



# Generation and characterization of distonic dehydrophenoxide radical anions under electrospray and atmospheric pressure chemical ionizations

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## ABSTRACT

We have explored the possibilities of generating radical anions under electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) conditions. By using different sets of *ortho*-, *meta*-, and *para*-isomers of nitrobenzoic acids, methylphenols, and nitrophenols, and *m*-, and *p*-isomers of hydroxybenzaldehydes and hydroxyacetophenones as the precursor molecules, we have successfully generated the isomeric distonic dehydrophenoxide radical anions ( $m/z$  92) using the ESI process by applying relatively high capillary voltages, the in-source dissociation (ISD) condition. Under the same conditions, the *o*-hydroxybenzaldehyde and the *o*-hydroxyacetophenone yielded the even-electron dehydrophenoxide anion ( $m/z$  93) due to the well-known *ortho*-effect. The distonic phenoxide radical anions at  $m/z$  92 were also generated under APCI-ISD conditions by using *m*- and *p*-isomers of nitrobenzaldehydes and nitroacetophenones. While the *o*-nitrobenzaldehyde and the *o*-nitroacetophenone mainly yielded the phenoxide anion at  $m/z$  93, due to the *ortho*-effect. The collision-induced dissociation (CID) experiments of all the anionic precursor molecules formed from either ESI or APCI produced comparable mass spectra as those observed in the ESI-ISD or the APCI-ISD experiments. The radical anions at  $m/z$  92 reacted with  $\text{CO}_2$  and  $\text{O}_2$  to form the  $\text{CO}_2$  adduct and the oxygen atom abstraction product, respectively, revealing the dual-character of the distonic radical anions, the phenide ion and the phenyl radical. Computational studies support the results of the ion-molecule reactions.

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## 1. Introduction

The chemistry of distonic ions has been a subject of mass spectrometry studies for many years. “Distonic ions” refer to a special group of radical ions with distinct and spatially separated charge and radical sites [1]. This type of radical ions possesses dual reactivity with the potential to undergo both ionic and radical reactions. If the charge sites are sufficiently inert, the radical sites can be used as probes for radical reactivity. In this way, distonic ions provide unique systems to study the chemistry of free radicals using mass spectrometry techniques [2–5]. With the pioneering work of Kenttämaa and coworkers as well as other researchers, numerous studies have been directed toward charge-inert radical cations [3,6–12]. At the same time, the studies of distonic radical anions have also yielded interesting results [4,5,13–18]. The fascinating benzyne radical anions were synthesized by Squires, Wenthold and coworkers in a flowing afterglow-triple quadrupole instrument.

They characterized the structures of *o*-, *m*-, and *p*-benzyne radical anions via step-wise ion-molecule and radical-molecule reactions with  $\text{CO}_2$  and  $\text{NO}_2$ , respectively, to produce nitrobenzoates. The relative proton affinities of the nitrobenzoates were then compared to those of authentic isomeric nitrobenzoates [19–21]. Using electrospray ionization (ESI) coupled with collision-induced dissociation (CID), Kass and coworkers successfully generated the same set of isomeric benzyne radical anions from isomeric benzenedicarboxylic acids in a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer [17]. Recently, Blanksby and coworkers generated carboxylate-admantyl radical anion in an ESI-linear quadrupole ion trap mass spectrometer. The structurally unusual bridge-head radical readily reacted with oxygen molecules to produce peroxy radical in the gas-phase [22]. The same group also reported the observation of carboxylate-cyclohexyl distonic radical anions and these radical ions also reacted with oxygen molecules readily [23].

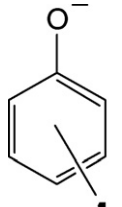
The distonic dehydrophenoxide radical anions ( $\text{C}_6\text{H}_4\text{O}^{\bullet-}$ ,  $m/z$  92) were first observed by Bowie and coworkers in the CID experiments of isomeric *o*-, *m*-, and *p*-alkyl substituted phenoxide anions [24]. Later on, Kass and coworkers also generated these radical anions through CID experiments with *o*-, *m*-, and *p*-nitrobenzoate [18]. In these experiments, the nitrobenzoate first lost the  $\text{CO}_2$

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molecule to form nitrophenide anion, and the nitrophenide ion lost NO (an odd electron species) to produce the dehydrophenoxide radical anion. The radical site is expected to be on the benzene ring and is located regio-specifically. The structures of these radical ions were characterized through a series of ion–molecule and radical–molecule reactions in an FTICR instrument. The thermochemical properties of these radical ions were also determined [18]. In a recent study, Attygalle and coworkers generated the same set of phenoxide radical anions from isomeric hydroxyphenyl carbaldehydes and ketones via ESI and CID experiments in a triple-quadrupole mass spectrometer. They observed that the deprotonated precursor molecules readily lost either the HCO radical (may be via two steps) or the RCO radical to produce the phenoxide radical anions at  $m/z$  92. The proposed mechanisms were studied using isotope labeling experiments [25].



$m/z$  92

We discovered that an odd electron anion at  $m/z$  92 could also be formed in the negative ESI process from ionization of isomeric *o*-, *m*-, and *p*-nitrobenzoic acids. Could this odd-electron ion have the structure of dehydrophenoxide radical anion? This seemingly violates the “even-electron rule”, considering that ESI is a soft-ionization process and the ions generated are expected to be stable even-electron species [26,27]. Recently, Cole and coworkers reported the observation of an odd-electron phenoxide radical anion from low-energy CID of an alkoxy-phenoxide generated under negative ESI. The observation of the radical anion at near 0 eV collision energy suggested that the formation of this ion is an energy efficient channel [27]. This also indicated that this odd-electron ion might be generated in the ESI process under certain instrument conditions.

The generation of radical anions during the ESI process may have potential applications for electron-transfer dissociation (ETD) of gas-phase peptides. ETD is a relatively new type of gas-phase ion-ion reaction [28,29]. The basic principle of ETD involves the generation of reagent ions (anions) followed by ion–ion reactions between the reagent ions and multiply charged peptide ions (cations) to form peptide radical ions in the mass spectrometer. These nascent radical ions dissociate readily to yield sequence-informative fragment ions. Compared to conventional dissociation methods, such as CID, ETD has the advantage of the preservation of structural information of precursor ions with labile functional groups, like phosphorylation. Studies have shown that electron transfer induced fragmentation prefers to cleave the N–C $_{\alpha}$  bonds of the peptide backbone over the labile side chain of the post-translational modification (PTM). Therefore, ETD is particularly useful in characterizing the sequences of peptides with PTMs [28,30,31]. However, efficient ETD needs “good” reagent ions. The good reagent ions should be readily generated with strong ion signals, should have high tendency to transfer electrons to peptides and should have minimum tendency for proton transfer reactions [32]. The usual reagent ions used in ETD experiments are radical anions generated through chemical ionization of vaporized neutral precursor molecules by using a heated ion source [28,32,33]. The reagent ions generated in this way require the precursor molecules to be readily vaporized, which would limit the  $m/z$  values of the reagent ions. McLuckey and coworkers devel-

oped a dual-ESI source coupled with trap-type mass analyzers. This technique allows the generation and trapping of ions with opposite polarity simultaneously [29]. They have demonstrated the formation of a new class of efficient ETD reagent anions, carbanions, via ESI followed by CID. For example, the 9-anthracenecarboxylate formed from ESI undergoes CID by loss of CO<sub>2</sub> and lead to the formation of anthracenide [34]. The applications of the dual-ESI technique could be greatly broadened if the ETD reagent ions could be generated directed from ESI or APCI processes.

We have explored the possibilities of generating radical anions via ESI and atmospheric pressure chemical ionization (APCI) conditions using a variety of precursor molecules. We have found that dehydrophenoxide radical anions could be generated from a series of substituted phenols, and nitrobenzaldehydes and nitroacetophenones, as well as nitrobenzoic acid under certain ESI or APCI conditions. In this paper, we describe the results from these studies.

## 2. Experimental

### 2.1. Mass spectrometry

The mass spectrometry measurements were carried out using a triple-quadrupole mass spectrometer (Varian 320L and Varian 1200L, Varian Inc., Walnut Creek, CA) located in the Mass Spectrometry Facility of the Chemistry Department at the University of the Pacific. The instrument is equipped with two switchable ion sources, ESI and APCI. Data acquisition was controlled with the MS Workstation software package (version 6.9). For both negative mode ESI and APCI experiments, nitrogen was used as the desolvation gas and compressed air was used as the nebulization gas. The ion source and drying (desolvation) gas temperatures were kept at 50 °C and 300 °C, respectively. The pressures of the nebulization gas and drying gas were 50 and 18 psi, respectively. The ESI needle voltage was maintained at –4.5 kV. The APCI corona needle current and voltage were set at 5  $\mu$ A and –5.0 kV, respectively, and the auxiliary gas (vaporizer gas) was set at 500 °C. To perform the in-source dissociation (ISD) with the ions generated in either ESI or APCI experiments, the capillary voltage was varied in the range of 10–120 V. The ion guide chamber has about 1 mTorr nitrogen gas. Ions generated in the ESI or APCI source are thermalized by multiple collisions with the nitrogen molecules in the ion guide. The CID experiments were performed in the collision chamber of the triple quadrupole with argon as the collision gas. The pressure of argon gas is around 0.5 mTorr. For carrying out ion–molecule reactions, the precursor ions were isolated and transmitted into the collision chamber. The collision gas was replaced with reagent gas, such as CO<sub>2</sub>, and the collision energy was kept relatively low (0–2 eV). The reaction products were analyzed with the third quadrupole.

Similar experiments (ESI-ISD, CID, and ion–molecule reactions) were also performed previously in a Quattro triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an ESI source. Spectra were acquired using the Masslynx software (version 3.2). The typical operating conditions included a capillary voltage at –3.5 kV and cone voltage at 10–50 V. The source and desolvation temperatures were kept relative high at 150 °C and 300 °C, respectively. Nitrogen was used as the nebulization and desolvation gas. For ion–molecule reaction experiments, CO<sub>2</sub> gas was used as the collision gas and the pressure inside the collision cell was  $9 \times 10^{-4}$  mbar (0.7 mTorr) and the collision energy was set at 0 eV. For CID experiments, argon was used as the collision gas and the pressure was maintained at  $3 \times 10^{-4}$  mbar (0.2 mTorr). High resolution mass spectra were recorded on a QSTAR XL mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA). The typical operating Cap voltage was –4.0 kV and declustering potential was greater than 50 eV.

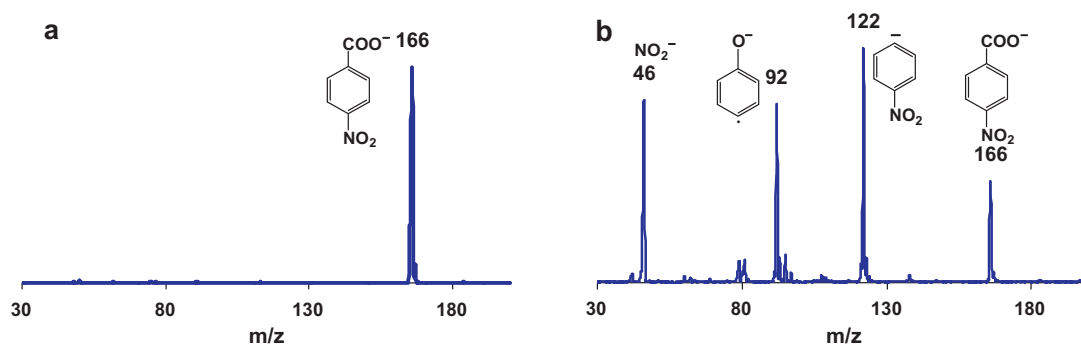


Fig. 1. Mass spectra recorded under ESI-ISD conditions for *p*-nitrobenzoic acid at (a) capillary voltage of 20 V, and (b) capillary voltage of 80 V.

All the compounds in this study were commercially available (Sigma–Aldrich, St. Louis, MO) and were used without further purification. Stock solutions ( $\sim 10^{-3}$  M) of all the compounds were made in HPLC-grade methanol and water with a 50:50 (v:v) ratio. Stock solutions were diluted with methanol and water (50:50) to achieve a final concentration of  $10^{-4}$ – $10^{-5}$  M to be used as the sample solution. The sample solution was introduced into the ESI or APCI source by an infusion pump at a flow rate of 10–20  $\mu\text{L}/\text{min}$ .

## 2.2. Computational method

All computations were performed using the Gaussian 03 suite of programs [35]. The geometry optimizations, frequencies, and energies were all calculated at the B3LYP/6-31+G(d) level of theory. The spin unrestricted UB3LYP/6-31+G(d) was used for all odd-electron species. All structures were optimized to energy minima as reflected by all real frequencies.

## 3. Results and discussion

### 3.1. Nitrobenzoic acids

The three isomeric *o*-, *m*-, and *p*-nitrobenzoic acids, **2**, were examined under ESI negative ion condition by varying the capillary voltages. All three compounds behave similarly. At a low capillary voltage (<20 V), only the signal corresponding to the deprotonated precursor molecule, **3** at *m/z* 166, was observed. Upon increasing the capillary voltage, several smaller ions were also observed at *m/z* 122, *m/z* 92 and *m/z* 46. Two representative spectra are shown in Fig. 1. The complete set of ESI-ISD spectra is provided in supplemental information. The proposed reaction scheme is shown in Scheme 1A. The smaller ions are apparently the fragments from **3**. The process may be described as in-source dissociation (ISD). The ion at *m/z* 122 is the primary fragment and is likely the nitrophenide anion (**4**) formed by  $\text{CO}_2$  loss from **3**. The loss of  $\text{CO}_2$  is a common fragmentation process observed from aromatic carboxylic acids under CID conditions [18,36–38]. The ion at *m/z* 46 represents  $\text{NO}_2^-$ , which is a characteristic fragment ion for nitroaromatic compounds [39]. The ion at *m/z* 92 is an interesting fragment ion, which corresponds to the loss of 74 mass units from **3**. Apparently, the ion at *m/z* 92 contains an unpaired electron and is a radical anion,  $\text{C}_6\text{H}_4\text{O}^{\bullet-}$ .

In order to confirm the ion at *m/z* 92 is a fragment from **3**, we performed several CID experiments on the ions formed in the ESI source. The dissociation of the ion at *m/z* 166 (**3**) in the collision cell yielded a CID spectrum (Fig. 2a) qualitatively similar to that generated from the ESI-ISD process (Fig. 1b). The peak at *m/z* 92 is clearly seen in this CID spectrum. The dissociation of the ion at *m/z* 122 (**4**) in the collision cell also yielded the ion at *m/z* 92 (Fig. 2b). The CID experiments suggest that the ion at *m/z* 92 was formed by the loss

of the odd-electron species, NO, from the nitrophenide anion (**4**). This means that the ion at *m/z* 92 is indeed a radical anion. We further performed precursor ion scan experiment. The result showed that both *m/z* 166 and *m/z* 122 were the precursor ions for *m/z* 92. The mechanism for the formation of *m/z* 92 in the collision cell is shown in Scheme 1B. In a previous study, Kass and coworkers have reported the generation of *o*-, *m*-, and *p*-dehydrophenoxide radical anions (*m/z* 92) from isomeric nitrobenzoates by using the CID experiments in an FT-ICR instrument [18]. Their studies suggested that the radical anions were formed in the ICR cell in two sequential steps, the loss of  $\text{CO}_2$  to yield nitrophenide anions followed by the loss of NO.

The results show that the same radical anions at *m/z* 92 that were observed in the CID experiments can also form in the ESI experiments under ISD conditions. As shown in Scheme 1A, the carboxylate anion (**3**) was generated directly from ESI. Upon increasing the capillary voltage, the ISD process took place. The carboxylate anion first loses the  $\text{CO}_2$  unit to form the phenide anion (**4**). A portion of the phenide anion carries excess energy and continues to fragment by losing the NO radical, and the radical anion at *m/z* 92 (**1**) is formed.

### 3.2. Substituted phenols

We extended the ESI-ISD experiments to a series of substituted phenols. Bowie and coworkers have reported the generation of radical anions from alkyl-substituted phenoxide anions. They found that the alkyl-phenoxides could readily lose alkyl radicals in the CID experiments [24]. We first selected a set of isomeric cresols, the *o*-, *m*-, and *p*-methylphenol (**5**). Under mild ESI condition (low capillary voltages), the methylphenoxide anions (**6**) could easily form. Upon increasing the capillary voltage, the expected radical anions at *m/z* 92 were observed for all three isomeric methylphenols. The spectrum for the *p*-isomer is shown in Fig. 3a. The peak at *m/z* 106 is likely the result of losing a hydrogen atom from deprotonated cresol [24]. The formation of the radical anion would involve direct loss of methyl radical ( $\text{CH}_3^{\bullet}$ ) from **6**, Scheme 2A. The elemental composition of the ion at *m/z* 92 was confirmed by high resolution mass spectrometry (HRMS) data. We also performed the CID experiments on methylphenoxide anions (**6**), and the results were similar to those observed in the ESI-ISD experiments.

With these encouraging results, we selected three other sets of phenol derivatives, nitrophenols (**7**), hydroxybenzaldehydes (**8**), and hydroxyacetophenones (**9**). All these isomeric compounds gave abundant signals corresponding to deprotonated phenols in the ESI experiments under low capillary voltages. At higher capillary voltages (>60 V) all compounds, except the *o*-hydroxybenzaldehyde and the *o*-hydroxyacetophenone, yielded a common ion at *m/z* 92. Selected spectra are shown in Fig. 3(a–f). The complete set of ESI-ISD spectra is provided in supplemental information.

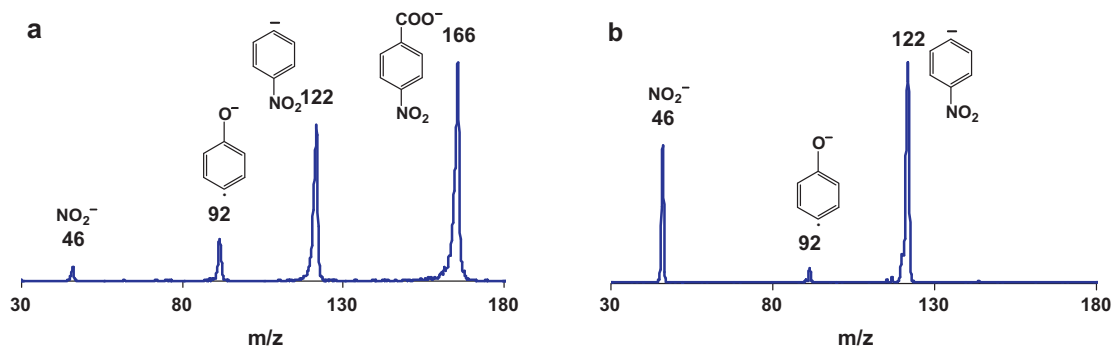
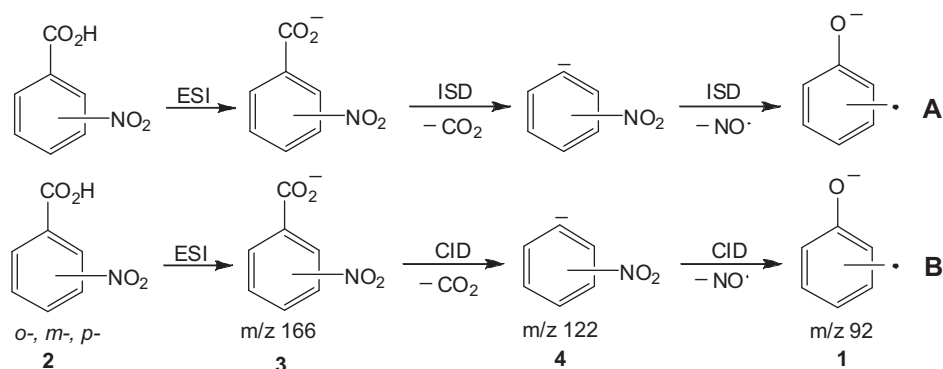
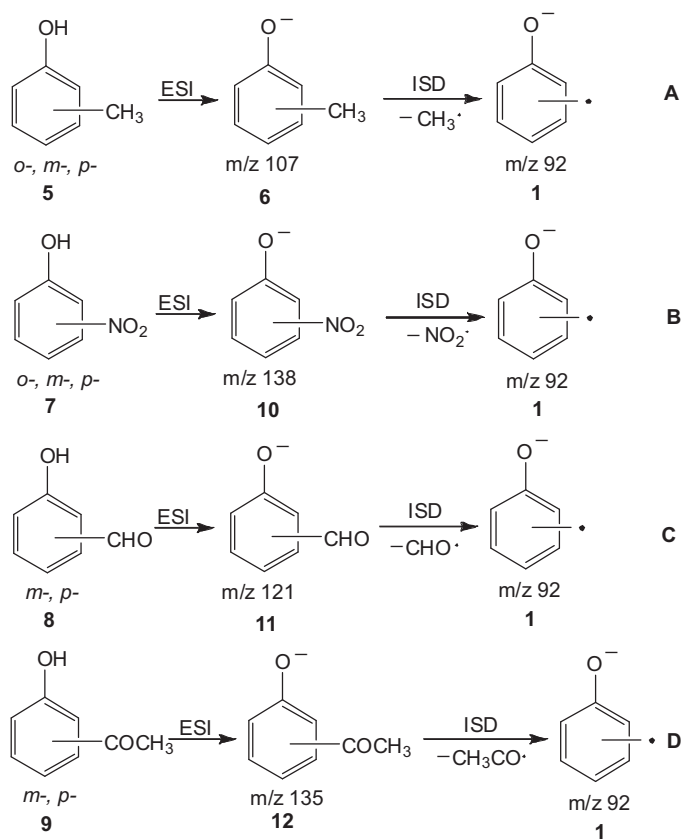


Fig. 2. CID spectra of (a) *p*-nitrobenzoate at *m/z* 166 with collision energy at 30 V, and (b) *p*-nitrophenide at *m/z* 122 with collision energy at 15 V.

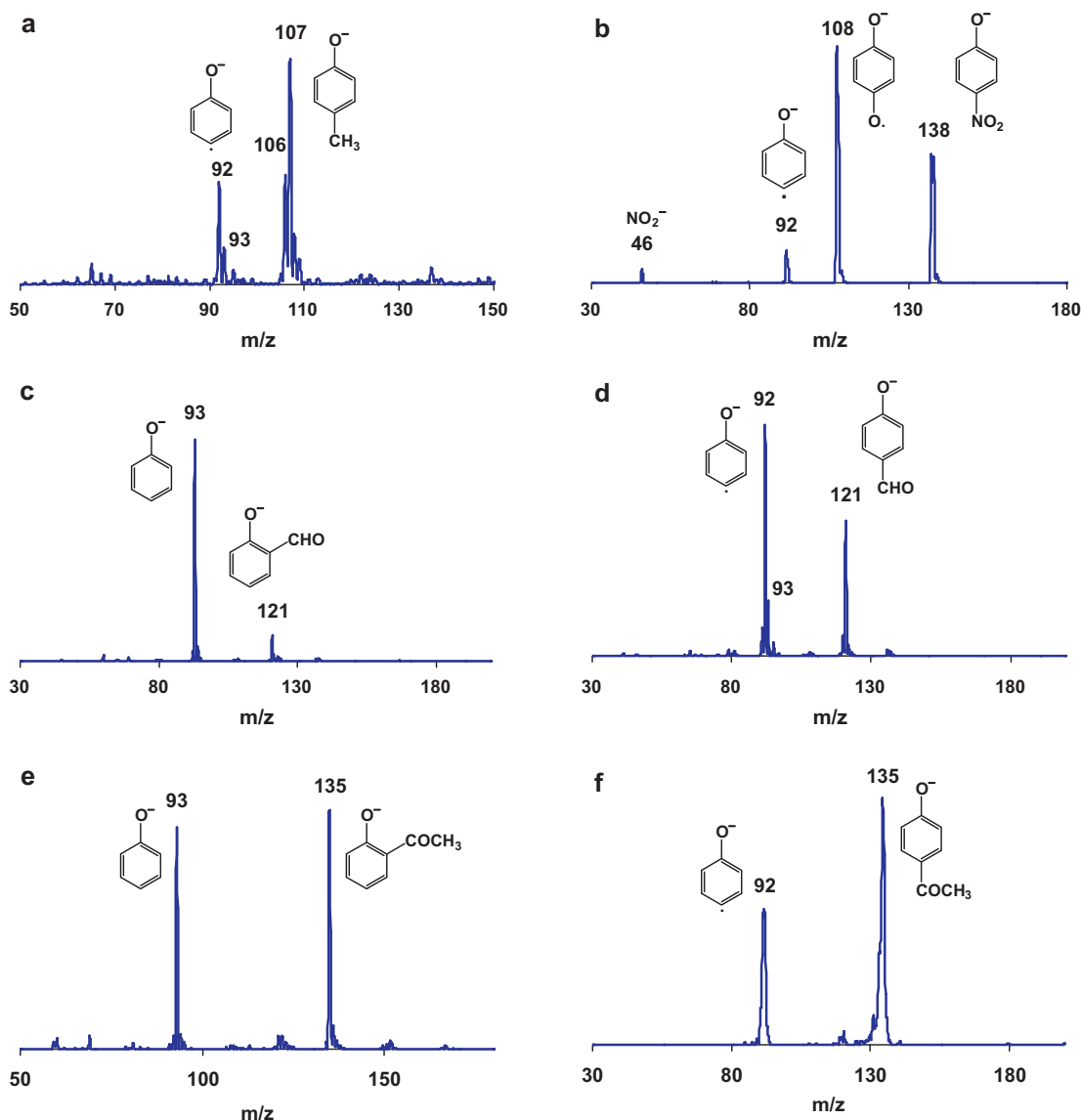


Scheme 2.

The three isomeric nitrophenols (**7**) gave similar ESI-ISD spectra with varied ion intensities at a particular capillary voltage. Fig. 3b shows the spectrum for the *p*-isomer. The peak at *m/z* 138 is for the precursor ion, the nitrophenoxide anion (**10**). On the lower mass side, besides the peak at *m/z* 92, a peak at *m/z* 108 is also observed. The ion at *m/z* 108 is likely a radical anion ( $^{\bullet}\text{O}-\text{C}_6\text{H}_4-\text{O}^{\bullet}$ ) formed by losing the NO radical from **10**. The expected radical anion at *m/z* 92 is likely the result of losing  $\text{NO}_2$  (an odd electron species) directly from **10**. The proposed mechanism is shown in Scheme 2B. All the fragment ions observed in the ESI spectra are also observed in the CID experiments from dissociation of the corresponding nitrophenoxide anions.

The *m*-, and *p*-hydroxybenzaldehydes (**8**) and hydroxyacetophenones (**9**) yielded comparable ESI-ISD spectra (Fig. 3d and f). The radical anion at *m/z* 92 was the major fragment ion for all these compounds. The elemental composition of the ion at *m/z* 92 was confirmed by HRMS experiments. The radical anion was likely formed by direct loss of  $\text{CHO}^{\bullet}$  and  $\text{CH}_3\text{CO}^{\bullet}$  from the corresponding precursor ions, **11** and **12**, respectively. The proposed mechanisms are shown in Scheme 2C and D, respectively. The CID experiments with **11** and **12** yielded comparable spectra as those observed in the ESI-ISD experiments. The results from the CID experiments agree well with those reported by Attygalle and coworkers [25].

The *o*-hydroxybenzaldehyde (**13**) and the *o*-hydroxyacetophenone (**14**) did not yield the expected ion at *m/z* 92, instead, a strong signal at *m/z* 93 was observed for both compounds (Fig. 3c and e). The ion at *m/z* 93 should be an even-electron anion,  $\text{C}_6\text{H}_5\text{O}^-$  (**15**). This ion is likely formed by the loss of CO from the deprotonated *o*-hydroxybenzaldehyde and the loss of  $\text{CH}_2\text{CO}$  from the deprotonated *o*-hydroxyacetophenone to yield a carbanion followed by proton shift to give the phenoxide anion (Scheme 3A). The structure of  $\text{CH}_2\text{CO}$  is likely to be  $\text{H}_2\text{C}=\text{C}=\text{O}$ . Alternatively, ion **15** may be formed via the process



**Fig. 3.** Mass spectra recorded under ESI-ISD conditions for (a) *p*-methylphenol, (b) *p*-nitrophenol, (c) *o*-hydroxybenzaldehyde, (d) *p*-hydroxybenzaldehyde, (e) *o*-hydroxyacetophenone, and (f) *p*-hydroxyacetophenone.

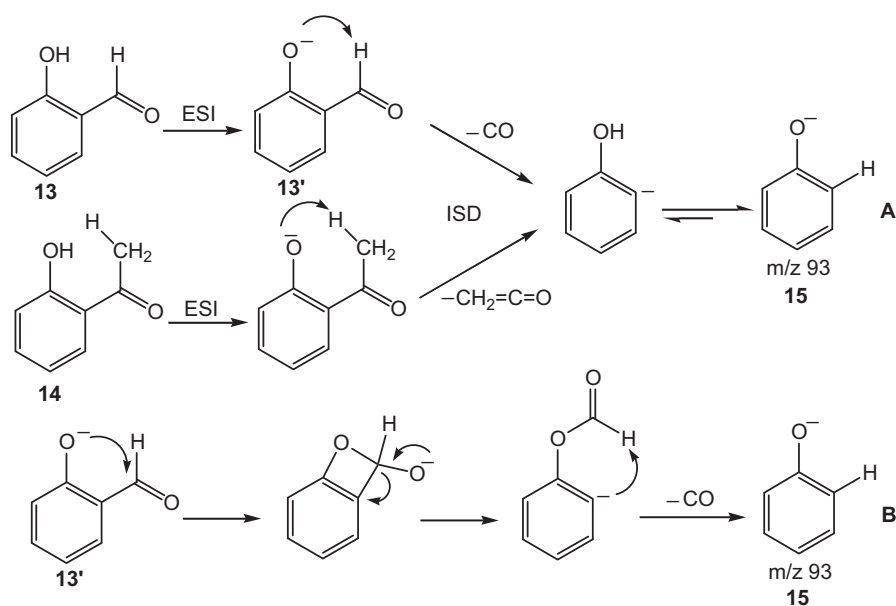
shown in (Scheme 3B) [25]. The formation of the anion at  $m/z$  93 is best described with *ortho*-effect [26]. The similar *ortho*-effect has been reported in the literature [8,25,40]. In addition to the mechanisms shown in Scheme 3, the deprotonated **13** may lose CO and form **15** directly without proton transfer. By carefully examining the spectra of *m*- and *p*-isomers of hydroxybenzaldehydes (Fig. 3d) and hydroxyacetophenones (Fig. 3f), a relatively weaker peak at  $m/z$  93 could be observed as well. The formation of the ion at  $m/z$  93 is likely a charge-remote fragmentation by the loss of CO from **11** and the loss of  $\text{CH}_2\text{CO}$  from **12**. Attygalle and coworkers have studied the mechanisms of similar fragmentations by isotopic labeling coupled with CID experiments [25].

#### 4. Nitrobenzaldehydes and nitroacetophenones

With the successful ESI-ISD experiments, we moved on to explore the possibilities of generation of radical anions by using the atmospheric pressure chemical ionization (APCI) under ISD conditions. We chose isomeric nitrobenzaldehydes (**16**) and nitroacetophenones (**17**) for this study. These two groups of

compounds do not have acidic hydrogens and are ionized inefficiently in negative mode ESI experiments. However, they form abundant molecular anions,  $\text{M}^{\bullet-}$ , in negative APCI experiments. By increasing the capillary voltage to 80–100V, a few characteristic fragment ions were observed for all these compounds. The radical anion at  $m/z$  92 was common for all *m*- and *p*-isomers, while the ion at  $m/z$  93 was observed for the two *o*-isomers. Selected spectra are shown in Fig. 4. The complete set of APCI-ISD spectra is provided in supplemental information.

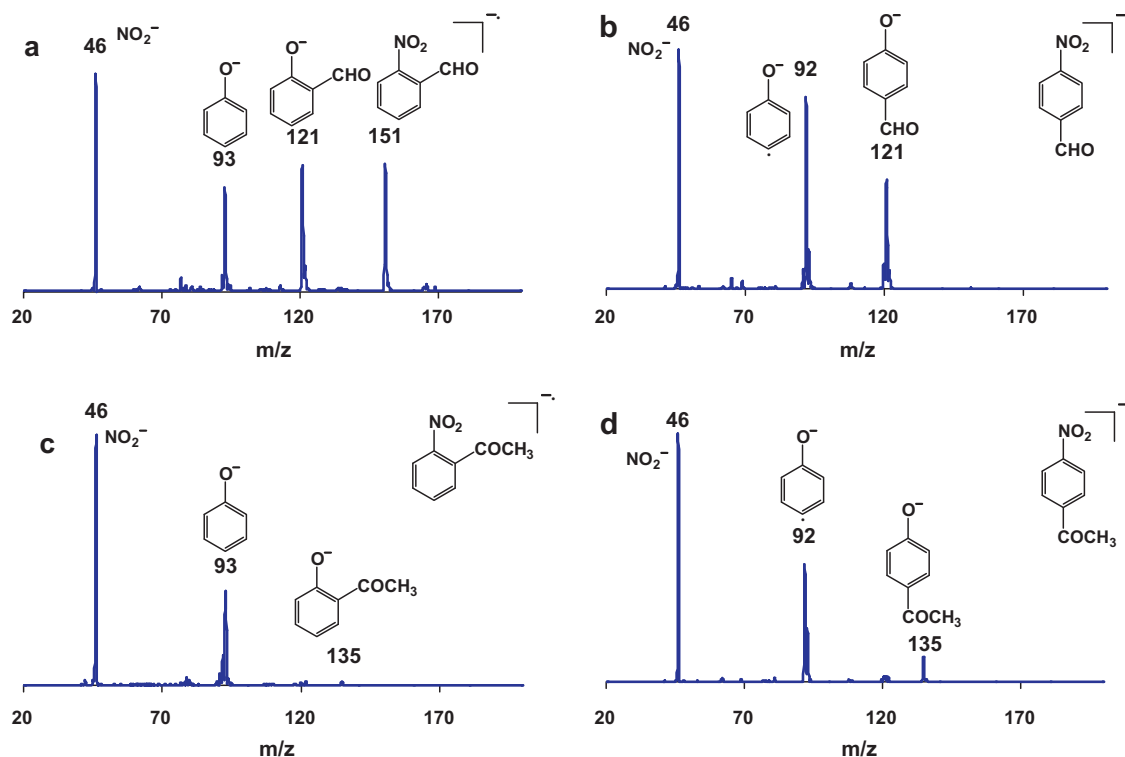
In Fig. 4a, the formation of the ion at  $m/z$  93 from *o*-nitrobenzaldehyde is likely the result from two elimination steps, the elimination of the NO radical from the molecular anion ( $m/z$  151) to yield the phenoxide anion ( $m/z$  121) followed by removal of CO. The latter step is promoted by the *ortho*-effect (Scheme 4A). In the case of *m*- and *p*-nitrobenzaldehydes (Fig. 4b), the radical anion at  $m/z$  92 was formed by eliminating the NO radical from the molecular anion ( $m/z$  151) to yield phenoxide anion ( $m/z$  121) followed by ejecting  $\text{CHO}\bullet$  radical (Scheme 4B). Such loss of NO was reported earlier in the CID studies on nitroaromatic compounds [18]. The APCI-ISD spectra of isomeric nitroacetophenones mirror those of nitroben-



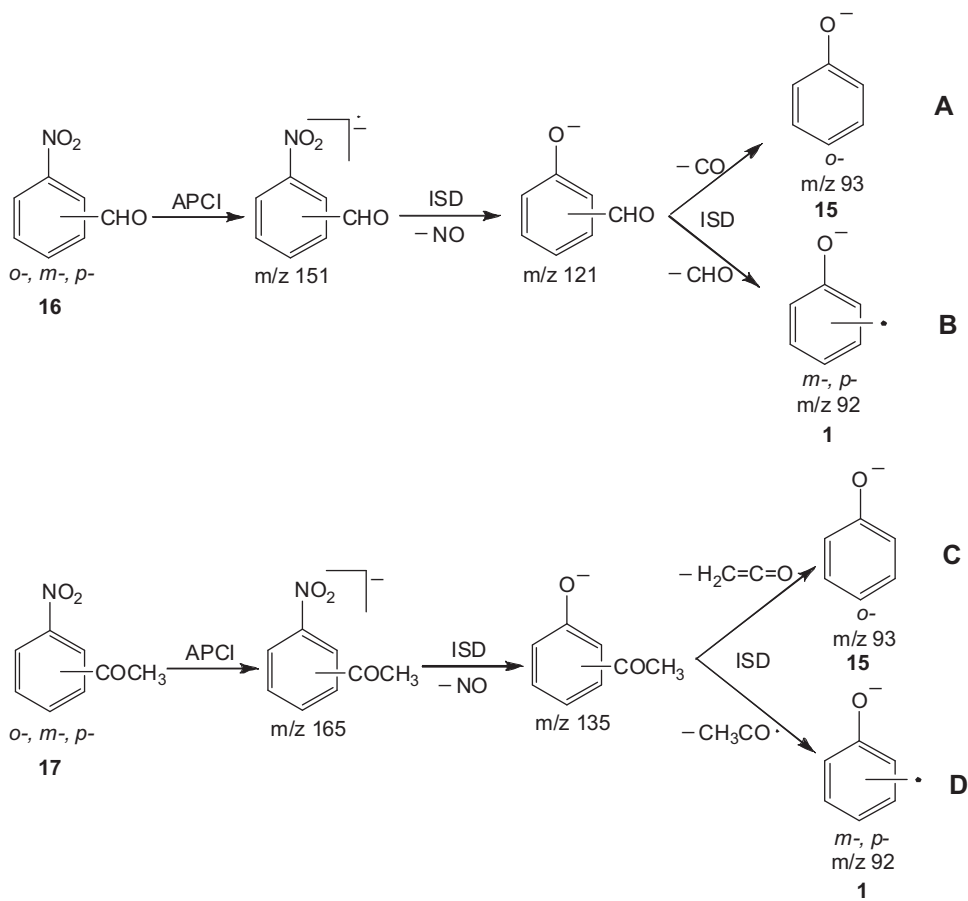
zaldehydes. For the *o*-nitroacetophenone (Fig. 4c), the molecular anion (*m/z* 165) loses an NO to form the acetophenoxide anion (*m/z* 135). Then the acetophenoxide anion loses  $\text{H}_2\text{C}=\text{C}=\text{O}$  to give the ion at *m/z* 93, which is promoted by the *ortho*-effect (Scheme 4C). While for the *m*- and *p*-nitroacetophenone (Fig. 4d), the acetophenoxide anion (*m/z* 135) eliminates the  $\text{CH}_3\text{CO}\cdot$  radical to yield the radical anion at *m/z* 92 (Scheme 4D). Clearly, radical anions can be generated under suitable APCI-ISD conditions. It should be pointed out that the peaks corresponding to the precursor radical anions are not shown in some of the spectra in Fig. 4, due to the efficient fragmentations of the precursor ions at higher capillary voltages.

## 5. Ion molecule reactions

In order to further confirm the structures of the radical anions at *m/z* 92 ( $\text{C}_6\text{H}_4\text{O}^{\cdot-}$ ) and the anion at *m/z* 93 ( $\text{C}_6\text{H}_5\text{O}^-$ ), we performed ion–molecule reactions. The ion at *m/z* 92 was formed via step-wise mechanisms with the radical site and the charge site residing on different atoms, and therefore this ion is a distonic radical anion. Since oxygen is more electronegative than carbon, the most possible structure should have the negative charge on oxygen and the radical site on the benzene ring. The ion at *m/z* 93 was produced largely due to the *ortho*-effect. The structure of this



**Fig. 4.** Mass spectra recorded under APCI-ISD conditions for (a) *o*-nitrobenzaldehyde, (b) *p*-nitrobenzaldehyde, (c) *o*-nitroacetophenone, and (d) *p*-nitroacetophenone.



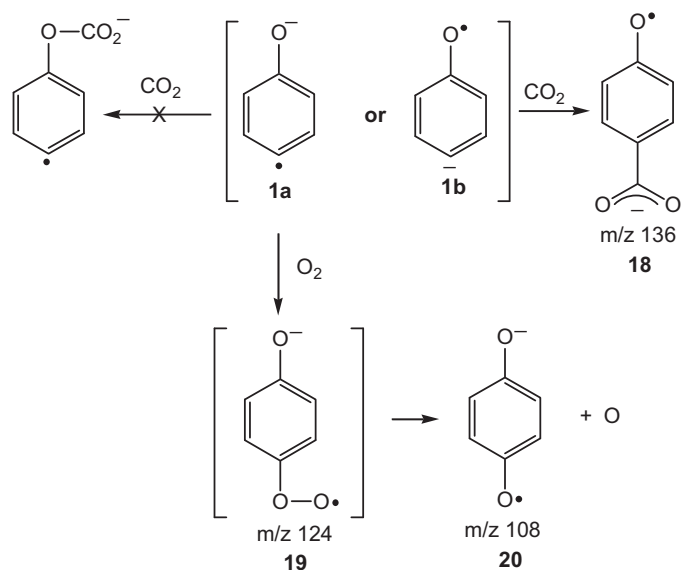
Scheme 4.

anion is likely a phenoxide with the negative charge on the oxygen atom.

Carbanions are known to react with  $\text{CO}_2$  to yield carboxylate anions [20,41–44]. The studies on benzyne radical anions by Squires, Wenthold and coworkers showed that  $\text{CO}_2$  reacted with the anionic site of the benzyne radical anion to produce distonic benzoate radical while keeping the radical site intact [20]. In the work of evaluation of the structures of decarboxylated anions from isomerically substituted benzoates, Kumar, Vairamani and coworkers demonstrated that the selective ion–molecule reactions between carbanions and  $\text{CO}_2$  in the collision cell of a triple quadrupole mass spectrometer could be used to probe the structures of isomeric carbanions [37,38]. The radical site of the distonic radical ions is also known to react with  $\text{O}_2$  to form adducts [3,12,22,23,45]. The addition of  $\text{O}_2$  is highly exothermic (>100 kcal/mol). Multiple collisions are necessary to remove the excess energy [22]. A recent report shows that the distonic pyridine radical cations ( $^+\text{Pyd}^\bullet$ ) undergo radical–molecule reaction with  $\text{O}_2$  to give the  $\text{O}_2$  adduct ( $^+\text{Pyd}-\text{OO}^\bullet$ ) and the oxygen atom abstraction product ( $^+\text{Pyd}-\text{O}^\bullet$ ). The formations of these two types of products are exothermic by about 200 kcal/mol and <10 kcal/mol, respectively. The ejection of an oxygen atom from  $^+\text{Pyd}-\text{OO}^\bullet$  to yield  $^+\text{Pyd}-\text{O}^\bullet$  is a way to remove the excess energy associated with the formation of the former product [46].

We examined the radical anion (**1**) and the phenoxide anion (**15**) by allowing the ions to react with  $\text{CO}_2$  and/or  $\text{O}_2$  in the collision cell of the triple quadrupole instrument. As expected, no additional product was observed for the phenoxide anion (**15**). The distonic radical anion (**1**) may be described by two distinct electronic structures, the phenoxide ion–phenyl radical (Scheme 5, **1a**)

or the phenoxyl radical–phenide ion (Scheme 5, **1b**). In **1a**, the unpaired electron is localized in the plane of the  $\sigma$ -system and the charge is in the  $\pi$ -system, while **1b** has the charge in the  $\sigma$ -system and the unpaired electron in the  $\pi$ -system. The radical anion (**1**) with such a structure that composes of phenoxide anion and phenyl radical (**1a**) is not expected to react with  $\text{CO}_2$ . Surprisingly, all the



Scheme 5.

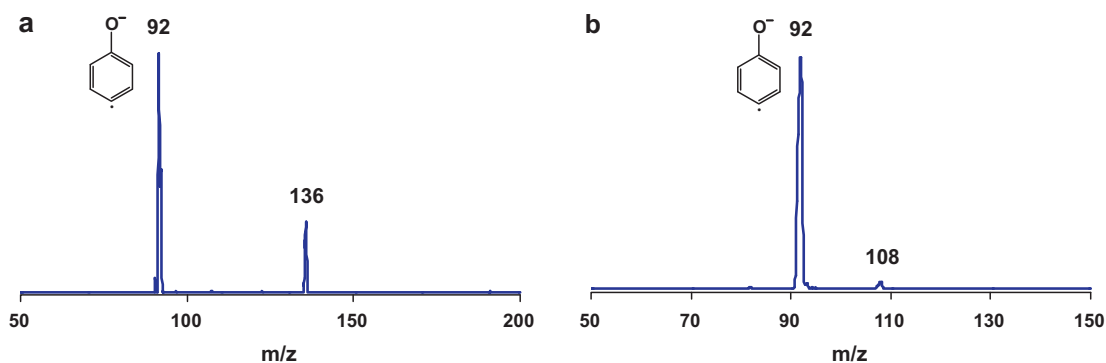


Fig. 5. Spectra for the ion-molecule reactions between phenoxide radical anion at  $m/z$  92 and (a)  $\text{CO}_2$ , and (b)  $\text{O}_2$ .

radical anions at  $m/z$  92 generated from different precursor compounds reacted with  $\text{CO}_2$  to form a common adduct ion at  $m/z$  136 in varying yields. A representative mass spectrum is shown in Fig. 5a. The adduct formation implies that the radical anion has a carbanionic character such that the negative charge is on the benzene ring while the radical site is on the oxygen, the phenide radical anion (**1b**). Although **1a** is a more favored structure than **1b**, it is this phenide ion (**1b**) that leads to the reaction with  $\text{CO}_2$ . The radical anion also reacted with  $\text{O}_2$  to produce a weak signal at  $m/z$  108 that would be the oxygen atom abstraction product. The  $\text{O}_2$  adduct (**19**) was not observed. A spectrum is shown in Fig. 5b. This reaction suggested the phenyl radical character, **1a** in Scheme 5. The results from the ion-molecule/radical-molecule reactions reveal the dual-character of the radical anion at  $m/z$  92.

## 6. Computational studies

In order to gain further understanding of the results from the ion-molecule reactions, we performed computational studies. The geometries, electronic structures and energies of the phenoxide radical anions and related molecules and ions were calculated at the B3LYP/6-31+G(d) level of theory. The electron density distributions suggest that the radical ions are best described as phenoxide radical anion (**1a** in Scheme 5), rather than phenide radical anion (**1b** in Scheme 5). The enthalpy changes (at 298 K) for the ion-molecule reactions were derived using the thermochemical quantities obtained from calculations. The formation of the  $\text{CO}_2$  adduct (**18**) was calculated to be exothermic by 43 kcal/mol. The formation of the oxygen molecule adduct (**19**) would be exothermic by 103 kcal/mol and the formation of the oxygen atom abstraction product (**20**) was exothermic by 9 kcal/mol. The absence of the oxygen molecule adduct (**19**) in the ion-molecule reaction may be explained by the high exothermicity of this reaction. The large excess energy would make this adduct too short-lived to be observed. However, by ejecting one oxygen atom, a great amount of excess energy would be carried away, and hence the oxygen atom abstract product could be observed. The possible structures of adding the  $\text{CO}_2$  and the  $\text{O}_2$  to the oxygen site of the phenoxide radical anion were also calculated. The results suggest that these types of structures are unstable. The computational studies further support the dual-character of the distonic radical anion.

## 7. Conclusions

We have explored the possibilities of making radical anions under ESI and APCI processes. The isomeric distonic dehydrophenoxide radical anions were successfully generated in the ESI and APCI processes by using relatively high capillary voltages,

the in-source dissociation condition. The radical anions can be formed from various isomerically (especially the *meta*- and *para*-isomers) substituted aromatic compounds, including substituted phenols, nitrobenzaldehyde and nitroacetophenone, as well as nitrobenzoic acid. Among these compounds, some *ortho*-isomers formed even-electron dehydrophenoxide anion due to *ortho*-effect. The dehydrophenoxide radical anions were also formed in the collision cell of a triple-quadrupole instrument from the CID experiments of all the corresponding anionic precursor molecules. The ion-molecule reactions with  $\text{CO}_2$  and  $\text{O}_2$  in combination with computational studies reveal the dual-character of the dehydrophenoxide radical anion, the phenide ion and phenyl radical. The formation of suitable radical anions in the ion source could be used as reagents ions in ETD experiments. We are in the process of evaluating a group of larger aromatic compounds that have potentials to form radical anions under ESI-ISD or APCI-ISD conditions.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2010.10.025.

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