Title: **CID fragmentation of deprotonated N-acyl aromatic sulfonamides. Smiles-type and nitrogen-oxygen rearrangements**

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Abstract: The NIST tandem mass spectral library (2020 version) includes over 800 aromatic sulfonamides. In negative mode, upon collisional activation most benzenesulfonamides lose a neutral SO_2 molecule leading to an anilide anion ($C_6H_5NH^-$, *m/z* 92). However, for deprotonated N-benzoyl aromatic sulfonamides, the phenoxide ion ($C_6H_5O^-$, *m/z* 93.0343) is the principal product ion. A variety of N-acyl benzenesulfonamide derivatives were also found to overwhelmingly produce the phenoxide ion as the most intense product ion. A mechanism is proposed in which, at low energy, a carbonyl oxygen atom (C=O) is transferred to benzene ring, known as a Smiles-type rearrangement (the amide oxygen atom attacks the arylsulfonyl group at the ipso position), in parallel and determining the reaction at high energy a nitrogen-oxygen rearrangement mechanism leads to the formation of the phenoxide ion. Tandem mass spectra of deprotonated N-benzoyl-^{18}O-benzenesulfonamide and N-thiobenzoyl-p-toluenesulfonamide confirmed the rearrangement since base peaks at *m/z* 95.0384 and *m/z* 123.0270 which correspond to an ^{18}O phenoxide ion ($[C_6H_5{}^{18}O]^-$) and a 4-methylbenzenethiolate anion ($[CH_3C_6H_4S]^-$) were observed, respectively. The parallel mechanism is supported by the strong correlation between the observed product ion intensities and the corresponding activation energies obtained by Density Functional Theory calculations. This is an example of a relatively simple ion with a complex path to fragmentation, being a cautionary tale for indiscriminate use of in-silico spectra in place of actual measurement.

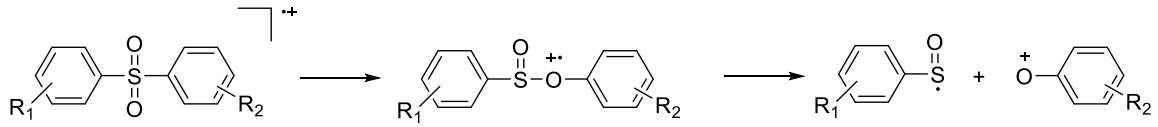
Introduction

Aromatic sulfonamides and their derivatives play an important role as organic intermediates for drug synthesis in the pharmaceutical industry and have been widely used as antibacterial compounds for the treatment of diseases in livestock production since their antibacterial activity was first discovered in 1935.[1] Consequently, the increased use of sulfonamides causes residue accumulation in dairy, eggs and meat, which leads to the antibiotic resistance of many bacteria.[2] Liquid chromatography in conjunction with electrospray tandem mass spectrometry (LC-MS/MS) provides an excellent analytical tool for characterization and quantification of these sulfonamide compounds.[3-5] In order to enhance unknown compounds analysis by mass spectrometry, spectral libraries were employed for fast, reliable identification of compounds which have been measured by instruments with different fragmentation methods. For instance, NIST tandem MS library provides reference mass spectral data for the identification of various compounds through the fragmentation of their ions generated by electrospray ionization and the current version (2020) includes 1.3 M spectra of 186 K precursor ions from 31 K chemical compounds.[6] We have measured the tandem mass spectra of many aromatic sulfonamides with Higher-energy Collision Dissociation (HCD) and ion trap fragmentations in positive and negative modes and these reference

spectra have been included into the latest version of the NIST tandem MS library. Of these aromatic sulfonamides, N-acyl sulfonamides represent an important structural motif in drug synthesis as several drugs including inhibitors of tRNA synthetases function as antibacterial agents and therapeutic agents for Alzheimer's disease.[7-8]

In general, deprotonated aromatic sulfonamides are prone to lose a neutral sulfur dioxide molecule to form an anilide anion in gas phase.[9-10] But a deprotonated N-benzoyl aromatic sulfonamide was found to generate a phenoxide ion instead which corresponds to a peak at *m/z* 93 as recorded in the tandem mass spectrum. This required an oxygen transfer to the benzene ring is an unusual rearrangement in collision induced dissociation (CID). Few papers reported similar rearrangements, all of which occurred within fragmentation of protonated sulfonamides. Jiang and coworkers[11] reported a novel oxygen transfer pathway in the dissociation of protonated N-phenyl p-toluenesulfonamides, whereby an oxygen atom of sulfonyl group is transferred to the ortho, meta, or para position of the aniline ring in the CID process. Zu[12] reported an unprecedented reaction via N-O exchange in the CID experiments of protonated N-(3-aminophenyl)benzamide. The carbonyl oxygen was transferred to the aniline ring by a water molecule migration rearrangement. The authors stated that the finding was the first report of the N-O exchange pathway of protonated N-(3-aminophenyl)benzamides observed through MS/MS experiments.

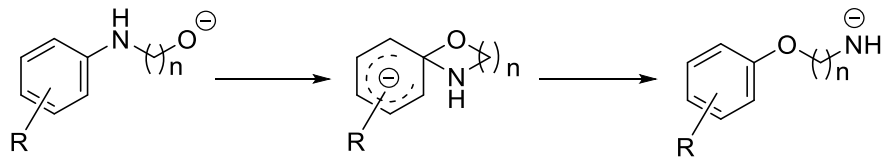
In electron ionization, there are some reports about the sulfonyl oxygen transfer to benzene ring. EI mass spectra of diaryl sulfones and alkyl aryl sulfones have been interpreted partly in terms of a rearrangement in which an aryl group migrates from sulfur to oxygen. This rearrangement is also known as Meyerson's rearrangement (Scheme 1).[13-15]



Scheme 1. Meyerson rearrangement

However, migrations of carbonyl oxygens are rare. Recently, Irikura and Todua[16] studied the fragmentation process of N-acylarylsulfonamide radical cations under electron ionization conditions and concluded that it follows a Smiles-type rearrangement. The authors showed the amide oxygen atom of N-acylarylsulfonamides attacks the arylsulfonyl group at the ipso position, displacing a molecule of SO_2. In synthetic organic chemistry, the Smiles rearrangement reaction

has been known for several decades, a simplified version of the base-catalyzed Smiles rearrangement[17] is presented in Scheme 2. In LC-MS/MS field, Bowie reported the gas-phase Smiles rearrangement of $ArX(CH_2)_nO^-$ (X = O or S) ions with a joint theoretical and experimental approach, the extent of the Smiles process decreases as n increases. [18-19]



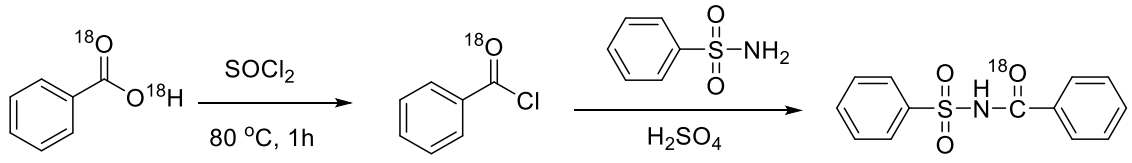
Scheme 2. Base-catalyzed Smiles rearrangement

While the above mass spectrometry studies dealt with protonated and radical cation of sulfonamides, we investigated the fragmentation of deprotonated N-acyl aromatic sulfonamides and their derivatives in electrospray ionization and propose a concomitant Smiles and N-O rearrangement mechanisms which involve a carbonyl oxygen migrating to a benzene ring.

Experimental

Materials

Acetonitrile and water (HPLC grade) were purchased from Honeywell - Burdick & Jackson (Muskegon, MI, USA). Formic acid solution (50 % in water) was purchased from Fluka (Charlotte, NC, USA). Thionyl chloride and benzenesulfonamide was purchased from Sigma-Aldrich (St. Louis, MO, USA). $^{18}O_2$-labeled benzoic acid was synthesized according to literature.[20]



Scheme 3. Synthesis of N-benzoyl-^{18}O-benzenesulfonamide

Preparation of benzoyl-^{18}O-chloride

To a 5 mL glass vial was added $^{18}O_2$-labeled benzoic acid (20 mg, 0.16 mmol). Thionyl chloride (100 µL) was added to the vial and heated to 80 ℃ for 1 h. The reaction mixture was cooled to room temperature and dried under nitrogen gas (Scheme 3).

General procedure for preparation of N-acyl aromatic sulfonamides [21]

Under a nitrogen atmosphere a solution of benzenesulfonamide (20 mg, 0.13 mmol) and acyl chloride (0.19 mmol) in acetonitrile (50 µL) was stirred at 60 ℃ and treated with 98 % sulfuric acid (0.5 µL). The solution was maintained at 60 ℃ for 1 h. The solvent was removed under nitrogen and the residue was dried in vacuum.

Mass Spectrometry

N-Acyl aromatic sulfonamides were dissolved in acetonitrile/water/formic acid (50 : 50 : 0.1, v:v:v) at a concentration of about 0.1 mg/mL and directly infused into an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts) via a nano electrospray source. Ion trap (IT-CID and FT-CID) and HCD (Higher Energy Collision Dissociation) spectra were acquired in the negative mode. The gases N_2 (99.999 %) and He (99.999 %) were utilized as collision gases for the HCD and IT spectra, respectively. Ion trap spectra were acquired at a normalized collision energy (NCE) of 35 %. The HCD spectra were collected by using various normalized collision energies. The resolution for MS^2 was set at 30,000. Spectra were acquired in 'profile' mode for both FT-CID and HCD.

Computational methods

Several plausible reaction mechanisms for the fragmentation of deprotonated N-benzoylbenzenesulfonamide were examined using the semiempirical PM3[22-24] potential energy surface. Then, Density Functional Theory (DFT) calculations were performed on the most satisfactory mechanisms using the hybrid density functional method B3LYP[25-26] in conjunction with the Pople's basis set[27] [6-311++g(d,p)] as implemented in Gaussian 09[28]. Frequency analysis at the same level of theory was used to identify the optimized structures as minima or transition structures on the potential energy surface. Intrinsic reaction coordinate (IRC) calculations[29] were performed to confirm that specific transition states were connected to the designated local minima. These calculations have been used in previous studies of ion fragmentation of small molecules under CID conditions[30-32].

Results and discussion

*Comparison of mass spectra of N-phenylbenzenesulfonamide **1a** with N-benzoylbenzenesulfonamide **1b***

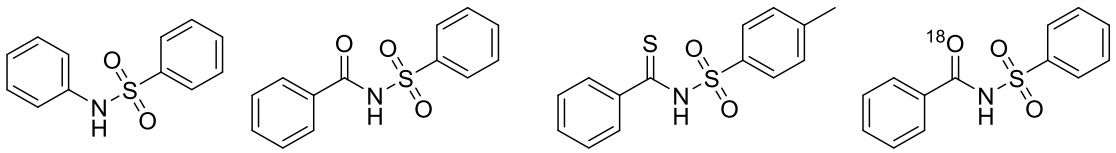


Figure 1. Molecular structures of *N*-phenyl benzenesulfonamide ***1a***, N-benzoylbenzenesulfonamide ***1b***, N-thiobenzoylbenzenesulfonamide ***1c*** and N-benzoyl-^{18}O-benzenesulfonamide compound ***1d***.

Aromatic sulfonamides derived from primary amines tend to lose a proton to generate gaseous anions under appropriate ESI conditions. However, compared with studies of protonated forms, few investigations have been carried out on the fragmentation of deprotonated sulfonamides. Generally, such fragmentations are characterized as loss of a neutral sulfur dioxide molecule by an intramolecular rearrangement process and subsequent fragmentation to form an anilide anion at *m/z* 92. For example, in 2013, Attygalle[3] reported that deprotonated *N*-phenyl benzenesulfonamide ***1a*** (Figure 1), at *m/z* 232, undergoes the loss of a neutral SO_2 to form the peak at *m/z* 168 which further fragments to generate the ion peak at *m/z* 166 by expelling a H_2 molecule (Figure ***2a***). Alternatively, the *m/z* 232 precursor may also lose a benzyne and undergo subsequent sulfur dioxide elimination to afford the anilide anion of *m/z* 92 which is the second highest peak.

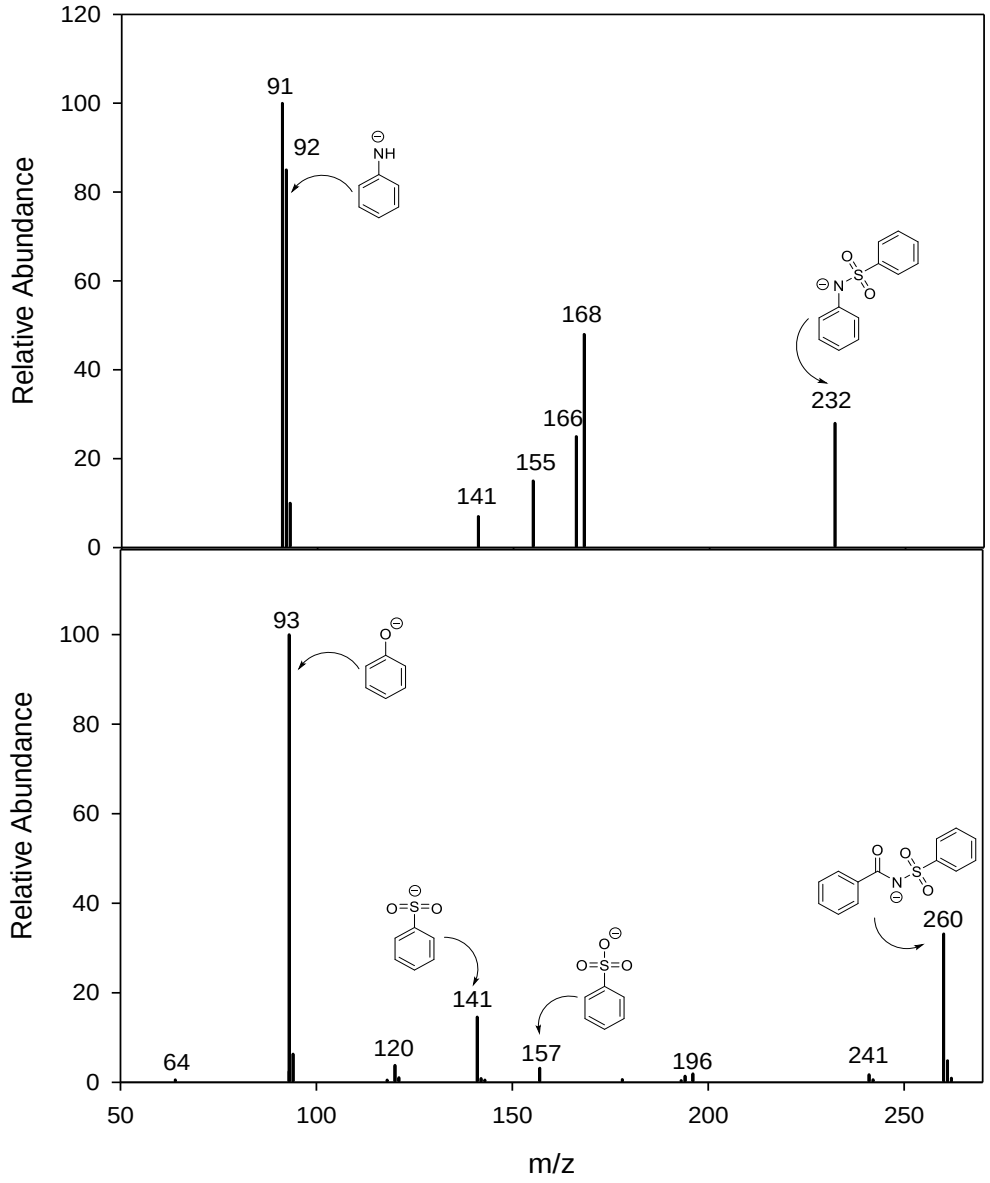


Figure 2. a) Tandem mass spectrumof deprotonated N-phenyl benzenesulfonamide ***1a*** (precursor, *m/z* 232, top), b) Tandem mass spectrum of deprotonated N-benzoylbenzenesulfonamide ***1b*** (precursor, *m/z* 260, bottom).

When a carbonyl group is introduced into the benzenesulfonamide molecule, i.e. N-benzoylbenzenesulfonamide ***1b***, the CID spectrum shows the phenoxide ion of *m/z* 93.0343 as the most abundant ion and there is no anilide peak at *m/z* 92 (Figure ***2b***). In addition to the phenoxide

ion peak and the deprotonated ***1b*** precursor peak at *m/z* 260, five distinctive product ion peaks are observed in the ESI tandem mass spectrum: 1) the ion of *m/z* 241 ($[C_{13}H_7NO_2S]^{-\cdot}$, fragment ion after loss of a H_2O molecule and a hydrogen radical from precursor); 2) the ion of *m/z* 196 ($[C_{13}H_{10}NO]^-$, fragment ion after loss of a SO_2 molecule from precursor); 3) the ion of *m/z* 157 ($[C_6H_5O_3S]^-$, a benzenesulfonate ion); 4) the ion of *m/z* 141 ($[C_6H_5O_2S]^-$, a benzenesulfinate ion); 5) the ion of *m/z* 120 ($[C_6H_5CONH]^-$, a benzamide anion formed via cleavage of sulfonamide bond).

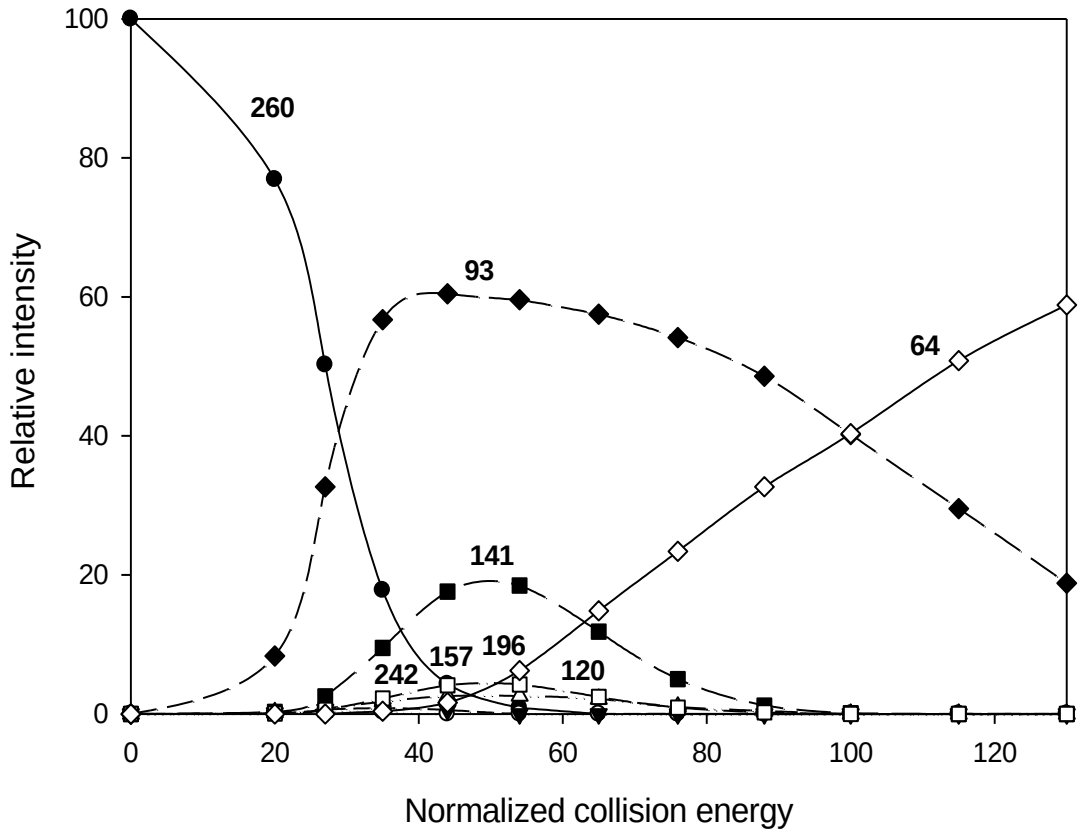
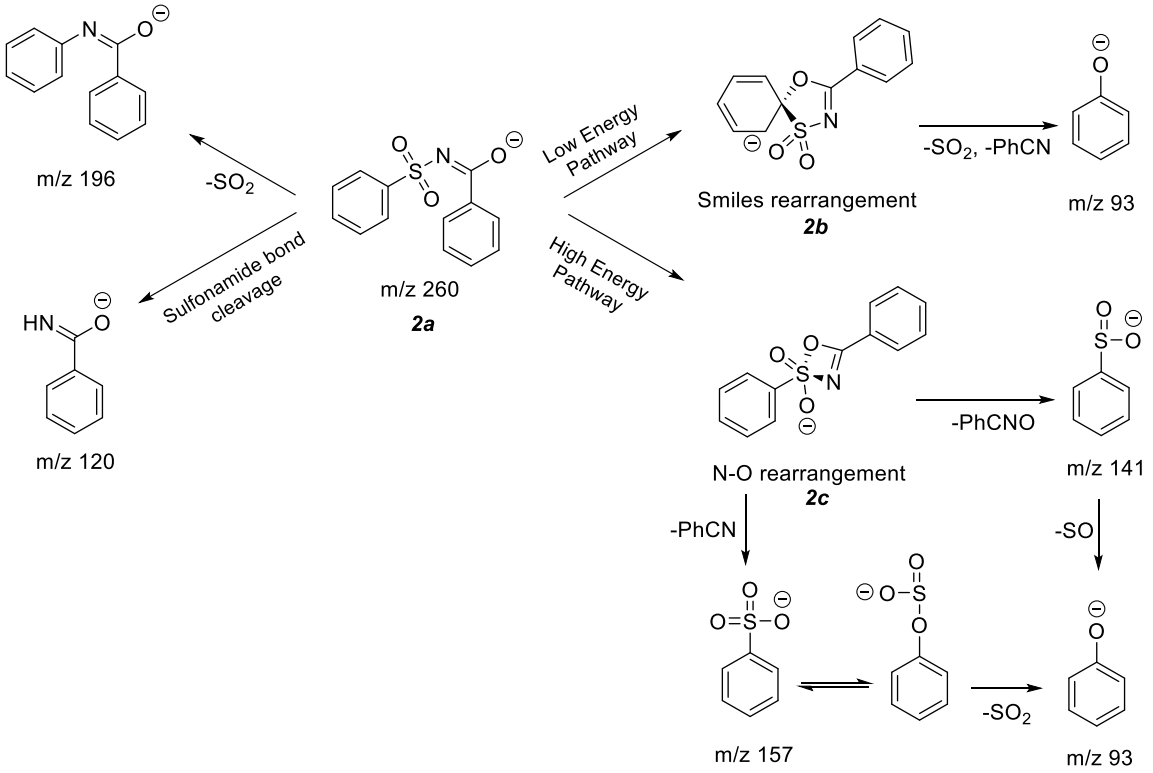


Figure 3. Relative product ion abundances as a function of normalized collision energies in the HCD tandem mass spectrum of deprotonated N-benzoylbenzenesulfonamide ***1b*** in the orbitrap instrument. Abundances are normalized to total ion current. Each curve is identified by the m/z value of the corresponding product ion (*m/z* 260 is the precursor ion).

Figure **3** shows the dependence of the relative intensities of the main peaks in the spectrum of deprotonated ***1b*** on HCD collision energy in the Orbitrap mass spectrometer. The first peak to appear at low collision energy is the phenoxide ion at *m/z* 93. At the next higher energy, its relative intensity increases to 33 %, while the other peaks at *m/z* 141 and *m/z* 157 begin to appear

concurrently. The intensity of the *m/z* 93 peak reaches its maximum level at 59 % under a collision energy of 44 % and maintains the highest level across most of the energy range. This phenomenon that the phenoxide ion dominates in the spectrum of ***1b*** indicates that the oxygen transfer rearrangement to benzene ring occurs rapidly and is energetically favorable. The ions at *m/z* 141 and at *m/z* 157 reach their maximum abundances at about the same collision energy, but the value for the former ion (relative intensity 18 %) is nearly seven times greater than that of the latter ion (relative intensity 2 %), i.e., the formation of benzenesulfinate ion is more prominent than that of benzenesulfonate ion. The peak at *m/z* 64 appears at much higher energy and is ascribed to sulfur dioxide radical anion as confirmed by high accuracy HCD spectra. It is noteworthy that the *m/z* 157 ion is an unexpected product ion since an additional oxygen atom is attached to the benzenesulfonyl group to form the benzenesulfonate ion which can then fragment to generate the ion of *m/z* 93 by a neutral loss of SO_2 molecule, as confirmed by MS^3 experiments.[33] This pathway for formation of the phenoxide anion represents another unique oxygen transfer rearrangement as it cannot account for the observed rapid formation of phenoxide at low collision energies as shown in Figure 3. Since N-benzoyl benzenesulfonamide ***1b*** contains an additional carbonyl group as compared to N-phenyl benzenesulfonamide ***1a***, we assume that the oxygen atom of the carbonyl group is transferred to the benzenesulfonyl moiety to form the benzenesulfonate ion at *m/z* 157.



Scheme 4. Proposed mechanism to rationalize the formation of the phenoxide ion at *m/z* 93 and other product ions from deprotonated N-benzoyl benzenesulfonamide.

Reaction mechanism

Based on these findings, we propose the Smiles-type and N-O rearrangement mechanisms to explain how the oxygen atom was transferred to form the unexpected fragment ions. As shown in Scheme 4, when N-benzoyl benzenesulfonamide ***1b*** is subjected to ionization within the ESI source in negative mode it loses a proton from the amide NH to produce the precursor ion ***2a*** at *m/z* 260. Since the negative charge may be shifted from the nitrogen atom to the amide oxygen atom, which attacks the arylsulfonyl group at the ipso position via a five-membered ring intermediate ***2b***, it is a Smiles-type rearrangement with a low energy pathway. Subsequent rapid neutral losses of SO_2 and benzonitrile result in the most abundant phenoxide ion at *m/z* 93. N-O rearrangement takes place with a high energy pathway as the amide oxygen anion attacks the sulfur atom via an intramolecular nucleophilic reaction to generate the four-membered ring intermediate anion ***2c***, which undergoes loss of a neutral benzonitrile (C_6H_5CN) molecule under collision conditions to form the benzenesulfonate ion at *m/z* 157, whose MS^2 spectrum shows rapid loss of a neutral SO_2 molecule to produce the phenoxide ion at *m/z* 93. The intermediate anion ***2c*** may also generate the benzenesulfinate ion at *m/z* 141, which further fragments to form the phenoxide ion by loss of a neutral SO via a Meyerson-type rearrangement.[15]

*Tandem mass spectra of N-thiobenzoylbenzenesulfonamide **1c***

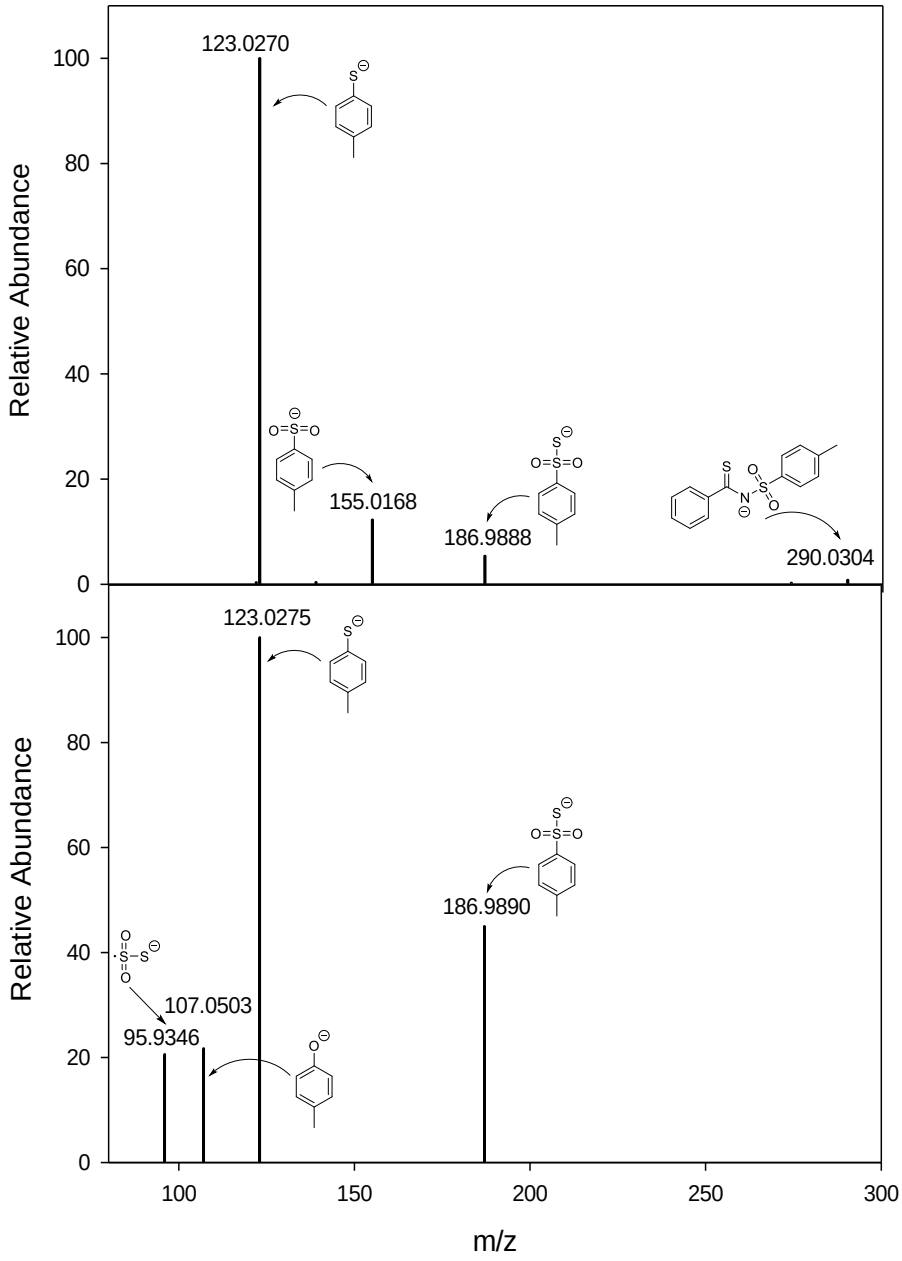


Figure 4. a) Tandem mass spectrum of deprotonated N-thiobenzoyl-p-toluenesulfonamide ***1c*** (precursor, *m/z* 290.0304, top), b) MS^3 spectrum of its product ion at *m/z* 186.9888 (bottom).

To support this hypothesis, we searched for similar compounds in the newly released NIST tandem library and found a variety of sulfonamide derivatives. One of the search results is a compound ***1c***, where the benzoyl group of ***1b*** is replaced with a thiobenzoyl group (Figure 1), which allows us to investigate whether the sulfur atom could be transferred to the ipso position or the sulfonyl group

and subsequently fragments to form the thiophenol anion in the collision cell. The deprotonated ***1c*** ion was subjected to fragmention in ion trap at collision energy of 35 %, followed by analysis in Orbitrap analyzer, and the resulting spectrum is shown in Figure ***4a***. The most abundant ion peak is at *m/z* 123.0270 which corresponds to 4-methylbenzenethiolate anion ($[CH_3C_6H_4S]^-$), where the sulfur atom in C=S is migrated to the toluene ring. Similar to the spectrum of compound ***1b***, the toluenesulfinate ion ($[C_7H_7SO_2]^-$) at *m/z* 155.0168 is generated with a relative abundance of 18 % comparable to that of benzenesulfinate ion at *m/z* 141 mentioned before. We also noticed the benzenesulfonate-like anion ($[C_7H_7S_2O_2]^-$) at *m/z* 186.9888, where a sulfur atom is attached to the sulfonyl group to form S-S bond as shown in Figure ***4a***. MS^3 experiments were carried out to further fragment the $C_7H_7S_2O_2^-$ anion in ion trap and subsequently analyzed in Orbitrap, the acquired spectrum exhibits the most intense peak at *m/z* 123.0275, indicating this ion can originate from the $C_7H_7S_2O_2^-$ ion by loss of a SO_2 molecule (Figure ***4b***). Methylphenoxide ion peak at *m/z* 107.0503 along with a radical anion S_2O_2 peak at *m/z* 95.9346 were also found with relative lower intensities of 21% and 20%, respectively.

Tandem mass spectra of N-benzoyl-^{18}O-benzenesulfonamide compound ***1d***

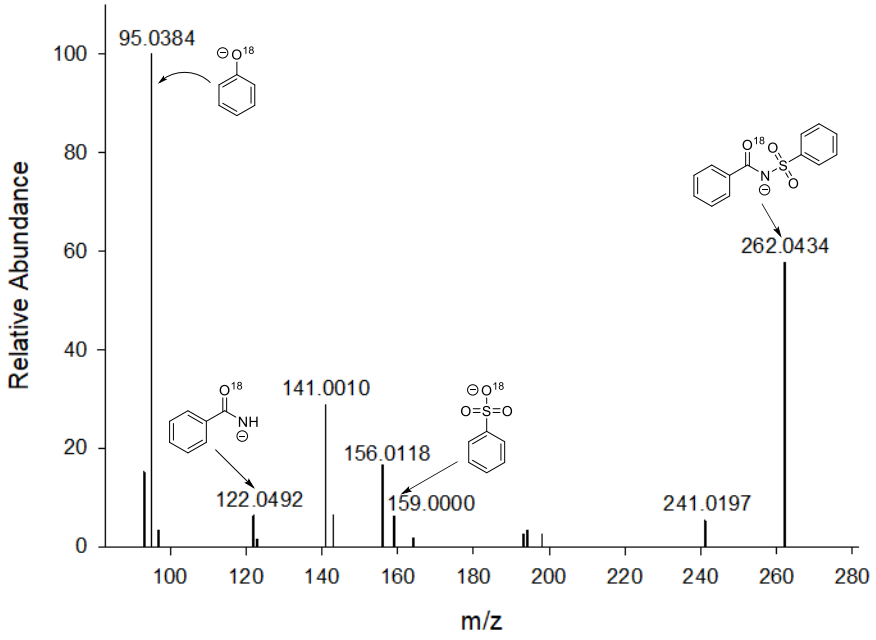


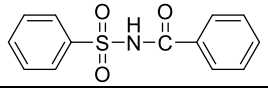
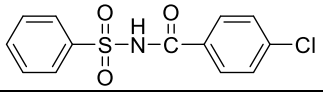
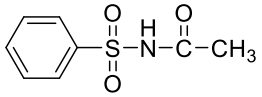
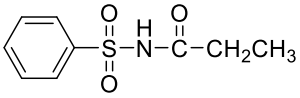
Figure 5. HCD tandem mass spectrum of deprotonated N-benzoyl-^{18}O-benzenesulfonamide ***1d*** (precursor, *m/z* 262.0434).

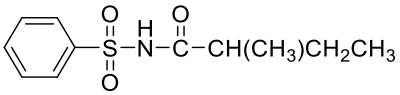
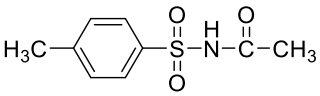
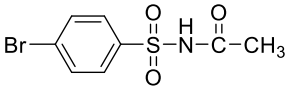
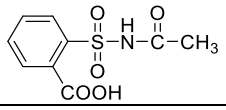
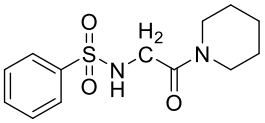
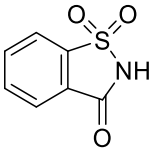
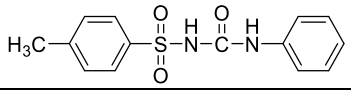
In order to further support the hypothesis that the oxygen atom of the observed phenoxide ion is derived from the carbonyl group and not from the sulfonyl group, we synthesized N-benzoyl-^{18}O-benzenesulfonamide compound ***1d*** via reaction of benzoyl-^{18}O-chloride with benzenesulfonamide. HCD tandem mass spectrum of the N-benzoyl-^{18}O-benzenesulfonamide shows a most intense peak at *m/z* 95.0384 which corresponds to an ^{18}O-phenoxide ion accompanied by the phenoxide ion at *m/z* 93.0304 with a relative abundance of 18 % because of the above mentioned Meyerson-type rearrangement (Figure **5**). The ions of *m/z* 122.0492 ($[C_7H_6N^{18}O]^-$) and *m/z* 159.0000 ($[C_6H_5O_2S^{18}O]^-$) were also observed with relative lower intensities. The phenomenon that these *m/z* values shift two mass units upward is consistent with our suggestion that carbonyl oxygen atom is transferred to benzene ring and sulfur atom via the Smiles-type and N-O rearrangement. It should be noted that other product ion peaks at *m/z* 141.0010 and *m/z* 156.0118 without the ^{18}O atom were also present in the spectrum of ***1d***, which correspond to the respective benzenesulfinate ion ($[C_6H_5O_2S]^-$) and benzenesulfonamide anion ($[C_6H_5O_2SNH]^-$).

Tandem mass spectra of other N-acyl aromatic sulfonamides

To extend the application of the amide oxygen transfer mechanism to other compounds, we examined a number of N-acyl aromatic sulfonamides. Some typical compounds and their product ion abundances normalized to total ion current are listed in Table 1.

Table 1. Various compounds which exhibit the Smiles-type and N-O exchange rearrangements under ion trap collision at collision energy 35 % and their product ion abundances.

Compound	Structure	Observed rearrangement product ion	Abundance normalized to total ion current (100%)
N-benzoylbenzenesulfonamide, ***1b***		$C_6H_5O^-$	38
N-(4-chloro-benzoyl)benzenesulfonamide, ***3***		$C_6H_5O^-$	64
N-acetylbenzenesulfonamide, ***4***		$C_6H_5O^-$	57
N-propionylbenzenesulfonamide, ***5***		$C_6H_5O^-$	75

N-(2-methyl-butanoyl)benzenesulfonamide, ***6***		$C_6H_5O^-$	81
N-acetyl-4-methylbenzenesulfonamide, ***7***		$C_7H_7O^-$	35
N-acetyl-4-bromobenzenesulfonamide, ***8***		$BrC_6H_4O^-$	66
2-(N-acetylsulfamoyl)benzoic acid, ***9***		$C_6H_5O^-$	23
2-Oxo-S-phenyl-2-(piperidin-1-yl)ethane-1-sulfonamido, ***10***		$C_6H_5O^-$	2
1, 1-dioxo-1, 2-benzothiazol-3-one, ***11***		-	-
3-(4-Methylbenzenesulfonyl)-1-phenylurea, ***12***		-	-

N-acyl benzenesulfonamide derivatives: for N-benzoylbenzenesulfonamide ***1b***, phenoxide ion abundance was calculated as 38 %. N-(4-chloro-benzoyl)benzenesulfonamide ***3***, where a hydrogen atom on the benzoyl moiety is substituted by a chlorine atom, shows a dramatic increase in the extent of the rearrangement with the abundance value at 64 %. When replacing the benzoyl with an aliphatic acyl group like acetyl, the resulting N-acetylbenzenesulfonamide ***4*** undergoes fragmentation to generate the phenoxide ion with moderate abundance (57 %). Similar mechanisms with larger aliphatic acyl groups, such as propionyl ***5*** and pentanoyl ***6***, afford higher product ion abundances as 75 % and 81 %, respectively.

N-acetyl substituted-benzenesulfonamide derivatives: substitution effects on the benzene ring of benzenesulfonyl also were investigated, N-acetyl-4-methylbenzenesulfonamide ***7*** and N-acetyl-4-bromobenzenesulfonamide ***8*** provide their corresponding product ions with abundances at 35 % and 66 %, respectively. The results may be rationalized by the ability of the Br to stabilize the negative charge on oxygen atom of the product ion. However, the carboxyl group on the benzene ring for 2-(N-acetylsulfamoyl)benzoic acid ***9*** provides an abundance of the phenoxide ion at only 23 %. The main reason is that proton loss from the carboxyl group of compound ***9*** within the ESI

source is very facile in negative mode, resulting in the subsequent loss of a neutral carbon dioxide as the predominant pathway.

Other benzenesulfonamide derivatives: The existence of amide bond ($CONH_2$) in compound structure is critical for giving rise to the rearrangement reaction, in the case of 2-oxo-S-phenyl-2-(piperidin-1-yl)ethane-1-sulfonamido ***10*** where a CH_2 is inserted into the amide bond, only 2 % abundance of rearrangement ion was observed. Annular sulfonamide like 1,1-dioxo-1,2-benzothiazol-3-one ***11*** does not show the rearrangement behavior due to stability of the five-membered ring. Even when the normalized collision energy is raised to 100 no pronounced product ion peaks are observed. Sulfonylurea compound ***12*** also does not give the rearrangement product ion, instead forming the benzenesulfonamide and anilide anion as the dominated peaks such as N-phenyl benzenesulfonamide ***1a*** does.

Theoretical calculations

As pointed out by Herman and Harrison,[34] at low internal energies, the relative rates of competing fragmentation reactions are determined largely by their relative activation energies. Hence, intrinsic reaction coordinate and activation energy calculations were used to assess the feasibility of parallel rearrangement reactions involved in the fragmentation of sulfonamides. Reactions with multiple product channels has been studied before using the direct-measurement kinetics method and theoretical calculations.[35]

In this work, there are several experimental observations that are not compatible with a single fragmentation pathway. For example, Figure 3 shows that the ion at *m/z* 93 ($C_6H_6O^-$) appears first and it seems to be produced directly from the parent ion in the initial stages. This observation goes along with a Smiles-type rearrangement; however, the Smiles rearrangement cannot explain the intense peaks at *m/z* 157 and *m/z* 141 or the intense peak at *m/z* 93 in the spectrum of the labelled compound (Figure 5). On the other hand, a N-O rearrangement mechanism is appropriate to explain the product ions at *m/z* 157 and *m/z* 141 and it is expected to contribute marginally to the intensity of the product ion at *m/z* 93. Also, there is no evidence that the peak at *m/z* 141 is produced by the loss of an oxygen atom from benzenesulfonate (*m/z* 157). The production of these ions runs parallel at a rate that is approximately an order of magnitude higher for the product ion at *m/z* 141. It seems that both ions can be produced from the same transition state of the N-O rearrangement depending

on the cleavage site to form sulfonate ion or sulfinate ion. Based on this experimental evidence, it is likely that the observed fragment ions are produced by two parallel rearrangements and each rearrangement reaction contributes mainly to the intensity of one product ion. In the following this is proved in two steps, 1) by testing the feasibility of the rearrangement reactions using IRC calculations and then 2) by correlating the experimental intensities and the activation energies in a quantitative manner.

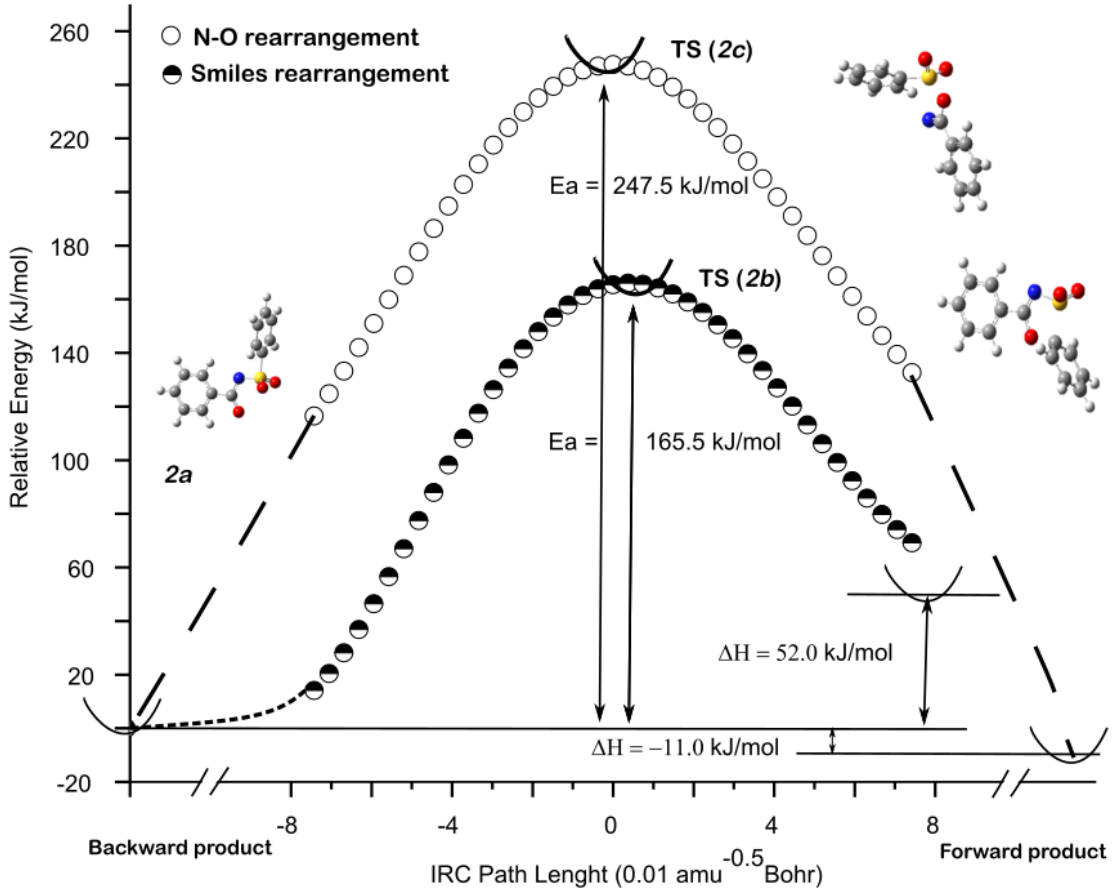


Figure 6. Intrinsic Reaction Coordinate (IRC) diagram of the N-O rearrangement and Smiles rearrangement showing the chemical structures involved in the process, the activation energy (Ea) and the thermal result of the reaction. Ea and ΔH were estimated from the electronic energies, other contributions are too small to be of any significance. The dashed lines do not represent calculated points and only serve to guide the eye.

PM3 semiempirical relaxed scans[36] were performed to explore the potential energy surface using deprotonated N-benzoylbenzenesulfonamide ***2a*** as a model ion followed by DFT calculations at the critical points. An IRC calculation was performed to confirm the located TS saddle point lies on the minimum energy path between the assumed minima (***2a*** and the rearranged ion ***2c***). The forty-points IRCs plot in Figure 6 shows the N-O rearrangement is possible, and it produces a physically reasonable activation energy of 247.5 kJ/mol. Also note that the rearrangement is kinetically controlled because both isomers are almost equally thermodynamically stable. The reaction is barely exothermic with the rearranged ion ***2c*** being 11 kJ/mol more stable than the original anion ***2a***. These same calculations were performed for the different rearrangement reactions of the parent ion.

Figure 6 also shows the IRC path for the Smiles-type rearrangement. The activation energy for this process is 165.5 kJ/mol, significantly lower than for the N-O rearrangement, so this mechanism dominates the low-energy end of the spectrum (initial stages), although the intermediate product ***2b*** (fragment ion + neutral) is thermodynamically less stable by 52.0 kJ/mol. Therefore, the spectrum of ***2a*** is the result of at least two major rearrangements of the parent ion, a Smiles-type rearrangement and a N-O rearrangement. (The 3D structures of the transition state and minima are shown in Figure 6, and their electronic energies are given in Table S1 of the supporting information). In addition, Meyerson's rearrangement directly originated from ***2a*** provides a significant contribution to the intensity of the ion at *m/z* 93 in a more convoluted way [see discussion below]. The spectrum also shows some small peaks from the sulfonamide-bond fragmentation and other structural rearrangements of the parent ion.

By combining the information in Figure 2b and Figure 5, the contribution of different rearrangements to the intensity of each peak in the spectrum was estimated. For example, at low energy, the peak at *m/z* 93 can be generated from a parent ion that experiences a Smile-type rearrangement and with a minor contribution of a Meyerson rearrangement. The Meyerson rearrangement does not involve the carbonyl oxygen, so its contribution to the intensity of the peak at *m/z* 93 is approximately 18 % according to the labelled spectrum (Figure 5). It is worth noting that the Meyerson rearrangement proceeds with negligible activation energy, however, the activation energy of the rearranged ion to produce the fragment ion at *m/z* 93 is 269.2 kJ/mol, significantly higher than the activation energy for the formation of products from the Smiles-type or N-O rearrangements. At high energy, a major contribution of Meyerson rearrangement to the spectrum of ***2a*** is via the fragmentation of the sulfonate ion produced by the N-O rearrangement as discussed in reference 27. The peaks at *m/z* 157 and *m/z* 141 are almost exclusively produced from

the N-O-rearranged ion depending on the cleavage site, either on the left or right of the carbonyl oxygen. Figure 2b shows the intensity of peaks at *m/z* 157 and *m/z* 141 are approximately 7 % and 15 % of the base peak, respectively. The peak at *m/z* 120 is originated from the direct cleavage of the sulfonamide bond with a contribution of 6 %. The activation energy of the sulfonamide bond breaking is relatively high, 334 kJ/mol, and it explains its marginal contribution to the spectrum. It is worth mentioning that there is a small peak at *m/z* 241 that seems to be originated from the losses of atomic hydrogen and water producing a radical anion. Theoretical calculations of open-shell species require higher levels of theory and are beyond the scope of this paper.

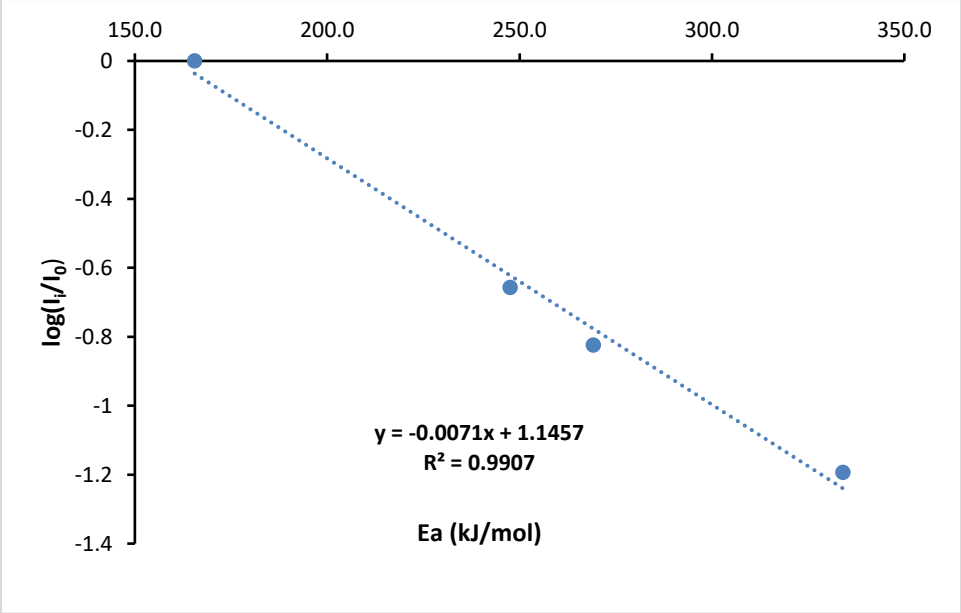


Figure 7. Plot of $log\left(\frac{I_i}{I_0}\right)$ versus (Ea_i) according to equation 2 of the supporting information. The plot uses base 10 logarithm instead of base *e* logarithm. The contributions to peak intensity values are listed and explained in Table S2. The activation energies were also derived from the electronic energies listed in Table S2.

A plot of $ln\left(\frac{I_i}{I_0}\right)$ versus (Ea_i) (see Supporting Information for a rationale for this graph) was drawn using the estimated experimental values of peak intensities and the calculated activation energies. (The activation energies were calculated using the electronic energies listed in Table S1 of the supporting information). The linear relationship shown in Figure 7 represents a validation of the assumption that the most relevant peaks in the spectrum of ***2a*** originate from different rearrangements of the parent ion (see also Table S2 in supporting information). Calculations also

suggest that the rearrangement reactions considered here have activation energies that are considerably smaller than the direct breaking of the sulfonamide bond.

In an attempt to test the performance of in-silico software regarding complex mechanisms like the fragmentation of ***2a*** discussed in this work, we used the Competitive Fragmentation Modeling-ID software or in short CFM-ID developed by the University of Alberta[37] for predicting the spectra of N-benzoylbenzenesulfonamide. CFM-ID was not able to predict the major peaks associated with either the N/O or the Smiles rearrangement reactions (see Figure S2).

Conclusions

Unlike deprotonated *N*-phenyl benzenesulfonamide which generally loses SO_2 to form the anilide anion, deprotonated N-benzoyl benzenesulfonamide mainly undergoes a Smiles-type *ipso* rearrangement to form the phenoxide ion as the most intense peak. At higher energies a novel N-O rearrangement produces benzenesulfonate and benzenesulfinate ions. Specifically, the Smiles-type rearrangement involves attack of the amide oxygen anion on the arylsulfonyl group at the *ipso* position followed by losses of SO_2 and PhCN to form the phenoxide ion. The N-O rearrangement occurs through an intramolecular nucleophilic reaction of the amide oxygen with the sulfur atom to generate the benzenesulfonate ion by loss of PhCN, which can further lose SO_2 to form the phenoxide ion. The N-O rearrangement also may yield the benzenesulfinate ion which can lose SO to form the phenoxide ion. The proposed mechanisms are supported by substituting the carbonyl of *N*-benzoyl benzenesulfonamide with a thiocarbonyl group experiment and by ^{18}O isotopic labeling N-benzoyl-^{18}O-benzenesulfonamide experiment which lead up to form the corresponding benzenethiolate anion and ^{18}O phenoxide ion. The strong correlation between product ion intensities and the corresponding calculated activation energies suggests that the fragmentation of N-benzoylbenzenesulfonamide experiences the two major parallel rearrangement reactions, the Smiles-type and N-O rearrangement, and also minor contributions from the Meyerson rearrangement reaction and the direct breaking of the sulfonamide bond. This parallel mechanism is so compelling that should be regarded as an example of the limitations of in-silico modeling and the ever-increasing need for building experimental spectral libraries. We also found certain compounds which demonstrate the Smiles-type and N-O rearrangements in their spectra and this rule may be incorporated into NIST MS interpreter[38] and other fragmentation modeling software to enhance spectrum evaluation and thereby improve the quality of the library.

Acknowledgments

The author would like to thank Dr. Karl K. Irikura of the National Institute of Standards and Technology for his advice and comments. Certain commercial equipment, instruments, or materials are identified in this document. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.

Supporting Information

The supporting information contains 1) An analytical derivation of the correlation between peak intensity and activation energy as shown in Figure 7. 2) Details about the ab initio calculations. 3) The PM3 Potential Energy Surface (PES) of the N-O rearrangement reaction (Figure S1). 4) Structural and energetic data related to the optimized geometries obtained using ab initio methods (Table S1). 5) Calculated activation energies for the rearrangement reactions (Table S2). 6). A head-to-tail comparison between the predicted CFM-ID spectrum and the NIST library spectrum (Figure S2).

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For Table of Contents Use Only

Title: CID fragmentation of deprotonated N-acyl aromatic sulfonamides. Smiles-type and nitrogen-oxygen rearrangements

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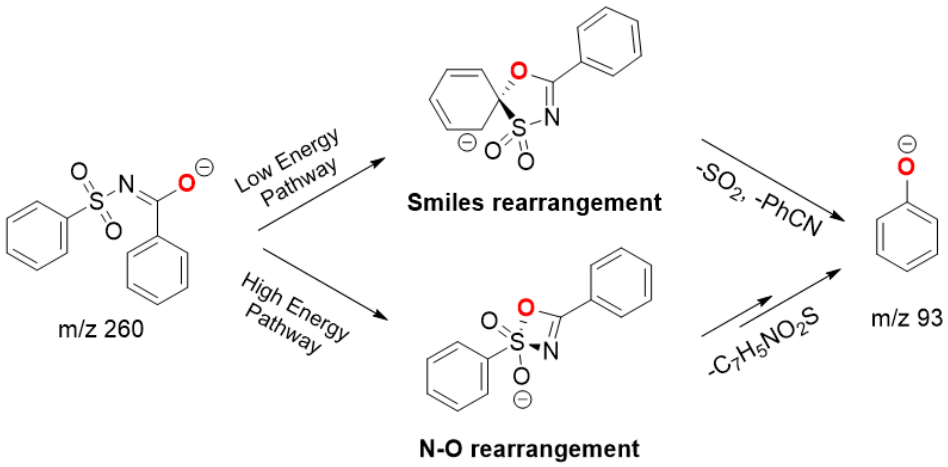

Proposed mechanism to rationalize the formation of the phenoxide ion at *m/z* 93 from deprotonated N-benzoyl benzenesulfonamide.