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Fourier-transform ion cyclotron resonance mass spectrometric studies of elimination reactions of anionic bases with metabolites of a fluorinated anesthetic agent: towards modeling bioactivation in the gas phase

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Abstract

Sevoflurane (fluoromethyl 2,2,2-trifluoro-[2,2,2-trifluoromethyl]ethyl ether) is a volatile anesthetic agent that is widely used in the U.S. and abroad. Sevoflurane undergoes degradation in the anesthetic circuit to form 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A). As it is metabolized, Compound A alkylates the cysteine side chain in the tripeptide glutathione, which acts as a sort of scavenger for xenobiotics such as Compound A. The *S*-alkylated glutathione or glutathione *S* conjugate loses its C-terminal and N-terminal residues as it is further metabolized. This leaves a cysteine *S* conjugate of Compound A. The cysteine conjugate undergoes bioactivation by an enzyme known as β-lyase to produce nephrotoxic metabolites. Although Compound A is nephrotoxic in rats, Compound A-associated nephrotoxicity has not been observed in the human clinical use of sevoflurane, apparently because β-lyase activities are much lower in human kidney tissue than in rat kidney tissue. Since β-lyase reacts with carbonyl compounds by mechanisms involving deprotonation of the α-carbon, the reactions of Compound A-derived cysteine conjugates with the basic anionic species hydroxide, methoxide, and ethoxide were examined by Fourier-transform ion cyclotron resonance mass spectrometry. The anionic bases examined react with the cysteine conjugates by an initial deprotonation of the α-carbon to form an enolate intermediate followed by elimination of either a thiolate anion or of HF. Since the HF elimination leads to CF_2 loss, it is suggested that the F atom eliminated as HF comes from a CF_3 group. Collision-induced dissociation (CID) of the product ions suggested structures consistent with this overall mechanistic picture. It is evident from these results that the same mechanism by which other cysteine conjugates are bioactivated could operate in the case of Compound A. That is, deprotonation to form enolate intermediates could lead to the release of very reactive species that might inactivate enzymes by alkylating them or otherwise reacting irreversibly with them. The thiolate product could alkylate an enzyme under appropriate conditions. Condensed-phase hydrolysis of the thiolate product could produce 2-(fluoromethoxy)-3,3,3-trifluoropropionic acid, a known metabolite of Compound A. The HF loss channel produces not only HF, but also CF_2, a very reactive species. Evidence is noted that a closely related enzyme substrate system reacts to release fluoride in condensed phases suggesting that the activation of a CF_3 observed here could be a more general process. (Int J Mass Spectrom 195/196 (2000) 203–213) © 2000 Elsevier Science B.V.

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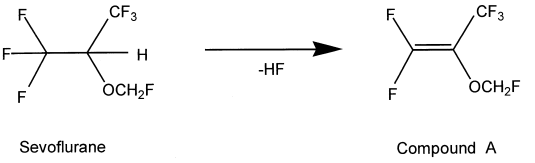
1. Introduction

A number of relatively simple eliminations involving alkyl halides [1–7], ethers [8], and thioethers [9,10] have been examined in the gas phase and characterized in some detail. These studies suggest that the mechanisms of these simple gas-phase eliminations closely resemble the mechanisms of similar condensed-phase processes. This, in turn, suggests that gas-phase studies might be useful in elucidating mechanisms of more complex eliminations. In the gas phase, anionic bases typically react with esters to form enolates as terminal products [11,12]. Recently, however, we reported the observation of elimination reactions of *S*-alkylated cysteine ester derivatives. It was found that gas-phase reactions of hydroxide and alkoxide with the alkylated cysteine derivatives led to the formation of enolate transient intermediates that eliminated thiolate and thioketene. These findings support the postulated mechanism of the toxicological bioactivation of the alkylated cysteine derivatives by enzymes known to deprotonate esters [13]. We report here the study of the elimination reactions of *S*-alkylated cysteine derivatives analogous to cysteine conjugates formed in the metabolism of Compound A, a degradation product of the widely used anesthetic sevoflurane. The anesthetic is degraded in the anesthesia circuit to form Compound A, a species that is nephrotoxic in rats but not in humans. The study reveals possible bioactivation pathways for these cysteine conjugates, pathways that might lead to cytotoxic species. This study, therefore, is an effort to use ion–molecule reactions to model specific biological chemistry rather than to model a general reaction type. It is hoped that this will encourage a wider use of ion–molecule reaction studies in the examination of such specific biological processes.

As stated above, the widely used anesthetic sevoflurane (fluoromethyl 2,2,2-trifluoro-[2,2,2-trifluoromethyl]ethyl ether undergoes degradation in the anesthetic circuit to form 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (Compound A) as shown in Scheme 1 [14–16]. The toxicity of Compound A in rats is well established [17–21]. Compound A *S*-alkylates glutathione (Glu-Cys-Gly) forming glutathione conjugates which are enzymatically converted to *S*-alkylated cysteine conjugates. Under the action of enzymes such as β-lyase these conjugates give nephrotoxic metabolites. Compound A-derived glutathione conjugates are excreted in the bile of rats given Compound A and the corresponding N-acetylated cysteine conjugates (mercapturates) are excreted both in the urine of rats given Compound A and in the urine of human subjects anesthetized with sevoflurane [22–24]. Also, 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid, a metabolite characteristic of the β-lyase-dependent metabolism of Compound A, is present in the urine of rats given Compound A and in the urine of human subjects anesthetized with sevoflurane and is formed in vitro during the biotransformation of the Compound A-derived cysteine *S*-conjugate *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-L-cysteine [24–26]. Although Compound A is nephrotoxic in rats, Compound A-associated nephrotoxicity has not been observed in the human clinical use of sevoflurane, apparently because β-lyase activities are much lower in human kidney tissue than in rat kidney tissue [27,28].



Scheme 1.

The specific objective of these studies was to investigate the formation of reactive intermediates and products derived from Compound A-derived cysteine *S* conjugates. In these experiments, the products formed from fully blocked Compound A-derived cysteine *S* conjugates were examined by Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). β-lyase is one of a group of pyridoxal

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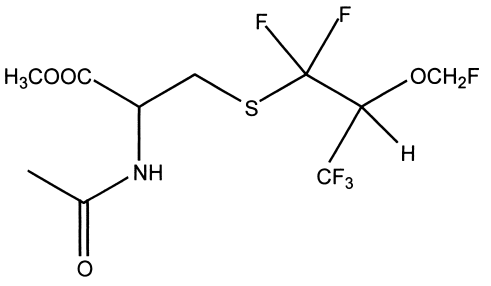
Dedicated to Bob Squires for his many seminal contributions to mass spectrometry and ion chemistry.



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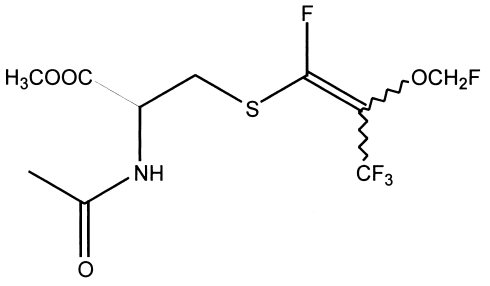

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Fig. 1. Structures of cysteine *S* conjugates **1** (methyl *S*-[2-fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine and **2** (methyl *S*-[2-fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine.

phosphate-dependent enzymes that reacts with carbonyl compounds by mechanisms involving deprotonation of the α-carbon. We therefore examined the reactions of Compound A-derived cysteine conjugates with the basic anionic species: hydroxide, methoxide, and ethoxide.

2. Experimental

2.1. Materials and methods

2.1.1. Syntheses

Methyl *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L cysteine **1** (Fig. 1). *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine

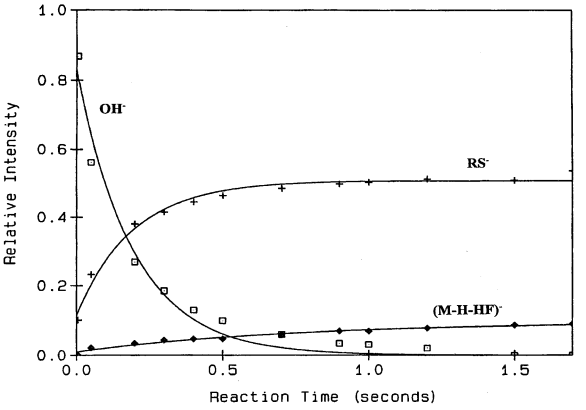


Fig. 2. The reaction of OH^- with **2**. OH^- was produced from H_2O by a 0.1 s 7 eV electron beam pulse on 1×10^{-6} Torr of H_2O. The cysteine conjugate, introduced on a probe through a vacuum lock to a pressure of approximately 10^{-7} Torr, reacts with OH^- to form RS^- and other minor ions. Only two product ions are shown in this kinetic plot, the other minor ions are not included to avoid the confusion of too many incidental curves. Nominally R = (·CF=C(OCH_2F)CF_3).

was provided by Abbott Laboratories. An ethereal solution of diazomethane was added to 0.15 mmol (52 mg) of the mercapturic acid until all compound was dissolved. The ether was evaporated in vacuo to give a pale yellow oil. The crude product was loaded onto a silica gel column, which was eluted with ethyl acetate/hexane (9:1) to give methyl *S*-[2-fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine as colorless oil (50 mg, 94%): GC-MS, t_R 17.9 min, *m/z* 357.

Methyl *S*-[2-fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine **2** (Fig. 1). 2-(fluoromethoxy)-1,1,3,3,3-pentafluoro-1-propene (1.6 mL, 12.96 mmol) and *N*-acetyl-L-cysteine methyl ester (1.77 g, 10.8 mmol) were dissolved in 25 mL THF containing triethylamine (3.0 mL, 22.0 mmol) and allowed to react at 0 °C for 30 min. The solvent was evaporated, 30 mL of water was added, and the mixture was brought to pH 2 by addition of concentrated HCl. The mixture was extracted with ethyl acetate (3×50 mL). The organic layers were combined and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to give a yellow, viscous oil. The crude product was loaded onto a silica gel column and eluted with ethyl acetate/dichloromethane (4:6). ^{19}F nuclear magnetic reso-

Table 1
FT-ICR-MS analysis of distribution of products formed by gas-phase reaction of hydroxide, methoxide, or ethoxide with methyl *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **1**

Base	$(M–R)^-$ *m/z* 176	$(RS–HF)^-$ *m/z* 193	RS^- *m/z* 213	$([X]–CH_2CO–HF)^-$ *m/z* 254	$([X]–CF_2)^-$ *m/z* 266	$([X]–CH_2CO)^-$ *m/z* 274	$(M–H–(HF)_2)^-$ *m/z* 316
HO^-	8	35	9	5	9	11	23
CH_3O^-	6	30	6	4	6	12	34
$C_2H_5O^-$	4	32	6	3	4	11	40

[a] X = $(M–H–(HF)_2)^-$ or *m/z* 316.
[b] R is nominally ($\cdot CF_2CH(OCH_2F)CF_3$) or *m/z* 181.

nance (NMR) spectroscopic analysis showed that the product was a 1:1 mixture of methyl *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **1** and methyl *S*-[2-fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenyl]-*N*-acetyl-L-cysteine **2**. Elution of the column with ethyl acetate/dichloromethane (1:1) gave a yellow solid that contained about 10% methyl *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **1**. A sample was purified for analysis by TLC (ethyl acetate/dichloromethane; 1:1; R_f = 0.28). ^{1}H NMR (d_6-acetone): δ 7.75 (bs, 1H, NH), 5.75 (d, 2H, J = 54 Hz,–OCH_2F), 4.88–4.98 (m, 1H,–SCH_2CH), 3.46–3.78 (m, 2H,–SCH_2CH), 2.20 (s, 3H, $NHC(=O)CH_3$). ^{19}F NMR (d_6-acetone): 10.8 (d, 3F,–CF_3), −32.20 (m, 1F, −S (F−)C=C–CF_3(–OCH_2F)); −74.80 (t, 1F, J = 54 Hz,–OCH_2F).

2.2. Instrumental analyses

2.2.1. NMR

^{1}H and ^{19}F NMR spectra were recorded with a Bruker 270 MHz spectrometer operating at 270 MHz for ^{1}H and 254 MHz for ^{19}F. Chemical shifts δ are reported in parts per million (ppm). The solvent resonance at 2.1 ppm was used as the internal standard for ^{1}H NMR spectra when acetone-d_6 was the solvent. Trifluoroacetamide (δ = 0.0 ppm) was used as the external standard for ^{19}F NMR spectra.

2.2.2. GC-MS

Mass spectra were recorded with a Hewlett-Packard model 5890 gas chromatograph (25 m × 0.2 mm, 0.5-μm film thickness, HP-1 cross-linked methyl siloxane column) coupled to a Hewlett-Packard model 5972 mass selective detector; the injector and transfer-line temperatures were 200 °C and 240 °C, respectively. The mercapturic acid methyl esters were analyzed with a temperature program of 50 °C for 1 min followed by a linear gradient of 10 °C/min to 240 °C.

2.2.3. FT-ICR-MS

The cysteine conjugates were introduced on an unheated probe into the vacuum chamber of a FTMS-2000 (Finnigan FTMS, Madison, WI) dual-cell Fourier-transform ion cyclotron resonance spectrometer. Reactant ions were formed by 7 eV electron impact on water vapor (OH^-) or a mixture of water vapor and methanol vapor (CH_3O^-), or a mixture of water vapor

Table 2
FT-ICR-MS analysis of distribution of products formed by gas-phase reaction of hydroxide, methoxide, or ethoxide with methyl *S*-[2-(fluoromethoxy)-1,3,3,3-tetafluoro-1-propenyl]-*N*-acetyl-L-cysteine **2**

Base	$(RS–F)^-$ *m/z* 174	RS^- *m/z* 193	$([X]–CH_2CO–HF)^-$ *m/z* 254	$([X]–CF_2)^-$ [a] *m/z* 266	$([X]–CH_2CO)^-$ [a] *m/z* 274	$(M–H–(HF))^-$ [a] *m/z* 316
HO^-	15	52	9	7	12	5
CH_3O^-	8	44	10	14	16	8
$C_2H_5O^-$	8	43	10	13	14	12

[a] X = $(M–H–(HF))^-$ or *m/z* 316.
[b] R is nominally ($\cdot CF \cdot C(OCH_2F)CF_3$) or *m/z* 161.

and ethanol vapor ($C_2H_5O^-$). The total nominal pressure in the chamber was approximately 10^{-7} Torr. After pulsing the electron gun, all ions but the reactant ions of interest were ejected from the ion trapping cell. Mass spectra were obtained at various times to obtain kinetic data on the ion–molecule reactions of the various reactant ions with the cysteine *S* conjugates.

Collision-induced dissociation spectra were obtained by adding argon gas to the cell to a total pressure of 10^{-6} Torr, allowing enough reaction time for the original reactant ions to disappear, ejecting all of the ions from the trap but the ion of interest, exciting the ion of interest to a selected kinetic energy, allowing time for collisions, and obtaining a spectrum of the parent ion and fragment ions.

3. Results

The distribution of products from the ion–molecule reactions of cysteine *S* conjugates **1** and **2** with the three different reactant ions are given in Tables 1 and 2, respectively. The variation with reaction time of the relative abundances of the reactant OH^- ions and the major product ions formed from conjugate **2** is shown in Fig. 2. Collision-induced decomposition spectra of the product ions at *m/z* 193 and *m/z* 316 are shown in Figs. 3–4 and are summarized in Tables 3 and 4.

4. Discussion

4.1. Ion molecule reactions

The temporal variation of the major ions in a typical system is shown in Fig. 2. A reaction scheme consistent with the results is shown in Scheme 2. The branching ratios shown in the scheme are for reaction with ethoxide. Branching ratios for reaction of OH^- and CH_3O^- are given in Table 2. The reactions of conjugate **1** are similar, and the differences are discussed below. The results can be reasonably well accounted for by the initial transfer of the enolic proton to the base followed by either loss of the 2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenylthiolate, RS^- (*m/z* 193), or loss of HF to give an ion of *m/z* 316. A less important channel involves formation of a radical anion at *m/z* 174, which corresponds to 2-(fluoromethoxy)-1,3,3,3-tetrafluoro-1-propenylthiolate minus a fluorine atom.

In the absence of solvent, the exothermicity of the initial proton transfer to the base is partitioned into ionic and neutral products. The energetic enolate product decomposes unimolecularly and rapidly and is, therefore, not observed in the spectra. The energy partitioning is such that some of the *m/z* 316 product has sufficient energy to decompose further, whereas some is stable and is observed in the spectrum. Hence, the bracketed *m/z* 316 species shown in Scheme 2 is given a double superscript (^{*,0}). The loss of CF_2 from the *m/z* 316 ion supports the structure proposed for the *m/z* 316 ion shown in Scheme 2. The loss of ketene (CH_2CO), presumably from the *N*-acyl group, competes with the loss of CF_2. The loss of the ketene is probably intramolecularly base catalyzed by the CF_2 group, because ketene loss does not accompany the loss of CF_2. The product formed by the loss of ketene can lose HF to form a highly conjugated anion of *m/z* 254, as shown in Scheme 2. All of these unimolecular processes are much faster than the time scale of the experiment (detection time >100 μs) and faster than the time between collisions (tens to hundreds of ms).

The variation of the product distributions among the three different bases indicates that HF loss is the lowest energy process. The formation of *m/z* 193 (RS^-) ion from conjugate **2** decreases in importance on going from OH^- to CH_3O^- to $C_2H_5O^-$. The decomposition of *m/z* 316 ion to give ions of *m/z* 274, *m/z* 266, and *m/z* 254 is also less important for the weaker bases. The *m/z* 193 (RS^-) product is still important in the reaction of the weakest base, however, suggesting the two major channels, loss of HF and formation of RS^-, are close in energy.

The reactions of conjugate **1** with the three bases gave the same products (see Table 1) as the reactions of conjugate **2** with two exceptions: little 2-(fluoromethoxy)-1,1,3,3,3-propenethiolate (*m/z* 213) was detected, but this species lost HF readily so that the ion at *m/z* 193 was the major product as was the case with conjugate **2**. The other exception is that an ion at *m/z*

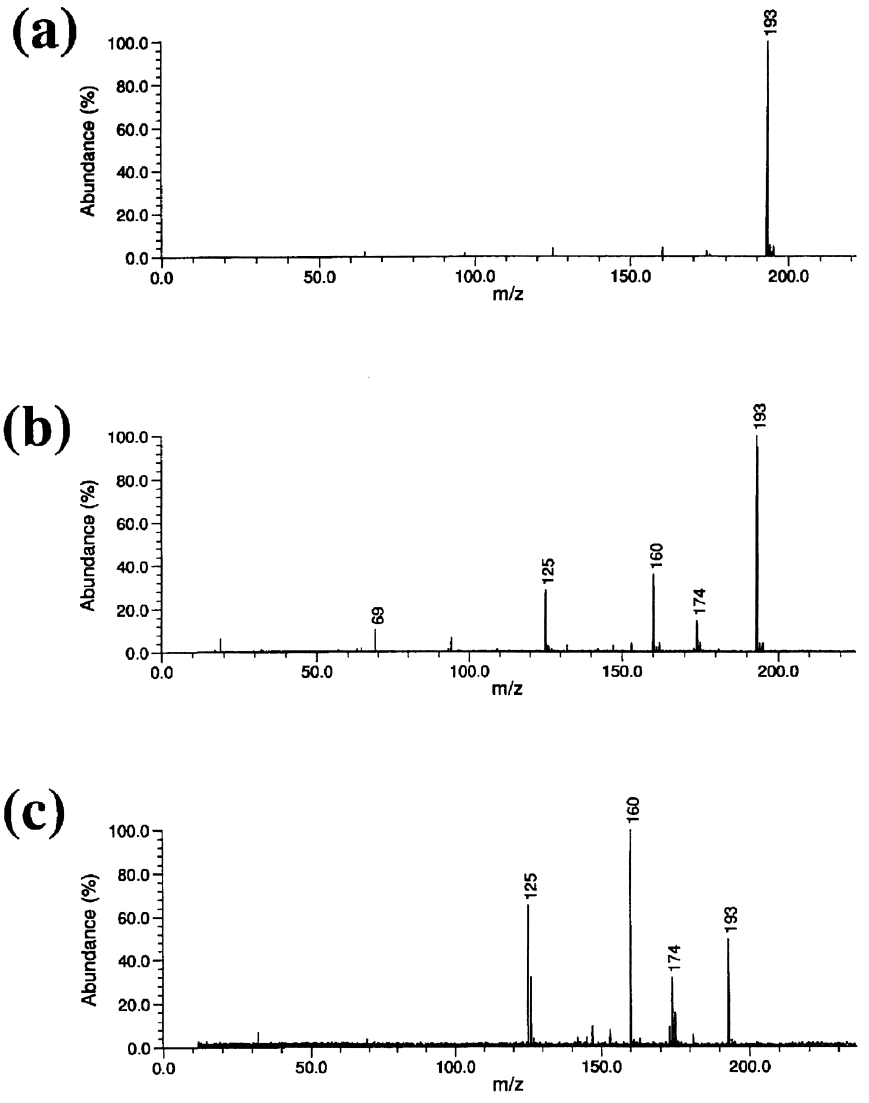


Fig. 3. The collision-induced dissociation of thiolate RS^- *m/z* 193, formed as in Fig. 2. The thiolate is isolated, accelerated to various kinetic energies, and allowed to collide with CO_2 gas pulsed into the apparatus with a computer controlled solenoid valve. Spectra (a), (b), and (c) of the residual RS^- and collision produced fragment ions are obtained at laboratory kinetic energies of 0 eV (isolation), 9.75 eV, and 196 eV, respectively.

176 instead of an ion at *m/z* 174 was observed. This product is unique in that it does not originate with attack of the base at the enolic proton. Rather, the base must attack the proton on carbon 2 of 2-(fluoromethoxy)-1,1,3,3,3-propylthiolate, which results in the formation of 2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropene that is lost as a neutral species and leaves the cysteine thiolate ion $(M–R)^-$ as the product at *m/z* 176. The variation of the product distributions of conjugate **1** with the basicity of the reactant ions were similar to that of conjugate **2**. The ion at *m/z* 316 became more important than the ions at *m/z* 213 and *m/z* 193 as the basicity of the reactant ion decreased. The extent to which the ion at *m/z* 316 decomposed also decreased as the basicity of the reactant ion decreased.

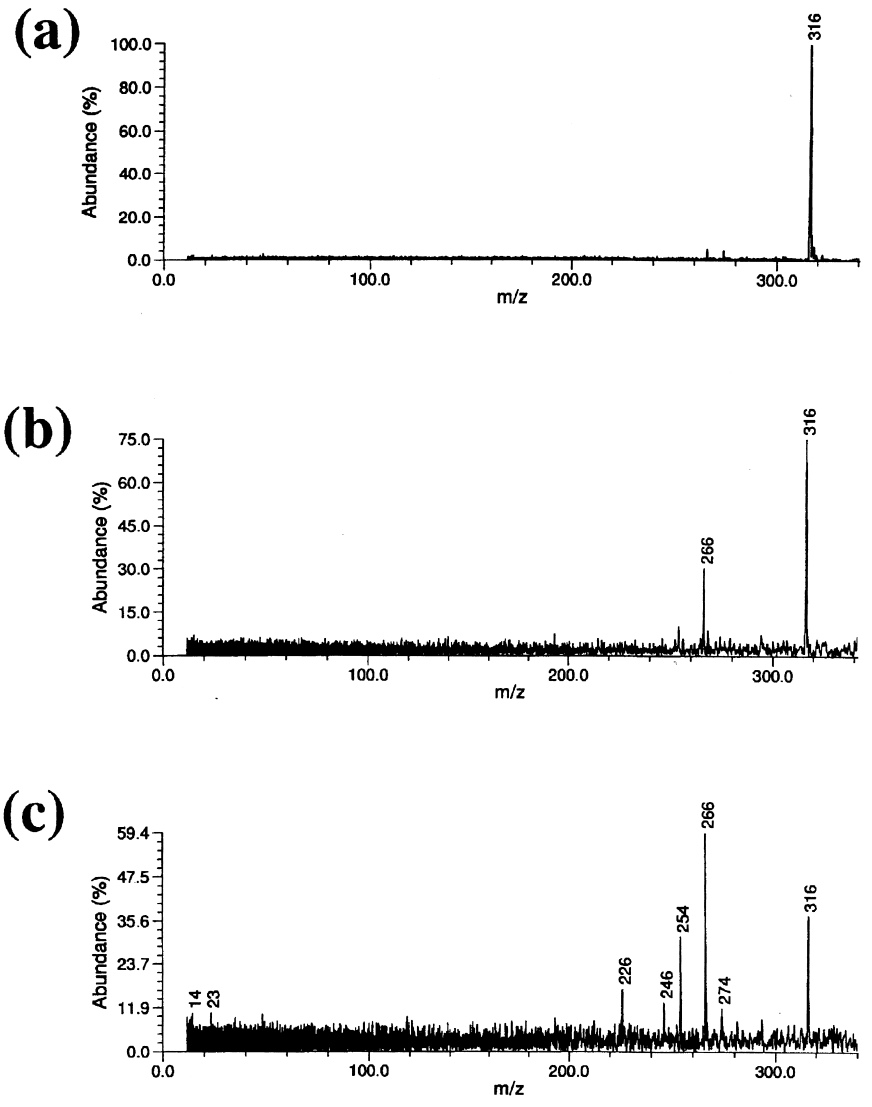


Fig. 4. The collision-induced dissociation of *m/z* 316 $(M–H–HF)^-$, formed as in Fig. 2. The $(M–H–HF)^-$ is isolated, accelerated to various kinetic energies, and allowed to collide with CO_2 gas pulsed into the apparatus with a computer controlled solenoid valve. Spectra (a), (b), and (c) of *m/z* 316 and collision produced fragment ions are obtained at laboratory kinetic energies of 0 eV (isolation), 1.875 eV, and 3.0 eV, respectively.

In solution, the thiolate product at *m/z* 193 formed from **1** and **2** could give rise to the Compound A metabolite 2-(fluoromethoxy)-3,3,3-trifluoropropionic acid [24–26] on hydrolysis. This confirms a parallel between the gas-phase processes under examination here and the bioactivation of Compound A.

The apparent activation of the CF_3 group to form *m/z* 316 is an interesting and unusual feature of the reactions of gas phase bases with **1** and **2**. It is a major channel, and the further loss of CF_2 helps confirm that the initial HF loss involves a fluorine on the CF_3 group. Not only is the CF_2 loss product at *m/z* 266 observed among the ion–molecule reaction products, but CID on an isolated *m/z* 316 ion produce predom-

Table 3
Distribution of products formed by the collision-induced dissociation of *m/z* 193 fragment formed from methyl *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **1** and methyl *S*-[2-(fluoromethoxy)-1,3,3,3-tetafluoro-1-propenyl]-*N*-acetyl-L-cysteine **2**

	Conjugate **1**		Conjugate **2**	
Fragment ion	(b) E_{com} = 1.7 eV	(c) E_{com} = 34 eV	(b) E_{com} = 1.7 eV	(c) E_{com} = 34 eV
m/z 160	0.58	0.54	0.56	0.61
m/z 145	0.12	0.22	0.00	0.00
m/z 125	0.30	0.24	0.44	0.39

inantly *m/z* 266 as discussed below. Thus there can be little doubt that the F atom lost as HF to form *m/z* 316 in the ion–molecule reaction originates with the CF_3 group in **1** and **2**. The action of medium-chain acyl-CoA dehydrogenase on the desamino cysteine *S* conjugate **3** (Fig. 5) has been examined by Baker-Malcolm and Thorpe [29]. The medium-chain acyl-CoA dehydrogenase and β-lyase are enzymes that react with carbonyl substrates by mechanisms thought to involve deprotonation of the α-carbon. Incubation of desamino conjugate **3**, which has an allylic CF_3 group analogous to that in **2**, with the acyl-CoA dehydrogenase produces a significant concentration of fluoride anion as detected by NMR. Of course in **3**, the CF_3 group is the only possible source of F. This indicates an enzyme activation of a CF_3 similar to the process leading to the *m/z* 316 ion in the present gas-phase study verifying the relevence of the gas-phase model.

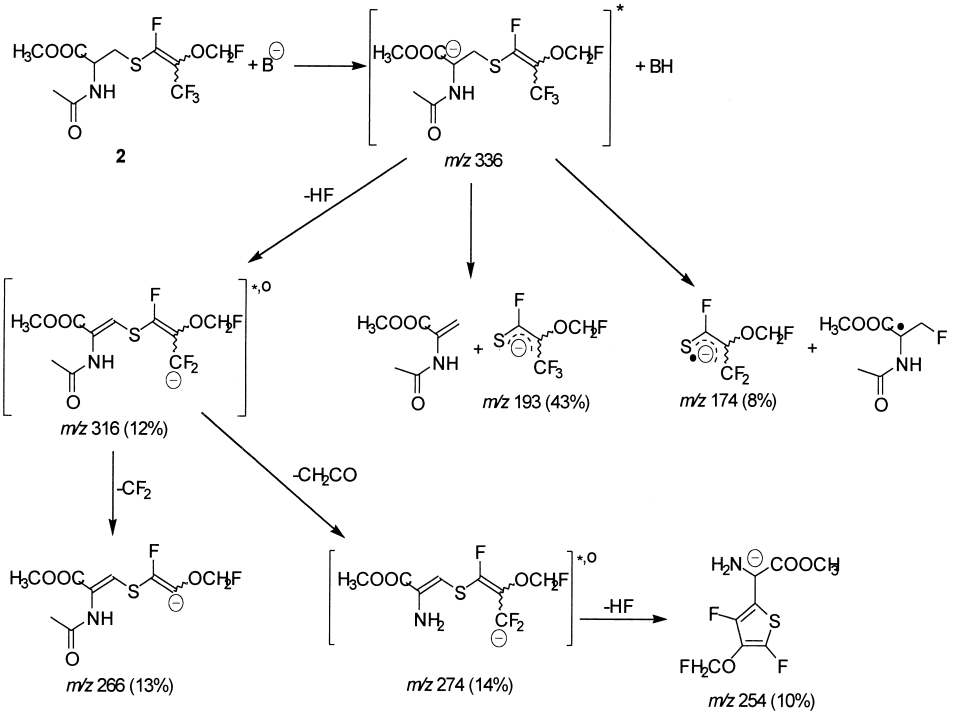
4.2. Collision-induced dissociation

As is evident from Fig. 4 and Table 4, CID of the ion at *m/z* 316 gave the same products (*m/z* 266, *m/z* 274, and *m/z* 254) that were observed in the ion–molecule reactions as shown in Scheme 2. These products were formed at very low collision energies, indicating that the stable ion of *m/z* 316 formed in the ion–molecule reactions has almost enough energy to decompose. This is consistent with the characterization suggested above about the partitioning of the exothermicity of the original proton-transfer reaction. Much of that energy is retained in the initially formed enolate so that the enolate decomposes rapidly to give products with substantial internal energy. Some of the reaction energy is partitioned into the various neutral products, indicating that not all of the *m/z* 316 ions, for example, have the same energy. Some ions have enough energy to decompose, whereas others lack sufficient energy to decompose.

Collisional activation of the stable ion at *m/z* 316 produced minor products in addition to the CF_2 loss product at *m/z* 266. In addition to the ion–molecule reaction products, CID of *m/z* 316 gave ions at *m/z* 246 and *m/z* 226, which correspond to loss of one and two molecules, respectively, of HF from the ion at *m/z* 266. It seems probable that even if the ion at *m/z* 266

Table 4
Distribution of products formed by the collision-induced dissociation of *m/z* 316 fragment formed from methyl *S*-[2-(fluoromethoxy)-1,1,3,3,3-pentafluoropropyl]-*N*-acetyl-L-cysteine **1** and methyl *S*-[2-(fluoromethoxy)-1,3,3,3-tetafluoro-1-propenyl]-*N*-acetyl-L-cysteine **2**

	Conjugate **1**		Conjugate **2**	
Fragment ion	(b) E_{com} = 0.41 eV	(c) E_{com} = 0.56 eV	(b) E_{com} = 0.32 eV	(c) E_{com} = 0.52 eV
m/z 193	0.00	0.00	0.00	0.091
m/z 226	0.093	0.16	0.099	0.11
m/z 246	0.097	0.11	0.14	0.10
m/z 254	0.24	0.22	0.21	0.21
m/z 266	0.49	0.45	0.43	0.38
m/z 274	0.00	0.069	0.14	0.11



Scheme 2.

was initially formed with the structure shown in Scheme 1, the vinylic carbanion would attack an electrophilic site in the molecule closing a ring. The carbonyl carbons would both be electrophilic, but the loss of one and two molecules of HF on CID indicates only extensive rearrangement and does not provide definitive information about the structure of the ion at *m/z* 266.

As is evident from Tables 3 and 4, it takes higher energy collisions to induce the fragmentation of *m/z* 193. It gives three fragments: *m/z* 160 corresponding to the loss of SH, *m/z* 145 corresponding to the loss of CHFO, and *m/z* 125 corresponding to the loss of CHFO and HF. Scheme 3 suggests structures for *m/z* 193 that could lead to these losses. Particularly interesting is the absence of the *m/z* 145 ion in the CID of the *m/z* 193 formed from conjugate **2**.

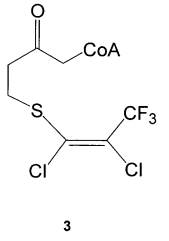
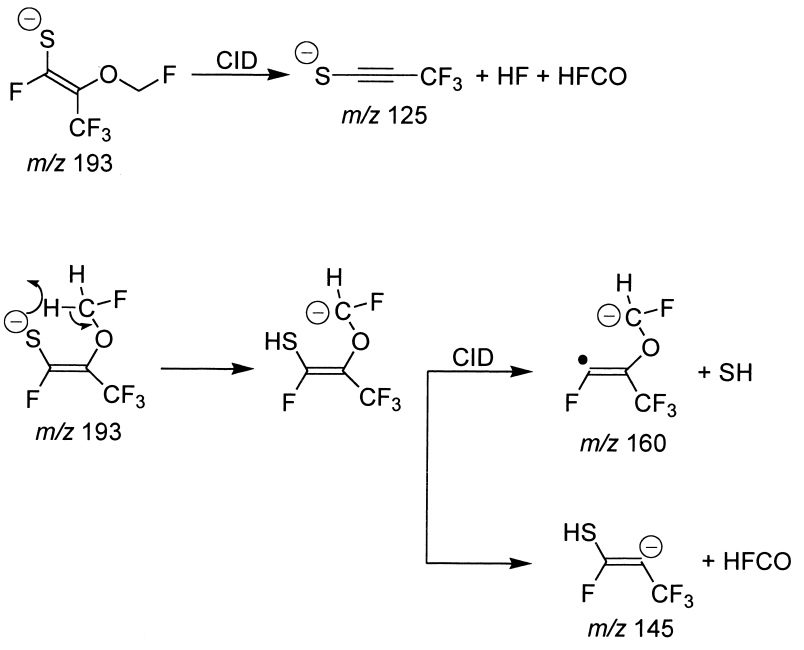


Fig. 5. Structure of desamino cysteine *S* conjugate **3**.

5. Conclusions

The anionic bases examined react with cysteine conjugates **1** and **2** by initial deprotonation of the α-carbon to form an enolate intermediate followed by elimination of either a thiolate anion or of two HF molecules (from **1**) or one HF molecule (from **2**). Since the HF elimination leads to CF_2 loss, it is suggested that a F atom eliminated as HF comes from the CF_3 group. CID of the product ions suggested structures consistent with this overall mechanistic



Scheme 3.

picture. It is evident from these results that the same mechanism by which other cysteine conjugates are bioactivated could operate in the case of Compound A. That is, deprotonation to form enolate intermediates could lead to the release of very reactive species that might inactivate enzymes by alkylating them or otherwise reacting irreversibly with them. A unique feature of the Compound A chemistry is the lability of the F atoms in the CF_3 group. In the gas phase, at least, this results in the formation of HF and CF_2. We note that the desamino cysteine conjugate **3**, with a structure similar to the Compound A conjugate, produces a significant NMR signal for F^- on incubation with medium-chain acyl-CoA dehydrogenase, suggesting activation of the CF_3 group. The acyl-CoA dehydrogenases and β-lyase both operate by mechanisms that are thought to involve deprotonation of the α-carbon in CoA thioesters and cysteine conjugates, respectively.

The elimination of thiolate from the intermediate enolate could also parallel an enzymatic process. The known Compound A metabolite, 2-(fluoromethoxy)-3,3,3-trifluoropropanoic acid, could result from hydrolysis of the thiolate (*m/z* 193).

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