# Mutagenic Heterocyclic Nitrogen Compounds Related to Protein Pyrolysates. V. Electron Impact Fragmentation of L-Glutamic Acid and Related Dipyrido[1,2-*a*:3′,2′-*d*]imidazoles



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Six dipyrido[1,2-*a*:3′,2′-*d*]imidazole derivatives related to glutamic acid pyrolysates have been studied by mass spectrometry. The data indicate that there are certain ions which are characteristic of the fragmentation of this family of compounds under electron impact. These compounds should thus be amenable to analysis if they were produced during the combustion of foods. In addition, electronolysis of L-glutamic acid was also studied: the fragmentation pattern of the latter has shown the formation of some known dipyridoimidazoles for temperatures higher than 100°. Similarities between pyrolysis and electronolysis of this acid are discussed.

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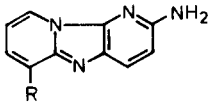
In recent years, it has been found from epidemiological and experimental data that some new chemical agents could play an important role, in our environment, on the incidence of cancer. In particular, it has been shown recently that pyrolysis products from charred parts of cooked foods (meat, fish, bakery, cereals, *etc.*) or certain proteins and amino acids were highly mutagens [1-7].

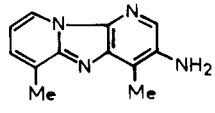
Two heterocyclic amines, 2-amino-6-methyldipyrido-[1,2-*a*:3′,2′-*d*]imidazole (**1**) and 2-aminodipyrido[1,2-*a*:-3′2′-*d*]imidazole (**2**) were found in glutamic acid pyrosylates [4]. These amines, respectively designated as Glu-P-1 and Glu-P-2, also formed in the combustion of sardine, dried squid and of casein [8,9] and are carcinogenic in both mice and rats [10,11].

Pyrolysis products of glutamic acid appear as very important in respect to the environmental carcinogenesis since this amino acid is universally present in food.

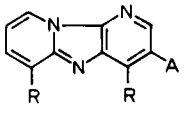
Recently, we have reported the chemical synthesis of various derivatives of the dipyrido[1,2-*a*:3′,2′-*d*]imidazole ring [12], their mutagenicity towards S. *Typhimurium* [13] and their biochemical effects on rat liver microsomes [14].



1 R = $CH_3$ : Glu-P-1
2 R = H : Glu-P-2



3 Glu-P-3



4 R = A = H
5 R = Me, A = H
6 R = Me, A = NH−$COCH_3$
7 R = Me, A = $NO_2$

Chart 1

Moreover, structural modifications of DNA by covalent binding of Glu-P-1 and Glu-P-3 (3-amino-4,6-dimethyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (**3**) were also demonstrated [15-17].

In order to look the possibility of selective detection and identification of these chemicals by mass spectrometry, we studied the behaviour under impact electronic in the mass spectrograph of six dipyrido[1,2-*a*:3′,2′-*d*]imidazole derivatives showed in the Chart 1.

We also investigated the behaviour of L-glutamic acid under electron impact at different temperatures in the range from 60 to 350°.

Table I

Significant Peaks Observed in the Mass Spectra of Dipyridoimidazole **4** [a]

| m/e | Relative Intensity % | Nature of the Ion |
|---|---|---|
| 170 | 18.9 | $M^+ + 1$ |
| 169 | 100 | $M^+$ |
| 168 | 8.7 | $M^+ - 1$ |
| 143 | 8.3 | |
| 142 | 17.8 | $[M - HCN]^+$ |
| 115 | 10.2 | $[M - 2HCN]^+$ |
| 104 | 5.5 | |
| 103 | 7.2 | |
| 79 | 8.8 | $[Py]^+$ [b] |
| 78 | 73.3 | $[Py]^+$ [b] |
| 77 | 5.9 | |
| 76 | 10.9 | |
| 64 | 10.3 | |
| 52 | 26.2 | |
| 51 | 59.6 | $[Py - HCN]^+$ [b] |
| 50 | 23.1 | |

[a] Peaks with relative intensities >5% have only been reported because those with lower values are not directly relevant to the fragmentation process. [b] Py = pyridine.

Mass Spectral Analysis.

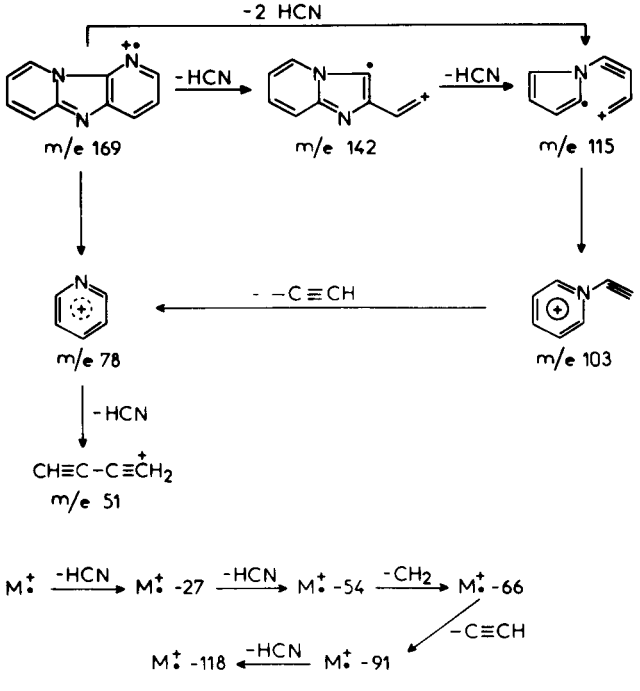
1.Dipyrido[1,2-*a*:3',2'-*d*]imidazole (**4**).

Mass spectral data of this compound and the more plausible nature of the main ions formed are summarized in Table 1.

The fragmentation pattern of the heterocycle [4] is relatively simple. It shows a behaviour similar to that other nitrogen containing compounds [18,23,24]. The molecular ion (which is also the base peak) extrudes hydrogen cyanide to give the fragment $C_9H_6N_2$, m/e 142, which in turn, loses a second molecule HCN to give the ion at m/e 115. The two other main peaks are the peak at m/e 78 which likely corresponds to the ion pyridinium ($Py^+$) and that at m/e 51 ($C_4H_3^+$) which is formed from the former by loss of HCN.

The presence of three nitrogen atoms in the heterocycle makes difficult the determination of the fragmentation pattern, however, on the basis of some data of the literature [18,20,23,24] the Scheme 1 seems the most plausible.

Scheme 1




2. 4,6-Dimethyldipyrido[1,2-*a*:3',2'-*d*]imidazole (**5**).

The fragmentation pattern of this compound is quite similar to that of the heterocycle parent **4** though a weaker intensity of the different ions formed from the molecular ion (the base peak) suggests a greater stability of **5** under electron impact. As it is shown in Table 2, one observes the loss of HCN from ($M^+ - 1$) but the loss of 2 HCN from the molecular ion is less important. The major fragmentation pattern consists in the formation of methylpyridinium ion (m/e 92, I% 17.5) and the ion at m/e 65 (I% 20.6) by loss of HCN from $MePy^+$. As in the fragmentation pattern of **4**, it can be noticed the presence of two peaks respectively at m/e 78 and m/e 51 corresponding to the pyridinium ion ($Py^+$) and the ion ($Py^+ - HCN$).

Table 2

Mass Spectral Data of Compound **5**

| m/e | Relative Intensity % | Nature of the Ions |
|---|---|---|
| 198 | 12.7 | $M^+ + 1$ |
| 197 | 100 | $M^+$ |
| 196 | 41.6 | $M^+ - 1$ |
| 182 | 6.3 | $[M - 15]^+$ (loss of Me ?) |
| 169 | 5.2 | [M - HCNH] |
| 142 | 1.2 | $[M - 2HCN]^+$ |
| 98 | 3.1 | $M^{2+}$ |
| 93 | 5.7 | $[MePy]^+$ [a] |
| 92 | 17.5 | $[MePy]^+$ [a] |
| 78 | 6.7 | $[Py]^+$ |
| 65 | 20.6 | $[MePy^+ - HCN]$ [a] |
| 51 | 8.6 | $[Py^+ - HCN]$ |

[a] MePy = methylpyridine.

3. 2-Aminopyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-2).

The fragmentation pattern of Glu-P-2 presents a certain degree of complexicity due to the presence of the amino function and involves some rearrangements. Nevertheless, analysis of spectral data (Table 3) shows the main fragments corresponding to the mode of fragmentation of the heterocyclic ring examined above. As it is shown in Table 3, it can be noted i) The loss of one and two HCN from the molecuar ion to give the ions with m/e 157 (I% 10.7) and

Table 3

Mass Spectral Data of Glu-P-2

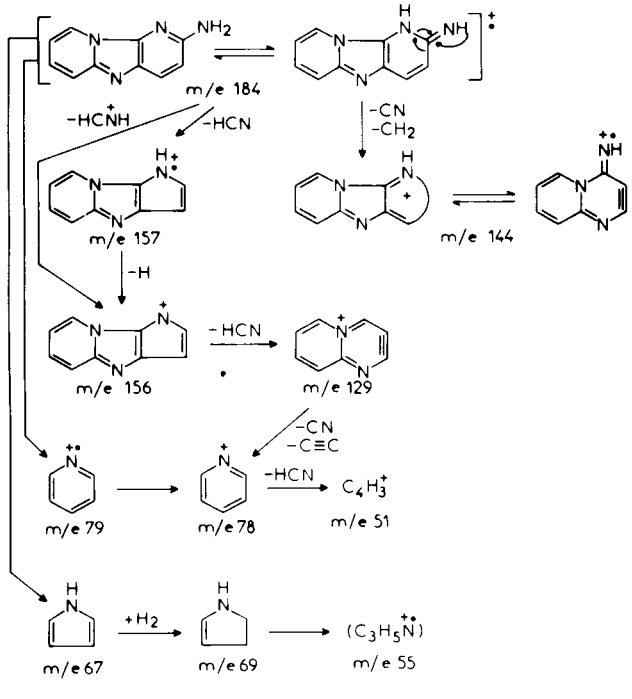
| m/e | Relative Intensity % | Nature of the Ions |
|---|---|---|
| 185 | 15.0 | $M^+ + 1$ |
| 184 | 86.7 | $M^+$ |
| 157 | 10.7 | $[M - HCN]^+$ |
| 156 | 8.4 | [M - HCNH] |
| 144 | 11.8 | $M^+ - [CN + CH_2]$ |
| 129 | 12.4 | $[M - 2HCN]^+ - 1$ |
| 105 | 12.8 | $[M - 79]^+$ loss of Py ? |
| 101 | 10.1 | 129 - HCN ? |
| 97 | 72.9 | $C_4H_7N_3^+$ |
| 95 | 50.0 | |
| 93 | 23.7 | $M^2 + 1$ |
| 84 | 50.9 | |
| 83 | 73.2 | $C_4H_7N_2^+$ |
| 79 | 91 | $[Py]^+$ |
| 78 | 39.4 | $[Py]^+$ |
| 69 | 90.5 | $C_4H_7\dot{N}$ (dihydroindole) |
| 68 | 25 | |
| 67 | 41 | |
| 55 | 100 | $C_3H_5\dot{N}$ |
| 54 | 19.2 | |
| 51 | 12.8 | $[C_4H_3]^+$ |

m/e 129 (I% 12.4). ii)The formation of the ions pyridine (m/e 79, I% 91), pyridinium (m/e 78, I% 39.4) and $(C_4H_3)^+$ (m/e 51, I% 12.8). The great abundance of the ion pyridine may be due to the McLafferty rearrangement [21] consisting of a transfer of an hydrogen probably from the amino group.

Moreover, other fragments having relatively high intensities, due to the presence of amino group, are involved in the fragmentation. Thus, the base peak with m/e 55 corresponding to the formula $(C_3H_5N)^+$ can be formed from the molecular ion (Scheme 2): this latter undergoes some fragmentations with rearrangements which lead to the fragment m/e 55 *via* the ion dihydroindole (m/e 69, I% 90.5). Two other fragments are of interest: peak at m/e 97 (I% ≈ 73) corresponding to the formula $C_4H_7N_3$ which extrudes probably a nitrogen atom to give the second peak with m/e 83 (I% 73). Formation of these fragments is more complex and results from various reactions of rearrangement of some intermediate species in the fragmentation pathways.

Scheme 2 shows the probable structure of the main fragments and the modes of formation the most plausible.

Scheme 2




It can be noticed that we did not observe the loss of $NH_2$ or NH from the molecular ion.

4. 3-Amino-4,6-dimethyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-3).

Glu-P-3 presents two minor structural differences with Glu-P-2: the presence of two additional methyl groups in the positions 4 and 6 of the ring and the position of the amino group which is on position 3 in Glu-P-3 while it is on position 2 in Glu-P-2.

The fragmentation pattern of Glu-P-3 presents similar fragments to that encountered in the mass spectrum of Glu-P-2. Thus, as it is shown in table 4, it can be noted the presence of the ions corresponding to $(M - HCN)^+$, $(M - 2HCN)^+$, $(MePy)^+$, $(MePy)^+$, $(MePy - HCN)^+$, $(Py)^+$, $(Py)^+$, $(C_4H_3)^+$ and also the peaks with m/e 97 and m/e 83.

It is interesting to note the similarity of behaviour of Glu-P-2 and Glu-P-3 under electron impact. In this respect, it seems that amino derivatives of dipyrido[1,2-*a*:3',2'-*d*]imidazole undergo a specific mode of electronolytic decomposition independently of the position of the amino group on the ring. The presence and the position of methyl substituents do not seem to influence the electronolysis of these compounds.

5. *N*-3-Acetylamino-4,6-dimethyldipyrido[1,2-*a*:3',2'-*d*]imidazole (**6**).

The fragmentation pattern of this acetamide shows two characteristic facts: i) the presence in the spectrum of a peak at m/e 295 (I% 6.6) corresponding to a diacetylated ion $[Ar\text{-}N(COCH_3)_2]^+$ whose formation results probably from a rearrangement from the molecular ion; ii) the formation of Glu-P-3 (m/e 212, base peak) from the ion parent by loss of $CH_2=CO$.

Table 4

Mass Spectral Data of Glu-P-3

| m/e | Relative Intensity % | Nature of the Ions |
|---|---|---|
| 213 | 18.5 | $M^+ + 1$ |
| 212 | 100 | $M^+$ |
| 211 | 20.3 | $M^+ - 1$ |
| 197 | 13 | $[M - Me]^+$ |
| 185 | 5.3 | $[M - HCN]^+$ |
| 184 | 9.2 | $M^+ - HCNH$ |
| 172 | 17.9 | $M^+ - (CN + CH_2)$ |
| 157 | 4.4 | $[M - 2HCN]^+ - 1$ |
| 149 | 24.2 | |
| 106 | 10.2 | $M^{2+}$ |
| 97 | 17.0 | $C_4H_7N_3$ |
| 93 | 29.3 | $[MePy]^+$ |
| 92 | 41.9 | $MePy^+$ |
| 83 | 22.8 | $C_4H_7N_2^+$ |
| 79 | 9.7 | $Py^+$ |
| 78 | 13.3 | $Py^+$ |
| 69 | 66.9 | $C_4H_7N$ |
| 67 | 25.7 | |
| 65 | 34.45 | $[MePy - HCN]^+$ |
| 55 | 55.6 | $C_3H_5N$ |
| 51 | 10.7 | $[C_4H_3]^+$ |

From the latter ion with m/e 212, the fragmentation pattern is similar to that Glu-P-3 (data not shown).

6. 4,6-Dimethyl-3-nitrodipyrido[1,2-*a*:3',2'-*d*]imidazole (**7**).

This compound, whose spectra are analyzed in Table 5, undergoes, in a first step, a mode of decomposition char-

acteristic to the nitro aromatic compounds [22]: the molecular ion extrudes both NO and $NO_2$ to give the fragments with m/e 216 (I% 24.5) and m/e 196 (I% 52) which constitute two of the major fragments. Moreover the ion with m/e 197 $(Ar\text{-}H)^+$ undergoes the same fragmentation pattern that the compound **5**, (4,6-dimethyldipyrido[1,2-*a*:3',2'-*d*]-imidazole) analysed above in Table 2.

It seems however, worth mentioning that the presence of the nitro group diminishes the stability of the molecule under electron impact since the base peak corresponds, here, to the methylpyridinium ion (m/e 92).

Table 5

Mass Spectral Data of Nitro Compound **7**

| m/e | Relative Intensity % | Nature of the Ions |
|---|---|---|
| 243 | 16.1 | $M^+ + 1$ |
| 242 | 55 | $M^{+\cdot}$ |
| 226 | 5 | $[M - O]^+$ |
| 212 | 24.5 | $[M - NO]^+$ |
| 197 | 10.8 | $ArH^{+\cdot}$ |
| 196 | 52 | $[M - NO_2]^+ \rightleftharpoons Ar^+$ |
| 169 | 14.9 | $[Ar - HCN]^+$ |
| 142 | 4.5 | $[ArH - 2HCN]^+$ |
| 93 | 9.0 | $MePy^{+\cdot}$ |
| 92 | 100 | $MePy^+$ |
| 65 | 81.5 | $C_6H_5^+$ |
| 51 | 16.5 | $C_4H_3^+$ |

Based on above analysis of the mass spectral data of the six dipyridoimidazole derivatives, it should be to point out the characteristic features: 1) The formation, of two ions, from the molecular ion, corresponding to the loss of one and two molecules of HCN; 2) A common fragmentation pattern with the formation of two main ions with m/e 78 and m/e 51 (and m/e 92 and m/e 65 for methylated derivatives). 3) The formation, in the case of amino derivatives, of four other ions respectively at m/e 97, m/e 83, m/e 69 and m/e 55.

7. L-Glutamic Acid.

The pattern of electron impact fragmentation of amino acids has been investigated mostly in their volatile derivatives (as *e.g.* alkylesters, alkylsilyl derivatives *etc.*) [25]. Free amino acids, because of their zwitterion character, have a very low pressure and frequently decompose if heated to the temperatures required for vaporisation [26]. Nevertheless, it has appeared interesting to us to study the fragmentation pattern of free glutamic acid under electron impact in order to attempt a comparison between the behaviour of this amino acid in pyrolytic and electronolytic conditions. The possibility for glutamic acid to undergo various decomposition in the inlet system of mass spectrometer can eventually lead to some species of ions identifiable with the products of glutamic acid pyrolysates or the characteristic fragments of their mass spectra. If such is the case, the analysis of mass spectral data will bring some informations on the modes of formation of Glu-P-1 and its analogs.

For this purpose, we have investigated the fragmentation of L-glutamic acid in a gradient of temperatures from 60 to 350° and that of L-glutamic acid pyrolysate.

At a temperature lower than 100° (sufficiently weak to avoid all phenomenon of thermolysis) the fragmentation pattern of L-glutamic acid (Table 6) is relatively simple. The essential features of the spectra consist in the loss, from the molecular ion which corresponds to the base peak, of CO, (CO + CN) and (2CO + CN) to lead the fragments with m/e 119 (I% 51.3), m/e 93 (I% 53.3) and m/e 65 (I% 44.3). Formation of these ions is the result of rearrangements consisting of hydrogen and $OH^-$ transfert.

Table 6

Mass Spectral Data at 83°C of L-Glutamic Acid.

| m/e | Relative Intensity % | Nature of the Ions |
|---|---|---|
| 148 | 23.2 | $M^+ + 1$ |
| 147 | 100 | $M^{+\cdot}$ |
| 146 | 35.6 | $M^+ - 1$ |
| 120 | 6.4 | |
| 119 | 51.3 | $[M - CO^+]$ |
| 118 | 3.4 | |
| 105 | 3.4 | $[M - CO_2]^{+\cdot}$ |
| 93 | 53.3 | $[M - [CN + CO)]^+$ |
| 92 | 37.8 | $[M - [HCN + CO)]^+$ |
| 78 | 5.9 | |
| 66 | 19.3 | |
| 65 | 44.3 | $[M - (CN + 2C0)]^+$ |
| 64 | 16.5 | |
| 51 | 12.7 | |

Increasing the temperature in the inlet system of the instrument provokes considerable modifications in the fragmentation process which occurs with a greater degree of complexity. As it is shown in Table 7, mass spectral data of L-glutamic acid for temperatures higher than 100° (even for temperatures lower than the temperature of decomposition of glutamic acid , *i.e.* 249°) present striking differences with data listed in Table 6 and indicate the formation of multiple ions. Some of these ions present a higher molecular weight than that of the parent compound (m/e 147 whose the relative intensity of the corresponding peak is weak in all cases).

However, it can be observed, in general, in all spectra, the presence of the same fragments as the temperature varies from 100 to 350°, the only difference is the relative abundance of the individual fragments. Table 7 shows the mass spectral data of L-glutamic acid obtained at 133° (A), 195° (B), 219° (C), 317° (D), 322° (E) and 343° (F). In addition, we have also added the mass spectral data of the pyrolysate product of L-glutamic acid.

Table 7

Mass Spectral Data of L-Glutamic Acid at Different Temperatures Higher than 100° [a]

| m/e | Relative Intensities A (133°) | B (195°) | C (219°) | D (377°) | E (322°) | F (343°) | Pyrolyzate |
|---|---|---|---|---|---|---|---|
| 213 | 2.3 | 3.0 | 4.7 | 3.9 | 4.9 | 2.4 | 0.3 |
| 199 | 1.5 | 3.0 | 4.1 | 3.7 | 2.1 | 2.4 | 0.5 |
| 185 | 3.1 | 4.3 | 5.3 | 5.4 | 3.1 | 4.2 | 0.5 |
| 171 | 2.9 | 4.5 | 5.0 | 5.1 | 5.5 | 5.5 | 0.3 |
| 149 | 11.3 | 4.0 | 5.2 | 5.3 | 5.3 | 4.6 | 0.2 |
| 147 | 3.0 | 4.0 | 4.2 | 6.7 | 6.7 | 4.2 | 0.0 |
| 137 | 6.0 | 5.2 | 6.1 | 5.3 | 1.2 | 4.4 | 0.2 |
| 129 | 7.1 | 12.3 | 19.0 | 11.8 | 11.0 | 14.6 | 3.9 |
| 111 | 13.0 | 23.0 | 21.5 | 21.1 | 10.5 | 19.5 | 4.9 |
| 97 | 53.2 | 62.3 | 44.3 | 56.6 | 16.1 | 32.1 | 1.4 |
| 84 | 32.4 | 36.1 | 100.0 | 52.8 | 30.5 | 33.5 | 100.0 |
| 83 | 63.1 | 65.2 | 49.4 | 93.7 | 93.2 | 88.3 | 12.6 |
| 69 | 100.0 | 74.7 | 53.3 | 76.8 | 5.0 | 46.5 | 4.4 |
| 55 | 68.2 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 15.6 |
| 51 | 1.9 | 6.8 | 9.8 | 8.8 | 17.7 | 16.1 | 0.8 |

[a] A, B, C, D, E, F are the different spectra analysed. The number in parenthesis indicates the corresponding temperature.

The mechanisms involved in the formation of these ions are complex, but probably reflect the occurence of reactions similar to those which occur in the thermolysis of L-glutamic acid.

We briefly summarize the important points.

First, one does not observe in the spectral data listed in Table 7 any characteristic peak of the fragmentation pattern of L-glutamic acid recorded at weak temperature (83°).

Secondly, whereas the base peak in the latter case corresponds to the molecular ion (m/e 147), in the case of spectra recorded at higher temperature the base peak corresponds, in general way, to the fragment with m/e 55. Moreover, the main peaks observed in all spectra correspond to the fragments with m/e 97, m/e 83, m/e 69, m/e 55. These fragments, absent in the mass spectrum of L-glutamic acid at 83° are, on the contrary, present in the mass spectrum of Glu-P-2 and Glu-P-3.

Thirdly, it is worth to note in all spectra recorded at temperatures higher than 100°, the presence of peaks with m/e 213, m/e 199, m/e 185 and m/e 171. Although their relative intensities are relatively weak (2-5%), the presence of these peaks is of great interest. Effectively, these peaks probably correspond to the molecular ions of Glu-P-3 (m/e 213, $M^+ + 1$), Glu-P-1 (m/e 199, $M^+ + 1$), Glu-P-2 (m/e 185, $M^+ + 1$) and of the non-substituted ring (m/e 171, $M^+ + 1$). It is not a simple coincidence since the presence, in spectral data (Table 7), of the ions with m/e 97, m/e 83, m/e 69, m/e 55 and m/e 51 is relevent with the possible formation of such molecular species during the electronolysis of glutamic acid in the described conditions. These latter species, highly unstables under electron impact, undergo the fragmentation of Glu-P-1, Glu-P-2, Glu-P-3 and the dipyridoimidazole ring to give the characteristic ions as indicated above.

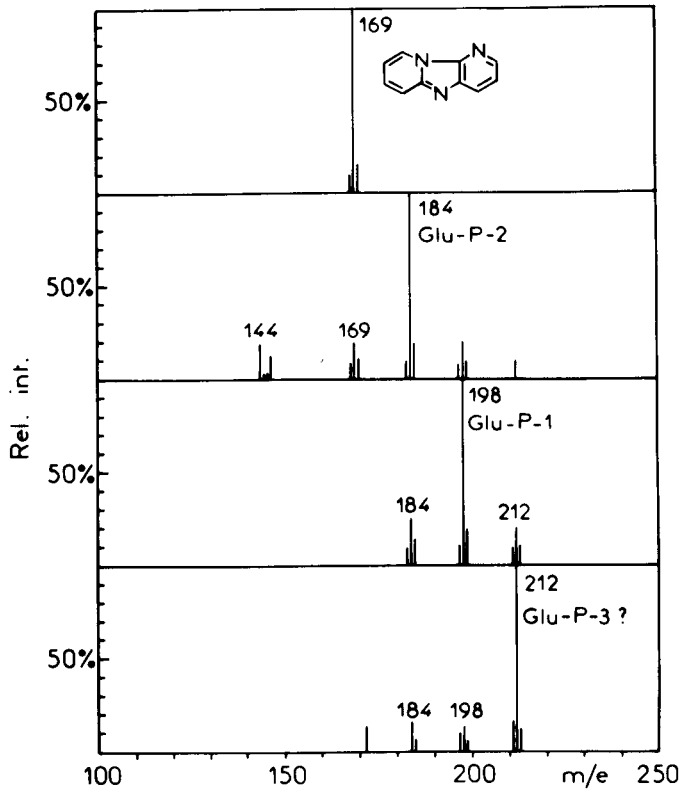



Figure 1

The occurence of this process has been substantiated by a comparison of spectral data of the pyrolysate products of glutamic acid (last right column of Table 7) with that of A to F. Both spectra are practically identical except that in

the case of the spectrum of the pyrolysate, the relative abundance of the ions are weaker. However, it should be noted that the base peak, in this spectrum, is the one with m/e 84. The corresponding ion is also present in all spectra A to F as a prominent peak. In addition, the formation of the heterocyclic fragments was confirmed by accurate analysis of the glutamic acid pyrolysate using a combination of gas chromatography and mass spectrometry (gc-ms). From the gc effluents the main fragment ions pointed out above were investigated and monitored sequentially in the mass spectrometer as it is shown in Fig. 1.

In conclusion, the results obtained in this study have shown that the fragmentation pathways of dipyrido[1,2-*a*:3',2'-*d*]imidazole derivatives lead to the formation of characteristic ions and suggest that the mass spectrometry can be used for the identification of such compounds. Moreover, analysis of mass spectra of L-glumatic acid monitored in the range of temperatures between 60 and 350° has shown evidence of the formation of ions revealing the transitory existence of dipyridoimidazole ring, Glu-P-1, Glu-P-2 and Glu-P-3 (or an isomer). The formation of these products proceed from some complex phenomenons such as molecular rearrangements subsequently to the decomposition of L-glutamic acid.

Similar rearrangements have been described in the literature [27-32]. These rearrangements are visualized as preceeding *via* a multicenter transition state similar to that in the Mc Lafferty rearrangement [21]. An interesting example is the formation of cyclohexanone azine ($M^+ = 220$) from the mass fragmentation of cyclohexanone semicarbazone ($M^+ = 155$); moreover considerable interest has been attached in the electron-impact-induced migration of some alkyl, aryl and other functional groups and rearrangments in mass spectrometry [30-32].

These results suggest that the behaviour of L-glutamic acid in the mass spectrograph for temperatures higher 100° (even lower than the decomposition point of this amino acid *ca* 247-249°) is similar to its behaviour in the thermolys reaction by heating at 400°. Glu-P-1 and Glu-P-2 had been already isolated from the pyrolysis products of L-glutamic acid by chromatography process. Our study in mass spectrometry demonstrates, in addition, the likely formation of the unsubstituted ring dipyrido[1,2-*a*:3',2'-*d*]-imidazole and an other amine which could be Glu-P-3 a compound whose strongly genotoxic properties have been already demonstrated [16].

Finally, as a result of the present study, the application of mass spectrometry for detection and identification of dipyrido - imidazoles in certain mixtures as charred parts of food appears as a convenient and valid method.

## EXPERIMENTAL

Mass spectra were recorded with a Ribermag MS R 10-10 a quadripole apparatus equipped with a single ion source working under electron impact ionization by differential vacuum pumping and with dual disc data acquisition and processing SIDAR computer.

Ionizing potential and ionizing current were 70 eV and 210 μA, respectively. Samples were analyzed by direct introduction through a heated inlet system at *ca* 150°.

Analyses in gc-ms was performed with the Ribermag R 10-10 combined with a Girdel Chromatograph using a glass column (2 mm × 3 m) packed with 3% OV-17 on chromosorb (60/80 mesh). The temperatures of column oven, injection port and separator were maintained at 190°, 240° and 250° respectively.

The compounds studied are already described by us in this journal [12] except for Glu-P-3 described elsewhere [16]. L-glutamic acid was purchased from Merck and was chromatographically pure. Pyrolysis was performed by heating of L-glutamic acid (2 g) at 400° for 15 minutes. Pyrolysate product was introduced directly in the mass spectrograph in its solid form or in solution in pyridine in the chromatograph.

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