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Atmospheric Pressure Chemical Ionization Tandem Mass Spectra of α- and β-Aspartame

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Atmospheric pressure chemical ionization tandem mass spectrometry was used to study the gas-phase reactions of protonated α- and β-aspartame. The results show that under collision-induced dissociation conditions, these ions undergo dehydration to form anhydro derivatives in addition to carbon–carbon bond cleavages. The fragment ions formed from these dissociation pathways can be used to distinguish the two isomers. Specifically, ions at *m/z* 175 and 88 are unique to protonated α-aspartame whereas ions at *m/z* 249, 189 and 74 are unique to protonated β-aspartame. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Aspartame (**1**, L-α-aspartyl-L-phenylalanine methyl ester, Fig. 1), marketed under the trade name Nutrasweet, is a well known artificial sweetener.[1–4] It has been used in many products, including pharmaceuticals.[5,6]

Aspartame and its degradation products in solution have been extensively studied. As can be seen in **1**, aspartame contains two hydrolytically sensitive bonds, the ester and the peptide bonds. The ester bond is hydrolytically more labile than the peptide bond.[6] Three major degradation products of aspartame were observed in solution, namely (5-benzyl-3,6-dioxopiperazin-2-yl)acetic acid (diketopiperazine (DKP), **2**, Fig. 1), L-aspartyl-L-phenylalanine (**3**, Fig. 1) and phenylalanine (**4**, Fig. 1).[7–10] Of these, **2** and **3** are formed from the breaking of the ester bond while **4** is formed from the breaking of both the peptide and the ester bonds. Below pH 3 aspartame mainly undergoes hydrolysis to **3**, whereas above pH 6 it mainly undergoes cyclization to form **2**.

The degradation of aspartame has also been studied in the dry state. When heated in an acidified–lyophilized state at 110 °C for 24 h in vacuum, aspartame formed many degradation products.[11] In addition to the well characterized degradates (**2**–**4**), aspartame was observed to dehydrate to form an anhydro derivative (**5**, Fig. 1), which has not been observed in solution so far.

In spite of extensive studies of aspartame in solution and in the solid state, the chemistry of aspartame in the gas phase has not been well studied. Owing to the recent development of the API (atmospheric pressure ionization) technique in mass spectrometry (MS), studies of thermally labile, low-volatile compounds, such as aspartame, by MS have become feasible.[12] Recently, a study of analytical conditions for the determination of aspartame by liquid chromatography/electrospray (ES) MS has been reported.[13] Different degrees of aspartame fragmentation were obtained by varying the electrospray spraying capillary voltage. However, no detailed analysis of the fragmentation pattern was given. Here, we report a study of protonated aspartame in the gas phase by the use of APCI (atmospheric pressure chemical ionization) tandem mass spectrometry (MS/MS). This study demonstrates that after collision-induced dissociation (CID) by argon in the gas phase, protonated aspartame undergoes dehydration to form an anhydro derivative (**5**) in addition to carbon–carbon bond cleavages.

α-Aspartame has a positional isomer, β-aspartame (**6**, Fig. 1). β-Aspartame is considered to be a decomposition product of α-aspartame.[10] It would be significant to distinguish them in the study of aspartame chemistry. Since MS has great potential to distinguish isomers, and especially MS/MS has been actively used for distinguishing saturated and unsaturated isomeric molecular ions,[14] MS/MS was applied in the present study to probe the differences in the gas-phase reactions of protonated α- and β-aspartame. It was observed that under the same APCI/MS/MS and CID conditions, protonated α- and β-aspartame show characteristic fragmentation patterns in both the dehydration and the carbon–carbon bond cleavages that are distinguishable. Since α- and β-aspartame are typical derivatives of α- and β-dipeptides, this study is significant for MS/MS studies of α- and β-peptides in general.

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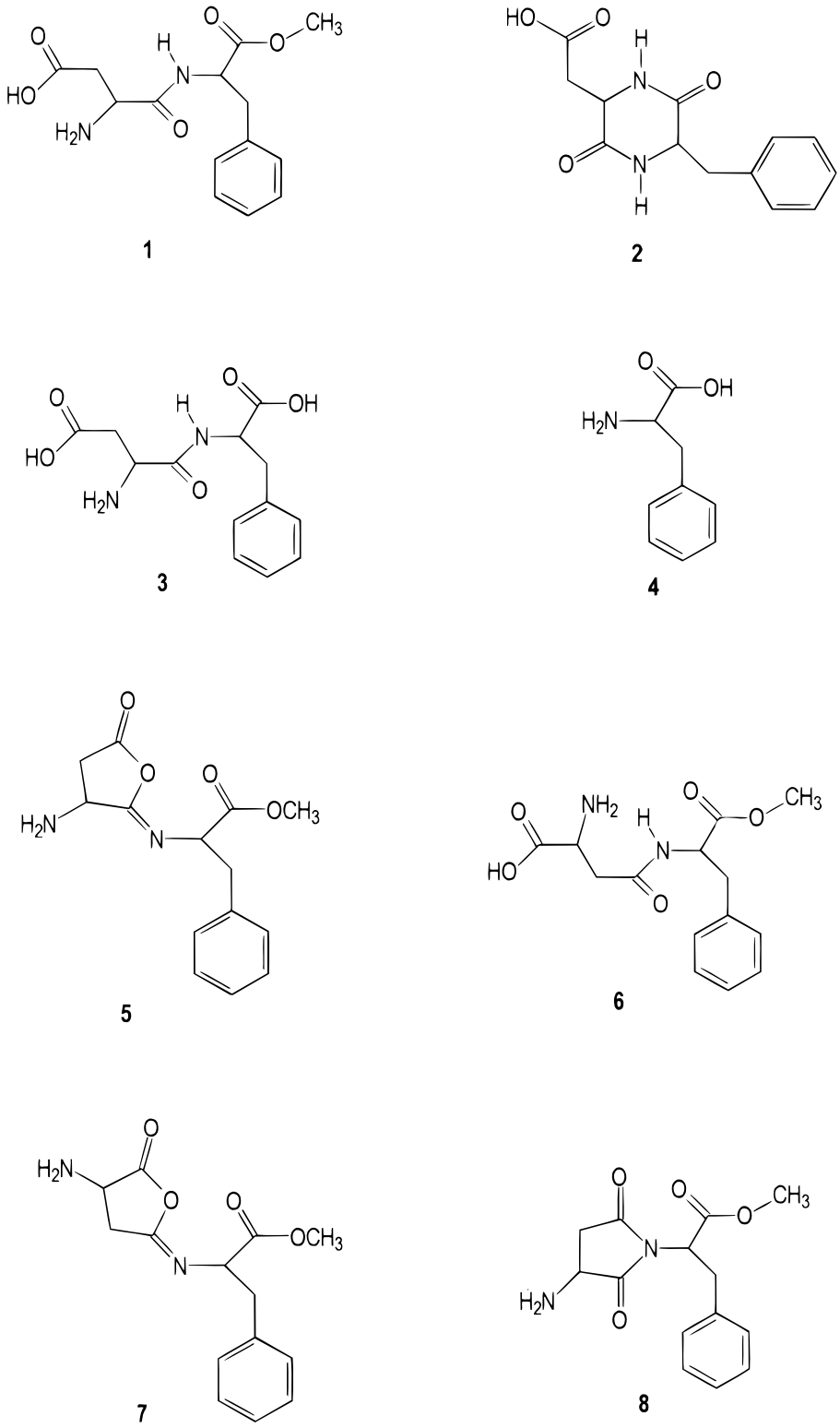


Figure 1. Structures of α-aspartame (**1**), diketopiperazine (**2**), ASP-PHE (**3**), phenylalanine (**4**), anhydro derivative (**5**), β-aspartame (**6**), anhydro derivative (**7**) and pyrrolidinedione (**8**).

EXPERIMENTAL

Samples

Aspartame (α-aspartame), β-aspartame and L-aspartyl-L-phenylalanine (ASP-PHE) were purchased from Sigma (Milano, Italy) and were used as received. Their standard solutions were made by dissolving 20 mg of each compound in 200 ml of water.

Flow Injection MS and MS/MS

An HP 1090 liquid chromatograph and a Finnigan

MAT TSQ 7000 mass spectrometer were interfaced by a Finnigan MAT APCI source operated in the positive ionization mode. Flow injection parameters were a flow rate of 0.5 ml min^{-1}, a carrier stream of 20% acetonitrile–80% 0.02 M NH_4OAc and an injection volume of 20 μl. The APCI parameters were vaporizer 500 °C, capillary 200 °C, sheath gas (N_2) flow meter 50 psi, auxiliary gas (N_2) flow meter 30 units and corona setting, 5 μA. Parameters for CID were collision gas argon at a pressure of 1.5 mTorr (1 Torr = 133.3 Pa), collision energy 20 eV and MS/MS correction factor 0%. The protonated molecular ions of α- and β-aspartame and ASP-PHE at *m/z* 295, 295 and 281, respectively, were selected by Q1 while Q3 was scanned repetitively from *m/z* 50 to 350 in 1 s.

RESULTS AND DISCUSSION

MS/MS of the protonated α-aspartame ion

Under the experimental conditions, the mass spectrum of protonated aspartame (APM) was dominated by the parent ion at *m/z* 295 with little fragmentation (not shown). A typical product ion spectrum of this ion is shown in Fig. 2. This spectrum is rather complicated. These ions are divided into two groups. The proposed dissociation pathways for the two groups of ions are shown in Schemes 1 and 2, respectively. Some product ions in Scheme 1 are referred to as 'a-type,' 'y-type' and 'z-type' ions. Such nomenclature refers to ions formed from protonated peptides.[15] Since aspartame is a derivative of the dipeptide ASP-PHE, the nomenclature is also used for its product ions.

Scheme 1 shows the dissociation pathways resulting in ions at *m/z* 235, 218, 180, 175, 163, 120 and 88. The ions at *m/z* 235 and *m/z* 88 (a_1) are formed by carbon–carbon bond cleavages according to α-scissions assuming the amine group is protonated. The ion at *m/z* 175 is formed by loss of an $HCOOCH_3$ group from the *m/z* 235 ion. As shown, two structures have been proposed for the ions at *m/z* 235. When assuming the amide group is protonated, the alternative ion at *m/z* 235 (a_2) can be formed by carbon–carbon bond cleavage according to α-scission. The ion at *m/z* 218 is formed from the *m/z* 235 ion by deamination. The ions at *m/z* 180 (y_1) and *m/z* 163 (z_1) are postulated to be formed when the amide group is protonated. The ion at *m/z* 120 is formed by loss of an $HCOOCH_3$ group from the *m/z* 180 ion. The ions at *m/z* 235 and 180 are the most abundant ions in this group.

The second group of ions, shown in Scheme 2, contains ions at *m/z* 277, 260, 245, 228, 217 and 200. The overall abundance of these ions is much lower than that of the first group of ions (the overall abundance ratio of the first group to the second group is about 4:1). The ion at *m/z* 277 is the key to understanding the formation of these ions since they are formed consecutively from this ion, as depicted. The ion at *m/z* 277 is suggested to be the anhydro derivative **5**. This species was identified by fast atom bombardment (FAB) MS in a study of aspartame in an acidified–lyophilized state,[11] in which the characteristic fragment ions of this species at *m/z* 245 and 217 were also reported. As shown in Scheme 2, the ions at *m/z* 260, 245 and 217 are formed by deamination, the loss of a $HOCH_3$ and the loss of a $HCOOCH_3$ from the *m/z* 277 ion, respectively. The ions at *m/z* 260 and 245 underwent further dissociation. The ion at *m/z* 228 is formed from deamination from the ion at *m/z* 245 and the ion at *m/z* 200 is formed from the loss of an $HCOOCH_3$ group from the ion at *m/z* 260. The ion at *m/z* 260 is the primary ion in this group. In

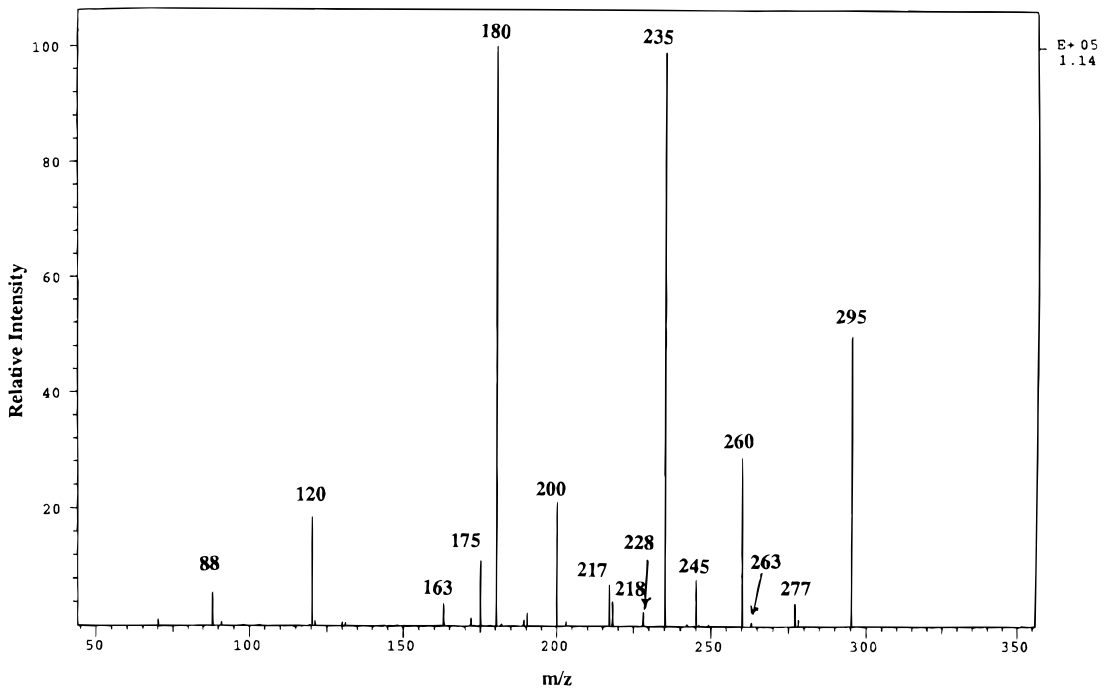
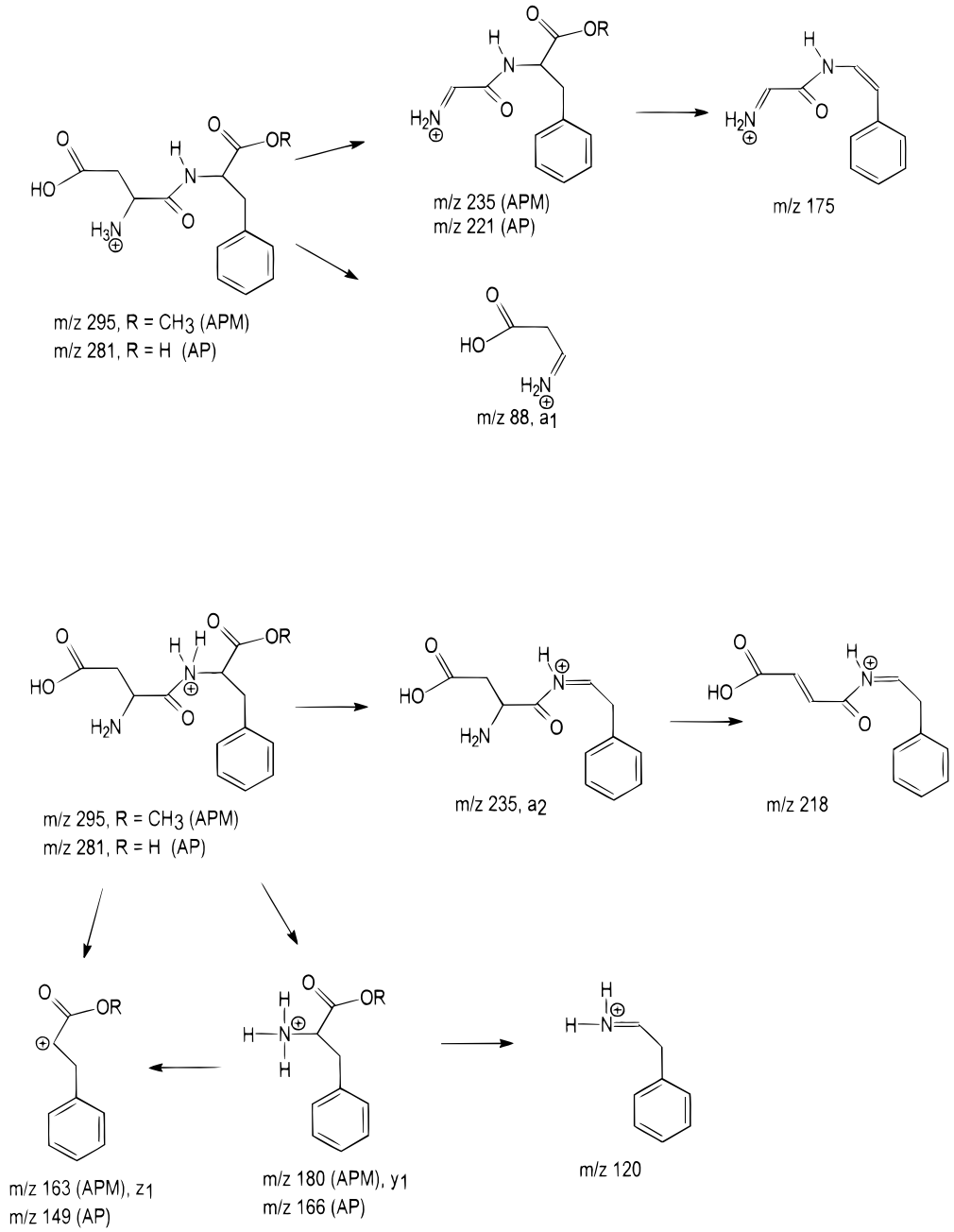


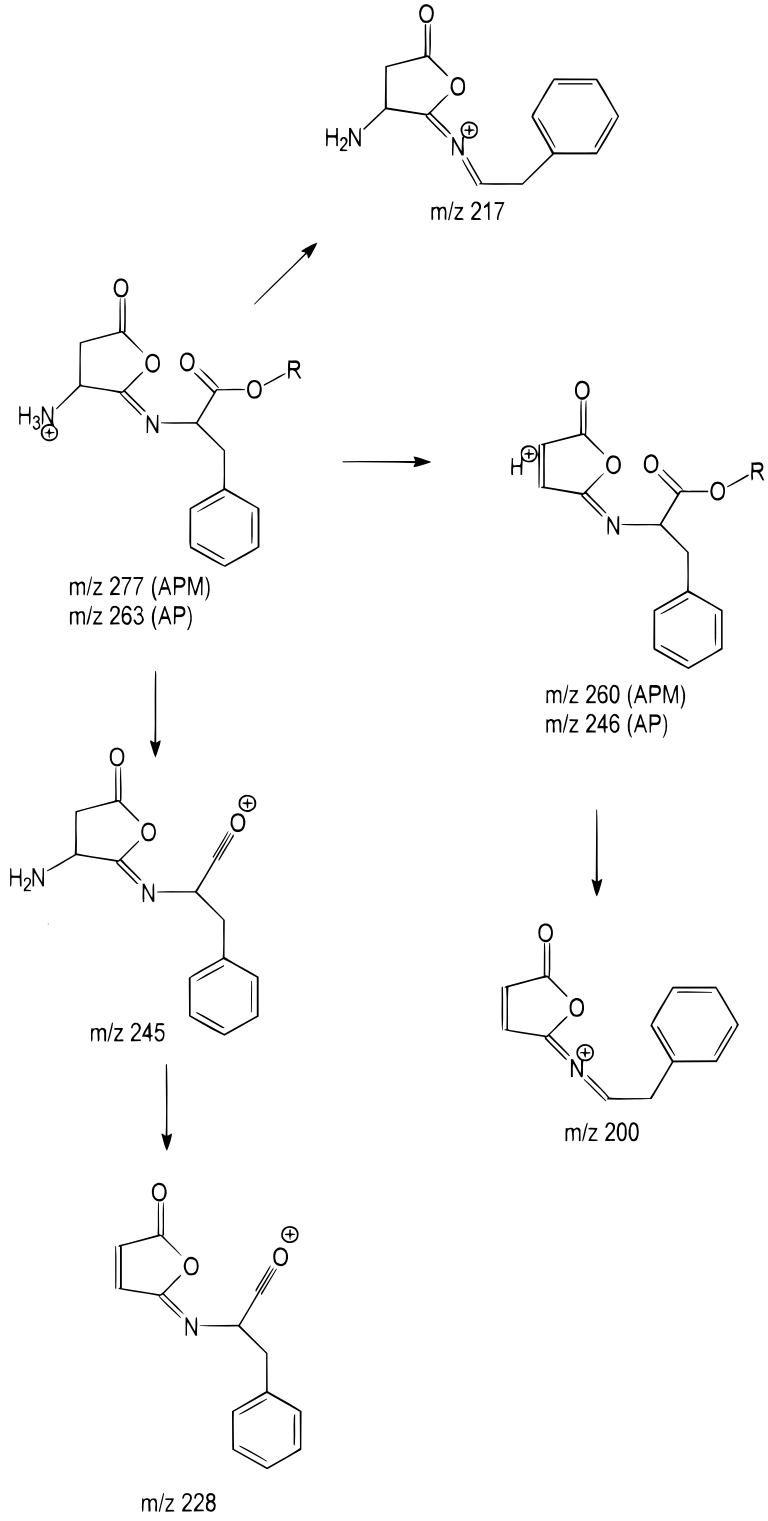
Figure 2. Product ion spectrum of protonated α-aspartame obtained under the CID conditions described in the Experimental section.



Scheme 1. Dissociations of protonated *α*-aspartame (APM) and ASP-PHE (AP).

addition to these ions, there is a very minor ion at *m/z* 263 in Fig. 2. It is assigned to protonated aspartame-DKP formed by cyclization by the loss of an $HOCH_3$ group from protonated aspartame.

Further studies of the product ion spectra of protonated aspartame were conducted by varying collision energy from 0 to 40 eV. Below 10 eV, the protonated aspartame ion showed very little fragmentation. With increasing collision energy to 20 eV, many product ions were formed. As described above, for the first group of ions, the ions at *m/z* 235 and 180 were the most abundant ions at this collision energy. On increasing collision energy to 30 eV, the ions at *m/z* 235 and 180 decreased whereas those at *m/z* 120, 163, 175 and 88 increased. The ion at *m/z* 120 became the most abundant ion. With further increasing collision energy to 40 eV, the ion at *m/z* 120 remained the most abundant ion while all the other ions decreased (the relative abundances of these ions were all $<15\%$). For the second group of ions, the ion at *m/z* 260 was the primary ion at a collision energy of 20 eV. On increasing collision energy to 30 eV, this ion and the ions at *m/z* 245, 228 and 217 decreased whereas the ion at *m/z* 200 increased and became the primary ion. On increasing the collision energy to 40 eV, all the ions in the second group decreased with the ion at *m/z* 200 still being the primary ion. In general, the ratio of the overall abundance of the first group to that of the second group increased with increasing collision energy. At a collision energy of 20 eV, the ratio was about 4:1, as mentioned above. It became about 5:1 and 10:1 at collision energies of 30 and 40 eV, respectively. These results are in agreement with the dissociation pathways proposed in Schemes 1 and 2.



Scheme 2. Dissociation of the protonated anhydro derivative ions formed from dehydration of protonated α-aspartame (APM) and ASP-PHE (AP).

In summary, protonated α-aspartame undergoes dehydration (Scheme 2) in addition to fragmentation by carbon–carbon bond cleavages (Schemes 1) under CID conditions.

MS/MS of the protonated ASP-PHE ion

In order to support the analysis of the product ion spectrum of protonated α-aspartame, its hydrolysis product, ASP-PHE (**3**), was studied under the same conditions. The mass spectrum of protonated ASP-PHE was dominated by the parent ion at *m/z* 281 with little fragmentation (not shown). A representative product ion spectrum of this ion is shown in Fig. 3. The spectrum of ASP-PHE can be similarly interpreted by two groups of ions as those of α-aspartame. Every analysis of the

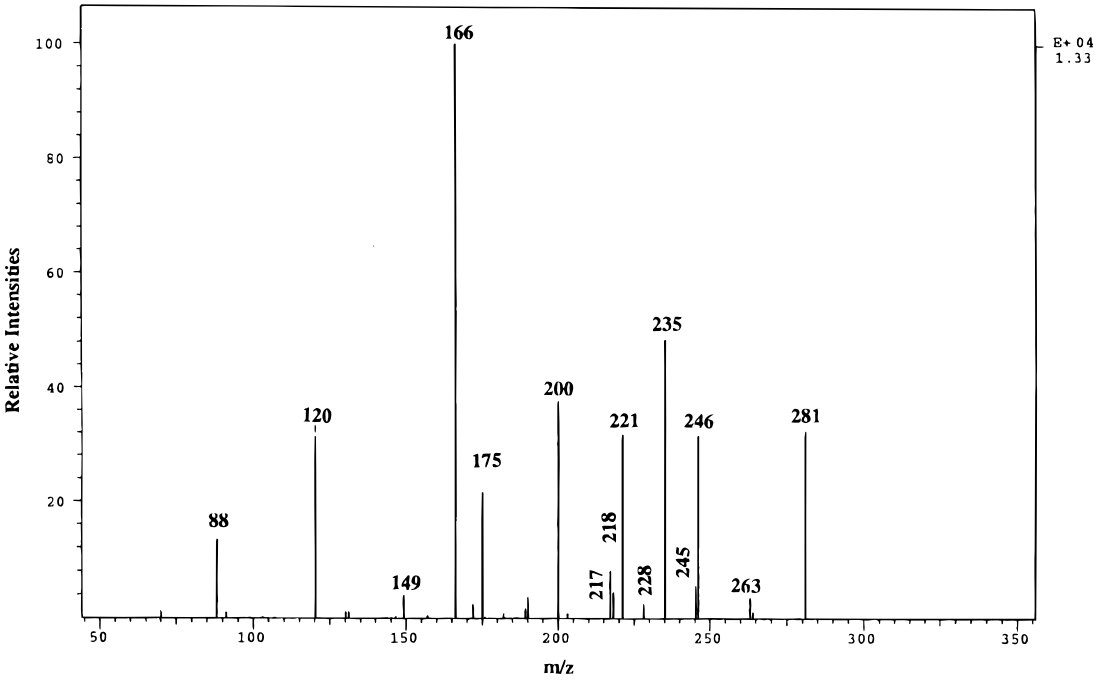


Figure 3. Product ion spectrum of protonated ASP-PHE obtained under the CID conditions described in the Experimental section.

product ion spectrum of protonated α-aspartame can be applied to that of protonated ASP-PHE after replacing the methyl ester group of aspartame with the carboxyl group of ASP-PHE, as shown in Schemes 1 and 2. It should be pointed out that ions at both *m/z* 221 and 235 were observed, supporting the contention that there are two structures for the *m/z* 235 ions from aspartame (see Scheme 1). In conclusion, the parallel results for α-aspartame and ASP-PHE strongly support the analysis of the product ion spectrum of protonated α-aspartame.

MS/MS of the protonated β-aspartame ion

β-Aspartame (**6**), a positional isomer of α-aspartame, was studied under the same conditions. The molecular mass of β-aspartame is the same as that of α-aspartame, and the mass spectrum showed the parent ion at *m/z* 295 with little fragmentation (not shown). A representative product ion spectrum of this ion is shown in Fig. 4. These ions can also be divided into two groups. The first group, shown in Scheme 3, is predominant. It contains eight ions at *m/z* 249, 235, 218, 189, 180, 163, 120

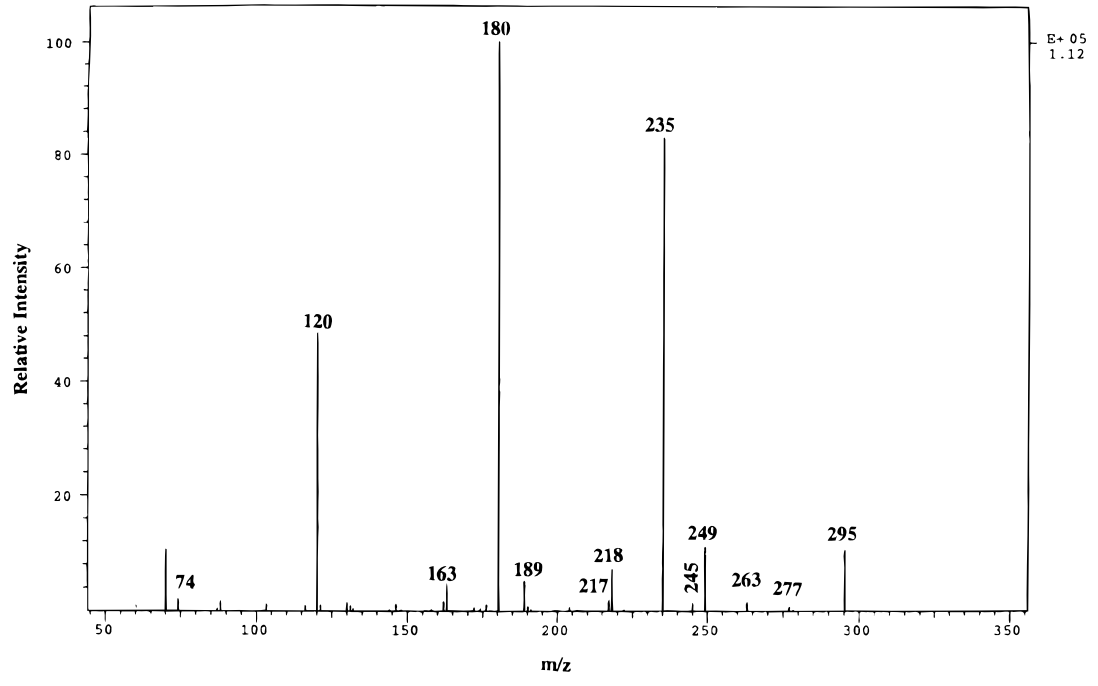
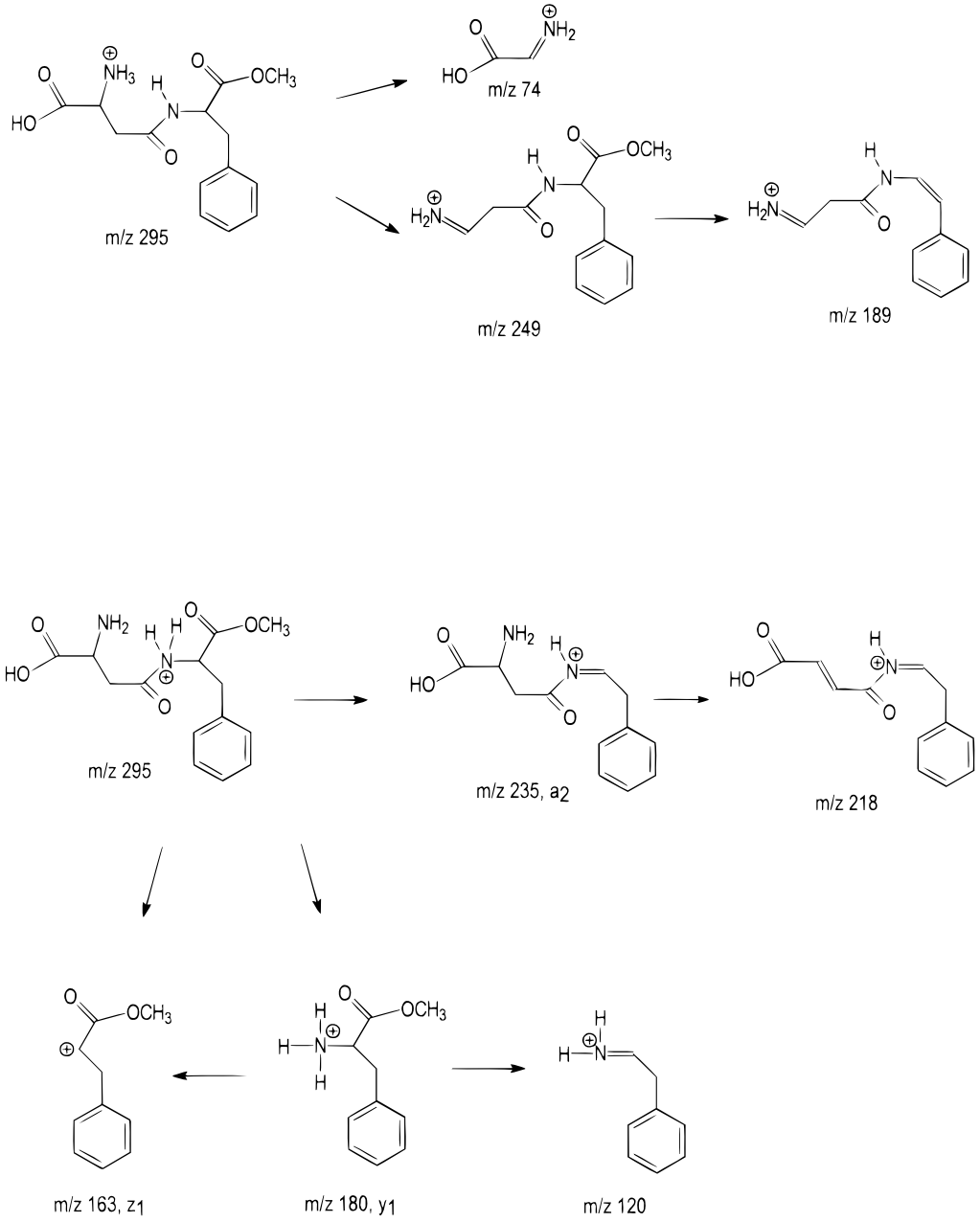
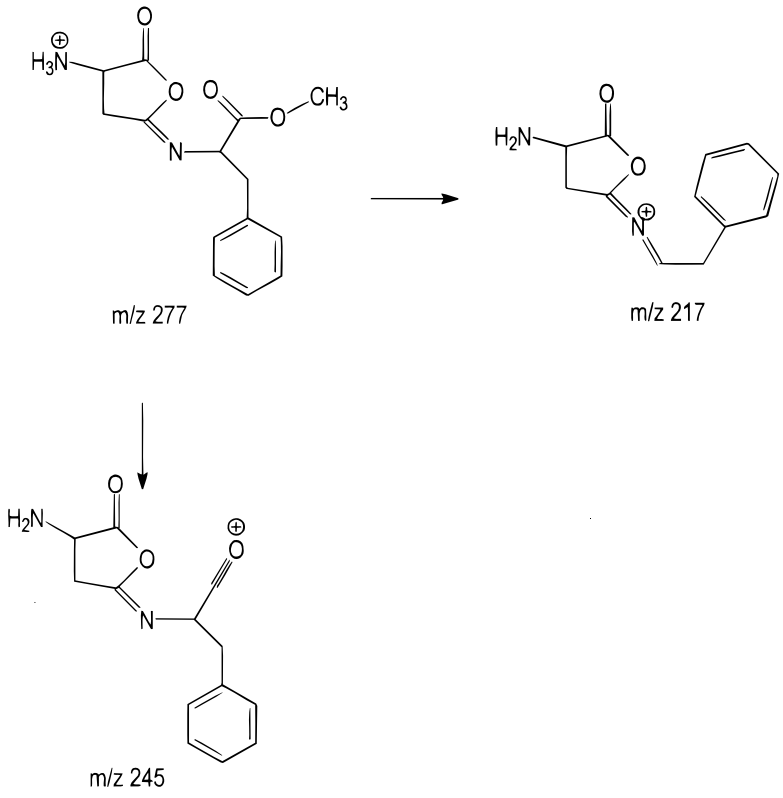


Figure 4. Product ion spectrum of protonated β-aspartame obtained under the CID conditions described in the Experimental section.



Scheme 3. Dissociation of protonated β-aspartame.

and 74. In this group, the ions at *m/z* 235, 218, 180, 163 and 120 are formed when the amide group is protonated. They are not affected by the different position of the amine group in β-aspartame as compared with α-aspartame. The different position of the amine group affects the formation of the ions at *m/z* 249, 189 and 74, formed after the amine group is protonated. As shown in Scheme 3, the ions at *m/z* 249 and 74 are formed by carbon–carbon bond cleavages according to α-scissions when the amine group is protonated. The ion at *m/z* 249 increases by 14 u and the ion at *m/z* 74 decreases by 14 u from the corresponding two ions for α-aspartame (namely the ions at *m/z* 235 and 88), reflecting the different positions of the amine groups in the two isomers. Subsequent loss of $HCOOCH_3$ from the ion at *m/z* 249 produces the ion at *m/z* 189. It also increases by 14 u from the corresponding ion at *m/z* 175 for α-aspartame. The ions at *m/z* 249, 189 and 77 are unique to β-aspartame while those at *m/z* 175 and 88 are unique to α-aspartame. They can be used to distinguish the two isomeric aspartames. The most abundant ions of the first group for β-aspartame are the ions at *m/z* 235 and 180. The second group of ions formed from protonated β-aspartame ion are very weak, and mainly consist of the ions at *m/z* 277, 245 and 217 (the ratio of the overall abundance of the first group to that of the second group is $>50:1$). The ion at *m/z* 277 is proposed to be the anhydro derivative **7** formed by dehydration as shown in Scheme 4. The ions at *m/z* 245 and 217 are formed from **7** by the loss of $HOCH_3$ and $HCOOCH_3$, respectively. The anhydro derivative **7** is an isomer of **5**. It appears that the two ions **5** and **7** and also the ions formed consecutively from them had very different abundance distributions. The overall abundance of **7** and its fragment ions was much lower than that of **5**. In particular, the ions formed from deamination observed



Scheme 4. Dissociation of the protonated anhydro derivative ion formed from dehydration of protonated β-aspartame.

for **5** were not observed for **7**. Presumably, their relative abundances were too low to be observed for **7** under the experimental conditions. In addition to the two groups, there is an ion at *m/z* 263, which is a very minor species and assigned to a seven-membered cyclization product, formed by the loss of $HOCH_3$ between the amine group and the methyl ester group.

Structures of the dehydrated aspartame ions

As described above, under the CID conditions, protonated α- and β-aspartame undergo dehydration to form anhydro derivatives in addition to carbon–carbon bond cleavages. The anhydro derivatives are quasi-molecular ions $[M + H]^+$ at *m/z* 277, which differ from the aspartame ions by 18 u, consistent with dehydration of the aspartame ions. The proposed structure of the anhydro derivative from the α-aspartame ion is **5** and that from the β-aspartame ion is **7**. They are formed by the nucleophilic attack of the oxygen of the amide group on the carbon of the carboxyl group in aspartames and subsequent loss of a water molecule. The anhydro derivative **5** was characterized by FABMS and photoacoustic infrared spectrometry in the study of degradation products of aspartame in an acidified–lyophilized state.[11] It was only formed under acidic conditions. The instability of aspartame under acidic conditions is attributed to protonation of the carboxyl group since OH^- is a much better leaving group that O^-. The protonated aspartame ion in the gas phase is similar to its acidified–lyophilized state, and therefore when it gains energy from collision in the gas phase it can undergo the same dehydration to form **5**. According to the *m/z* 277 ion, there is another possible structure of the dehydration product, the pyrrolidinedione structure **8**, which is formed by cyclization with loss of water between the phenylalanine amino group and the terminal carboxyl group. The structure **8** was suggested as an intermediate between the conversion of α- and β-aspartame in a recent MS determination of aspartame decomposition products in aqueous solutions.[16] In the present study, if **8** was the structure of the ion at *m/z* 277, both α- and β-aspartame would form the same ion of **8**. This cannot explain the large difference in relative abundances of the ions formed consecutively from the ions at *m/z* 277 for the protonated α- and β-aspartame ions under the same experimental conditions. Hence **8** is excluded. Further argument to support this is that since the oxygen of the amide group in aspartame is more electronegative than that of the nitrogen of the amide group, in the gas phase where there is no solvent influence, it is more likely that the oxygen attacks the carboxyl group followed by dehydration to form **5** (or **7**) than the nitrogen attacks the carboxyl group to form **8**.[17]

CONCLUSION

APCI/MS/MS study of protonated α- and β-aspartame shows that under CID conditions they undergo dehydration ($-H_2O$) to form anhydro derivatives in addition to carbon–carbon bond cleavages. They can be distinguished by the fragment ions formed from these dissociation pathways. Specifically, ions at *m/z* 175 and 88 are unique to protonated α-aspartame while ions at *m/z* 249, 189 and 74 are unique to protonated β-aspartame.

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REFERENCES

1. R. E. Baldwin and B. M. Korschgen, *J. Food Sci.* **44**, 938 (1979).
2. M. R. Cloninger and R. E. Baldwin, *Science* **170**, 81 (1970).
3. M. R. Cloninger and R. E. Baldwin, *J. Food Sci.* **39**, 347 (1974).
4. B. E. Homler, *Food Technol.* **38**, 50 (1984).
5. *The Nutrasweet Breakthrough*. Technical Bulletin, G. D. Searle & Co., Skokie, IL (1982).
6. B. Homler, in *Low-Calorie Products, Conference, 25–26 March 1987*, p. 113. Elsevier Applied Science, London (1988).
7. I. Furda, P. D. Malizia, M. G. Kolor and P. J. Vernieri, *J. Agric. Food Chem.* **23**, 340 (1975).
8. M. F. Boehm and J. L. Bada, *Proc. Natl. Acad. Sci. USA* **81**, 5263 (1984).
9. W. Tsang, M. A. Clarke and F. W. Parrish, *J. Agric. Food. Chem.* **33**, 734 (1985).
10. J. Prodolliet and M. Bruelhard, *J. Assoc. Off. Anal. Chem.* **76**, 275 (1993).
11. D. J. Graves and S. Luo, *J. Agric. Food Chem.* **35**, 439 (1987).
12. J. R. Chapman, *Practical Organic Mass Spectrometry*. Wiley, New York (1993).
13. G. C. Galletti, P. Bocchini, A. M. Gioacchini and A. Roda, *Rapid Commun. Mass Spectrom.* **10**, 1153 (1996).
14. J. S. Splitter and F. Turecek (Eds), *Application of Mass Spectrometry to Organic Stereochemistry*. VCH, New York (1994).
15. R. S. Johnson, S. A. Martin and K. Biemann, *Int. J. Mass Spectrom. Ion Processes* **86**, 137 (1988).
16. J. A. Stamp and T. P. Labuza, *Food Addit. Contam.* **6**, 397 (1989).
17. J. March, *Advanced Organic Chemistry*, 3rd edn, p. 325. Wiley, New York (1985).