Available online at www.sciencedirect.com
ScienceDirect
International Journal of Mass Spectrometry 255–256 (2006) 111–122
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Fragmentation reactions of deprotonated peptides containing aspartic acid

Alex G. Harrison *, Alex B. Young

Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ont., Canada M5S 3H6

Received 20 October 2005; received in revised form 13 December 2005; accepted 27 December 2005
Available online 30 January 2006

In appreciation of the outstanding contributions of Diethard Bohme to gas-phase ion chemistry.

Abstract

The fragmentation reactions of deprotonated peptides containing aspartic acid have been elucidated using MS^2 and MS^3 experiments and accurate mass measurements where necessary. The disposition of labile (N– and O– bonded) hydrogens in the fragmentation products has been studied by exchanging the labile hydrogens for deuterium whereby the $[M–D]^-$ ion is formed on electrospray ionization. α-Aspartyl and β-aspartyl dipeptides give very similar fragment ion spectra on collisional activation, involving for both species primarily formation of the y_1 ion and loss of H_2O from $[M–H]^-$ followed by further fragmentation, thus precluding the distinction of the isomeric species by negative ion tandem mass spectrometry. Dipeptides of sequence H–Xxx–Asp–OH give characteristic spectra different from the α- and β-isomers. For larger peptides containing aspartic acid a common fragmentation reaction involves nominal cleavage of the N–C bond N-terminal to the aspartic acid residue to form a c ion (deprotonated amino acid amide (c_1) or peptide amide (c_n)) and the complimentary product involving elimination of a neutral amino acid amide or peptide amide. When aspartic acid is in the C-terminal position this fragmentation reaction occurs from the $[M–H]^-$ ion while when the aspartic acid is not in the C-terminal position the fragmentation reaction occurs mainly from the $[M–H–H_2O]^-$ ion. The products of this N–C bond cleavage reaction serve to identify the position of the aspartic acid residue in the peptide.

Keywords: Deprotonated peptides; Aspartic acid; Tandem mass spectrometry; Fragmentation mechanisms

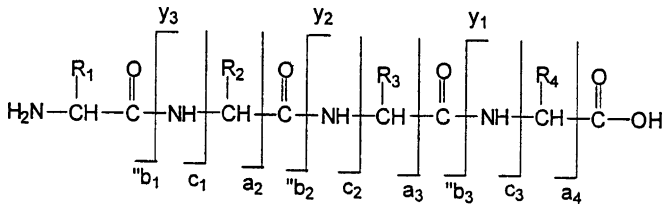
1. Introduction

The use of collision-induced dissociation (CID) studies in tandem mass spectrometry to identify and to provide the sequence of the amino acids present in a peptide is well established [1–4]. The majority of these CID studies have been carried out on protonated or multiply-protonated peptides. As a result of many studies the main fragmentation channels have been established and considerable progress has been made in understanding, in detail, the mechanistic aspects of the fragmentation of protonated peptides; these studies have been the subject of several recent reviews [5–10]. Tandem mass spectrometric studies of deprotonated peptides are less common and as a result less is known concerning the detailed fragmentation modes of the deprotonated species. In early studies, Bowie and co-workers have reported the high-energy (keV) CID of mostly deprotonated dipeptides and tripeptides; the mechanistic information obtained from these studies has been summarized [11,12]. More recently, Bowie and co-workers have carried out extensive studies of the low-energy (eV) CID of larger deprotonated peptides and have shown that useful structural and sequence information can be obtained which often is complimentary to that obtained by positive ion studies; these studies recently have been reviewed [13]. Cassady and co-workers [14–17] also have explored the low-energy fragmentation reactions of a variety of larger deprotonated peptides while Marzluff et al. [18] have explored the lowest energy fragmentation pathways of a variety of small deprotonated peptides using Fourier-transform mass spectrometric techniques.

The studies of Bowie and co-workers [11–13] have shown that there often are fragmentation reactions which are specific to particular side chain groups in the peptide. In the absence of such specific side-chain effects, the fragment ions observed on collisional activation of deprotonated peptides can be illustrated schematically as shown in Scheme 1 using nomenclature [16,19] adapted from that used for protonated peptide fragmen-

* Corresponding author. Tel.: +1 416 978 3577.
E-mail address: aharriso@chem.utoronto.ca (A.G. Harrison).

1387-3806/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2005.12.037



Scheme 1.

tation [20,21]; this is well-illustrated by the results obtained [19] for deprotonated peptides containing only H or alkyl side chains under low-energy collisional activation. However, we have observed that fragmentation of deprotonated peptides containing phenylalanine [22], glutamic acid [23] or proline [24] show atypical fragmentation reactions dependent on the specific amino acid residue present. At the same time our studies have shown that more extensive and, often, sequence-specific fragmentation is observed under low-energy CID than that observed under high-energy CID conditions [11,12]. In the present study we have examined the fragmentation of small deprotonated peptides containing aspartic acid and we compare our results with the results obtained by Bowie and co-workers [25,26]. Aspartic acid also is of interest since both α-aspartyl and β-aspartyl derivatives are possible and there is considerable interest [27–31] in distinguishing between the isomeric species; Bowie did not examine β-aspartyl derivatives.

2. Experimental

Low-energy CID studies were carried out using an electrospray/quadrupole mass spectrometer (VG Platform, Micromass, Manchester, UK) with CID in the interface between the atmospheric pressure source and the quadrupole mass analyzer, so-called cone-voltage CID. It has been clearly established [32–36]

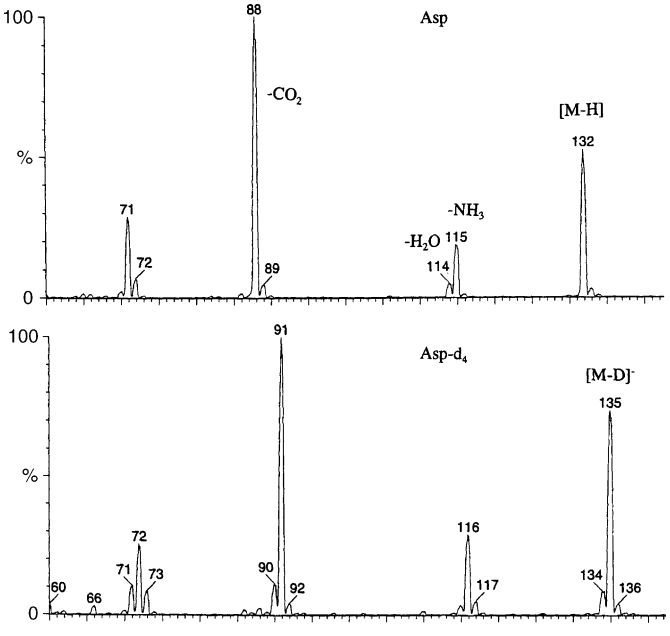
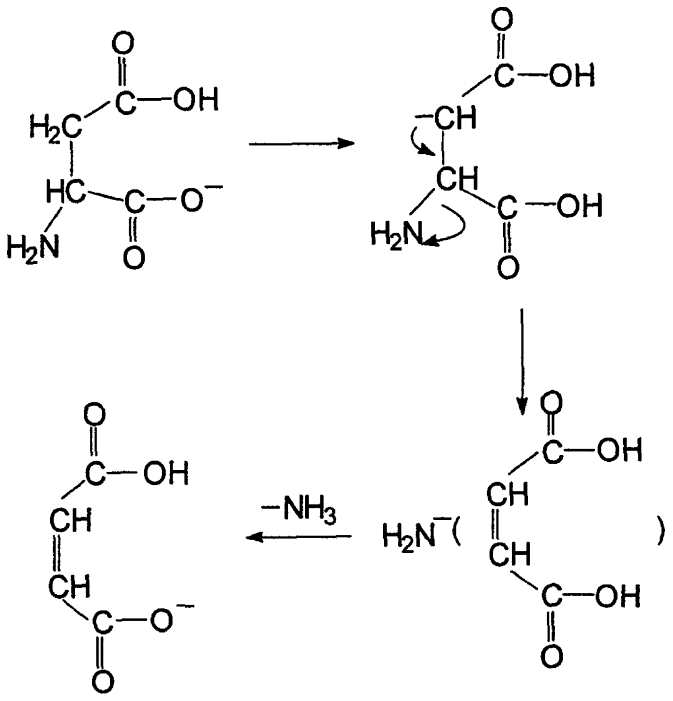


Fig. 1. Comparison of CID mass spectra of deprotonated Asp and $[M-D]^-$ ion of Asp-d_4. 40 V cone voltage.



Scheme 2.

that CID in this fashion produces mass spectra very similar to those obtained by low-energy CID in quadrupole collision cells. Since there is no mass selection of the precursor ion to be studied this approach is only viable when there is one dominant primary ion; such was the case in the present studies. The results are presented in the following either as CID mass spectra at a set cone voltage or as breakdown graphs expressing relative fragment ion intensities as a function of the cone voltage, a measure of the collision energy in the interface region. MS^2 and MS^3 experiments also were carried out using an electrospray/quadrupole/time-of-flight (QqToF) mass spectrometer (QStar, MDS Sciex, Concord, Canada). In the MS^3 experiments CID in the interface region produced fragment ions, that of interest being mass-selected by the quadrupole mass analyzer (Q) to undergo collisional activation in the quadrupole collision cell (q), with the ionic fragmentation products being analyzed by the time-of-flight analyzer. The QqToF instrument was operated under conditions

Table 1
CID mass spectra of anions derived from H—Asp—OMe (20 eV collision energy)

m/z	$[M-H]^-$ (*m/z* 146)	$[M-H-NH_3]^-$ (*m/z* 129)	$[M-H-CH_3OH]^-$ (*m/z* 114)
146	3.0		
129	18.9	9.8	
114	95.1		7.5
101		5.9	
86	13.6		
85	36.0	100	
83		11.7	
72	44.3		37.2
71	91.3	17.6	100
70	100		5.6

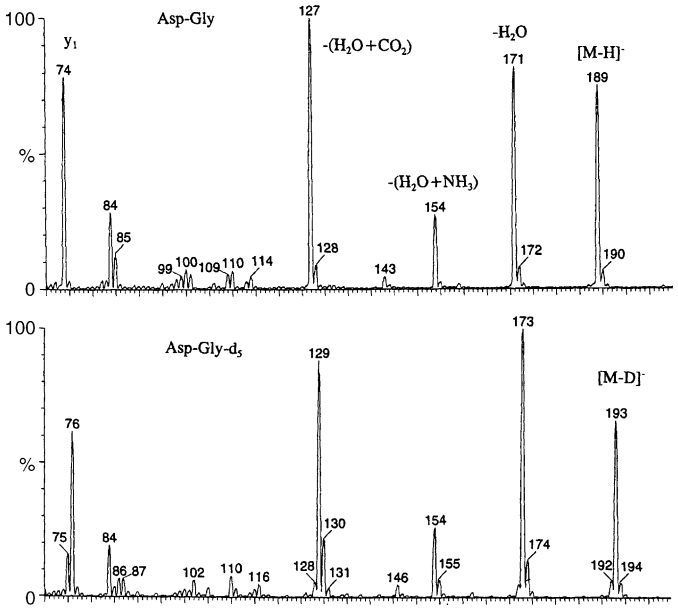


Fig. 2. Comparison of CID mass spectra of deprotonated Asp—Gly and $[M-D]^-$ ion of Asp—Gly-d_5. 40 V cone voltage.

which permitted accurate mass measurements to confirm elemental compositions where necessary.

Ionization was by electrospray on both instruments. For the single-quadrupole instrument the sample was dissolved in 1:1 CH_3CN:1% aqueous NH_3 and introduced into the ion source at a flow rate of 30 $\mu L\,min^{-1}$ with nitrogen being used as both nebulizing and drying gas. The use of 1:1 CD_3CN:1% ND_3 in D_2O as solvent resulted in exchange of all labile hydrogens for deuterium and formation of the $[M-D]^-$ ion on ionization, thus allowing study of the disposition of the labile hydrogens in the fragment ions observed. For the QqToF

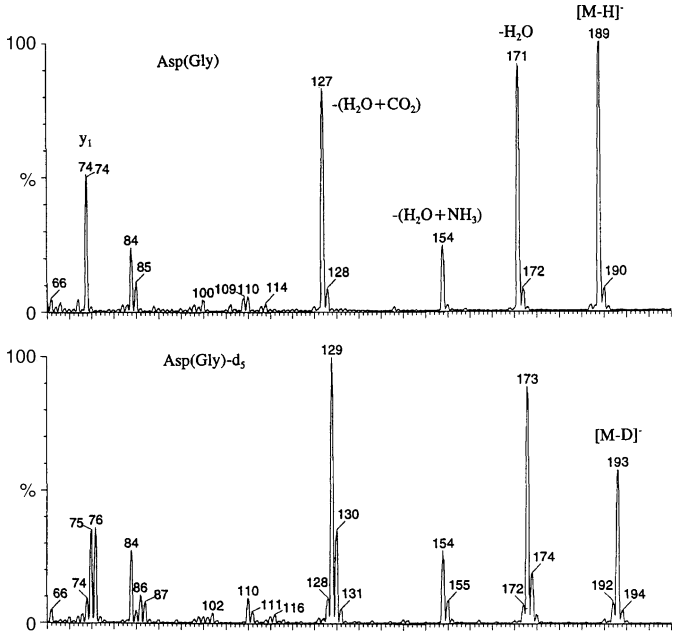


Fig. 3. Comparison of CID mass spectra of deprotonated Asp(Gly) and $[M-D]^-$ ion of Asp(Gly)-d_5. 40 V cone voltage.

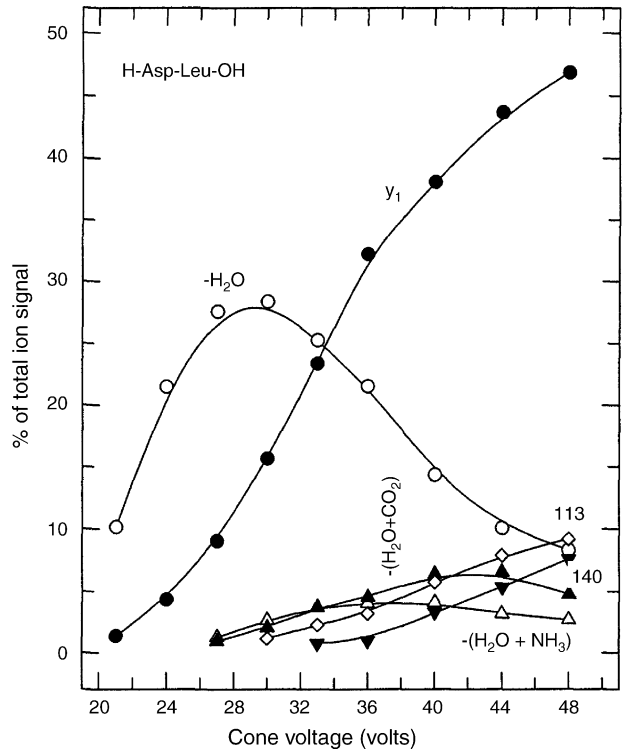


Fig. 4. Breakdown graph for deprotonated Asp—Leu. $[M-H]^-$ ion (*m/z* 245) not shown.

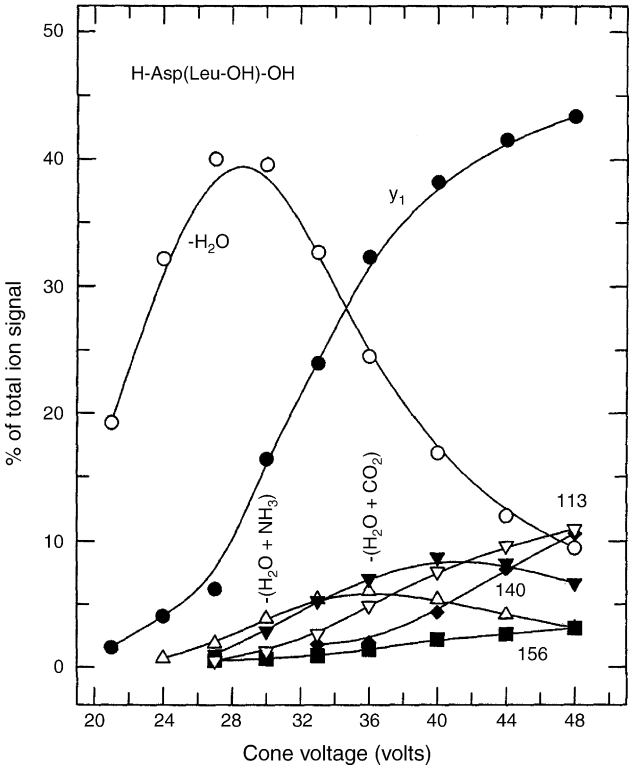
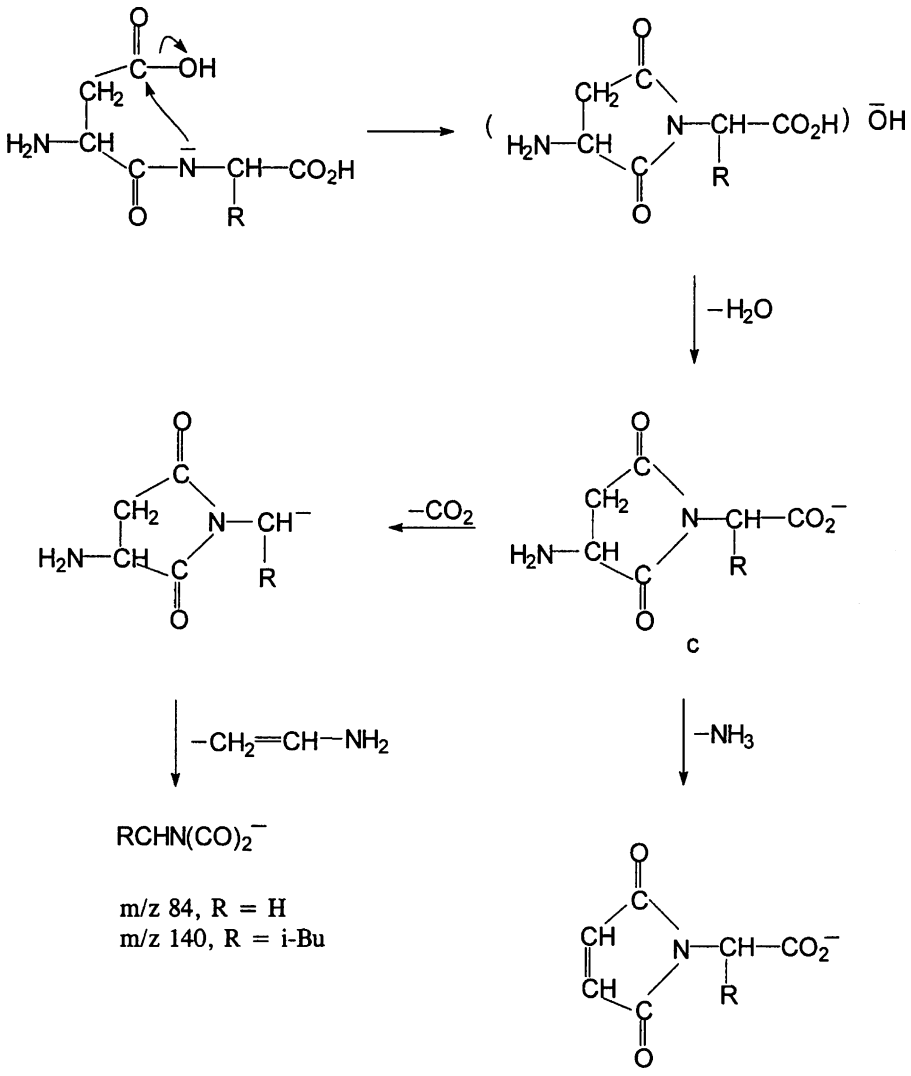


Fig. 5. Breakdown graph for deprotonated Asp(Leu). $[M-H]^-$ ion (*m/z* 245) not shown.



Scheme 3.

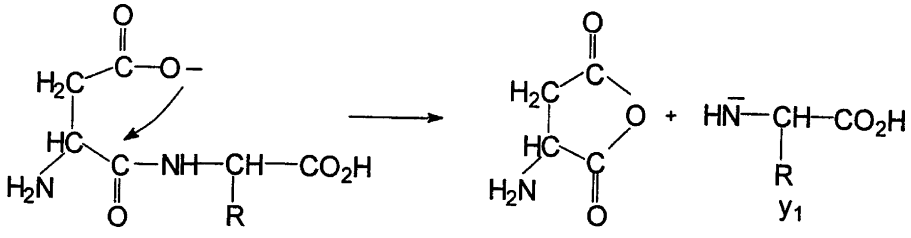
instrument the peptide was dissolved in 1:1 CH_3OH:0.1% aqueous NH_3 and introduced into the ion source at a flow rate of 80 μL min^{-1}. Nitrogen was used as nebulizing and drying gas and as collision gas in the quadrupole collision cell.

All peptide samples were obtained from Bachem Biosciences (King of Prussia, PA) and were used as received. The sample of H–Asp–OCH_3 contained a minor impurity of H–Asp–OH consequently experiments were carried out only on the QqToF instrument where mass selection of the ion to be studied was possible.

3. Results and discussion

3.1. Aspartic acid and methyl aspartate

Fig. 1 compares the CID mass spectrum of deprotonated aspartic acid with that obtained for the $[M–D]^-$ ion of the acid in which the labile hydrogens has been exchanged for deuterium. The major primary fragmentation reaction involves loss of CO_2 from $[M–H]^-$ to form *m/z* 88, with minor elimination of NH_3 (*m/z* 115) and even more minor elimination of H_2O (*m/z* 114). In high-energy CID studies Bowie and co-workers [37] observed



Scheme 4.

these fragmentation reactions but with a much more pronounced loss of H_2O and minor loss of CO from the $[M–H]^-$ ion. MS^3 studies showed that the *m/z* 115 ion eliminated CO_2 to form *m/z* 71, in agreement with the work of Bowie. The labelling results show that the ammonia lost incorporates two labile hydrogens (those bonded to N?) plus one hydrogen initially bonded to carbon and presumably gives deprotonated maleic (or fumaric) acid as a product. The elimination of NH_3 appears to be a direct 1,2-elimination; a charge-directed mechanism, such as illustrated in Scheme 2 should lead to appreciable loss of ND_3 for the labelled compound since one of the carboxyl groups bears a deuterium.

A much more abundant *m/z* 114 ion is formed by loss of CH_3OH from deprotonated H–Asp–OMe. The CID spectra obtained on the QqToF instrument for $[M–H]^-$, $[M–H–NH_3]^-$ and $[M–H–CH_3OH]^-$ are presented in Table 1. The major primary fragmentation reactions of the deprotonated ester involve loss of NH_3 and loss of CH_3OH, although there may be a direct route from the deprotonated species to *m/z* 70. The major fragmentation route of $[M–H–NH_3]^-$ involves loss of CO_2 to form *m/z* 85 as confirmed by accurate mass measurements. The $[M–H–CH_3OH]^-$ (*m/z* 114) fragments to form *m/z* 72 ($[C_2H_2NO_2]^-$), *m/z* 71 ($[C_2HNO_2]^-$) and *m/z* 70 ($[C_3H_4NO]^-$, involving loss of CH_2CO, CH_3CO and CO_2 respectively, in agreement with the earlier study of Bowie and co-workers [37].

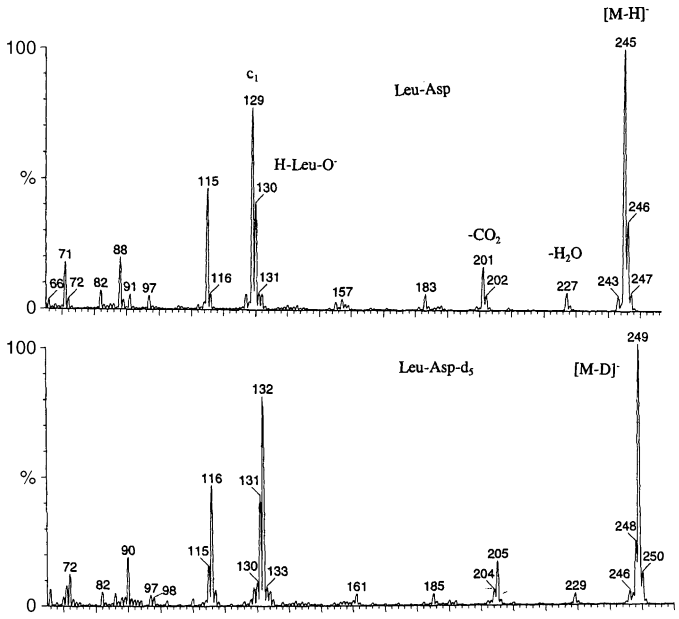
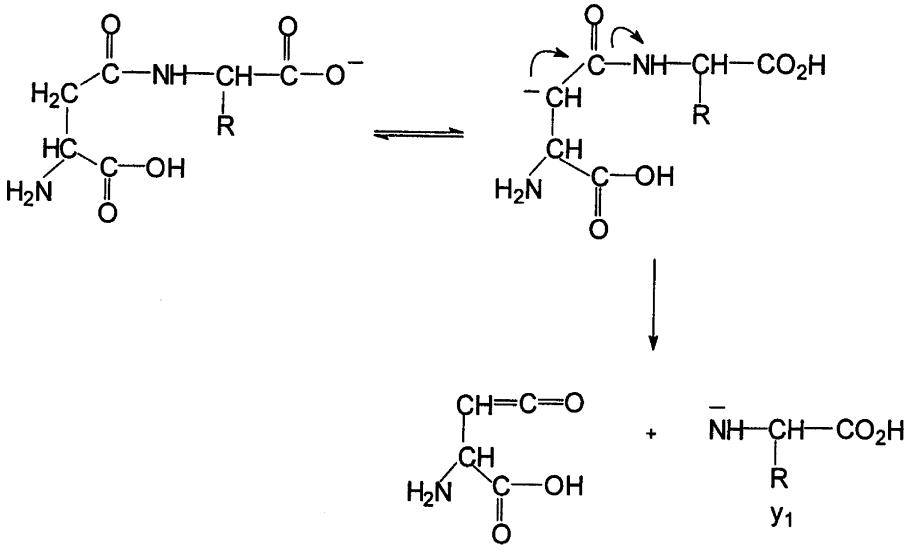


Fig. 6. Comparison of CID mass spectra of deprotonated Leu—Asp and $[M—D]^-$ ion of Leu—Asp-d_5. 48 V cone voltage.

3.2. Dipeptides

Figs. 2 and 3 show the CID mass spectra obtained by cone-voltage CID for deprotonated H–Asp–Gly–OH and H–Asp(Gly–OH)–OH and compares the spectra with those obtained for the $[M–D]^-$ ion of the peptides in which the labile hydrogens were exchanged for deuterium. The first observation is that the spectra of the α- and β-dipeptides are very similar thus precluding the possibility of distinguishing the different linkages by negative ion tandem mass spectrometry. This is in contrast to positive ion tandem mass spectrometry which does provide distinction between the isomeric dipeptides [30] and negative ion tandem mass spectrometry which does provide distinction between α- and γ-glutamyl dipeptides [23]. The major primary fragmentation of $[M–H]^-$ at low collision energies involves elimination of H_2O although formation of the y_1 ion becomes the dominant fragmentation reaction at higher collision energies. This is shown clearly by the breakdown graphs for deprotonated H–Asp–Leu–OH and H–Asp(Leu–OH)–OH presented in Figs. 4 and 5. Bowie and co-workers [25] have reported that the major fragmentation reaction of deprotonated α-aspartyl dipeptides in high-energy CID involved loss of water with minor formation of the y_1 ion. The labelling results (Figs. 2 and 3) show that primarily labile hydrogens are involved in the water-loss reaction although there appears to be minor loss of HDO from the deuterated species (similar results, not shown, were obtained



Scheme 5.

Table 2
CID mass spectra of anions derived from H–Leu–Asp–OH (20 eV collision energy)

m/z	$[M-H]^-$ (*m/z* 245)	$[M-H-H_2O]^-$ (*m/z* 227)	$[M-H-CO_2]^-$ (*m/z* 201)
245	100		
227	12.1	100	
209	7.9	13.3	
201	91.1		100
183	7.4	93.3	8.4
157	8.6		8.4
130	43.5		
129	72.2	20.0	84.3
115	27.5		
97		13.3	
88	15.7		30.1
71	4.7		

for the dipeptides containing leucine). Fragmentation of the $[M-H-H_2O]^-$ ion involves loss of NH_3 (*m/z* 154, Figs. 2 and 3) and loss of CO_2 (*m/z* 127, Figs. 2 and 3). MS^3 experiments showed that the *m/z* 127 ion from H–Asp–Gly–OH formed *m/z* 85 (loss of CH_2CO) and *m/z* 84 (loss of $CH_2{=}CHNH_2$). The major fragmentation pathways triggered by the initial loss of H_2O are summarized in Scheme 3 for H–Asp–Xxx–OH. In this scheme and several further schemes ion-neutral complexes are proposed to be involved. There is substantial evidence [38,39] for such complexes in the fragmentation of gaseous anions although concerted reactions cannot be excluded in some cases. Initial loss of H_2O from deprotonated H–Asp(Xxx–OH)–OH leads to the same ion *c* and, thus, to similar CID mass spectra for the isomeric dipeptides. A fragmentation product unique to the dipeptides containing leucine is observed at *m/z* 113 and corresponds to elimination of C_5H_{10} from the $[M-H-H_2O-CO_2]^-$ ion; this fragmentation product shifted in part to *m/z* 115 and, in part, to *m/z* 116 when the labile hydrogens were exchanged for deuterium.

A subtle difference in the fragmentation of the deprotonated α- and β-dipeptides is the labile hydrogen retention in the y_1 ion. As Fig. 2 shows the y_1 ion derived from H–Asp–Gly–OH shows largely incorporation of two labile hydrogens in the y_1 ion while H–Asp(Gly–OH)–OH shows (Fig. 3) almost equal retention of one and of two labile hydrogens in the y_1 product ion. Similar results, not shown, were obtained for H–Asp–Leu–OH and H–Asp(Leu–OH)–OH. A pathway which leads to incorporation of two labile hydrogens in y_1 is shown in Scheme 4 for an α-dipeptide; a similar fragmentation reaction can be written for the β-dipeptide in both cases leading to an aminosuccinic anhydride neutral. An alternative pathway which leads to incorporation of only one labile hydrogen in the y_1 ion is shown in Scheme 5 for the β-dipeptide. An equivalent pathway is less

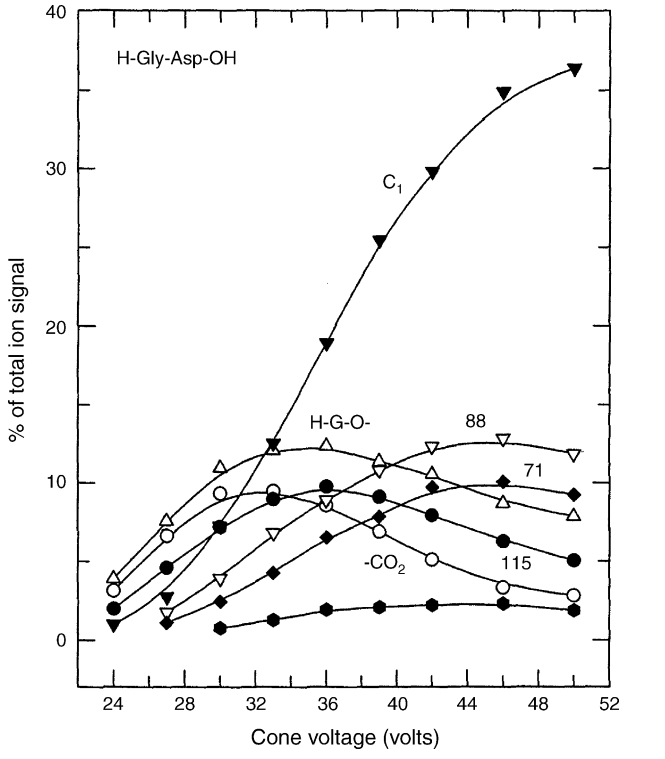
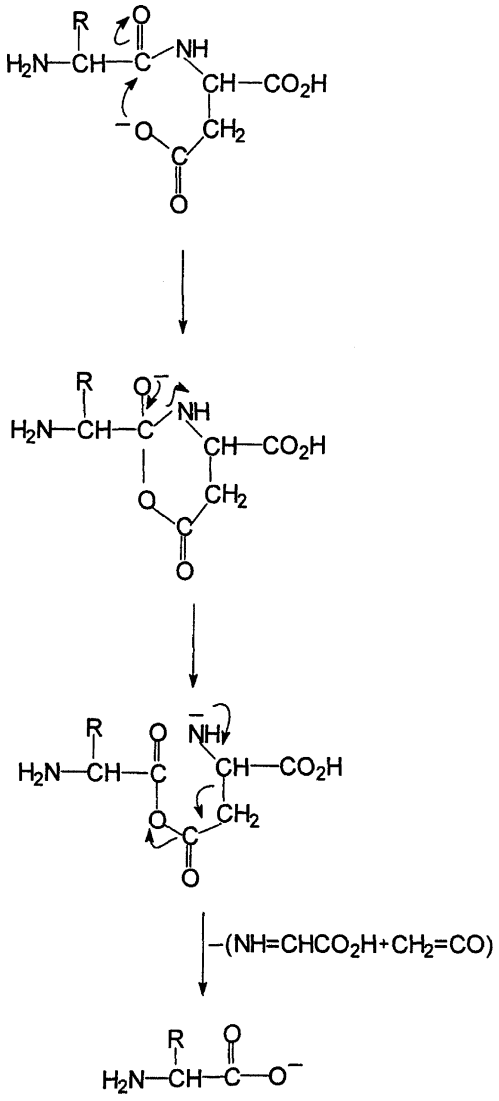
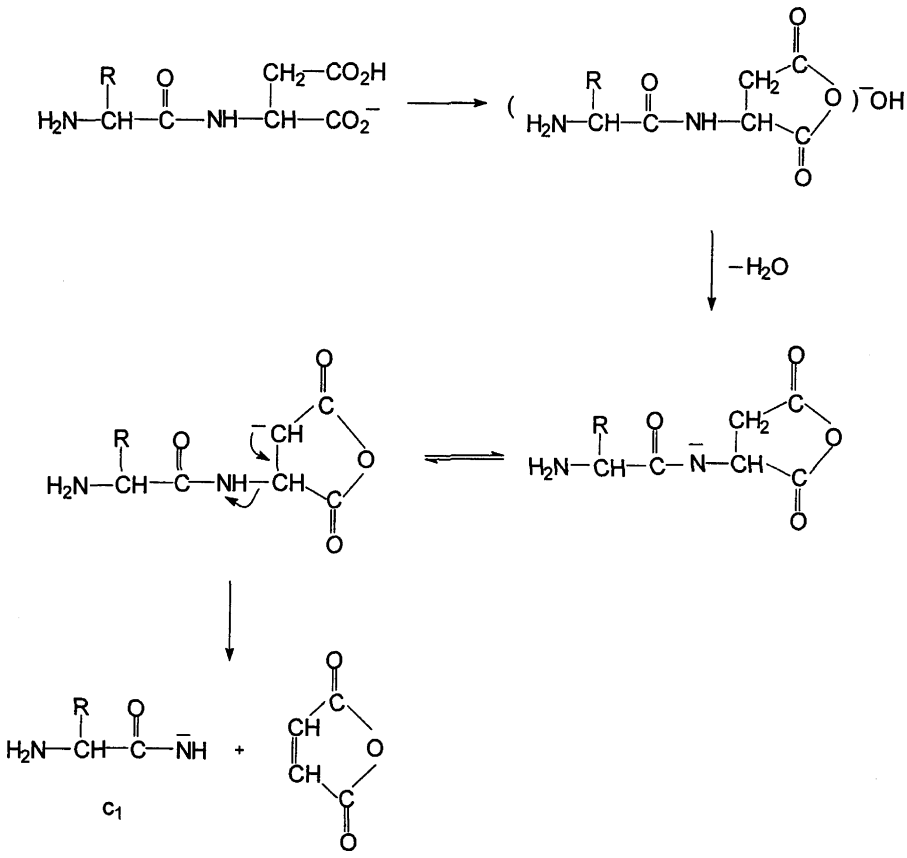


Fig. 7. Breakdown graph for deprotonated Gly–Asp. $[M-H]^-$ ion (*m/z* 189) not shown.



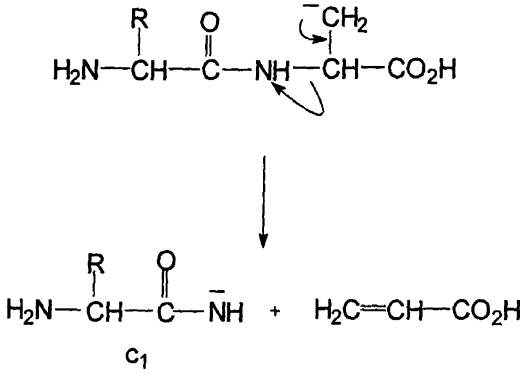
Scheme 6.



Scheme 7.

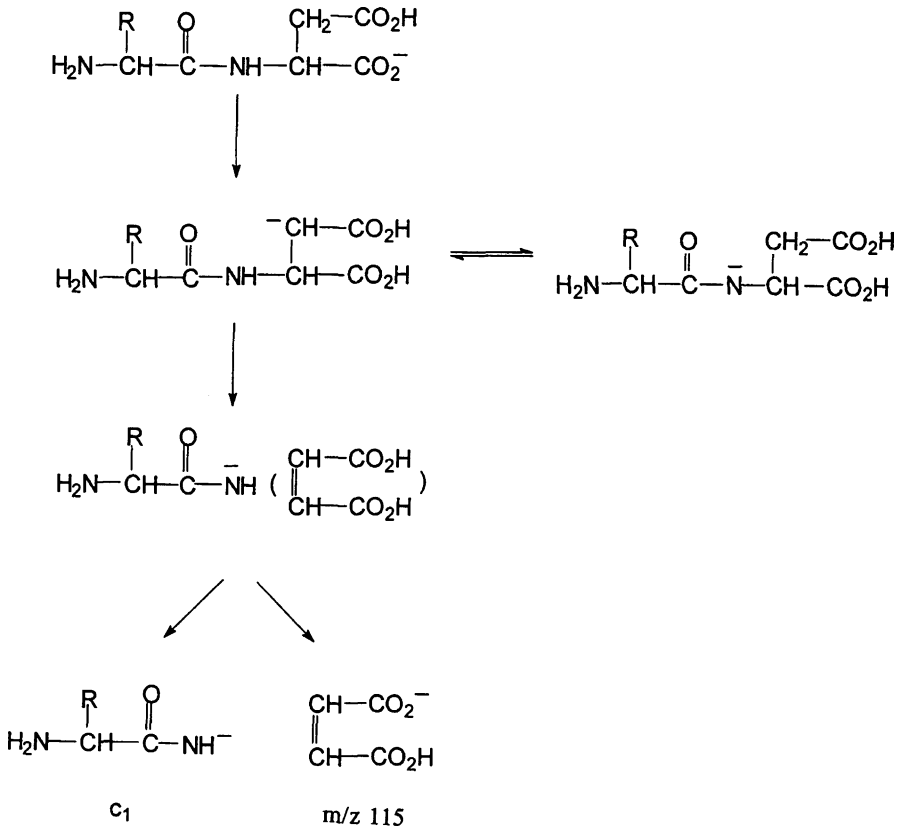
likely for the α-dipeptide since it would involve abstraction of the hydrogen on the α-carbon which is made less acidic by the amino substituent.

Fig. 6 compares the CID spectrum of deprotonated H–Leu–Asp–OH with that of the $[M–D]^-$ ion of the dipeptide in which the labile hydrogens were exchanged for deuterium, while Table 2 records the CID spectra obtained on the QToF instrument for three ions derived from H–Leu–Asp–OH. The energy dependence of the fragmentation reactions are illustrated by the breakdown graph for deprotonated H–Gly–Asp–OH, as obtained by cone-voltage CID and shown in Fig. 7. The spectra observed are quite different from the spectra of the α- and β-aspartyl dipeptides discussed above. Bowie and co-workers [25] observed mainly loss of H_2O and loss of CO_2 in the high-energy CID of deprotonated H–Leu–Asp–OH, with minor formation of *m/z* 130 (deprotonated leucine) and *m/z* 115. The breakdown graph of Fig. 7 shows that there are four fragmentation pathways of $[M–H]^-$ at the lowest collision energies, formation of deprotonated glycine (*m/z* 74), loss of CO_2, formation of *m/z* 115 (elimination of neutral glycinamide) and formation of the c_1 ion (deprotonated glycinamide), with the latter product becoming the dominant fragmentation product at higher collision energies. The signal for loss of H_2O never amounted to more than 2% of the total ionization for either deprotonated H–Gly–Asp–OH or H–Leu–Asp–OH.



Scheme 8.

The deprotonated N-terminal amino acid (*m/z* 130, Fig. 6 and *m/z* 74, Fig. 7) has also been observed by Bowie and co-workers [25]. A plausible fragmentation mechanism is shown in Scheme 6. Bowie and co-workers have proposed that the initial cyclic intermediate fragments to the carboxylate anion and a β-lactam; we propose instead fragmentation to an imine and ketene rather than the lactam. The mechanism proposed in Scheme 6 is similar to that proposed by Gronert and co-workers [40] for elimination of the C-terminus amino acid residue from metal-cationated peptides. Although it is not entirely clear from Fig. 6, it appears that the major part of the ion signal incorporates two labile hydrogens, as expected. There are several pathways to the c_1 ion. As the results in Table 2 show, one pathway is by fragmentation of the $[M–H–H_2O]^-$ ion; a possible mechanism is shown



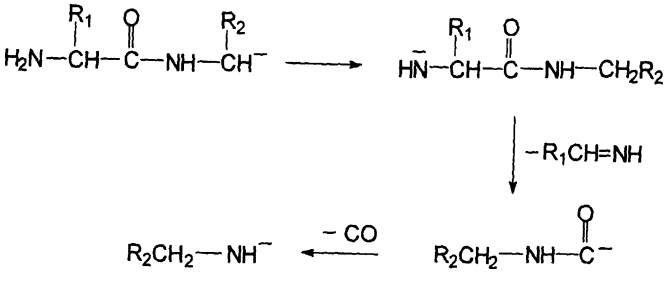
Scheme 9.

in Scheme 7 and involves elimination of maleic anhydride from $[M–H–H_2O]^-$. A second pathway is by fragmentation of the $[M–H–CO_2]^-$ ion; as outlined in Scheme 8, a pathway involving elimination of acrylic acid from $[M–H–CO_2]^-$ is the likely route. It also is likely that c_1 is formed directly from $[M–H]^-$. A pathway which leads to either the c_1 ion or to *m/z* 115 (deprotonated maleic or fumaric acid) is illustrated in Scheme 9. This fragmentation mode is similar to that reported by Steinborner and Bowie [26] for fragmentation of larger deprotonated peptides containing aspartic acid.

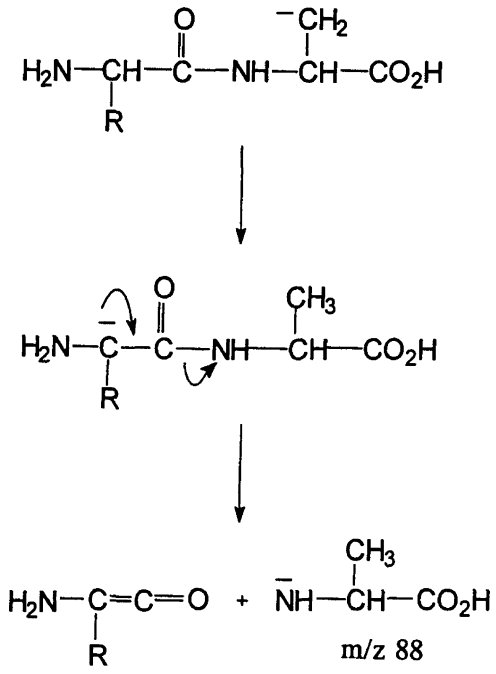
A further fragment ion observed for both H–Leu–Asp–OH and H–Gly–Asp–OH is that of *m/z* 88; the MS^3 results of Table 2 show that this fragment arises by nominal elimination of the N-terminus amino acid residue from $[M–H–CO_2]^-$. Styles and O'Hair [41] first reported elimination of the glycine residue from the a_2 ($[M–H–CO_2]^-$) ion of glycylglycine; this fragmentation mode was investigated, in detail, both experimentally and computationally by Chass et al. [42]. For the systems studied earlier, the fragmentation occurs in the step-wise fashion indicated in Scheme 10 with the acyl ion observed as an intermediate. This pathway, as applied to the a_2 ion of H–Xxx–Asp–OH where the α-carboxyl group is lost in forming $[M–H–CO_2]^-$, would lead to retention of three labile hydrogens in the product ion because of the presence of the β-COOH group. However, as is evident from Fig. 6, the fragment ion contains only two labile hydrogens. Thus, a pathway involving initial loss of CO_2 from the β-carboxyl group is proposed in Scheme 11 and results in retention of two labile hydrogens in the ionic product.

3.3. Larger peptides

Fig. 8 presents the breakdown graph obtained by cone-voltage CID for deprotonated H–Gly–Ala–Asp–OH. The major primary fragmentation products are deprotonated H–Gly–Ala–OH (*m/z* 145), the c_2 ion (*m/z* 144) and *m/z* 115 (deprotonated maleic or fumaric acid), along with more minor yields of the y_1 ion (*m/z* 132) and the a_3 ion (*m/z* 216). The formation of deprotonated H–Gly–Ala–OH can be accommodated by a pathway analogous to Scheme 6; this species fragments further to form *m/z* 88 (deprotonated alanine) and *m/z* 73 (deprotonated glycinamide) as observed earlier [42]. The formation of the c_2 ion (*m/z* 144)



Scheme 10.



Scheme 11.

and the ion of *m/z* 115 can be rationalized by the pathway illustrated in Scheme 9. The *m/z* 115 product fragments further by elimination of CO_2 to form *m/z* 71.

Fig. 9 compares the CID mass spectrum of deprotonated H–Gly–Asp–Gly–OH with that of the $[M–D]^-$ ion of the

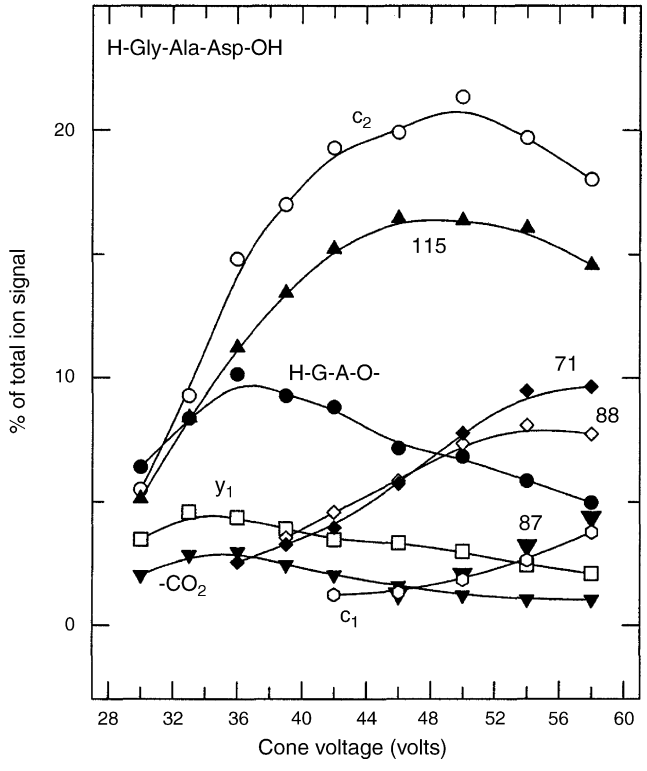


Fig. 8. Breakdown graph for deprotonated Gly—Ala—Asp. $[M–H]^-$ ion (*m/z* 260) not shown.

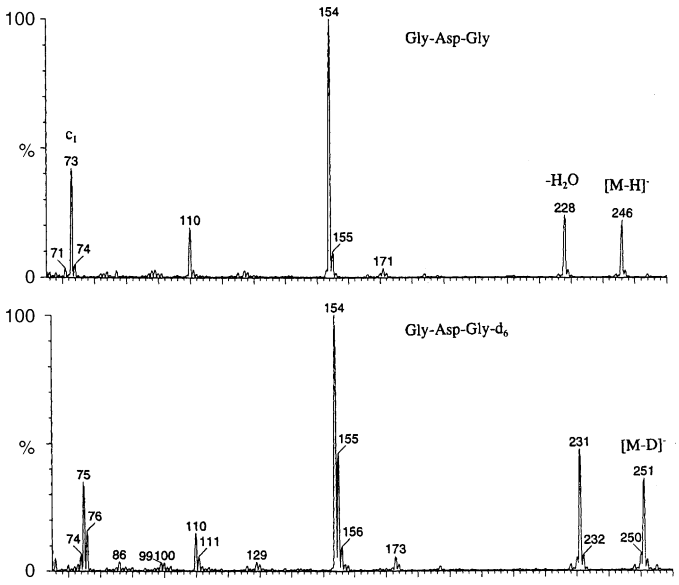


Fig. 9. Comparison of CID mass spectra of deprotonated Gly—Asp—Gly and $[M–D]^-$ ion of Gly—Asp—Gly-d_6. 40 V cone voltage.

tripeptide in which the labile hydrogens were exchanged for deuterium while Fig. 10 shows a similar comparison for deprotonated H–Gly–Gly–Asp–Ala–OH. Both spectra show substantial loss of H_2O from $[M–H]^-$, incorporating almost exclusively labile hydrogens; energy-resolved mass spectra showed that this was the dominant fragmentation mode at low internal energies. For H–Gly–Asp–Gly–OH the base peak at *m/z* 154 corresponds to elimination of glycinamide from $[M–H–H_2O]^-$ while *m/z* 73 (the c_1 ion) is the complementary product, deprotonated glycinamide. Similarly, the *m/z* 168 product of Fig. 10 represents the loss of neutral glycylglycinamide from $[M–H–H_2O]^-$ while *m/z* 130 (the c_2 ion) is the complementary product, deprotonated glycylglycinamide. MS^3 studies of the $[M–H–H_2O]^-$ (*m/z* 299)

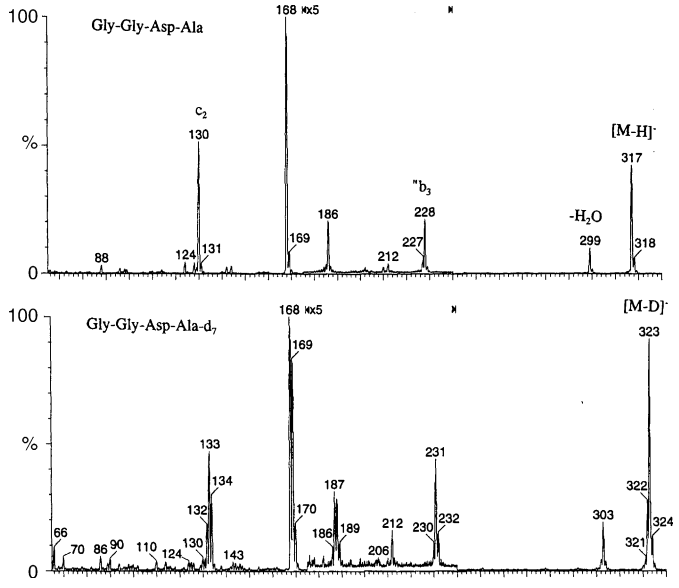
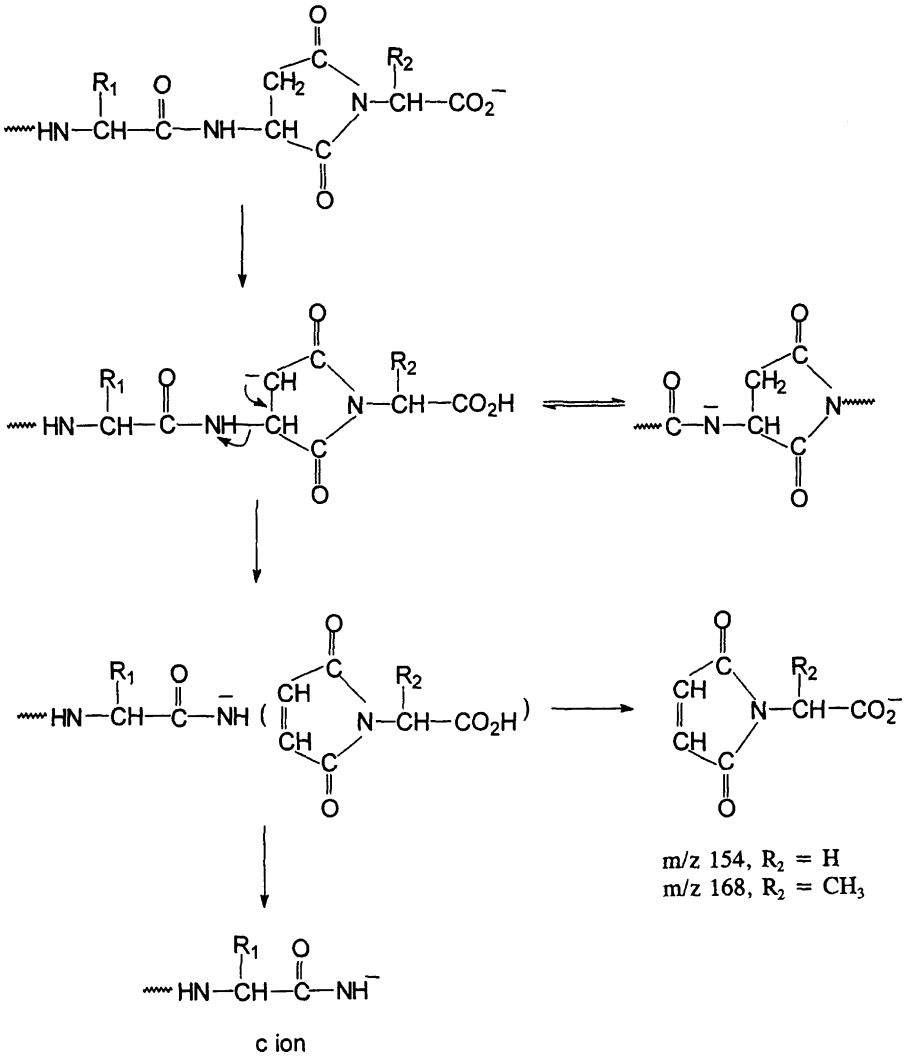


Fig. 10. Comparison of CID mass spectra of deprotonated Gly—Gly—Asp—Ala and $[M–D]^-$ ion of Gly—Gly—Asp—Ala-d_7. 42 V cone voltage. Note the X5 amplification *m/z* 175 to *m/z* 240.

ion of H–Gly–Gly–Asp–Ala–OH showed formation of both *m/z* 168 and *m/z* 130 with the *m/z* 130/*m/z* 168 ratio increasing with increasing collision energy. Further fragmentation of *m/z* 168 by loss of CO_2 and by loss of $CO_2 + CH_3$ and fragmentation of *m/z* 130 by loss of H_2O was observed at higher collision energies. Thus, for both peptides the major fragmentation of $[M–H–H_2O]^-$ involves cleavage of the N–C bond N-terminal to the aspartic acid residue to eliminate an amino acid or peptide amide or to form the appropriate deprotonated amide. In their study of larger deprotonated peptides containing aspartic acid Steinborner and Bowie [26] have observed a similar fragmentation occurring mainly from the $[M–H]^-$ ion but also from the $[M–H–H_2O]^-$ ion. For $[M–H]^-$ ions, the reaction leading to elimination of a neutral amide has been called a γ-cleavage while formation of the deprotonated amide has been called a δ-cleavage; calculations on a model system showed that the γ-cleavage reaction is thermochemically favoured [13]. One should note the weak signal at *m/z* 186 in Fig. 10 which corresponds to elimination of glycylglycinamide from $[M–H]^-$, the fragmentation reaction observed by Steinborner and Bowie. As discussed above, the main fragmentation of the $[M–H]^-$ ion of H–Gly–Ala–Asp–OH involves cleavage of the N–C bond to form a deprotonated peptide amide and the complementary product deprotonated maleic/fumaric acid. However, it is clear that, for the smaller peptides studied in the present work, N–C bond cleavage occurs mainly from the $[M–H–H_2O]^-$ ion when the aspartic acid is not in the C-terminal position.

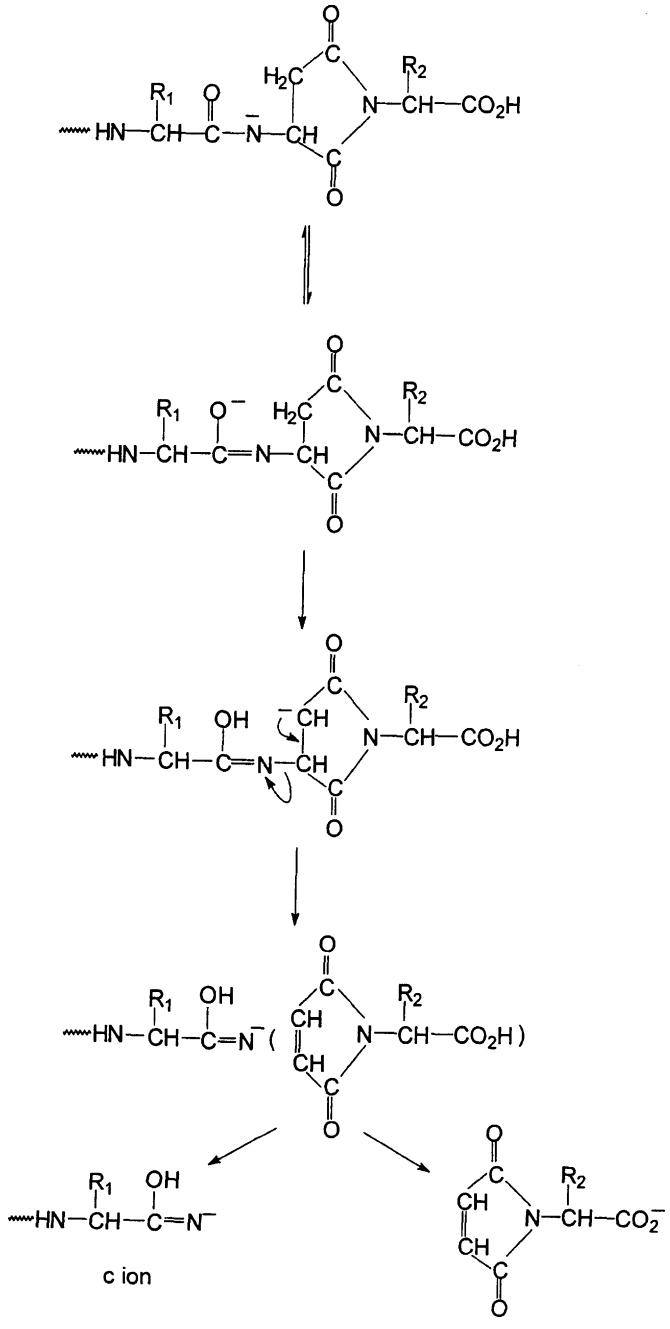
The labelling results show that the *m/z* 154 product (Fig. 9) incorporates to a significant extent one labile hydrogen as does the *m/z* 168 product of Fig. 10. The c_1 ion of Fig. 9 shows incorporation of a total of two or of three labile hydrogens while the c_2 ion of Fig. 10 incorporates a total of three or of four labile hydrogens. A plausible pathway, applicable to both peptides, is shown in Scheme 12 where hydrogen interchange (H/D exchange for the labelled peptides) between the amide nitrogen and the β-CH_2 group is proposed to rationalize the labelling results. This mechanism (without the hydrogen interchange) is very similar to that proposed by Steinborner and Bowie [26] for fragmentation of $[M–H]^-$ ions. The *m/z* 186 ion of Fig. 10 shows incorporation of one or of two labile hydrogens; this is compatible with the Steinborner and Bowie mechanism as long as one allows hydrogen exchange between the amide nitrogen



Scheme 12.

and the β-CH_2 group. The MS^3 studies showed that the c_2 ion derived from H–Gly–Gly–Asp–Ala–OH fragmented by loss of H_2O. This fragmentation of c_2 ions has been observed earlier [22] for c_2 ions derived from peptides containing phenylalanine and was interpreted in terms of formation, in part, of the enol form of the deprotonated peptide amide. An analogous pathway leading to the enol form is possible in this case as well as illustrated in Scheme 13. It is likely that both the pathways of Schemes 12 and 13 are operative.

Fig. 11 shows the breakdown graph obtained for deprotonated H–Asp–Val–Tyr–OH. Loss of NH_3, loss of H_2O and loss of $H_2O + NH_3$ are observed as low-energy fragmentation products



Scheme 13.

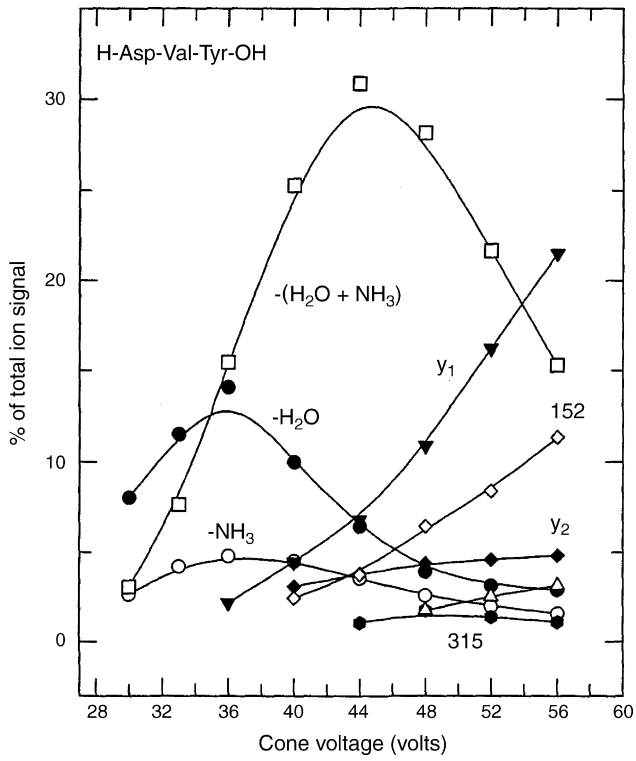


Fig. 11. Breakdown graph for deprotonated Asp—Val—Tyr. $[M—H]^-$ ion (*m/z* 394) not shown.

with loss of $H_2O + NH_3$ dominating much of the breakdown graph. The y_1 ion (deprotonated tyrosine) becomes a prominent ion at higher collision energies as does a product of *m/z* 152. MS^3 studies showed that the major fragmentation reaction of $[M–H–H_2O]^-$ was loss of NH_3 although both $[M–H–H_2O]^-$ and $[M–H–H_2O–NH_3]^-$ did fragment, in part, to form the y_1 ion. The *m/z* 152 product appeared to arise by fragmentation of $[M–H]^-$ although the detailed pathway is not clear.

4. Conclusions

The present work to some extent confirms the earlier observations of Bowie and co-workers [25,26] but also extends these observations and conclusions to a considerable extent. The use of deuterium labelling and MS^3 studies has provided further details concerning fragmentation mechanisms. As we have observed in earlier studies [19,22–24], low-energy (eV) collisional activation provides more extensive sequence-informative fragmentation than observed in the high-energy (keV) collisional activation studies of Bowie. One of the more important observations is that CID of the $[M–H]^-$ ions of α-aspartyl and β-aspartyl dipeptides give very similar fragment ion spectra thus precluding their distinction by negative ion tandem mass spectrometry; this probably also extends to larger α- and β-aspartyl peptides since the initial elimination of H_2O forms a common species for both isomers. Bowie and co-workers did not study β-aspartyl derivatives. A characteristic fragmentation reaction of deprotonated aspartic acid derivatives involves cleavage of the N–C bond N-terminal to the aspartic acid residue to from a deprotonated peptide amide or to eliminate a neu-

tral peptide amide. Although this reaction has been identified for larger peptides containing aspartic acid [26] it has not been characterized for smaller deprotonated peptides. The present work has shown that, for deprotonated peptides containing a C-terminal aspartic acid residue, such as H–Xxx–Asp–OH or H–Xxx–Yyy–Asp–OH, the N–C bond cleavage occurs from the $[M–H]^-$ ion to form the appropriate c ion or the complimentary product, deprotonated maleic acid (Scheme 9). However, for peptides with the sequence H–Yyy–Asp–Zzz–OH or H–Xxx–Yyy–Asp–Zzz–OH the N–C bond cleavage occurs primarily from the $[M–H–H_2O]^-$ ion to form the appropriate c ion or a deprotonated N-substituted maleimide as shown in Schemes 12 and 13. The products of this N–C bond cleavage serve to identify the position of the aspartic acid residue in the peptide.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council (Canada) through a research grant to AGH and an equipment grant to the Department of Chemistry which made possible the acquisition of the QStar.

References

[1] D.F. Hunt, J.R. Yates III, J. Shabanowitz, S. Winston, J.R. Hauer, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 6233.
[2] K. Biemann, Methods Enzymol. 193 (1990) 455.
[3] K. Biemann, in: T. Matsuo, R.M. Caprioli, M.L. Gross, Y. Seyama (Eds.), Biological Mass Spectrometry: Present and Future, Wiley, New York, 1993, p. 276.
[4] I. Papayannopoulos, Mass Spectrom. Rev. 14 (1995) 49.
[5] R.A.J. O'Hair, J. Mass Spectrom. 35 (2000) 1377.
[6] A. Schlosser, W.D. Lehmann, J. Mass Spectrom. 35 (2000) 1382.
[7] M.J. Polce, D. Ren, C. Wesdemiotis, J. Mass Spectrom. 35 (2000) 1391.
[8] V.H. Wysocki, G. Tsaprailis, L.L. Smith, L.A. Breci, J. Mass Spectrom. 35 (2000) 1399.
[9] T. Vaisar, in: N.M.M. Nibbering (Ed.), Encyclopedia of Mass Spectrometry, vol. 4, Elsevier, New York, 2005, p. 752.
[10] B. Paizs, S. Suhai, Mass Spectrom. Rev. 24 (2005) 508.
[11] R.J. Waugh, J.H. Bowie, Rapid Commun. Mass Spectrom. 8 (1994) 169.
[12] A.M. Bradford, R.J. Waugh, J.H. Bowie, Rapid Commun. Mass Spectrom. 9 (1995) 677.
[13] J.H. Bowie, C.S. Brinkworth, S. Dua, Mass Spectrom. Rev. 21 (2002) 87;
J.H. Bowie, C.S. Brinkworth, in: N.M.M. Nibbering (Ed.), Encyclopedia of Mass Spectometry, vol. 4, Elsevier, New York, 2005, p. 424.
[14] J. Jai-nkuknan, C.J. Cassady, Rapid Commun. Mass Spectrom. 10 (1996) 1678.
[15] J. Jai-nkuknan, C.J. Cassady, Anal. Chem. 70 (1998) 5122.
[16] N.P. Ewing, C.J. Cassady, J. Am. Soc. Mass Spectrom. 12 (2001) 105.
[17] N.L. Clipston, J. Jai-nkuknan, C.J. Cassady, Int. J. Mass Spectrom. 222 (2003) 363.
[18] E.J. Marzluff, S. Campbell, M.T. Rodgers, J.L. Beauchamp, J. Am. Chem. Soc. 116 (1994) 7787.
[19] A.G. Harrison, J. Am. Soc. Mass Spectrom. 13 (2002) 1.
[20] P. Roepstorff, J. Fohlman, Biomed. Mass Spectrom. 11 (1984) 601.
[21] K. Biemann, Biomed. Environ. Mass Spectrom. 16 (1988) 99.
[22] A.G. Harrison, J. Am. Soc. Mass Spectrom. 13 (2002) 1242.
[23] A.G. Harrison, J. Mass Spectrom. 39 (2004) 136.
[24] A.G. Harrison, A.B. Young, J. Mass Spectrom. 40 (2005) 1173.
[25] R.J. Waugh, J.H. Bowie, R.N. Hayes, Org. Mass Spectrom. 26 (1991) 250.
[26] S.T. Steinborner, J.H. Bowie, Rapid. Commun. Mass Spectrom. 11 (1997) 253.
[27] J.R. Lloyd, M.L. Cotter, D. Ohori, D.L. Doyle, Biomed. Environ. Mass Spectrom. 15 (1988) 399.
[28] C. Castet, C. Engalbal, P. Fulcrond, J.L. Guichon, J. Martinez, J.-L. Aubagnac, Rapid Commun. Mass Spectrom. 10 (1996) 1934.
[29] R.J. Waugh, S.T. Steinborner, J.H. Bowie, J.C. Wallace, M.J. Tyler, P. Hu, Aust. J. Chem. 48 (1995) 1981.
[30] A.G. Harrison, Y.-P. Tu, J. Mass Spectrom. 33 (1998) 532.
[31] L.J. González, T. Shimazu, Y. Satami, L. Betancourt, V. Besada, G. Padrón, R. Orlando, T. Shirasawa, Y. Shimonishi, T. Tabao, Rapid Commun. Mass Spectrom. 14 (2000) 2092.
[32] A.G. Harrison, Rapid Commun. Mass Spectrom. 13 (1999) 1663.
[33] W.D. van Dongen, J.I.T. van Wijk, B.M. Green, W. Heerma, J. Haverkamp, Rapid Commun. Mass Spectrom. 13 (1999) 1712.
[34] A.G. Harrison, J. Mass Spectrom. 34 (1999) 1153.
[35] J. Makowiecki, A. Tolonen, J. Uusitalo, J. Jalonen, Rapid Commun. Mass Spectrom. 15 (2001) 1506.
[36] C. Buré, C. Lange, Curr. Org. Chem. 7 (2003) 1613.
[37] M. Eckersley, J.H. Bowie, R.N. Hayes, Int. J. Mass Spectrom. Ion Process. 93 (1989) 199.
[38] J.H. Bowie, Mass Spectrom. Rev. 9 (1990) 349.
[39] J.H. Bowie, N.M.M. Nibbering (Eds.), Encyclopedia of Mass Spectrometry, vol. 4, Elsevier, New York, 2005, p. 443.
[40] W.Y. Feng, S. Gronert, K.A. Fletcher, A. Warres, C.B. Lebrilla, Int. J. Mass Spectrom. 222 (2003) 117.
[41] M.L. Styles, R.A.J. O'Hair, Rapid Commun. Mass Spectrom. 12 (1998) 809.
[42] G.A. Chass, C.N.J. Marai, A.G. Harrison, I.G. Csizmadia, J. Phys. Chem. A 106 (2002) 9695.