RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2010; **24**: 1707–1716
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.4569

RCM

Gas-phase formation of protonated benzene during collision-induced dissociation of certain protonated mono-substituted aromatic molecules produced in electrospray ionization

Min Li*, Mingxiang Lin and Abu M. Rustum
Global Quality Services – Analytical Sciences, Merck & Co. Inc., 1011 Morris Ave, Union, NJ 07083, USA

Received 23 February 2010; Revised 3 April 2010; Accepted 5 April 2010

Protonated benzene, $C_6H_7^+$, has been studied extensively to understand the structure and energy of a protonated organic molecule in the gas phase. The formation of $C_6H_7^+$ is either through direct protonation of benzene, i.e., chemical ionization, or through fragmentation of certain radical cations produced from electron ionization or photon ionization. We report a novel observation of $C_6H_7^+$ as a product ion formed in the collision-induced dissociation (CID) of protonated benzamide and related molecules produced via electrospray ionization (ESI). The formation of $C_6H_7^+$ from these even-electron precursor ions during the CID process, which has not been previously reported, is proposed to occur from the protonated molecules via a proton migration in a five-membered ring intermediate followed by the cleavage of the mono-substituent C–C bond and concurrent formation of an ion-molecule complex. This unique mechanism has been scrutinized by examining some deuterated molecules and a series of structurally related model compounds. This finding provides a convenient mean to generate $C_6H_7^+$, a reactive intermediate of considerable interest, for further physical or chemical investigation. Further studies indicate that the occurrence of $C_6H_7^+$ in liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) appears to be a rather common phenomenon for many compounds that contain 'benzoyl-type' moieties. Hence, the observation of the $C_6H_7^+$ ion in LC/ESI-MS/MS can be used as an informative fragmentation pathway which should facilitate the identification of a great number of compounds containing the 'benzoyl-type' and similar structural features. These compounds are frequently present in food and pharmaceutical products as leachable impurities that require strict control and rapid elucidation of their identities. Copyright © 2010 John Wiley & Sons, Ltd.

Protonated benzene, $C_6H_7^+$, is a prototype organic cation that has been studied extensively as a model to understand the structure and energy of a protonated organic molecule in the gas phase.[1] The formation and structure, as well as the reactivity and thermodynamic properties, of $C_6H_7^+$ have been investigated both experimentally and theoretically.[2–10] The formation of $C_6H_7^+$ is reported to occur either through direct protonation of benzene via chemical ionization or through fragmentation of certain radical cations produced from electron ionization or photon ionization. For instance, $C_6H_7^+$ can be generated from gas-phase proton transfer from CH_5^+ and $C_2H_5^+$ to benzene,[2] i.e., chemical ionization of benzene. Protonated benzene can also be produced as a fragment ion from the electron ionization (EI) of *O*-toluidine and *N*-methylaniline,[3] or benzylamine.[4] Recently, $C_6H_7^+$ was observed in the product ions of an EI-generated phenylarsenium radical cation through mass-analyzed ion kinetic energy (MIKE) spectrometry and collision-induced dissociation (CID).[11] The $C_6H_7^+$ ion could also be formed from the photon ionization of carboxymethyl cyclohexa-2,5-diene.[5] Upon its formation, $C_6H_7^+$ can be mass-selected and studied by various spectroscopic techniques or allowed to react with different molecules in the gas phase to probe its structure and reactivity. Nevertheless, $C_6H_7^+$ has never been observed as a product ion in LC/ESI-MS/MS, despite the widespread use of the technique. We report a novel observation of $C_6H_7^+$ from the CID of protonated benzamide (and related compounds), an even-electron precursor ion produced via electrospray ionization (ESI) in a typical LC/ESI-MS/MS experiment. The most plausible formation mechanism of $C_6H_7^+$ is proposed; the results obtained from various experiments, including deuterium labeling of the benzamide molecule and examination of the fragmentation of a series of model compounds that are structurally related to benzamide, are consistent with the proposed mechanism.

**Correspondence to*: M. Li, Global Quality Services – Analytical Sciences, Merck & Co. Inc., 1011 Morris Ave, Union, NJ 07083, USA.
E-mail: minli88@yahoo.com

RCM

EXPERIMENTAL

Materials

All chemicals except *N*-cyclohexylbenzamide were obtained from Sigma-Aldrich (St. Louis, MO, USA). *N*-Cyclohexylbenzamide-d_5 was prepared by mixing equal molar amounts of cyclohexylamine, benzoyl-d_5 chloride and triethylamine in methylene chloride at room temperature; the reaction was completed instantly.

Mass spectrometric analysis

Mass spectrometric analysis was primarily performed on a Waters (Milford, MA, USA) Q-Tof Premier quadrupole time-of-flight mass spectrometer operating in electrospray positive ion mode. Analyte solutions were prepared in 50:50 methanol and water mixture, while deuterated compounds were dissolved in 50:50 deuterated methanol (CH_3OD) and deuterated water (D_2O). Sample solutions were infused via the embedded syringe pump at a flow rate of 5 μL/min into the ESI source. The ESI source was operated with the following parameters: electrospray voltage 3.5 kV, cone voltage 35 V, source temperature 100°C, desolvation temperature 250°C, cone gas flow rate 60 L/h and desolvation gas flow rate 600 L/h. The TOF mass analyzer was operated in V mode at a rate of 1 scan/s and 0.1 s inter-scan time. Mass calibration was performed using a sodium cesium iodide solution. For MS/MS experiments, the quadrupole mass selection window width was set to 1 *m*/*z* unit and the lowest mono-isotopoic peaks of appropriate analyte ions were selected as precursor ions. Argon was used as the collision gas and the collision energy was set to 10 eV for most MS/MS experiments unless indicated otherwise. When in-source fragmentation was needed, the cone voltage was increased to 50 V to induce additional fragmentation in the source region. To evaluate the impact of the collision gas and to compare the fragmentation patterns between different types of instruments, a few selected MS/MS experiments were also carried out on a ThermoScientific (San Jose, CA, USA) LTQ linear ion trap mass spectrometer. The ESI source of the LTQ was operated with the ESI voltage set to 4.0 kV and the capillary temperature set to 300°C. The MS/MS data were collected with an isolation width of 1 *m*/*z* unit. Helium was used as the collision gas and the normalized collision energy (collision energy normalized across the mass range scanned) was set to 35%. The data presented in the following Results and Discussion section were acquired from the Q-Tof mass spectrometer unless indicated otherwise.

RESULTS AND DISCUSSION

Formation of protonated benzene, $C_6H_7^+$ (*m*/*z* 79), from benzamide and a proposed formation mechanism

CID of protonated benzamide (*m*/*z* 122) yielded three major product ions: *m*/*z* 105, 79 and 77 (Fig. 1(a)). The structures of benzamide and all other molecules examined in this study are summarized in Table 1, along with the assignments of the product ions observed from the CID of each precursor ion. Proposed fragmentation pathways leading to the formation of the three major product ions from protonated benzamide are given in Scheme 1. The formation of the ions at *m*/*z* 105 and 77 can be readily explained. The protonated benzamide (**I**) can easily lose NH_3 at elevated collision energy, resulting in the *m*/*z* 105 ion ($C_6H_5CO^+$). The latter ion can further undergo a decarbonylation process to form the phenyl cation $C_6H_5^+$, the *m*/*z* 77 ion (pathway a, Scheme 1). On the other hand, the occurrence of the *m*/*z* 79 ion as a major product ion appeared somewhat puzzling initially, as there have been no reports of such an event in the CID of an even-electron precursor ion. After carefully reviewing all the data, it occurred to us that *m*/*z* 79 might be protonated benzene, $C_6H_7^+$. The formation of $C_6H_7^+$ cannot be explained by a single bond cleavage from protonated benzamide (**I**). To explain this unusual formation, a novel mechanism starting from a five-membered ring intermediate (**Ia**) was proposed (pathway b): upon activation by CID, one proton transfers from the protonated amide nitrogen to the *ortho*-position of the benzene ring. This is followed by cleavage of the C–C bond between the carbonyl and the benzene ring, resulting in the formation of an ion-molecule complex (**I_b**) consisting of a neutral benzene molecule and a $O{=}C{=}NH_2^+$ ion (Scheme 1). This [benzene·$O{=}C{=}NH_2^+$] complex can be regarded as a proton-bound dimer of benzene and isocyanic acid (O=C=NH), which could have three outcomes: (1) it can revert back to **Ia**; (2) it can dissociate with a proton transfer from $O{=}C{=}NH_2^+$ to benzene, producing protonated benzene (*m*/*z* 79) and neutral isocyanic acid; and (3) it can dissociate without proton transfer, leading to the formation of protonated isocyanic acid $O{=}C{=}NH_2^+$ (*m*/*z* 44) and neutral benzene.

Design of various experiments to verify the proposed mechanism for the formation of protonated benzene

In order for the proposed mechanism to be operative, two requirements must be met: (1) there should be a hydrogen atom available on the amide nitrogen to migrate as a proton to the *ortho*-position of the benzene ring, and (2) the benzene formed in the complex **I_b** should be able to abstract a proton from $O{=}C{=}NH_2^+$. Thus, the end result is that two protons are transferred from the amide nitrogen to form the resulting $C_6H_7^+$. To verify this hypothesis, deuterium labels were introduced on the benzene ring and the amide nitrogen, respectively, in order to examine if proton transfer in the five-membered ring intermediate did indeed occur during the CID of protonated benzamide. In addition, the fragmentation behavior of three groups of structural analogs of benzamide was examined to probe the impact of different functional groups in benzamide on the formation of protonated benzene. First, *N*,*N*-dimethylbenzamide that contains no amide hydrogen was examined; in this case, no hydrogen is available for the initial proton migration. Hence, formation of protonated benzene would not be expected from *N*,*N*-dimethylbenzamide. Second, the oxygen in the benzamide carbonyl group was replaced with other heteroatoms (X) to assess the impact of the electron-withdrawing capability of the C=X bond on the formation of the critical five-membered ring intermediate (**Ia**). Third, different substituents were introduced into the benzene ring to modify the proton affinity of the benzene moiety and thus to evaluate its impact

DOI: 10.1002/rcm

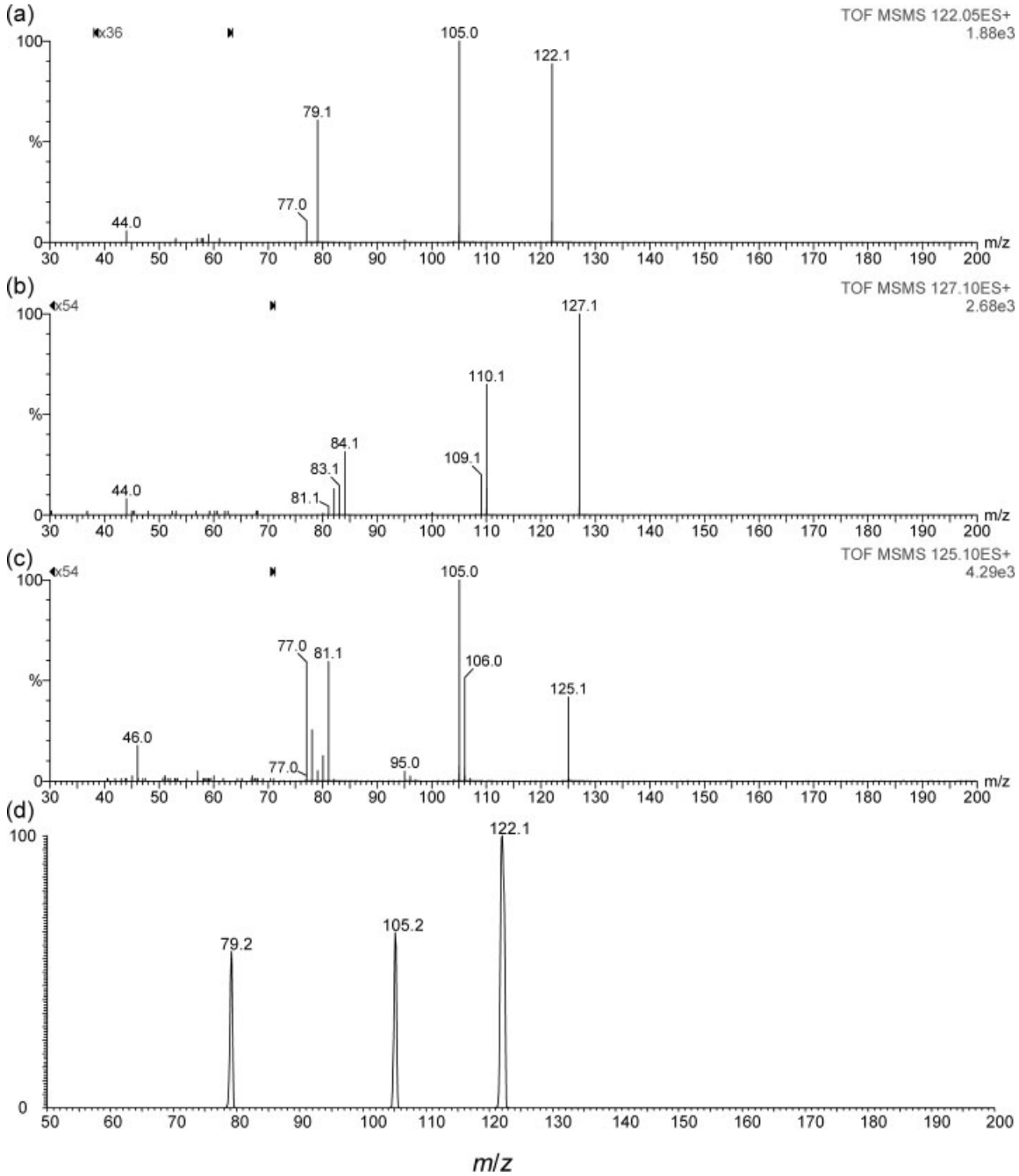


Figure 1. Collision-induced dissociation product ion spectra of (a) protonated benzamide (*m/z* 122), (b) protonated benzamide-d_5 (*m/z* 127), which was generated as an in-source fragment ion of *N*-cyclohexylbenzamide-d_5, and (c) the benzamide-*N*-d_3 cation (*m/z* 125) generated from ESI of benzamide-*N*-d_2 in deuterated solvent. (d) CID product ion spectrum of protonated benzamide obtained from the LTQ.

on the proton transfer step in forming the corresponding protonated benzene derivatives from $\mathbf{I_b}$-like intermediates.

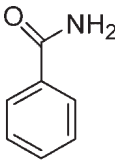
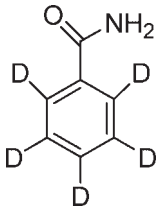
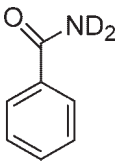
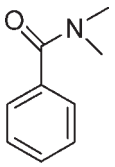
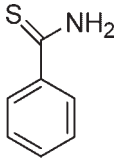
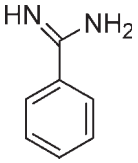
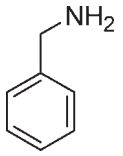
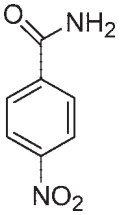
Deuterium-labeling experiments with benzamide-d_5 and benzamide-*N*-d_3

Two complementary deuterium-labeled benzamide molecules, benzamide-d_5 with all five aromatic hydrogens replaced by deuterium atoms and benzamide-*N*-d_3 with three amide hydrogens (including one hydrogen gained in solution during ESI) labeled by deuterium atoms, were examined to probe the proposed initial proton migration and subsequent proton transfer during the formation of $C_6H_7^+$. In the case of benzamide-d_5, protonated benzamide-d_5 (*m/z* 127) was generated as a product ion of *N*-cyclohexylbenzamide-d_5. In the proposed fragmentation pathway of benzamide-d_5 (Scheme 2), protonated benzamide-d_5 was produced as an in-source fragment ion of *N*-cyclohexylbenzamide-d_5 after eliminating cyclohexene. The same fragmentation pathways as had been observed in protonated benzamide were observed in protonated benzamide-d_5 (Fig. 1(b)). A direct neutral loss of NH_3 from protonated benzamide-d_5 resulted in $C_6D_5CO^+$ (*m/z* 110), which further lost CO to produce the benzene-d_5 cation $C_6D_5^+$ (*m/z* 82). The protonated benzene observed from benzamide-d_5 was observed at *m/z* 84

DOI: 10.1002/rcm

RCM

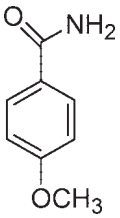
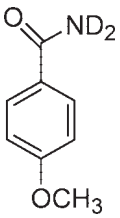
Table 1. Summary of product ions observed from collision-induced dissociation of protonated benzamide and its related molecules

Compound	Structure	Product ions observed from protonated molecules: *m/z* (assignment)	Comment	Figure
Benzamide		105 ($C_6H_5CO^+$), 79 ($C_6H_7^+$), 77 ($C_6H_5^+$), 44 ($O{=}C{=}NH_2^+$)	Protonated benzene was observed.	1a
Benzamide-d_5		110 ($C_6D_5CO^+$), 109 ($C_6D_4HCO^+$), 84 ($C_6D_5H_2^+$), 83 ($C_6D_4H_3^+$), 82 ($C_6D_5^+$), 81 ($C_6D_4H^+$), 44 ($O{=}C{=}NH_2^+$)	Protonated benzene was observed as $C_6D_5H_2^+$ and $C_6H_4H_3^+$.	1b
Benzamide-*N*-d_2		106 ($C_6H_4DCO^+$), 105 ($C_6H_5CO^+$), 81 ($C_6H_5D_2^+$), 78 ($C_6H_4D^+$), 77 ($C_6H_5^+$), 46 ($O{=}C{=}ND_2^+$)	Deuterated benzene $C_6H_5D_2^+$ was observed.	1c
N,*N*-Dimethylbenzamide		105 ($C_6H_5CO^+$), 77 ($C_6H_5^+$), 72 [$(CH_3)_2NCO^+$]	Protonated benzene was not observed.	2a
Thiobenzamide		121 ($C_6H_5CS^+$), 79 ($C_6H_7^+$), 77 ($C_6H_5^+$), 60 ($S{=}C{=}NH_2^+$)	Protonated benzene (*m/z* 79) and $S{=}C{=}NH_2^+$ (*m/z* 60) were observed.	2b
Benzamidine		104 ($C_6H_5C{=}NH^+$), 77 ($C_6H_5^+$)	Protonated benzene was not observed.	2c
Benzylamine		91 ($C_7H_7^+$), 65 ($C_5H_5^+$)	Protonated benzene was not observed.	2d
4-Nitrobenzamide		150 ($NO_2C_6H_4CO^+$), 137 ($HOC_6H_4CONH_2^+$), 124 ($NO_2C_6H_6^+$), 121 ($C_6H_4CONH_3^+$), 120 ($OC_6H_4CO^+$), 109 ($HOC_6H_4NH_2^+$), 104 ($C_6H_4CO^+$)	Protonated nitrobenzene was observed at *m/z* 124.	3a, 3b

(*Continues*)

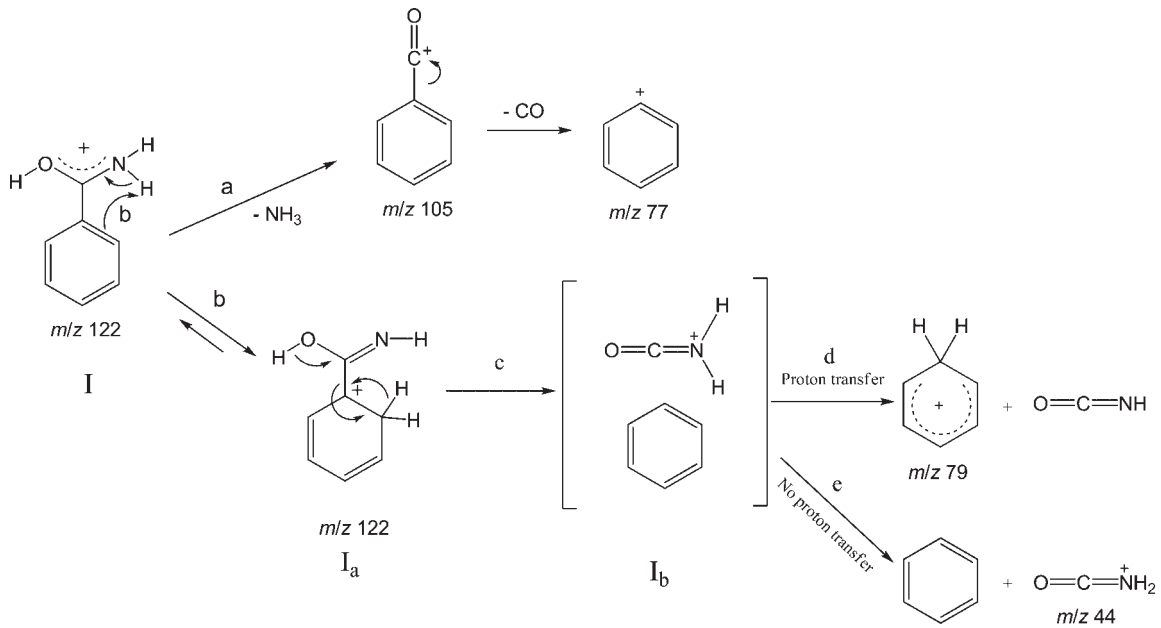
DOI: 10.1002/rcm

Table 1. (Continued)

Compound	Structure	Product ions observed from protonated molecules: *m/z* (assignment)	Comment	Figure
4-Methoxybenzamide		135 ($CH_3OC_6H_4CO^+$), 109 ($CH_3OC_6H_6^+$), 94 ($C_6H_6O^+$)	Protonated 4-methoxybenzene was observed at *m/z* 109.	3c, 3d
4-Methoxybenzamide-*N*-d_2		136 ($CH_3OC_6H_3DCO^+$), 135 ($CH_3OC_6H_4CO^+$), 111 ($CH_3OC_6H_4D_2^+$), 96 ($C_6H_4D_2O^+$)	Deuterated 4-methoxybenzene was observed at *m/z* 111.	Not shown

(pathway A). In addition, another set of product ions was observed at *m/z* 109, 81 and 83, which were 1 *m/z* unit less than the three expected ions, i.e., loss of NH_3 (*m/z* 110), loss of NH_3 and CO (*m/z* 82) and $C_6D_5H_2^+$ (*m/z* 84). These ions can be explained as resulting from an intramolecular hydrogen/deuterium (H/D) exchange process prior to or during the CID of protonated benzamide-d_5. A deuterium in the benzene ring of **II**, probably the most acidic deuterium on the *ortho*-position, can exchange with a hydrogen atom on the amide nitrogen through pathways B1/B2 to produce **III** (Scheme 2), which then fragments to give $C_6D_4HCO^+$ (*m/z* 109), $C_6D_4H^+$ (*m/z* 81), and protonated benzene-d_4 (*m/z* 83).

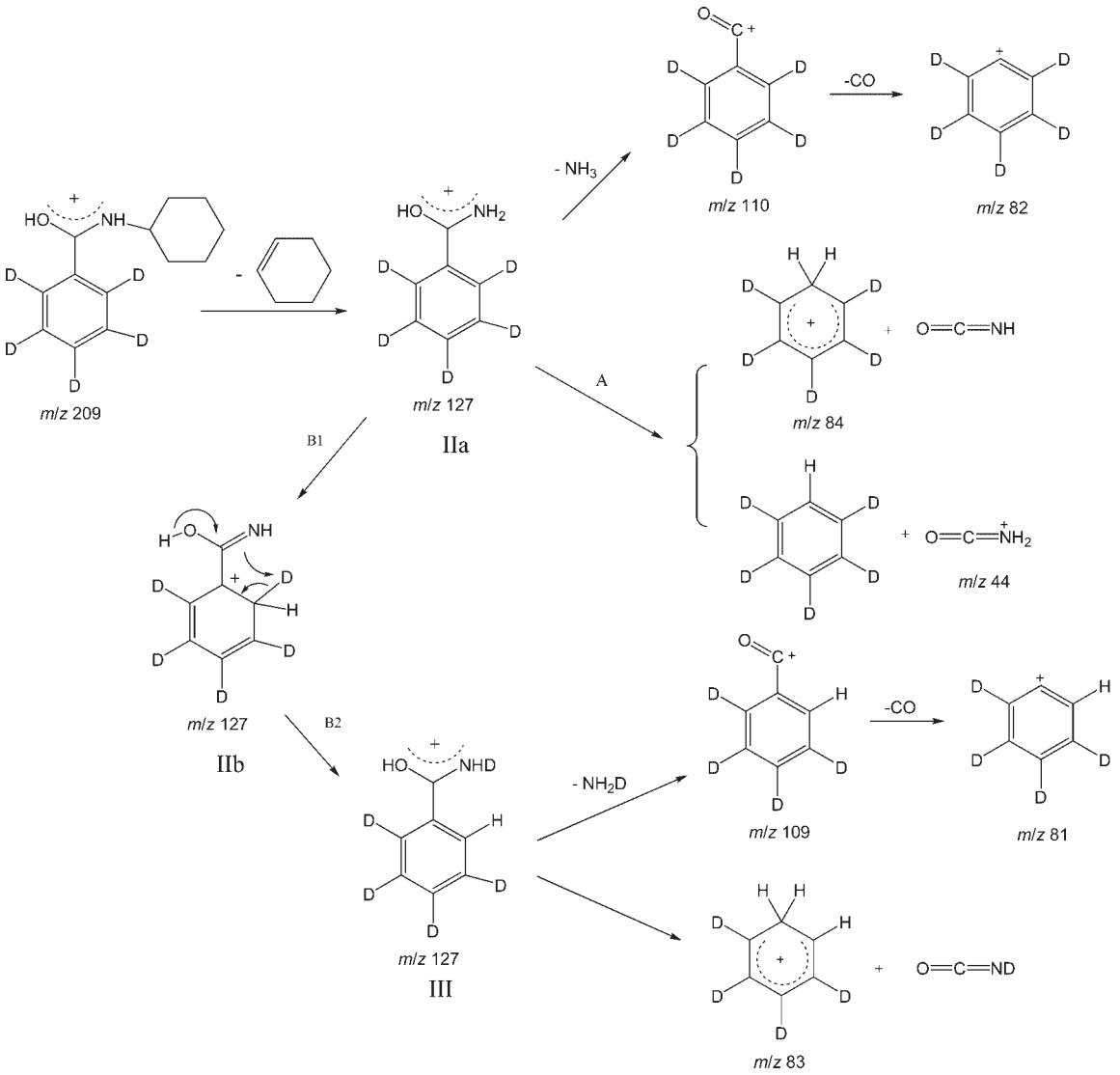
In another set of experiments, the benzamide-*N*-d_3 cation (**IV**) was generated from ESI of benzamide-*N*-d_2 in a completely deuterated solvent. Fragmentation of **IV** yielded the ions corresponding to those observed from protonated benzamide-d_5 with the predicted mass shifts (Scheme 3). The neutral loss of ND_3 from benzamide-*N*-d_3 (**IV**) resulted in $C_6H_5CO^+$ (*m/z* 105), which further fragmented to the phenyl cation, $C_6H_5^+$ (*m/z* 77) (Fig. 1(c)). Deuterated benzene was observed as $C_6H_5D_2^+$ (*m/z* 81), with the two deuterium atoms that originated from the deuterated amide nitrogen in **IV**. The proton-competing isocyanic acid cation was observed as $O{=}C{=}ND_2^+$ (*m/z* 46). An intramolecular H/D exchange process similar to that for benzamide-d_5 occurred for benzamide-*N*-d_3 (**IV**), leading to the formation of **V**. Subsequent fragmentation of **V** produced the ND_2H neutral loss product $C_6H_4DCO^+$ at *m/z* 106 and the benzene-*d* cation $C_6H_4D^+$ at *m/z* 78. The fragmentation behavior



Scheme 1. Proposed mechanism for the formation of protonated benzene ($C_6H_7^+$) and other product ions during CID of electrospray-generated protonated benzamide.

DOI: 10.1002/rcm

RCM



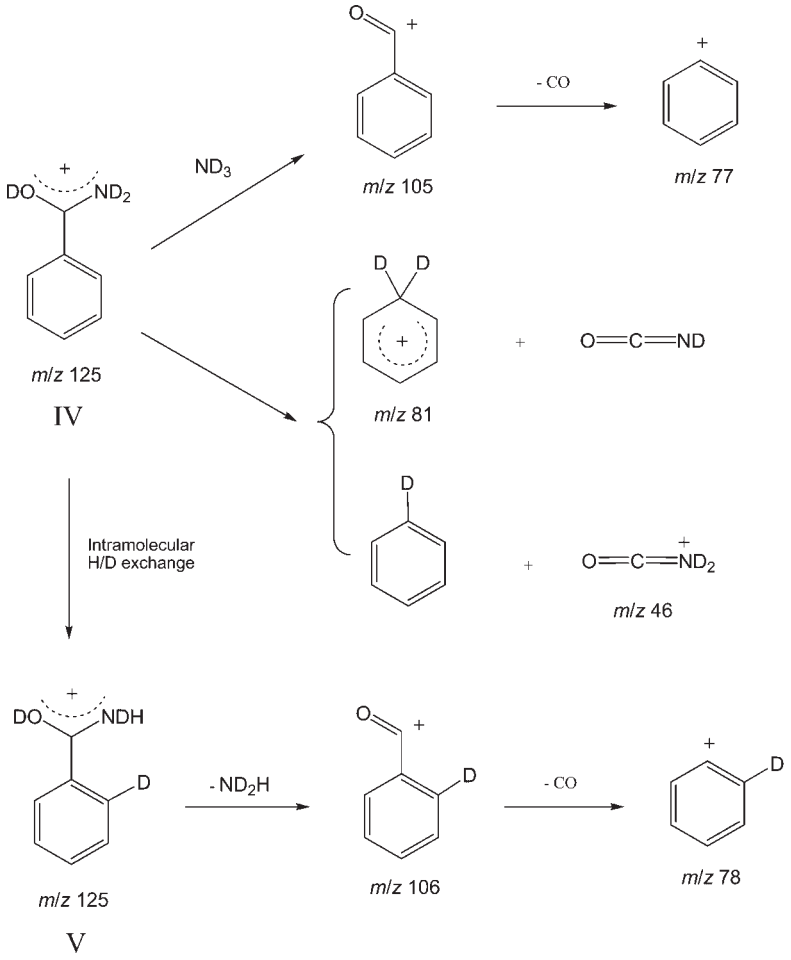
Scheme 2. Proposed fragmentation mechanism of protonated benzamide-d_5 under CID.

observed in the above studies of the two complementary deuterium-labeled benzamide molecules, benzamide-d_5 and benzamide-*N*-d_3, provided conclusive evidence that the amide hydrogen atoms are the source of the two hydrogens required to form protonated benzene as proposed in Scheme 1.

Protonated benzene obtained on different types of mass spectrometers using different collision gases

In addition to the MS/MS experiments performed on the Q-Tof mass spectrometer where argon was used as the collision gas, the fragmentation of protonated benzamide was also examined on a LTQ mass spectrometer where helium was used as the collision gas, to evaluate the impact of the collision gas and other instrumental factors on the formation of $C_6H_7^+$. The CID of protonated benzamide in the LTQ yielded two major product ions: $C_6H_5CO^+$ at *m/z* 105 and $C_6H_7^+$ at *m/z* 79 (Fig. 1(d)). Therefore, it can be concluded that the type of the inert collision gas used is not critical for the formation of $C_6H_7^+$. Compared with the result obtained from the Q-Tof (Fig. 1(a)), the phenyl cation ($C_6H_5^+$, *m/z* 77) was not observed in the MS^2 spectrum of protonated benzamide obtained from the LTQ. This difference may be due to the somewhat different collision modes used in the two instruments. In the LTQ, only the targeted precursor ion is activated by resonance excitation during an MS^2 experiment. The primary product ions formed in LTQ are not activated again; thus no further fragmentation can be produced. This explains why $C_6H_5^+$ was not observed in the MS^2 spectrum of protonated benzamide in the LTQ due to it being a secondary product ion from $C_6H_5CO^+$. An MS^3 experiment on $C_6H_5CO^+$ performed in the LTQ did indeed produce $C_6H_5^+$ (data not shown). On the other hand, in a CID experiment carried out in a tandem-in-space mass spectrometer (such as a triple-quadrupole type instrument, or the Q-Tof in this case), primary product ions can undergo additional collision activation resulting in secondary and further product ions. The fact that protonated benzene was observed from protonated benzamide in the LTQ not only confirms that $C_6H_7^+$ (*m/z* 79) is a primary product ion, which is consistent with the proposed mechanism, but also indicates that the formation

DOI: 10.1002/rcm



Scheme 3. Proposed fragmentation mechanism of protonated benzamide-*N*-d_3 under CID.

of $C_6H_7^+$ is determined by the intrinsic chemical property of the precursor ion, which is independent of the instruments used.

Studies in the formation of protonated benzene from structural analogs of benzamide

N,N-*Dimethylbenzamide*

Based on the proposed mechanism, *N,N*-dimethylbenzamide, which does not contain any exchangeable hydrogen on the amide nitrogen, should not be capable of forming $C_6H_7^+$. Indeed, the CID of protonated *N,N*-dimethylbenzamide yielded three major ions (Fig. 2(a)): $C_6H_5CO^+$ (*m/z* 105), the phenyl cation $C_6H_5^+$ (*m/z* 77), and $(CH_3)_2NCO$ (*m/z* 72). As expected, no protonated benzene, $C_6H_7^+$, was observed.

Thiobenzamide, benzamidine and benzylamine

To further verify other aspects of the proposed mechanism, additional structural analogs of benzamide were examined to investigate the impact of modifying certain moieties of the molecule on the formation of $C_6H_7^+$. Another critical step in the proposed mechanism is the cleavage of the carbonyl benzene C–C bond, which should occur concertedly with the proton migration but precede the formation of the presumed proton-bound dimer of benzene and isocyanic acid (**I$_b$**). The C–C bond connecting the carbonyl group to the benzene ring is one of the two weakest bonds in benzamide due to the presence of the electron-withdrawing carbonyl group.[12] Substituting the carbonyl oxygen with other heteroatoms (X) possessing less electronegativity may have an effect on the C–C bond cleavage, which would in turn affect the formation of $C_6H_7^+$ from these benzamide analogs. Therefore, thiobenzamide and benzamidine which contain C=S and C=N in place of the C=O, respectively, were chosen as the model compounds to test this hypothesis. In addition, benzylamine, which has a CH_2-NH_2 single bond instead of a double C=X bond, was also tested. The CID of thiobenzamide led to direct neutral loss of NH_3 to form $C_6H_5CS^+$ (*m/z* 121), and a subsequent C=S loss to yield the phenyl cation (*m/z* 77), as shown in Fig. 2(b). Although both $C_6H_7^+$ (*m/z* 79) and protonated isothiocyanic acid ($S{=}C{=}NH_2^+$, *m/z* 60) were observed, the intensity ratio of $C_6H_7^+$ (*m/z* 79) to the phenyl cation (*m/z* 77) was less than 1, while this ratio was greater than 1 in the case of benzamide (Fig. 1(a)). This result suggests that the pathway to form $C_6H_7^+$ from protonated thiobenzamide is less favored than the competing pathway of NH_3 neutral loss. In the case of benzamidine, the formation of $C_6H_7^+$ was completely suppressed. The neutral loss products, $C_6H_5C{=}NH^+$ (*m/z* 104) and the phenyl cation (*m/z* 77), were the two dominant ions observed (Fig. 2(c)); neither $C_6H_7^+$ nor $HN{=}CNH_3^+$ was observed. Collectively, these results appear to indicate that the C–C bonds between

DOI: 10.1002/rcm

RCM

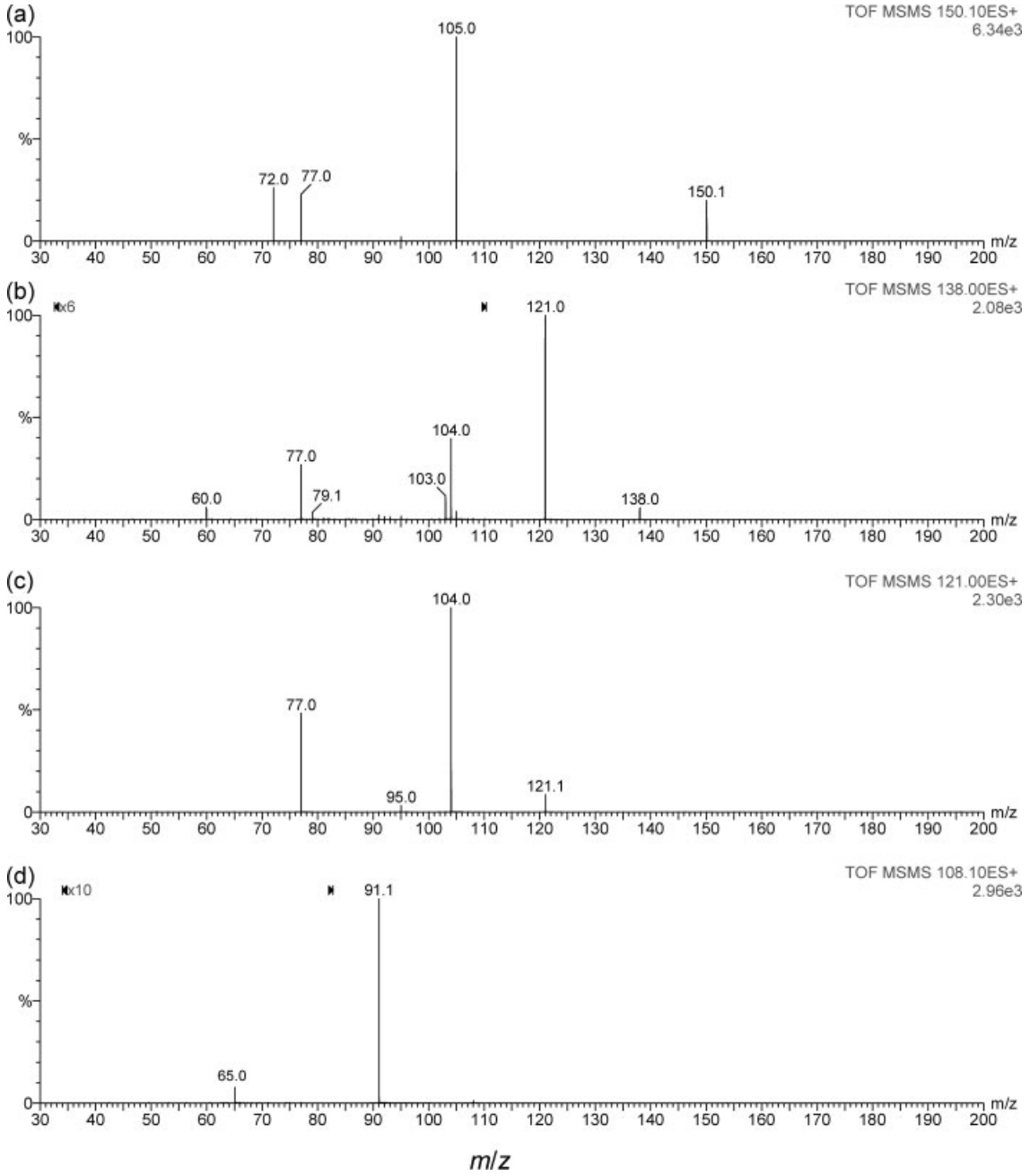


Figure 2. Collision-induced dissociation product ion spectra of protonated (a) *N*,*N*-dimethylbenzamide (*m/z* 150), (b) thiobenzamide (*m/z* 138), (c) benzamidine (*m/z* 121), and (d) benzylamine (*m/z* 108).

the benzene ring and the C=X group in both thiobenzamide and benzamidine are increasingly stronger than the one in benzamide, causing the two benzamide analogs to be less likely to proceed via the five-membered ring mechanism since cleavage of this C–C bond is a critical step, which will probably occur concertedly with the proton migration in the proposed mechanism (Scheme 1). On the other hand, CID of protonated benzylamine did not yield protonated benzene, which is not unexpected (Fig. 2(d)). This probably can be attributed to the following two factors: (1) no electron-withdrawing double bond is present to activate the C–C bond connected to the benzene ring in benzylamine, and/or (2) neutral loss of NH_3 from protonated benzylamine should produce a very stable benzylium/tropylium ion ($C_7H_7^+$, *m/z* 91). The comparative study of the three model compounds suggests that the electron-withdrawing C=O group attached to the benzene ring in benzamide decreases the carbonyl benzene C–C bond order[12] and thus renders the formation of $C_6H_7^+$ quite efficient.

4-Nitrobenzamide and 4-methoxybenzamide

Another factor affecting the formation of $C_6H_7^+$ in the proposed mechanism is the proton transfer step (pathway d, Scheme 1) which takes place after cleavage of the carbonyl benzene C–C bond and concomitant proton migration leading to the formation of the proton-bound dimer (pathway c). The proposed proton transfer step is essential to the formation of $C_6H_7^+$ and the efficiency of this step should be

DOI: 10.1002/rcm

determined by the relative proton affinities of the two entities in the proton-bound dimer ($\mathbf{I_b}$). In the case of benzamide, the two entities competing for the proton in the proton-bound dimer are benzene and isocyanic acid (O=C=NH). Their proton affinities are approximately the same: with benzene at 179.3 kcal/mol and O=C=NH at 180 kcal/mol.[13] As a result, both $C_6H_7^+$ and protonated isocyanic acid were observed from benzamide, although the intensity of the $C_6H_7^+$ ion was greater than that of protonated isocyanic acid. This observation is consistent with the proposed mechanism. Therefore, it can be predicted that, if a substituted benzene moiety possesses a higher proton affinity than isocyanic acid, the formation of $O{=}C{=}NH_2^+$ (m/z 44) would be less favored or even completely suppressed. To test this hypothesis, two benzamide derivatives, 4-nitrobenzamide and 4-methoxybenzamide, were examined. The proton affinity of nitrobenzene is 191.3 kcal/mol, which indicates that proton transfer from protonated nitrobenzene to O=C=NH is endothermic by about 11 kcal/mol, i.e., the formation of $O{=}C{=}NH_2^+$ from protonated 4-nitrobenzamide is less favored than from protonated benzamide. The experimental results obtained from 4-nitrobenzamide were indeed consistent with this prediction. In the product ion spectrum of protonated 4-nitrobenzamide (Fig. 3(a)), protonated nitrobenzene was observed at m/z 124 with 10 eV collision energy, while no $O{=}C{=}NH_2^+$ ion (m/z 44) was observed. Other major ions observed from CID of protonated 4-nitrobenzamide resulted from known fragmentation pathways and the assignments of

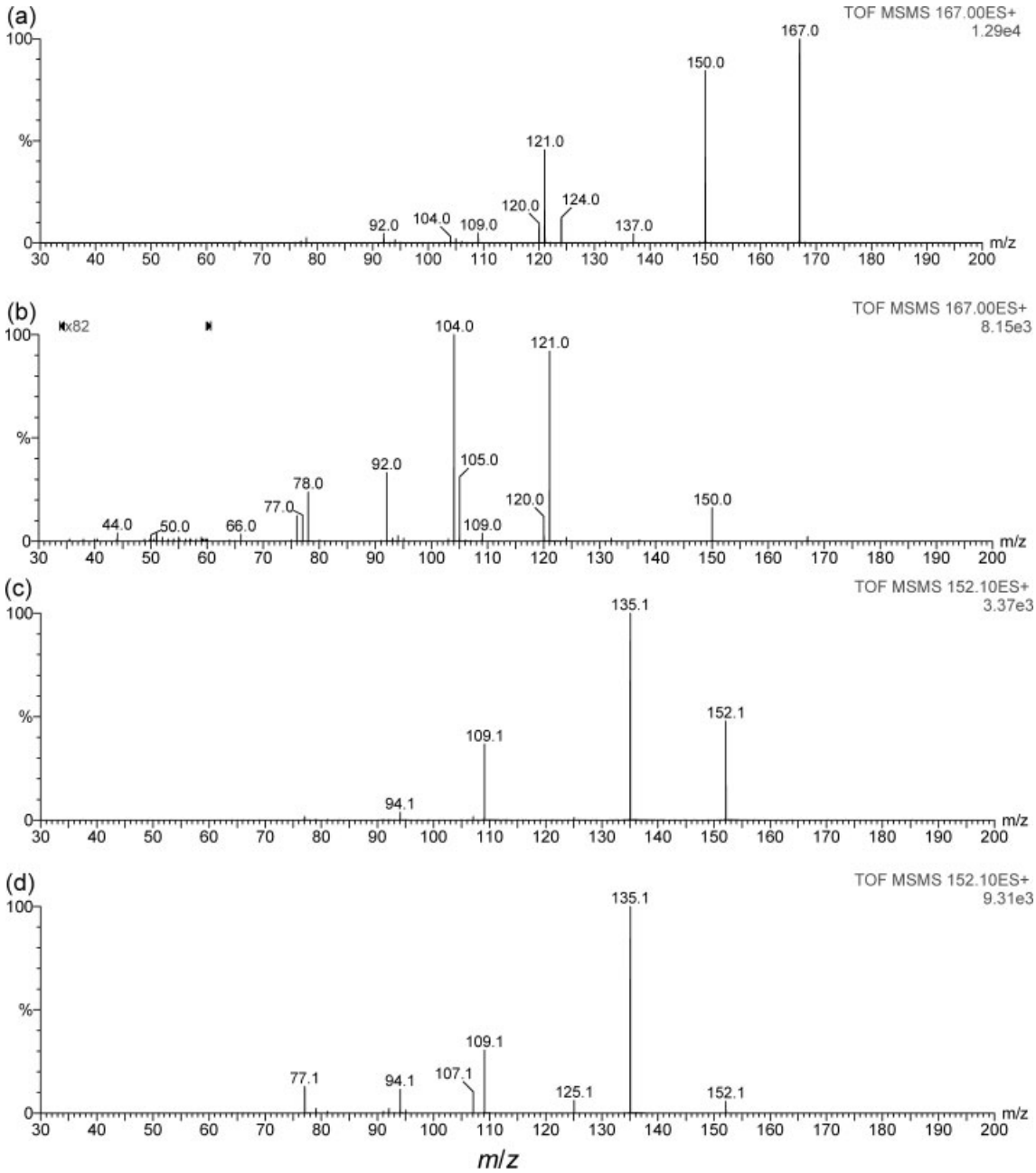


Figure 3. Collision-induced dissociation product ion spectra of protonated 4-nitrobenzamide at (a) 10 eV collision energy and (b) 15 eV collision energy, and 4-methoxybenzamide at (c) 10 eV collision energy and (d) 15 eV collision energy.

DOI: 10.1002/rcm

RCM

these ions are summarized in Table 1. When the collision energy was increased from 10 to 15 eV, the intensity of protonated nitrobenzene decreased and a trace amount of *m/z* 44 was observed (Fig. 3(b)), suggesting that endothermic proton transfer took place at the higher collision energy, leading to the formation of $O{=}C{=}NH_2^+$. The proton affinity of 4-methoxybenzamide is 200.7 kcal/mol, which is about 21 kcal/mol greater than that of O=C=NH. Following the same rationale outlined above, it can be predicted that the formation of protonated methoxybenzene should be even more favored than the formation of protonated nitrobenzene, which should result in further suppression of $O{=}C{=}NH_2^+$. Again, the experiment results agreed well with this prediction: protonated methoxybenzene (*m/z* 109) was observed as one of the major product ions (Fig. 3(c)), while $O{=}C{=}NH_2^+$ (*m/z* 44) was completely absent even at higher collision energy (Fig. 3(d)). Furthermore, additional product ions of protonated 4-methoxybenzamide-*N*-d_3 generated from ESI of 4-methoxybenzamide in fully deuterated solvents are consistent with the proposed mechanism with the corresponding mass shifts due to the methoxyl group and/or H/D exchange. These results suggest that the proton transfer step, proposed in the protonated benzene ($C_6H_7^+$) formation mechanism, is the final critical step towards the formation of $C_6H_7^+$. Although the ion energies in a CID process may not be controlled accurately, the qualitative comparison results obtained in this study are consistent with the intrinsic properties of the protonated molecules in the gas phase.

CONCLUSIONS

Protonated benzene ($C_6H_7^+$) has been observed in LC/ESI-MS/MS during the CID of the protonated benzamides, an event that has not previously been reported despite the widespread use of the technique. The formation of $C_6H_7^+$ is proposed as a fragmentation product of the protonated molecule via a proton migration and concomitant C–C bond breakage in a five-membered ring intermediate followed by a proton transfer process. These three important aspects of the proposed mechanism – proton migration, concomitant breakage of the carbonyl benzene C–C bond, and the subsequent proton transfer – were examined and challenged by the design of three sets of experiments. All the results unambiguously indicate that $C_6H_7^+$ is formed through the CID of the electrospray-generated protonated benzamide (and the precursor ions of certain structurally related compounds). This finding provides a convenient means to generate $C_6H_7^+$, a reactive intermediate of much interest, for further physical or chemical investigation. In addition, the observation of $C_6H_7^+$ (*m/z* 79) can be used as an informative diagnostic ion to facilitate the identification of unknown species containing the 'benzoyl-type' structural feature. Our further studies indicate that the formation of $C_6H_7^+$ in the CID of electrospray-generated ions appears to be a common phenomenon for a great number of compounds that contain 'benzoyl-type' or similar structural features. These compounds are frequently present in food and pharmaceutical products as leachable impurities that require strict control and rapid elucidation of their identities.

REFERENCES

1. Cacace F. *Acc. Chem. Res.* 1988; **21**: 215.
2. Jones W, Boissel P, Chiavarino B, Crestoni ME, Fornarini S, Lemaire J, Maitre P. *Angew. Chem. Int. Ed.* 2003; **42**: 2057.
3. Selim ETM, Rabbih MA, Fahmey MA. *Org. Mass Spectrom.* 1992; **27**: 919.
4. Bruins AP, Nibbering NMM. *Org. Mass Spectrom.* 1976; **11**: 950.
5. Schröder D, Loos J, Schwarz H, Thissen R, Dutuit O. *J. Phys. Chem. A* 2004; **108**: 9931.
6. Fornarini S, Crestoni ME. *Acc. Chem. Res.* 1998; **31**: 827.
7. Bouchoux G, Yáñez M, Mó O. *Int. J. Mass Spectrom.* 1999; **185/186/187**: 241.
8. Freiser BS, Woodin RL, Beauchamp JL. *J. Am. Chem. Soc.* 1975; **97**: 6893.
9. Kuck VD, Ingeman S, Konig LJ, Grutzmacher HF, Nibbering NMM. *Angew. Chem.* 1985; **97**: 691.
10. Holman RW, Gross ML. *J. Am. Chem. Soc.* 1989; **111**: 3560.
11. Letzel M, Kirchhoff D, Grützmacher HF, Stein D, Grützmacher H. *Dalton Trans.* 2006; 2008.
12. Al-Awadi NA. *J. Chem. Soc., Perkin Trans. II* 1990; **12**: 2187.
13. All proton affinities referred to in this study were taken from the NIST Chemistry WebBook, NIST Standard Reference Database Number 69. Available: http://webbook.nist.gov/chemistry/.

DOI: 10.1002/rcm