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# Gas-Phase Meerwein Reaction of Epoxides with Protonated Acetonitrile Generated by Atmospheric Pressure Ionizations

Lianming Wu, David Q. Liu, and Alireza S. Kord

Analytical Sciences, GlaxoSmithKline, King of Prussia, Pennsylvania, USA

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Ethyltrihydroimidazolium ion can be generated by protonation of acetonitrile (when used as the LC-MS mobile phase) under the conditions of atmospheric pressure ionizations, including electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) as well as atmospheric pressure photoionization (APPI). Ethyltrihydroimidazolium ion ( $\text{CH}_3 - \overset{+}{\text{C}} \equiv \text{NH}$  and its canonical form  $\text{CH}_3 - \overset{+}{\text{C}} = \text{NH}$ ) is shown to efficiently undergo the gas-phase Meerwein reaction with epoxides. This reaction proceeds by the initial formation of an oxonium ion followed by three-to-five-membered ring expansion via an intramolecular nucleophilic attack to yield the Meerwein reaction products. The density functional theory (DFT) calculations at the B3LYP/6-311+G(d,p) level show that the gas-phase Meerwein reaction is thermodynamically favorable. Collision-induced dissociation (CID) of the Meerwein reaction products yields the net oxygen-by-nitrogen replacement of epoxides with a characteristic mass shift of 1 Da, providing evidence for the cyclic nature of the gas-phase Meerwein reaction products. The gas-phase Meerwein reaction offers a novel and fast LC-MS approach for the direct analysis of epoxides that might be of genotoxic concern during drug development. Understanding and utilizing this unique gas-phase ion/molecule reaction, the sensitivity and selectivity for quantitation of epoxides can be enhanced. (J Am Soc Mass Spectrom 2010, 21, 1802–1813) © 2010 American Society for Mass Spectrometry

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Gas-phase ion/molecule reactions are fast, efficient, and highly sensitive to the fine structural variations such as relative positions of functional groups. Their product distributions often provide diagnostic information for structural characterization of both reactant ions and neutral molecules [1–4]. Ion/molecule reactions have also contributed significantly to the elucidation of organic reaction mechanisms, helping to bridge the gap between gas-phase ion chemistry and condensed-phase organic chemistry. It also provides access to key properties and intrinsic reactivities of a great variety of solvent- and counterion-free gaseous ions and, as such, constitutes a powerful tool for chemical studies. Many classic organic reactions have been studied in the gas phase using the mass spectrometric methods [5–14]. Recently, with the advent of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI), mass spectrometry has been widely used as an analytical tool for the study of atmospheric pressure ion/molecule reactions. These gas-phase reactions can even yield detailed information regarding the individual reaction step of the catalytic cycles, and thus allow the study of transient

intermediates that have not been accessible previously by the condensed-phase techniques [15, 16].

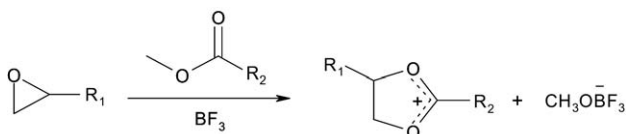
Epoxides are common reagents or key intermediates in a wide variety of synthetically important condensed-phase reactions, including asymmetric synthesis via the opening of the oxirane ring [17–19]. For example, Meerwein first reported his eponymous reaction in 1955 as a ring-expansion reaction occurring in a solution of epoxides and esters in the presence of  $\text{BF}_3$  (Scheme 1) [20].

In the gas phase, the Meerwein reaction with epoxides is found to be one of the most favored reactions of gaseous acylium ions (Scheme 2). The reaction proceeds by an initial O-acylation followed by an intramolecular nucleophilic attack. This results in three-to-five-membered ring expansion to yield cyclic 1,3-dioxolanylium ions [21–23]. This structure-selective reaction, which displays many features common to those of transacetalization reactions of acylium ions with acetals and analogs [1], has been demonstrated to be very useful for both characterization and selective screening of specific compounds such as warfare agents [24].

Some epoxides are considered to be genotoxic [25] since they can form DNA adducts [26]. The regulatory agencies pay increasing attention to genotoxic impurities (GTIs), including epoxides in pharmaceuticals, requiring their control to the low parts per million (ppm) levels [27]. Based on thresholds of toxicologic concern of GTIs during drug development, testing potential

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Address reprint requests to Dr. L. Wu, Analytical Sciences, GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA. E-mail: lianming.2.wu@gsk.com



**Scheme 1.** Solution-phase Meerwein reaction of epoxides with esters.

GTIs, such as epoxides at ppm levels, demand for sensitive and specific methods. In this paper, we report the gas-phase Meerwein reaction of several epoxides with ethylnitrilium ion (by protonation of acetonitrile) generated in the atmospheric pressure ionization (API) sources and the application of these in-source reaction products as a novel approach to the sensitive and selective analysis of epoxides.

## Experimental

### Chemicals

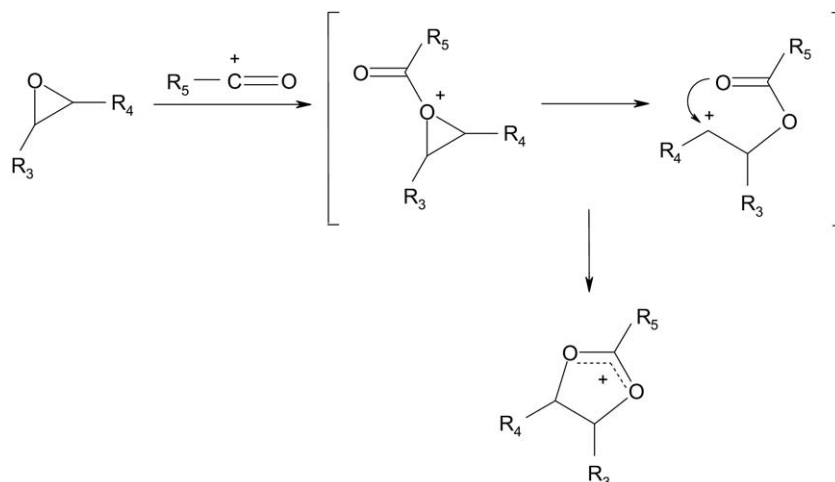
Four epoxides in this study are listed in Figure 1. Epoxide **I** ((R)-2-((5-bromo-2,3-difluorophenoxy)methyl)oxirane), **II** ((2R,2'R)-2,2'-(5-bromo-2-fluoro-1,3-phenylene)bis(oxy)bis(methylene)dioxirane) and **IV** (2-((S)-1-((R)-oxiran-2-yl)allyl)isoindoline-1,3-dione) were synthesized at GalxoSmithKline (King of Prussia, PA, USA). Epoxide **III** ((R)-2-(chloromethyl)oxirane), formic acid (99.0% purity), and deuterium oxide (deuterated water, 99% deuterium atom) were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA) and used without further purification. A solution of 0.01 M sodium formate was supplied by Waters Corporation (Milford, MA, USA). The HPLC-grade solvents including water (H<sub>2</sub>O), acetonitrile (CH<sub>3</sub>CN) and methanol (CH<sub>3</sub>OH) were purchased from Burdick and Jackson (Morristown, NJ, USA).

### HPLC Separation Conditions

The chromatographic separation was achieved using a Luna C18 column (50 × 2.0 mm, 3 μm; Phenomenex, Torrance, CA, USA) with a column temperature kept at 40 °C. Mobile phases A and B are 0.1% formic acid (FA) in water and 0.1% FA in acetonitrile, respectively. The HPLC gradient was ramped from 25% B to 75% B linearly in 4 min. When performing the hydrogen/deuterium (H/D) exchange experiments, 0.1% FA in deuterated water (D<sub>2</sub>O) was used replacing mobile phase A [28]. The mobile phase flow rate was set at 1 mL/min, of which 250 μL/min was split into the API source of a mass spectrometer by an Accurate splitter (LC Packings, Sunnyvale, CA, USA).

### Accurate MS and MS<sup>2</sup> Analysis on a Q-TOF Mass Spectrometer

The exact mass measurements and MS<sup>2</sup> experiments were performed on a Q-TOF Premier quadrupole orthogonal acceleration time-of-flight mass spectrometer with LockSpray controlled by MassLynx 4.1 software (Waters Corporation, Manchester, UK). The ESI source was operated in the positive ion mode with a spray voltage of −3.5 kV, the source and desolvation gas temperatures of 120 and 300 °C, an argon collision gas flow rate of 0.45 mL/min in the T-Wave guide Mark II collision cell, a collision energy of 12–20 eV for the collision-induced dissociation (CID) experiments, a desolvation gas flow rate of 600 L/hour, and a sample cone voltage of 30 V. The LockSpray source was operated under identical conditions except with a flow rate at 3 μL/min for infusion of a leucine-enkephalin (Leu-Enk) solution. For the APCI source, the corona discharge needle voltage was set to −3 kV. For the atmospheric pressure photoionization (APPI) source, the Krypton (Kr) lamp was used to generate 10.6 eV photons. The Q-TOF instrument was calibrated with a



**Scheme 2.** Gas-phase Meerwein reaction of epoxides with acylium ions.

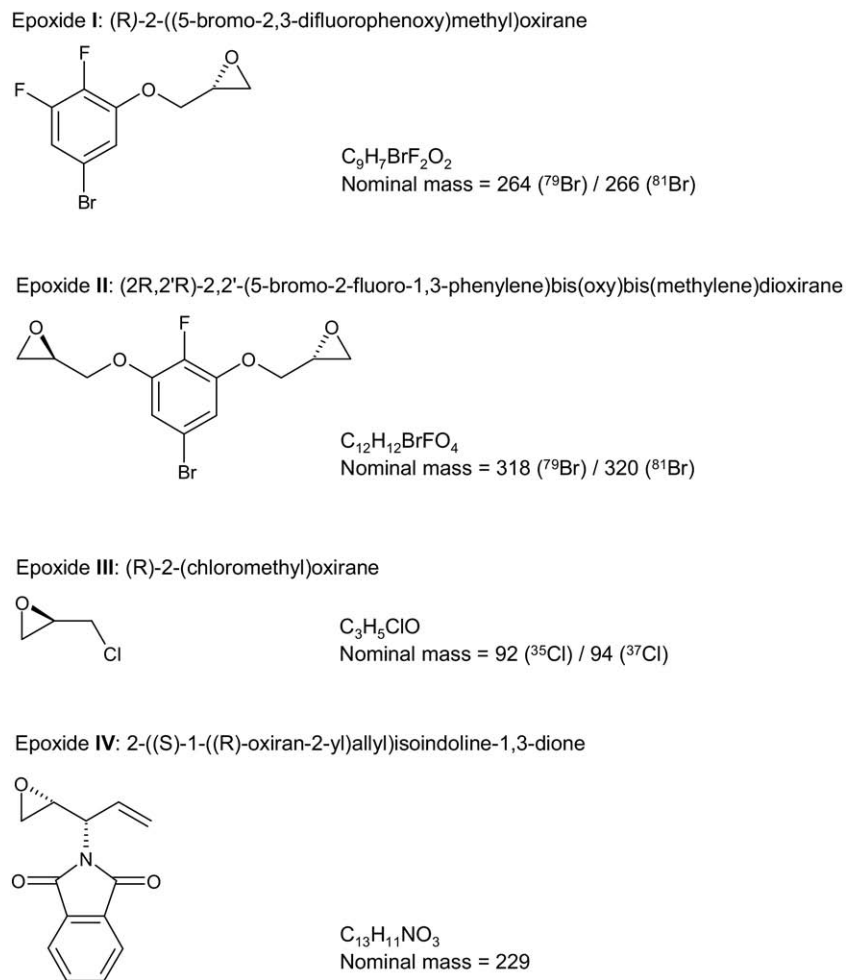


Figure 1. Structures of the four epoxides used in the study.

sodium formate solution at 0.01 mg/mL. All masses were corrected by the internal reference ion of  $m/z$  556.2771 (protonated Leu-Enk) that was introduced via the Lockspray source.

#### Data-Dependent $MS^2$ , $MS^3$ , and $MS^4$ Experiments on a Linear Ion Trap Mass Spectrometer

The data-dependent  $MS^2$ ,  $MS^3$ , and  $MS^4$  experiments were performed on a Thermo Electron linear ion trap LTQ mass spectrometer controlled by Xcalibur 1.4 software (San Jose, CA, USA). The ESI source was operated in the positive ion mode under the following conditions: spray voltage,  $-4.0$  kV; heated capillary temperature,  $300$  °C; capillary voltage,  $30$  V; tube lens offset voltage,  $30$  V; nitrogen sheath gas flow rate,  $30$  arbitrary units. In the full-scan  $MS^2$ ,  $MS^3$ , and  $MS^4$  modes, the precursor ion of interest was isolated by applying multiple frequency waveforms to remove undesired ions through broadband excitation. The isolated ions were then subjected to a supplementary alternating current (AC) potential for resonant excitation to cause CID. The Mathieu  $q_z$  values chosen for the resonance

excitation and ejection were  $0.25$  and  $0.88$ , respectively. The activation time used was  $30$  ms with helium as the collision gas. The normalized activation energy was set to  $25\%$  of  $0$ – $5$  V peak-to-peak potential.

#### Single Ion Monitoring (SIM) and Multiple Reaction Monitoring (MRM) on a Triple Quadrupole Mass Spectrometer

For the quantitative analysis, the SIM and MRM experiments were performed on an Agilent 6410 triple quadrupole mass spectrometer equipped with an Agilent 1200 HPLC system, which was controlled by MassHunter Workstation software (Agilent Technologies, Santa Clara, CA, USA). The ESI source was operated in the positive ion mode under the following conditions: spray voltage,  $-4.0$  kV; nitrogen drying gas flow rate and temperature,  $12$  L/min and  $300$  °C, respectively; nitrogen nebulizer gas pressure,  $45$  psi; dwell time,  $880$  ms; CID collision gas, nitrogen; collision energy for MRM,  $15$  eV.

### Theoretical Calculations

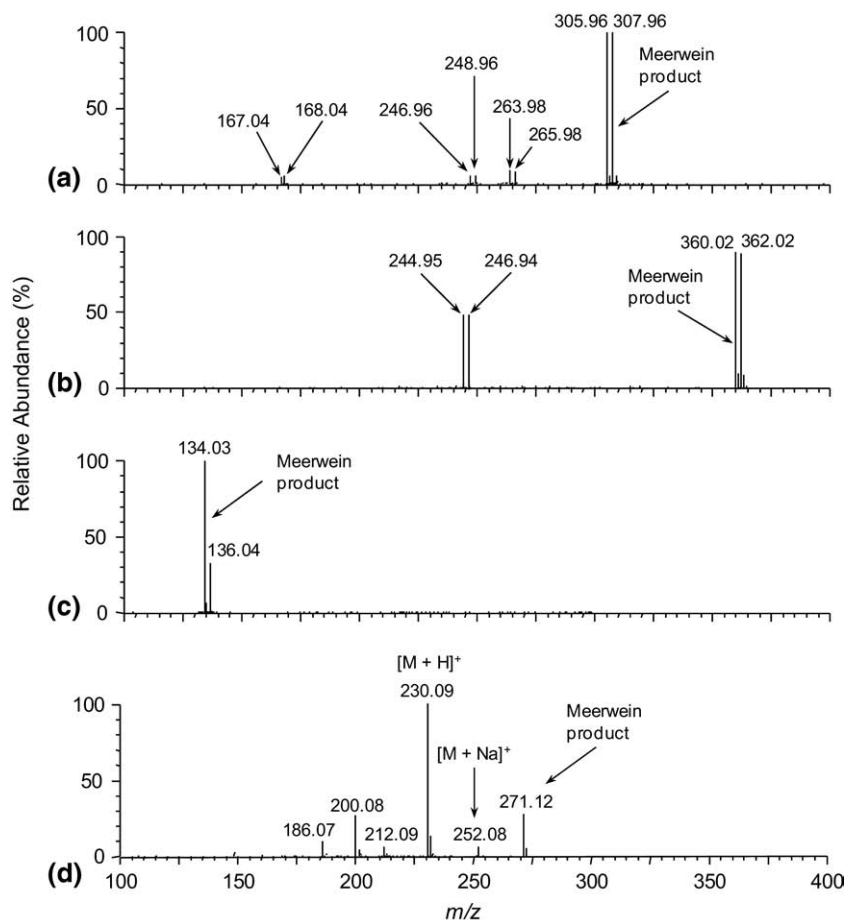
Theoretical calculations were performed based on density functional theory (DFT) using Becke's three-parameter hybrid functional with the Lee, Yang, and Parr correlation (B3LYP) and the 6-311+G(d,p) basis set [29]. Harmonic vibrational frequencies were calculated to characterize the stationary points and to obtain the zero-point vibrational energies (ZPVEs), which were scaled by a factor of 0.96 and incorporated in the final proton affinity (PA) calculations [30]. The Gaussian 2003 program (rev. D.02) was used for all calculations [31].

## Results and Discussion

### Atmospheric Pressure Gas-Phase Meerwein Reaction of Epoxides with Ethylnitrilium Ion

Acetonitrile, one of the most commonly used organic mobile phases in the LC-MS analysis, can form ethylnitrilium ion via protonation under typical ESI, APCI, and APPI conditions. Ethylnitrilium ion ( $\text{CH}_3 - \text{C} \equiv \text{NH}^+$  and its canonical form  $\text{CH}_3 - \text{C} = \text{NH}^+$ ) resembles acylium ions that have been known to

efficiently undergo the gas-phase Meerwein reaction with epoxides in the gas phase [21, 22]. The formation of the Meerwein reaction products of epoxides **I–IV** with ethylnitrilium ion were confirmed by the exact mass measurements within an error of 3.6 ppm. An appropriate atmospheric pressure environment facilitates efficient low-energy ion/molecule reactions in that a reactant ion and its products can be stabilized due to collisional cooling by the surrounding neutral molecules such as nitrogen gas in the API sources. As displayed in Figure 2, three epoxides (**I**, **II**, and **III**) selected in this study exhibit the favorable Meerwein reaction with ethylnitrilium ion under the ESI condition, probably as the result of collisional cooling of the reactant ion and the Meerwein products. Similarly, the Meerwein reaction occurs for all these three epoxides under the APCI and APPI conditions (spectra not shown). Of the two epoxide moieties in **II**, only one was observed to efficiently undergo the Meerwein reaction since it is unlikely for the associated Meerwein reaction product to proceed a further Meerwein reaction with ethylnitrilium ion via the cation ion/cation ion reaction. In the H/D exchange experiment using  $\text{D}_2\text{O}$  instead of



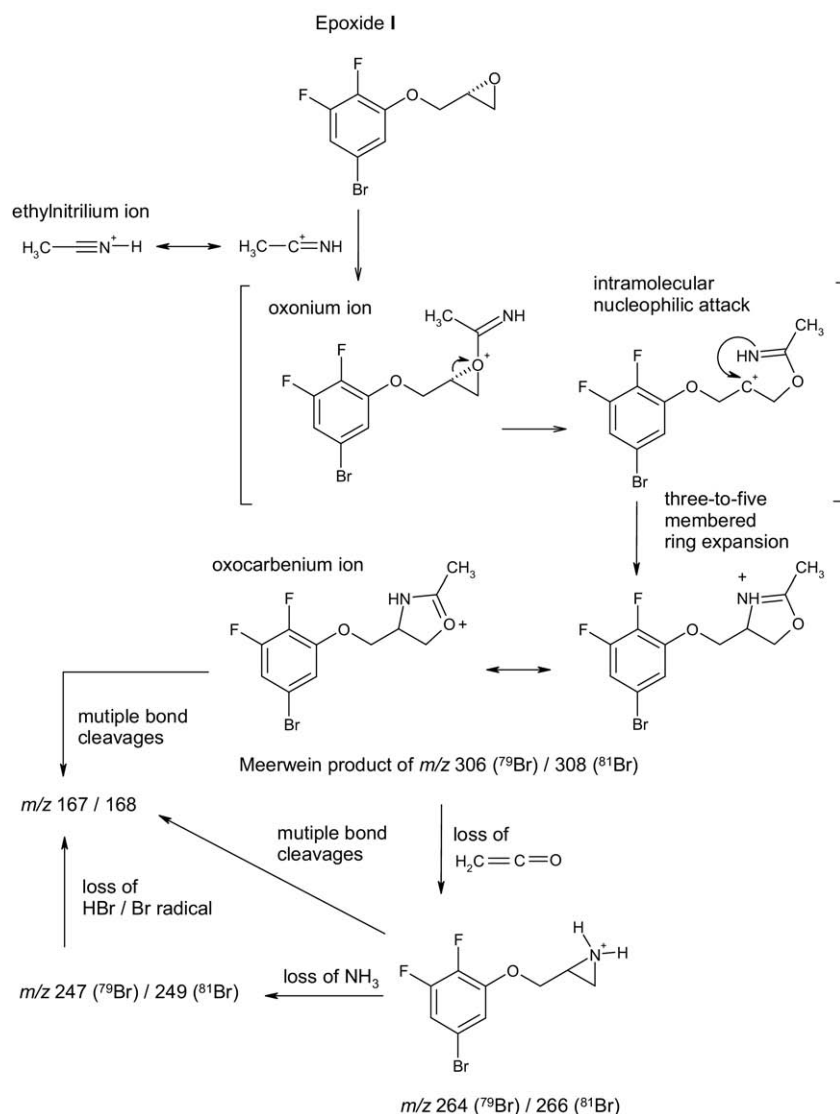
**Figure 2.** ESI-MS spectra of epoxides (a) **I**, (b) **II**, (c) **III**, and (d) **IV** that undergo the atmospheric pressure gas-phase Meerwein reaction with ethylnitrilium ion.

H<sub>2</sub>O as the mobile phase, the protonated molecule of acetonitrile becomes the deuterated species. Since epoxides **I**, **II**, and **III** have no exchangeable proton in their structures, their Meerwein reaction products are expected to exhibit an increase of 1 Da mass unit after H/D. This was confirmed by the experimental results supporting the cyclic structure of the gas-phase Meerwein reaction products [23].

There are some in-source fragmentation ions in the MS spectra of epoxides **I** and **II** (Figure 2a and b). For example, epoxide **I** exhibits the in-source fragmentation ions of *m/z* 264 (<sup>79</sup>Br) and 266 (<sup>81</sup>Br) as the result of the characteristic net oxygen-by-nitrogen replacement in the molecule (Scheme 3). These ions exhibit the further loss of a molecule of NH<sub>3</sub> to yield the ions of *m/z* 247 (<sup>79</sup>Br) and 249 (<sup>81</sup>Br). Epoxide **II**, on the other hand, does not give rise to such an oxygen-by-nitrogen replacement in the ESI source; instead, it undergoes a simple

cleavage of the C–O bond at the side chain affording the in-source fragmentation ions of *m/z* 245 (<sup>79</sup>Br) and 247 (<sup>81</sup>Br). As for epoxide **III** (Figure 2c), no in-source fragment ion was observable since it is a relatively small molecule. Epoxide **IV** mainly yields the proton transfer product along with a significant amount of the gas-phase Meerwein reaction product in addition to some in-source fragment ions (Figure 2d). This is probably due to that the structurally unspecific proton-transfer reaction competes with the structurally specific Meerwein reaction based on the calculated PAs as discussed below.

The calculated PAs of epoxides **I–IV** and CH<sub>3</sub>CN are listed in Table 1. Using DFT at the B3LYP/6-311+G(d,p) level, the calculated PA for CH<sub>3</sub>CN as 188.06 kcal/mol shows an excellent agreement with the experimentally measured data of 188.2 kcal/mol using the kinetic method [32]. This verifies that the chosen



**Scheme 3.** Gas-phase Meerwein reaction of epoxide **I** with ethylnitrilium ion and its major fragmentation pathways.

**Table 1.** The calculated proton affinities (PAs) of epoxides I–IV and CH<sub>3</sub>CN

Compound	$\Delta H$ (kcal/mol) <sup>a</sup>	$\Delta ZPVE$ (kcal/mol) <sup>b</sup>	PA (kcal/mol) <sup>c</sup>
Epoxide I	–187.31	7.41	180.20
Epoxide II	–176.82	7.34	169.78
Epoxide III	–190.39	7.67	183.02
Epoxide IV	–219.24	7.84	211.71
CH <sub>3</sub> CN	–194.42	6.63	188.06 <sup>d</sup>

<sup>a</sup> $\Delta H$  is the enthalpy change for the protonation reaction  $M(g) + H^+(g) = MH^+(g)$  by taking the enthalpy of a proton as 1.482 kcal/mol into account.

<sup>b</sup> $\Delta ZPVE$  is the difference of zero-point vibrational energies for the above protonation reaction.

<sup>c</sup>PA =  $-(\Delta H) - \Delta ZPVE \times 0.96$ .

<sup>d</sup>The measured PA of CH<sub>3</sub>CN by the kinetic method is 188.2 kcal/mol (see reference [32]), which shows an excellent agreement with the calculated PA.

level of theory is appropriate for the calculations of PAs of epoxides I–IV. Acetonitrile, which is the solvent to generate ethylnitrilium ion by protonation, appears to have higher PA than those of epoxides I–III (Table 1). This indicates that the proton transfer reaction is not favored for these compounds. As expected, the structurally unspecific reaction giving rise to the proton transfer or hydride abstraction product was not observed. In contrast, epoxide IV that contains a tertiary amine is expected and confirmed by the theoretical calculation to have higher PA than acetonitrile. Thus it mainly undergoes the proton transfer reaction rather than the gas-phase Meerwein reaction. This is supported by the experimental data (Figure 2d). In summary, a favorable gas-phase Meerwein reaction of epoxides is expected if the epoxide of interest has a lower PA than acetonitrile.

For comparison, ionization and ion/molecule reactions of epoxides I–IV were examined in methanol (instead of acetonitrile), another commonly used organic mobile phase in the LC-MS analysis. The results are summarized in Table 2. As expected, the gas-phase Meerwein reaction does not occur due to lacking the source of ethylnitrilium ion in absence of acetonitrile. Epoxides I, II, and IV afford protonated molecules and/or sodiated species under the ESI, APCI, and APPI conditions, while epoxide III fails to ionize by either protonation or sodiation. Epoxides I and II exhibit more favorable formation of the sodiated species rather than the protonated molecules, probably due to their low PAs but high sodium affinities and strong cation– $\pi$  interaction between the phenyl ring and sodium cation [33, 34]. When a compound has a high PA, protonation will compete with sodiation and this is the case for epoxide IV, which affords the intense protonated molecule in addition to the sodiated species. However, detailed discussion about the favorable formation of sodiated species of epoxides during APCI and APPI when using the CH<sub>3</sub>OH mobile phase is beyond the scope of this study.

**Table 2.** Major ions formed during ESI, APCI, and APPI MS using CH<sub>3</sub>CN and CH<sub>3</sub>OH as the mobile phases

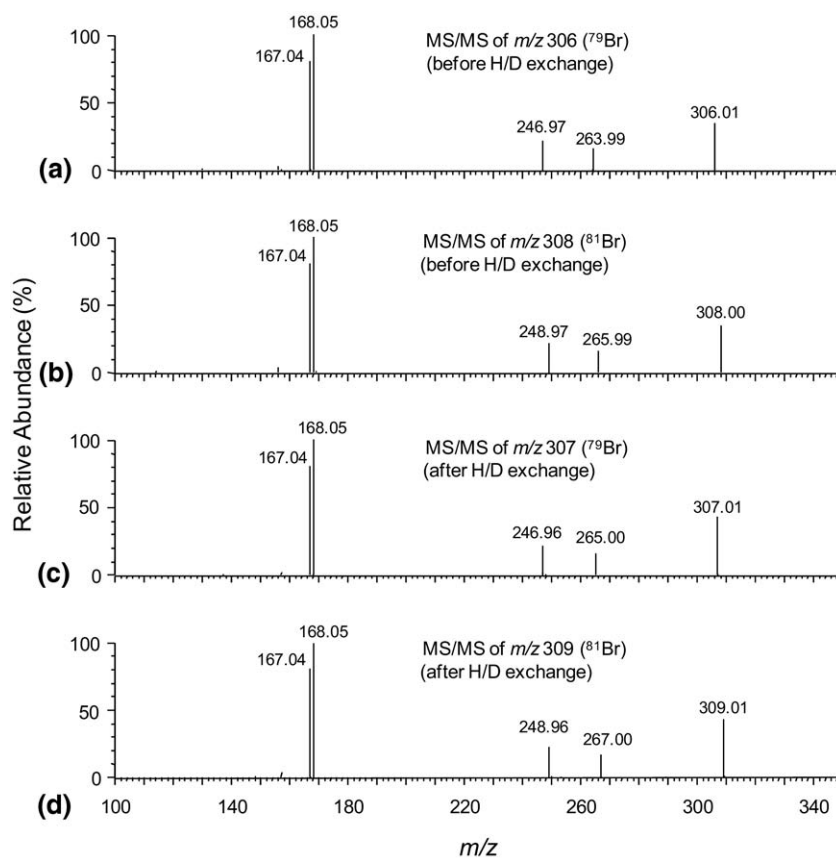
Epoxide	Major ions					
	Organic mobile phase: CH <sub>3</sub> CN		Organic mobile phase: CH <sub>3</sub> OH			
	ESI	APCI	APPI	ESI	APCI	APPI
I	Meerwein products	Meerwein products	Meerwein products	Sodiated species	Sodiated species	Sodiated species
II	Meerwein products	Meerwein products	Meerwein products	Sodiated species	Sodiated species	Sodiated species
III	Meerwein products	Meerwein products	Meerwein products	Not detected	Not detected	Not detected
IV	Protonated/sodiated species and Meerwein products	Protonated/sodiated species and Meerwein products	Protonated/sodiated species and Meerwein products	Protonated/sodiated species	Protonated/sodiated species	Protonated/sodiated species

### CID of the Meerwein Reaction Products

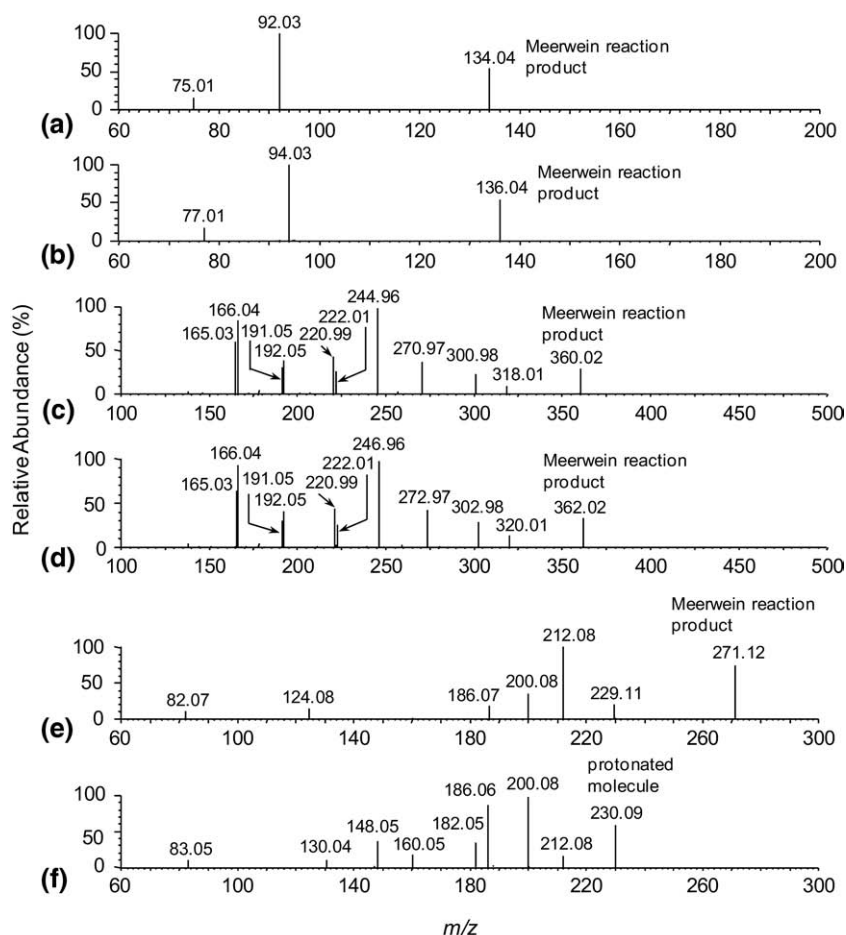
The gas-phase Meerwein reaction products resulting from the reaction of epoxides with ethylnitrilium ion were mass-selected and subjected to CID. To obtain unambiguous structural assignments for epoxides I–III, the precursor ions corresponding to the contributions from the mono isotopes of  $^{79}\text{Br}$  and  $^{81}\text{Br}$  or  $^{35}\text{Cl}$  and  $^{37}\text{Cl}$  before and after the H/D exchange were individually selected for the CID experiments. For epoxide I, the MS/MS spectra were obtained for the precursor ions of  $m/z$  306 ( $^{79}\text{Br}$ )/308 ( $^{81}\text{Br}$ ) (Figure 3a and b) and  $m/z$  307 ( $^{79}\text{Br}$ )/309 ( $^{81}\text{Br}$ ) (Figure 3c and d), which correspond to the protonated and deuterated molecules, respectively. These MS<sup>2</sup> data show one fragmentation pathway via the elimination of ethenone that leads to the net oxygen-by-nitrogen replacement within the molecule of epoxide I. This replacement results in a characteristic mass shift of 1 Da lower than the original epoxide, providing evidence for the cyclic nature of the gas-phase Meerwein product; otherwise CID would reveal the loss of acetonitrile rather than ethenone. Furthermore, as depicted in Scheme 3, the possible pathways that yield the major fragment ions of  $m/z$  167/168 are: (1) the Meerwein product of epoxide I undergoes the multiple bond cleavages and (2) the MS<sup>2</sup> product ion of  $m/z$  264 ( $^{79}\text{Br}$ ) or 266 ( $^{81}\text{Br}$ ) (via the elimination of ethenone) undergoes further fragmentation via the losses of NH<sub>3</sub> and HBr/Br radical. To test this hypoth-

esis, MS<sup>2</sup> of  $m/z$  306 ( $^{79}\text{Br}$ ) or 308 ( $^{81}\text{Br}$ ) and MS<sup>3</sup> of  $m/z$  264 ( $^{79}\text{Br}$ ) (306 → 264) or 266 ( $^{81}\text{Br}$ ) (308 → 266) as well as MS<sup>4</sup> of  $m/z$  247 ( $^{79}\text{Br}$ ) (306 → 264 → 247) or  $m/z$  249 ( $^{81}\text{Br}$ ) (308 → 266 → 249) were performed on the linear ion trap mass spectrometer under the resonant excitation (to avoid further fragmentation). It was found that the MS<sup>2</sup>, MS<sup>3</sup>, and MS<sup>4</sup> experiments can afford the fragment ions of  $m/z$  167/168, supporting the proposed fragmentation pathways occurring in the Q-TOF mass spectrometer as described in Scheme 3. It is worth noting that such fragmentation pathways were also observed to occur in the API sources, although the level of fragmentation was much less. As for epoxide III, fragmentation of its Meerwein reaction product predominantly undergoes the elimination of ethenone followed by a further loss of NH<sub>3</sub> (Figure 4a and b).

Increased structural complexity with the presence of other functional groups or substituents (when placed at positions that favor specific bond cleavages) induces other competitive dissociations. This is exemplified by the fragmentation of the Meerwein product of epoxide II (Figure 4c and d) with a more complex MS/MS spectrum consisting of a set of structurally characteristic fragment ions. Similar to epoxides I and III, the elimination of ethenone was observed to give rise to the characteristic product ion of  $m/z$  318 ( $^{79}\text{Br}$ ) or 320 ( $^{81}\text{Br}$ ) that undergoes a further loss of NH<sub>3</sub> to yield the product ion of  $m/z$  301 ( $^{79}\text{Br}$ ) or 303 ( $^{81}\text{Br}$ ). There is a



**Figure 3.** MS/MS spectra of the Meerwein reaction product of epoxide I of (a)  $m/z$  306 ( $^{79}\text{Br}$ ) in H<sub>2</sub>O, (b)  $m/z$  308 ( $^{81}\text{Br}$ ) in H<sub>2</sub>O, (c)  $m/z$  307 ( $^{79}\text{Br}$ ) in D<sub>2</sub>O, and (d)  $m/z$  309 ( $^{81}\text{Br}$ ) in D<sub>2</sub>O.



**Figure 4.** MS/MS spectra of the Meerwein reaction products of epoxide **III** of (a)  $m/z$  134 (<sup>35</sup>Cl) and (b)  $m/z$  136 (<sup>37</sup>Cl), epoxide **II** of (c)  $m/z$  360 (<sup>79</sup>Br) and (d)  $m/z$  362 (<sup>81</sup>Br), and epoxide **IV** of (e)  $m/z$  271 as well as the protonated molecule of epoxide **IV** of (f)  $m/z$  230.

fragmentation pathway possibly via the C–O bond cleavage of the side chain generating the major fragment ion of  $m/z$  245 (<sup>79</sup>Br) or 247 (<sup>81</sup>Br) (Scheme 4). In addition, the further fragmentation via the sequential losses of HBr and Br radical were observed for the fragment ions of  $m/z$  301 (<sup>79</sup>Br) or 303 (<sup>81</sup>Br),  $m/z$  271 (<sup>79</sup>Br) or 273 (<sup>81</sup>Br), and  $m/z$  245 (<sup>79</sup>Br) or 247 (<sup>81</sup>Br) to yield the ion pairs of  $m/z$  221/222, 191/192, and 165/166, respectively.

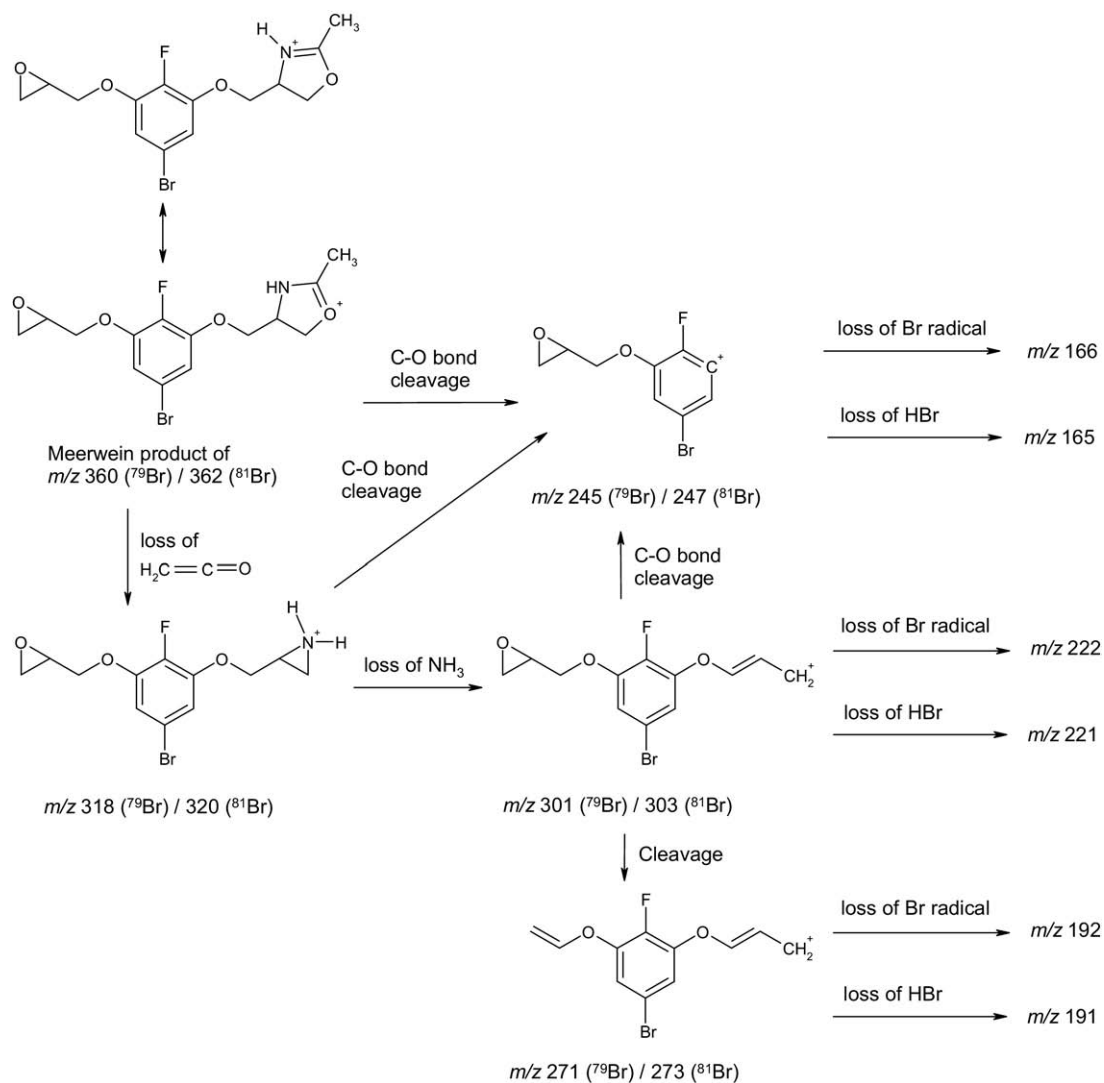
Epoxide **IV** can form both the gas-phase Meerwein reaction product ( $m/z$  271) and the protonated molecule ( $m/z$  230). Both of which were subject to CID and the MS<sup>2</sup> spectra are displayed in Figure 4e and f, respectively. Akin to epoxides **I–III**, CID of the Meerwein reaction product of epoxide **IV** ( $m/z$  271) gives rise to the product ion of  $m/z$  229 via the elimination of ethenone. As shown in Scheme 5, the other fragmentation pathways corresponding to  $m/z$  271 (e.g., giving the fragment ion of  $m/z$  124) and  $m/z$  230 (e.g., yielding the fragment ions of  $m/z$  182, 160, 148, and 130) appear to be quite different. This is probably due to the charge-remote fragmentation that occurs to the

Meerwein reaction product with the fixed charge versus the mobile proton (charge) in the protonated molecule [35].

#### Energetics of the Gas-Phase Meerwein Reaction

Taking epoxide **I** as an example, the energetics of the gas-phase Meerwein reaction with ethylnitrilium ion was calculated and the potential energy surface diagram is depicted in Figure 5. Similar to acylium and thioacylium ions [21], the Meerwein reaction with ethylnitrilium ion is highly exothermic (by  $-75.76$  kcal/mol) and hence the reaction is thermodynamically favorable. This is possibly owing to the more stable five-membered ring of the Meerwein reaction product than the three-membered ring of epoxide **I** (reactant). The calculated energies (relative to the energy of the reactant) of intermediates **A** and **B** are  $-12.96$  and  $10.84$  kcal/mol, respectively. This indicates that the energy required for the occurrence of the Meerwein reaction is relatively small. In contrast, the dissociation of the Meerwein reaction product needs to overcome  $58.04$





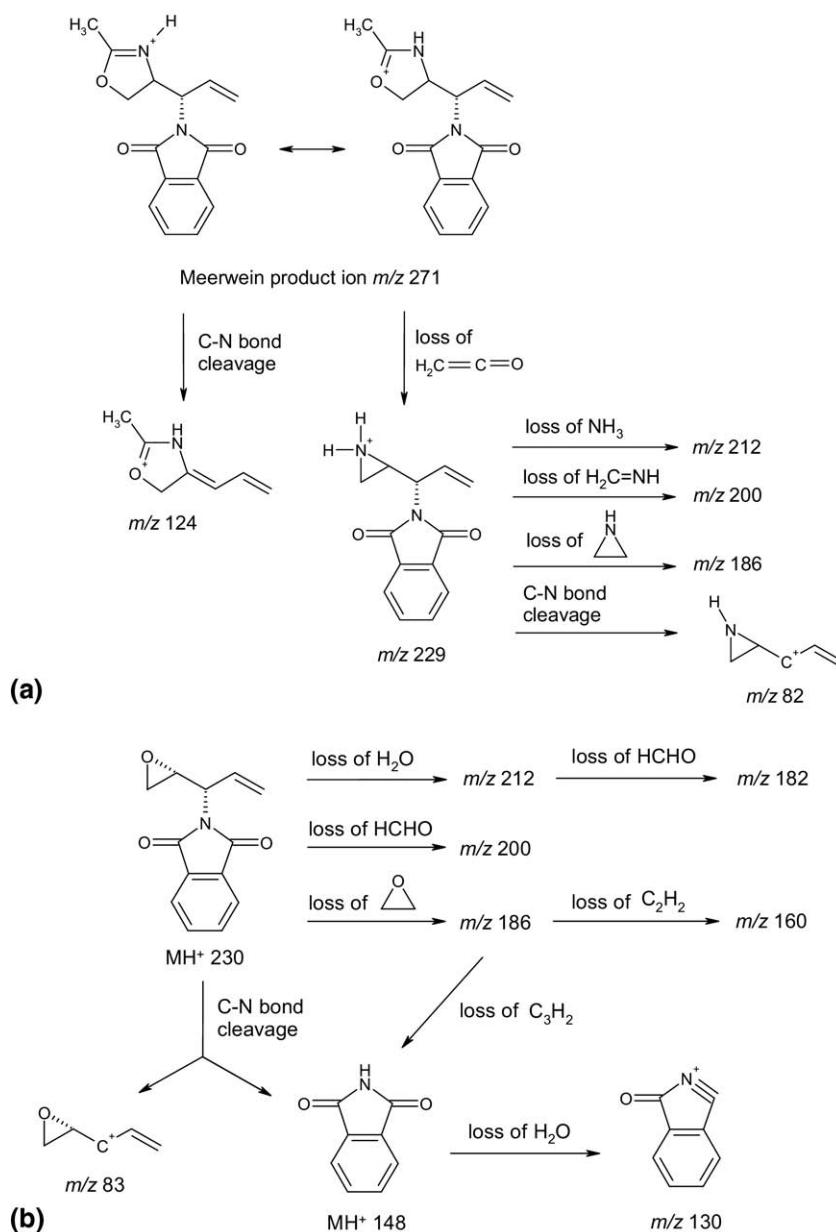
**Scheme 4.** Major fragmentation pathways of the gas-phase Meerwein reaction product of epoxide II with ethylnitrium ion.

kcal/mol energy barrier to give rise to the net oxygen-by-nitrogen replacement product of  $m/z$  264 ( $^{79}\text{Br}$ ) or 266 ( $^{81}\text{Br}$ ) along with ethenone. The theoretical calculations also suggest that the gas-phase route to convert epoxides into their nitrogen analogs via the elimination of ethenone upon CID is a more thermodynamically favorable process (by  $-17.72$  kcal/mol) than reverting to the original epoxide I. The calculated energetics for promoting the net oxygen-by-nitrogen replacement is in agreement with the reported value ( $-16.8$  kcal/mol) for leading to the net oxygen-by-sulfur replacement [21].

#### Potential Applications in Quantitative Analysis of Epoxides Using the Gas-Phase Meerwein Reaction

Although a number of GC-MS and LC-UV-MS methods have been developed for analysis of genotoxic epoxides, obtaining a highly sensitive and selective method for quantitation of such class of GTIs at trace levels (ppm)

in drug substances and products is still an ongoing challenge [36]. Recently, a chemical derivatization approach was reported where epoxides can be converted into tertiary amines by reacting with dimethylamine (DMA) as the derivatization agent [37]. The advantage of the method is that it not only introduces an ionizable site into the analyte for protonation but also stabilizes certain epoxides if unstable. Chemical derivatization has been used successfully to quantify several epoxides in various active pharmaceutical ingredients. The gas-phase Meerwein reaction of epoxides with ethylnitrium ion generated in the API-MS sources, on the other hand, offers an alternative approach enabling direct and selective analysis of epoxides. It takes advantage of the use of acetonitrile in the LC-MS mobile phase, thus eliminating the extra chemical derivatization step. The feasibility has been demonstrated herein using epoxide I as the model compound. A solution of epoxide I at a

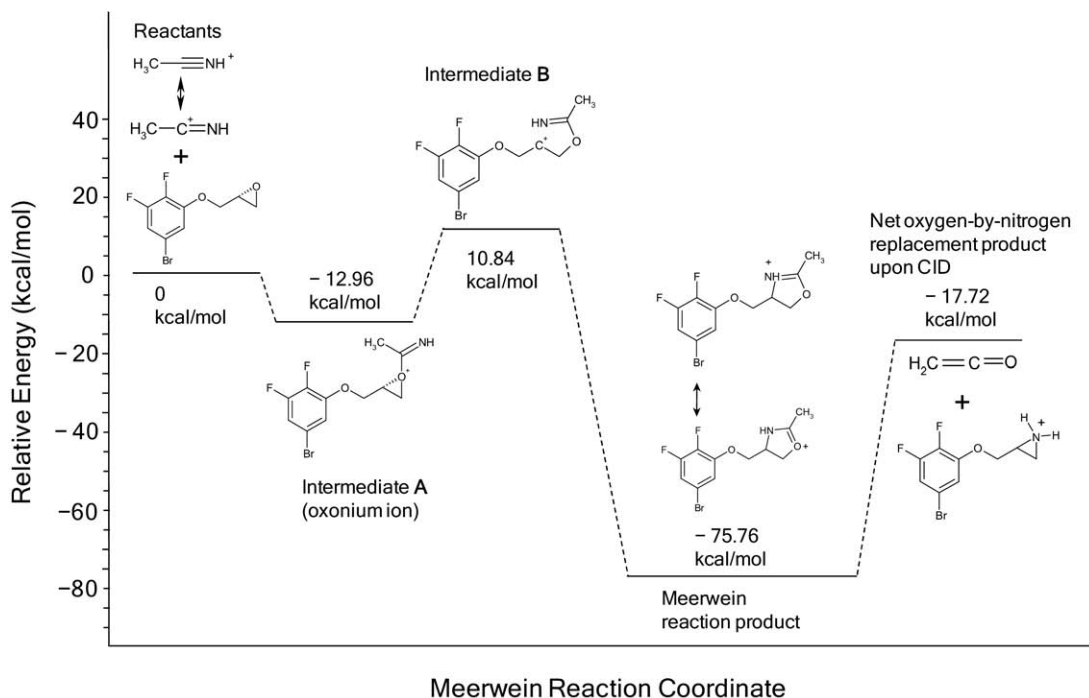


**Scheme 5.** Major fragmentation pathways of (a) the gas-phase Meerwein reaction product of epoxide IV with ethylnitrilium ion and (b) the protonated molecule of epoxide IV.

concentration level as low as 0.8 ng/mL was detected with a signal-to-noise ratio (S/N) of 21 (Figure 6). A linear calibration curve from 0.8 to 40 ng/mL was achieved ( $R^2 > 0.994$ , data not shown) using the SIM detection method by monitoring the Meerwein reaction product of I [ $m/z$  306 ( $^{79}\text{Br}$ ) or 308 ( $^{81}\text{Br}$ )]. Moreover, if the MRM detection is employed on a triple quadrupole instrument, one can take the advantage of the characteristic fragment pathways (e.g., that lead to the net oxygen-by-nitrogen replacement upon CID of the Meerwein reaction product) to enhance the detection selectivity. Similar sensitivity and linearity ( $R^2 > 0.996$ ) by utilizing the MRM transition  $m/z$  306  $\rightarrow$  264 ( $^{79}\text{Br}$ ) or  $m/z$  308  $\rightarrow$  266 ( $^{81}\text{Br}$ ) were achieved (data not shown).

## Conclusions

Epoxides are shown to efficiently undergo of the gas-phase Meerwein reaction with ethylnitrilium ion generated during the LC-MS analysis under the typical conditions of ESI, APCI, and APPI, resulting in a gain of 42 Da to the epoxides in the mass spectra. This should not be erroneously interpreted as a  $\text{CH}_3\text{CN}$ -adduct ion although it has the same  $m/z$ . This Meerwein reaction proceeds by the initial formation of an oxonium ion followed by rapid ring opening, and then ring closure that is promoted by an intramolecular nucleophilic attack. These steps result in three-to-five-membered ring expansion to yield the Meerwein reaction product.



**Figure 5.** The DFT calculated potential energy surface diagram for the gas-phase Meerwein reaction of epoxide I with ethylnitrilium ion followed by the elimination of ethenone upon CID.

The theoretical calculations show that the gas-phase Meerwein reaction is highly exothermic (e.g., by  $-75.6$  kcal/mol for epoxide I); therefore, it is thermodynamically favorable. When the reaction product is subjected to CID, ethenone is eliminated, constituting a gas-phase route to convert epoxides into their nitrogen analogs. This net oxygen-by-nitrogen replacement results in a characteristic mass shift of 1 Da, providing evidence for the cyclic nature of the gas-phase Meerwein products. Understanding of such a gas-phase ion/molecule reaction can help improve the sensitivity and selectivity for the detection of epoxides. The gas-phase Meerwein reaction of epoxides with ethylnitrilium ion not only

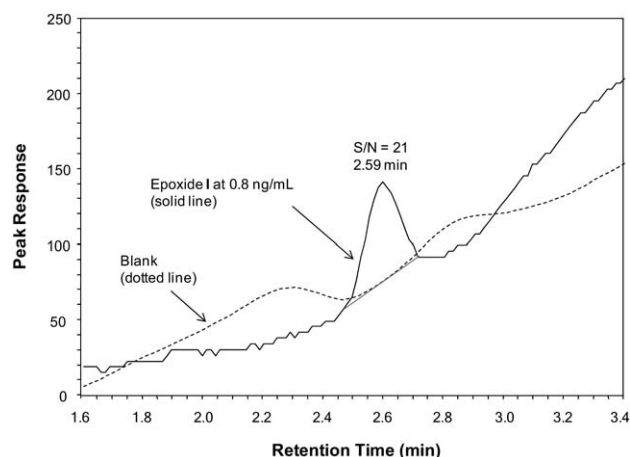
provides a means for structural identification but also offers a novel and fast approach for the direct LC-MS analysis of epoxide pharmaceutical impurities that are of genotoxic concern. A real world application for the quantitation by SIM and MRM is yet to be demonstrated but the proof of concept with respect to detecting epoxide I at sub-ng/mL concentration has been achieved.

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**Figure 6.** Overlay chromatograms of the standard of epoxide I at 0.8 ng/mL and the blank monitored by SIM at  $m/z$  306 ( $^{79}\text{Br}$ ).

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