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Novel Mechanisms for the Decomposition of 1-(Amino acid)-1-deoxy-D-fructoses (Amadori Compounds): A Mass Spectrometric Approach

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ABSTRACT

*A study of the mechanisms involved in unimolecular mass spectral fragmentations of 1-(amino acid)-1-deoxy-*D*-fructoses (Amadori rearrangement products, or ARPs) has led to proposals for a number of new mechanistic routes to Maillard reaction products. One of the proposed mechanisms involves decomposition by initial dehydration or dehydroxylation directly from the cyclic hemiketal forms of the ARPs rather than from the open chain carbonyl forms. Another proposed mechanism involves formation of the pyrylium ion which could be the key intermediate in the formation of polymeric material. A number of other important Maillard reaction heterocycles could also be formed via the reactive pyrylium ion. The effect of variation of the ARP amino acid on 1,2- and 2,3-type enolization products was demonstrated. Intramolecular nucleophilic reactions observed in the mass spectral fragmentations also gave clues to the mechanisms of formation of compounds such as β-carbolenes from the tryptophan ARP.*

INTRODUCTION

The importance of 1-(amino acid)-1-deoxy-D-fructoses (1 in Fig. 1) in food and biological systems is well established. In food systems, heat treatment or storage, for long periods of time, leads to the decomposition of Amadori rearrangement products (ARPs) giving rise to a multitude of heterocyclic

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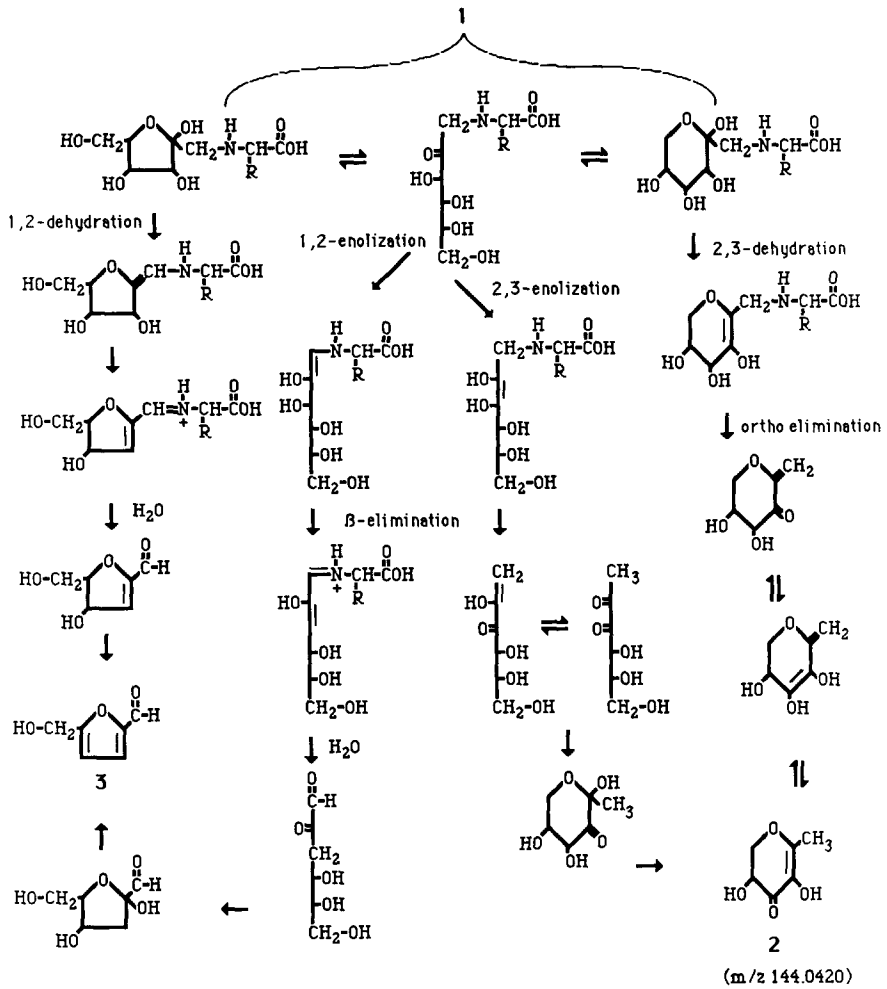

Fig. 1. Formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (2) and 5-hydroxymethyl-2-furfural (3) via the open chain and cyclic mechanisms from ARPs (1).

compounds that may play a significant role in flavour and colour production (Nursten, 1980). In biological systems, the formation of ARPs leads to non-enzymatic post translational modification of proteins, as in the case of haemoglobin A (Flückiger & Winterhalter, 1976).

From the standpoint of colour and flavour production, the importance of decomposition patterns of ARPs becomes evident, since, by controlling the relative concentrations of a given amino acid or the addition of a given ARP to a food system, the production of specific flavour compounds can be controlled. It has also been proposed (Reynolds, 1963) that the basicity of the amino acid moiety influences the pathway by which ARPs undergo decomposition. According to this hypothesis, the relative amounts of 1,2-

and 2,3-enolizations (Fig. 1) occurring during degradation depend on the basicity of the amino acid substituent and evidence suggests that degradation through 1,2-enolization is the main pathway to colour production, whereas 2,3-enolization is more important in flavour production (Reynolds, 1970).

However, our present knowledge of the mechanism of decomposition of ARPs is still fragmentary and there is no satisfactory scheme to date that can account for all the laboratory observations. The reason for this lack of a comprehensive view is the diversity of products formed in low concentrations and the quick subsequent reactions and decompositions of these compounds.

In addition there is no universal agreement about the conditions of decomposition; each researcher defines his own and, since the kind and the range of products obtained depends on the reaction conditions, the results obtained in different laboratories are not comparable. Many compounds that have been isolated so far from different model systems can be due to sugar or amino acid decompositions alone rather than the result of the Maillard reaction. Therefore, a systematic approach is essential in order to assign a definite compound to a specific precursor ARP.

TABLE 1
Relative Amounts of m/z 144·042 0 in Mass Spectra of ARPs

Amino acid	*m/z 144·042 0 peak as percentage of the total ion current of peaks greater than 50 mass* ($\sum_{50}$)
Glutamic acid methyl ester	8·06
Lysine (Difructosyl)	7·74
Proline	6·27
Lysine (*N*ε-Formyl)	5·25
Alanine	3·56
Valine	1·18
Glycine	0·90
Leucine	0·84
Threonine	0·71
Methionine	0·45
Hydroxyproline	0·28
Isoleucine	0·23
*N*α-Lysine	0
*N*ε-Lysine	0
Serine	0
Histidine	0
Phenylalanine	0
Tryptophan	0

TABLE 2
Relative Amounts of All A_3 Species for ARPs

Amino acid	*All A_3 peaks as percentage of the total ion current of peaks greater than 50 Mass ($\sum_{50}$)*
Glycine	6·39
Valine	3·58
Isoleucine	3·49
Methionine	3·30
Hydroxyproline	3·02
Leucine	2·90
Threonine	2·27
Alanine	2·26
Glutamic acid methyl ester	0·97
Tryptophan	0·71
Proline	0·64
Serine	0·25
*N*ε-Lysine	0·23
Lysine (Difructosyl)	0
*N*α-Lysine	0
Lysine (*N*ε-Formyl)	0
Histidine	0
Phenylalanine	0

Since the initial products formed from the ARPs are mainly the results of their unimolecular decompositions, we decided to study the unimolecular decompositions of different ARPs (ARPs given in Tables 1 and 2). To do this we chose to study ARPs under electron impact mass spectrometric conditions where the high vacuum conditions used ensure unimolecular reactions. Accurate mass determinations indicate the products formed and, after careful analysis of the data obtained, general patterns of decompositions emerged, upon which we based our conclusions.

EXPERIMENTAL

Instrumentation

The high resolution electron impact mass spectra of ARPs were determined on an Associated Electrical Industries (AEI, Manchester, Great Britain) MS-50, high performance double-focussing mass spectrometer with Nier-Johnson geometry. The ionization energy was 70 eV and the peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15 000.

The temperature in the ion source was varied between 150 and 250°C depending on the volatility of the particular compound. The samples were introduced directly into the ion source (quartz probe) through a vacuum lock system, the pressure inside was 2×10^{-7} torr and the accelerating voltage was 8000 V.

The data were analyzed by a DS-55 (Kratos), a computer-based data acquisition and analysis system for mass spectrometry. The system consisted of a minicomputer with a custom, high speed data acquisition interface and a set of programs for collecting, analyzing and reporting mass spectrometric data.

The structures of fragment ions were determined, according to mass spectrometric ion fragmentation mechanisms explained in detail by McLafferty (1980).

General procedure for the synthesis of Amadori products

The method used was a general procedure of synthesis based on the methods of Sgarbier *et al.* (1973) and Lee & Liau (1971).

1.0 g of the L-amino acid and 15·0 g of D-glucose were refluxed for 3 h in 500 ml of anhydrous methanol in a 1-litre round bottom flask. Unreacted D-glucose (if any) was filtered and the filtrate was evaporated *in vacuo* at 40°C. The residue was dissolved in a minimum amount of water and directly applied on a column (6 cm by 12 cm) of Dowex 50X4-400 ion-exchange resin (H^+ form) and eluted with water until the eluent was negative to the triphenyl tetrazolium chloride (TTC) test.

After the separation of excess sugar, the column was eluted with 5N ammonium hydroxide solution until the eluent was negative to the Elson–Morgan test (a specific test for amino sugars). The eluted ammonium hydroxide solution was evaporated under the fume hood in an evaporating dish. The residue was dissolved in 25 ml water and decolorized (room temperature) with charcoal several times until the solution was colourless. The filtrate from the last decolorization was evaporated again under the fume hood.

To further separate the Amadori product from the free amino acid contamination, the amorphous solid was dissolved in a minimum amount of water and then loaded on a cellulose column (2 cm by 20 cm). The column was packed with Whatman CF_{11} fibrous powder suspended in water-saturated *n*-butanol and eluted with the same solvent. The fractions were collected with an automatic fraction collector (1 ml/min) until the eluent was negative to the Elson–Morgan test. The purity of the fractions was tested by thin-layer chromatography (TLC, with cellulose; solvent system of *n*-butanol:water:acetic acid, 12:5:3 v/v; ninhydrin spray).

All the fractions that showed one spot on TLC were combined, treated with charcoal and the filtrate evaporated. The remaining white solid was crystallized several times from solvents recommended in the literature. Melting points and ^{1}H-NMR data were compared with those reported in the literature (Hodge & Fisher, 1963; Röper *et al.*, 1983).

Synthesis of *N*α, *N*ε-di-(deoxy-1-D-fructosyl)-L-lysine

The general procedure was followed, except that a catalytic amount of ethyl malonate was added to the refluxing mixture of lysine and D-glucose.

Synthesis of *N*ε-(deoxy-1-D-fructosyl)-L-lysine, *N*α-(deoxy-1-D-fructosyl)-L-lysine and *N*ε-formyl, *N*α-(deoxy-1-D-fructosyl)-L-lysine

Same procedure as that of Finot & Mauron (1969) was followed.

Chemical tests

TTC test for carbohydrates (Horn et al., *1968)*
To one drop of sample were added four drops of TTC reagent (3% methanol solution of 2,3,5-triphenyl-2H-tetrazolium chloride) and three drops of 6N sodium hydroxide. The mixture was shaken and allowed to stand at room temperature. A pink to brick red colour was a positive test.

Ninhydrin test for amino acids (Horn et al., *1968)*
To one drop of sample were added four drops of ninhydrin reagent (4% methanol solution of ninhydrin) and 1 ml of propionate buffer (199·2 ml of propionic acid added slowly with stirring to a cool solution of 67·2 g sodium hydroxide in 150 ml water, then diluted to 400 ml with water). The mixture was shaken and heated to 100°C for 30 min. A positive test was a blue–violet colour.

Elson–Morgan test for ARPs (Elson & Morgan, 1933)
To 1 ml of sample was added 1 ml of acetyl acetone solution (1 ml of acetyl acetone was dissolved in 50 ml of 0·5N sodium carbonate solution; prepared fresh daily), followed by heating for 15 min in a boiling water bath. After heating, the tube was cooled and 1 ml ethanol and 1 ml of Ehrlich reagent (1% acidified methanol solution of 4-dimethylamino benzaldehyde) were added. The appearance of a red colour and evolution of carbon dioxide was a positive test.

RESULTS AND DISCUSSION

There are two main reasons why Amadori rearrangement products (ARPs) lend themselves so readily to mass spectrometric investigation. First the sugar and amino acid moieties of the ARPs have a great capability for stabilizing a positive charge through the formation of oxonium and imminium ions (Fig. 2, the symbols used in this Figure are explained below in the valine ARP example). Secondly, in most cases the amino acid moiety contains bonds that are especially prone to cleavage, thus giving rise to intense fragmentation ions. ARPs, being complex polyfunctional molecules, contain several possible sites for the localized charge. However, keeping in mind that they can be considered as either *N*-substituted amino acids or as derivatives of D-fructose, it can be assumed that both the ring oxygen and the amino acid nitrogen can trigger fragmentations characteristic of fructose and the specific amino acid. The relative importance of each fragmentation pattern is determined by the ionization energies of the ring oxygen compared to that of the amino acid nitrogen and by the complexity of the side chain of

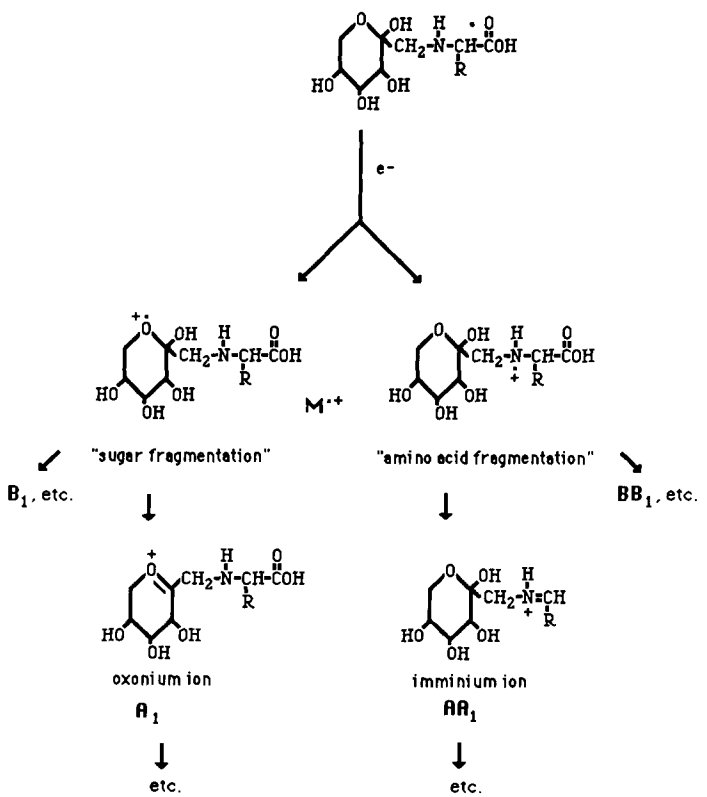

Fig. 2. Sugar and amino acid fragmentations in the mass spectra of ARPs.

the amino acid. Therefore, fragmentations initiated by the fructose moiety will be termed 'sugar fragmentation patterns' and those initiated by the amino acid moiety will be termed 'amino acid fragmentation patterns' (Fig. 2).

In the following discussion we will explain some of the mechanistic controversy that now involves the Maillard reaction and how the mass spectral data from the ARPs illustrate the preference for specific degradation pathways. The voluminous detailed fragmentation of each ARP will not be presented here (detailed fragmentations are given elsewhere, Yaylayan, 1986). An example of some of the general fragmentation patterns observed and their suspected origins will be illustrated below using a typical ARP, valine ARP, as an example. However, since the purpose of this paper is to highlight mechanistic implications of these fragmentations, only pertinent data will be presented for other ARPs.

Fragmentations of valine ARP under electron impact

The symbolism used for the individual ARP fragmentations involved initially using the nomenclature of Chizhov & Kotchetkov (1966) for carbohydrate molecule fragmentations. Thus an ion generation related by a common origin in the sugar moiety is denoted by capital letters (A_i, B_i, C_i, etc.). To denote ions related by a common origin in the amino acid moiety, we decided to use double capital letters (AA_i, BB_i, CC_i, etc.; see Fig. 3 for parent ions for valine ARP). The numbers after the DD_1 symbol are the

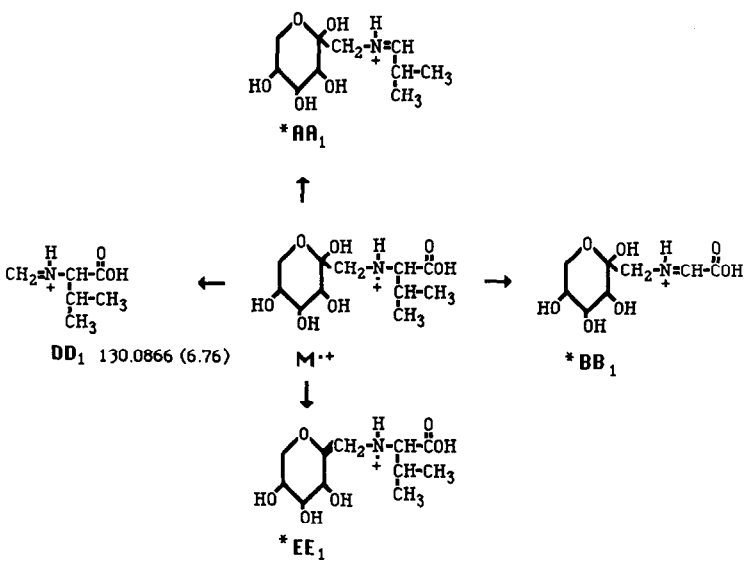

Fig. 3. Initial amino acid mass spectral fragmentations for valine ARP.

TABLE 3
Ions Observed in the Mass Spectrum of Valine ARP

Ion symbol	*m/z* (% *Relative intensity*)
A_1	262·127 2 (0·96)
A_2	244·117 5 (1·88)
$AA_1{}^{l}$	216·123 1 (3·75)
$AA_1{}^{m}$	198·111 6 (1·21)
E_2g	186·112 7 (4·31)
A_3g	182·117 5 (1·00)
$AA_2{}^{l}$	174·076 6 (11·04)
$E_3{}^{g}$	168·102 4 (1·56)
$AA_2{}^{m}$	156·065 7 (6·77)
$A_2{}^{d}$	144·065 5 (1·71)
—	144·042 0 (10·00)
$AA_2{}^{n}$	138·055 3 (6·31)
$A_1{}^{a}$	133·050 0 (3·57)
DD_1	130·086 6 (6·76)
dDD_2	128·071 1 (27·26)
$A_3{}^{d}$	126·055 6 (16·45)
L_2 and/or $lL_2{}^{a}$	119·034 4 (13·03)
$A_2{}^{a}/E_2{}^{c}$	115·039 2 (1·91)
—	114·091 6 (1·08)
$E_3{}^{d}$	112·039 8 (2·14)
$A_3{}^{c}$	111·044 5 (5·03)
—	100·076 6 (29·27)
C_1/P_1	104·047 2 (1·18)
$bB_2{}^{c}/P_2$	103·039 5 (2·42)
$lL_3{}^{a}$	102·031 2 (2·93)
EE_4	101·059 7 (1·54)
$E_2{}^{a}/L_3$	101·023 8 (11·68)
—	98·060 5 (1·28)
$A_3{}^{a}/E_3{}^{c}$	97·028 9 (6·36)
$D_1{}^{c}$	91·039 4 (3·30)
B_2	89·023 9 (2·14)
C_2	87·044 8 (1·65)
DD_1	86·096 9 (5·60)
DD_2	85·088 7 (12·13)
DD_3	84·081 5 (35·25)
—	84·045 1 (37·06)
$EE_3{}^{i}$	82·065 7 (8·90)
—	75·032 2 (11·21)
$hH_1{}^{c}$	74·037 1 (4·77)
pP_2	74·039 6 (9·25)
$BB_2{}^{l}$	74·024 6 (28·13)
—	73·085 2 (4·42)
$F_1/hH_2{}^{a}$	73·029 4 (15·34)
—	72·081 7 (100)

(*continued*)

TABLE 3—*contd*

Ion symbol	*m/z* (% *Relative intensity*)
F_2	72·021 5 (3·09)
—	70·065 7 (20·58)
C_3	69·034 3 (3·39)
J_1[c]	61·031 0 (12·10)
H_1	60·023 4 (8·44)
H_2	59·015 7 (1·22)
EE_4[g]	57·072 6 (11·33)
bI_2	57·036 3 (9·32)
aI_2	56·028 2 (5·97)
aAA_2	55·056 6 (51·37)
I_2	55·020 3 (10·96)

accurate mass and percentage relative intensity for this ion in parentheses. Note that in the case of the valine ARP there is no CC series which would involve cleavage in the amino acid side chain carbon bonds since other more favourable fragmentations predominate.

Subscript numerals corresponded to the number of steps needed for the transformation of the parent ion to the given fragment. An asterisk before a symbol indicates that a parent ion was not observed in the mass spectrum, although subsequent daughter ions were observed. Thus for the valine ARP, although parent ions AA_1, BB_1 and EE_1 are not seen in the mass spectrum, subsequent fragmentation leads to a number of daughter ions that are observed (see Table 3).

A lower case letter on the left of the symbol of the fragment (aA, bBB, etc.) indicates an alternate route of fragmentation from the same molecular ion.

TABLE 4
Common Transformations Noted in the Mass Spectra of ARPs

Letter code	*Change observed*
a	Replacement of entire side chain at the anomeric carbon with a hydrogen
c	Same as 'a' but a methyl group remains
d	Same as 'a' but a methylene amino group remains
g	Loss of carbon dioxide
i	Loss of carbon dioxide with concomitant loss of a hydrogen molecule
l	Loss of water molecule
m	Loss of two water molecules
n	Loss of three water molecules

a, c and d are rearrangements with details on mechanism reported in Yaylayan (1986).

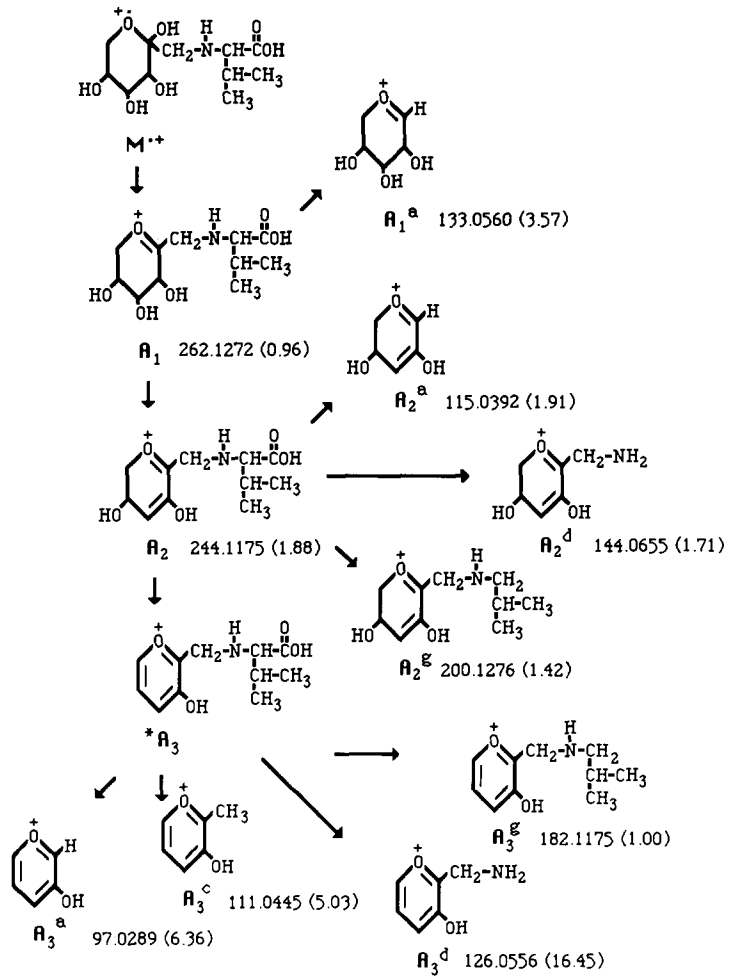

Fig. 4. The A series mass spectral fragmentations of the pyranose form of valine ARP.

Common transformations were also given lower case letters (a, c, d, g, etc., see Table 4) which were then incorporated into the symbols devised previously for identification of ion peaks, as superscripts. Thus the designation A_3^a indicates that the corresponding fragment ion originated from the sugar moiety by pathway A, with three consecutive steps from the parent molecule and that it had undergone the rearrangement a, which represents replacement of the entire side chain at the anomeric carbon with a hydrogen. All other fragment ions not originating from known carbohydrate or amino acid fragmentation routes, were designated by their *m/z* values.

Thus an example of the fragmentations of valine ARP in the important A series for the pyranoid form is given in Fig. 4. Again, the numbers

associated with each ion represent the accurate mass of the ion followed, in parentheses, by the percentage relative intensity of the ion. A similar 'A series' scheme can be shown starting with the furanoid form of valine ARP. Of course, the mass spectrum cannot distinguish between structural isomers (for example, pyranose and furanose forms with identical molecular formula). Finally, using the above notation and calculating the most likely mechanisms for all the peaks in the valine ARP mass spectrum, a chart such as the one given in Table 3 results. To all the fragments shown in Table 3, plausible structures have been assigned with appropriate mechanisms of formation. Peaks associated with symbols are products of common mechanistic pathways, found in all ARPs, whereas those without symbols are mostly the result of unique fragmentations of the particular ARP.

Basicity and 1,2- versus 2,3-enolization

There are conflicting reports in the literature regarding the influence of the amino acid moiety, in terms of enolizations, on the subsequent reaction of ARPs. According to Birch *et al.* (1984), there is no overall relationship between the basicity of the amino acid moiety and the quantities of the expected products from the pyrolysate of the ARP. On the other hand, according to Hodge & Mills (1976), basic amines will prefer 1,2-enolization and strongly basic amines will undergo 2,3-enolization. According to the former view, the primary event in the decomposition of ARPs is the scission of the carbon—nitrogen bond at C-1 of the sugar, releasing the free amine; then the resultant fructosyl moiety may degrade via 1,2- or 2,3-enol intermediates, without the influence of the amino acid moiety. However, Hodge & Mills (1976) concluded that the ARPs undergo 1,2- or 2,3-enolizations while the amino acid moiety is attached. The formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (2, Fig. 1) in these systems is evidence for the operation of the 2,3-enolization pathway, whereas the isolation of 2-furaldehydes (such as hydroxymethylfurfural, 3 in Fig. 1) and *N*-substituted pyrroles, indicates that 1,2-enolization has occurred. However, it is very difficult to determine the relative importance of the two pathways since some products can be formed by either route. One reason for this confusion is the use of pK_b values as basicity indicators while studying pyrolytic reactions, which are gas phase reactions. In aqueous solutions the basicities of amines (2° amine > 3° amine) are different from basicities in the gas phase due to solvent effects (Brauman *et al.*, 1971).

According to Anet (1964), a 2-ketose may enolize in two ways: by the loss of a proton from either C-1 or C-3. A β-elimination can then take place from C-3, in the first case, and, in the second, from either C-1 or C-4. Under alkaline conditions, the enolizations are rapid and reversible, so that the

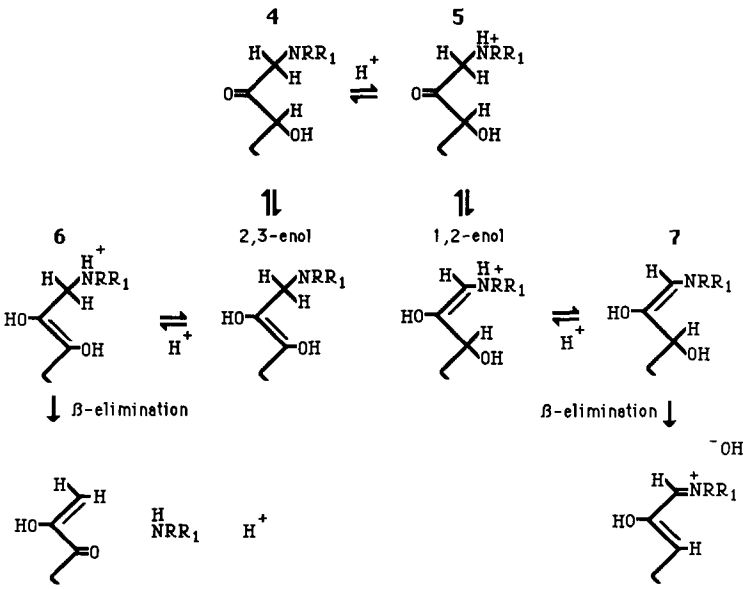

Fig. 5. 2,3- versus 1,2-enol formation in the open chain form of an ARP.

direction of the overall reaction depends on the relative ease of elimination of the functional groups at C-1, C-3 and C-4.

ARPs in acidic solution decompose by 1,2-enolization followed by the elimination of the functional group from C-3. However, under weakly alkaline conditions those compounds derived from strongly basic secondary amines, such as morpholine, proline, etc., eliminate the amine from C-1, with 2,3-enolization (Simon, 1962). Degradation products arising from 2,3-enolization with elimination from C-4 have not been observed with ARPs.

For enolization to take place, ARPs must be in one of the keto forms (4 or 5 in Fig. 5). In the free-base form, 4, the electron withdrawing effect from the nitrogen atom is less at C-1 than the electron withdrawing effect of the oxygen at C-3 and 1,2-enolization is more difficult. This effect is more important with strongly basic amines and may be negligible with weakly basic amines. Therefore, under alkaline solution conditions ARPs derived from strong bases undergo 2,3-enolization. After the 2,3-enolization, elimination is favoured through the protonated form (6 in Fig. 5) which is at a higher concentration for the more basic amino acids and favoured by an excess of groups that can donate the required hydrogen ion. Hence, the degradations involving 2,3-enolization with amine elimination are best carried out in the presence of amine salts with excess amine (Hodge & Nelson, 1961).

Under acidic solution conditions, the ARP is in the salt form (5 in Fig. 5); 1,2-enolization is assisted by the withdrawal of electrons from C-1 by the

positively charged nitrogen atom. This effect is strongest with derivatives of weak bases. Protonation of the carbonyl group would accelerate the reaction. The elimination from C-3 is assisted by the amines being in the free-base form (7 in Fig. 5). Therefore, the determining factor for the future course of the degradation of ARPs is the pH of the medium. At alkaline pHs strongly basic ARPs will undergo 2,3-enolization, whereas at highly acidic pHs ARPs with weakly basic amino acids will mostly undergo 1,2-enolization.

In order to ascertain the effect of the basicity of amino acid moieties in the degradation of Amadori compounds, irrespective of the pH of the medium, studies of the fragmentation of these products should be carried out in the gaseous phase (pyrolysis, electron impact). Since the method chosen was electron impact mass spectroscopy, a relationship should be found between gas phase basicities of amino acids and their ionization energies. According to Morishima *et al.* (1974), a relationship does exist between ionization energies, basicities and the hybridized nature of the lone pair electrons in amines. According to this study, as the per cent 's character' of the lone pair electrons of nitrogen increases, the ionization energy increases with subsequent decrease in basicity. Theoretically, therefore, those ARPs that show dominant 'amino acid fragments' should have relatively lower ionization energies for the nitrogen atom and hence will have a higher gas phase basicity and *vice versa.*

The assumption that ARPs can undergo decomposition by 1,2- or 2,3-enolizations is based on the Lobrey de Bruyn–Alberda van Ekenstein transformation (Speck, 1958). This transformation is subject to general acid-base catalysis and, although its highest rate is in alkaline solution, it also takes place under neutral and acidic conditions, but at very slow rates. The open chain form of the compound must be present for 1,2- and 2,3-enolizations to occur. However, according to ^{13}C-NMR studies, 98% (64% β-pyranose, 15% α-pyranose, 15% β-furanose, 6% α-furanose) of the Amadori compounds (regardless of the kind of amino acid) exist in cyclic forms and only 2% are in the open chain form. Therefore, it seems surprising that open chain forms alone are used to describe the decomposition of ARPs. High pH conditions are required for attaining reasonable reaction rates, via the open chain ARP forms, especially with 1,2- and 2,3-enolizations, and in food systems these pHs are seldom achieved. In addition, the same products can be envisaged to be formed by the more concentrated cyclic forms in alternate routes shown in Fig. 1. Therefore, a new reaction mechanism (Fig. 6) is suggested which is based on the fragmentations observed in the mass spectra of ARPs taking into account ^{13}C-NMR data. The general fragmentations shown were observed in the mass spectra of all the ARPs studied.

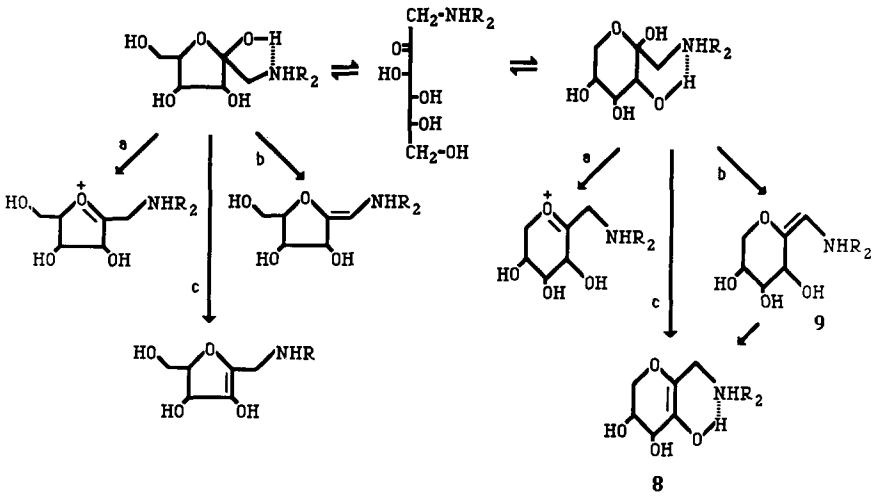

Fig. 6. Decomposition routes for ARPs via cyclic forms.

According to this mechanism, acyclic Amadori products may decompose by enolization at two positions, 1,2 and 2,3, whereas cyclic forms decompose by 1,2- or 2,3-dehydrations and O,2-dehydroxylation at the anomeric hydroxyl group. The driving force for path a (Fig. 6) is the formation of a stable oxonium ion. In the mass spectrometer this path becomes dominant when the molecular ion is formed at the ring oxygen and, since five-membered oxonium ions are more planar than six-membered ones, it is expected that the furanoid oxonium ion will be formed at a faster rate.

Paths b and c (Fig. 6) cannot be distinguished by mass spectroscopy. However, the 2,3-dehydration path is expected to be preferable over 1,2-dehydration for two reasons. First, compound 8 (in Fig. 6) is more stable than 9, since it is a product of Saytzef elimination (more substituted double bond). Secondly, ^{13}C-NMR, circular dichroism and X-ray analysis of a large number of 1-deoxy-2-ketose sugar derivatives (Mester *et al.*, 1979) indicate the presence of a hydrogen bond between the C-3 hydroxyl and the nitrogen of the amino acid (in the case of the furanoid form, the hydrogen bond is between the anomeric hydroxyl of C-2 and the nitrogen of the amino acid). Therefore, 2,3-dehydration preserves this hydrogen bond, whereas 1,2-dehydration breaks it by freezing the –NH–CH(COOH)–R_1 group away from the C-3 hydroxyl hydrogen. In the furanoid form, both 1,2- and 2,3-dehydrations are unable to preserve the hydrogen bonding; therefore, both paths may be operative in certain cases.

It follows that O,2-dehydroxylation (path a) can be initiated in the mass spectrometer by the formation of the molecular ion at the ring oxygen, and in food systems by highly polar conditions. Path a corresponds to the 'sugar fragmentation pattern'.

1,2-Dehydrations might be initiated in the mass spectrometer by the formation of the molecular ion at the amino acid nitrogen due to the electron withdrawing effect of a positively charged nitrogen atom. However, the 1,2-dehydration product would probably isomerize into the more stable 2,3-dehydration product in the pyranose case, to restore the six-membered ring hydrogen bond. Paths b and c constitute 'amino acid fragmentation patterns'.

In the mass spectrometer, amino acid nitrogens with relatively low ionization potentials (having high gas phase basicities and low solution phase basicities) and protonated amino acid nitrogen species might prefer the 'amino acid fragmentation pattern' relative to other ARPs.

'Amino acid' fragmentations

(*a*) 2,3-*dehydrations*

In Fig. 1, the 2,3-dehydration path produces compound 2. Since compound 2 appears at *m/z* 144·042 0 in the majority of the mass spectra of ARPs, we can consider per cent total ionization of *m/z* 144·042 0 as a measure of the tendency of ARPs to undergo 2,3-dehydration path (see Table 1). Note that in this Table the *m/z* peak of 144·042 0 is presented as total ion current of peaks greater than 50 mass, for better comparison between mass spectral results of the individual ARPs. There is a significant difference between relative amounts of *m/z* 144·042 0 in the first four Amadori compounds, and the rest (Table 1).

The presence of fructosyl-proline in the top four ARPs can be explained by the fact that it is a 3° amine and hence possesses a high gas phase basicity and low ionization energy at the amino acid nitrogen. As a result, the 2,3-dehydration path will dominate. In fact, the common property underlying all of the ARPs showing significant *m/z* 144·042 0 peaks, is their ability to cyclize into five- or six-membered nitrogen-containing rings, thus converting them into 3° amines (Fig. 7). Numerous peaks containing the noted cyclization are seen in the mass spectra of these compounds.

The cyclized structures contain an ammonium ion which is more electron withdrawing than the radical ion of the secondary amine, thus favouring 2,3-dehydration. Cyclization is faster than the dehydration step. ARPs that cannot cyclize and contain 2° amines (valine, alanine, glycine) produce less of the 2,3-dehydration product because the radical cation that initiates 2,3-dehydration is less electron withdrawing than the ammonium ion.

Mills (1979) subjected *N*-methylglycine ARP (3° amine) to thermal degradation and isolated compound 2 (Fig. 1) in 63% yield from the distillate, whereas glycine ARP (2° amine, Birch *et al.*, 1980) gave, as the major component in the pyrolysate, compound 10 (Fig. 7), which is formed by a 'sugar fragmentation pattern'.

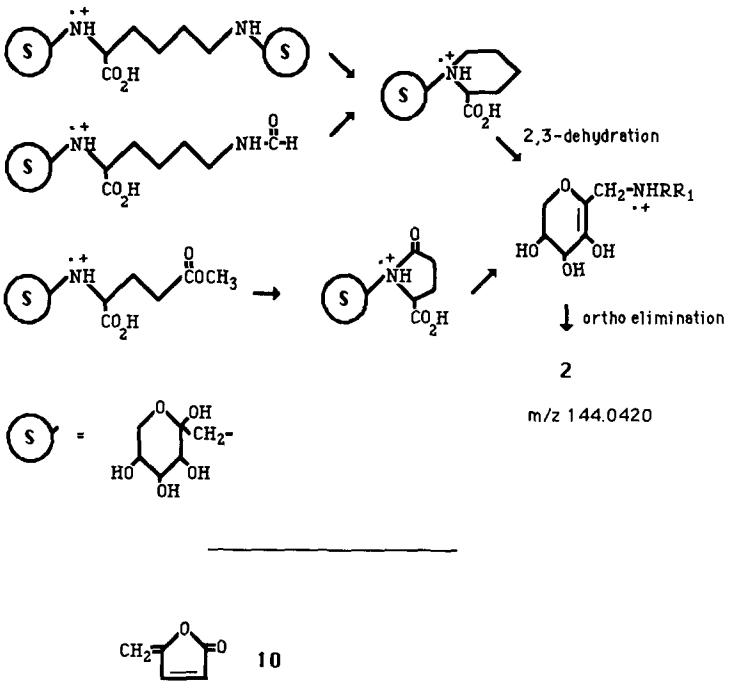

Fig. 7. Mass spectral fragmentations observed for ARPs forming the peak (2) at *m/z* 144·042 0.

(*b*) 1,2-*dehydrations*

As was stated earlier, the 2,3-dehydration path is preferable over the 1,2-dehydration path in the case of pyranoid forms, since it can preserve the hydrogen bond between the C-3 hydroxyl and the nitrogen of the amino acid. However, in the furanoid form, both 1,2- and 2,3-dehydrations are unable to preserve the hydrogen bonding; therefore, it is expected that 1,2-dehydration will be prominent in the furanoid forms whereas 2,3-dehydrations would predominate in the pyranoid forms.

According to scheme 2, the indicator compound for 1,2-dehydration is hydroxymethylfurfural (HMF, compound 3 in Fig. 1) which cannot be detected by mass spectrometry because it needs a hydrolytic (bimolecular) step for its formation; however, the precursor intermediates (of the types 11, 12, 13 in Fig. 8) can be detected in some of the ARP mass spectra. Table 5 gives some examples.

'Sugar' fragmentations

Many of the products of the decomposition of ARPs cannot be explained by the classical 1,2- and 2,3-enolization mechanism; however, the 'sugar fragmentation patterns' observed in the mass spectrometer can shed light on the formation of some of these compounds.

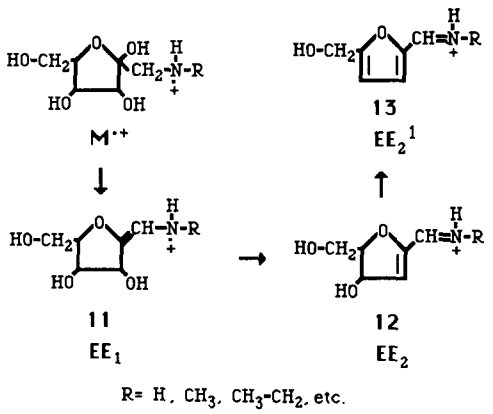

Fig. 8. Furan and pre-furan compounds found in the mass spectra of ARPs.

(a) Polymerizations

The importance of the O,2-dehydration pathway of ARPs for the formation of early Maillard polymers becomes evident if we consider the ionic nature of the products which serve as an initiator for chain polymerization or as a monomer for condensation or addition polymers.

Chain polymerizations require an initiator species with a reactive centre which can be either a free radical, cation or an anion. Polymerization occurs by the propagation of the reactive centre by the successive additions of large numbers of monomers in a chain reaction happening in a matter of a second or so.

The pyrylium ions formed from the ARPs (14 in Fig. 9) contain a nucleophilic centre, which can act as a monomer in a condensation or

TABLE 5

Relative Amounts of All Species 11, 12, 13 (Fig. 8) for ARPs

Amino acid	*Peaks as percentage of the total ion current of peaks greater than 50 mass* ($\sum_{50}$)		
	11	*12*	*13*
Glycine		0·10	0·46
Alanine		0·21	1·12
Valine	0·13	1·20	2·24
Leucine	0·27	0·60	1·49
Isoleucine	0·19	0·73	1·36
Serine			0·12
Threonine	0·06	1·38	0·60
Methionine	0·20		0·77

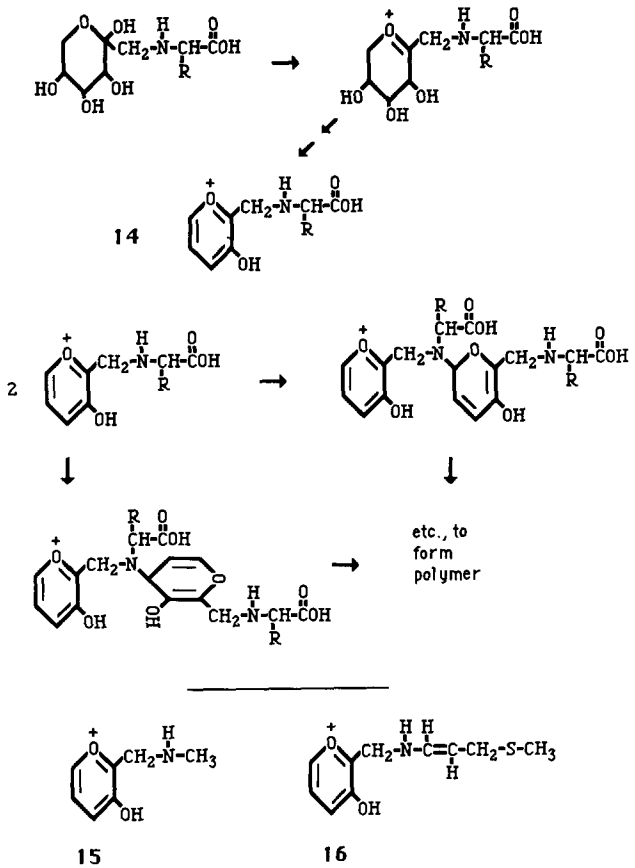

Fig. 9. Polymerization of pyrylium ions.

addition polymerization as shown in Fig. 9. In fact, pyrylium salts have been successfully used as cationic polymerization initiators (Bawn *et al.*, 1965) as well as initiators for stereospecific polymerization of 1,3-butadienes (Melega *et al.*, 1968).

Feather & Nelson (1984) isolated a water-soluble Maillard polymer (MW = 16 500) from a mixture of glucose and glycine, after refluxing for 8 h. Elemental analysis suggested that the polymer is composed of 1 mole of sugar, 1 mole of glycine, minus 3 moles of water. Studies using 90 atom % enriched D-glucose-1-^{13}C, glycine-1-^{13}C and glycine-2-^{13}C as precursors in the reaction and ^{13}C-NMR probes show that both carbon atoms of glycine are incorporated into the polymer and that the C-1 of D-glucose appears as a substituted methyl or methylene group. The NMR data further suggested that the main monomeric units are unreacted sugar and amino acid or Amadori derivatives.

Olsson *et al.* (1981) isolated the same polymer and reported that it showed

no UV absorbance at 230 or 280 nm, and that the ^{13}C-NMR spectrum of the polymer resembled that of the ARP derived from glucose and glycine. They also suggested that the polymer might well result from the dehydration and polymerization of the ARP. Similar results were obtained when glycine was replaced with methionine.

All the above data about the polymers can be incorporated into the polymerization reaction shown in Fig. 9, where the monomer (14) is the pyrylium ion.

The pyrylium ion, 14, does not appear in the mass spectrum for glycine, but the decarboxylated product does, at *m/z* 140·070 4 (15 in Fig. 9).

The same holds true in the methionine case, where the monomeric unit does not show in the mass spectrum, but its product (16 in Fig. 9) at *m/z* 212·071 3 does. Most other ARPs also exhibited pyrylium cationic species or A_3 species using our symbolic notation (Table 3). Lack of A_3 species for some of the ARPs does not mean that these ARPs are not capable of producing pyrylium ions. Since mass spectral reactions are kinetically controlled, other competing reactions occur more rapidly than production of pyrylium ions in these ARPs. For example, all lysine species fragment predominantly via cyclization (Fig. 7) and aromatic species fragment predominantly via species with conjugation to the aromatic groups.

The repeating unit in the early Maillard polymers might be attributed to the products obtained in a series of substituted pyrylium cations, known to possess pronounced electrophilic reactivity at positions 2, 4 and 6, which enables it to add nucleophiles in these positions. With basic nucleophiles (Balaban *et al.*, 1982), such as amines and cyanide, the addition occurs at positions 2 or 4 or 6 and a pyran is formed. The nucleophilic reactivity of 2- or 6-positions usually exceeds that of the 4-position.

The pyrans thus formed are susceptible to thermally allowed electrocyclic ring opening, giving rise to the conjugated products which then can recyclize

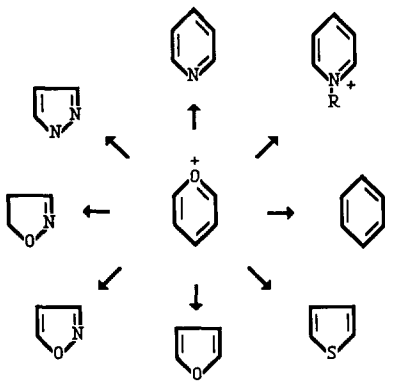

Fig. 10. Some products from further reaction of pyrylium ions.

regiospecifically, leading to new carbocyclic or heterocyclic systems, mostly aromatics with 5-, 6-, 7-membered rings such as benzenes, furans, isoxazolines, thiophenes, pyrazoles, pyridines, to name a few (Fig. 10).

If pyrylium cations are present in large quantities at the beginning of the Maillard reaction, and indirect experimental evidence suggests that they are, then the importance of these reactive intermediates becomes overwhelming in terms of production of various important heterocyclic compounds, during the Maillard reactions, and many unanswered questions about the Maillard reaction can be rationalized.

Research is under way, in this laboratory, to try to trap pyrylium cations in model systems and monitor their generation and disappearance during the course of the early Maillard reaction, to provide direct evidence for their presence.

(*b*) *Intramolecular nucleophilic reactions*

The intramolecular cyclization of the O,2-dehydroxylation products can yield fused heterocyclic compounds. This process will be illustrated by the tryptophan ARP, although a few other ARPs also show this tendency (histidine, proline). In the case of tryptophan, this process yields many derivatives of β-carboline (Fig. 11). Braütigan & Severin (1974) isolated many β-carboline derivatives from a model system consisting of tryptophan and glucose.

One mechanism observed in the mass spectrum involves intramolecular cyclization of the imminium ion (DD_1 ion illustrated in Fig. 11). In this mechanism, the driving force for the intramolecular cyclization is the electrophilic carbon atom of the imminium ion. A number of substituted β-carbolines are also seen in the mass spectrum of tryptophan ARP. These substituted β-carbolines are mostly furan derivatives.

Finally, it can be concluded that all of the mechanisms discussed above are possible to a greater or lesser extent in foods depending on the temperature conditions. Decompositions similar to the ones observed in our mass spectral study would be more likely to occur at higher temperatures (frying, baking, broiling, etc.), whereas, under milder conditions, the 1,2- and 2,3-enolizations of the open chain form might be expected to predominate.

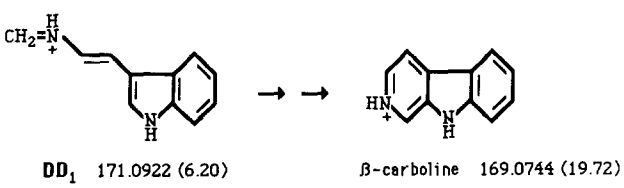

Fig. 11. β-Carboline formation.

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