Articles

Gas Phase Reactions of Cysteine with Charged Electrophiles: Regioselectivities of the Dimethylchlorinium Ion and the Methoxymethyl Cation[†,1]

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The gas phase reactions of cysteine with the dimethylchlorinium ion, $(CH_3)_2Cl^+$, and the methoxymethyl cation, $CH_3OCH_2^+$, have been studied by using chemical ionization tandem mass spectrometry (CI/MS/MS). The structures of the $[M + CH]^+$ and $[M + CH_3]^+$ ions in the CH_3Cl CI plasma and the $[M + CH]^+$, $[M + CH_3]^+$, and $[M + CH_3OCH_2]^+$ ions formed in the $(CH_3)_2O$ CI plasmas have been probed by using MS/MS techniques. Where possible, these MS/MS spectra were compared to those generated for ions of known structure (i.e., the $[M + H]^+$ ions of *N*-methylcysteine, *S*-methylcysteine, cysteine methyl ester, and (*R*)-thiozolidine-4-carboxylic acid, which were produced under isobutane CI conditions). Structure assignment of the $[M + CH_3]^+$ ions was also facilitated via the use of labeled cysteine, $H_2NCH(CD_2SH)CO_2H$, and via qualitative comparison of MS^3 spectra of the immonium and episulfonium ions, $[M + CH_3 - ROH - CO]^+$ and $[M + CH_3 - NH_2R]^+$, respectively, where R = H or CH_3, with those of the standard compounds. Taken together, the experimental data suggest that the regioselectivity for the formation of the $[M + CH_3]^+$ ions is very different for the two methyl cation donors ($CH_3OCH_2^+$ and $(CH_3)_2Cl^+$). For the case of the dimethylchlorinium ion, the data reveal that methylation occurs at all sites, with the degree of methylation at each site following the order N > S > O. In constrast, the degree of methylation at each site follows the order S > N > O for the methoxymethyl cation. Furthermore, the structures of various $[M + CH_3]^+$ and $[M + CH]^+$ ions derived from cysteine has been modeled by using *ab-initio* calculations at the MP2(FC)/6-31G*//HF/6-31G* level of theory. It was found that the *ab-initio* stability order for the $[M + CH_3]^+$ ions is N > S > O of C═O group > O of OH group, while the most stable $[M + CH]^+$ ion was found to be N-protonated (*R*)-thiozolidine-4-carboxylic acid.

Introduction

As part of our ongoing work into probing the gas phase reactivity of biological model compounds with various electrophilic species, we have examined the reactions of the simplest amino acid, glycine, with the following electrophiles: $(CH_3)_2Cl^+$,[2a] $CH_3OCH_2^+$,[2a] $(CH_2)_2X^+$ (X = Cl and Br),[2b] and NO^+.[2c] In addition, we have reported on the gas phase reactivity of a model for the nucleobase sites in DNA reacting with $(CH_3)_2Cl^+$ [3] as well as the reactions of multiply charged oligonucleotide anions reacting with Cl_3C^+ [4a] and $(CH_3)_3SiCl$.[4b]

Our approach in several of these studies[2,3] has been to use chemical ionization tandem mass spectrometry (CI/MS/MS)[5,6] together with isotopic labeling, independent synthesis of potential products, and *ab-initio* calculations to gain insights into the energetics and mechanisms of these processes. For example, we have shown that glycine reacts with the dimethylchlorinium ion in a regiospecific fashion via an S_N2 mechanism with the greater amount of methylation occurring at the nitrogen (eq 1), while the methoxymethyl cation (eq 2a) gave no observable methylation product but rather yielded a $[M + CH]^+$ ion via an addition/elimination pathway (eq 2b). MS/MS studies revealed the structure of the latter ion to be $[CH_2{=}NHCH_2CO_2H]^+$. Interestingly, the addition/elimination pathway (eq 2b) has a direct solution phase analogue.[7]

$$H_2NCH_2CO_2H + (CH_3)_2Cl^+ \longrightarrow [H_2NCH_2CO_2H + CH_3]^+ + CH_3Cl \quad (1)$$

$$H_2NCH_2CO_2H + CH_3OCH_2^+ \longrightarrow \begin{cases} [H_2NCH_2CO_2H + CH_3]^+ + CH_2O & (2a) \\ [H_2NCH_2CO_2H + CH]^+ + CH_3OH & (2b) \end{cases}$$

To gain further insights into the gas phase reactivity of the methoxymethyl cation, we have recently examined

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† Dedicated to Professor Charles H. DePuy, on the occasion of his 70th birthday.

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the kinetics of its reactions with 21 neutral nucleophiles using a FA apparatus.[8] We found that these reactions range from collision controlled to efficiencies as low as 0.006. Futhermore, $[M + CH]^+$ ion formation often dominates over $[M + CH_3]^+$ ion formation. For example, both H_3N and H_2S react with $CH_3OCH_2^+$ with an efficiency of 0.22 and yield the $[M + CH]^+$ ion as the major product ion.

In this paper we now turn our attention to the gas phase alkylation reactions of the amino acid cysteine, which is of particular interest due to the role that cysteine residues play in a range of biological processes.[9,10] For example, the cysteine thiol group in the ubiquitous tripeptide glutathione is involved in many biochemical reactions including the detoxification of foreign electrophilic compounds and reactive intermediates.[9] We note that the examination of the gas phase reactions of cysteine with the methoxymethyl cation and the dimethylchlorinium cation poses the following challenges compared to our previous studies on glycine: (1) the presence of four nucleophilic sites within the neutral increases the potential number of products, (2) a side chain fragmentation process can become significant thereby complicating the MS/MS spectra, and (3) the number of geometric conformations of product ions to be studied by using *ab initio* techniques increases.

Computational Methods

Structures of ions and neutrals were optimized at the Hartree-Fock level using GAUSSIAN 92[11] with the standard 6-31G* basis set.[12] All optimized structures were then subjected to frequency calculations with the same basis set, followed by a calculation of the correlated energy by using the MP2(FC)/6-31G* level of theory (FC = frozen core). Energies are corrected for zero-point vibrations scaled by 0.9.[13] In each case, a set of possible rotamers was explored.[14] Complete structural details and lists of vibrational frequencies for each of the lowest energy HF/6-31G* optimized conformers can be found in the supplementary material.

Experimental Section

All experiments were performed on a Fisons/VG (Manchester, UK) Autospec-Q instrument of E1BE2qQ geometry (where E = electric sector, B = magnetic sector, q = RF only quadrupole, and Q = quadrupole). MS/MS experiments were performed in the unimolecular and collision induced dissociation (CID) MIKES modes, in which the ion of interest was mass selected by using E1B and the metastable fragments were determined by scanning E2. In general, the CID spectra gave more abundant product ions and are those which are reported in this work. MS^3 experiments were performed in the CID mode by setting the ratio of the B/E1 to transmit a specific daughter ion and then scanning E2. All CID experiments were carried out by using xenon as the collision gas, set at a pressure to attenuate the precursor ion by 50%. Methylated cysteine ions, $[M + CH_3]^+$, were formed in the chemical ionization source by using either CH_3Cl or $(CH_3)_2O$ as the CI gas. $[M + CH]^+$ ions of cysteine were formed by using $(CH_3)_2O$ or CH_3Cl as the CI gas. In each instance the CI plasma conditions were optimized for the ion of interest (i.e., $(CH_3)_2Cl^+$ or $CH_3OCH_2^+$) before introducing cysteine through a heated direct insertion probe. The major ions present in the CI source as well as their modes of formation have been discussed previously.[2a] Typical source conditions were source temperature = 250 °C, electron energy = 70 eV, emission current = 200 mA, and source pressure was 6×10^{-5} mBarr, measured on the source ion gauge. The source CI mass spectra for each of the cysteine experiments are listed in the supplementary material.

S-Methylcysteine was formed via a known literature procedure (involving a sodium/liquid ammonia reduction of (*R*)-thiozolidine-4-carboxylic acid).[15] All other reagents were commercially available and were used without further purification. $H_2NCH(CD_2SH)CO_2H$ (98% D) was obtained from Cambridge Isotope Laboratories.

Results and Discussion

(A) CI/MS Studies of the Reaction of Cysteine with the Dimethylchlorinium Ion and the Methoxymethyl Cation. In order to determine the types of reactions that cysteine undergoes with both charged electrophiles, we have examined the CI/MS spectra of $H_2NCH(CH_2SH)CO_2H$ and $H_2NCH(CD_2SH)CO_2H$ (see Appendix for listings of these CI/MS) with the CI plasmas formed from CH_3Cl and $(CH_3)_2O$. In each instance quite a few product and fragment ions are observed and a detailed analysis of the formation of every one of them is beyond the scope of this paper. Instead we will focus on those product ions which arise from electrophilic modification of cysteine. Thus the key product ions formed via CH_3Cl CI/MS of cysteine are the $[M + H]^+$, $[M + CH]^+$, and $[M + CH_3]^+$ ions, while those formed in the $(CH_3)_2O$ CI plasma are the $[M + H]^+$, $[M + CH]^+$, $[M + CH_3]^+$, and $[M + CH_3OCH_2]^+$ ions. In both instances, the $[M + CH]^+$ ion was formed in a higher yield than the $[M + CH_3]^+$ ion.[16]

Given the limitations of CI/MS, it is impossible to unequivocally assign these product ions as arising from specific reagent ions present in the CI plasma. Nonetheless, by considering the known modes of reactivity of the three key reagent ions present in the CH_3Cl CI plasma, it seems likely that the $[M + H]^+$ ion arises via a reaction between cysteine and a proton donor such as CH_3ClH^+ (eq 3), while the $[M + CH]^+$ and $[M + CH_3]^+$ ions are likely to arise from cysteine reacting with CH_2Cl^+ via an addition/elimination reaction (eq 4)[17,18] and $(CH_3)_2Cl^+$ via a S_N2 reaction (eq 5).[19] Similarly, considering the known reactivity of $CH_3OCH_2^+$,[8] it seems likely that all three

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alkylated ions (i.e., $[M + CH]^+$, $[M + CH_3]^+$, and $[M + CH_3OCH_2]^+$) arise from its reactions with cysteine (eq 6).

$$M + CH_3ClH^+ \longrightarrow [M + H]^+ + CH_3Cl \quad (3)$$

$$M + CH_2Cl^+ \longrightarrow [M + CH]^+ + HCl \quad (4)$$

$$M + (CH_3)_2Cl^+ \longrightarrow [M + CH_3]^+ + CH_3Cl \quad (5)$$

$$M + CH_3OCH_2^+ \longrightarrow \begin{cases} [M + CH]^+ + CH_3OH & (6a) \\ [M + CH_3]^+ + CH_2O & (6b) \\ [M + CH_3OCH_2]^+ & (6c) \end{cases}$$

In order to probe whether the $[M + CH]^+$ and $[M + CH_3]^+$ ions arise from fragmentation reactions of the $[M + CH_3OCH_2]^+$ ion of cysteine, we have examined the MS/MS spectra of the $[M + CH_3OCH_2]^+$ ions of cysteine and its D_2 isotopomer. In each case, the only species which were formed from decomposing $[M + CH_3OCH_2]^+$ ions were the $[M + CH]^+$ ion as the major product (eq 7a) and the $[M + CH_3]^+$ ion as the minor product (eq 7a).[16]

$$[M + CH_3OCH_2]^+ \longrightarrow \begin{cases} [M + CH]^+ + CH_3OH & (7a) \\ [M + CH_3]^+ + CH_2O & (7b) \end{cases}$$

The next sections describe the results of studies undertaken to determine the structures of the $[M + CH]^+$ and $[M + CH_3]^+$ ions formed in the CH_3Cl and $(CH_3)_2O$ CI plasmas.

(B) *Ab-Initio* Studies of the Methylation of Cysteine at Various Sites. Given that both $CH_3OCH_2^+$ and $(CH_3)_2Cl^+$ form $[M + CH_3]^+$ ions with cysteine (see above), we wanted to make predicitions as to the thermodynamically favored sites of methylation of cysteine. As we have previously noted,[2a,c] the thermodynamic likelyhood of these S_N2 methylation reactions taking place depends on the relative methyl cation affinities (MCA's) of X (where X = OCH_2 and CH_3Cl) and cysteine. The MCA of a substrate X is defined by eq 8 and can be calculated from known experimental heats of formation (eq 9)[20] or via *ab-initio* calculations (eq 10).[2a,c]

$$CH_3X^+ \xrightarrow{MCA(X)} CH_3^+ + X \quad (8)$$

$$MCA(X) = \Delta H_f°(CH_3^+) + \Delta H_f°(X) - \Delta H_f°(CH_3X^+) \quad (9)$$

$$MCA(X) = E(CH_3^+) + E(X) - E(CH_3X^+) \quad (10)$$

Given the dearth of experimentally derived thermochemical information on MCA's of amino acids, we have performed *ab-initio* calculations on the $[M + CH_3]^+$ isomers (**A**, **B**, **C**, and **D**) that result from methylation at the nitrogen, at the sulfur, at the oxygen atom of the carbonyl group, and at the oxygen atom of the hydroxyl group. The starting geometries for each of the isomers was calculated at the HF/6-31G* level of theory. Conformation analysis of each isomer was performed by using a systematic search by manually generating a set of possible rotamers for each isomer.[14] In addition, vibrational frequency analysis and single point energies were computed for each conformation. We found a total of 20, 23, 33, and 21 conformers of **A**, **B**, **C**, and **D**, respectively.[21] The HF/6-31G* optimized geometries of the lowest energy rotamer for the four isomeric structures are shown in Figure 1, their full geometries (in cartesian coordinates) and vibrational frequencies are given in the Appendix, while their energies at various levels of theory are given in Table 1. Data on all the other higher energy conformers of **A**−**D** are available from the authors upon request.

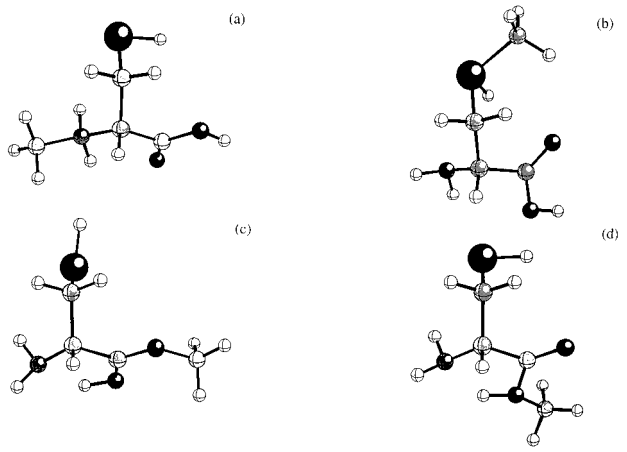
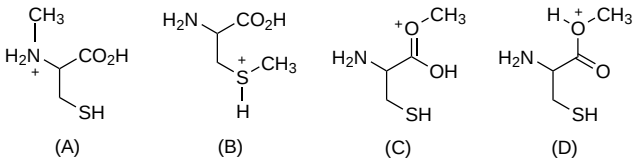


Figure 1. HF/6-31G* optimized structures of (a) N-methylated cysteine, (b) S-methylated cysteine, (c) CO-methylated cysteine, and (d) OH-methylated cysteine.



Using the data in Table 1, it is possible to calculate the MCA of the individual functional groups within cysteine (at 0 K) via eq 10, where $E(CH_3^+)$ is the total energy of the methyl cation (corrected for zero point energy vibrations), $E(X)$ is the total energy of cysteine (corrected for zero point energy vibrations), and $E(CH_3X^+)$

(17) For gas phase reactions of CH_2Cl^+, see: (a) Burinsky, D. J.; Campana, J. E. *Org. Mass Spectrom.* **1988**, *23*, 613. (b) Lane, D. C.; McGuire, M. *Org. Mass Spectrom.* **1983**, *18*, 494. (c) Isern-Fletcha, I.; Cooks, R. G.; Wood, K. V. *Int. J. Mass Spectrom. Ion Proc.* **1984**, *62*, 73. (d) Benezra, S. A.; Hoffmann, M. K.; Bursey, M. M. *J. Am. Chem. Soc.* **1995**, *117*, 2071. (e) Speranza, M.; Pepe, N.; Cipollini, R. *J. Chem. Soc. Perkin Trans. 2* **1979**, 1179. (f) Brodbelt, J. S.; Cooks, R. G. *Anal. Chim. Acta* **1988**, *206*, 239. (g) Karpas, Z.; Anicich, V. G.; Huntress, W. T. *Chem. Phys. Lett.* **1978**, *59*, 84. (h) Karpas, Z. *Chem. Phys. Lett.* **1985**, *120*, 53.

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(20) (a) For a discussion and compilation of MCA's, see: Bartmess, J. E. *Mass Spectrom. Rev.* **1989**, *8*, 297. (b) For experimental gas phase thermochemical data on ions and neutrals, see: *Gas-phase Ion and Neutral Thermochemistry*. Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin R. D.; Mallard, W. G. *J. Phys. Chem. Ref. Data* **1988**, *17*, Suppl. 1. (c) The experimental heat of formation of $ClCH_2^+$ is 227.0 kcal mol^{-1}: Holmes, J. L.; Lossing, F. P.; McFarlane, R. A. *Int. J. Mass Spectrom. Ion Proc.* **1988**, *86*, 209.

(21) We cannot guarantee that all possible conformations of each of these species have been found.

Table 1. *Ab-Initio* Total Energies and Zero Point Energies of the Isomeric $[M + CH_3]^+$ Ions of Cysteine

species	energies, hartrees			rel energies, kcal mol^{-1}	
	HF/6-31G*	MP2(fc)/6-31G*	ZPE[a]	MP2[b]	HF[c]
CH_3^+	−39.230 64	−39.325 14	0.027 31		
CH_3SH	−437.700 32	−437.952 35	0.044 67		
$(CH_3)_2SH^+$	−477.057 00	−477.437 00	0.083 65		
$NH_2CH(CH_2SH)CO_2H$	−719.373 02	−720.394 90	0.106 35		
$[CH_3NHCH(CH_2SH)CO_2H]^+$	−758.772 57	−759.922 30	0.147 11	0.0	0.0
$[NH_2CH(CH_2SHCH_3)CO_2H]^+$	−758.742 90	−759.891 98	0.144 69	17.1	17.5
$[NH_2CH(CH_2SH)C(OCH_3)OH]^+$	−758.744 72	−759.885 77	0.144 83	16.0	21.5
$[NH_2CH(CH_2SH)CO(HOCH_3)]^+$	−758.708 69	−759.859 85	0.143 20	37.6	36.7

[a] Corrected by 0.9. [b] At the MP2(fc)/6-31G*//HF/6-31G* + 0.9 ZPE level of theory. [c] At the HF/6-31G*//HF/6-31G* + 0.9 ZPE level of theory.

Table 2. Calculated Methyl Cation Affinities (MCA's) of the Various Nucleophilic Sites in Cysteine

nucleophile	methyl cation affinitiy (MCA), kcal mol^{-1} [a]		
	HF/6-31G*	MP2(FC)/6-31G*	exptl[b]
CH_3SH	71.8	92.8	99.8
nitrogen atom of NH_2 group[c]	97.6	118.5	*g*
sulfur atom of SH group[d]	80.5	101.0	*g*
oxygen atom of C=O group[e]	81.5	97.0	*g*
oxygen atom of OH group[f]	60.0	81.8	*g*

[a] All calculations were carried out with the energies given in Table 1 using eq 10 in the text. [b] Taken from ref 20a. [c] See Figure 1a for geometry. [d] See Figure 1c for geometry. [e] See Figure 1b for geometry. [f] See Figure 1d for geometry. [g] Unknown.

is the total energy of the methylated cysteine species (corrected for zero point energy vibrations). The *ab-initio* MCA's (at the MP2(FC)/6-31G*//HF/6-31G* level, in kcal mol^{-1}) thus obtained are listed in Table 2. Thus the thermodynamic order for methylation is as follows: nitrogen atom > sulfur atom > oxygen atom of the carbonyl group > the oxygen atom of the hydroxyl group. The MCA's of $(CH_3)_2Cl^+$ and $CH_3OCH_2^+$ are 62.0 and 78.3 kcal mol^{-1}, respectively.[2a] Thus methylation at all sites should be thermodynamically possible for both $(CH_3)_2Cl^+$ and $CH_3OCH_2^+$.

Although we cannot directly comment on the kinetics for methylation of the individual sites within cysteine, the rates of reactions of $(CH_3)_2Cl^+$ and $CH_3OCH_2^+$ with some simpler nucleophiles have been studied in the gas phase. Thus Sen Sharma and Kebarle have shown that the rate constants for the reactions of $(CH_3)_2Cl^+$ with nucleophiles increase approximately as the reaction exothermicity increases.[19a] The situation is somewhat more complicated for the kinetics of $CH_3OCH_2^+$ since $[M + CH]^+$ formation competes with $[M + CH_3]^+$ formation. Nonetheless, a similar general trend is observed. If the kinetics for the formation of the various isomeric $[M + CH_3]^+$ ions were to follow the thermodynamic MCA's of the various nucleophilic sites in cysteine, then methylation at nitrogen, sulfur, and oxygen should be observed. Experiments designed to examine the sites of methylation in cysteine are described in the next section.

(C) Experimental Studies of the Gas Phase Methylation of Cysteine. As noted in the Introduction, the MS/MS of electrophilically modified cysteine is potentially more complicated than that of glycine for two main reasons. First, the sulfhydryl group can change the fragmentation characteristics of ions derived from cysteine relative to their glycine counterparts. For example, while the formation of the immonium ion is the base peak in the MS/MS spectrum of the $[M + H]^+$ ion of glycine (eq 11), the major peak in the MS/MS spectrum of the $[M + H]^+$ ion of cysteine arises via the loss of ammonia to produce an episulfonium ion (eq 12).

$$[H_3NCH_2CO_2H]^+ \rightarrow [H_2NCH_2]^+ + H_2O + CO \quad (11)$$

$$[H_3NCH(CH_2SH)CO_2H]^+ \rightarrow [CH(CH_2SH)CO_2H]^+ + NH_3 \quad (12)$$

Second, the MS/MS spectra of alkylated cysteine ions can give rise to isomeric product ions which cannot be distinguished on the basis of their *m/z* ratios alone. For example, by examining the MS/MS spectra of the $[M + CH_3]^+$ ions of glycine we were readily able to distinguish the degree of methylation by direct comparison of the immonium ions formed via fragmentation. Thus the product due to methylation at oxygen yielded the immonium ion $H_2N{=}CH_2^+$ ($m/z = 30$, eq 13) while the product due to methylation at nitrogen yielded the immonium ion $CH_3NH{=}CH_2^+$ ($m/z = 44$, eq 14). This is not the case for cysteine: although the product due to methylation at oxygen is expected to yield the immonium ion $H_2N{=}CHCH_2SH^+$ ($m/z = 76$, eq 15), the ion corresponding to methylation at nitrogen produces an immonium ion $CH_3NH{=}CHCH_2SH^+$ (eq 16) which is isomeric (i.e., it has the same *m/z* of 90) to the immonium ion $H_2N{=}CHCH_2SCH_3^+$ resulting from methylation at sulfur (eq 17).

$$[H_2NCH_2CO_2CH_3 + H]^+ \rightarrow [H_2N{=}CH_2]^+ + CH_3OH + CO \quad (13)$$

$$[CH_3NHCH_2CO_2H + H]^+ \rightarrow [CH_3NH{=}CH_2]^+ + H_2O + CO \quad (14)$$

$$[H_2NCH(CH_2SH)CO_2CH_3 + H]^+ \rightarrow [H_2N{=}CHCH_2SH]^+ + CH_3 + CO \quad (15)$$

$$[CH_3NHCH(CH_2SH)CO_2H + H]^+ \rightarrow [CH_3NH{=}CHCH_2SH]^+ + H_2O + CO \quad (16)$$

$$[H_2NCH(CH_2SCH_3)CO_2H + H]^+ \rightarrow [H_2N{=}CHCH_2SCH_3]^+ + H_2O + CO \quad (17)$$

A similar situation arises for the formation of the episulfonium ions (which result from the loss of an amine, RNH_2, where R = H or CH_3) in the MS/MS spectra of the $[M + CH_3]^+$ ions of cysteine. Thus, although the product due to methylation at nitrogen is expected to yield the episulfonium ion $(HSCH_2CH)CO_2H^+$ ($m/z = 105$, eq 18), the ion corresponding to methylation at oxygen produces an episulfonium ion $(HSCH_2CH)CO_2CH_3^+$ (eq 19) which is isomeric (i.e., it has the same *m/z* of 119) to the episulfonium ion $(CH_3SCH_2CH)CO_2H^+$ resulting from

Table 3. CID MS/MS Spectra of $[M + CH_3]^+$ Ions of Cysteine and the $[M + H]^+$ Ions of Its Methylated Derivatives

precursor ion, *m/z*	daughter ions[d]
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136[a]	119(100), 105(25), 102(11), 90†(44), 88†(43), 87†(46), 76*(35), 70(5), 59*(49), 45*(21), 32†(15), 30†(9), 28†(8)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138[a]	121(100), 107(30), 104(17), 92(61), 89†(58), 88†(156), 78(49), 74*(12), 61*(60), 47*(33), 32†(19), 30*(12)
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136[b]	119(100), 107(7), 90*(67), 76†(10), 74†(11), 70†(5), 68(10), 63†(14), 61†(21), 59†(13), 47†(14), 45†(8), 30†(10), 28†(7)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138[b]	121(85), 109†(11), 104(8), 92*(100), 78†(9), 75†(9), 74†(10), 63†(29), 61*(21), 45*(27), 30†(9), 28†(7)
$[CH_3NHCH(CH_2SH)CO_2H + H]^+$, 136[c]	119(16), 105(65), 102(21), 94†(11), 90†(100), 88†(74), 87†(63), 76†(16), 74†(13), 59*(68), 45†(25), 43†(19), 32†(33), 30†(16), 28†(11)
$[H_2NCH(CH_2SCH_3)CO_2H + H]^+$, 136[c]	119(100), 94*(8), 90*(5), 74*(5), 61*(5), 45*(5)
$[H_2NCH(CH_2SH)CO_2CH_3 + H]^+$, 136[c]	119(100), 102(7), 88†(21), 87†(34), 76(75), 59*(26), 55(7), 44*(13)

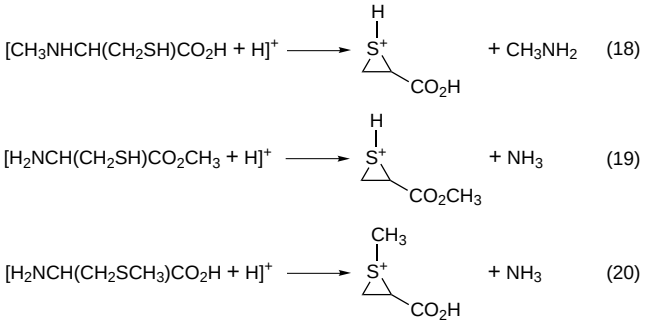
[a] Formed in the CH_3Cl CI plasma. [b] Formed in the $(CH_3)_2O$ CI plasma. [c] Formed in the isobutane CI plasma. [d] (†) Designates an unresolved ion. (*) Designates an unresolved multiplet of ions with the indicated ion having the highest intensity.

Table 4. CID MS/MS/MS Spectra of the Immonium Ions of Cysteine and Its Methylated Derivatives

precursor ion, parent *m/z*, daughter *m/z*	grandaughter ions[d]
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136, 76[a]	59(100), 47*(10), 43*(19)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138, 78[a]	61(100), 49*(7), 45*(20)
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136, 90[a]	75*(7), 59*(23), 57*(100), 47*(7), 45*(13), 42*(7), 28*(6)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138, 92[a]	61*(17), 59*(100), 49*(8), 46*(9), 42*(9), 28*(6)
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136, 76[b]	59(100), 47*(11), 45*(11), 43*(13), 28*(7)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138, 78[b]	61(100), 49*(20), 45*(28)
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136, 90[b]	74*(21), 63†(44), 61*(97), 44*(100), 28*(19)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138, 92[b]	74*(7), 63*(100), 44*(83), 28*(8)
$[CH_3NHCH(CH_2SH)CO_2H + H]^+$, 136, 90[c]	75†(5), 59†(13), 57*(100), 47*(5), 45*(7), 42*(9), 28*(5)
$[H_2NCH(CH_2SCH_3)CO_2H + H]^+$, 136, 90[c]	75*(100), 61(42), 58†(11), 45*(35), 43*(91), 30,(11), 28*(9)
$[H_2NCH(CH_2SH)CO_2CH_3 + H]^+$, 136, 76[c]	59(100), 48†(5), 47†(7), 45†(8), 43*(18), 28(5)

[a] Formed in the CH_3Cl CI plasma. [b] Formed in the $(CH_3)_2O$ CI plasma. [c] Formed in the isobutane CI plasma. [d] (†) Designates an unresolved ion. (*) Designates an unresolved multiplet of ions with the indicated ion having the highest intensity.

methylation at sulfur (eq 20).



Notwithstanding these difficulties with isomeric daughter ions, we have examined the MS/MS spectra of the various $[M + CH_3]^+$ ions of cysteine (Table 3). A consideration of the methylated "standards" reveals that despite the problems of isomeric daughter ions, their MS/MS spectra do differ from each other in terms of both the types of ions observed and their relative abundances. Thus the N-methylated standard has abundant ions at *m*/z 105 and 90, corresponding to the episulfonium and immonium ions, respectively. The S-methylated standard has abundant ions at *m/z* 119 and 76, corresponding to the episulfonium and immonium ions, respectively. Finally, the O-methylated standard has abundant ions at *m/z* 119 and 90, corresponding to the episulfonium and immonium ions respectively. A comparison of the MS/MS spectra of the $[M + CH_3]^+$ ions of cysteine formed in the CH_3Cl and $(CH_3)_2O$ plasmas reveals discernible differences. Indeed, when each of these $[M + CH_3]^+$ ions of cysteine is compared with those of the "standards", it becomes apparent that the MS/MS spectrum of the $[M + CH_3]^+$ ion of cysteine formed in the CH_3Cl plasma has the closest match to that of the N-methylated standard. In contrast, the MS/MS spectrum of the $[M + CH_3]^+$ ion of cysteine formed in the $(CH_3)_2O$ plasma has a closer match to that of the S-methylated standard.

In order to gain further insights into the site(s) of methylation, the MS^3 spectra for the product ions (i.e., the immonium ions and the episulfonium ions) formed in the MS/MS spectra of the $[M + CH_3]^+$ ions can be compared with the MS^3 data of independantly synthesized standards. Thus we have carried out MS^3 experiments of the structures of the immonium ions and episulfonium ions formed in the MS/MS spectra of the various $[M + CH_3]^+$ ions of cysteine. The data can be found in Tables 4 and 5, respectively.

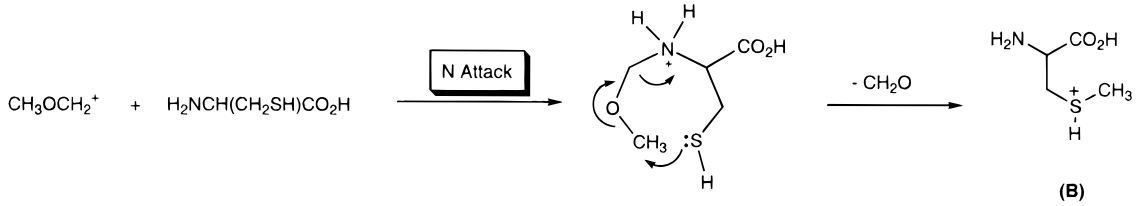
Consider first the immonium ions: thus the MS/MS/MS spectrum of immonium ion of the O-methylated standard (*m/z* 76) clearly matches with those of the immonium ions at *m/z* 76 from the CH_3Cl and $(CH_3)_2O$ plasmas. Thus these immonium ion "markers" clearly reveal that O-methylation occurs in *both* plasmas. Furthermore, the MS/MS/MS spectra of the isomeric immonium ions (*m/z* 90) of N-methylated and S-methylated cysteine display distinct differences: the N-methylated species gives an abundant ion at *m/z* 57 with another distinctive ion at *m/z* 42, while the S-methylated species gives an abundant ion at *m/z* 75 with other distinctive ions at *m/z* 61 and *m/z* 43. A comparison of the MS/MS/MS spectra of the immonium ions at *m/z* 90 formed in the CH_3Cl and $(CH_3)_2O$ plasmas reveals discernible differences. Indeed, when each of the MS/MS/MS spectra of the immonium ions at *m/z* 90 are compared with those of the "standards", it becomes apparent that the MS/MS/MS spectrum of the *m/z* 90 immonium ions originating from the $[M + CH_3]^+$ ion of cysteine formed in the CH_3Cl plasma has the closest match to that derived from the N-methylated standard. In contrast, MS/MS/MS spectrum of the *m/z* 90 immonium ions originating from the $[M + CH_3]^+$ ion of cysteine formed in the $(CH_3)_2O$ plasma

Table 5. CID MS/MS/MS Spectra of the Episulfonium Ions of Cysteine and Its Methylated Derivatives

precursor ion, parent *m/z*, daughter *m/z*	grandaughter ions[d]
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136, 105[a]	87(100), 59*(23), 45*(9)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138, 107[a]	89(100), 61*(26), 45*(5)
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136, 119[a]	91(27), 87(100), 75(19), 59*(49), 45*(16)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138, 121[a]	89(100), 75(10), 61*(32), 57†(16), 45*(7)
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136, 105[b]	87(100), 59*(29), 45*(10)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138, 107[b]	89(100), 61*(37), 45*(8)
$[H_2NCH(CH_2SH)CO_2H + CH_3]^+$, 136, 119[b]	103(32), 101*(9), 91*(70), 87*(63), 77†(55), 75†(49), 73†(100), 59*(51), 55†(23), 45*(52), 27*(11)
$[H_2NCH(CD_2SH)CO_2H + CH_3]^+$, 138, 121[b]	103(8), 101*(8), 93*(27), 89(44), 87(15), 77†(41), 75*(100), 61*(35), 57*(26), 45*(40), 29*(7)
$[H_2NCH(CH_2SH)CO_2H + H]^+$, 122, 105[c]	87(100), 59*(13), 45*(6)
$[H_2NCH(CH_2SH)CO_2H + H]^+$, 124, 107[c]	87(100), 59*(13), 45*(6)
$[CH_3NHCH(CH_2SH)CO_2H + H]^+$, 136, 105[c]	89(100), 61*(20), 45*(6)
$[H_2NCH(CH_2SCH_3)CO_2H + H]^+$, 136, 119[c]	103(25), 101*(7), 90*(70), 86(14), 77(46), 75†(43), 73†(100), 58*(49), 55†(26), 45*(72), 41(9), 27*(9)
$[H_2NCH(CH_2SH)CO_2CH_3 + H]^+$, 136, 119[c]	87(100), 59(25), 55(7), 45(5)

(a) Formed in the CH_3Cl CI plasma. (b) Formed in the $(CH_3)_2O$ CI plasma. (c) Formed in the isobutane CI plasma. (*) Designates an unresolved multiplet of ions with the indicated ion having the highest intensity. (†) Designates an unresolved ion.

Scheme 1



has a closer match to that derived from the S-methylated standard. Thus the immonium ion data reveal that O methylation occurs in both plasmas and that the preferred order of methylation in the CH_3Cl plasma is N > S, while that in the $(CH_3)_2O$ plasma is S > N. Furthermore, these MS/MS/MS results are consistent with the MS/MS data discussed above.

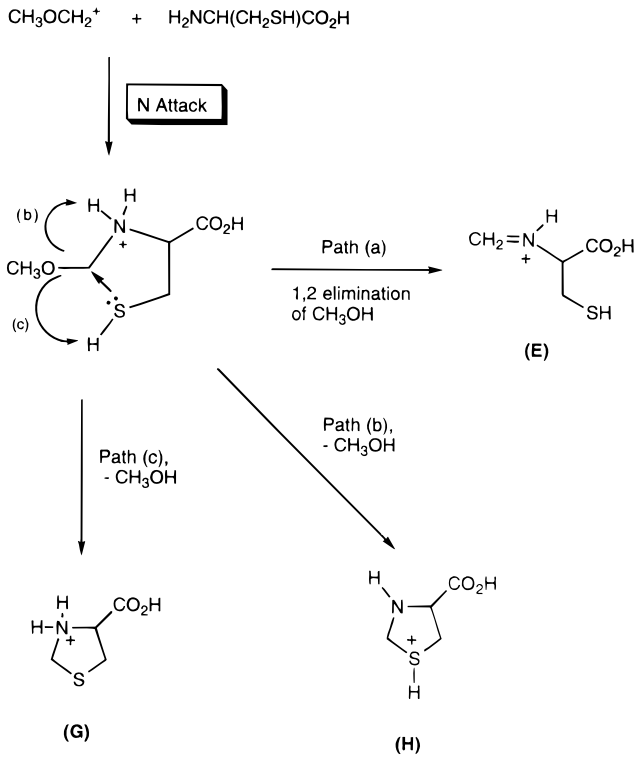
A similar analysis can be done by considering the episulfonium ions: thus the MS/MS/MS spectrum of episulfonium ion of the N-methylated standard (*m/z* 105) clearly matches with those of the episulfonium ions at *m/z* 105 from the CH_3Cl and $(CH_3)_2O$ plasmas. Thus these episulfonium ion "markers" clearly indicate that N-methylation occurs in *both* plasmas. Furthermore, the MS/MS/MS spectra of the isomeric episulfonium ions (*m/z* 119) of O-methylated and S-methylated cysteine display distinct differences: the O-methylated species gives an abundant ion at *m/z* 87 with another distinctive ion at *m/z* 59, while the S-methylated species gives an abundant ion at *m/z* 73 with other distinctive ions at *m/z* 58 and *m/z* 45. A comparison of the MS/MS/MS spectra of the episulfonium ions at *m/z* 119 formed in the CH_3Cl and $(CH_3)_2O$ plasmas reveal discernible differences. Indeed, when each of the MS/MS/MS spectra of the episulfonium ions at *m/z* 119 are compared with those of the "standards", it becomes apparent that the MS/MS/MS spectrum of the *m/z* 119 episulfonium ions originating from the $[M + CH_3]^+$ ion of cysteine formed in the CH_3Cl plasma has the closest match to that derived from the O-methylated standard. In contrast, MS/MS/MS spectrum of the *m/z* 119 episulfonium ions originating from the $[M + CH_3]^+$ ion of cysteine formed in the $(CH_3)_2O$ plasma has a closer match to that derived from the S-methylated standard. Thus the episulfonium ion data reveal that N-methylation occurs in both plasmas and that the preferred order of methylation in the CH_3Cl plasma is O > S, while that in the $(CH_3)_2O$ plasma is S > O.

Taken all together, the experimental data suggest that the regioselectivity for the formation of the $[M + CH_3]^+$ ions is very different for the two methyl cation donors ($CH_3OCH_2^+$ and $(CH_3)_2Cl^+$). For the case of the dimethylchlorinium ion, the data reveal that methylation occurs at all sites, with the degree of methylation at each site following the order N > O > S. In constrast, the degree of methylation at each site follows the order S > N > O for the methoxymethyl cation.

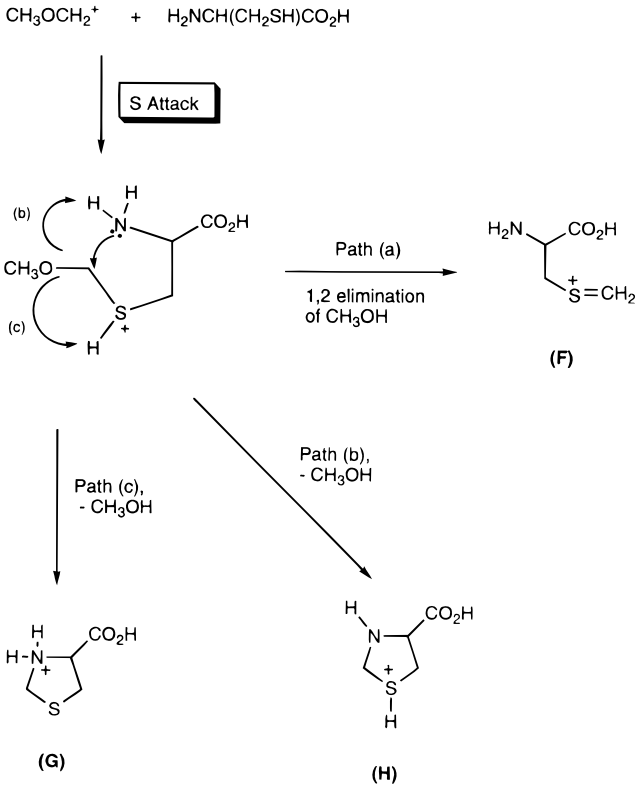
The seemingly anomalous experimental result obtained for the case of the $[M + CH_3]^+$ ion derived from the methoxymethyl cation suggests that preferential methylation on sulfur may be due to the initial formation of an energized $[M + CH_3OCH_2]^+$ ion (in which the methoxymethyl cation is bound to the nitrogen atom), which subsequently undergoes an intramolecular CH_3^+ transfer with concomitant loss of CH_2O (Scheme 1).

(D) *Ab-Initio* and Experimental Studies of the $[M + CH]^+$ Ions of Cysteine. What are the structures of the $[M + CH]^+$ ions of cysteine formed in the CH_3Cl and $(CH_3)_2OCI$ plasmas? In order to answer this question, it is worth considering the gas phase reactivity of the methoxymethyl cation and the chloromethyl cation, which are the electrophiles likely to be responsible for the formation of the $[M + CH]^+$ ions in the $(CH_3)_2O$ and CH_3Cl CI plasma, respectively. Thus using the FA technique, we have recently shown that both H_3N and H_2S react with $CH_3OCH_2^+$ with an efficiency of 0.22 and yield the $[M + CH]^+$ ion as the major product ion, while H_2O reacts with $CH_3OCH_2^+$ with an efficiency of 0.006 to yield no discernible trace of a $[M + CH]^+$ ion. Unfortunately the kinetics of the reactions of $ClCH_2^+$ have only been studied for two nucleophiles: H_2S and H_2O. Under ICR conditions, H_2S and H_2O react with $ClCH_2^+$ with efficiencies of 0.33 and 0.05, respectively, yielding the $[M + CH]^+$ ion as the only product ion. Finally, as noted in the introduction, our previous studies on glycine revealed the structure of the $[M + CH]^+$ ion (which was formed under very similar $(CH_3)_2O$ CI conditions to those used here) to be $[CH_2{=}NHCH_2CO_2H]^+$.

Scheme 2



Scheme 3



Thus two possible structures of the $[M + CH]^+$ ions are the two acyclic structures **E** and **F**, which can arise from initial attack by the electrophile $CH_2{=}X^+$ (X = Cl and OCH_3) onto the amino group (path A of Scheme 2) and the sulfhydryl group, respectively (path A of Scheme 3). It is also important to consider cyclic structures such as **G**−**J**, since several workers have shown that related species may be involved in the reactions of $CH_3OCH_2^+$ which result in the formation of $[M + CH]^+$ ions.[22] These

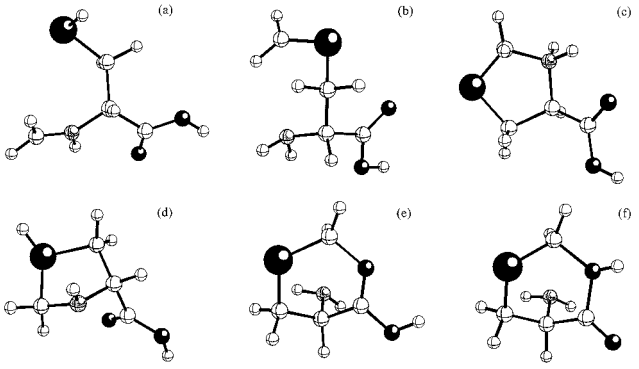
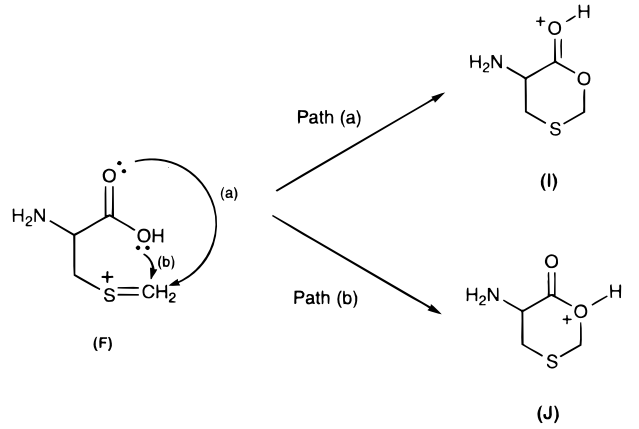


Figure 2. HF/6-31G* optimized structures of (a) $CH_2{=}NHCH(CH_2SH)CO_2H^+$, (b) $H_2NCH(CH_2S{=}CH_2)CO_2H^+$, (c) N-protonated thiozolidine-4-carboxylic acid, (d) S-protonated thiozolidine-4-carboxylic acid, (e) **I**, and (f) **J**.

Scheme 4



species may be formed directly (paths b and c of Schemes 2 and 3) or may be formed from their acyclic precursors (Scheme 4). Note that **G** and **H** are related to the parent neutral system thiozolidine-4-carboxylic acid.

Thus, in order to gain some insights into the thermochemistry associated with the formation of the various isomeric $[M + CH]^+$ ions, we have carried out *ab-initio* calculations on their structures, energies, and vibrational frequencies.[23] Once again several different conformations were considered for each species, and in each instance, more than one stable conformation was found (33 for **E**, 35 for **F**, 5 for **G**, 8 for **H**, 2 for **I**, 2 for **J**).[24] The HF/6-31G* optimized geometries of each of the the most stable conformers are shown in Figure 2; their full geometries (in cartesian coordinates) and vibrational frequencies are given in the Appendix, while their energies at various levels of theory are given in Table 6. Using the data from Table 6, we calculated the energetics associated with the formation of the various $[M + CH]^+$ ions from both the

(22) (a) Pau, J. K.; Kim, J. K.; Caserio, M. C. *J. Am. Chem. Soc.* **1978**, *100*, 3838. (b) Eichmann, E. S.; Brodbelt, J. S. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 97. (c) Eichmann, E. S.; Brodbelt, J. S. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 230.

(23) As we have noted previously, the MP2(FC)/6-31G*//HF/6-31G* + 0.9 ZPE level of theory gives reasonable thermochemistry when compared to experimentally derived thermochemistry. This is also true for the thermochemistry of $[M + CH]^+$ ion formation. Thus, the thermochemistry for the formation of $CH_2{=}NH_2^+$ is −16.0 kcal mol^{-1} (experimentally, see ref 2a) and −17.9 kcal mol^{-1} (*ab initio*, see ref 2a) for $CH_3OCH_2^+$ and −60.1 kcal mol^{-1} (experimentally, see refs 22b,c) and −63.9 kcal mol^{-1} (*ab initio*, see ref 2a) for $ClCH_2^+$.

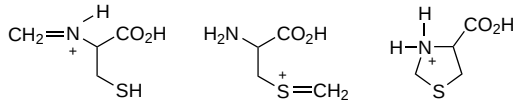
(24) One of the conformers of **H** underwent ring opening during the optimization process, yielding a confromer of **E** as the final optimized geometry. Although we have not searched for a transition state to this process, this suggests a low barrier to ring opening.

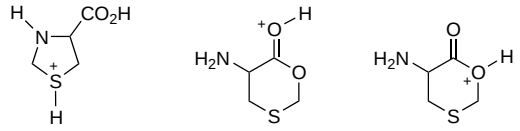
Table 6. Energies Associated with the Formation of [M + CH]$^+$ Ions in the Reaction of $H_2NCH_2CO_2H$ and $CH_3OCH_2^+$ (Schemes 2 and 3)

species	energies, hartrees[a] HF/6-31G*	MP2(FC)/6-31G*	ZPE[b]
$CH_3OCH_2^+$ [c]	−153.204 03	−153.618 73	0.066 75
$ClCH_2^+$ [d]	−498.149 92	−498.393 73	0.027 20
CH_3OH [e]	−115.035 42	−115.344 93	0.055 34
HCl [f]	−460.059 98	−460.192 24	0.006 53
$CH_2{=}NHCH(CH_2SH)CO_2H^+$ [g]	−757.585 51	−758.716 62	0.123 01
$H_2NCH(CH_2S{=}CH_2)CO_2H^+$ [h]	−757.547 79	−758.691 20	0.123 50
N-protonated thiozolidine-4-carboxylic acid [i]	−757.601 17	−758.746 16	0.128 91
S-protonated thiozolidine-4-carboxylic acid [j]	−757.582 15	−758.712 82	0.123 18
I [k]	−757.559 28	−758.694 81	0.126 86
J [l]	−757.520 54	−758.666 76	0.125 51

[a] All calculations were carried out on the HF/6−31G* optimized geometries. [b] Scaled by 0.9. [c] Taken from ref 2a. [d] See supplementary material for geometry. [e] Taken from ref 2a. [f] Taken from ref 2b. [g] See Figure 2a for geometry. [h] See Figure 2b for geometry. [i] See Figure 2c for geometry. [j] See Figure 2d for geometry. [k] See Figure 2e for geometry. [l] See Figure 2f for geometry.

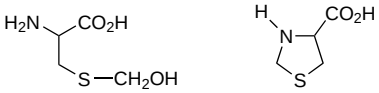
reactions of $CH_3OCH_2^+$ and $ClCH_2^+$ with cysteine (cf. eqs 4 and 6a). An examination of these data, which is given in Table 7, reveals that in each case, the formation of the [M + CH]$^+$ ions from the reaction of $ClCH_2^+$ is more exothermic than the reaction of $CH_3OCH_2^+$. The thermodynamically favored order for the [M + CH]$^+$ product ions (at the MP2(FC)/6-31G*//HF/6-31G* level of theory) is **G** > **E** > **H** > **I** > **F** > **J**. Furthermore, with the exception of the reaction between cysteine and $CH_3OCH_2^+$ to yield **J**, each of the isomeric [M + CH]$^+$ ions is a thermodynamically viable product.



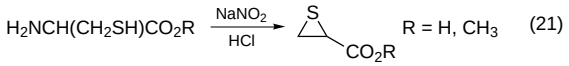


To gain insights into the structures of the ions formed in both the CH_3Cl and $(CH_3)_2O$ CI plasmas, we have carried out several MS/MS experiments, the results of which are given in Table 8. The MS/MS spectra of both [M + CH]$^+$ ions of cysteine formed in the CH_3Cl and $(CH_3)_2O$ CI plasmas are virtually identical to that of the [M + H]$^+$ ion of thiozolidine-4-carboxylic acid formed in the isobutane plasma. This suggest that these ions have similar structures which fragment via the same pathways. Thus the thermodynamically favored product **G** seems a likely candidate, although we cannot rule out the possibility of other structures for these [M + CH]$^+$ ions.

(E) Comparisons between Gas Phase and Solution Phase Reactivity of Cysteine. Although the reactions that we have studied in the gas phase have not been carried out in the solution phase, some indirect analogies can be made. For example, Tome and Naulet have studied the reactions of various amino acids with formaldehyde under acid catalyzed conditions. They found that cysteine reacts rapidly with formaldehyde to form the *S*-hydroxymethyl derivative **K**, which subsequently undergoes a cylization reaction to form the cyclic species **L**.



Regarding the fragmentation reactions of the various [M + CH_3]$^+$ ions of cysteine, there is an analogy to the key reaction channel involving elimination of ammonia to form the episulfonium ions. For example, cysteine and its esters undergo deaminative cyclizations to give thiiranecarboxylic acids when treated with sodium nitrite−hydrochloric acid (eq 21).[25] In this case, the amino group of cysteine is converted into a better leaving group (N_2), thereby facilitating this cyclization reaction.



Conclusions

This is the first study on the electrophilic modification of cysteine by organic electrophiles in the gas phase. Methylation at nitrogen and oxygen is observed, in direct analogy to our previous results for glycine. In addition, methylation is observed at sulfur, which is consistent with the solution phase reactivity of cysteine. In seems

Table 7. Thermodynamics for the Formation of Various [M + CH]$^+$ Ions of Cysteine

reaction	level of theory	thermochemistry, kcal mol^{-1},[a] for formation of **E**	**F**	**G**	**H**	**I**	**J**
cysteine + $CH_3OCH_2^+$	HF	−23.5	0.5	−29.6	−21.3	−4.6	18.8
	MP2	−26.1	−9.8	−40.9	−23.6	−10.0	6.8
cysteine + CH_2Cl^+	HF	−78.6	−54.6	−84.7	−76.4	−59.7	−36.2
	MP2	−76.7	−60.4	−91.5	−74.2	−60.6	−43.8

[a] All calculations were carried out with the energies given in Tables 1 and 6; see text for discussion.

Table 8. CID MS/MS Spectra of [M + CH]$^+$ Ions of Cysteine and the [M + H]$^+$ Ions of Thiozolidine-4-carboxylic Acid

precursor ion, *m/z*	daughter ions[d]
[$H_2NCH(CH_2SH)CO_2H$ + CH]$^+$, 134[a]	117(6), 106(2), 100(1), 88(100), 74(1), 69*, 61*, 59*, 45*,43*, 30*, 28*
[$H_2NCH(CD_2SH)CO_2H$ + CH]$^+$, 136[a]	119(5), 108(2), 102*, 90(100), 75*, 69*, 61*, 59*, 45*, 30*, 28*
[$H_2NCH(CH_2SH)CO_2H$ + CH]$^+$, 134[b]	117(5), 106(1.5), 100*, 88(100), 74*, 69*, 61*, 59*, 45*, 43*, 30*, 28*
[$H_2NCH(CD_2SH)CO_2H$ + CH]$^+$, 136[b]	119(3), 108(3), 102*, 90(100), 75*, 69*, 61*, 59*, 45*, 30*, 28*
[thiozolidine-4-carboxylic acid + H]$^+$, 134[c]	117(3), 106(2), 100*, 88(100), 74(1), 69*, 61(1), 59(1), 45*, 43*, 30*, 28*

[a] Formed in the CH_3Cl CI plasma. [b] Formed in the $(CH_3)_2O$ CI plasma. [c] Formed in the isobutane CI plasma. [d] (*) Designates an ion with an intensity of less than 1%, but greater than 0.1%.

likely that other amino acids possessing nucleophilic side chains (such as serine, lysine, and arginine) will undergo alkylation at the side chain site. We are currently investigating the use of gas phase ion−molecule reactions between organic electrophiles and simple biomolecules as a means of identifying structure. One such example, which involves using the methoxymethyl cation to cleave peptide bonds, is under investigation and our results will be reported in due course.

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Supporting Information Available: Listing of source CI mass spectra (Appendix 1) and optimized geometries in the form of Cartesian coordinates and vibrational frequencies of all structures at the HF/6-31G* level (Appendix 2) (22 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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