reaction, which can be used as a diagnostic marker to differentiate *ortho*-dehydroanilinium radical cation from its isomeric counterparts.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jasms.9b00108.

Figures and tables showing additional mass spectral, ion mobility, and computational data.

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Notes

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