Research Article

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Formation of the bisulfite anion (HSO_3^-, *m/z* 81) upon collision-induced dissociation of anions derived from organic sulfonic acids

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In the negative-ion collision-induced dissociation mass spectra of most organic sulfonates, the base peak is observed at *m/z* 80 for the sulfur trioxide radical anion ($SO_3^{\cdot-}$). In contrast, the product-ion spectra of a few sulfonates, such as cysteic acid, aminomethanesulfonate, and 2-phenylethanesulfonate, show the base peak at *m/z* 81 for the bisulfite anion (HSO_3^-). An investigation with an extensive variety of sulfonates revealed that the presence of a hydrogen atom at the β-position relative to the sulfur atom is a prerequisite for the formation of the bisulfite anion. The formation of HSO_3^- is highly favored when the atom at the β-position is nitrogen, or the leaving neutral species is a highly conjugated molecule such as styrene or acrylic acid. Deuterium-exchange experiments with aminomethanesulfonate demonstrated that the hydrogen for HSO_3^- formation is transferred from the β-position. The presence of a peak at *m/z* 80 in the spectrum of 2-sulfoacetic acid, in contrast to a peak at *m/z* 81 in that of 3-sulfopropanoic acid, corroborated the proposed hydrogen transfer mechanism. For diacidic compounds, such as 4-sulfobutanoic acid and cysteic acid, the *m/z* 81 ion can be formed by an alternative mechanism, in which the negative charge of the carboxylate moiety attacks the α-carbon relative to the sulfur atom. Experiments conducted with deuterium-exchanged and deuterium-labeled analogs of sulfocarboxylic acids demonstrated that the formation of the bisulfite anion resulted either from a hydrogen transfer from the β-carbon, or from a direct attack by the carboxylate moiety on the α-carbon. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: anions; sulfonates; fragmentation; CID; gaseous ions; bisulfite

INTRODUCTION

Sulfonic acids and their derivatives play an important role in pharmaceutical and industrial chemical manufacturing.[1] For example, aliphatic and aromatic sulfonates are frequently utilized as anionic surfactants in detergents.[2–6] Substituted aromatic and aliphatic sulfonates are also widely used in the chemical industry for the production of pharmaceuticals and dyes.[7–10] Conjugate bases of sulfonic acids, such as benzenesulfonate (besylate), methanesulfonate (mesylate), ethanesulfonate (esylate), and camphorsulfonate (camsylate), provide suitable anionic counterions, which are sometimes necessary for salt formation of certain pharmacologically active ingredients (APIs).[11,12] However, many sulfonic acid esters are genotoxic; at least one drug (nelfinavir mesylate) has been withdrawn from the market because of such complications.[13–16] In organic chemistry, low-molecular weight sulfonic acids, such as methanesulfonic acid, trifluoromethanesulfonic acid, and *p*-toluenesulfonic acid, are often used as acid catalysts for esterification, alkylation, and condensation reactions.[17–19]

The anionic nature of sulfonates renders them ideal candidates for analysis by negative-ion electrospray ionization mass spectrometry. Although studies have been carried out on fragmentation pathways of simple sulfonates, the intricate details of their fragmentation mechanisms are not very well understood.[20–28] Negative-ion mass spectra of alkyl and aryl sulfonates indicate that the major product ion produced by collision-induced fragmentation is the sulfur trioxide radical anion ($SO_3^{\cdot-}$, *m/z* 80).[20–28] On the other hand, some sulfonates are known to give the bisulfite anion (HSO_3^-, *m/z* 81).[20,28] For example, in the collision-induced dissociation (CID) mass spectra of fluorotelomer sulfonates, the base peak is observed at *m/z* 81 indicating the formation of the bisulfite anion.[28] In addition, we observed that the base peak in the CID mass spectrum of cysteic acid is at *m/z* 81. However, in a previous publication, the formation of an ion of *m/z* 80 had been attributed as the major fragmentation pathway of cysteic acid.[25,26] In this paper, we report the results about our investigations on the fragmentation mechanisms of organic sulfonates.

EXPERIMENTAL

Materials

Methanol [high-performance liquid chromatography (HPLC) grade], acetonitrile (HPLC grade), hydrochloric acid (ACS grade), formic acid (88%, ACS grade), and ammonium hydroxide (ACS grade) were purchased from Pharmco-AAPER (Brookefield, CT, USA). Methanesulfonic acid (**1**), ethanesulfonic acid (**2**), benzenesulfonic acid (**5**), *p*-toluenesulfonic acid (**6**), *p*-aminobenzenesulfonic

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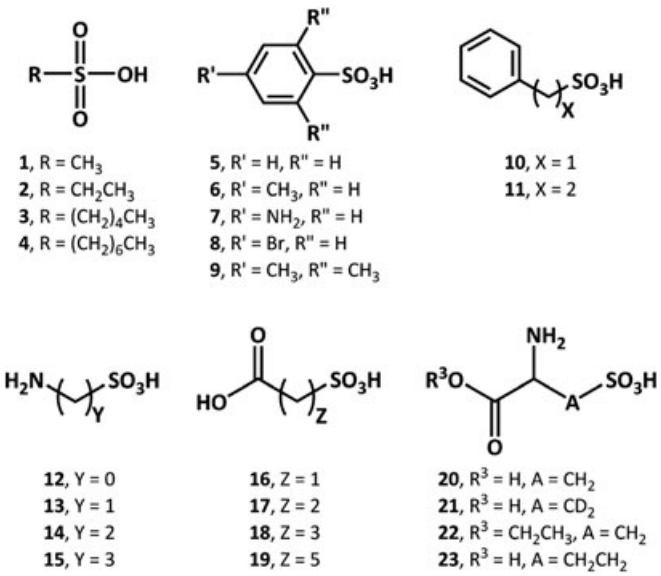
acid (**7**), *p*-bromobenzenesulfonic acid (**8**), sulfamic acid (**12**), aminomethanesulfonic acid (**13**), 2-aminoethanesulfonic acid (taurine, **14**), 3-aminopropanesulfonic acid (**15**), L-homocysteic acid (**23**), 1-bromopentane, 1-bromoheptane, benzyl bromide, 2-phenylethyl bromide, mesitylene, bromoacetic acid, 3-bromopropanoic acid, 4-bromobutanoic acid, 6-bromohexanoic acid, L-cysteine, L-cysteine ethyl ester, and [$^2H_4$]ammonium deuteroxide were purchased from Sigma-Aldrich Co. (Milwaukee, WI, USA). Anhydrous sodium sulfite, fuming sulfuric acid (20% SO_3), and hydrogen peroxide (30%) were purchased from Fisher Chemical Co. (Fairlawn, NJ, USA). L-[3,3-$^2H_2$] Cysteine was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). The silica gel (particle size: 32–63 μm) used for flash chromatography was obtained from Selecto Scientific Inc. (Suwanee, GA, USA).



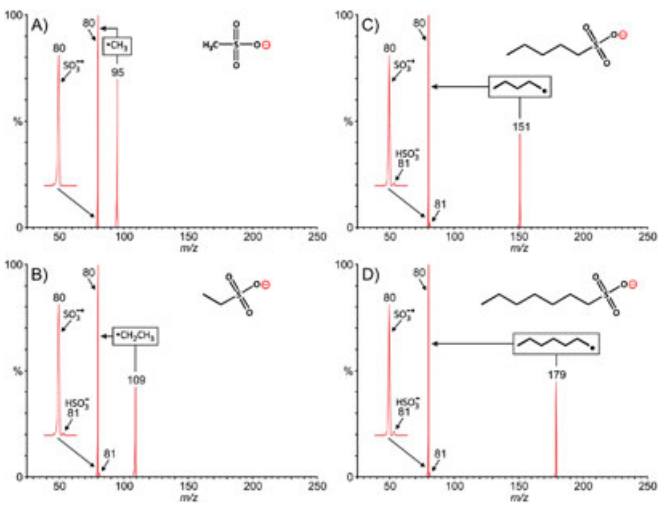


Figure 1. Product-ion spectra of anions derived from methanesulfonic acid (*m/z* 95) (A), ethanesulfonic acid (*m/z* 109) (B), pentanesulfonic acid (*m/z* 151) (C), and heptanesulfonic acid (*m/z* 179) (D) recorded on an API-3000 tandem mass spectrometer at laboratory-frame collision energy of 25 eV.

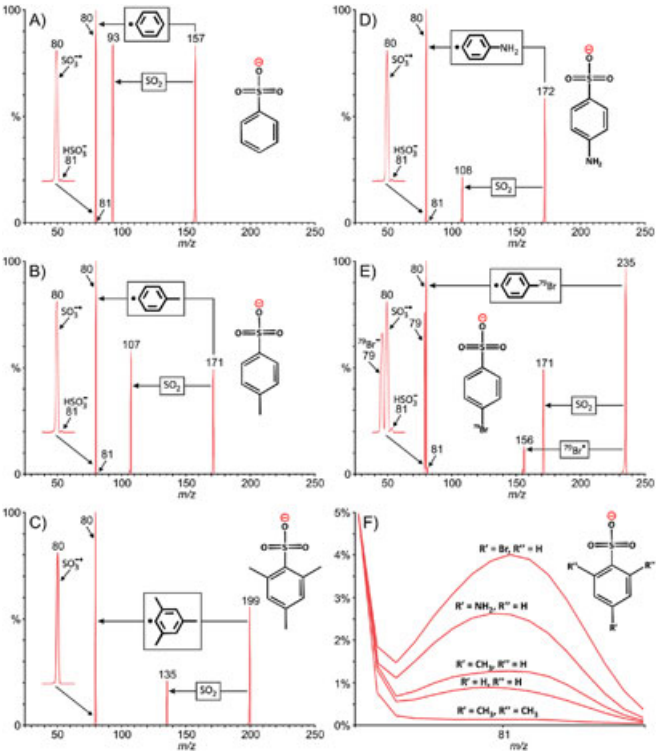
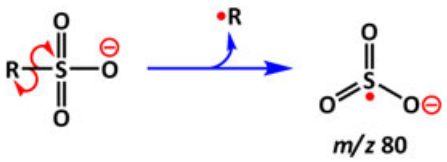
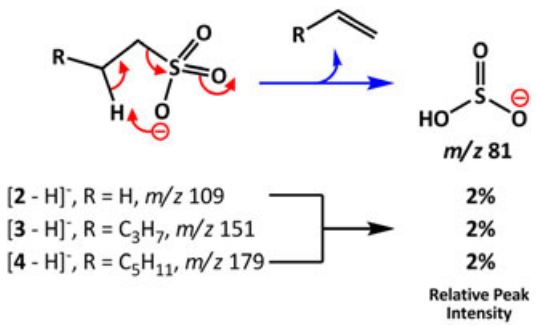


Figure 2. Product-ion spectra of anions derived from benzenesulfonic acid (*m/z* 157) (A), *p*-toluenesulfonic acid (*m/z* 171) (B), 2-mesitylenesulfonic acid (*m/z* 199) (C), *p*-aminobenzenesulfonic acid (*m/z* 172) (D), *p*-bromobenzenesulfonic acid (*m/z* 235) (E), and an overlay of the *m/z* 81 peak from the five aforementioned compounds (F) recorded on an API-3000 tandem mass spectrometer at laboratory-frame collision energy of 25 eV.



Scheme 1. Fragmentation mechanism for the formation of the sulfur trioxide radical anion (*m/z* 80, $SO_3^{\cdot-}$).



Scheme 2. Proposed mechanism for the formation of the bisulfite anion (*m/z* 81, HSO_3^-) upon fragmentation of deprotonated alkyl sulfonic acids **2–4**.

Synthesis of alkyl sulfonic acids

Pentanesulfonic acid (**3**), heptanesulfonic acid (**4**), phenylmethanesulfonic acid (**10**), and 2-phenylethanesulfonic acid (**11**) were synthesized from 1-bromopentane, 1-bromoheptane, benzyl

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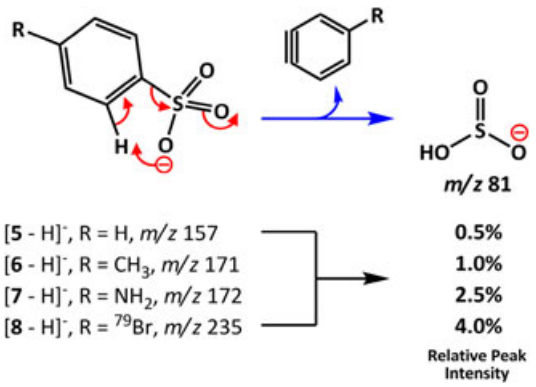
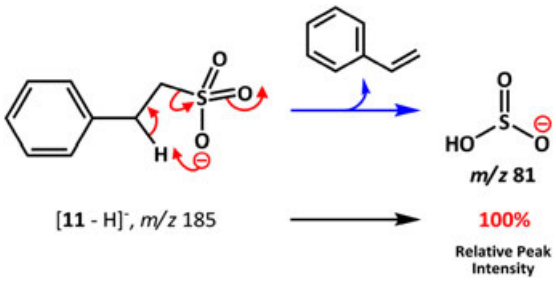


Scheme 3. Proposed mechanism for the formation of the bisulfite anion (*m/z* 81, HSO_3^-) upon fragmentation of deprotonated aromatic sulfonic acids **5–8**.



Scheme 4. Proposed mechanism for the formation of the bisulfite anion (*m/z* 81, HSO_3^-) upon fragmentation of 2-phenylethanesulfonate ([**11** – H]$^-$).

bromide, and 2-phenylethyl bromide, respectively, by Strecker sulfite alkylation.[29,30] 1-Bromopentane (50 mg, 0.331 mmol), 1-bromoheptane (50 mg, 0.279 mmol), benzyl bromide (50 mg, 0.292 mmol), or 2-phenylethyl bromide (50 mg, 0.270 mmol), was refluxed with an aqueous solution of sodium sulfite (1.0 M; 10 ml) at 110 °C for 12 h. The reaction mixture was then acidified with conc. HCl to a pH of 1.0, and the resulting sulfonic acids were purified by flash chromatography (32–63 μm of silica gel) using methanol as the eluting solvent. The sulfonic acids in methanol were dried under a stream N_2 and were crystalline in nature.

Synthesis of 2-mesitylenesulfonic acid

2-Mesitylenesulfonic acid (**9**) was synthesized from mesitylene by aromatic sulfonation.[31] Mesitylene (50 mg, 0.417 mmol) was vigorously shaken with conc. fuming sulfuric acid (0.5 ml) until the organic layer had completely disappeared and a yellowish solution resulted. This solution was then poured into 10 ml of ice water. After an hour at room temperature, vacuum filtration was used to collect the precipitated 2-mesitylenesulfonic acid crystals. The white crystalline product was washed with 10 ml of cold water (~5 °C) and 10 ml of cold methanol (~5 °C). The resulting product was air-dried and used as obtained.

Synthesis of sulfocarboxylic acids

2-Sulfoacetic acid (**16**), 3-sulfopropanoic acid (**17**), 4-sulfobutanoic acid (**18**), and 6-sulfohexanoic acid (**19**) were also synthesized by the Strecker sulfite alkylation.[29,30] Bromoacetic acid (50 mg, 0.360 mmol), 3-bromopropanoic acid (50 mg, 0.327 mmol), 4-bromobutanoic acid (50 mg, 0.299 mmol), or 6-bromohexanoic acid (50 mg, 0.256 mmol), was refluxed with an aqueous solution of sodium sulfite (1.0 M; 10 ml) at 110 °C for 12 h. The reaction mixture was then acidified with conc. HCl to a pH of 1.0, and the resulting sulfocarboxylic acids were purified by flash chromatography (32–63 μm of silica gel) using methanol as the eluting solvent. The methanolic extracts were dried under a stream N_2, and the compounds obtained were crystalline in nature.

Oxidation of cysteine and its analogs to cysteic acid

L-Cysteine, L-[3,3-$^2H_2$]cysteine, and L-cysteine ethyl ester were oxidized by performic acid to L-cysteic acid (**20**), L-[3,3-$^2H_2$] cysteic acid (**21**), and L-cysteic acid ethyl ester (**22**), respectively.[32] The performic acid reagent was prepared by admixing H_2O_2 (30%, 10 ml) and formic acid (88%, 90 ml). The solution was kept at room temperature for 30 min to maximize performic acid formation. L-Cysteine (50 mg, 0.413 mmol), L-cysteine ethyl ester (50 mg, 0.336 mmol), or L-[3,3-$^2H_2$]cysteine (5 mg, 0.041 mmol), was dissolved in the performic acid solution, and the mixture was kept at room temperature for 30 min. The reaction mixture was dried in an Eppendorf Vacufuge® concentrator (Eppendorf, Hauppauge, NY, USA) at 60 °C for 2 h, and crystalline cysteic acids were recovered.

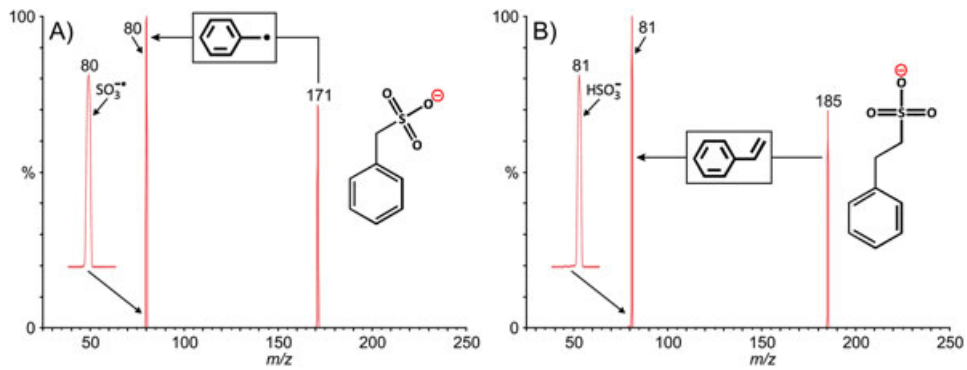


Figure 3. Product-ion spectra of anions derived from phenylmethanesulfonic acid (*m/z* 171) (A), and 2-phenylethanesulfonic acid (*m/z* 185) (B) recorded on an API-3000 tandem mass spectrometer at laboratory-frame collision energy of 25 eV.

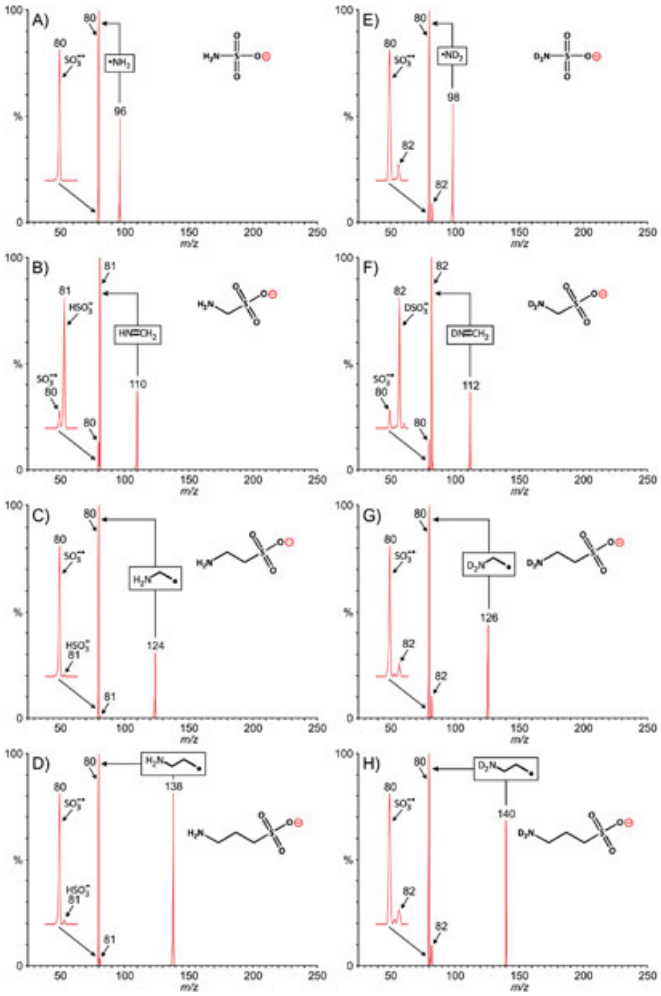
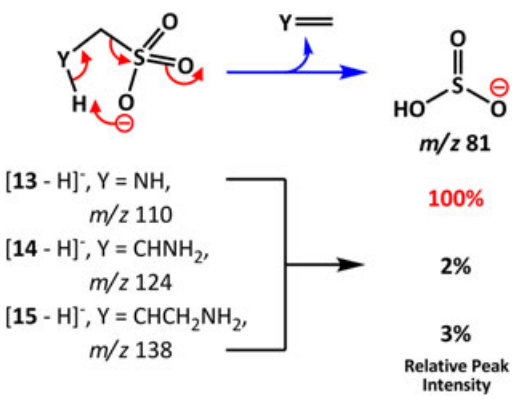


Figure 4. Product-ion spectra of anions derived from sulfamic acid (*m/z* 96) (A), aminomethanesulfonic acid (*m/z* 110) (B), 2-aminoethanesulfonic acid (*m/z* 124) (C), 3-aminopropanesulfonic acid (*m/z* 138) (D), sulfamic acid in D_2O (*m/z* 98*) (E), aminomethanesulfonic acid in D_2O (*m/z* 112*) (F), 2-aminoethanesulfonic acid in D_2O (*m/z* 126*) (G), and 3-aminopropanesulfonic acid in D_2O (*m/z* 140*) (H) recorded on an API-3000 tandem mass spectrometer at laboratory-frame collision energy of 25 eV. (*Note that isotopologues bearing either two deuterium atoms or one ^{34}S atom are isobaric; therefore, ions isolated at unit-resolution should be composites).



Scheme 5. Proposed mechanism for the formation of the bisulfite anion (*m/z* 81, HSO_3^-) upon fragmentation of deprotonated aminoalkylsulfonic acids **13–15**.

Mass spectrometry

The negative-ion CID mass spectra were acquired on an Applied Biosystems API 3000 (Concord, ON, Canada) triple quadrupole mass spectrometer equipped with a TurboIonSpray® source. The source temperature was held at 50 °C. Both the nebulizer gas (N_2) and curtain gas (N_2) flow rate settings were at 15 (arbitrary units). The drying gas (N_2) was maintained at a flow rate of 1 l/min at 60 psi. The ionspray voltage was held at −4500 V, and a declustering potential of −50 V was used. The collision gas (N_2) pressure in the collision cell was maintained at 2.1×10^{-5} Torr, and the collision energy was varied between 20 and 30 eV. Each of the compounds **1–23** was diluted to a concentration of 1 μg/ml in a 1 : 1 mixture of acetonitrile-water with 1% v/v ammonia. For deuterium-exchanged experiments, samples were dissolved in a 1 : 1 mixture of acetonitrile-deuterium oxide with 1% v/v deuteriated ammonia. Samples were introduced into the source by infusion at a flow rate of 10 μl/min.

RESULTS AND DISCUSSION

Aliphatic and aromatic sulfonates are ideal candidates for negative-ion electrospray ionization mass spectrometry because of their acidic nature and the ability to readily form the corresponding conjugate bases in solution. Thus, anions derived from sulfonic acids have been widely used for their qualitative and quantitative determinations.[28,33–38] The product-ion mass spectra of anions derived from aliphatic and aromatic sulfonic acids (**1–9**) show an intense signal at *m/z* 80, representing the sulfur trioxide radical anion ($SO_3^{\cdot-}$) resulting from a homolytic cleavage of the precursor (Figs 1 and 2, Scheme 1). However, the mass spectra of aliphatic sulfonates with alkyl chains containing two or more carbons also show a minor peak at *m/z* 81 for the bisulfite anion (HSO_3^-) (Fig. 1 (B,C, and D)). Although the relative intensity of the peak for the *m/z* 81 ion is only about 2%, the presence of this peak is intriguing because these anions do not bear any exchangeable hydrogen atoms. Because the peak at *m/z* 81 is only observed in the mass spectra of sulfonates with alkyl chains containing two or more carbons, we concluded that the fragmentation process may be best represented as a cyclic *syn*-elimination mechanism (Scheme 2), similar to the Cope amine *N*-oxide elimination.[39–42] In a previous publication, it has been conclusively demonstrated that the formation of the bisulfate anion (HSO_4^-, *m/z* 97) upon CID of organic sulfates also occurs via a similar mechanism.[43,44]

In the case of aromatic sulfonates, the neutral loss of an SO_2 (Fig. 2(A–E)) has been studied in detail by Binkley *et al.*[24] Furthermore, the *m/z* 81 peak is also observed in the mass spectra of aromatic sulfonates, such as benzenesulfonate ([**5** – H]⁻, Fig. 2(A)), *p*-toluenesulfonate ([**6** – H]⁻, Fig. 2(B)), *p*-aminobenzenesulfonate ([**7** – H]⁻, Fig. 2(D)), and *p*-bromobenzenesulfonate ([**8** – H]⁻, Fig. 2(E)). A mechanism, similar to that given in Scheme 2, whereby the hydrogen atom at the *ortho*-position is transferred via the cyclic *syn*-elimination, to eliminate a benzyne derivative is envisaged for the formation of the bisulfite anion from aromatic sulfonates (Scheme 3). This cyclic *syn*-elimination mechanism was supported by the observation that the *m/z* 81 peak was not observed in the mass spectrum of 2-mesitylenesulfonate ([**9** – H]⁻, Fig. 2(C)). From the few examples we have investigated that the properties of the substituent group at the *para* position appear to have an effect

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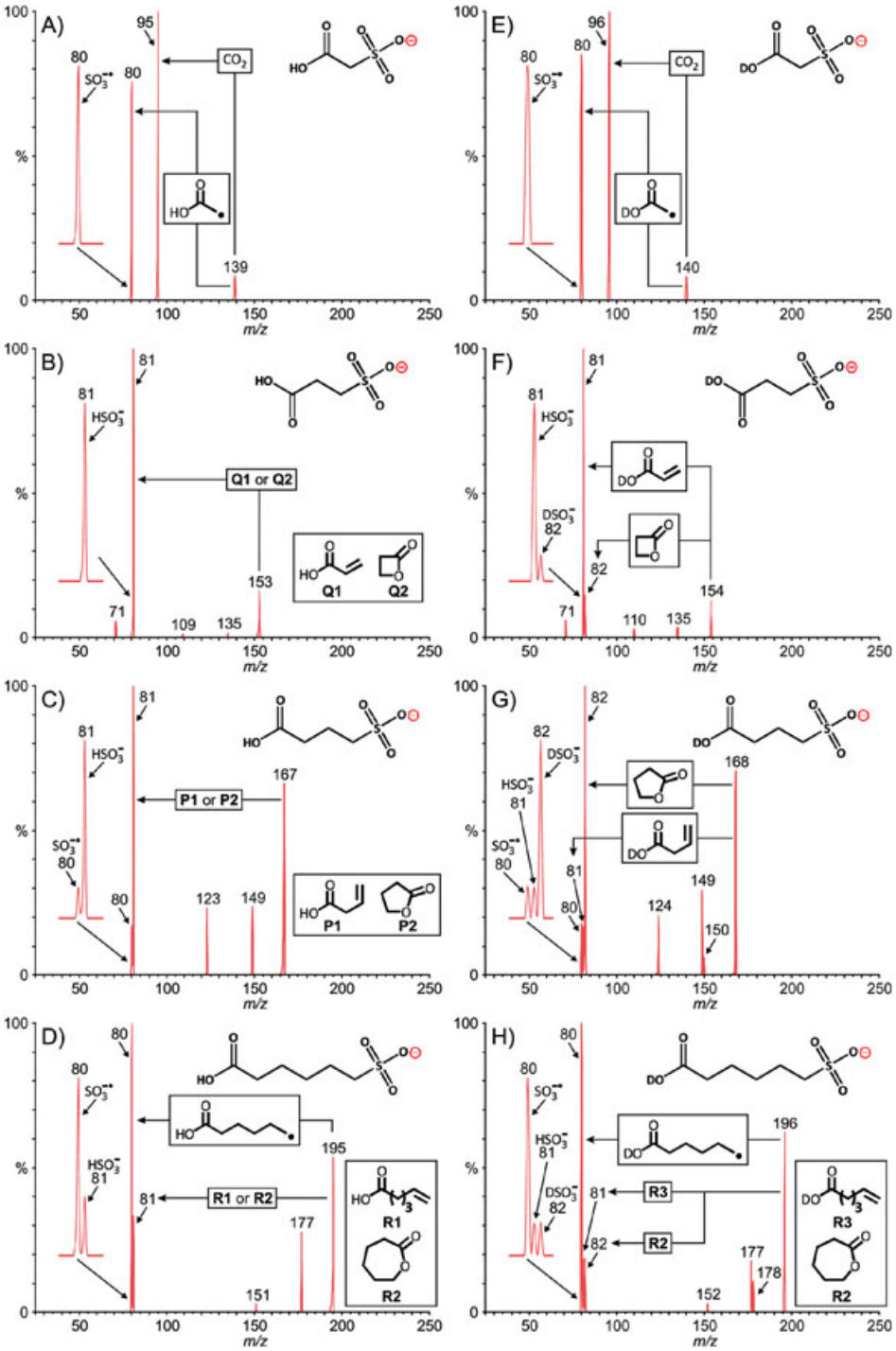
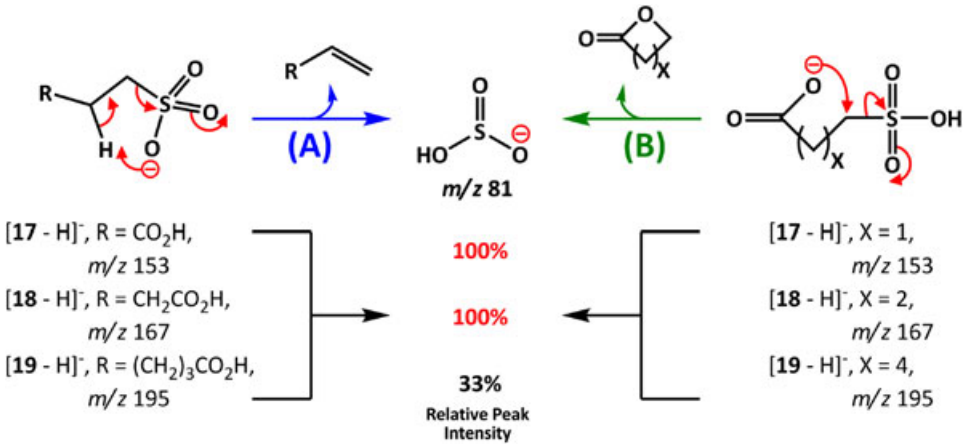


Figure 5. Product-ion spectra of anions derived from 2-sulfoacetic acid (*m/z* 139) (A), 3-sulfopropanoic acid (*m/z* 153) (B), 4-sulfobutanoic acid (*m/z* 167) (C), 6-sulfohexanoic acid (*m/z* 195) (D), 2-sulfoacetic acid in D_2O (*m/z* 140) (E), 3-sulfopropanoic acid in D_2O (*m/z* 154) (F), 4-sulfobutanoic acid in D_2O (*m/z* 168) (G), and 6-sulfohexanoic acid in D_2O (*m/z* 196) (H) recorded on an API-3000 tandem mass spectrometer at laboratory-frame collision energy of 25 eV.

on the formation of the bisulfite anion (Fig. 2(F)). Moreover, the *m/z* 81 peak was not observed in the mass spectrum of phenylmethanesulfonate ([**10** – H]$^-$, Fig. 3(A)), further validating the cyclic *syn*-elimination mechanism, because the *β*-carbon, relative to the sulfur atom, does not have any hydrogens. On the other hand, the *m/z* 81 peak is the base peak in the spectrum of 2-phenylethanesulfonate ([**11** – H]$^-$, Fig. 3(B), Scheme 4). It seems that the elimination of a neutral styrene molecule is a highly favorable pathway, similar to the results described by Cope *et al.* for the thermal decomposition of amine *N*-oxide elimination.[40]

Further support for the cyclic *syn*-elimination mechanism was obtained by the CID experiments conducted with aminomethanesulfonate ([**13** – H]$^-$, Fig. 4(B), Scheme 5). The spectrum of



Scheme 6. Proposed mechanism for the formation of the bisulfite anion (*m/z* 81, HSO_3^-) upon fragmentation deprotonated sulfocarboxylic acids **17–19**.

aminomethanesulfonate ([**13** – H]$^-$) showed the base peak at *m/z* 81. When the same compound was sprayed as a D_2O solution, a peak was observed at *m/z* 82, which was attributed to the DSO_3^- anion (Fig. 4(F)). This observation indicated that the hydrogen transfer occurs from the β-position for the bisulfite anion formation. However, if the amino group is moved further away from the sulfonate moiety, then the intensity of the *m/z* 81 peak is reduced to 2%, and the *m/z* 80 peak once again becomes the base peak (Fig. 4(C and D)). In addition, the *m/z* 81 peak is not observed in the mass spectrum of sulfamate ([**12** – H]$^-$, Fig. 4(A)). Although a peak at *m/z* 82 (~10%) is also observed in the spectra of deuteriated sulfamic acid (Fig. 4(E)), deuteriated 2-aminoethanesulfonic acid (Fig. 4(G)), and deuteriated 3-aminopropanesulfonic acid (Fig. 4(H)), this peak was attributed to the $^{34}SO_3^-$ ion and not to a DSO_3^-, because the intensity of the *m/z* 81 peak, in the spectra (Fig. 4(A, C, and D)) of the non-deuteriated analogs, is very low (Note that the *m/z* 98, 126, and 140 ions isolated at unit-resolution are composites of isotopologues bearing either two deuterium atoms or one ^{34}S atom).

Further information about the hydrogen transfer mechanism was obtained from the investigations carried out with sulfocarboxylic acids (**16–19**). As shown in Fig. 5(A), peaks for two major product ions (*m/z* 95 and *m/z* 80) are observed in the mass spectrum of deprotonated 2-sulfoacetic acid ([**16** – H]$^-$). The *m/z* 95 peak represents an ion that originates from a decarboxylation of the anion of 2-sulfoacetic acid ([**16** – H]$^-$), and the *m/z* 80 peak represents the sulfur trioxide radical anion ($SO_3^{\cdot-}$). Compared with that of 2-sulfoacetic acid ([**16** – H]$^-$), the spectrum of 3-sulfopropanoic acid ([**17** – H]$^-$) shows that base peak is at *m/z* 81 (Fig. 5(B)). In fact, the addition of an extra methylene group between the carboxylic acid and the sulfonic acid moieties results in the exclusive formation of the bisulfite anion (HSO_3^-). However, when the 3-sulfopropanoic acid ([**17** – H]$^-$) is sprayed in D_2O, a peak (~15%) for the deuteriated bisulfite anion (DSO_3^-) is also observed (Fig. 5(F)). Therefore, two separate mechanisms are expected to participate in the formation of the bisulfite anion (Schemes 6(A and B)). For 3-sulfopropanoic acid ([**17** – H]$^-$), the primary mechanism is a cyclic *syn*-elimination wherein the charge on the sulfonate oxygen attacks the hydrogen attached to the β-carbon, resulting in the elimination of a neutral acrylic acid molecule (Scheme 6(A)). The secondary mechanism for 3-sulfopropanoic acid ([**17** – H]$^-$) is driven by the charge on the carboxylate, which attacks the α-carbon resulting in the elimination of a neutral β-propiolactone molecule (Scheme 6(B)). Because the intensity of the *m/z* 81 peak is so large compared with that of *m/z* 82 (Fig. 5(F)), it is evident that the sulfonate charge-mediated pathway (Scheme 6(A)) is highly favored. The base peak of the spectra of both 3-sulfopropanoic acid ([**17** – H]$^-$) and 4-sulfobutanoic acid ([**18** – H]$^-$) is *m/z* 81 (Figure 5(B and C)). However, the CID mass spectrum of deuterium-exchanged 4-sulfobutanoic acid showed the base peak at *m/z* 82, which revealed that the carboxylate charge-mediated pathway is favored for 4-sulfobutanoic acid ([**18** – H]$^-$) (Fig. 5(G)). In this case, the *m/z* 82 peak is much more intense than that at *m/z* 81, indicating that the formation and elimination of the γ-butyrolactone is the favored pathway (Scheme 6(B)), although the *syn*-elimination mechanism also plays a role (Scheme 6(A)). As the carboxylate moiety is placed farther away from the sulfonate, for example in the case of 6-sulfohexanoic acid ([**19** – H]$^-$), the formation of the sulfur trioxide radical anion becomes the primary fragmentation pathway (Fig. 5(D)). The CID mass spectrum of the anion derived from deuterium-exchanged 6-sulfohexanoic acid shows peaks at both *m/z* 81 and 82, indicating that both pathways are equally favored in this case (Fig. 5(H)). Other fragmentation pathways for anions derived from sulfocarboxylic acids consist of a loss of a neutral H_2O or CO_2 molecule probably proceed via fragmentation mechanisms similar to those detailed by Grossert *et al.* for dicarboxylic acids.[45]

Cysteic acid (**20**) is the oxidized form of the amino acid cysteine. It is an intermediate in the metabolism of cysteine and is a precursor of taurine (**14**).[32] The mass spectrometric fragmentation pathways of anions derived from cysteic acid ([**20** – H]$^-$) have been previously discussed by Smith *et al.*[25,26] Their results were based on mass-analyzed ion kinetic energy spectra obtained from fast atom bombardment MS/MS experiments. According to their results, cysteic acid ([**20** – H]$^-$) fragments similar to other sulfonates and a peak at *m/z* 80 for the sulfur trioxide radical anion ($SO_3^{\cdot-}$) had been reported.[25,26] However, our low-energy collision-induced fragmentation spectra recorded from cysteic acid ([**20** – H]$^-$) showed only a peak at *m/z* 81 in that region (Fig. 6(A)). A detailed evaluation of CID mass spectra of the deuterium-exchanged cysteic acid indicated that the two separate fragmentation pathways proposed for the sulfocarboxylic acids

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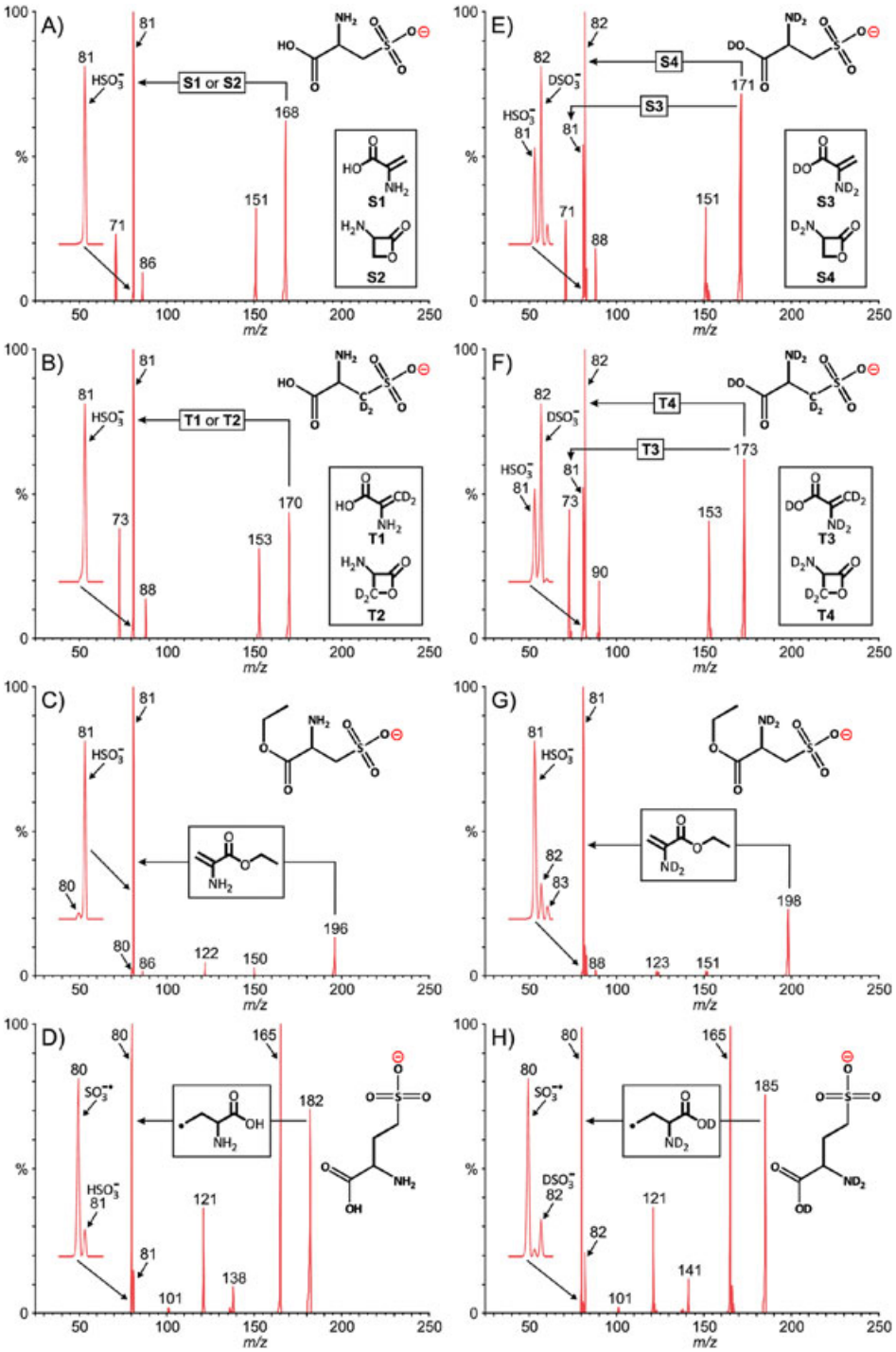
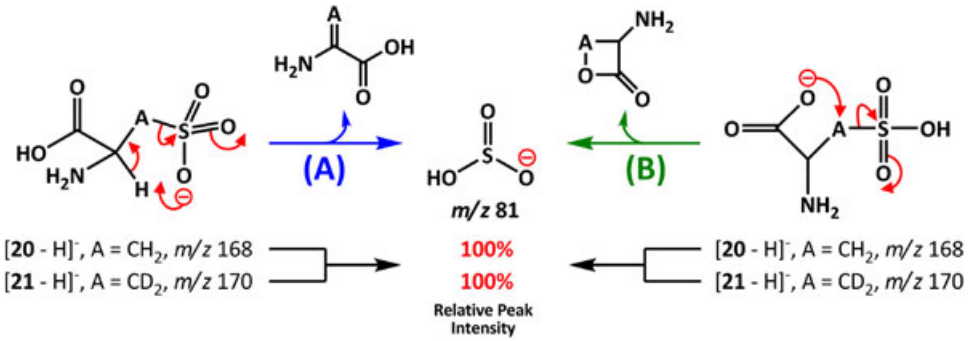
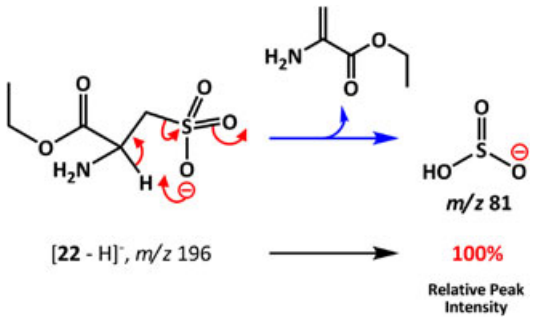


Figure 6. Product-ion spectra of anions derived from cysteic acid (*m/z* 168) (A), [3,3-$^2H_2$]-cysteic acid (*m/z* 170) (B), cysteic acid ethyl ester (*m/z* 196) (C), homocysteic acid (*m/z* 182) (D), cysteic acid in D_2O (*m/z* 171) (E), [3,3-$^2H_2$]-cysteic acid in D_2O (*m/z* 173) (F), cysteic acid ethyl ester in D_2O (*m/z* 198) (G), and homocysteic acid in D_2O (*m/z* 185) (H) recorded on an API-3000 tandem mass spectrometer at laboratory-frame collision energy of 25 eV.

(Scheme 6) are also valid for cysteic acid (Fig. 6). In this case, the carboxylate-mediated charge-directed attack on the α-carbon (Scheme 7(B)) is more favorable than the sulfonate-based charge-mediated attack on the hydrogen on the β-carbon (Scheme 7(A)). The CID mass spectra of anions derived from [3,3-$^2H_2$]cysteic acid ([**21** – H]$^-$) and deuterium-exchanged [3,3-$^2H_2$]cysteic acid support the conclusion that the sulfonate group exclusively attacks the β-carbon to form the bisulfite anion (Fig. 6(B and F)). The CID mass spectra of cysteic acid ethyl ester ([**22** – H]$^-$) and deuterium-exchanged cysteic acid ethyl ester further corroborated the sulfonate charge-mediated *syn*-elimination mechanism (Fig. 6(C and G)). In both these spectra, the base peak is observed at *m/z* 81, indicating that the sulfonate charge-mediated pathway is the only accessible pathway



Scheme 7. Proposed mechanism for the formation of the bisulfite anion (*m/z* 81, HSO_3^-) upon fragmentation of deprotonated cysteic acid (**20**) and [3,3-$^2H_2$]cysteic acid (**21**).



Scheme 8. Proposed mechanism for the formation of the bisulfite anion (*m/z* 81, HSO_3^-) upon fragmentation of deprotonated cysteic acid ethyl ester (**22**).

(unlike in the carboxylate group, the ester moiety cannot bear a negative charge) (Scheme 8).

Homocysteic acid (**23**) is a gliotransmitter, known to function as an endogenous *N*-methyl-(D)-aspartic acid receptor agonist.[46] High concentrations of homocysteic acid may cause hyperhomocysteinemia, which can often lead to vascular and neuronal lesions.[46] In the case of homocysteic acid ([**23** – H]$^-$), the base peak is observed at *m/z* 80 with *m/z* 81 at 15% relative intensity (Fig. 6(D)). However, the mass spectrum of the deuterium-exchanged homocysteic acid shows a peak at *m/z* 82, revealing that the bisulfite anion is mostly a result of the carboxylate charge-mediated pathway (Figure 6(H), Scheme 9(B)).

CONCLUSIONS

Although the major product ion upon fragmentation of many sulfonates is the sulfur trioxide radical anion ($SO_3^{\cdot-}$, *m/z* 80), in certain cases, the major product ion is the bisulfite anion (HSO_3^-, *m/z* 81). The data are summarized in Table 1. In fact, in the negative-ion CID mass spectra of anions derived from 2-phenylethanesulfonic acid ([**11** – H]$^-$), aminomethanesulfonic acid ([**13** – H]$^-$), 3-sulfopropanoic acid ([**17** – H]$^-$), 4-sulfobutanoic acid ([**18** – H]$^-$), and cysteic acid ([**20** – H]$^-$), the base peak is observed at *m/z* 81. In the absence of a secondary deprotonation site, the formation of the bisulfite is facilitated by a site-specific hydrogen transfer from the *β*-carbon via a cyclic *syn*-elimination. Furthermore, upon introduction of a second deprotonation site, two separate pathways, the aforementioned *syn*-elimination pathway and a carboxylate charge-mediated pathway, compete for the formation of the bisulfite anion. In the case of the sulfonate charge-mediated pathway, a hydrogen atom is exclusively abstracted from the *β*-carbon for the formation of the bisulfite anion.

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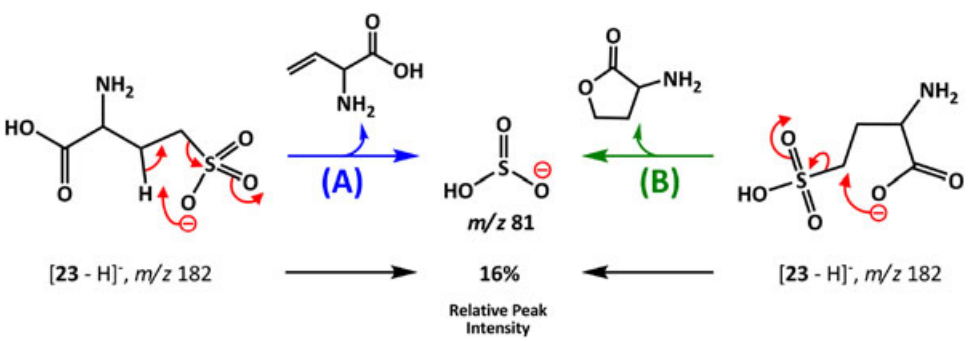

Scheme 9. Proposed mechanism for the formation of the bisulfite anion (*m/z* 81, HSO_3^-) upon fragmentation of deprotonated homocysteic acid (**23**).

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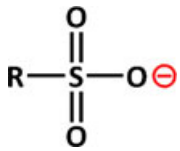
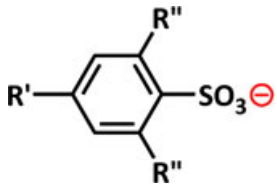
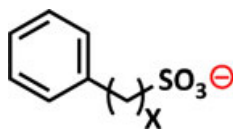
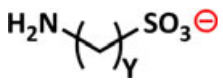
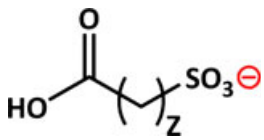
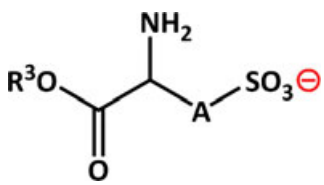
Table 1. A summary of the relative intensities of *m/z* 80 and 81 peaks in CID spectra of some organic sulfonates

Parent ion	[M – H]⁻ (m/z)	*m/z* 80 relative intensity (%)	*m/z* 81 relative intensity (%)
			
R = CH_3	[**1** – H]⁻ (*m/z* 95)	100	0
R = CH_2CH_3	[**2** – H]⁻ (*m/z* 109)	100	2
R = *n*-Pentyl	[**3** – H]⁻ (*m/z* 151)	100	2
R = *n*-Heptyl	[**4** – H]⁻ (*m/z* 179)	100	2
			
R′ = H, R″ = H	[**5** – H]⁻ (*m/z* 157)	100	0.5
R′ = CH_3, R″ = H	[**6** – H]⁻ (*m/z* 171)	100	1
R′ = NH_2, R″ = H	[**7** – H]⁻ (*m/z* 172)	100	2.5
R′ = ^{79}Br, R″ = H	[**8** – H]⁻ (*m/z* 235)	100	4
R′ = CH_3, R″ = CH_3	[**9** – H]⁻ (*m/z* 199)	100	0
			
X = 1	[**10** – H]⁻ (*m/z* 171)	100	0
X = 2	[**11** – H]⁻ (*m/z* 185)	0	100
			
Y = 0	[**12** – H]⁻ (*m/z* 96)	100	0
Y = 1	[**13** – H]⁻ (*m/z* 110)	13	100
Y = 2	[**14** – H]⁻ (*m/z* 124)	100	2
Y = 3	[**15** – H]⁻ (*m/z* 138)	100	3
			
Z = 1	[**16** – H]⁻ (*m/z* 139)	80	0
Z = 2	[**17** – H]⁻ (*m/z* 153)	0	100
Z = 3	[**18** – H]⁻ (*m/z* 167)	17	100
Z = 5	[**19** – H]⁻ (*m/z* 195)	100	33
			
R^3 = H, A = CH_2	[**20** – H]⁻ (*m/z* 168)	0	100
R^3 = H, A = CD_2	[**21** – H]⁻ (*m/z* 170)	0	100
R^3 = CH_2CH_3, A = CH_2	[**22** – H]⁻ (*m/z* 196)	4	100
R^3 = H, A = CH_2CH_2	[**23** – H]⁻ (*m/z* 182)	100	16

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