Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Mass Spectrometry 249–250 (2006) 21–30
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

# The mass spectrometry-induced cyclization of protonated *N*-[2-(benzoyloxy)phenyl]-benzamide: A gas-phase analog of a solution reaction

Joseph T. Moolayil [a], M. George [a,*], R. Srinivas [b], N.S. Swamy [b], Amber L. Russell [c], Daryl E. Giblin [c,*], Michael L. Gross [c]

[a] *Department of Chemistry, Sacred Heart College, Thevara, Cochin, Kerala 682013, India*
[b] *National Center for Mass Spectrometry, Indian Institute of Chemical Technology, Hyderabad, India*
[c] *Department of Chemistry, Washington University, One Brookings, Drive, St. Louis, MO, USA*

Received 16 November 2005; received in revised form 5 January 2006; accepted 5 January 2006
Available online 14 February 2006

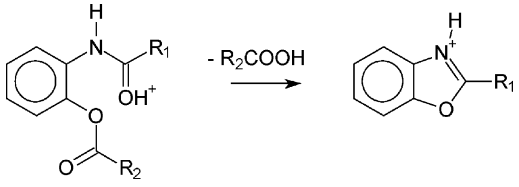
**Abstract**

A cyclization of *N*-[2-(benzoyloxy)phenyl]-benzamide, accompanied by the elimination of a molecule of benzoic acid, takes place in a mass spectrometer upon protonation by fast atom bombardment (FAB) or electrospray ionization (ESI). The elimination of benzoic acid from the $[M + H]^+$ yields protonated 2-phenylbenzoxazole by a process that is analogous to the acid catalyzed cyclization of *N*-[2-(benzoyloxy)phenyl]-benzamide in solution. A similar elimination of benzoic acid occurs from the molecular radical cation produced by electron ionization (EI). The proposed cyclization and elimination of benzoic acid are supported by accurate mass measurements, product-ion spectra in tandem mass spectrometry (MS/MS), mass spectra of reference compounds, isotopic labeling, and molecular modeling by density functional theory.


*Keywords:* Solution analogy; Cyclization; Fast atom bombardment; Electrospray ionization; Density functional theory

## 1. Introduction

*N*-[2-(Benzoyloxy)phenyl]-benzamide, in the presence of an acid catalyst [1], cyclizes in solution to yield 2-phenylbenzoxazole (Eq. (1)). This cyclization reaction is an effective means for the synthesis [2] of 2-phenylbenzoxazole and substituted analogs. When mixed *N*,*O*-diacylated compounds are used as substrates, they produce mixtures of 2-substituted benzoxazoles [3]; the major product is the benzoxazole in which the acyl group on the nitrogen is retained. The proposed mechanism of the reaction involves protonation of the substrate followed by cyclization to a seven-membered ring intermediate, which decomposes by ring-opening and elimination of a molecule of benzoic acid ($R_2 = C_6H_5$, Eq. (1))




(1)

Acid-catalyzed reactions involving eliminations such as these may be also readily studied in the gas-phase using mass spectrometric and theoretical methods. Ionization methods such as fast atom bombardment (FAB), electrospray ionization (ESI), and chemical ionization (CI) readily generate protonated molecules or $[M + H]^+$ ions. Although mass spectrometry is an important tool for study of these processes, the mechanisms may be different due to the absence of a solvent. We identified this system as one that may be useful in extending our knowledge of analogies between gas-phase and solution elimination reactions under acid catalysis.

Although the examples of gas-phase analogies for solution reactions are too large to review here, we draw attention

* Corresponding authors.

1387-3806/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2006.01.014

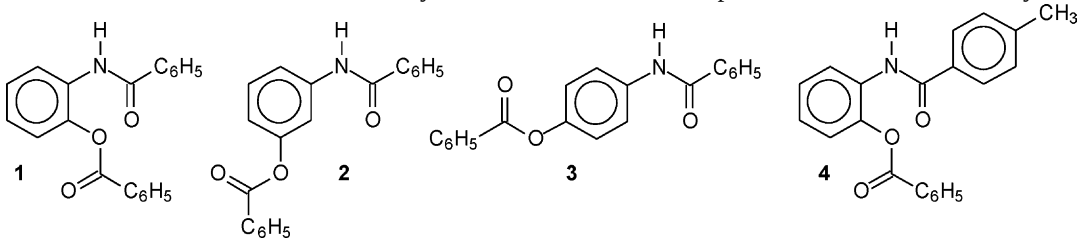
to some studies demonstrating cyclization reactions. Recent examples by Eberlin and co-workers [4] and by Cooks and co-workers [5] demonstrate that gas-phase "synthetic chemistry" is of current interest. Eberlin found that, in analogy with solution chemistry, various acylium ions react readily with benzonitrile by cyclization via a double nitrile addition to form aromatic 1,3,5-oxadiazinium ions, whereas Cooks showed that arylnitrenium ions formed in a mass spectrometer ion source react with ethyl vinyl ether and 1,3-dioxolanes to afford, via formation of new C–N bonds, a gas-phase "synthesis" of indoles and benzomorpholines. Another example is of reductive elimination from 2-nitro-2′-hydroxy-5′-acetylazobenzene to afford 2-(2′-hydroxy-5′-acetylphenyl)benzotriazole [6]. A gas-phase reaction closely related to the Dieckmann condensation, not seen in solution, is cyclization of diesters bearing α-alkyl substituents by elimination of a molecule of a dialkyl ether [7]. This work demonstrates that a new reaction that builds on older chemistry can be uncovered by mass spectrometric studies. One of the early examples of the value of metastable-ion analysis is the 1978 study by Glish and Cooks [8] of a gas-phase analogy for the Fischer indole synthesis, exemplified by the acid-catalyzed elimination of $NH_3$ from protonated phenylhydrazones.

Cycloadditions of gas-phase ions and their connection to solution chemistry has been of interest for other 30 years, starting with work from this laboratory on the cyclization of styrene radical cations [9,10]. A fine review of this subject was recently prepared by Eberlin [11].

The reaction reported here is an example of a proximity effect called the "ortho effect", for which there are numerous reports [12]; ortho effects are a specific example of general proximity effects [13]. The abundance of these rearrangements upon EI is probably due to the high-energy states for the odd-electron ions compared to the relatively low energy state of even-electron (closed-shell) ions generated by FAB or ESI. Despite the significant difference between radical cations and closed-shell species, rearrangements in EI mass spectrometry do have close parallels with rearrangements in the condensed phase. The analogies pointed to a design for syntheses of heterocyclic organic compounds [14,15], to the discovery of new synthesis procedures for heterocycles [16,17], and to a direct connection between intramolecular cyclization of pyridine derivatives occurring in solution and in the gas-phase under EI conditions [18].

An analogous proximity effect is manifest by protonated 2,2′-diacetoxybiphenyl generated by FAB. This closed-shell $[M+H]^+$ ion undergoes cyclization due to the interaction of the two acetyl groups followed by elimination of acetic acid to afford a cyclic product-ion [19]. A similar elimination of acetic acid from the $[M+H]^+$ ion of acetylated hyperacine, a marine natural product, helped to establish its structure [20]. In addition, the successive elimination of two OH radicals from protonated aromatic nitro compounds [21] follows the cyclization of the protonated molecules, which can be generated by FAB ionization.

Returning to the "gas-phase synthetic reaction" that is subject of this study, we synthesized *N*-[2-(benzoyloxy)phenyl]-benzamides (compounds **1**–**4**) and investigated them by a variety of mass spectrometric approaches to test whether facile cyclizations occur in the gas-phase upon protonation of the compounds by FAB and ESI, as well as upon removal of an electron by EI.




We are pleased to dedicate this article to the memory of Professor Chava Lifshitz, who spent part of her career studying elimination reactions. Notable are her studies of the eliminations of $C_2$ and similar fragments from aromatic and fullerene compounds [22,23], the exhaustive elimination of Cl from various compounds [24], and the expulsion of small molecules from peptide ions [25]. Eliminations of $CH_3$ in competition with $CH_4$ were important to her in the demonstration of non-ergodic processes [26].

## 2. Experimental

### 2.1. Synthesis

Compounds **1**–**3** were synthesized by benzoylation of the appropriate amino-phenols as reported in the literature [27,28]. The products obtained were collected by filtration and purified by recrystallization from methanol. Compound **4** was obtained by the reaction of 4-methybenzoyl chloride with excess of 2-aminophenol under acidic conditions followed by reaction with benzoyl chloride in the presence of sodium hydroxide. 2-Phenylbenzoxazole was obtained by the acid catalyzed cyclization of compound **1** using toluene-4-sulphonic acid, as previously reported [1]. Physical constants, $^1$H NMR, IR and mass spectral data were consistent with the identity of each compound.

### 2.2. Mass spectrometry

Tandem mass-spectrometry experiments (MS/MS) on FAB-, CI- or EI-produced ions were conducted on a VG ZAB-T four-sector mass spectrometer of BEBE design [29]. MS1 is standard high-resolving power, double-focusing mass spectrometer (ZAB) of reverse geometry. Although MS2 had a Mattauch–Herzog-type design, incorporating a standard magnet and a planar electrostatic analyzer having an inhomogeneous electric field to permit use of an array detector, a single-point

detector was used for these studies. For FAB, samples were dissolved in methanol and a 1-μL aliquot was loaded on the probe along with 1 μL of the matrix, 3-nitrobenzyl alcohol. A $Cs^+$ ion gun operated at 30 keV was used to desorb the ions, which were accelerated to 8 kV. Samples were introduced through direct-probe insertion for EI and CI experiments. For CI experiments, $CH_4$ or $CD_4$ was used to generate the CI reagent ions and produce $[M+H]^+$ or $[M+D]^+$ ions, respectively. High mass resolving power (~8000) EI mass spectra were recorded by using a VG-ZAB SE, a reverse-geometry, Nier–Johnson mass spectrometer operated at 8 kV acceleration voltage and 70 eV ionizing energy. Perfluorophenanthrene was used as mass-calibration standard for full-scan mass spectra.

The dissociation of the precursor ion, by either metastable-ion (MI) or high-energy (4 keV) collisionally activated dissociation (CAD) with helium as collision gas, was studied in the third field-free region. Sufficient helium gas was added to the collision cell to decrease the main-beam intensity by 30%. Both MS1 and MS2 of the mass spectrometer were operated at a mass resolving power of 1000. Typically 10–20 scans were signal averaged for each spectrum. Data acquisition and workup were accomplished by using a VAX 3100 workstation equipped with OPUS software.

High mass-resolving-power FAB measurements were conducted with a Kratos MS-50 triple analyzer tandem mass spectrometer [30] equipped with a standard Kratos FAB source and an Ion Tech saddle-field atom gun. The atom beam was 6–7 keV argon atoms generated with a gun current of 1 mA. A mixture of CsI and glycerol was used to produce reference-mass ions for the peak-match mode experiments.

The ESI experiments, both MS and low-energy CAD (MS/MS), were conducted by using a Micromass Q-TOF-Ultima instrument operated in the positive ion mode. The needle voltage was 3 kV, and the cone voltage was 90 V. The temperatures of the source block and for desolvation were 90 and 150 °C, respectively. The samples were dissolved in 1:1 mixture of acetonitrile and water. The samples were introduced by direct infusion at a flow rate of 10 μL/min. All parameters (e.g., aperture to the TOF, transport voltage, offset voltages) were optimized to achieve maximum sensitivity and a mass resolving power of 15,000 (full-width at half-maximum).

The CAD experiments of ESI-produced ions were carried out by mass selecting the precursor ion using the quadruple analyzer and recording the product-ions by using the time-of-flight analyzer operating at a mass resolving power of 15,000 ('W' mode). The energies for colliding the ions under study were of the order of 7–9 eV with argon as the collision gas. The accurate masses of the product-ions were determined by using the precursor ion as the internal mass standard. An ESI-MS/MS/MS experiment (low-energy CA) was performed on a Finnigan LCQ Classic ion-trap mass spectrometer. The samples, dissolved in 1:1 mixture of acetonitrile and water, were introduced by direct infusion at a flow rate of 10 μL/min.

### 2.3. Theoretical calculations

Proposed reaction mechanisms with consequent structures of intermediates and the heats of formation/reaction were evaluated and calculated by molecular modeling of the precursor ions, proposed intermediates, and products. Owing to the large size of the ions, the initial survey calculations were performed by using the PM3 [31] semi-empirical algorithm, which was obtained as part of the Spartan'02 for Linux package (Wavefunction Inc.). The starting point of the investigation was the proposed solution mechanism [3], which was modified and expanded for the gas-phase process. Furthermore, we limited the theoretical calculations to ions from the unsubstituted *ortho*-diphenyl compound **1**.

Second stage calculations were by density functional theory (DFT), which required less computational overhead than formal ab initio methods and yet incorporated dynamic correlation, had little spin contamination [32], and usually performed adequately giving proper geometries, energies, and frequencies [33]. DFT was part of the Gaussian'98 or Gaussian'03 suites (Gaussian Inc.) [34,35].

Minima and transition states were optimized to the DFT level of B3LYP/6-311+G(2d,p)//B3LYP/6-31G(d,p), confirmed by vibrational frequency analysis, and scaled zero-point and thermal-energy corrections for standard temperature and pressure were applied [36]. Connections of transition states were confirmed by projections of the normal variable associated with imaginary frequency or by path calculations. The heats of formation or reaction are reported as enthalpies relative to the ion that represents the starting point for the mechanistic scheme. It must be noted that these calculations yield information about the potential-energy surface, but ultimately fragmentation patterns are determined by kinetic processes.

## 3. Results and discussion

### 3.1. Experimental observations

Fast atom bombardment of compound **1** affords a protonated molecule, $[M+H]^+$ of *m*/*z* 318, as well as fragment ions of *m*/*z* 196 and 105 (Table 1). The formation of the benzoyl ion of *m*/*z* 105 is not surprising given that compound **1** is a benzoate. The formation of the ion of *m*/*z* 196, involving the elimination of a molecule of benzoic acid, however, is of considerably more interest. Accurate mass measurement revealed that the *m*/*z* 196 ion has the formula $C_{13}H_{10}NO$ (measured mass = 196.0762, calculated mass = 196.0752). The MI and CAD spectra (Table 1) of the $[M+H]^+$ ions show that fragmentation gives abundant product-ions of *m*/*z* 196 and 105, indicating that the elimination of benzoic acid is a facile dissociation process of the precursor. The uniqueness of the process for the *ortho*-isomer is revealed by the fact that the CAD and MI mass spectra of the *meta*- and *para*-isomers of the $[M+H]^+$ ions provide no evidence for the elimination of benzoic acid to give the ion of *m*/*z* 196. However, the spectra do show the production of *m*/*z* 105 ions (benzoyl ions) (see Table 1). The low abundance ion at *m*/*z* 213 is observed under conditions of FAB MI and high-energy CAD,

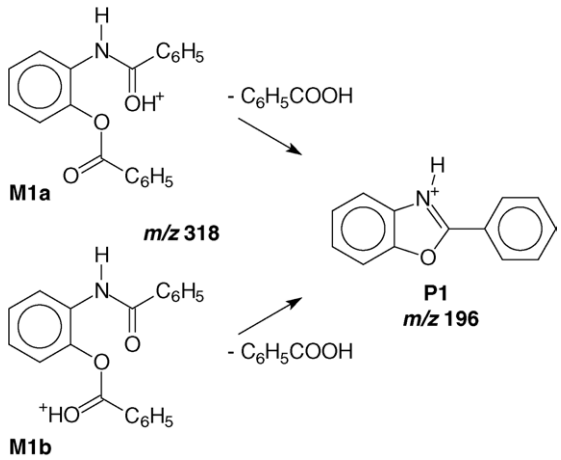
Table 1
Partial FAB mass spectra, metastable-ion, and high-energy CAD spectra of compounds **1**–**3**

| Fragmentation mode | Relative abundances (%) of ions | | | | |
|---|---|---|---|---|---|
| | $[M+H]^+$, *m/z* 318 | *m/z* 213 | *m/z* 196 | *m/z* 195 | *m/z* 105 |
| **1** | | | | | |
| MS[a] | 62 | – | 7 | 16 | 100 |
| MI | | 2 | 61 | – | 100 |
| CAD | | 2 | 12 | – | 100 |
| **2** | | | | | |
| MS | 100 | – | – | – | 90 |
| MI | | 5 | | | 100 |
| CAD | | 4 | | | 100 |
| **3** | | | | | |
| MS | 95 | | – | – | 100 |
| MI | | 13 | | | 100 |
| CAD | | 11 | | | 100 |

[a] MS: mass spectra; MI: metastable-ion spectra; CAD: collisionally activated decomposition spectra.

but it is barely seen in a full-scan mass spectrum. This ion is not formed accompanying ESI MS or even upon low-energy CAD of ESI-produced ions. This ion is likely formed by charge transfer during the separation of *m/z* 105; its *m/z* corresponds to that for benzoyl radical loss.

The fragment ion of *m/z* 196 is formed from the *m/z* 318 precursor (M1a, M1b—protonated compound **1**) by elimination of presumably benzoic acid to give a product ion, protonated 2-phenylbenzoxazole, P1, as shown in Scheme 1. To confirm this structure assignment, we compared the CAD mass spectrum of P1 with that of the appropriate reference, protonated 2-phenylbenzoxazole (Fig. 1). The two spectra are similar, indicating that ion P1 likely has the assigned structure and that its formation is an intramolecular cyclization that occurs upon protonation of the precursor. (On the schemes, we use M, TS, P and Q to designated minima, transition states, ionic and neutral products, respectively, to distinguish from the designations of the neutral compounds.)




Scheme 1.

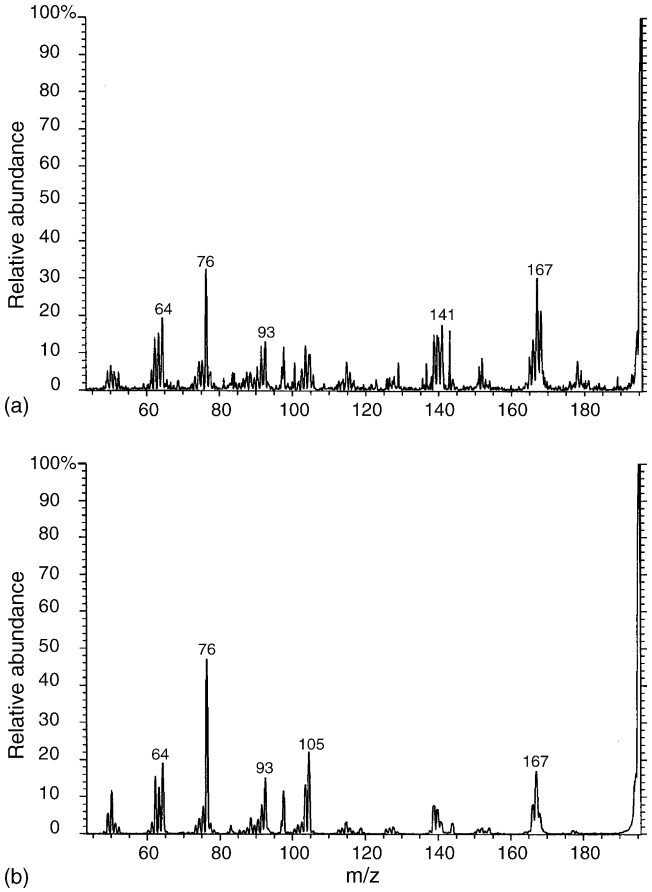



Fig. 1. CAD product-ion mass spectra of FAB-produced ions of *m/z* 196 from (a) compound **1** and from (b) the reference protonated 2-phenylbenzoxazole.

The ESI mass spectrum of compound **1** (Fig. 2a) shows that the protonated molecule, $[M+H]^+$, of *m/z* 318, formed by this method also fragments to give ions of *m/z* 196 and 105. The low-energy ESI CAD (Fig. 2b) mass spectrum of the $[M+H]^+$ also exhibited peaks corresponding to ions of *m/z* 196 (measured accurate mass = 196.0750, calculated mass = 196.0752) and 105 (benzoyl ion).

A comparison of the ESI CAD mass spectra of *m/z* 196, generated by collisional activation of *m/z* 318, and the $[M+H]^+$, *m/z* 196, of the 2-phenylbenzoxazole (Fig. 3) produced by ESI, are again similar, indicating that the loss of benzoic acid leads to a cyclic ion, having the 2-phenylbenzoxazole structure, as shown in Scheme 1. The losses of benzoic acid are competitive for both the ester and amide substitution sites.

To gain further insight into the mechanism for elimination of benzoic acid, we obtained the CAD spectrum of ESI-produced ions (Fig. 4) and the MI spectrum of FAB-produced $[M+H]^+$ ions (Fig. 5) from compound **4**, in which we placed a methyl group to label the benzoyl group attached to the amide. The product-ion spectrum (CAD) of ESI-produced $[M+H]^+$ of *m/z* 332 shows expulsion of both benzoic acid (to give an ion of *m/z* 210.0918, $C_{14}H_{12}NO^+$ calcd. 210.0919) and toluic acid (to give the *m/z* 196.0763 ion, $C_{13}H_{10}NO^+$ calcd. 196.0762). Furthermore, benzoyl (*m/z* 105) and toluoyl (*m/z* 119) ions are also generated in similar abundances; and these ions in turn

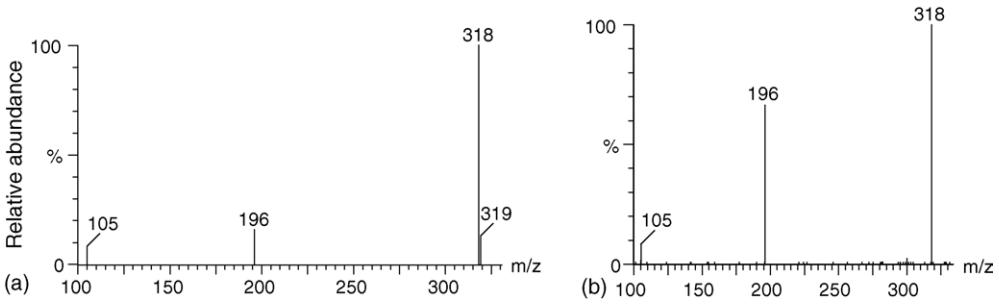



Fig. 2. Mass spectrum of ESI-produced ions (a) and the product-ion mass spectrum (CAD) of ESI-produced ions (b) from compound **1**.

lose CO to form *m*/*z* 77 and 91 ions, respectively (data not shown).

The observations indicate that a benzoyl group, attached either to the amide nitrogen or to the ester oxygen, can be eliminated as part of benzoic acid, suggesting that mechanistic symmetry is involved in both the elimination of benzoic acid and the formation of benzoyl ions from either site. This indicates that protonation can occur on both the ester carbonyl and the amide carbonyl groups.

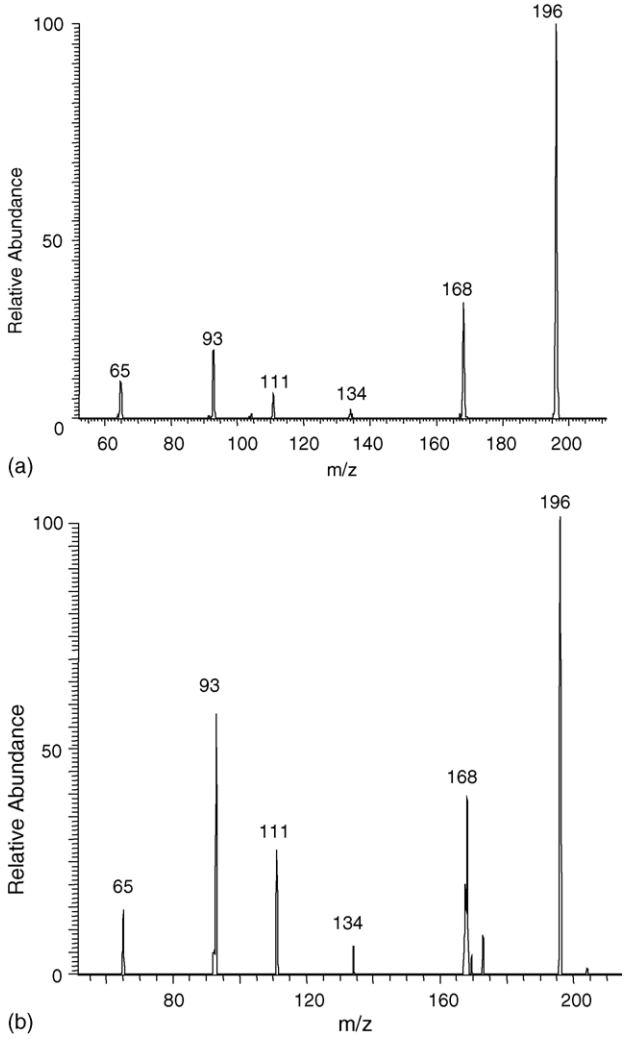



Fig. 3. The CAD spectra of ESI-produced *m*/*z* 196 ions obtained from (a) compound **1** by CAD and from (b) 2-phenylbenzoxazole.

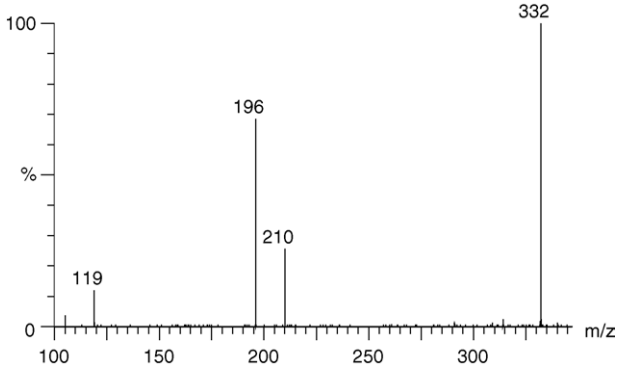



Fig. 4. ESI-CAD mass spectrum of $[M+H]^+$ of *m*/*z* 332 of compound **4.**

### *3.2. Theoretical calculations—fragmentation of protonated molecules*

We undertook theoretical calculations to aid in the elucidation of mechanisms of fragmentation and cyclization. Surprisingly, we were able to locate three different sets of feasible mechanisms by exploring the potential energy surface for the loss of benzoic acid from the $[M+H]^+$ ions derived from compound **1**. We chose compound **1** because, unlike empirical results, the fate of the individual benzoyl moieties can be tracked and the addition of a methyl group to the benzoyl unit to label it would

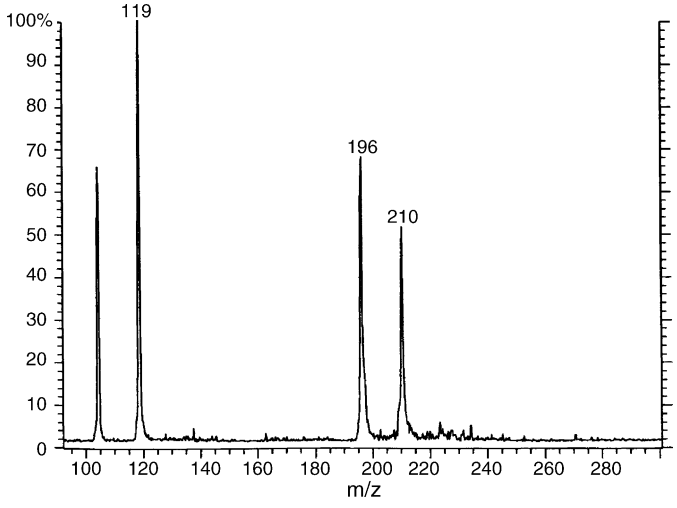
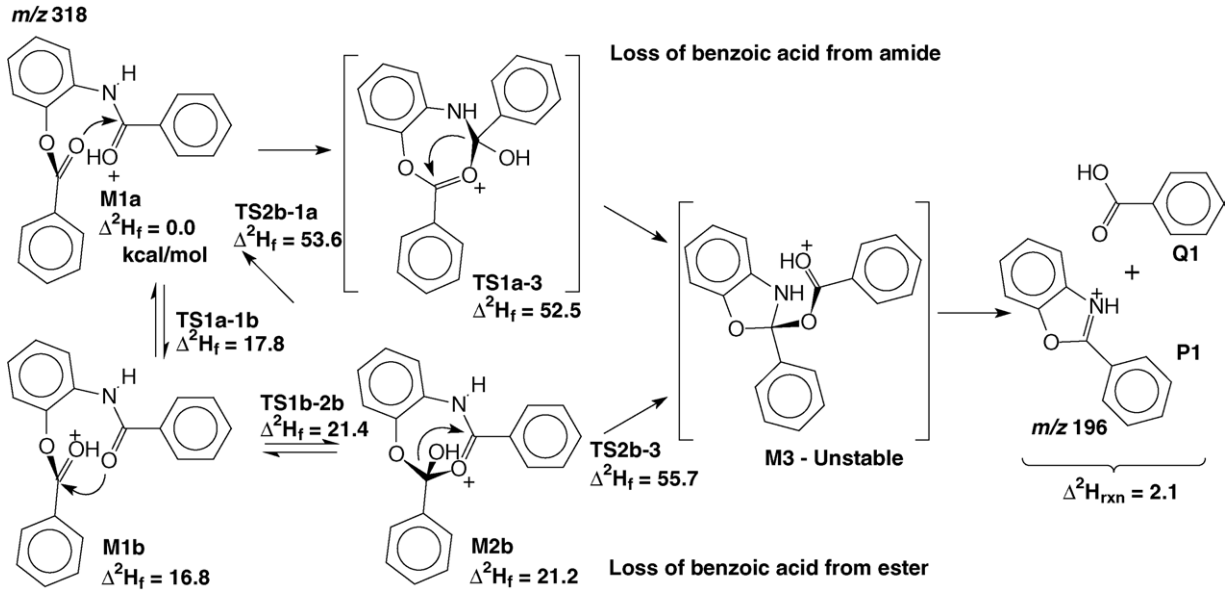



Fig. 5. FAB MI mass spectrum of $[M+H]^+$ of *m*/*z* 332 compound **4**.




Scheme 2. Proposed mechanisms for benzoic acid loss: seven-membered ring intermediates (energies in kcal/mol).

only add to the computational overhead without providing any more useful information. In our theory studies, we calculated the heats of formation/reaction of the $[M+H]^+$ ions, intermediates and products relative to that for the $[M+H]^+$ ion, M1a, the most stable of the initially protonated molecules, by using density functional theory (DFT) as described in Section 2. In general, our results show that for internal energies necessary to induce fragmentation, the ionizing proton can be promoted to either carbonyl oxygen in addition to the amide nitrogen. According to our calculations, movement of the proton from the amide carbonyl to the ester carbonyl requires an enthalpy change of nearly 17 kcal/mol, which is consistent with the 15–20 kcal/mol higher proton affinities of carboxylic acids and esters versus corresponding amides (see http://webbook.nist.gov/chemistry/).

The first set of proposed mechanisms proceeds through seven-membered ring intermediates formed by nucleophilic attack of the unpaired electrons of the unprotonated carbonyl oxygen upon the carbon of the protonated carbonyl (Scheme 2).

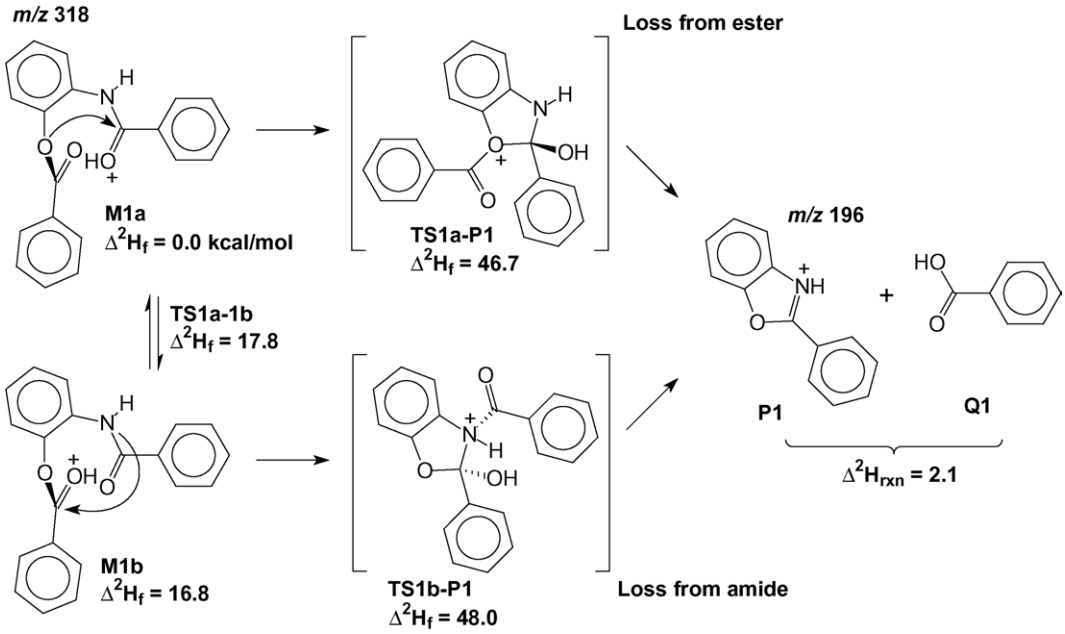
Elimination of benzoic acid from the ester occurs via a shallow intermediate (M2b), whereas the complementary elimination from the amide occurs via only an intermediate transition state (TS2a-3). The seven-member ring intermediates contract to an unstable five-member ring that decomposes producing benzoic acid (Q1) and the 2-phenylbenzoxazole $[M+H]^+$ ion (P1). We note that the greatest barriers to reaction (TS1a-3 and TS2b-3) are similar (3.2 kcal/mol difference); thus, losses of benzoic acid from both ester and amide moieties should be observed, and they are.

The second set of mechanisms involves the attack on the carbonyl carbon of the protonated moiety by the unpaired electrons on the uncharged ester oxygen or amide nitrogen (Scheme 3). In both cases, nucleophilic attack results in a five-membered ring transition state (TS1a-P1 or TS1b-P1), which decomposes to liberate benzoic acid (Q1) from the amide and form the 2-phenylbenzoxazole $[M+H]^+$ ion (P1). Both of these transition states present similar barriers to reaction (1.3 kcal/mol difference), which likely would lead to similar kinetics in product formation, in accord with experiment.

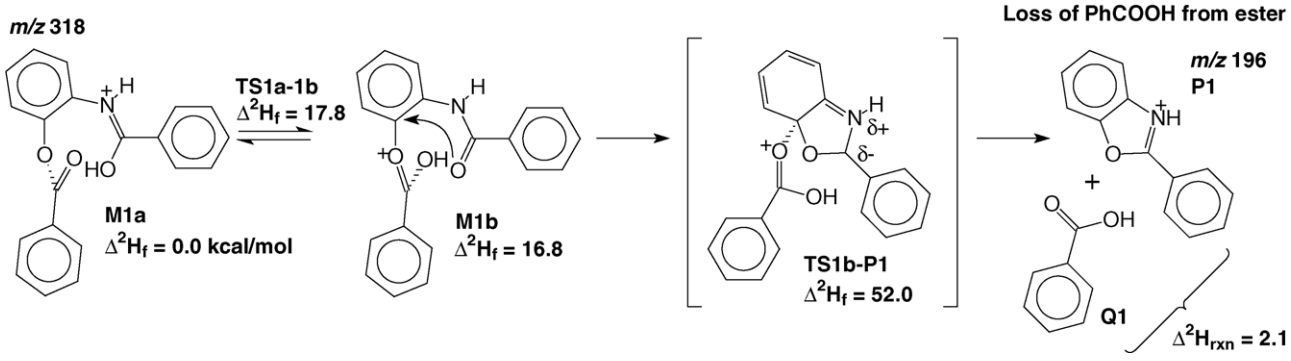
The third type of possible reaction mechanism involves aromatic nucleophilic substitution of the initially charged moiety, which displaces neutral benzoic acid from the ester side of M1b (Scheme 4). An analogous route leading to the elimination of the imine tautomer of benzamide from the amide side of M1a would generate a product-ion of *m/z* 197 (not shown). However, no *m/z* 197 ion can be detected, even in high-energy CAD experiments; the energy barrier associated with that transition state is 67.7 kcal/mol which is >14 kcal/mol greater than maximum barrier for competing pathways (Schemes 2 and 3 and Table 2), explaining the non-competitive nature of the imine elimination.

In the proposed mechanisms, there are only two active protons in the $[M+H]^+$ ions; thus, the ionizing proton has a 1 in 2 probability of being eliminated as part of the benzoic acid. When we made the $[M+D]^+$ by FAB of compound **1** by using 3-nitrobenzyl alcohol-OD as the matrix and obtained both its MI and high-energy CAD spectra, we found that intensity ratio of the *m/z* 196 and 197 peaks was 1:1, implying that the H/D ratio in the eliminated the benzoic acid is also 1:1 (data not shown). Thus, the aryl hydrogens are not involved in any active mechanism leading to the loss of benzoic acid.

The calculated relative heats of formation or reaction for the various schemes, summarized in Table 2 and shown on the schemes, and indicate that the proposed reaction mechanisms in Schemes 2–4 for the loss of benzoic acid and production of protonated 2-phenylbenzoxazole require similar investments of internal energy. We note that the overall enthalpy of reaction for the production of benzoic acid (Q1) and the 2-phenylbenzoxazole $[M+H]^+$ ion (P1) is modestly endothermic at 2.2 kcal/mol and plays no direct role in the energetics of the rate-limiting steps. The transition state barriers, however,




Scheme 3. Proposed mechanisms for benzoic acid loss via five-membered ring transition states.




Scheme 4. Proposed mechanism: displacement reaction.

are lowest for loss of benzoic acid from both amide and ester for Scheme 3. Thus, we expect the process in Scheme 3 to be the dominant mechanisms for the loss of benzoic acid from the $[M+H]^+$ ions of compound **1**. Furthermore, we expect that loss of toluic acid from the $[M+H]^+$ ions of compound **4** would entail similar energetics to the loss of benzoic acid in the prototype.

### 3.3. Fragmentation of the radical cation—theory and experiment

To investigate whether similar fragmentations and their mechanisms pertain to the radical cations, we turned to EI mass spectrometry. The EI mass spectrum of the *ortho*-isomer, com-

Table 2
The relative heats (enthalpies) of formation and reaction (in kcal/mol) for components in Schemes 2–4 computed relative to M1a

| Scheme 2 | | Scheme 3 | | Scheme 4 | |
|---|---|---|---|---|---|
| Tag | $\Delta^2H_f$ or $\Delta H_{rxn}$ | Tag | $\Delta^2H_f$ or $\Delta H_{rxn}$ | Tag | $\Delta^2H_f$ or $\Delta H_{rxn}$ |
| M1a | 0.0 | M1a | 0.0 | M1a | 0.0 |
| M1b | 16.8 | M1b | 16.8 | M1b | 16.8 |
| M2b | 21.2 | | | | |
| TS1a-1b | 17.8 | TS1a-1b | 17.8 | TS1a-1b | 17.8 |
| TS1a-3 | 52.5 | TS1a-P1 | 46.7 | TS1b-P1 | 52.0 |
| TS1b-2b | 21.4 | TS1b-P1 | 48.0 | | |
| TS2b-1a | 53.6 | | | | |
| TS2b-3 | 55.7 | | | | |
| P1 + Q1 | 2.1 | P1 + Q1 | 2.1 | P1 + Q1 | 2.1 |

Table 3
Partial EI mass spectra (MS) and metastable-ion (MI) and collisionally activated decomposition (CAD) product-ion mass spectra of the molecular radical cations of compounds **1**–**3**

| Compound | Relative abundance (%) | | |
|---|---|---|---|
| | $M^{\bullet+}$, *m/z* 317 | *m/z* 195 | *m/z* 105 |
| **1** | | | |
| MS | 0.3 | 60 | 100 |
| MI | | 100 | 40 |
| CAD | | 100 | 50 |
| **2** | | | |
| MS | 10 | – | 100 |
| CAD | | | 100 |
| **3** | | | |
| MS | 8 | – | 100 |
| CAD | | | 100 |

pound **1**, Table 3, shows the molecular radical cation of *m/z* 317 and abundant fragment ions of *m/z* 195 and 105, the latter is likely the benzoyl ion. The molecular formula of the *m/z* 195 ion is $C_{13}H_9NO$ (measured mass = 195.0677, calculated mass = 195.0684). Production of an ion of this molecular formula $[M - 122]^{\bullet+}$ occurs by the elimination of presumably benzoic acid. The metastable-ion (MI) mass spectrum of the *m/z* 317 ion clearly indicates the direct generation of this fragment ion is a low-energy process (Table 3). Moreover, the collision activated dissociation (CAD) product-ion spectrum also shows an abundant ion of *m/z* 195, supporting the conclusion that the loss of benzoic acid is the major dissociation pathway. The *meta*- and *para*-isomers (compounds **2** and **3**) do not show ions of *m/z* 195 either in their EI mass spectra, Table 3, or in their product-ion spectra obtained upon collisional activation.

The structure of the $[M -$ benzoic acid$]^{\bullet+}$ fragment ion is that of the molecular radical cation of 2-phenylbenzoxazole. This conclusion is based on a comparison of the CAD mass spectra of that molecular ion with that of the *m/z* 195 fragment ion derived from *m/z* 317 (see Fig. 6(a) and (b)). The two spectra are nearly identical.

The MI spectrum, Fig. 7, of compound **4**, which has a 4-methylbenzoyl group attached to the nitrogen and a benzoyl group attached to the oxygen shows that the predominant elimination is of benzoic acid. The trace of a product-ion from expulsion of *p*-toluic acid to give an ion of *m/z* 195 may not be significant, resulting instead from an impurity in the sample preparation.

We propose that the mechanism for the elimination of benzoic acid from the ester moiety of the molecular radical cation of compound **1** (M7 in Scheme 5) proceeds via nucleophilic aromatic substitution. This mechanism has some analogy to the scheme for loss of benzoic acid from the protonated molecule of compound **1** (Scheme 4). Only the loss of benzoic acid from the ester moiety can occur by this kind of mechanism. The most stable conformer of the radical cation of **1** is M7a (not shown), a conformer of M7. The overall reaction, from M7a to products P3 and Q1, is modestly endothermic at 2.8 kcal/mol. Moreover, the transition state barriers for these fragmentation pathways are

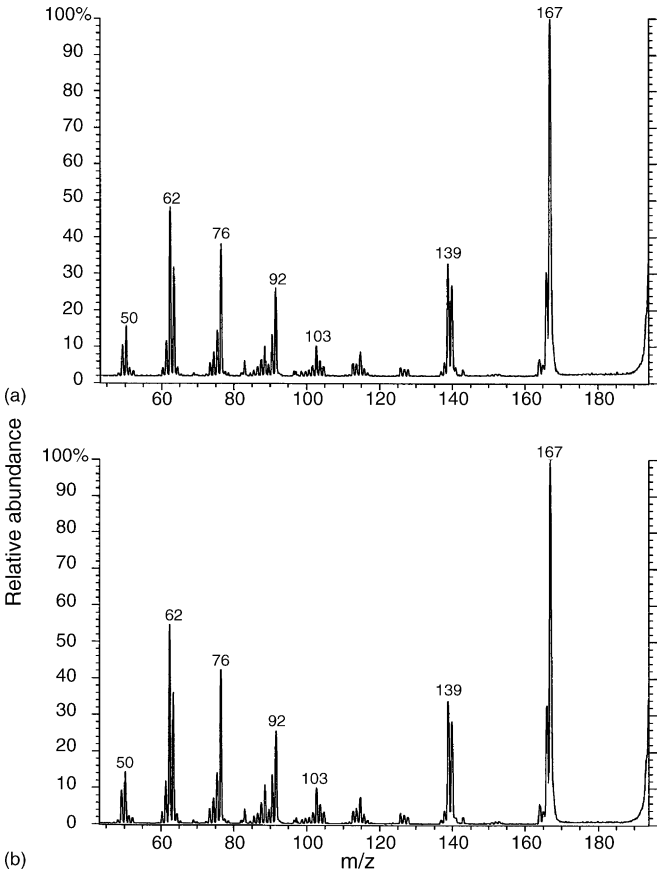



Fig. 6. CAD mass spectra of *m/z* 195 from (a) compound **1** and from (b) 2-phenylbenzoxazole.

lower that those for the fragmentation pathways of the $[M + H]^+$ ions (Table 4).

Another possible mechanisms for loss of benzoic acid would involve first the H transfer from the amide nitrogen to the ester carbonyl oxygen, followed by benzoic acid displacement resulting from attack by the amide carbonyl oxygen. However, calculations show that the transition state associated with the displacement presents a barrier of 41.7 kcal/mol compared with the maximum barriers of 25.3 and 28.6 kcal/mol (TS8a-8c and

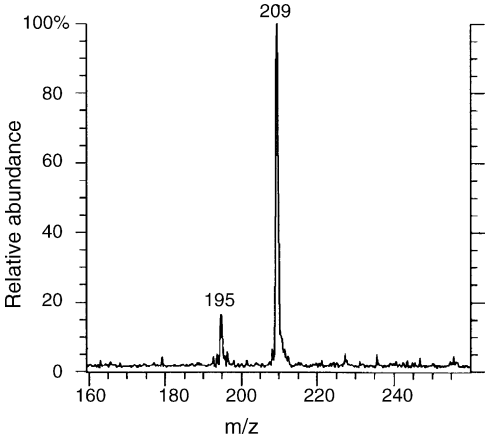
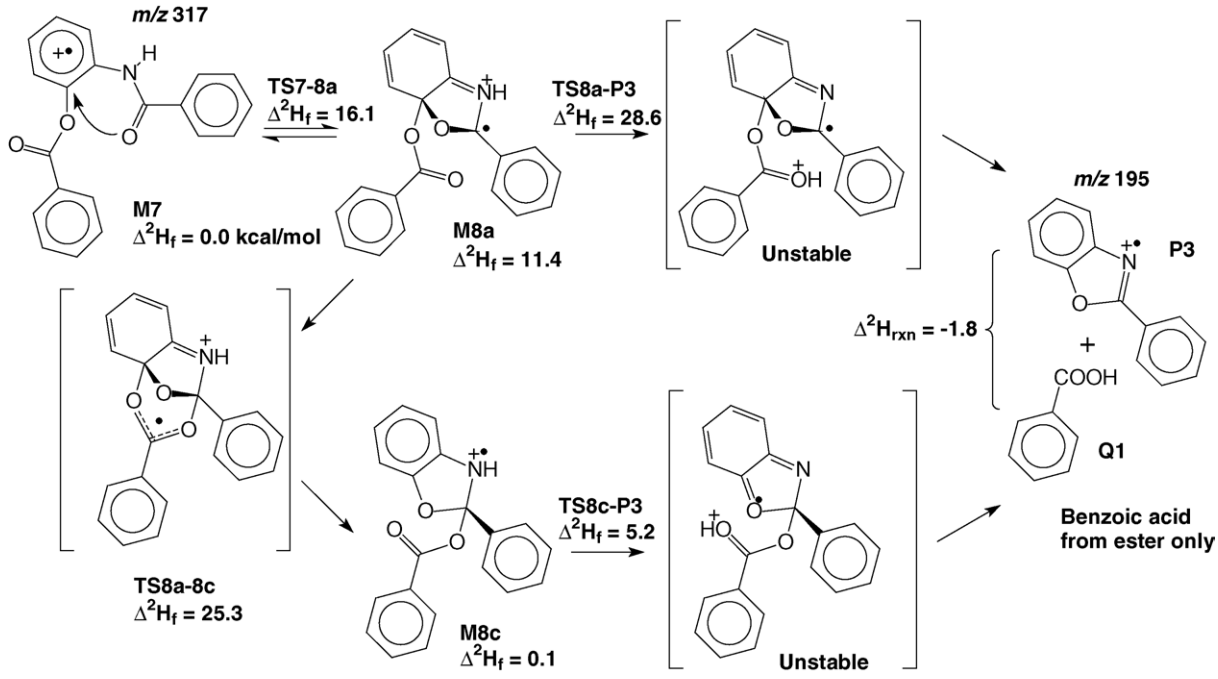



Fig. 7. MI spectrum of the molecular radical cation, $M^{\bullet+}$, of compound **4**.




Scheme 5. Proposed mechanism for loss of benzoic acid from the molecular radical cation.

Table 4
The relative heats of formation and reaction (kcal/mol) in Scheme 5

| Tag | Relative $\Delta^2 H_f/\Delta H_{rxn}$ |
|---|---|
| M7a | −4.6 |
| M7 | 0.0 |
| M8a | 11.4 |
| M8c | 0.1 |
| TS7-8a | 16.1 |
| TS8a-P3 | 28.6 |
| TS8a-8c | 25.3 |
| TS8c-P3 | 5.2 |
| P3 + Q1 | −1.8 |

TS8a-P3, respectively, Scheme 5) in the proposed scheme. Thus, we consider that route as non-competitive.

Although minor loss of toluic acid from the amide, if real, would involve another mechanism, it is likely that this signal arises from an isomeric impurity in the original sample.

## 4. Conclusion

The $[M+H]^+$ ions of *N*-[2-(benzoyloxy)phenyl]-benzamide produced by FAB, ESI, and CI and the $M^{\bullet+}$ by EI all undergo elimination of benzoic acid with cyclization, producing a fragment ion having the core structure of 2-phenylbenzoxazole irrespective of the method of ionization. The *meta*- and *para*-isomers do not undergo this cyclization or lose benzoic acid, underscoring a unique proximity effect. There is a clear analogy with solution synthetic chemistry: the *ortho*-isomer can also be cyclized upon protonation in solution, establishing once more that mass spectral processes can be an informative analogy with future predictive capability for solution processes.

## Acknowledgements

The authors J.T.M. and M.G. thank the Kerala State Council for Science Technology and Environment for financial assistance and Principal, S.H. College, Thevara for providing infrastructure. R.S. and N.S.S. thank Dr J.S. Yadav, Director, IICT, Hyderabad, for facilities and Dr M. Vairamani for cooperation. Research at Washington University was supported by the National Centers for Research Resources of the NIH, Grant P41RR00954.

## References

[1] M.R. DeLuca, S.M. Kerwin, Tetrahedron 53 (1997) 457.
[2] Y.H. So, J.P. Heeschen, J. Org. Chem. 62 (1997) 3552.
[3] M.R. DeLuca, I.B. Taraporewala, S.M. Kerwin, Heterocycles 51 (1999) 979.
[4] E.C. Meurer, L.A.B. Moraes, M.N. Eberlin, Int. J. Mass Spectrom. 212 (2001) 445.
[5] H. Chen, H. Chen, R.G. Cooks, H. Bagheri, J. Am. Soc. Mass Spectrom. 15 (2004) 1675.
[6] D. Kuila, S. Huang, Org. Mass Spectrom. 29 (1994) 226.
[7] D.J. Burinsky, R.G. Cooks, J. Org. Chem. 47 (1982) 4864.
[8] G.L. Glish, R.G. Cooks, J. Am. Chem. Soc. 100 (1978) 6720.
[9] C.L. Wilkins, M.L. Gross, J. Am. Chem. Soc. 93 (1971) 895.
[10] G.S. Groenewold, E.K. Chess, M.L. Gross, J. Am. Chem. Soc. 106 (1984) 539.
[11] M.N. Eberlin, Int. J. Mass Spectrom. 235 (2004) 263.
[12] (a) J.T. Bursey, M.M. Bursey, D.G.I. Kingston, Chem. Rev. 73 (1973) 191;
(b) H. Schwarz, Top. Curr. Chem. 73 (1978) 232;
(c) D.V. Ramana, N. Sundaram, M. George, Org. Mass Spectrom. 25 (1990) 161;
(d) D.V. Ramana, N. Sundaram, M. George, Org. Mass Spectrom. 24 (1989) 63;
(e) F.W. McLafferty, F. Turecek, Interpretation of Mass Spectra, 4th ed., University Science Books, Mill Valley, California, 1993, p. 77.

[13] (a) T.H. Morton, Tetrahedron 38 (1982) 3195;
(b) M.A. Trikoupis, J.K. Terlouw, P.C. Burgers, J. Am. Chem. Soc. 120 (1998) 12131;
(c) L.M. Fell, P.C. Burgers, P.J.A. Ruttink, J.K. Terlouw, Can. J. Chem. 76 (1998) 335;
(d) J. Byun, M.L. Gross, M. George, D.M. Parees, A.Z. Kamzelski, D.F.H. Swijter, D.A. Willcox, J. Mass Spectrom. 32 (1997) 71 (and references cited therein).
[14] O. Pentti, S. Geza, P. Kalevi, K. Mati, J. Am. Soc. Mass Spectrom. 5 (1994) 113.
[15] V. Pirjo, F. Ferenc, B. Gabor, P. Kalevi, J. Heterocyclic Chem. 26 (1989) 1453.
[16] D.V. Ramana, K. Kantharaj, Tetrahedron 50 (1994) 2485.
[17] D.V. Ramana, N. Sundaram, T.E. Yuvaraj, K.B.G. Babu, Indian J. Chem. 38B (1999) 905.
[18] A.T. Lebedev, N.K. Karakhanova, A.M. Sipyaguin, I.V. Efremov, N. Tretyakova, R. Hass, J. Mass Spectrom. 32 (1997) 728.
[19] M. Orlando, M. George, M.L. Gross, Org. Mass Spectrom. 28 (1993) 1184.
[20] N. Tao, M. Orlando, J.S. Hyon, M.L. Gross, P.S. Song, J. Am. Chem. Soc. 115 (1993) 2526.
[21] M.R.M. Domingues, M.G.O.S. Marques, C.M.A. Alonso, M.G.P.M.S. Neves, J.A.S. Cavaleiro, A.J. Ferrer-Correia, O.V. Nemirovskiy, M.L. Gross, J. Am. Soc. Mass Spectrom. 13 (2002) 1427.
[22] (a) W. Cui, B. Hadas, B. Cao, C. Lifshitz, J. Phys. Chem. A 104 (2000) 6339;
(b) Y. Ling, C. Lifshitz, Isr. J. Mass Spectrom. 32 (1997) 1219;
(c) Y. Gotkis, M. Oleinikova, M. Naor, C. Lifshitz, Isr. J. Phys. Chem. 97 (1993) 12282.
[23] (a) B. Cao, T. Peres, R.J. Cross Jr., M. Saunders, C. Lifshitz, J. Phys. Chem. A 109 (2005) 10257;
(b) B. Cao, T. Peres, R.J. Cross Jr., M. Saunders, C. Lifshitz, J. Phys. Chem. A 105 (2001) 2142;
(c) S. Matt, R. Parajuli, A. Stamatovic, P. Scheier, T.D. Mark, J. Laskin, C. Lifshitz, Eur. Mass Spectrom. 5 (1999) 477;
(d) J. Laskin, C. Lifshitz, Int. J. Mass Spectrom. Ion Processes 138 (1994) 95;
(e) C. Lifshitz, M. Iraqi, T. Peres, J.E. Fischer, Int. J. Mass Spectrom. Ion Processes 107 (1991) 565.
[24] (a) J. Sun, H.-F. Gruetzmacher, C. Lifshitz, Int. J. Mass Spectrom. Ion Processes 138 (1994) 49;
(b) C. Lifshitz, T. Peres, S. Kababia, I. Agranat, Int. J. Mass Spectrom. Ion Processes 82 (1988) 193.
[25] (a) B. Balta, V. Aviyente, C. Lifshitz, J. Am. Soc. Mass Spectrom. 14 (2003) 1192;
(b) B. Balta, M. Basma, V. Aviyente, C. Zhu, C. Lifshitz, Int. J. Mass Spectrom. 201 (2000) 69;
(c) Y. Ling, C. Lifshitz, Israel. J. Mass Spectrom. 33 (1998) 25.
[26] G. Depke, C. Lifshitz, H. Schwarz, E. Tzidony, Angew. Chem. 93 (1981) 824.
[27] R. Meirer, F. Bohler, Chem. Berg. 89 (1956) 2301.
[28] K. Venkataraman, D.R. Wagle, Tetrahedron Lett. 23 (1979) 3037.
[29] M.L. Gross, in: J.A. McCloskey (Ed.), Methods in Enzymology, vol. 193, Mass Spectrometry, Academic Press, San Diego, 1990, p. 131.
[30] M.L. Gross, P.A. Lyon, E.K. Chess, F.W. Crow, S. Evans, H. Tudge, Int. J. Mass Spectrom. Ion Phys. 42 (1982) 243.
[31] (a) J.J.P. Stewart, J. Comp. Chem. 10 (1989) 209;
(b) J.J.P. Stewart, J. Comp. Chem. 10 (1989) 221.
[32] (a) J.M. Wittbrodt, H.B. Schlegel, J. Chem. Phys. 105 (1996) 6574;
(b) J. Baker, A. Scheiner, J. Andzelm, J. Chem. Phys. Lett. 206 (1993) 380;
(c) G.J. Laming, N.C. Hardy, R.D. Amos, Mol. Phys. 80 (1993) 1121.
[33] A. Nicolaides, D.M. Smith, F. Jensen, L.J. Radom, J. Am. Chem. Soc. 119 (1997) 8083.
[34] (a) M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery Jr., R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G.A. Petersson, P.Y. Ayala, Q. Cui, K. Morokuma, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, J.L. Andres, C. Gonzalez, M. Head-Gordon, E.S. Replogle, J.A. Pople, Gaussian'98, Revision A. 6, Gaussian Inc., Pittsburgh, PA, 1998;
(b) M.J. Frisch, A. Frisch, Gaussian'98, User's Reference, Gaussian Inc., Pittsburgh, PA, 1999 (and references cited therein).
[35] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian'03, Revision C. 02, Gaussian Inc., Wallingford, CT, 2004.
[36] A.P. Scott, L. Radom, J. Phys. Chem. 100 (1996) 16502.