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RESEARCH ARTICLE

Experimental and Theoretical Studies on Gas-Phase Fragmentation Reactions of Protonated Methyl Benzoate: Concomitant Neutral Eliminations of Benzene, Carbon Dioxide, and Methanol

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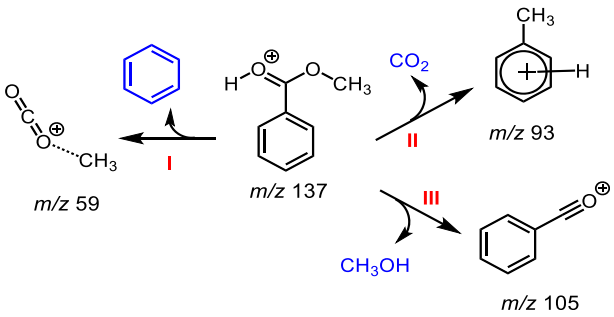

Abstract. Protonated methyl benzoate, upon activation, fragments by three distinct pathways. The *m/z* 137 ion for the protonated species generated by helium-plasma ionization (HePI) was mass-selected and subjected to collisional activation. In one fragmentation pathway, the protonated molecule generated a product ion of *m/z* 59 by eliminating a molecule of benzene (Pathway I). The *m/z* 59 ion (generally recognized as the methoxycarbonyl cation) produced in this way, then formed a methyl carbenium ion in situ by decarboxylation, which in turn evoked an electrophilic aromatic addition reaction on the benzene ring by a termolecular process to generate the toluenium cation (Pathway II). Moreover, protonated methyl benzoate undergoes also a methanol loss (Pathway III). However, it is not a simple removal of a methanol molecule after a protonation on the methoxy group. The incipient proton migrates to the ring and randomizes to a certain degree before a subsequent transfer of one of the ring protons to the alkoxy group for the concomitant methanol elimination. The spectrum recorded from deuteronated methyl benzoate showed two peaks at *m/z* 105 and 106 for the benzoyl cation at a ratio of 2:1, confirming the charge-imparting proton is mobile. However, the proton transfer from the benzenium intermediate to the methoxy group for the methanol loss occurs before achieving a complete state of scrambling.

Keywords: HepI, Helium-plasma ionization, Methyl benzoate, Fragmentation

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Introduction

Lewis acid-catalyzed electrophilic aromatic substitution is a classical reaction widely used in synthetic organic chemistry [1]. The reaction occurs via an initial adduct called the *Wheland* intermediate [2]. One of the simplest examples of this reaction is the generation of toluene from benzene under Friedel-Crafts conditions [3–5]. For this reaction, the electrophilic species, which is the methyl carbenium ion, is generated in situ and is added to the benzene ring to generate a toluenium ion [6, 7]. Analogous alkylations have been investigated in gas phase for many decades [8–16]. Some alkylation reactions have been conducted in gas phase with olefins and arenium ions generated by chemical ionization [17]. The mechanism of aromatic electrophilic substitution has been thoroughly scrutinized, and the role that arenium ions play as intermediates has been well established [18]. Particularly for gas-phase reactions, the intermediacy of complexes formed between arenium ions and neutral complexes has been well accepted [10, 19, 20]. The primary benefit of investigating ion–molecule interactions in the gas phase with mass-selected ions under mass spectrometric conditions is that such reactions can be thoroughly

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scrutinized without the impediments mediated by solvent and counterion effects [21–23]. As part of our pursuit on intricate details of gas-phase ion fragmentation mechanisms [24], we investigated protonation of methyl benzoate under helium-plasma ionization (HePI) conditions [25]. From the spectra recorded from protonated methyl benzoate, we noted a peak at *m/z* 93 for the toluenium ion that originates from an internal electrophilic aromatic substitution reaction.

Although chemical ionization of esters has been the subject of extensive studies [26, 27], detailed investigations have been carried out generally under negative-ion generating modes [28]. To rationalize the gas-phase dissociation of protonated esters, three major fragmentation pathways have been proposed [26]. Although the loss of an alcohol molecule by the acyl carbon-oxygen bond cleavage to generate an acylium cation is one of the major fragmentation pathways for most esters, it appears that for esters made from higher alcohols, the loss of an alkene molecule to form a protonated acid takes precedence. However, it is irrational to expect an alkene loss from methyl esters. In the third mechanism, the protonated ester eliminates the neutral acid and generates a carbocation. Such a mechanism is also not expected from methyl esters because the formation of a methyl carbenium ion is thermodynamically unfavorable. Thus, the second and third mechanisms do not contribute to fragmentation pathways of protonated methyl benzoate. In other words, the protonated methyl benzoate molecule neither fragments to generate protonated benzoic acid nor generates methyl cation by a direct cleavage mechanism; instead, it loses a molecule of methanol as envisaged by the first mechanism. However, we noted that it is not a simple and straightforward reaction. The results of our detailed study are presented here.

Experimental

Materials

All chemicals including methyl benzoate, [2,3,4,5,6-$^{2}H_5$]benzoic acid, [carboxyl-^{13}C]benzoic acid, methanol-d_4, D_2O (99.9 atom % D), and $H_2{}^{18}O$ (97 atom % ^{18}O) were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO) and used without further purification. Structures of all synthetic products were confirmed by mass spectrometry. EI mass spectra (70 eV) were recorded by GC–MS.

Mass Spectrometry

A Micromass Quattro Ultima triple-quadrupole tandem mass spectrometer (Manchester, UK) was used to obtain all collision-induced dissociation mass spectra. Gaseous ions from samples were generated by HePI procedures [25]. A stream (~30 mL min^{-1}) of high-purity helium (99.999%) was passed through the metal capillary held typically at about 3.6 kV. The source temperature was held at 100 °C. The hot (100 °C) desolvation gas (N_2), at a flow of 90 L/h, was used to heat the samples. Typically, the cone voltage for the ion transfer was set at 10 V. For CID experiments, the pressure of the argon in the collision cell was held at 1.16×10^{-4} mbar, and the laboratory-frame collision energy was kept at about 15 eV (unless otherwise stated). Liquid samples (about 1–5 μL) were mixed with silica gel (200 μm; 5 mg) and added to the bottom of a one-side-sealed glass tube (1.7 o.d. × 1.3 i.d. × 11.0 mm), and the tube was attached to the inner side of the cylindrical glass enclosure with the aid of a small wad of "museum putty." For gas-phase deuterium-exchange experiments, a cotton swab soaked in D_2O (20–30 μL) was inserted into the source enclosure through a hole in the front panel of the source. A stream of hot nitrogen (150 °C) via the "desolvation gas" line was deployed to facilitate the evaporation of D_2O from the cotton swab. Accurate mass product-ion spectra were recorded on a Synapt G2 HDMS (Waters Corp., MA) mass spectrometer equipped with a HePI source. Nitrogen was used as the nebulizer, desolvation, and cone gases. Mass calibration (*m/z* 10–1200) was performed using a solution of sodium formate (100 ppm). Mass spectra were acquired in the positive-ion mode over a range of *m/z* 10–150. The capillary voltage was set at 3.00 kV; sampling cone, 11.0 V; extraction cone, 1.5 V; desolvation-gas flow rate, 370 L/h; Vernier-probe adjuster position, 5.92 mm; trap collision energy, 2 eV; transfer collision energy, 11.8 eV. The source and desolvation-gas temperatures were held at 80 and 100 °C, respectively.

Chemical Synthesis

Synthesis of [$^{2}H_3$]Methyl Benzoate

Methanol-d_4 (5 μL) was mixed with benzoic acid (10 mg), dicyclohexylcarbodiimide (10 mg), and a catalytic amount of 4-dimethylaminopyridine in dichloromethane (1 mL) at room temperature. The mixture was heated at 50 °C for 1 h and flashed through a silica gel column with hexane/diethyl ether (5:5). 70-eV EI-MS, *m/z* (%) 139($M^{+\cdot}$, 41), 106(9), 105(100), 77(68), 51(42), 50(25).

Methyl [2,3,4,5,6 -$^{2}H_5$]Benzoate

To a solution of [2,3,4,5,6-$^{2}H_5$]benzoic acid (1.2 mg) in Et_2O (1 mL), methanol (5 μL) and *p*-toluene sulfonic acid (1 mg) were added, and the mixture was stirred overnight at 60 °C. After adding H_2O (1 mL), the reaction mixture was extracted three times with diethyl ether (5 mL × 3). The desired product was isolated as described above. 70-eV EI-MS, *m/z* (%) 141($M^{+\cdot}$, 38), 110(100), 82(66), 54(34).

Methyl [Carboxyl-^{13}C]benzoate

This compound was synthesized starting from [carboxyl-^{13}C]benzoic acid, by a procedure similar to that described for methyl [2,3,4,5,6 -$^{2}H_5$]benzoate. 70-eV EI-MS, *m/z* (%) 137($M^{+\cdot}$, 32), 106(100), 77(77), 51(37), 50(21).

[$^{18}O_2$]Benzoic Acid

To a solution of benzoic acid (1 mg) in ^{18}O-water (95%, 50 μL), concentrated H_2SO_4 (1 μL) was added, and the mixture was stirred overnight at 60 °C. The mixture was extracted three

times with Et_2O (0.5 mL × 3). The ether layers were separated, combined, and evaporated to obtain the desired product.

Methyl [Carbonyl-^{18}O]Benzoate

This compound was synthesized in a manner similar to that for methyl [2,3,4,5,6 -$^{2}H_5$]benzoate, starting from [$^{18}O_2$]benzoic acid. 70-eV EI-MS, *m/z* (%) 138($M^{+\cdot}$, 16), 137(4), 136(27), 108(4), 107(50), 106(7), 105(78), 77(100), 51(59), 50(33).

Computational Methods

All calculations were done using Gaussian 09 [29]. Geometries of all species involved in several possible pathways were fully optimized by using the widely used B3LYP [30, 31] method with a large 6–311++G(2d,2p) basis set. Frequency calculations were done at the same level to verify the nature of each stationary state on the potential energy surface, i.e., reactants/products are associated with all positive vibrational frequencies, and transition state (TS) is associated with only one imaginary frequency, for which the normal vibrational mode corresponds to the expected bond formation/breaking movements in a specific reaction pathway. Gibbs free energies were calculated at room temperature and ambient pressure.

Results and Discussion

Helium-plasma ionization (HePI) provides a potent way of forming protonated molecules even from gaseous substances [25]. For example, methyl benzoate vapor when subjected to HePI generates an ion of *m/z* 137 for the protonated species (Fig. 1a). The determination of the precise protonation sites of gas-phase aromatic molecules has attracted considerable attention of researchers for many years [32–36]. Thus, the first question we wanted to address in this study was the initial protonation site of methyl benzoate.

In addition to the four different ring sites, there are two other positions in methyl benzoate that could undergo protonation (Table 1). The relative Gibbs free energy values calculated for the optimized structures for the putative positive ions showed that the carbonyl-oxygen is the thermodynamically most favorable protonation site (Table 1).

The computational results predicted that the carbonyl-oxygen to be the most favorable site to accept the incipient proton. It also indicated that the protonation on carbonyl-oxygen lengthens the C=O double bond to 1.293 Å with a concomitant shortening of the $C^{carboxyl}$—O^{alkoxy} single bond to 1.268 Å (Supporting Information, Fig. S1a). Moreover, once protonated on the carbonyl-oxygen, the carboxylate group is positioned closer to the phenyl ring to maximize intramolecular interactions. The calculation results also showed that the protonation at the alkoxy oxygen site leads to an immediate dissociation of the bond between this oxygen atom and the carbonyl-carbon since the bond length is greatly elongated to 2.043 Å (Fig. S1b). Thus, the molecule should immediately lose methanol to form the *m/z* 105 species, upon protonation on the methoxy group. Moreover, the high Gibbs free energies obtained for the four ring-protonated isomers indicate that direct ring protonation is disfavored, which agrees with the experimental observation that protonated methyl benzoate when exposed to D_2O vapor undergoes essentially only one H/D exchange (Fig. 1a, b). It is well known that the benzenium ion rapidly interacts with water [21, 33, 37–39]. In other words, if the charge-imparting proton migrates rapidly to the ring to form a benzenium ion, more than one H/D exchanges are expected when methyl benzoate is exposed to D_2O in a HePI source. For example, such a tendency is witnessed with protonated methyl phenylacetate, which undergoes at least 3–4 H/D exchanges under similar ion-source conditions (Fig. 1a, d). Proton shuttling mechanisms mediated by water within the timeframe of acquiring mass spectra have been well investigated [40, 41]. The suggestions we made on charge-imparting proton migrations are supported by our computational results which indicated that the ring protonation in methyl phenylacetate is not as prohibitive as that observed for methyl benzoate. For methyl benzoate, a protonation at any ring site of the molecule increases its relative energy to a higher level than that predicted for the alkoxy protonation. Thus, it is not surprising that protonated methyl phenylacetate undergoes more than one H/D exchanges because a protonation on any ring carbon of methyl phenylacetate is favored over a protonation on its alkoxyl oxygen atom (Table 1). Moreover, the Gibbs free energy barrier that the proton must surmount for a transfer from the carbonyl-oxygen site of methyl phenylacetate to the ring ortho position via the six-membered transition state (TS 2a-2c2) is relatively low (5.76 kcal/mol) compared to 37.18 kcal/mol required to reach the TS 1a-1c2 transition state of protonated methyl benzoate (Fig. S2).

The mass-selected *m/z* 137 ion, upon collisional activation, fragments by several different pathways. The base peak of the spectrum was observed at *m/z* 59 (Fig. 2a). For the formation of the *m/z* 59 ion, a molecule of benzene must be eliminated from the protonated precursor molecule. Accurate mass measurements confirmed the elementary composition of *m/z* 59 ion to be $C_2H_3O_2$ (Fig. S3). Spectra recorded from protonated methyl [*carboxyl*-^{13}C]benzoate (Fig. 2b), methyl [*carbonyl*-^{18}O]benzoate (Fig. 2c), [$^{2}H_3$]methyl benzoate (Fig. 2e), and methyl [2,3,4,5,6-$^{2}H_5$]benzoate (Fig. 2f) showed peaks at *m/z* 60, 61, 62, and 59, respectively, which indicated that the carbonyl-carbon and all the three hydrogen atoms on the methyl group are retained when the *m/z* 59 product ion is formed. Furthermore, gas-phase H/D exchange experiments showed that the incipient proton that bestows the charge to the molecule is specifically lost with the elimination of the benzene molecule (Fig. 2d). Based on this supportive data, the elemental composition of the *m/z* 59 ion was further confirmed to be $C_2H_3O_2$. The *m/z* 59 ion is a frequently encountered fragment in the mass spectra of carboxylic compounds [42]. Many familiar, and some unfamiliar, isomeric structures are possible for this *m/z* 59 ion [43–46]. The structures of isomeric $[C_2H_3O_2]^+$ ions have been investigated extensively using computational methods [44, 47]. Blanchette et al. considered 11 isomeric

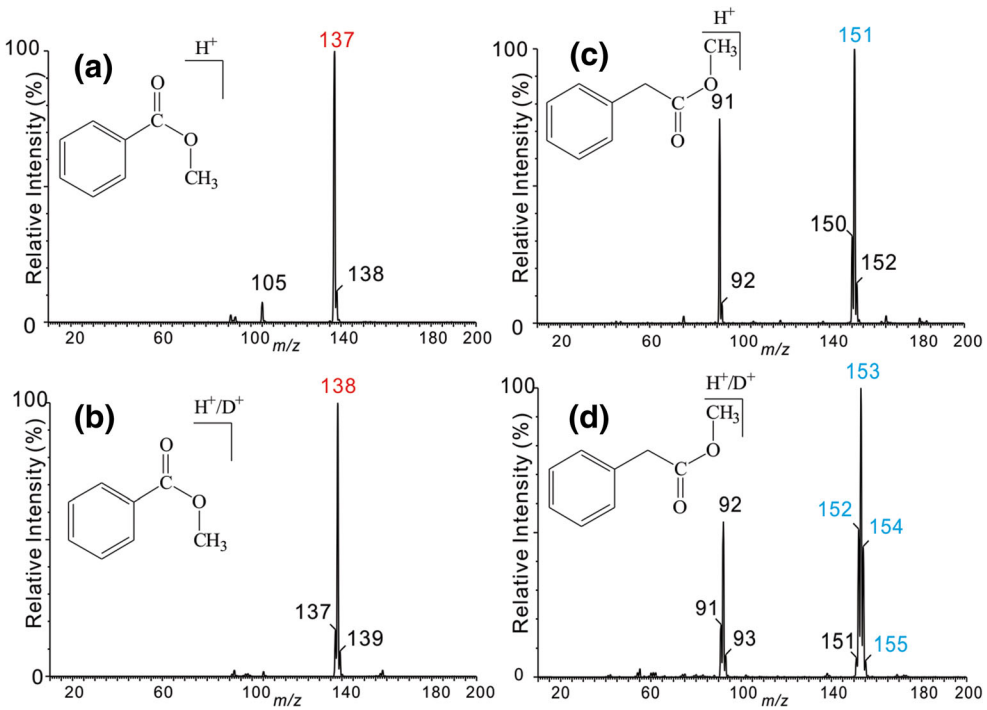


Figure 1. A unit-mass resolution MS^1 spectra recorded from samples of methyl benzoate (**a**) and methyl phenylacetate (**c**) placed in the HePI source, at a cone voltage setting of 10 V. After 10 s of data acquisition, a cotton swab soaked in D_2O was introduced to the enclosed HePI source and spectra were re-recorded (**b**, **d**)

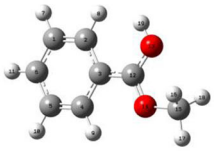
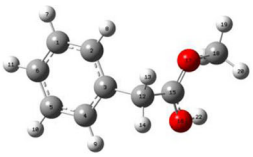
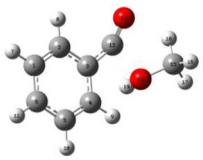
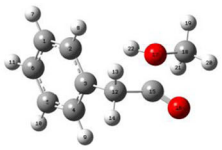
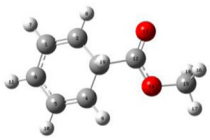
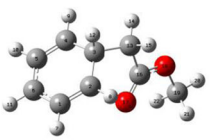
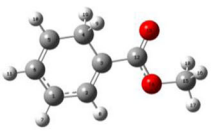
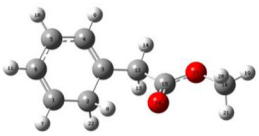
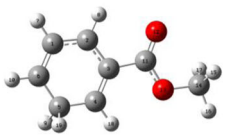
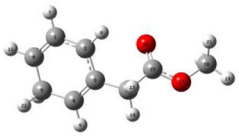
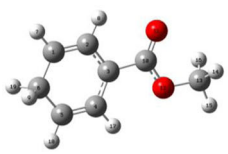
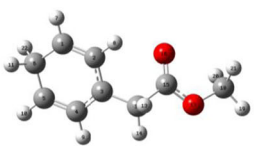
structures and concluded that the methoxycarbonyl cation to be the thermodynamically most stable species [44].

The most interesting peak in the product–ion spectrum of protonated methyl benzoate is that observed at *m/z* 93. To generate an *m/z* 93 ion, a molecule of carbon dioxide should be eliminated from the precursor *m/z* 137 ion. The loss of CO_2 was confirmed by the spectra acquired from isotope-labeled methyl benzoates. The spectra recorded from protonated methyl [*carbonyl*-^{18}O]benzoate (Fig. 2c) or methyl [*carboxyl*-^{13}C]benzoate (Fig. 2b) showed a peak at *m/z* 93 corresponding to a loss of $CO^{18}O$ or $^{13}CO_2$, respectively. This result indicated that the atoms required for the loss of a 44-Da neutral molecule from protonated methyl benzoate are the carbonyl-carbon atom and the two oxygen atoms.

To eliminate a carbon dioxide molecule from the precursor ion, the methyl group must be transferred to the phenyl ring by a specific rearrangement. To rationalize the formation of both the methoxycarbonyl (*m/z* 59) and the $C_7H_9^+$ *m/z* 93 cations, we propose the fragmentation mechanism illustrated in Scheme 1. Upon activation of the precursor ion, the charge-imparting proton attached to the carbonyl-oxygen (1a) is initially transferred to the *ortho*-ring position via a five-membered transition state (TS 1a-1c2) (Fig. 3). The proton is then relocated to the *ipso*-ring carbon, and the intermediate formed in this way (1c1) can then undergo fragmentation by two channels (Scheme 1). In one pathway, the bond between *ipso*-ring carbon and the carbonyl-carbon elongates and breaks leading to the formation of the *m/z* 59 ion by losing a benzene molecule. Alternatively, the methoxycarbonyl cation (*m/z* 59) and the benzene molecule generated by the initial fragmentation can remain associated as a transient termolecular complex (TS 1c1-4). The methyl cation in the complex, then acts as an electrophilic reagent and attacks the benzene ring to generate the *m/z* 93 toluenium cation (Scheme 1). Such addition products are not usually observed in low-pressure collision cells. However, Giacomello and Pepi [11] had demonstrated that when benzene is exposed to the methoxycarbonyl cation generated from the dissociation of methyl chloroformate in an ICR cell at relatively high pressure (0.2–0.3 Torr), an electrophilic reaction takes place to generate an *m/z* 93 ion. Herein, we have shown that a similar reaction takes place intramolecularly in which the methoxycarbonyl cation acts as a methylcarbenium ion donor in a manner that resembles the well-known Friedel–Crafts substitution mechanism [3, 5].

Gibbs free energy calculations indicated that the energy demand for the generation of the *m/z* 59 ion (39.4 kcal mol^{-1}) is only slightly higher than that needed for the *m/z* 93 ion formation (TS 1c1-4, 39.07 kcal mol^{-1}) (Fig. 3). At a first glance, this result appeared to be incongruent with the experimental result that the intensity of *m/z* 93 peak is much weaker than that at *m/z* 59. However, because the *m/z* 93 ion can further fragment to form *m/z* 91 and 77 ions [49, 50], it is evident that the intensity of *m/z* 93, 91, and 77 peaks taken together is similar to that of the *m/z* 59 ion. In fact, this result is in agreement with the observation that the *m/z* 93 ion is formed at lower collision energies than those needed for the *m/z* 59 ion generation because the formation energy for *m/z* 93 ion is somewhat lower than that of the *m/z* 59 ion (Fig. S4).

Table 1. Optimized structures and relative Gibbs free energies (in kcal/mol) of methyl benzoate and methyl phenylacetate protonated at different sites, computed for 298.15 K and 1 atm by the density functional theory method B3LYP using a 6-311++G(2d,2p) basis set

	Optimized structures and computed relative Gibbs free energies (in kcal/mol)	
Protonation site	**$PhCO_2Me$**	**$PhCH_2CO_2Me$**
Carbonyl oxygen	0.00  **1a**	0.00  **2a**
Alkoxy oxygen	17.40  **1b**	11.02  **2b**
Ph-1 (*ipso carbon*)	27.78  **1c1**	12.66  **2c1**
Ph-2 (*ortho carbon*)	23.41  **1c2**	4.67  **2c2**
Ph-3 (*meta carbon*)	23.66  **1c3**	8.86  **2c3**
Ph-4 (*para carbon*)	24.38  **1c4**	6.34  **2c4**

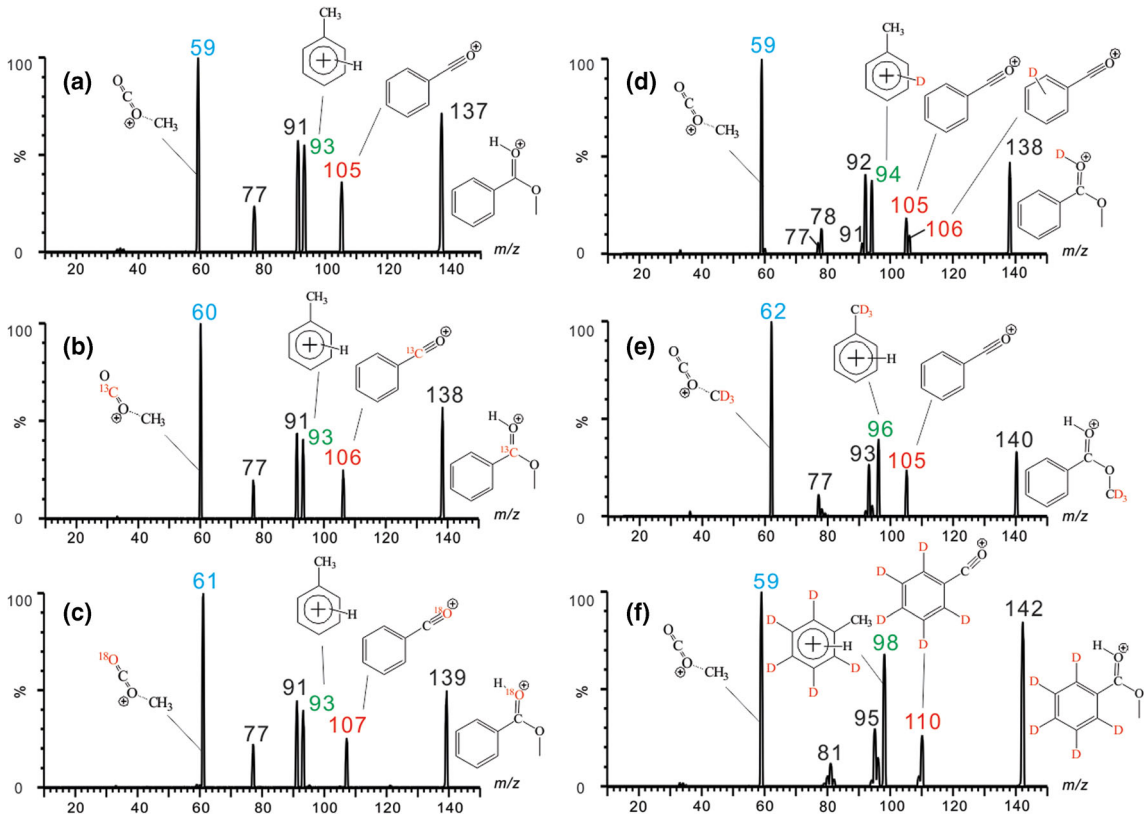
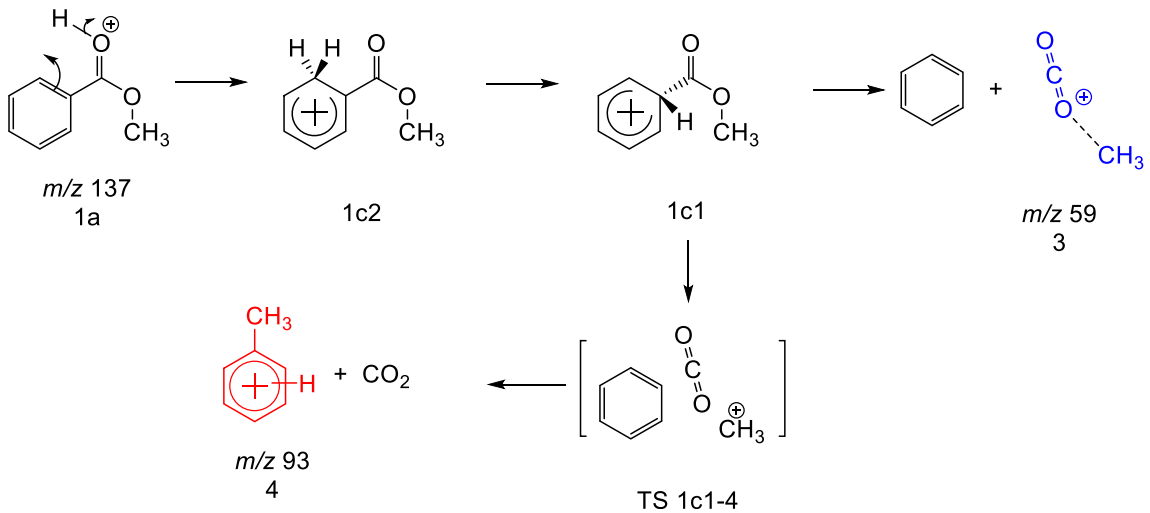


Figure 2. Unit-mass resolution product-ion spectra of protonated (**a**) methyl benzoate, (**b**) methyl [*carboxyl*-^{13}C]benzoate, (**c**) methyl [*carbonyl*-^{18}O]benzoate, (**d**) deuteronated methyl benzoate (generated by exposing methyl benzoate to D_2O vapor in the source; *m/z* 138), and those of (**e**) protonated [$^2H_3$]methyl benzoate and (**f**) protonated methyl [2,3,4,5,6-$^2H_5$]benzoate at a laboratory-frame collision energy settings of 15 eV, recorded on a Quattro Ultima mass spectrometer

The CID spectrum of protonated methyl benzoate also shows a prominent peak at *m/z* 105 (Fig. 2a), which represents a methanol loss. The fragmentation pathway leading to this apparently simple elimination is however not a straightforward reaction. If the charge-imparting proton is simply removed together with the methoxy group without the intervention of a ring intermediate, then a CH_3OD molecule is expected to be removed directly from deuteron-charged methyl benzoate. However, the spectrum recorded from the mass-selected monodeuterio isotopologue of protonated methyl benzoate (*m/z* 138) showed two peaks of 2:1 relative intensity at *m/z* 105 and 106 (Fig. 2d). Moreover, the spectrum recorded from *m/z* 142 ion of protonated methyl [2,3,4,5,6-$^2H_5$]benzoate showed two peaks at *m/z* 109 and



Scheme 1. Proposed fragmentation pathway for the formation of Toluenium ions (*m/z* 93) and *m/z* 59 methoxycarbonyl cation

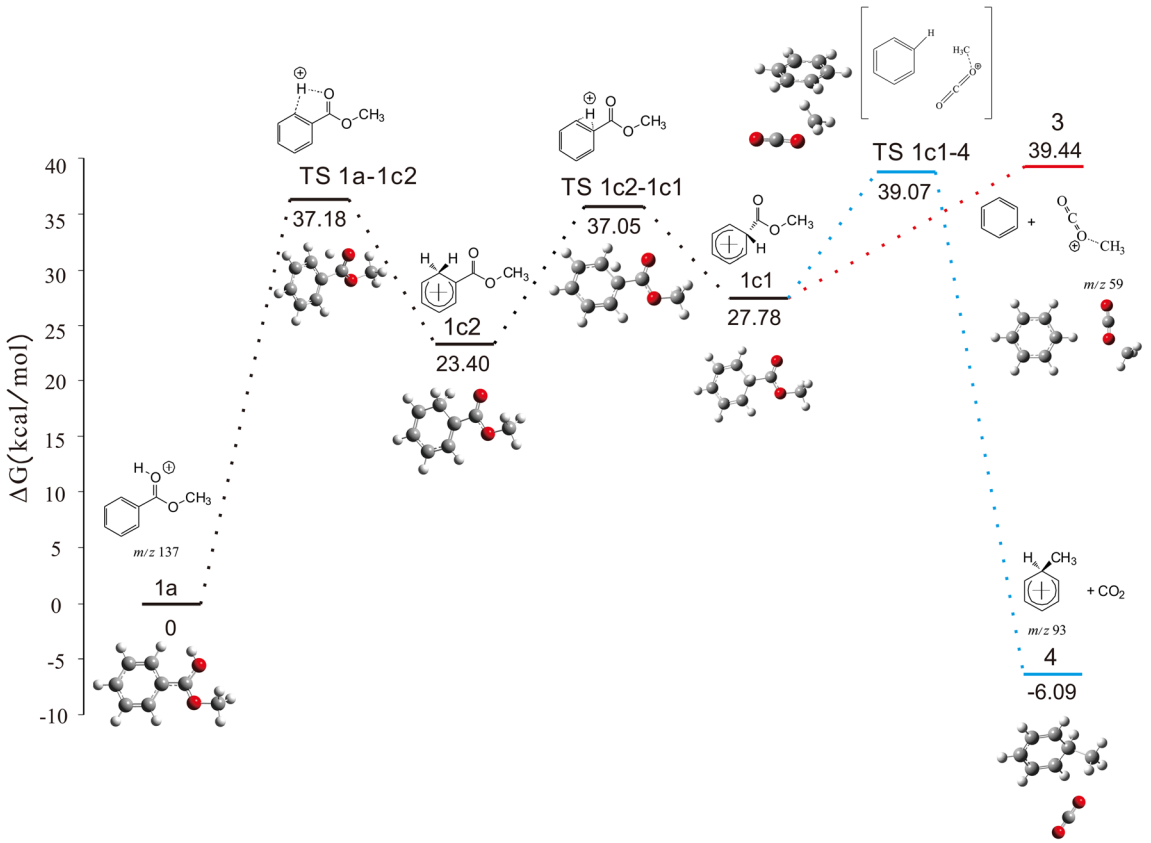
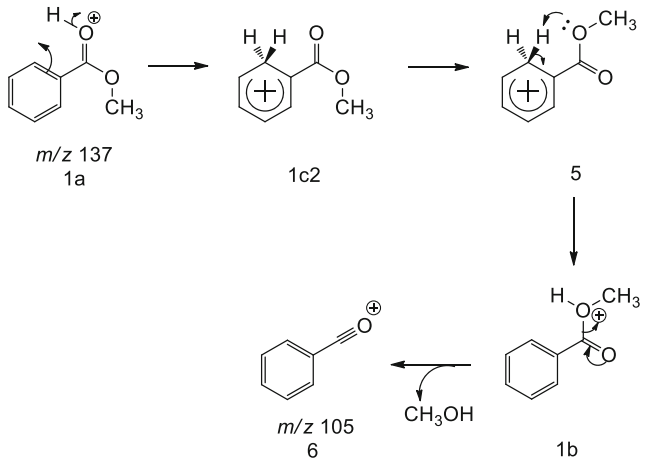


Figure 3. Relative Gibbs free energies [in kcal/mol computed for 298.15 K and 1 atm by the density functional theory method B3LYP using a 6-311+ +G(2d,2p) basis set] and molecular structures of energy-optimized product ions and transition states associated with the generation of *m/z* 59 and 93 ions from protonated methyl benzoate

110 (Fig. 2f). These results confirmed that the methanol loss is not strictly confined to the transactions that take place at the peripheral methoxycarbonyl functionality. The charge-imparting proton ambulates to the ring and randomizes before a subsequent transfer of one of the ring protons to the methoxy group for the concomitant methanol elimination. Previously, we observed an analogous methanol loss from protonated methyl dihydrocinnamate [47]. However, the benzenium ion intermediate generated from protonated methyl dihydrocinnamate underwent near statistical randomization before the subsequent transfer of one of the ring protons to the methoxy group. Not only the overall intermolecular H/D exchange process of protonated methyl benzoate, but also the intramolecular scrambling of the protons in the benzenium ion intermediates is sluggish compared to those processes of methyl dihydrocinnamate. If there were a complete scrambling after the charge-imparting proton is transferred, then the *m/z* 105:106 peak ratio in Fig. 2d should have been 1:5. The randomization process is known to proceed by a "ring walk" mechanism in protonated methyl benzoate, which is a slow process compared to that of protonated benzene, which is more-or-less instantaneous [33–38]. According to Kuck [21], the hydrogen ring walk in substituted benzenium ions is markedly affected by the nature of the ring substituents. Apparently, the methoxycarbonyl group does not accelerate the randomization process. Various types of scrambling mechanisms in gaseous ions have been reviewed by Kuck [48].

The mechanism we propose to rationalize the methanol loss is illustrated in Scheme 2. Prior to the elimination of a molecule of methanol, the incipient proton on the carbonyl group is transferred to the ring via a five-membered transition state (TS 1a-1c2) (Fig. S5). However, before the protons attain a completely randomized state in the substituted benzenium ion 1c2, the methoxycarbonyl group pivots and forms another intermediate (5). A proton is then transferred from the benzenium intermediate 5 to the methoxy oxygen (5 → 1b; Scheme 2). Because a protonation of the methoxy oxygen is dissociative, it leads to an immediate cleavage of the bond between this oxygen and the carbonyl-carbon. The proposed mechanism is supported by computational data which indicated an elongation of the bond between carbonyl-carbon and methoxy oxygen upon protonation of the methoxy group 2.043 Å (Fig. S1b).

From the spectra recorded from the isotope-labeled compounds, it is evident that the incoming proton is preferentially eliminated during the methanol loss process. The spectra recorded for protonated methyl [2,3,4,5,6-$^{2}H_5$]benzoate (Fig. 2f) and methyl benzoate exposed in D_2O vapor (Fig. 2d) showed that loss of CH_3OH and CH_3OD, respectively, clearly prevail.



Scheme 2. Proposed fragmentation pathways for the formation of benzoyl cation by methanol loss from protonated methyl benzoate

Incorporation of a hydrogen or a deuterium atom from the phenyl ring provides only a minor contribution to the methanol loss in both cases. Thus, the rate of proton back transfer from the *ortho*-carbon to the methoxy group (5) must be faster than the randomization rate of ring hydrogens in the benzenium ion intermediate 1c2. In addition, the spectrum acquired from protonated methyl [*carbonyl*-^{18}O]benzoate (Fig. 2c) showed only one peak at *m/z* 107 for the specific loss of [^{16}O]methanol originating exclusively from the methoxy group. In other words, this observation shows that scrambling between the oxygen atoms of methoxy and carbonyl groups does not occur in protonated methyl benzoate.

Our proposed fragmentation mechanism was also supported by Gaussian calculation results. Computations suggest that the energy demand for the generation of the *m/z* 105 ion is higher than that needed for the *m/z* 59 ion formation by 6.08 kcal mol^{-1} (TS 1c2-5, 45.52 kcal mol^{-1}) (Fig. S5). This result agrees with the experimental data that the intensity of *m/z* 59 is about two to three times higher than that of the *m/z* 105 ion (Fig. 2a and S4).

The spectrum of protonated methyl benzoate shows also a peak at *m/z* 77 for the phenyl cation, which could originate from two different pathways. Either the benzoyl cation formed by the methanol loss could eliminate a carbon monoxide molecule, or the toluenium cation formed by the CO_2 loss could lose a molecule of methane to form the *m/z* 77 ion.

If the benzoyl cation is the sole precursor for the phenyl cation, then the relative intensity ratio of *m/z* 105 and 106 peaks from the product-ion spectrum of the *m/z* 138 ion generated from methyl benzoate species exposed to D_2O vapor (Fig. 2d) should be reflected on those obtained for the *m/z* 77 and 78 peaks. However, the relative intensity ratio of *m/z* 105 and 106 peaks is about 2:1 and the relative intensity ratio of *m/z* 77 and 78 peaks is 1:2. It is well known that a loss of methane from toluenium ion also lead to the formation of the phenyl cation [9, 49]. Our spectrum for protonated [^{2}H$_3$]methyl benzoate

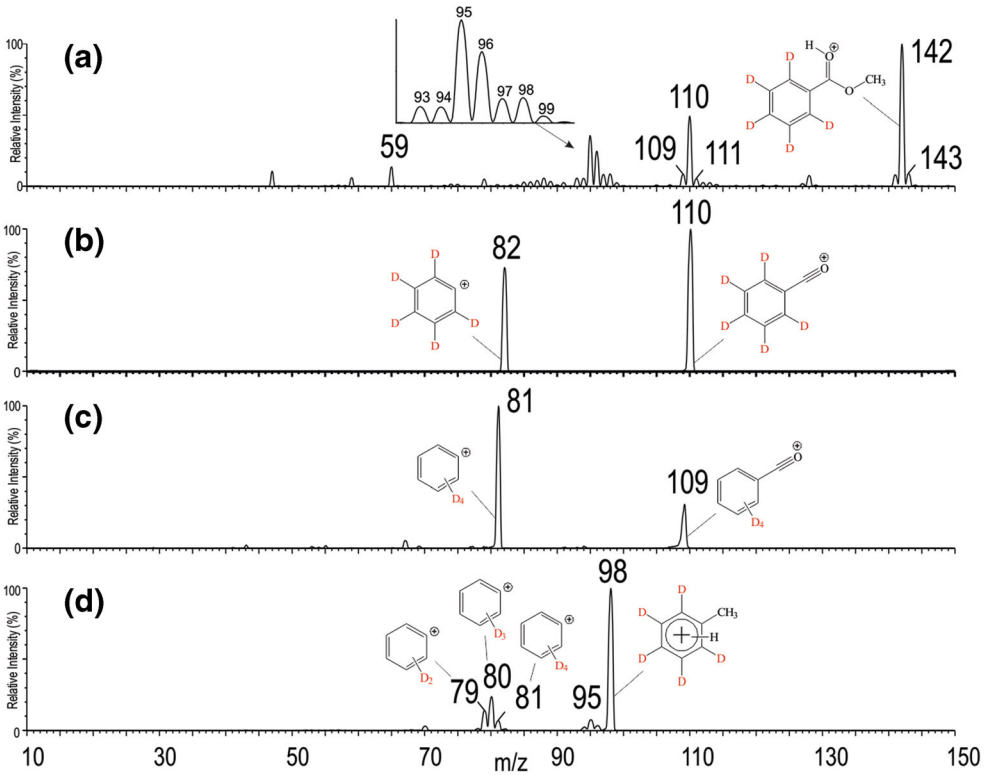


Figure 4. A unit-resolution MS^1 spectrum recorded from a sample of methyl [2,3,4,5,6-^{2}H$_5$]benzoate placed in the HePI source at a cone voltage setting of 98 V (**a**), and collision-induced (15 eV) product-ion spectra (MS^2) of *m/z* 110 (**b**), *m/z* 109 (**c**), and *m/z* 98 (**d**) ions generated from protonated methyl [2,3,4,5,6-^{2}H$_5$]benzoate by in-source fragmentation at a cone-voltage setting of 11, 17, and 11 eV, respectively

(Fig. 2e) corroborates the generalization that the methyl group and one hydrogen atom in the aromatic ring are eliminated for the methane loss. Because the hydrogens in methyl group also can undergo scrambling with the ring hydrogens, a series of peaks for phenyl cations with different numbers of deuterium atoms were noted from the spectra of protonated [$^2H_3$]methyl benzoate (Fig. 2e) and methyl [2,3,4,5,6-$^2H_5$]benzoate (Fig. 2f). Moreover, the product-ion spectrum of pentadeuteriotoluenuim ion (*m/z* 98, Fig. 4d), which was generated in the ion source from protonated methyl [2,3,4,5,6-$^2H_5$]benzoate by in-source fragmentation, showed peaks at *m/z* 79, 80, and 81 for CHD_3, CH_2D_2, and CH_3D losses, respectively. This result confirmed that the ring hydrogens can undergo scrambling with the methyl group hydrogens before the methane loss occurs. Analogously, the peaks at *m/z* 78 and 79 in the spectrum of protonated [$^2H_3$]methyl benzoate (Fig. 2e) correspond specifically to this methane loss mechanism.

Furthermore, the spectrum of protonated methyl benzoate also shows an intense peak at *m/z* 91, which can be attributed to the well-known dihydrogen loss from the toluenium ion (Fig. 2a) [9, 49, 50]. As reported by Kuck et al. [49], the methyl hydrogen atoms do not scramble completely with the ring hydrogens before H_2 or CH_4 is eliminated. Our experimental results from protonated [$^2H_3$]methyl benzoate (Fig. 2e) and methyl [2,3,4,5,6-$^2H_5$]benzoate agree well with the Kuck's mechanism.

Experimental data acquired from protonated methyl [*carbonyl*-^{18}O]benzoate (Fig. 2c) and methyl [*carboxyl*-^{13}C]benzoate (Fig. 2b) confirmed the origins of the carbon and oxygen atoms for the carbon monoxide loss because each spectrum showed only one peak representing the $C^{18}O$ or ^{13}CO loss, respectively. With this CO-loss mechanism, it is obvious that among all four phenyl cation products (*m/z* 79, 80, 81, and 82, Fig. 2f) of protonated methyl [2,3,4,5,6-$^2H_5$]benzoate, only the *m/z* 81 and 82 ions originate from a 28-Da neutral loss from the precursor *m/z* 109 and 110 ions. This hypothesis was supported by the product-ion spectra of *m/z* 110, 109 ions (Fig. 4b, c), which were generated in-source from protonated methyl [2,3,4,5,6-$^2H_5$]benzoate. Each respective spectrum showed only one peak either at *m/z* 82 or 81. Analogously, in the three-peak cluster for phenyl cations in the spectrum of protonated [$^2H_3$]methyl benzoate (Fig. 2e), only that at *m/z* 77 represents the CO loss from the benzoyl cation.

Conclusions

In this research, we have provided an example of an internal methylation reaction of benzene during the fragmentation of protonated methyl benzoate. For the formation of *m/z* 93 ion, protonated methyl benzoate undergoes a decarboxylation through a complex formed between a benzene molecule and a methoxycarbonyl cation (*m/z* 59). Also, we proposed a flip-flop fragmentation pathway to rationalize the methanol loss. For this pathway, the incipient proton initially attached to the carbonyl-oxygen ambulates to the phenyl ring and then relocates to the methoxy group to be eliminated as methanol.

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