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Dimethyl Ether Chemical Ionization Mass Spectrometry of α-Amino Acids

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Dimethyl ether (DME) is a useful reagent gas for the characterization of a variety of diverse biologically important or environmentally significant classes of compounds. In this work the gas-phase ion–molecule reactions of DME with 23 α-amino acids were investigated and the collision-induced dissociation (CID) fragmentations of the protonated molecules and their most prominent adduct ions were studied. The identities and relative abundances of the adduct ions varied widely and, not unexpectedly, were dependent on the nature of the R substituent in $H_2NCH(R)CO_2H$. With a few exceptions, notably serine and threonine, protonated molecules and $[M + 13]^+$ adduct ions were highly abundant, and in most cases methoxymethylene cations $[M + 45]^+$ were also prominent. The solvated methoxymethylene cation $[M + 91]^+$ was seen in very modest abundance or not at all, except for serine and threonine, when it was the most abundant. CID fragmentations of the protonated molecules generated in the DME plasma showed similar characteristics to those generated by fast atom bombardment in that sequential elimination of H_2O and CO to the iminium ion was the predominant process in the majority of cases, and was also accompanied by the loss of NH_3 in the cases of cysteine, glutamine, ornithine and lysine. Loss of NH_3 alone was the predominant process for tryptophan, and for arginine and methionine the fragmentations were dominated by the guanidino and methylthio substituents, respectively. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Methane chemical ionization (CI) mass spectra of the biologically important α-amino acids were first reported 27 years ago and were interpreted in terms of protonation at a specific site in the molecule followed by fragmentation reactions depending on the site of protonation.[1] Later studies with hydrogen and isobutane in addition to methane provided evidence for other low-energy fragmentation pathways.[2–4] Of the many ionization methods developed for the mass spectrometry of thermally labile and involatile molecules, fast atom bombardment (FAB) has been most widely applied to study the fragmentation characteristics of molecular ion species of a variety of amino acids under both low- and high-energy conditions.[5,6] The low-energy metastable ion fragmentation and high-energy collision-induced dissociation (CID) fragmentation reactions of both the $[MH]^+$ and $[M - H]^-$ species of 24 amino acids using mass-analyzed ion kinetic energy spectroscopy (MIKES)[7] were first reported, but no detailed fragmentation mechanisms were suggested.[5] The recent comprehensive study of fragmentation of FAB-generated protonated α-amino acids by Harrison and co-workers[6] utilized an energy-resolved mass spectrometric approach, in which the observed energy dependence of the CID fragmentations allowed for the elucidation of mechanistic pathways for many of the amino acids. Other investigations of the fragmentation of protonated individual amino acids included those of arginine, produced by field desorption and by FAB,[8] methionine, produced by FAB,[9] and leucine and isoleucine, produced by FAB[10] and by plasma desorption and methane and ammonia CIMS.[11]

Dimethyl ether (DME), an unusual positive ion reagent for CIMS, was first investigated by Keough[12] for differentiation among functional group isomers by specific ion–molecule reactions. Since then, in the past 5–6 years DME has been extensively used for the characterization of a variety of classes of organic compounds, and was the topic of a recent review.[13] Ion–molecule reactions of DME gave rise to fragment–molecule adducts which were especially useful in differentiation among isomeric substituted aromatics.[14–18] DME CIMS has further been applied to such diverse biologically important or environmentally significant classes of compounds as benzodiazepines,[19] cinchona alkaloids,[20] trichothecene biotoxins,[21] nucleic acid bases and nucleosides,[22] nucleoside antibiotics,[23] nitramine munitions[18,24] and polynuclear aromatic hydrocarbons.[25]

A rationale for the application of DME CIMS to individual amino acids was suggested by the ongoing work of O'Hair and co-workers in which the gas-phase

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reactions of the simplest amino acid, glycine, with a variety of electrophiles including the dimethylchloronium ion $(CH_3)_2Cl^+$ and the methoxymethylene cation $CH_3OCH_2^+$ were studied.[26] The former reaction was a non-regioselective S_N2 process leading to methylation at both nitrogen and oxygen, whereas the latter was a regiospecific pathway involving addition at nitrogen followed by elimination of methanol to form a $[M + 13]^+$ adduct. In the more complex case of cysteine, published recently,[27] for both electrophiles the predominant pathway was addition/elimination. It was shown that for both electrophiles methylation took place at all three possible sites to different degrees: for dimethylchloronium ion the extents of methylation decreased in the order N > S > O, whereas for methoxymethylene cation the order S > N > O was followed. Hence it appeared likely that other amino acids with nucleophilic side-chains would undergo gas-phase alkylation with electrophilic reagents at the side-chain site. In this work we investigated the gas-phase ion–molecule reactions of DME with 23 α-amino acids and studied the CID fragmentations of the protonated molecules and of the most prominent fragment–molecule adducts in each case.

EXPERIMENTAL

The instrument used was a Finnigan MAT TSQ-70B with a 20 kV dynode detector. Operating parameters were source temperature 80 °C, source pressure 6–7 Torr (1 Torr = 133.3 Pa) (measured by a Convectron gauge), electron energy 70 eV, emission current 400 μA, dynode 15 kV, electrometer amplifier gain 10^{-4} mA V^{-1} and scan time 0.5 s. For the CID experiments argon was the collision gas at 2 mTorr collision cell pressure, the analyte was accelerated to ~30–40 eV and the electrometer amplifier gain was reduced to 10^{-5} mA V^{-1}. Samples (0.1–0.5 μg) were introduced into the source by direct exposure desorption from a rhenium filament heated at 16–17 °C s^{-1}. Relative abundances were unchanged over this sample size range, and were essentially identical over a pressure range of 3–7 Torr. The amino acids were purchased from Sigma Chemical and DME reagent gas from US Airgas.

RESULTS AND DISCUSSION

Principal adduct ions

The identities and relative abundances of adduct ions observed for the amino acids, displayed in Table 1, varied widely and, not unexpectedly, were dependent on the nature of the R substituent in $H_2NCH(R)CO_2H$. With a few exceptions, notably serine and threonine, protonated molecules and $[M + 13]^+$ adduct ions were highly abundant, and in most cases methoxymethylene cations $[M + 45]^+$ were also prominent. The solvated methoxymethylene cation $[M + 91]^+$ was seen in very low abundance or not at all, except for serine and threonine, where it was the most abundant adduct ion. In

Table 1. Dimethyl ether PCI mass spectra of α-amino acids [*m/z* and relative abundances (%) of principal ions]

Compound	$[MH]^+$	$[M+13]^+$	$[M+15]^+$	$[M+45]^+$	$[M+47]^+$	$[M+57]^+$	$[M+59]^+$	$[M+91]^+$	$[M+93]^+$	$[2M+H]^+$
Gly	—[a]	—[a]	—[a]	120 (19)	122 (100)	132 (48)	134 (71)	166 (10)	168 (9)	151 (18)
Ala	—[a]	102 (100)	—	134 (29)	136 (87)	146 (20)	148 (67)	180 (14)	182 (7)	179 (8)
Pro	116 (94)[b]	128 (83)	130 (30)	160 (100)	162 (32)	172 (56)	174 (12)	206 (12)	—	231 (22)
Val	118 (56)[c]	130 (100)	—	162 (79)	164 (41)	174 (80)	176 (36)	208 (14)	—	—
Leu	132 (70)	144 (100)	—	176 (51)	178 (46)	188 (17)	190 (28)	222 (7)	—	263 (1)
Ile	132 (79)	144 (100)	—	176 (63)	178 (49)	188 (5)	190 (18)	222 (7)	—	263 (2)
Ser	106 (3)	118 (5)	—	150 (19)	152 (46)	162 (3)	164 (68)	196 (100)	198 (54)	211 (15)
Thr	120 (5)	132 (7)	—	164 (39)	166 (60)	176 (7)	178 (55)	210 (100)	212 (38)	239 (6)
Hyp	132 (73)	144 (24)	146 (4)	176 (100)	178 (10)	—	—	222 (2)	—	263 (1)
Asp	134 (83)	146 (100)	—	178 (32)	180 (38)	—	192 (11)	224 (3)	—	267 (2)
Glu	148 (97)	160 (100)	—	192 (45)	194 (11)	—	206 (1)	238 (1)	—	295 (4)
Asn	133 (87)	145 (100)	—	177 (36)	179 (24)	—	191 (6)	223 (1)	—	265 (5)
Gln[d]	147 (100)	159 (93)	161 (9)	191 (66)	193 (5)	—	—	—	—	293 (5)
Arg[e]	175 (100)	187 (8)	189 (7)	219 (51)	—	—	—	—	—	—
Lys	147 (100)	159 (95)	161 (15)	191 (76)	—	—	—	—	—	293 (43)
Orn	133 (100)	145 (72)	147 (11)	177 (51)	—	—	—	—	—	265 (1)
His	156 (95)	168 (23)	170 (15)	200 (100)	—	—	—	—	—	311 (11)
Phe	166 (100)	178 (97)	—	210 (92)	212 (77)	—	224 (12)	256 (12)	—	331 (12)
Tyr	182 (100)	194 (95)	—	226 (81)	228 (37)	—	240 (5)	272 (4)	—	363 (5)
Trp	205 (100)	217 (57)	—	249 (37)	251 (9)	—	263 (3)	—	—	—
Cys	122 (85)	134 (100)	136 (10)	166 (78)	168 (99)	—	180 (88)	212 (14)	214 (8)	243 (20)
Met	150 (93)	162 (100)	164 (11)	194 (79)	196 (44)	—	—	240 (4)	—	299 (7)
$[Cys]_2$	241 (100)	253 (37)	255 (23)	285 (95)	287 (55)	—	—	—	—	—

[a] Not determined.
[b] $[M - H]^+$ (92%) was also observed.
[c] $[M - H]^+$ (46%) was also observed.
[d] In addition, $[M + 28]^+$ and $[M + 30]^+$ adduct ions were seen in very high abundances (99 and 96%, respectively).
[e] In addition, the $[M + 28]^+$ adduct was seen in high abundance (77%).

contrast, for hydroxyproline $[M + 45]^+$ was highest in abundance while $[M + 91]^+$ amounted to little more than a trace. Enhanced stability of the solvated cations $[M + 91]^+$ had not been observed in previously reported studies of DME mass spectra of simple alcohols,[28] diols[29] and diol ethers[30] or of complex polyfunctional polyhydroxylated species.[20–23] The only previously documented instances of enhanced stability of solvated methoxymethylene cations were unrelated and were due to proximity effects of adjacent nitro substituents in nitroaromatic isomers[17,18] and in the nitramines RDX and HMX.[18,24]

Methoxymethylene cations $[M + 45]^+$ are well known to undergo fragmentation by two major pathways: loss of CH_2O followed by transfer of methyl to give $[M + 15]^+$ adducts, and loss of CH_3OH containing one hydrogen derived from the substrate, resulting in $[M + 13]^+$ adducts.[13] A third pathway involving loss of C_2H_4O and/or CH_3OCH_2 to give protonated molecules and molecular ions, respectively, has been documented for polynuclear aromatic hydrocarbons.[25] The protonated DME adducts $[M + 47]^+$ have been shown to be loosely bonded complexes which fragment readily and exclusively to the respective protonated molecules.[14,31] Except for the basic amino acids (arginine, lysine, ornithine and histidine), $[M + 47]^+$ adducts were present in amounts varying from 100 to a few per cent (Table 1). The solvated protonated counterparts $[M + 93]^+$ were seen in significant abundances only for serine and threonine.

It should be mentioned that at source pressures > 0.4 Torr, the solvated ions $C_2H_5O\ C_2H_6O^+$ (m/z 91) and $C_2H_7O\ C_2H_6O^+$ (m/z 93) were predominant in the DME plasma;[12,13] consequently, it was not possible to measure protonated molecules for glycine and alanine or $[M + 13]^+$ or $[M + 15]^+$ adducts for glycine.

Protonated dimers or $[2M + H]^+$ adducts, well characterized in early methane CI of amino acids,[2] were observed in moderate abundances in most cases but, in agreement with the earlier work, only on the initial scan.

Trends in the formation of $[M + 13]^+$ and $[M + 15]^+$ adducts should be noted. The former predominated throughout; indeed, in the majority of cases $[M + 15]^+$ was not observed. For cysteine the relative abundances were 100 and 10, in keeping with the results of O'Hair and co-workers.[27] These researchers have also documented that $[M + 13]^+$ adducts are the predominant products of reaction of methoxymethylene with a variety of neutral nucleophiles such as NH_3 and H_2S. Unfortunately, except for proline, the relative abundances of the $[M + 15]^+$ adducts of the amino acids were too small for CID measurements for comparison with the patterns of their $[M + H]^+$ and $[M + 13]^+$ counterparts.

Regarding the site of alkylation to form the $[M + 45]^+$, $[M + 15]^+$ and $[M + 13]^+$ adducts, amino acids with nucleophilic side-chain substituents most probably alkylate at that site, as was seen for cysteine. Those with alkyl and aromatic side-chains most probably undergo a regiospecific alkylation at nitrogen as was observed for glycine.[26] It may be significant that whereas $[M + 13]^+$ adducts were generally very abundant, the less abundant $[M + 15]^+$ adduct ions were, with the exception of proline, seen only for amino acids with nucleophilic side-chain substituents.

In addition to the protonated molecule and $[M + 13]^+$, two unusual adduct ions, to our knowledge not previously reported in DME mass spectrometry, dominated the ion profile for glutamine. These were $[M + 28]^+$ and $[M + 30]^+$, which corresponded formally to $[M + CO]^+$ and $[M + CH_2O]^+$, respectively. With the exception of asparagine, which gave rise to the $[M + 28]^+$ adduct in high abundance, this phenomenon was not observed for any other amino acid.

CID fragmentations of protonated molecules

Fragmentation of protonated amino acids by elimination of the elements of H_2, C and O_2 to iminium ions has been studied mechanistically for at least 20 years under a wide variety of experimental and energetic conditions,[4,5,11,32] Sequential elimination of H_2O and CO is generally believed to be the major process, and for simplicity we shall use this terminology throughout. CID fragmentations of the protonated molecules of aliphatic amino acids generated in the DME plasma, summarised in Table 2, gave rise to the respective iminium ions as the major products. No fragmentations with loss of NH_3 or H_2O were observed, in agreement with the recent FAB generated study[6] and in contrast to the earlier one.[5] Rather, substantial amounts of a fragment due to loss of NH_3 in addition to $H_2O + CO$ were observed for valine and isoleucine but not for leucine or proline. It has been well documented that CID fragmentation of the iminium ions derived from leucine and isoleucine proceeded by different mechanisms to give distinctly different product ions: in the former case the most abundant ion (m/z 44) resulted from McLafferty rearrangement with loss of propene, whereas in the latter case loss of NH_3 to give a product ion of m/z 69 was predominant.[6,10,11] These characteristics have found important application in the structural determination of unknown peptides.[33,34] Hence it is likely that the abundant $C_4H_7^+$ cation (m/z 55) observed on CID of DME-protonated valine was derived by loss of NH_3 from the iminium ion by a process similar to that observed for isoleucine.

Of the amino acids bearing a hydroxyl or carboxyl substituent in the side-chain, serine and threonine did not give rise to sufficient amounts of protonated molecules for CID studies (Table 1). CID fragmentations of protonated molecules in the other three cases are summarized in Table 3. For hydroxyproline the iminium

Table 2. CID fragmentation of protonated aliphatic α-amino acids

Compound	Neutral loss [m/z and relative abundance (%)] $H_2O + CO$	$H_2O + CO + NH_3$
Pro	70 (100)	—
Val	72 (100)	55 (61)
Leu	86 (100)	—
Ile	86 (100)	69 (14)

Table 3. CID fragmentation of protonated acidic and hydroxylated α-amino acids

Compound	Neutral loss [*m/z* and relative abundance (%)]			
	H_2O	$H_2O + CO$	$2H_2O + CO$	$H_2O + CH_2CO$
Asp	116 (19)	88 (100)	—	74 (85)
Glu	130 (48)	102 (57)	84 (100)	—
Hyp	—	86 (100)	68 (88)	—

ion was the most abundant CID product, but the ion resulting from further loss of the 4-hydroxyl substituent was also present in nearly equal abundance.

The CID product distributions for protonated aspartic acid and glutamic acid were significantly different, indicating that these homologous ions fragmented by different pathways. For aspartic acid an important product ion, nearly as abundant as the iminium cation, was observed at *m/z* 74 and was not seen among the product ions of protonated glutamic acid. In addition, a homologous ion (*m/z* 88) was not among the products of the latter. The *m/z* 74 ion corresponded to loss of H_2O and CH_2CO to a stable species, $[HOOCCH{=}NH_2]^+$. The energy-resolved CID experiments of Harrison and co-workers[6] have shown that for protonated aspartic acid molecules generated by FAB, the ion at *m/z* 116 resulting from initial loss of H_2O from the side-chain carbohydroxy group, which predominated at low collision energies, gave rise to the *m/z* 74 species which became the base peak at higher collision energies. The predominant product ion arising from CID of DME-protonated glutamic acid was due to loss of $2H_2O + CO$, and the corresponding homologue was not seen among the CID products of DME-protonated aspartic acid (Table 3). The iminium ion of *m/z* 102 resulting from loss of $H_2O + CO$ and an ion of *m/z* 130 due to loss of a single H_2O were also prominent. These results are consistent with the pathway suggested for FAB-protonated glutamic acid,[6] whereby dehydration to pyroglutamic acid (*m/z* 130) was followed by loss of $H_2O + CO$ to a cyclic iminium ion of *m/z* 84.

CID product distributions for the two amidic amino acids, aspargine and glutamine, like those of their carbohydroxy relatives described above, differed significantly and indicated that the two homologous ions fragmented by different pathways which were analogous to those observed for aspartic and glutamic acids. For asparagine (Table 4) the most abundant product ion was $[HOOCCH{=}NH_2]^+$ (*m/z* 74), most probably resulting from successive losses of ammonia and ketene. The iminium ion of *m/z* 87 was also a major product, and the ion of *m/z* 116 due to loss of NH_3 was present in modest abundance, as had been observed for aspartic acid. In contrast, for glutamine (Table 4) the ion due to loss of NH_3 was nearly as abundant as the predominant ion (*m/z* 84). These results suggested a pathway similar to that for glutamic acid, where deamination to pyroglutamic acid was followed by loss of $H_2O + CO$ to a cyclic iminium ion.

Table 4. CID fragmentation of protonated amidic α-amino acids

Compound	Neutral loss [*m/z* and relative abundance (%)]			
	NH_3	$H_2O + CO$	$H_2O + CO + NH_3$	$NH_3 + CH_2CO$
Asn	116 (24)	87 (84)	—	74 (100)
Gln	130 (80)	101 (35)	84 (100)	—

Of the basic amino acids, protonated arginine not unexpectedly showed the most complex fragmentation pattern (see Table 5), although we did not observe any of the fragment ions of $m/z > 116$ which have been reported by other investigators under different conditions, namely *m/z* 158 ($-NH_3$),[5,6,8] 157 ($-H_2O$)[5,6] and 130.[5,6,35] Moderate amounts of the ion of *m/z* 116 due to loss of guanidine with formation of protonated proline were seen. Fragmentation of the latter with loss of $H_2O + CO$ to the cyclic iminium ion of *m/z* 70 has been observed in this and earlier[6] work. The most abundant CID product of DME-protonated arginine was the guanidinium ion (*m/z* 60). Fragmentations of protonated lysine and ornithine resulted in modest abundances of ions resulting from loss of NH_3, and in both cases the products resulting from further loss of $H_2O + CO$ were predominant. We did not observe the loss of water reported previously for FAB-protonated lysine[6,36] and ornithine.[5,6] For protonated histidine loss of $H_2O + CO$ was also the predominant process, and in this case we did not observe prior losses of NH_3 or H_2O. An additional minor product ion of *m/z* 83 might be formulated as a protonated methylimidazole.

CID product ion distributions for the DME-protonated aromatic amino acids are summarized in Table 6. For phenylalanine loss of $H_2O + CO$ to the iminium ion predominated and only a trace of the ion due to loss of NH_3 was seen. The ion of *m/z* 74 ($[HO_2CCH{=}NH_2]^+$) resulting from formal loss of toluene was also a minor product. For tyrosine, in addition to the predominant ion due to loss of $H_2O + CO$ and that due to loss of NH_3, significant abundances of two ions which had been observed previously as products of FAB-protonated tyrosine[5,6] were also present. While the nature of the more abundant ion (*m/z* 123) is not readily apparent, that of *m/z* 119 was attributable to losses of $NH_3 + H_2O + CO$. For DME-protonated tryptophan, loss of NH_3 was predominant and formation of the iminium ion was less important. The origin of the four additional product ions (see Table 6) is not

Table 5. CID fragmentation of protonated basic α-amino acids

Compound	Fragment ion [*m/z* and relative abundance (%)]	Identity or neutral loss assignment
Arg	116 (23)	$-(H_2N)_2C{=}NH$
	84 (10)	
	70 (69)	$-[H_2O + CO + (H_2N)_2C{=}NH]$
	60 (100)	$(H_2N)_3C^+$
Lys	130 (18)	$-NH_3$
	84 (100)	$-[H_2O + CO + NH_3]$
Orn	116 (14)	$-NH_3$
	70 (100)	$-[H_2O + CO + NH_3]$
His	110 (100)	$-[H_2O + CO]$
	95 (16)	
	83 (10)	

Table 6. CID fragmentation of protonated aromatic α-amino acids

Compound	Neutral loss [*m/z* and relative abundance (%)] NH_3	$H_2O + CO$	Other ions [*m/z* and relative abundance (%)]
Phe	149 (1)	120 (100)	74 (6): $[HO_2CCH{=}NH_2]^+$
Tyr	165 (11)	136 (100)	123 (26), 119 (16), 91 (13)
Trp	188 (100)	159 (15)	146 (75), 132 (33), 131 (30), 118 (41)

clear, but it may be significant that the most abundant of them, at *m/z* 146, is related to the precursor ion by a loss of 59 u, as is the unidentified product ion of *m/z* 123 from protonated tyrosine.

Fragmentation characteristics of the DME-generated protonated sulfur-containing amino acids are listed in Table 7. For cysteine the predominant processes were loss of $H_2O + CO$ to the iminium ion followed by loss of NH_3 to the most abundant product ion (*m/z* 59). Product ions due to loss of NH_3 and further loss of H_2O were also important. Similar processes have been documented for FAB-protonated cysteine.[6] Fragmentation of protonated methionine, on the other hand, gave rise to some unusual product ions and was dominated by the presence of the methylthio group.[9,37] The iminium ion resulting from loss of $H_2O + CO$ was seen in only modest abundance, and the product resulting from further loss of CH_3SH was by far the most abundant ion. The product due to further loss of NH_3 from the iminium ion was not seen. Ions due to single losses of NH_3 and CH_3SH from the protonated molecule were observed in approximately equal abundances, and in neither case was a product ion corresponding to an additional loss of H_2O seen. The ion $[HO_2CCH{=}NH_2]^+$ of *m/z* 74 was also present and was attributed to loss of ethyl methyl sulfide from the protonated molecule. Fragmentation of the protonated molecule to give the sulfonium ion $[CH_3SCH_2]^+$ was also a significant process.

Fragmentation of the protonated dimer cystine gave rise to a major product ion of *m/z* 152 and two other ions of approximately equal abundance of *m/z* 122 and 120. The latter two clearly constituted protonated and deprotonated cysteine, respectively. The predominant product ion, $[C_3H_6NO_2S_2]^+$, corresponded formally to addition of sulfur to deprotonated cysteine and could be formulated as the heterocycle **1**, derived from the protonated molecule by rearrangement with loss of alanine.

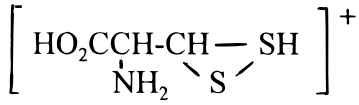

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Table 7. CID fragmentation of protonated sulfur-containing α-amino acids

Compound	Fragment ion [*m/z* and relative abundance (%)]	Identity or neutral loss assignment
Cys	105 (23)	$-NH_3$
	87 (12)	$-[NH_3 + H_2O]$
	76 (86)	$-[H_2O + CO]$
	59 (100)	$-[H_2O + CO + NH_3]$
Met	133 (4)	$-NH_3$
	104 (17)	$-[H_2O + CO]$
	102 (5)	$-CH_3SH$
	74 (6)	$[HO_2CCH{=}NH_2]^+$
	61 (14)	$[CH_3SCH_2]^+$
	56 (100)	$-[H_2O + CO + CH_3SH]$
$(Cys)_2$	152 (100)	$[C_3H_6NO_2S_2]^+$
	122 (44)	$[CysH]^+$
	120 (42)	$[Cys - H]^+$

CID-fragmentations of $[M + 45]^+$ adduct ions

Of the two major fragmentation processes observed previously for $[M + 45]^+$ adducts, only loss of CH_3OH was important, and loss of CH_2O was seen as a minor process for the adducts of proline and hydroxyproline only. Further fragmentations of the $[M + 45]^+$ adducts were complex and the identities of the neutral(s) lost could not be assigned unambiguously. The results are summarized in Tables 8–10. For the adducts of the majority of aliphatic, hydroxylic and acidic amino acids (Table 8), the most prominent product ion was due to loss of *m/z* 78, and loss of CH_3OH was also important. Exceptions were proline, hydroxyproline and leucine. For the adducts of phenylalanine, tyrosine and cysteine (Table 9), by far the most abundant product ion was also that due to loss of *m/z* 78, and loss of CH_3OH was observed to a lesser extent.

The amidic and basic amino acid adducts, with the exception of that of asparagine, did not give rise to appreciable losses of CH_3OH and showed some characteristic fragmentations not observed in other cases (Table 10). The asparagine adduct fragmented principally with loss of *m/z* 93, whereas for arginine loss of *m/z* 91 predominated. The other three basic amino acid adducts (histidine, ornithine and lysine) also showed substantial fragmentation with loss of *m/z* 93. For the adducts of histidine and glutamine the predominant product ions corresponded to losses of *m/z* 76 and 77, respectively. The ornithine and lysine adducts fragmented principally with loss of *m/z* 107, and this fragmentation was also observed to be substantial for arginine and glutamine. No loss of *m/z* 107, but a major loss of *m/z* 105, was seen for the histidine adduct. In this context it should be noted that the only product ion observed for the tryptophan adduct corresponded to loss of *m/z* 105, and similarly for the methionine adduct the only product ion was due to loss of *m/z* 106.

The cystine adduct gave rise to a major product ion of *m/z* 154 (Table 9), which corresponded to two more

Table 8. CID fragmentation of $[M + 45]^+$ adducts of aliphatic, hydroxylic and acidic α-amino acids

	Neutral loss [*m/z* and relative abundance (%)]							
Compound	CH_2O	CH_3OH	−46	−60	−78	−90	−92	Other ions [neutral loss]
Gly	—	88 (12)	74 (21)	60 (34)	42 (66)	—	—	45 (100)
Ala	—	102 (21)	88 (4)	74 (38)	56 (100)	—	—	45 (87)
Pro	130 (4)	128 (63)	114 (4)	100 (37)	—	70 (100)	68 (2)	
Val	—	130 (31)	116 (3)	102 (40)	84 (100)	72 (15)	70 (3)	
Leu	—	144 (3)	—	—	—	—	—	88 (100) [−88]
Ile	—	144 (28)	130 (3)	116 (55)	98 (100)	86 (20)	—	88 (46) [−88]
Ser	—	118 (75)	—	90 (58)	72 (100)	60 (37)	—	
Thr	—	132 (37)	—	104 (11)	86 (100)	74 (21)	—	88 (58) [−76]
Hyp	146 (6)	144 (100)	130 (3)	116 (52)	—	86 (92)	—	
Asp	—	146 (58)	132 (22)	118 (26)	100 (83)	88 (31)	86 (100)	128 (84)[a]
Glu	—	160 (18)	—	—	114 (100)	102 (6)	100 (2)	142 (7)[a]
								84 (52) [−108]

[a] $-[CH_3OH + H_2O]$.

hydrogen atoms than were present in the cyclic sulfonium cation **1**, which was the predominant product observed for the protonated molecule. Protonated cysteine was also a minor product of the $[M + 45]^+$ adduct.

CID fragmentations of $[M + 13]^+$ adduct ions

The fragmentations of these adducts, summarized in Tables 11–13, were also complex and widely variable, but showed some characteristics similar to those of the protonated molecules. For many of the aliphatic, aromatic and acidic amino acid adducts, loss of CO $+ H_2O$ was an important process, and loss of CO alone was also frequently observed. Losses of H_2O alone were seen for aspartic and glutamic acids (Table 11) and of NH_3 alone for ornithine and lysine (Table 12). Asparagine, but not glutamine, gave rise to losses of NH_3 and CO both alone and concomitantly.

Further fragmentations of the $[M + 13]^+$ adducts of aspartic and glutamic acids were dissimilar and were analogous to those of the protonated molecules (see

Table 9. CID fragmentation of $[M + 45]^+$ adducts of aromatic and sulfur-containing α-amino acids

	Neutral losses [*m/z* and relative abundance (%)]				
Compound	CH_3OH	−78	−90	−92	Other ions [neutral loss]
Phe	178 (2)	132 (100)	120 (3)	—	
Tyr	194 (3)	148 (100)	136 (4)	—	
Trp	—	—	—	—	144 (100) [−105]
Cys	134 (16)	88 (100)	—	74 (9)	120 (1) [−46]
Met	162 (1)	—	—	—	88 (100) [−106]
$[Cys]_2$	—	—	—	—	154 (100) [−139][a]

[a] The fragment ion corresponding to protonated cysteine at *m/z* 122 was also observed in moderate abundance (35%).

Table 10. CID fragmentation of $[M + 45]^+$ adducts of amidic and basic α-amino acids

	Neutral losses [*m/z* and relative abundance (%)]						
Compound	CH_3OH	−49	−76	−77	−93	−107	Other ions [neutral loss]
Asn	145 (46)	—	—	—	84 (100)	—	117 (32) [−60]
Gln	—	142 (49)	—	114 (100)	—	84 (42)	130 (98) [−61]
Arg	—	—	143 (21)	—	—	112 (86)	128 (100) [−91]
His	—	—	124 (100)	—	107 (37)	—	154 (2) [−46]
							122 (9) [−78]
							95 (88) [−105]
Orn	145 (2)	128 (73)	—	100 (29)	84 (52)	70 (100)	160 (8) [NH_3]
							127 (73) [−50]
							115 (93) [−62]
							99 (13) [−78]
Lys	159 (3)	142 (22)	—	114 (6)	98 (79)	84 (100)	128 (14) [−63]
							129 (6) [−62]

Table 11. CID fragmentation of $[M + 13]^+$ adducts of aliphatic, hydroxylic and acidic α-amino acids

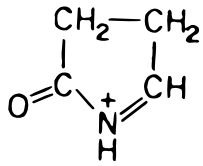
	Neutral losses [*m/z* and relative abundance (%)]							
Compound	H_2O	CO	$CO + H_2O$	−56	−58	−74	−76	Other ions [neutral loss]
Pro	—	100 (50)	—	—	70 (100)	—	—	
Val	—	102 (29)	84 (100)	—	72 (17)	56 (46)	—	67 (14) [−63]
								55 (53) [−75]
Leu	—	—	—	88 (100)	—	70 (3)	—	
Ile	—	116 (38)	98 (100)	88 (84)	86 (11)	70 (5)	—	
Hyp	—	116 (26)	—	—	86 (39)	—	68 (100)	
Asp	128 (38)	118 (12)	100 (54)	—	88 (25)	—	70 (11)	86 (100) [−60]
Glu	142 (7)	132 (3)	114 (46)	—	102 (7)	—	84 (100)	96 (15) [−64]
								68 (91) [−92]

Table 12. CID fragmentation of $[M + 13]^+$ adducts of amidic and basic α-amino acids

	Neutral losses [*m/z* and relative abundance (%)]								
Compound	NH_3	CO	$CO + NH_3$	$CO + H_2O$	−59	−61	−73	−75	−92
Asn	128 (38)	117 (10)	100 (19)	99 (14)	86 (97)	84 (100)	—	—	—
Gln	—	—	—	—	—	—	—	—	67 (100)
His	—	—	—[a]	—	—	107 (16)	95 (100)	—	—
Orn	128 (20)	—	—	—	—	84 (23)	—	70 (100)	—
Lys	142 (15)	—	—	—	—	98 (37)	—	84 (100)	—

[a] A product ion of *m/z* 124, corresponding to neutral loss of 44 u, was observed in high abundance (75%).

Table 3). The predominant product ion for aspartic acid (*m/z* 86) corresponded to loss of H_2O and CH_2CO, whereas that for glutamic acid corresponded to the heterocyclic cation **2**, which was also the most prominent product ion from the protonated molecule. The $[M + 13]^+$ adduct ions of ornithine and lysine also fragmented in a manner similar to the protonated molecules, giving rise to ions of *m/z* 70 and 84, respectively, as the predominant products.



2

Although it is not possible to make any structural assignments for the $[M + 45]^+$ or the $[M + 13]^+$ adducts on the basis of the observed CID fragmentations, it can be seen from the data summarized in Tables 8–13 that for 14 of the 17 amino acids for which comparisons of the product ion distributions of both $[M + 45]^+$ and $[M + 13]^+$ can be made, the *m/z* values of the most abundant product ions are identical in each case. Since one of the major fragmentation pathways for $[M + 45]^+$ adduct ions derived from a variety of organic substrates is known to involve loss of methanol containing a hydrogen atom derived from the substrate to give $[M + 13]^+$ adducts,[13,19] it is likely that for a majority of the amino acids, both further fragmentation of $[M + 45]^+$ and fragmentation of $[M + 13]^+$ take place according to similar processes. Glutamic acid, glutamine and hydroxyproline appear to be exceptions.

Table 13. CID fragmentation of $[M + 13]^+$ adducts of aromatic and sulfur-containing α-amino acids

	Neutral losses [*m/z* and relative abundance (%)]		
Compound	$CO + H_2O$	−73	−74
Phe	132 (100)	105 (5)	—
Tyr	148 (100)	—	—
Trp	—	144 (100)	—
Cys	88 (100)	—	—
Met	—	—	88 (100)

CONCLUSIONS

CID fragmentations of the protonated molecules were distinctive and useful in the characterization of isomers and homologues. Aspartic and glutamic acid showed significantly different fragmentation pathways, which were comparable to those observed for their FAB-generated counterparts,[6] and dramatic differences in the product ion distributions of asparagine and glutamine were attributable to fragmentation pathways analogous to those of the acid homologs. In contrast, ornithine and lysine behaved in a similar manner, and arginine showed complex fragmentations due to the guanidino group. The protonated dimer cystine, which had not been studied previously, gave rise to a unique sulfonium cation on fragmentation. CID fragmentations of the more prominent higher adduct ions were complex and involved multiple neutral losses.

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