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Ion Chemistry of Protonated Aspartic Acid Derivatives

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The fragmentation reactions of the protonated α-monomethyl and β-monomethyl esters of aspartic acid have been elucidated using low-energy collision-induced dissociation (CID) and metastable ion studies. It is shown that, following loss of H_2O from protonated H–Asp–OMe, there is a methoxy group migration which is followed by loss of CO from the α-carboxyl position. This migration also is observed for the $[MH^+ - NH_3]^+$ ion derived from H–Asn–OMe. The fragmentation reactions of a variety of protonated α- and β-dipeptides, H–Asp–Xxx–OR and H–Asp(Xxx)–OH, also have been elucidated. For both types of linkage facile formation of the y_1'' ion is observed, in line with earlier observations that protonated peptides undergo preferential cleavage at the C-terminus side of acidic residues. A characteristic reaction of protonated dipeptides with the β-linkage is elimination of $H_2O + CO$ from the α-carboxyl group of the aspartic acid residue. This is followed by elimination of the C-terminus amino acid to give a characteristic fragment ion at m/z 70, $H_2N^+=CH-CH=C=O$. Protonated dipeptides with the α-linkage show loss of H_2O from the aspartic acid β-carboxyl group. This water loss is followed by elimination of ketene or ammonia. These fragment ions, which are most abundant at low collision energies, serve to identify dipeptides with the α-linkage. © **1998 John Wiley & Sons, Ltd.**

KEYWORDS: fragmentation mechanisms; aspartic acid derivatives; collision-induced dissociation; isomer distinction

INTRODUCTION

Collision-induced dissociation (CID) of protonated peptides is frequently used as an approach to determining the amino acid sequence of the peptides.[1–3] Both low- and high-energy CID studies have been used to obtain this sequence information.[4] With the extensive coupling of electrospray and matrix-assisted laser desorption ionization sources to instruments incorporating low-energy quadrupole collision cells and with the increasing use of CID studies in quadrupole ion trap[5–10] and Fourier transform[11–15] mass spectrometers, there is a need for a better understanding of the low-energy fragmentation processes occurring. The fragmentation reactions of protonated peptides are determined in part by the identity of the constituent amino acids. We recently studied[16] the low-energy fragmentation reactions of protonated α-amino acids and have begun a systematic study of the fragmentation reactions of simple protonated peptides to compare the reactions occurring with those observed for the constituent amino acids. A recent paper reported on the fragmentation reactions of protonated lysine derivatives.[17] In the present paper we report a detailed study of the fragmentation reactions of simple protonated aspartic acid derivatives and compare the reactions observed with those of protonated aspartic acid itself. Aspartic acid also is of interest since both α-aspartyl and β-aspartyl derivatives are possible and there is considerable interest[18–20] in distinguishing between the isomeric species. We have addressed this issue with the study of a number of α- and β-aspartyl derivatives.

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EXPERIMENTAL

All experimental work was carried out using a ZAB-2FQ hybrid BEqQ mass spectrometer (VG Analytical, Manchester, UK), which has been described in detail previously.[21] Briefly, this instrument is a reversed-geometry (BE) double-focussing mass spectrometer that is followed by a deceleration lens system, an r.f.-only quadrupole collision cell (q) and a quadrupole mass analyzer (Q). For most of the work the ions of interest were prepared by fast atom bombardment (FAB) using an argon atom beam of 7–8 keV energy with the appropriate sample dissolved in a matrix consisting of thioglycerol-2,2′-dithioethanol (1 : 1) saturated with oxalic acid. In our experience this matrix gives more intense and longer-lasting ion signals than glycerol. Some experiments also were carried out using chemical ionization (CI) with CH_3OD as reagent gas; this reagent gives the MD^+ ion of the species in which all the labile hydrogens have been exchanged for deuterium.

To obtain relative abundances of fragment ions formed on the metastable ion time-scale, the precursor ion of interest (usually MH^+) was mass-selected by the BE double-focussing mass spectrometer at 6 keV ion kinetic energy, decelerated to 20–40 eV kinetic energy

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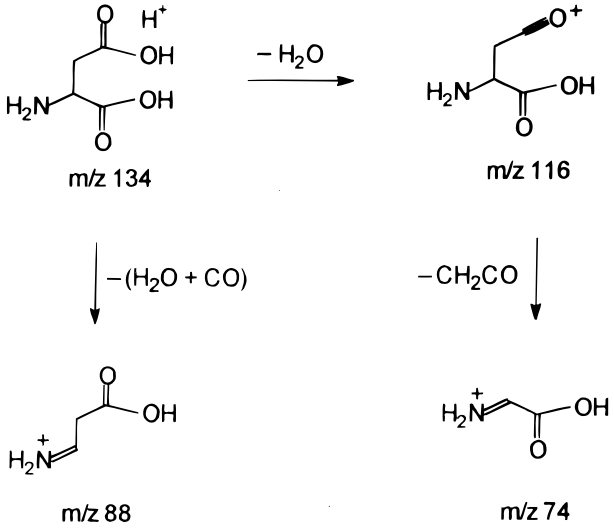

Scheme 1

and introduced into the r.f.-only quadrupole cell in the absence of collision gas. Low-energy CID studies were carried out in the same fashion but with addition of N_2 at an indicated pressure of $\sim 2 \times 10^{-7}$ Torr (1 Torr = 133.3 Pa) to the quadrupole collision cell. In the CID studies the incident ion energy typically was varied from 5 to 45 eV (laboratory scale). In both the unimolecular and CID studies, the ionic fragments were analyzed by scanning the final quadrupole Q; 20–30 2 s scans were accumulated on a multi-channel analyzer to improve the signal-to-noise ratio. The energy-resolved CID data are presented here in the form of breakdown graphs expressing the relative fragment ion signals as a function of the collision energy.

The aspartic acid derivatives studied were obtained from BACHEM Biosciences, King of Prussia, PA. H–Asn–OMe was prepared by esterification of asparagine with acidic methanol. The CH_3OD was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin.

RESULTS AND DISCUSSION

The low-energy fragmentation reactions of protonated aspartic acid have been studied previously.[16] On the metastable ion time-scale, loss of H_2O (83%) and H_2O + CO (17%) are observed. Under low-energy CID conditions loss of $H_2O + CH_2CO$ becomes the dominant

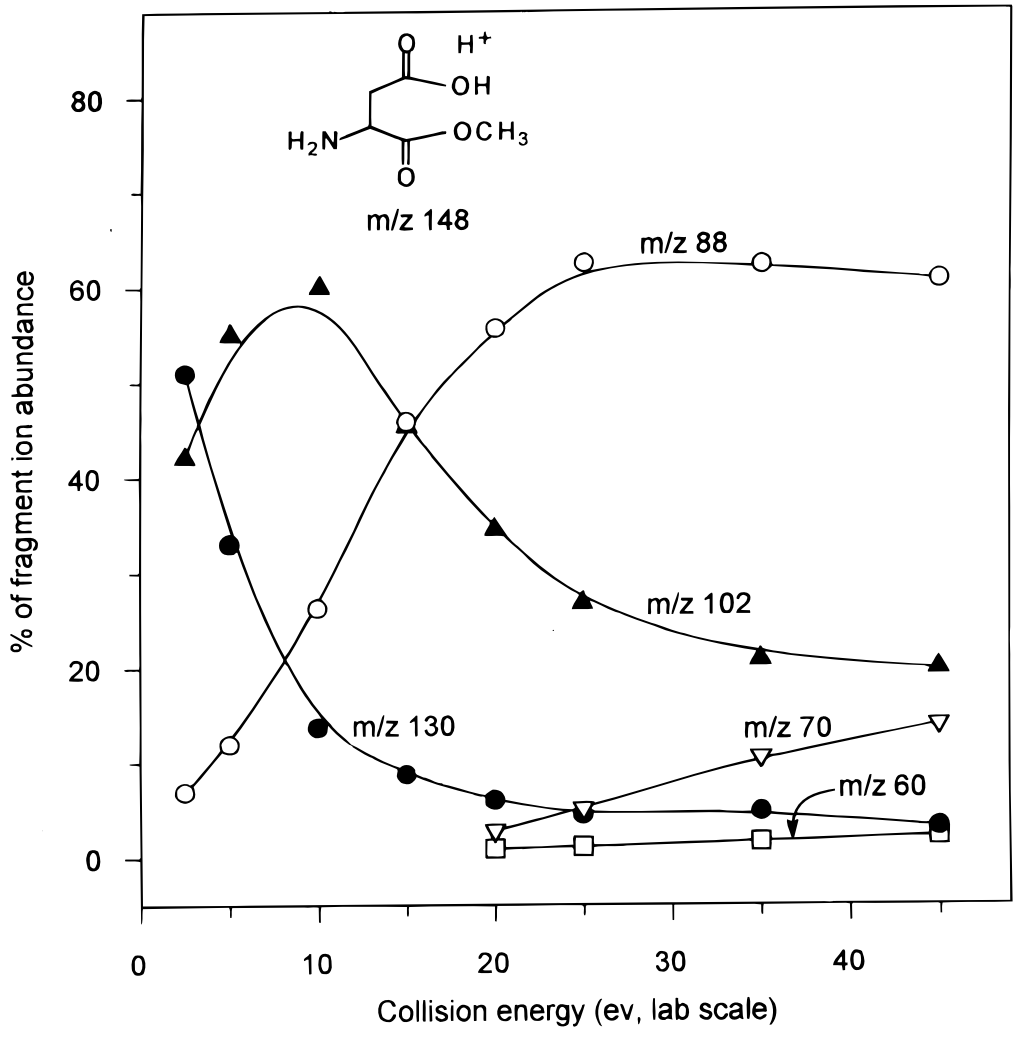

Figure 1. Breakdown graph for protonated H–Asp–OMe.

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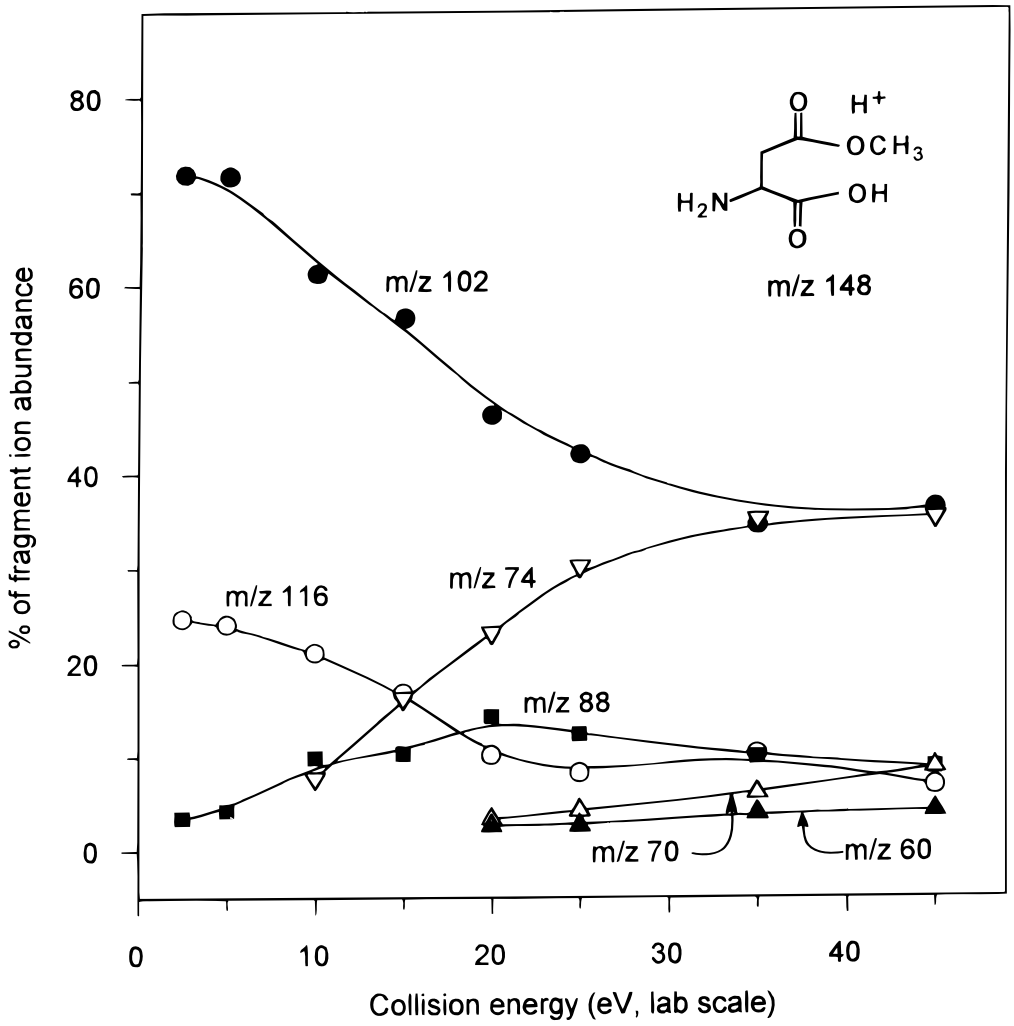

Figure 2. Breakdown graph for protonated H–Asp(OMe)–OH.

fragmentation reaction with loss of $H_2O + CO$ as the other significant fragmentation reaction, although loss of H_2O alone is still observed as a minor process. The interpretation provided[16] for these results is summarized in Scheme 1. Loss of $H_2O + CO$ was considered to involve the α-carboxyl group since there is considerable evidence[16,22–27] that the α-aminoacylium ion formed by loss of H_2O from the α-carboxyl group is unstable and spontaneously eliminates CO. It was further considered that loss of H_2O alone occurred from the β-carboxyl group and that, at higher internal energies, this loss of H_2O was followed by loss of ketene.

Further insight into these fragmentation reactions is achieved by studying the fragmentation reactions of the protonated α- and β-monomethyl esters, H–Asp–OMe and H–Asp(OME)–OH. The breakdown graphs for the MH^+ ion of these isomeric monoesters are shown in Figures 1 and 2. In metastable ion fragmentation reactions, the MH^+ ion of H–Asp–OMe showed dominant loss of H_2O (90%), in agreement with the conclusion[16] that the loss of H_2O from the MH^+ ion of aspartic acid involved loss from the β-carboxyl group. Minor metastable loss of $H_2O + CO$ (7%) and $CH_3OH + CO$ (3%) also was observed. As Figure 1 shows, at low collision energies a prominent ion signal is observed at *m/z* 102 corresponding to loss of $H_2O + CO$ from the MH^+ ion. This is unexpected since the earlier work[16] on protonated aspartic acid leads to the belief that loss of H_2O from the β-carboxyl group should be followed by loss of ketene (Scheme 1). This fragmentation reaction does occur as shown by the rapidly rising ion signal at *m/z* 88 as the collision energy is increased (Figure 1).

Fragmentation of the MD^+ ion of methyl aspartate-d_3 (prepared by CH_3OD CI) showed that the *m/z* 88 ion signal at 20 eV quadrupole energy was 60% – [$H_2O + CH_2CO$] and 40% – [$CH_3OH + CO$]. Further insight is provided from a study of the fragmentation of the MH^+ ion of H–Asn–OMe. In metastable ion fragmentation, this species showed 70% loss of NH_3, 24% loss of CH_3OH and 6% loss of $CH_3OH + CO$. The breakdown graph (Figure 3) shows a major ion signal at *m/z* 102 at low collision energies corresponding to loss of $NH_3 + CO$, analogous to the loss of $H_2O + CO$ from the MH^+ ion of H–Asp–OMe. At even higher collision energies, the breakdown graph for protonated asparagine methyl ester shows that the *m/z* 102 ion signal is surpassed by that at *m/z* 88 corresponding to loss of ketene from the $[MH - NH_3]^+$ ion.

In the absence of rearrangement, loss of CO from the $[MH - NH_3]^+$ ion of H–Asn–OMe and from the $[MH - H_2O]^+$ ion of H–Asp–OMe would lead to a high-

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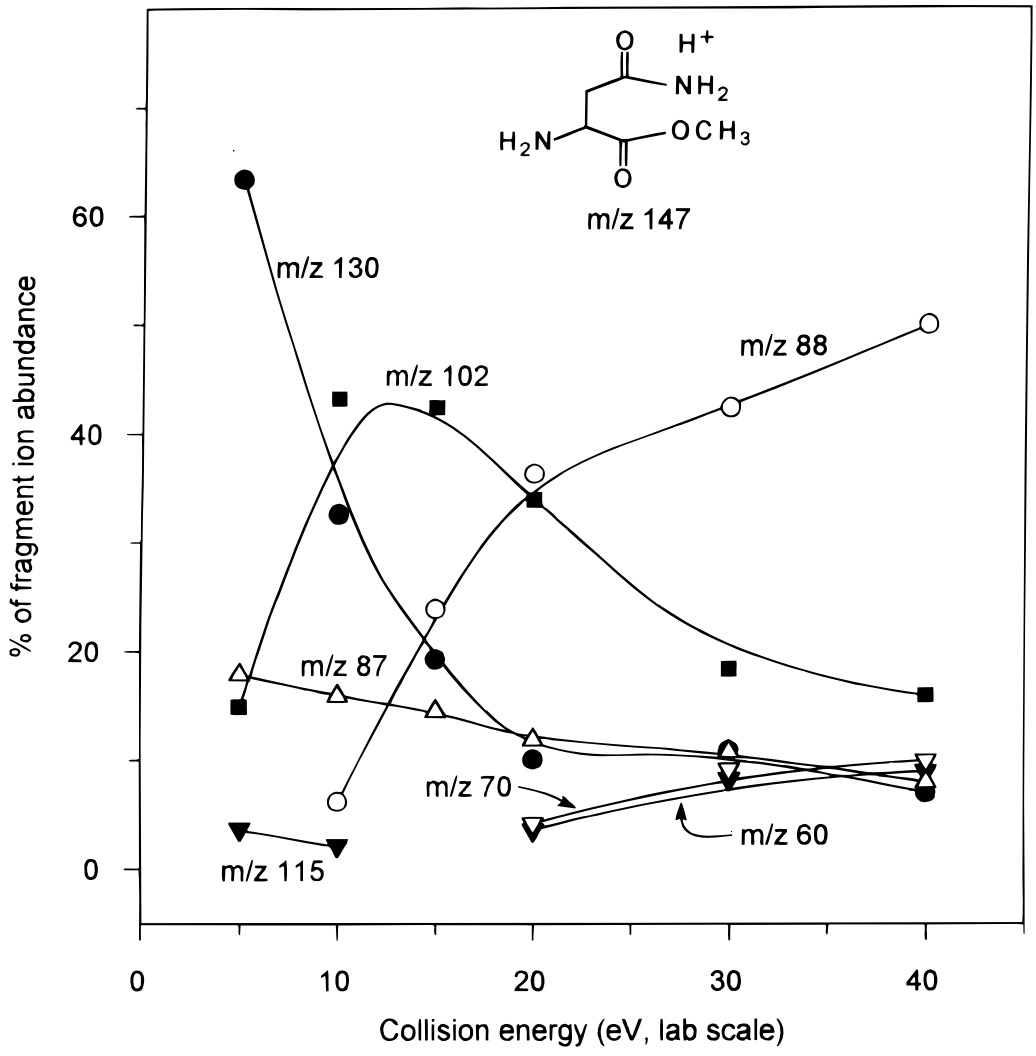
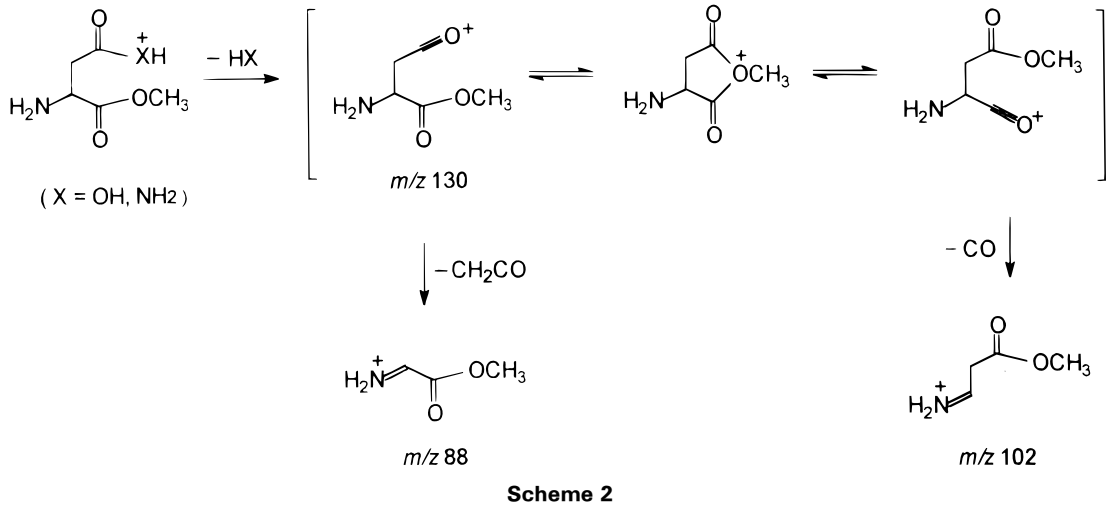


Figure 3. Breakdown graph for protonated H–Asn–OMe.

energy primary cation, an unlikely occurrence. Rather, we propose, as illustrated in Scheme 2, that, at low internal energies, there is a migration of the OCH_3 group from the α-position to the β-position through the intermediacy of a methyl-cationated succinic anhydride structure. As the internal energy is increased (by increasing the collision energy) the lifetime of the $[MH - HX]^+$ ion is decreased and simple bond fission by ketene elimination takes precedence over the rearrangement which leads to the ultimate elimination of CO. Interestingly, the rearrangement analogous to that of Scheme 2 is of much less importance in the fragmentation of protonated asparagine itself. Thus, at 20 eV collision energy, fragmentation of



Scheme 2

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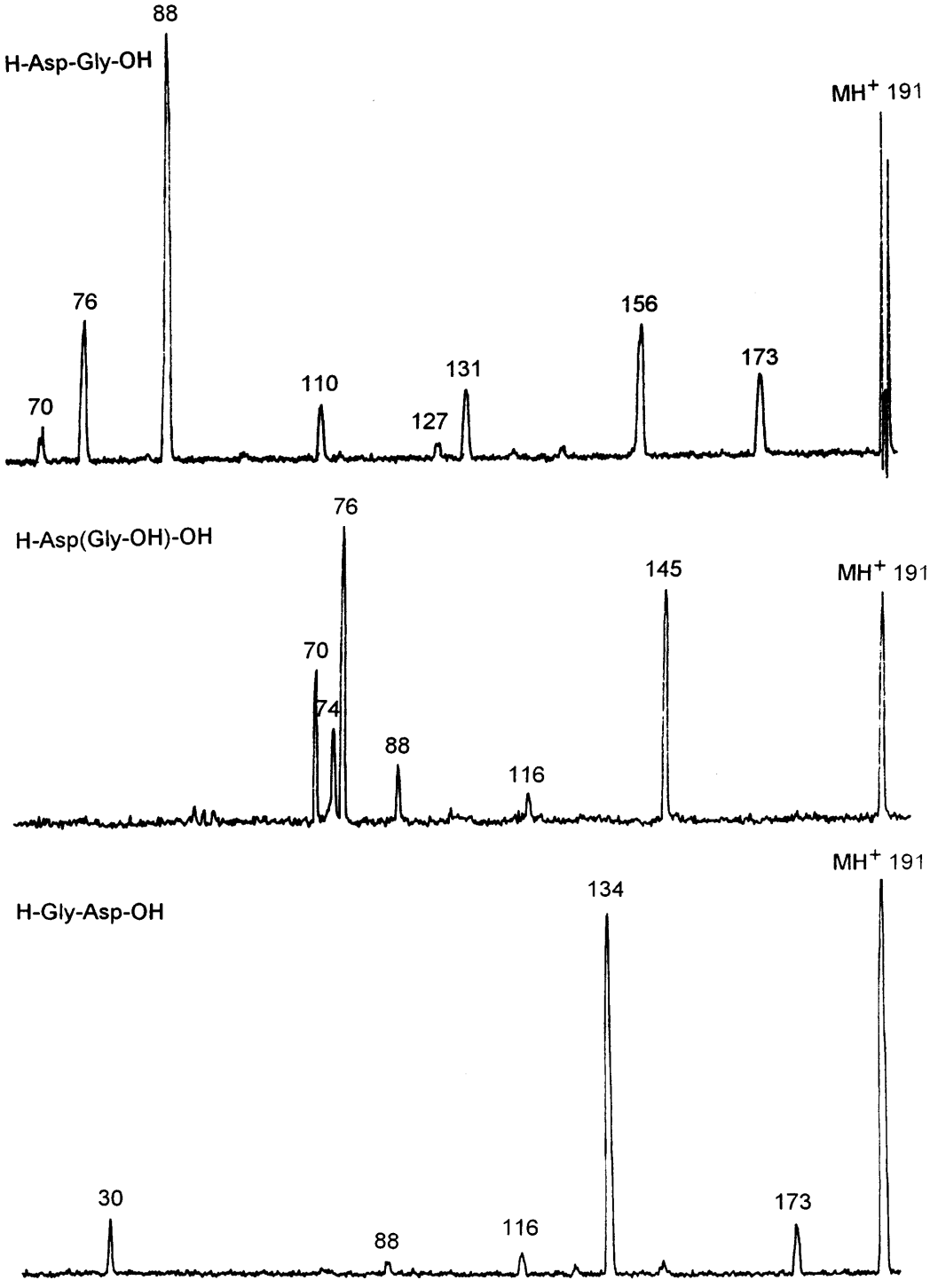

Figure 4. 20 eV CID mass spectra of protonated Asp/Gly dipeptides.

Table 1. Metastable ion fragmentation of protonated isomeric Asp–Gly and Asp–Leu dipeptides

	m/z (% fragment ion abundance)			
Peptide	$[MH - H_2O]^+$	$[MH - H_2O - CO]^+$	y_1''	a_1
H–Asp–Gly–OH	173 (100)			
H–Asp(Gly–OH)–OH	173 (28)	145 (72)		
H–Gly–Asp–OH	173 (60)		134 (40)	
H–Asp–Leu–OH	229 (60)	201 (26)	132 (14)	
H–Asp(Leu–OH)–OH	229 (26)	201 (63)	132 (11)	
H–Leu–Asp–OH	229 (4)		134 (2)	86 (94)

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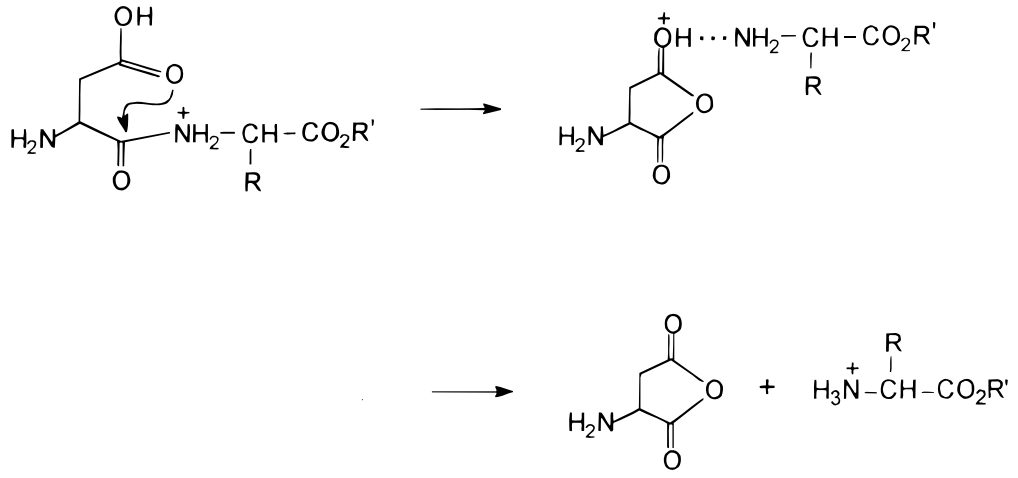


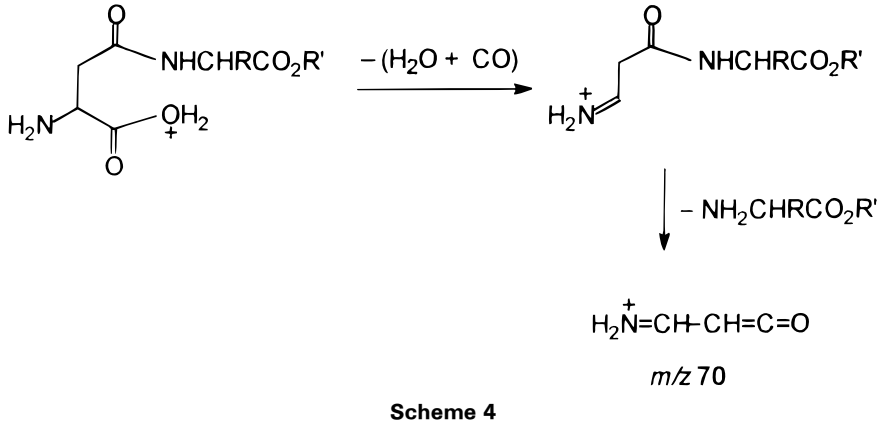
Scheme 3

protonated asparagine shows $-[NH_3 + CH_2CO]/-[NH_3 + CO] \approx 6$ compared to the ratio of *ca.* 1 for protonated asparagine methyl ester. Clearly, the hydroxyl group shows a much decreased tendency to migrate compared to the methoxy group. (The weak $[MH - NH_3 - CO]^+$ ion signal for protonated asparagine was not reported in our earlier study.[16])

The MH^+ ion of H–Asp(OMe)–OH showed dominant (60%) loss of $[H_2O + CO]$ in metastable ion fragmentation, with 22% loss of CH_3OH, 9% loss of H_2O and 3% loss of $[CH_3OH + CO]$. The breakdown graph for the MH^+ ion is shown in Figure 2; although loss of H_2O was observed in metastable ion fragmentation, this pathway was insignificant in the CID spectra even at the lowest collision energies. The reduced loss of CH_3OH from MH^+ of the β-ester compared to H_2O loss from the MH^+ of the α-ester is in line with observations[28] that H_2O is more readily lost from protonated molecules than CH_3OH. The observation of the ion signal at *m/z* 88 ($-[CH_3OH + CO]$), reaching ~15% of the total fragment ion signal in the CID spectrum, is again surprising but can be rationalized in terms of a hydroxyl group migration in the $[MH - CH_3OH]^+$ ion analogous to that depicted in Scheme 2.

Figure 4 compares the 20 eV CID mass spectra for the MH^+ ions of the three possible dipeptides containing aspartic acid and glycine, while Table 1 presents the relative ion signals for unimolecular fragmentation of the MH^+ ions on the metastable ion time scale. The three isomers are readily distinguished either from their metastable ion mass spectra or from their low-energy CID mass spectra of the respective MH^+ ions. Under CID conditions protonated H–Gly–Asp–OH fragments predominantly to give protonated aspartic acid (*m/z* 134) with minor ion signals corresponding to $[MH - H_2O]^+$ (b_2 ion) and *m/z* 30 (a_1 ion). Protonated glycine (*m/z* 76) is a prominent ion signal in the CID mass spectra of protonated H–Asp–Gly–OH and H–Asp(GlyOH)–OH. This is in line with earlier observations[29–33] that protonated peptides show preferential fragmentation adjacent to acidic amino acid residues. In agreement with the proposal of Yu *et al.*,[29] we suggest that the protonated amino acid (y_1'' ion) arises as shown in Scheme 3 for the α-isomer, involving formation of a protonated succinic anhydride/amino acid ion/neutral complex within which proton transfer occurs to give the protonated amino acid. An analogous reaction can be written for formation of the y_1'' ion from the protonated β-isomer. The α-amino group is not essential to the reaction; thus, the MH^+ ion of protonated succinylphenylalanine methyl ester shows substantial formation of protonated phenylalanine methyl ester in both unimolecular metastable ion and CID mass spectra.

An ion which appears generally to be characteristic of the H–Asp(Xxx)–OH configuration is that at *m/z* 70 as seen in the CID spectrum of the MH^+ ion of H–Asp(Gly)–OH (Figure 4) and seen in other β-Asp dipeptides. A weak ion *m/z* 70 ion signal is observed in the fragmentation of protonated H–Asp–Gly–OH, presumably arising by loss of H_2O from the abundant aspartic



Scheme 4

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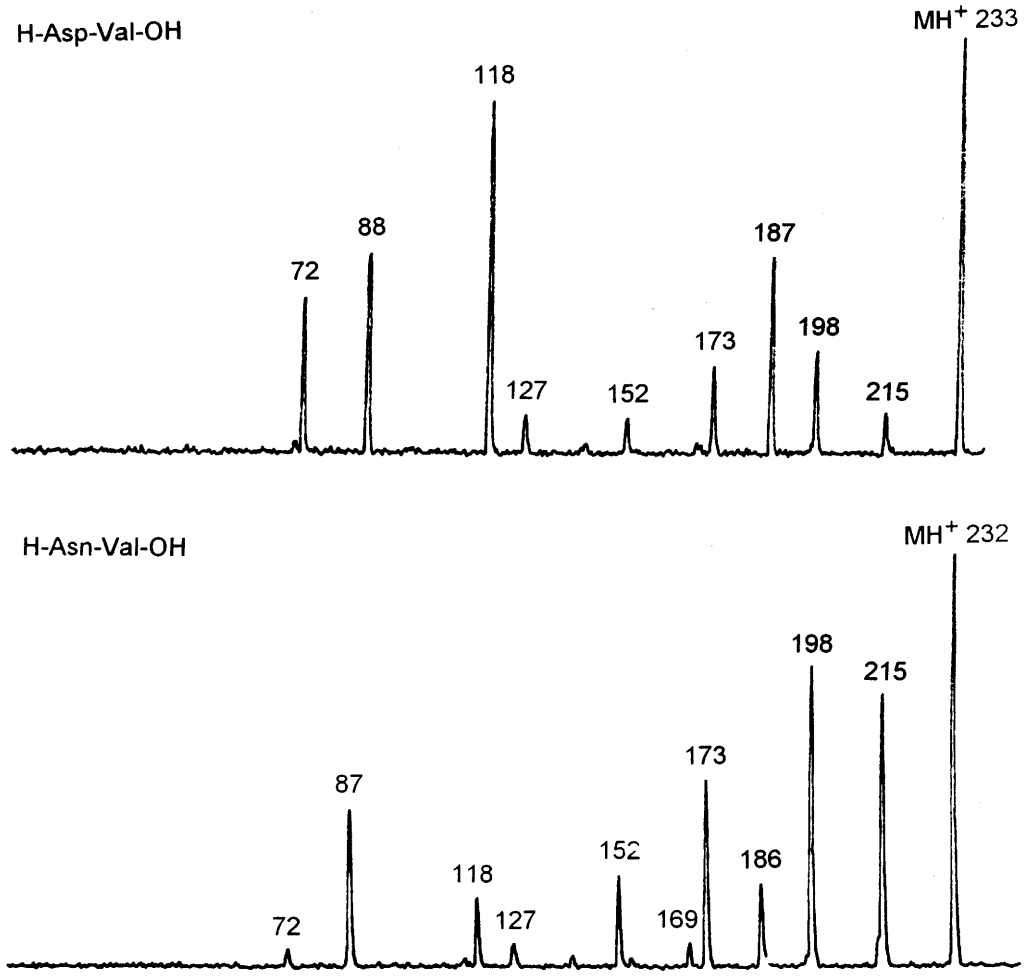
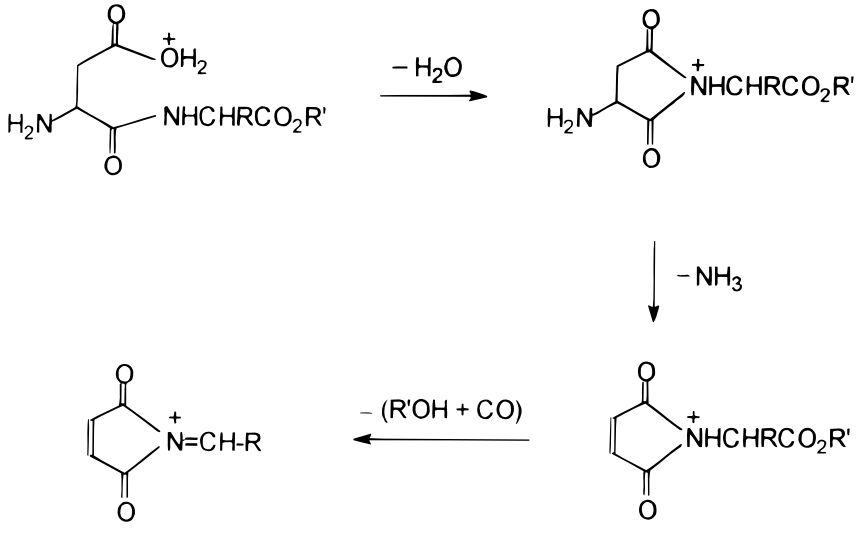


Figure 5. 20 eV CID mass spectra of protonated H–Asp–Val–OH and H–Asn–Val–OH.

acid immonium ion at *m/z* 88. Even in the case of the Asp/Gly combinations, the *m/z* 70/*m/z* 88 ratio serves to distinguish the α- and β-isomers. This *m/z* 70 ion from β-dipeptides appears to originate by loss of a neutral amino acid from the $[MH - H_2O - CO]^+$ ion (*m/z* 145 in Figure 4, middle) as outlined in Scheme 4. By contrast, the MH^+ ion of H–Asp–Gly–OH shows no signal for loss of $H_2O + CO$ but rather shows an abundant metastable ion signal corresponding to loss of H_2O (*m/z* 173). Loss of H_2O is followed by loss of ketene to give the ion at *m/z* 131 and also by loss of NH_3 to give the ion of *m/z* 156. The sequential loss of $H_2O + NH_3$ from protonated H–Asp–Gly–OCH_3 has been noted previously by Castet *et al.*[19] who postulated an acyclic structure for the product ion. A more likely interpretation is given in Scheme 5 involving formation



Scheme 5

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of a protonated N-substituted maleimide; the ion signal at *m/z* 110 can be rationalized, as shown, by loss of H_2O + CO from this structure.

A similar fragmentation sequence is much more prominent in the CID mass spectra of protonated asparagine derivatives. Figure 5 compares the 20 eV CID mass spectra of protonated H–Asp–Val–OH and protonated H–Asn–Val–OH. Although $[MH - H_2O - NH_3]^+$ (*m/z* 198) is clearly seen for H–Asp–Val–OH, the *m/z* 198 ion ($[MH - 2NH_3]^+$) is much more prominent in the CID spectrum of the asparagine derivative. In addition, *m/z* 173 ($[MH - NH_3 - CH_2CO]^+$) and *m/z* 152 ($[MH - 2NH_3 - H_2O - CO]^+$) are also more prominent for the asparagine derivative. For protonated H–Asn–Val–OH metastable ion fragmentation leads predominantly (93%) to loss of NH_3 while for the aspartic acid derivative the analogous loss of H_2O from the side chain carboxyl group accounts for less than 61% of the metastable ion signal (some of the metastable ion signal may correspond to loss of H_2O from the valine carboxyl group). Another noteworthy difference in the two spectra is the much more abundant signal for protonated valine (*m/z* 118) for the aspartic acid derivative; clearly, the carboxylic hydrogen of aspartic acid is much more readily transferred (Scheme 3) than the amidic hydrogen of asparagine.

The evolution of the characteristic fragmentation of the α- and β-dipeptides with internal energy is illustrated in Figures 6 and 7 for the MH^+ ions of H–Asp–Leu–OH and H–Asp(LeuOH)–OH, while the metastable ion data for Asp/Leu dipeptides are presented in Table 1. The MH^+ ion derived from H–Leu–Asp–OH gave predominantly *m/z* 86, the a_1 immonium ion derived from the N-terminal leucine at all collision energies. The characteristic $[MH - H_2O]^+$ (*m/z* 229) ion derived from the α-dipeptide and the fragments originating by further fragmentation of this ion (loss of NH_3 (*m/z* 212) and loss of ketene (*m/z* 187)) are prominent at low collision energy but decrease in importance with increasing collision energy, particularly $[MH - H_2O]^+$. At collision energies above 20–25 eV protonated leucine (*m/z* 132) and the immonium ions derived from aspartic acid (*m/z* 88) and from leucine (*m/z* 86) dominate the breakdown graph. The $[MH - H_2O - CO]^+$ ion (*m/z* 201) dominates the breakdown graph for the β-dipeptide at low collision energies. This fragmentation undoubtedly involves loss of H_2O + CO from both the α-carboxyl group of aspartic acid and the leucine car-

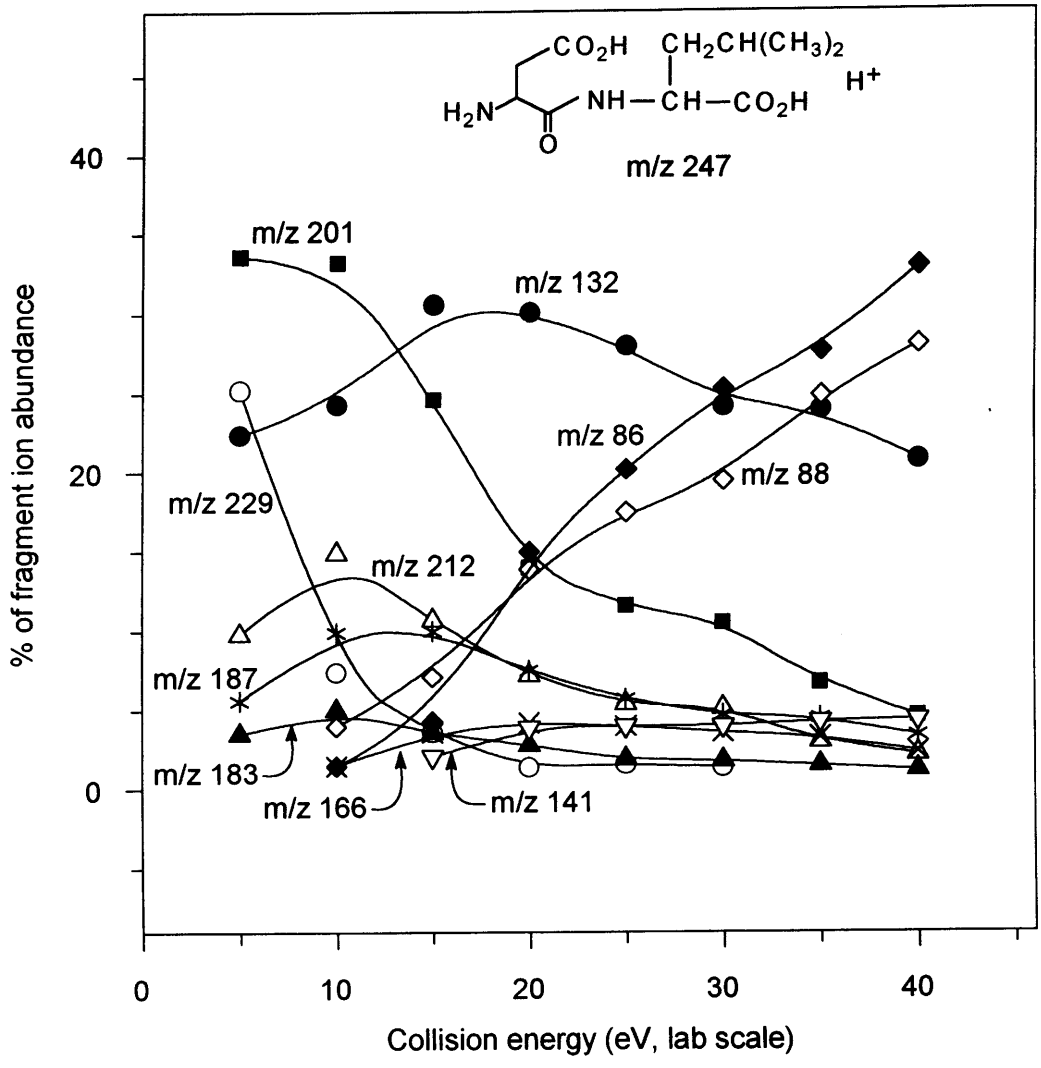

Figure 6. Breakdown graph for protonated H–Asp–Leu–OH.

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boxyl group. The $[MH - H_2O - CO]^+$ species arising from loss of the α-carboxyl group shows the characteristic fragmentation to yield *m/z* 70 by elimination of neutral leucine. The $[MH - H_2O - CO]^+$ arising by loss of the leucine carboxyl group appears to fragment further to give the leucine immonium ion, probably involving formation of a substituted succinic anhydride neutral (Scheme 5). The $[MH - H_2O - CO]^+$ seen in the spectrum of H–Asp–Leu–OH must involve loss of the leucine carboxyl group and a similar elimination of aminosuccinic anhydride from this ion can account for formation of the leucine immonium ion (*m/z* 86). For both dipeptides significant formation of protonated leucine (*m/z* 132) is observed (Scheme 3).

The 20 eV CID mass spectra of protonated H–Asp–Phe–OMe (aspartame) and protonated H–Asp(Phe–OMe)–OH (β-aspartame) are shown in Figure 8, while the relative fragment ion abundances observed on unimolecular metastable ion fragmentation are recorded in Table 2. Although $[MH - H_2O]^+$ (*m/z* 277) is of major importance in the metastable ion mass spectrum of aspartame, its abundance decreases rapidly in the CID mass spectra with increasing collision energy; the further fragmentation products arising by loss of NH_3

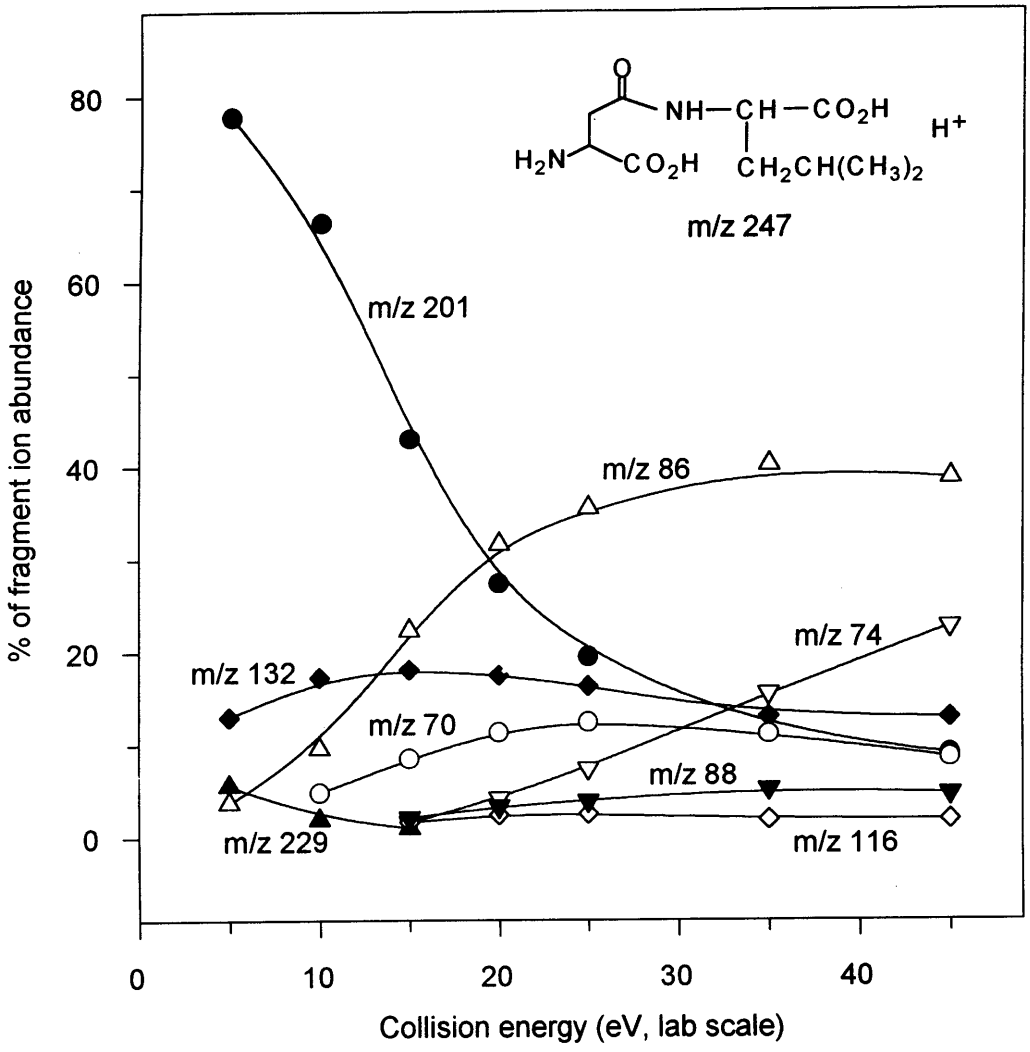

Figure 7. Breakdown graph for protonated H–Asp(Leu–OH)–OH.

Table 2. Metastable ion fragmentation of protonated H–Asp–Phe–OMe and H–Asp(PheOMe)–OH

Fragment Ion (*m/z*)	% fragment ion abundance H–Asp–Phe–OMe	H–Asp(PheOMe)–OH
$[MH^+ - H_2O]^+$ (277)	61	6
$[MH^+ - CH_3OH]^+$ (263)	27	28
$[MH^+ - H_2O - CO]^+$ (249)		29
$[MH^+ - CH_3OH - CO]^+$ (235)	3	21
y_1'' ion (180)	9	16

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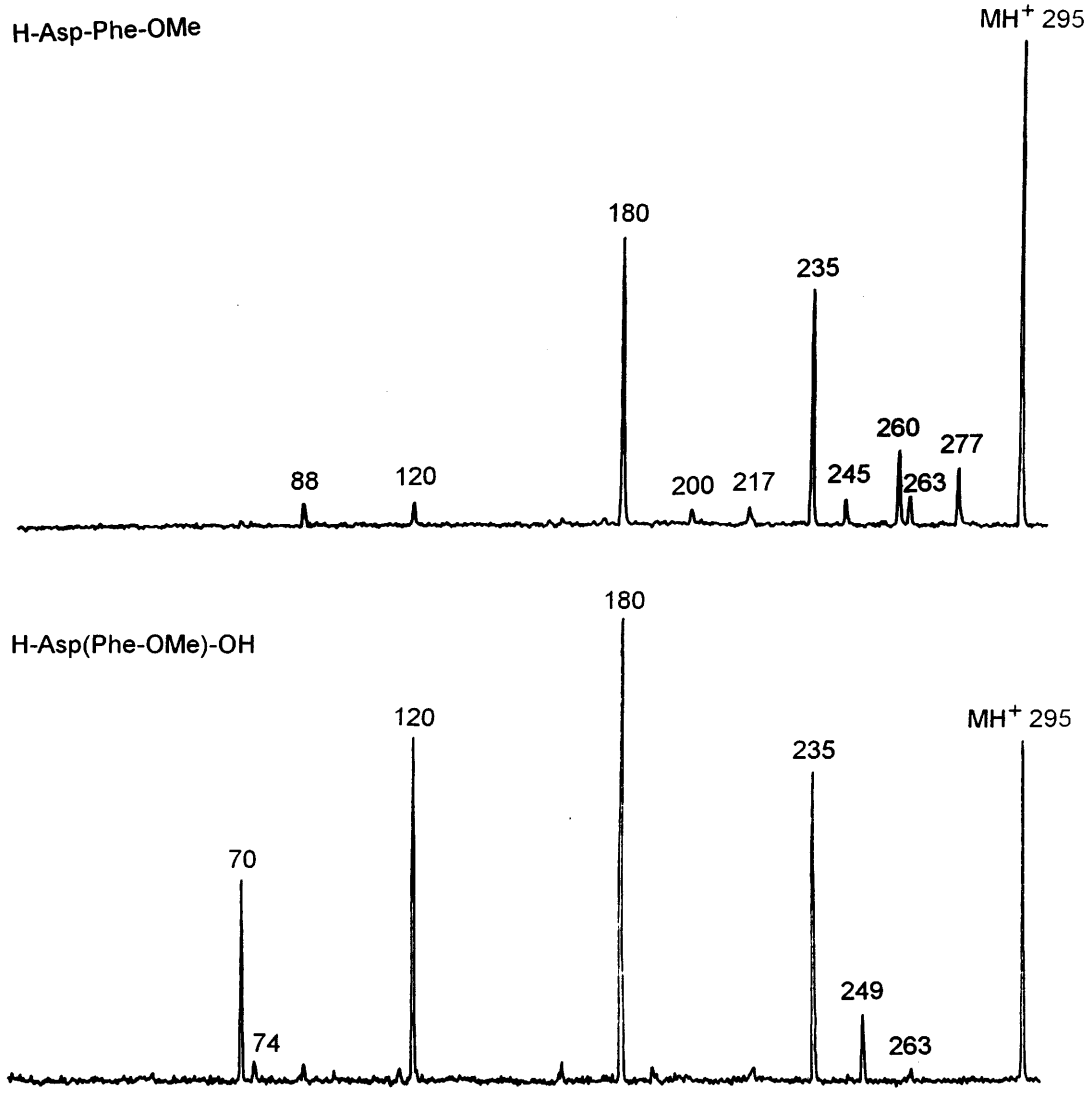

Figure 8. 20 eV CID mass spectra for protonated H–Asp–Phe–OMe and H–Asp(Phe–OMe)–OH.

(*m/z* 260) and ketene (*m/z* 235) from $[MH - H_2O]^+$ are observed in significant yield. The ion signal at *m/z* 235 undubtedly corresponds to both loss of $H_2O + CH_2CO$ and loss of $CH_3OH + CO$ from the MH^+ ion. Elimination of CH_3OH from protonated aspartame is a major metastable ion fragmentation route, although the ion signal (*m/z* 243) is weak in the CID mass spectra. Similarly, elimination of CH_3OH is a major metastable ion fragmentation reaction for protonated β-aspartame, although the signal has practically disappeared in the 20 eV CID spectrum, probably by elimination of CO to form the ion at *m/z* 235; this ion appears to fragment further by a reaction analogous to that depicted in Scheme 5 to give the phenylalanine immonium ion at *m/z* 120. Loss of $H_2O + CO$ from the free α-carboxyl group of β-aspartame is a significant fragmentation reaction under CID conditions and this ion decomposes further by elimination of phenylalanine methyl ester to give the ion at *m/z* 70, characteristic of the β-linkage. Both the protonated aspartames show formation of protonated phenylalanine methyl ester (*m/z* 180) in the metastable ion mass spectra and this y_1'' ion becomes the base peak in the 20 eV CID mass spectra, consistent with the general mechanism outlined in Scheme 3.

CONCLUSIONS

A detailed study of the fragmentation reactions of the isomeric monomethyl esters of aspartic acid has been carried out. A novel methoxy group migration is proposed to rationalize the observation of $H_2O + CO$ loss from protonated H–Asp–OMe. An analogous methoxy group migration is proposed to rationalize the observation of the loss of $NH_3 + CO$ from protonated H–Asn–OMe. The fragmentation reactions of a variety of dipeptides containing the α- and β-linkage to aspartic acid have been examined. It is shown that the isomers are readily distinguishable either from the metastable ion mass spectra or from the low-energy CID mass spectra of the MH^+ ions. A characteristic reaction for protonated dipeptides containing the β-linkage is loss of

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$H_2O + CO$ and this is followed by elimination of a neutral amino acid or ester to give a characteristic ion at m/z 70. For protonated dipeptides containing the α-linkage primary fragmentation is by elimination of H_2O and this is followed by elimination of ketene or ammonia, reactions which are not observed for dipeptides containing the β-linkage. It also appears that the aspartic acid immonium ion (m/z 88) is more abundant in the spectra of dipeptides with the α-linkage. For all protonated dipeptides facile formation of the y_1'' ion is observed consistent with earlier observations[29–33] of preferred cleavage of protonated peptides at acidic residues.

Acknowledgements

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