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RESEARCH ARTICLE

"Meta Elimination," a Diagnostic Fragmentation in Mass Spectrometry

Athula B. Attygalle, Upul Nishshanka, Carl S. Weisbecker

Center for Mass Spectrometry, Department of Chemistry, Chemical Biology, and Biomedical Engineering, Stevens Institute of Technology, Hoboken, NJ 07030, USA

Abstract

The diagnostic value of the "ortho effect" for unknown identification by mass spectrometry is well known. Here, we report the existence of a novel "meta effect," which adds to the repertoire of useful mass spectrometric fragmentation mechanisms. For example, the meta-specific elimination pathway described in this report enables unequivocal identification of meta isomers from ortho and para isomers of carboxyanilides. The reaction follows a specific path to eliminate a molecule of *meta*-benzyne, from the anion produced after the initial decarboxylation of the precursor. Consequently, in the CID spectra of carboxyanilides, a peak for the $(R\text{-}CO\text{-}NH)^-$ anion is observed only for the meta isomers. For example, the peaks observed at *m/z* 58, 86, 120, 128, and 170 from acetamido-, butamido-, benzamido, heptamido-, and decanamido-benzoates, respectively, were specific only to the spectra of meta isomers.

Key words: Ortho effect, Meta effect, Meta elimination, Collision-induced dissociation, Anthranilic acid, Anilides

Introduction

Ions derived from ortho-substituted aromatic compounds often produce spectra that are different from the spectra of their meta or para isomers [1]. This phenomenon, generally known as the "ortho effect," has been recognized as one of the diagnostically important mass spectral fragmentations. The mechanism of this process has been widely investigated for positively charged radical cations. For many radical cations, this is an outcome of a transfer of a hydrogen atom from a donor functional group, such as hydroxyl, amino, thiol, or even alkyl, to a polar functional group attached to the ortho position. The mechanism, initially presumed to proceed via a six-membered transition state [2], appears to follow a step-wise mechanism to eliminate a neutral molecule from the molecular ion [3–10]. In addition to what is observed directly from odd-electron molecular ions, this ortho-specific mechanism sometimes applies even to even-electron ions derived from molecular ions after an initial loss of a radical [11]. Recently, we reported that the EI spectra of *N*-formyl, *N*-acetyl, or *N*-benzoyl derivatives of haloanilines also show a dramatic ortho effect by a different mechanism [12]. For example, the spectra of derivatives of ortho isomers of chloro-, bromo-, or iodoanilines show a very prominent peak for the loss of the halogen atom, instead of an HX molecule usually expected from a conventional ortho elimination [12].

In contrast to radical ions produced by electron ionization, most of the modern desorption ionization methods produce even-electron gaseous ions. Recent investigations reveal that collision-induced fragmentation spectra of even-electron ions of substituted aromatic compounds also show peaks diagnostic of the ortho-substitution position [13–20].

Anthranilic acid is an important pharmacophore widely used in drug discovery programs. In our pursuit of finding intricate details of fragmentation mechanisms of anions, we recorded spectra of several anilides derived

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Correspondence to: Athula B. Attygalle; *e-mail:* Athula.Attygalle@stevens.edu

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from anthranilic acid and its ring isomers. Among the fragmentation pathways investigated, a mechanism specific to meta isomers was observed. Our conclusions reported here are supported by ab initio calculations and deuterium-labeling experiments.

Experimental

All chemicals, including 2-aminobenzoic acid, 3-aminobenzoic acid, and 4-aminobenzoic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Synthesized products were characterized using an API 2000 triple quadrupole mass spectrometer equipped with an ESI source. The collision-induced dissociation (CID) mass spectra were recorded on a Waters (Milford, MA, USA) Quattro Ultima mass spectrometer equipped with an electrospray ion source. Samples were infused as acetonitrile-water-NH_3 (50/50/0.01 % vol/vol) solutions at a flow rate of 10 μL min^{-1}. The source temperature was maintained at 100 °C. The argon gas pressure in the collision cell was adjusted to attenuate precursor ion transmission by about 30%–50%.

Synthesis of N-Acetyl Derivatives of Aminobenzoic Acids

Each aminobenzoic acid (50 mg, 0.368 mmol) in tetrahydrofuran (THF) was mixed with a mixture of acetic anhydride (1 mL) and pyridine (30 μL, 0.368 mmol), and the mixture was stirred for 1 h at RT. The reaction mixture was then acidified with 1% HCl and the final product was extracted into ethyl acetate.

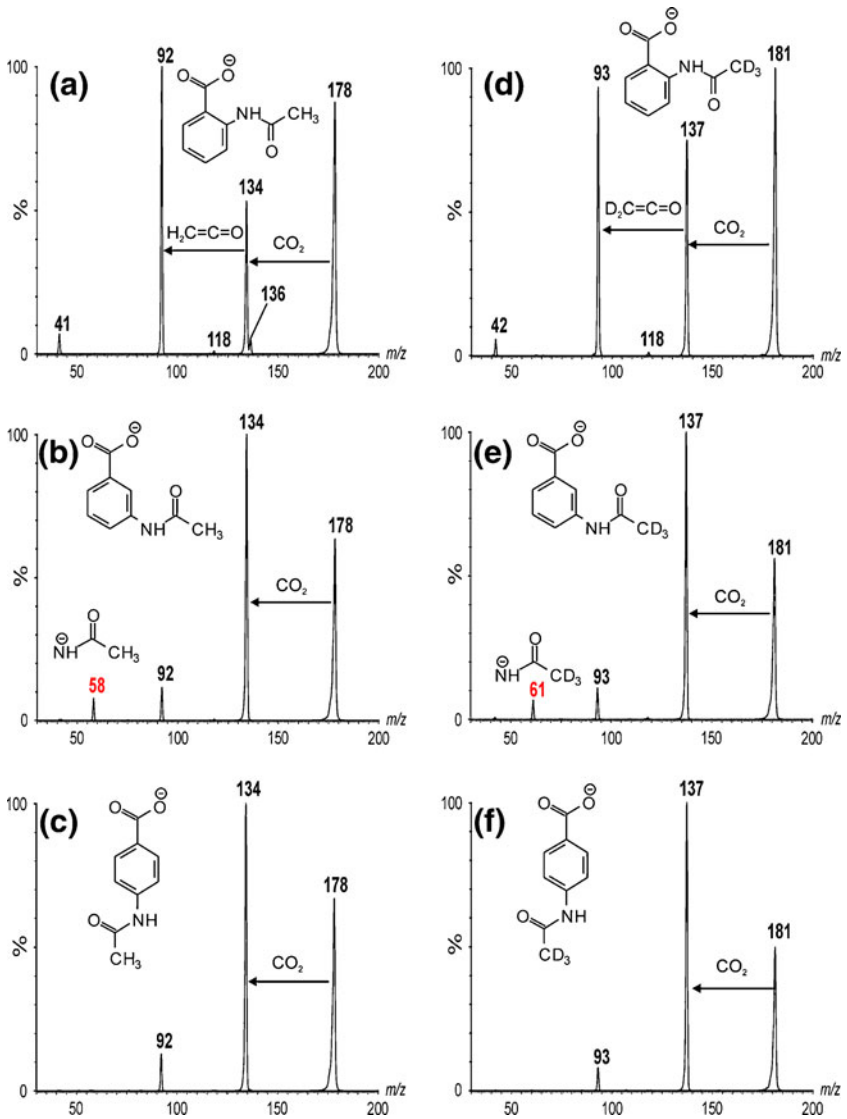


Figure 1. Unit–resolution product ion mass spectra of *m/z* 178 [(M – H)$^-$] ion derived from *ortho*- **(a)**, *meta*- **(b)**, and *para*-carboxyacetanilide **(c)**, and those of *m/z* 181 ion of *ortho*- **(d)**, *meta*- **(e)**, and *para*-carboxy[$^2H_3$]acetanilide **(f)** at a collision energy settings of 25 eV recorded on a Quattro Ultima mass spectrometer (all parameters, including collision gas pressure and skimmer-cone voltages, were kept identical)

General Synthesis of Other N-Acyl Derivatives of Aminocarboxylic Acids

Each carboxylic acid (50 mg) was dissolved in excess thionyl chloride (2 mL) and two drops of dimethylformamide (DMF). The mixture was kept 60 °C for 30 min and excess thionyl chloride was removed by a stream of N_2. The acid chlorides produced in this way were used to synthesize *N*-acyl derivatives of aminobenzoic acids.

Computational Methods

All calculations were performed using the Gaussian 03 W program package [21]. Calculations were performed using density functional theory using the B3LYP functional [22, 23] and 6-31 G(d) basis set for all structures, except the structures of neutral *meta*-benzyne and *para*-benzyne, which were calculated using the BLYP functional. Calculated structures were optimized to obtain stationary states. Frequency analysis of the optimized structures was used to distinguish energy minima from saddle points. Transition states were identified as first-order saddle points with one imaginary frequency. Zero-point energy corrections were made to the energies of stationary states. Zero-point energies obtained using the B3LYP functional were scaled by a factor 0.9804 [24]. Zero-point energies obtained using the BLYP functional were scaled by a factor of 1.009 [25].

The structures of *meta*- and *para*-benzyne have been extensively investigated, and the ground states of both

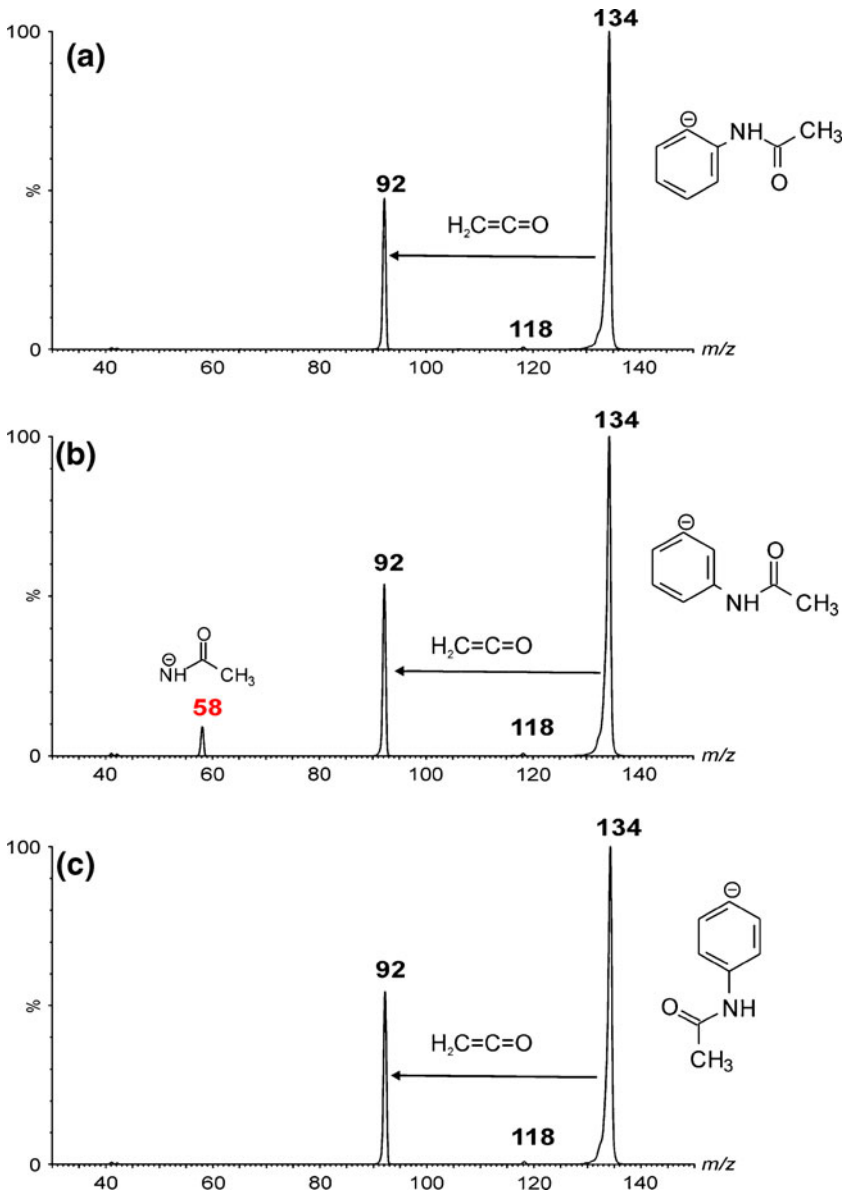
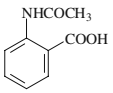
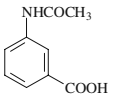
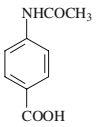
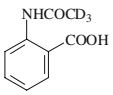
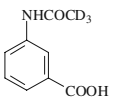
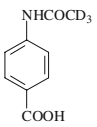


Figure 2. Product ion spectra of in-source-generated *m/z* 134 ion from 2-carboxyacetanilide **(a)**, 3-carboxyacetanilide **(b)**, and 4-carboxyacetanilide **(c)** recorded at a laboratory frame collision energy setting of 25 eV

Table 1. Collision-induced Dissociation Mass Spectra of some Carboxyanilides Recorded at a Laboratory-frame Collision Energy Setting of 25 eV

Compound (M)	$[M - H]^-$ *m/z* (%)	$[(M - H) - (CO_2)]^-$ *m/z* (%)	Ketenyl anion *m/z* (%)	Amide anion $^{\ominus}$NXCOR *m/z* (%)	Phenylazanide (or its hydrogen-shift isomeric structure) *m/z* (%)	Aminobenzoate *m/z* (%)
2-acetamidobenzoic acid 	178 (90)	134 (55)	41 (8)	58 (0) X = H R = CH_3	92 (100)	136 (5)
3-acetamidobenzoic acid 	178 (65)	134 (100)	41 (<1)	58 (9) X = H R = CH_3	92 (12)	136 (<0.5)
4-acetamidobenzoic acid 	178 (70)	134 (100)	41 (<1)	58 (0) X = H R = CH_3	92 (15)	136 (<0.5)
2-[2′,2′,2′-$^2H_3$]acetylaminobenzoic acid 	181 (98)	137[a](80)	42 (8)	61 (0) X = H R = CD_3	93 (95)	137[a] (?)
3-[2′,2′,2′-$^2H_3$]acetylaminobenzoic acid 	181 (55)	137[a](100)	42 (<1)	61 (9) X = H R = CD_3	93 (100)	137[a] (?)
4-[2′,2′,2′-$^2H_3$]acetylaminobenzoic acid 	181 (50)	137[a](100)	42 (8)	61 (0) X = H R = CD_3	93 (9)	137[a] (?)

molecules are known to be singlet states [26–28]. The singlet structure of *meta*-benzyne determined using B3LYP and similar hybrid functionals is known to have a short inter-atomic distance between the C1 and C3 atoms, suggesting a bicyclic structure with a σ bond between the two carbons [29]. However, the bicyclic structure is not congruent with experimental evidence on the *meta*-benzyne ground state from IR spectroscopy. Therefore, the structure of *meta*-benzyne versus its isomers is preferably calculated using the BLYP functional, which produces a more open ring structure with a larger C1–C3 inter-atomic distance. The structures of *meta*- and *para*-benzyne calculated for this paper were optimized using the restricted BLYP functional with a 6-31 G(d) basis set. The energies of these optimized molecules were then recalculated at the B3LYP/6-31 G(d) level for comparison with other calculated structures in an energy level diagram.

Table 1. (continued)

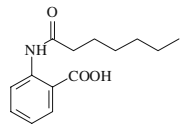
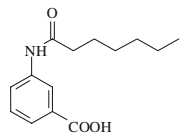
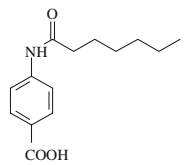
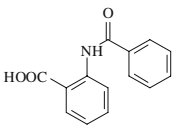
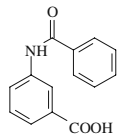
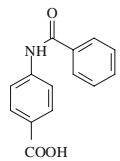
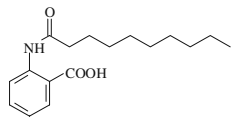
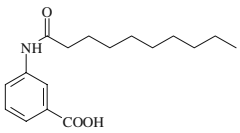
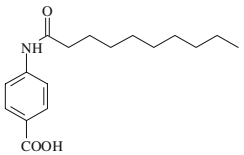
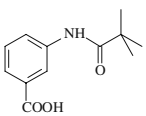
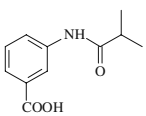
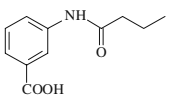
Compound (M)	$[M - H]^-$ *m/z* (%)	$[(M - H) - (CO_2)]^-$ *m/z* (%)	Ketenyl anion *m/z* (%)	Amide anion $\ominus$ NXCOR *m/z* (%)	Phenylazanide (or its hydrogen-shift isomeric structure) *m/z* (%)	Aminobenzoate *m/z* (%)
2-heptamidobenzoic acid 	248 (100)	204 (75)	111 (20)	128 (0) X = H R = $(CH_2)_5CH_3$	92 (70)	136 (25)
3-heptamidobenzoic acid 	248 (100)	204 (98)	111 (8)	128 (10) X = H R = $(CH_2)_5CH_3$	92 (28)	136 (<1)
4-heptamidobenzoic acid 	248 (90)	204 (100)	111 (2)	128 (0) X = H R = $(CH_2)_5CH_3$	92 (15)	136 (2)
2-benzamidobenzoic acid 	240 (90)	196 (100)	NA	120 (0) X = H R = (C_6H_5)	92 (0)	136 (0)
3-benzamidobenzoic acid 	240 (100)	196 (90)	NA	120 (20) X = H R = (C_6H_5)	92 (0)	136 (0)
4-benzamidobenzoic acid 	240 (100)	196 (50)	NA	120 (0) X = H R = (C_6H_5)	92 (0)	136 (0)
2-decanamidobenzoic acid 	290 (100)	246 (55)	153 (26)	170 (0) X = H R = $(CH_2)_8CH_3$	92 (55)	136 (35)

Table 1. (continued)

Compound (M)	$[M - H]^-$ *m/z* (%)	$[(M - H) - (CO_2)]^-$ *m/z* (%)	Ketenyl anion *m/z* (%)	Amide anion $\ominus$ NXCOR *m/z* (%)	Phenylazanide (or its hydrogen-shift isomeric structure) *m/z* (%)	Aminobenzoate *m/z* (%)
3-decanamidobenzoic acid 	290 (100)	246 (50)	153 (10)	170 (6) X = H R = $(CH_2)_8CH_3$	92 (18)	136 (<1)
4-decanamidobenzoic acid 	290 (100)	246 (50)	153 (5)	170 (0) X = H R = $(CH_2)_8CH_3$	92 (10)	136 (1)
3-[(2,2-dimethylpropanoyl)amino]-benzoic acid 	220 (75)	176 (100)	NA	100 (18) X = H R = $C(CH_3)_3$	92 (2)	136 (<1)
3-[(2-methylpropanoyl)amino]-benzoic acid 	206 (55)	162 (100)	69 (0)	86 (18) X = H R = $CH(CH_3)_2$	92 (8)	136 (<1)
3-butanamidobenzoic acid 	206 (50)	162 (100)	69 (0)	86 (15) X = H R = $(CH_2)_2CH_3$	92 (10)	136 (<1)

[a,b]=Composite peaks

Results and Discussion

Collision-induced dissociation mass spectra recorded from $(M - H)^-$ ions of ortho- meta, and para isomers of carboxyacetanilide are depicted in Figure 1. To our surprise, the spectrum recorded from *meta*-carboxyacetanilide (also known as *meta*-acetamidobenzoic acid) showed a small but highly significant peak at *m/z* 58, unique to the meta isomer

(Figure 1b). The major fragmentation pathways of anions derived from ring isomers of carboxyacetanilide can be attributed to consecutive losses of CO_2 and CH_2=C=O, or CH_2=C=O and CO_2 from the precursor anion of *m/z* 178 (Figure 1). However, tandem mass spectrometric experiments confirmed that only the ion at *m/z* 134, formed after the initial decarboxylation of the meta isomer fragments further to yield an *m/z* 58 ion (Figure 2), which in fact represents the anion of acetamide [H_3C – (CO) – NH^-]. The characterization of this ion was supported by the observation of a peak at *m/z* 61 in the spectrum of the ion at *m/z* 181 of 3-[2',2',2'-$^2H_3$]acetamidobenzoate (Figure 1e).

Evidently, the formation of an amide anion in this way is a general phenomenon because the spectra of several other *N*-acyl derivatives of *meta*-aminobenzoic acid also showed analogous peaks, which were absent in the spectra of corresponding ortho and para isomers (Table 1). For example, peaks specific for the meta isomer were observed at *m/z* 120 and 128 in the spectra of 3-carboxybenzanilide (a.k.a. 3-benzamidobenzoic acid; Figure 3) and 3-carboxyheptanilide (a.k.a. 3-heptamidobenzoic acid; Supplementary Figure S1), respectively. The relative intensity of the amide peak increased as the collision energy was increased. For example, see the CID spectrum and the plots of relative intensities versus laboratory-frame collision energies given in Figure 4 for 3-decanamidobenzoate.

To the best of our knowledge a peak specific for the meta isomer has not been previously recognized as a diagnostic marker in CID spectra. On one rare occasion, however, the formation of an *m/z* 81 peak for the HSO_3^- ion, specifically from the meta isomer has been reported in an investigation of spectra of sulfobenzoic acids [30].

For the formation of an ion at *m/z* 58 from *meta*-carboxyacetanilide, a molecule of *meta*-benzyne should be eliminated from the *m/z* 134 ion, generated after an initial loss of a CO_2 molecule from the *m/z* 178 precursor (Scheme 1). We reiterate that the product ion spectra recorded from the *m/z*

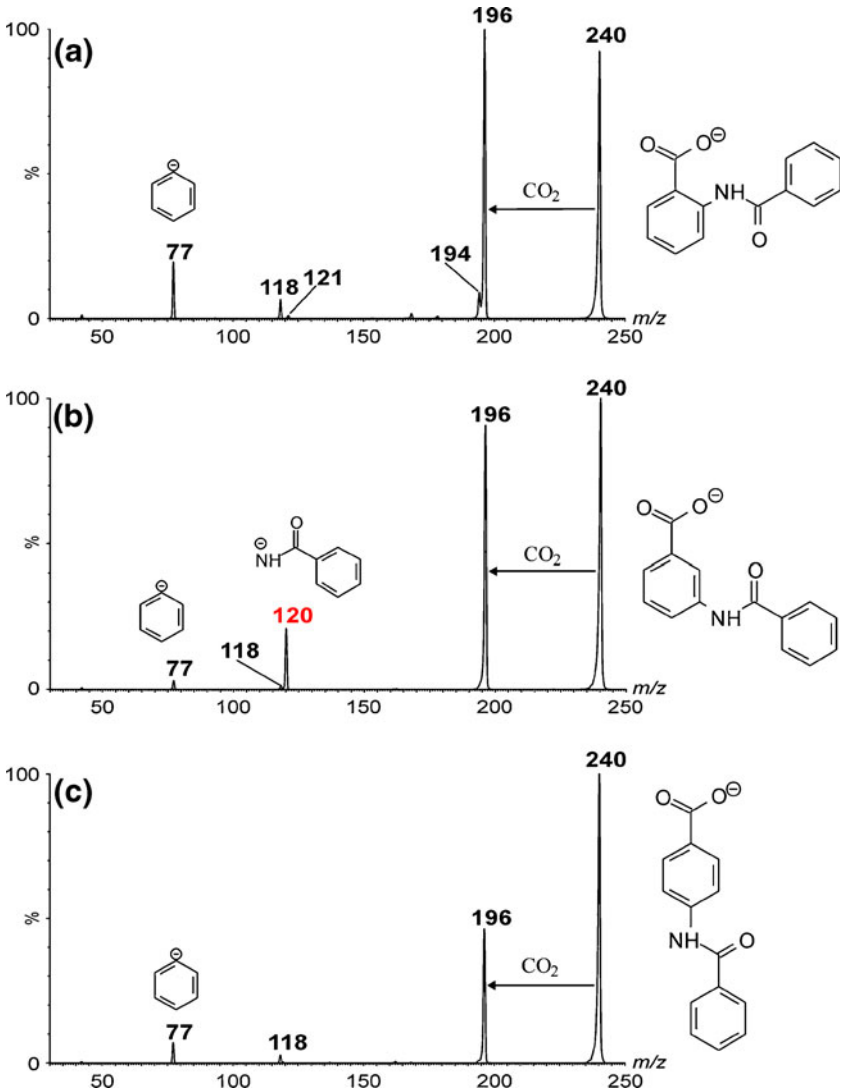


Figure 3. Product ion spectra generated from *m/z* 240 ion from 2-carboxybenzanilide **(a)**, 3-carboxybenzanilide **(b)**, and 4-carboxybenzanilide **(c)** recorded at a laboratory frame collision energy setting of 25 eV

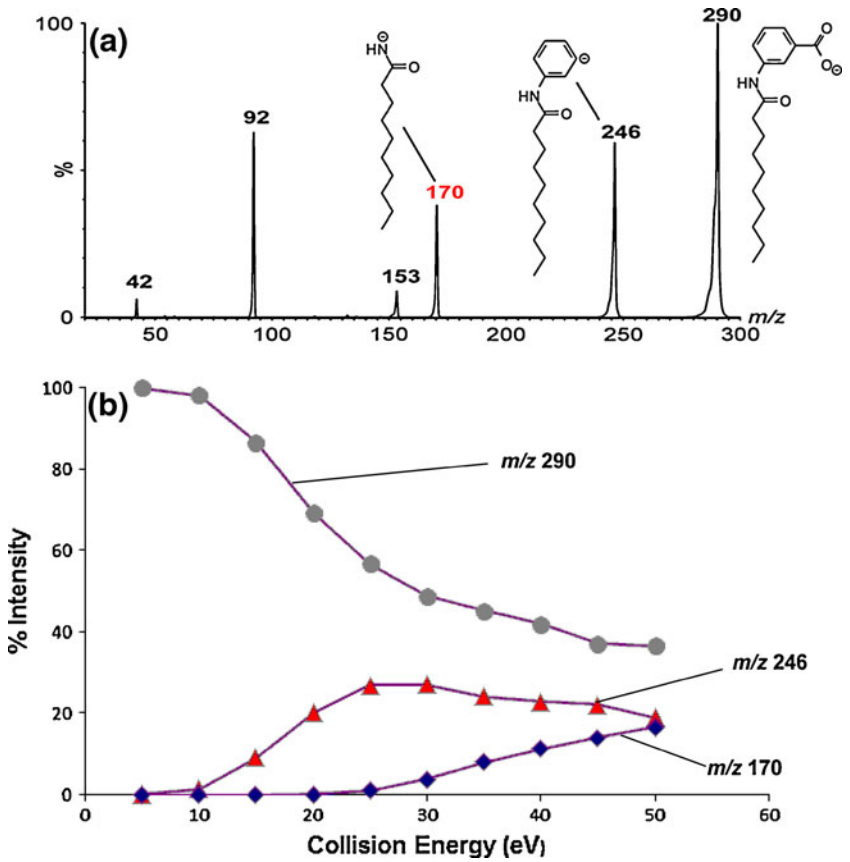
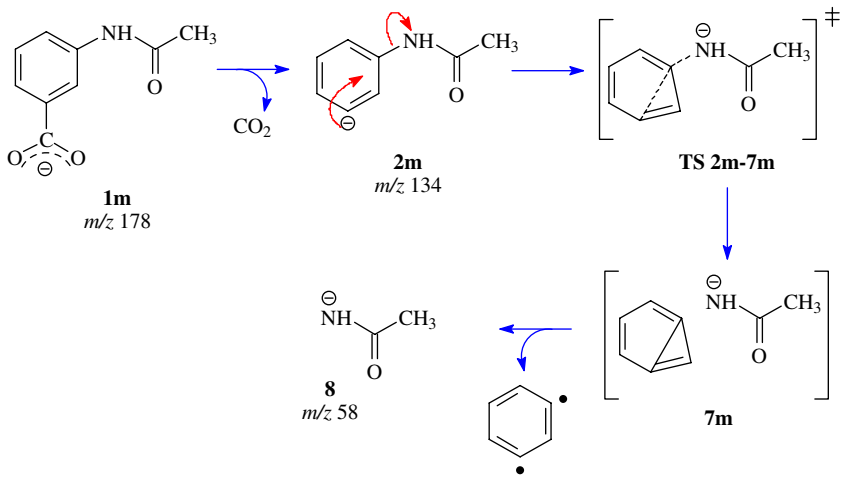


Figure 4. Unit–resolution product ion spectrum of *m/z* 290 [(M – H)$^-$] ion derived from 3-decanamidobenzoate at 40 eV collision energy **(a)**, and a plot of relative intensity versus laboratory-frame collision energy, for ions *m/z* 290 (●), 246 (▲), and 170 (◆) generated upon collision-induced dissociation **(b)**

134 ion from the ortho and para isomers, which are the hydrogen-shift isomers of that from the meta compound, fail to show a peak for the *m/z* 58 ion (Figure 2).

Density functional calculations carried out using 6-31 G (d) basis set indicated that the phenyl anion **2m** formed after the initial CO_2 loss from the meta isomer **1m** generates an intermediate via the transition state **TS-2 m-7 m** (Scheme 1). The ion-molecule complex **7 m** formed in this way can further dissociate to eliminate *meta*-benzyne to form the ion observed at *m/z* 58. Calculated relative energies of the intermediates and transition states are illustrated in Figure 5 (see Supplementary Figure S2 in the Electronic Supplementary Material for the energy-optimized structures). The structure of *m*-benzyne has been the topic of many investigations and much



Scheme 1. Proposed dissociation mechanism of *m*-carboxyacetanilide anion

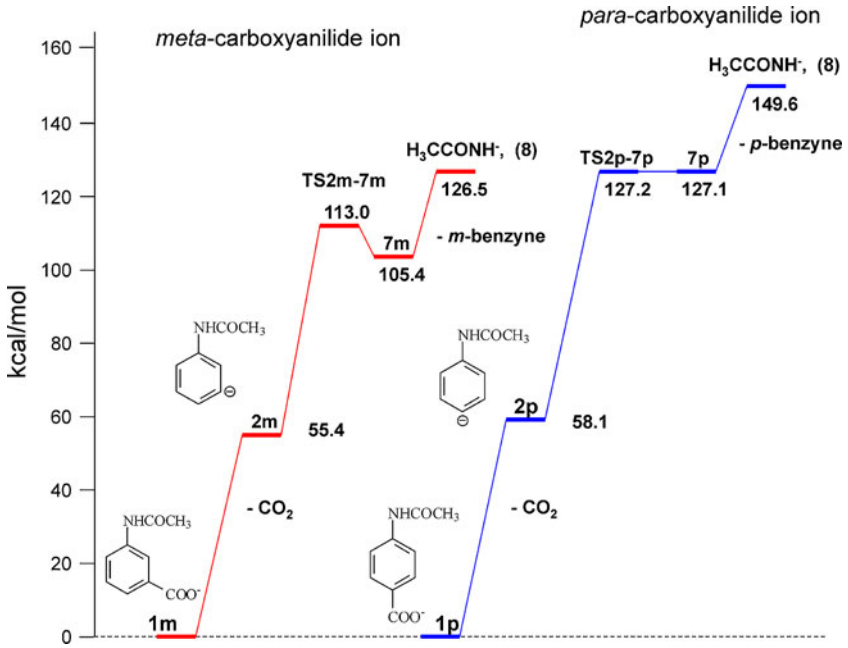


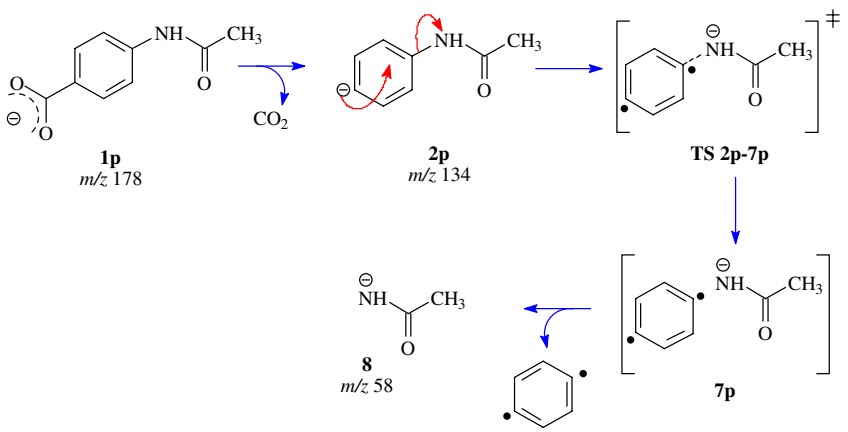
Figure 5. Energy profile for the fragmentation of *meta*- and *para*-carboxyacetanilide anions upon collisional activation (relative energies calculated using B3LYP functional and 6-31 G(d) basis set are given in kcal/mol)

debate [29, 31, 32]. Our results, which indicate that a *m*-benzyne is not a regular hexagon but a structure with a shorter distance between C1 and C3 positions, is congruent with other recent calculations [33] (Figure S2). In fact, the image recorded by scanning tunneling microscopy from chemisorbed *m*-benzyne on a Cu surface also agrees with the proposed structure [34].

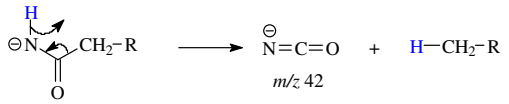
We also considered an alternative dissociation pathway involving an initial decarboxylation and a 1,2-hydrogen transfer that would lead to an expulsion of *o*-benzyne rather than *m*-benzyne. However, the energetic barriers for 1,2-hydrogen transfers in *m*- and *p*-benzynes that have been calculated by others groups are found to be excessively high [35]. There is little or no convincing evidence in literature to support that *m*- and *o*-benzynes isomerize via 1,2-hydrogen shifts. However, another indirect isomerization process which involves a ring-opening to form an ene-diyne intermediate has been proposed as more plausible pathway.

Although the para isomer can also fragment by a similar mechanism (Scheme 2, and Figure S3 in the Supplementary Information), calculations show that the overall process is more endothermic than that for the meta isomer (Figure 5). Thus, it is not surprising that a peak corresponding to an amide anion was not observed from the para isomers under typical laboratory-frame collision-energy conditions (10–40 eV). The ortho isomers also do not show a peak for the amide ions because ortho isomers have other favorable fragmentational channels, which are much less endothermic.

Moreover, the amide anions formed in this way can undergo further fragmentation by eliminating an alkane molecule (Figure S4 in the Supplementary Information, and Scheme 3). Indirectly, this fragmentation could also be



Scheme 2. Proposed dissociation of *para*-carboxyacetanilide anion [note that the fragmentation of *m/z* 134 ion given above is not an energetically favorable process, and a peak of any appreciable intensity is not observed at *m/z* 58]



Scheme 3. Fragmentation of amide anions derived from *meta*-carboxyacetanilide anions to give an *m/z* 42 product ion

considered a meta-specific pathway due to the fact that amide anions are formed under conditions described only from *meta*-carboxyacetanilides.

Conclusions

The experimental data presented here demonstrate the existence and utility of a novel “meta elimination,” which enables unequivocal distinguishing of meta isomers from those of ortho and para isomers of carboxyanilides. We envisage that this phenomenon can serve as a diagnostic maker of anions that bear a good leaving group at the meta position.

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