Pyrolysis/GC/MS Analysis of *N*-(1-Deoxy-D-fructos-1-yl)-L-phenylalanine: Identification of Novel Pyridine and Naphthalene Derivatives

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Pyrolysis/GC/MS has been employed to analyze phenylalanine specific products formed during Maillard reaction. Phenylalanine Amadori product and different model systems containing phenylalanine and glucose, ribose, or glycerladehyde were studied. Ribbon pyrolysis was used to study the effect of temperature (150, 200, 250 °C) on the efficiency of formation of initial pyrolysis products from phenylalanine and Amadori phenylalanine. Quartz tube pyrolysis was used at 250 °C to enhance the secondary reactions. To address specific mechanistic questions, [1-^{13}C]glucose was used. These studies revealed the formation of pyridine and naphthalene derivatives such as 3,5-diphenylpyridine, 1(2)-naphthaleneamine, *N*-methyl-1(2)-aminonaphthalene, 1-aminoanthracene, 2′-phenylpyrrolo[4,5-*a*]dihydronaphthalene, 1(2)-(*N*-phenethyl)naphthaleneamine, and 1(2)-(*N*-phenethyl-*N*-methyl)naphthaleneamine. The precursors for pyridine and naphthalene derivatives were verified by GC/MS identification of the target compounds in the reaction mixtures of the postulated precursors.

Keywords: *Amadori; decomposition mechanisms; Maillard reaction; ^{13}C-labeled glucose; phenylalanine; pyridine and naphthalene derivatives*

INTRODUCTION

Pyrolysis coupled with gas chromatography/mass spectrometry (Py/GC/MS) has been demonstrated to be a fast and convenient technique for the analysis of Maillard reaction products arising from the Amadori intermediate, especially with amino acids that are stable enough under pyrolysis conditions to react with the sugar rather than decompose (Huyghues-Despointes et al., 1994). Py/GC/MS analysis reduces reaction characterization to microscale level, which enables the efficient use of isotopically enriched compounds for mechanistic studies (Huyghues-Despointes and Yaylayan, 1995). In addition, different sample introduction techniques can reveal primary and secondary pyrolysis products. For example, quartz tube pyrolysis promotes bimolecular interactions, and ribbon pyrolysis produces initial degradation products (Huyghues-Despointes et al., 1994).

Phenylalanine and its corresponding Amadori compound (ARPP) thermally degrade at temperatures above 150 °C (Kato et al., 1971; Westphal et al., 1988). Kato et al. (1971) identified, in the volatiles generated from heating of phenylalanine at 300 °C, several alkyl-substituted benzene derivatives, aromatic amines, bibenzyl, stilbene, acetaldehyde, and aromatic aldehydes. Papadopoulou and Ames (1994) extracted and identified two nonvolatile products, *N*-(2-phenethyl)-3,4-diphenylpyrrole and *N*-(2-phenethyl)-3,4-diphenyl-3-pyrroline-2,5-dione, from phenylalanine heated at 210 °C in paraffin oil. According to Baltes and Mevissen (1988), under roasting conditions phenylalanine with a 6-fold excess of glucose produces 155 volatile components including furans, pyrones, pyrazines, pyrroles, aromatic compounds, and, in addition to parent pyridine, 4-methylpyridine and 3-phenylpyridine. In a subsequent study, Kunert-Kirchhoff and Baltes (1990) identified 58 phenylalanine specific products from the reaction of an equimolar mixture of glucose and phenylalanine at 120, 150, and 180 °C, among them, 3-(2′-furyl)-2-phenyl-2-propenales, phenylhydroxy ketones, benzylpyrazines, phenethylpyrazines, and phenethylamides, in addition to 2-[5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl]-3-phenylpropionic acid lactone. Sugimara et al. (1982) found mutagenic activity in phenylalanine pyrolysate and identified a pyridine derivative, 2-amino-5-phenylpyridine, arising from phenylalanine as mutagenic. In this paper, we report the effect of temperature on the products arising from pyrolysis of phenylalanine and its Amadori product, especially naphthalene derivatives, that have not been reported in phenylalanine model systems. The increased utilization of Maillard reaction mixtures to impart roasted flavors to microwaveable, vegetarian, and extruded foods has prompted regulatory agencies to focus their attention on their chemical composition (Manley, 1994).

MATERIALS AND METHODS

All reagents and chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). The synthesis of Amadori phenylalanine was performed according to the procedure of Sosnovsky et al. (1993).

Pyrolysis/GC/MS Analysis. A Hewlett-Packard GC/mass selective detector (5890 GC/5971B MSD) interfaced to a CDS Pyroprobe 2000 unit was used for the Py/GC/MS analysis. Two modes of sample introduction were used: (a) 1 mg equivalent of sample dissolved in deionized water was applied to the platinum filament with a total heating time (THT) of 20s (the water was evaporated using a nitrogen stream) or (b) 1−2 mg of solid samples was introduced inside a quartz tube (0.3 mm thickness), plugged with quartz wool, and inserted inside the coil probe with a THT of 20 s. The GC column flow rate was 0.8 mL/min for a split ratio of 92:1 and a septum purge of 3 mL/min. The pyroprobe interface temperature was set at 150, 200, or 250 °C depending on the analysis, and the Pyroprobe

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Table 1. Products Identified during Pyrolysis of Phenylalanine Model Systems, Using Quartz Tube at 250 °C for 20 s

	MW	Phe[a]	ARPP[a]	ARPP/Phe[a]	Glu/Phe[a]	Rib/Phe[a]	Gly/Phe[a]
furans							
2,2′-methylene-bis(5-methyl)furan	176	−[b]	−	−	+[b]	−	−
2-ethylfuran	96	−	+	−	−	−	−
2-furancarboxaldehyde	96	−	−	−	+	+	−
2,5-dimethylfuran	96	−	−	−	+	+	+
2-furanmethanol	98	−	+	−	+	+	−
1-(2-furanyl)ethanone	110	−	+	−	+	+	−
5-methyl-2-furancarboxaldehyde	110	−	+	−	+	−	+
3-phenylfuran	144	−	+	+	+	+	+
pyrazines							
3,4-dimethylpyrrolo[1,2-*a*]pyrazine	146	−	−	−	−	−	+
2,6-dimethylpyrazine	108	−	−	−	−	−	+
trimethylpyrazine	122	−	+	−	−	−	+
methylpyrazine	94	−	+	−	+	−	−
pyrroles							
2-methyl-1*H*-pyrrole	81	−	+	−	−	−	−
1-(1*H*-pyrrol-2-yl)ethanone	109	−	+	−	−	−	−
1-(2-furanylmethyl)-1*H*-pyrrole	147	−	−	−	−	+	−
aromatics							
1,1′-(1,2-ethanediyl)bis(benzene)	182	+	−	−	+	−	−
1-propynylbenzene	116	+	−	−	−	−	+
benzenemethanamine	107	+	−	+	−	−	−
phenethylamine	121	+	−	+	−	−	−
benzeneacetonitrile	117	+	+	−	−	−	−
benzenepropanitrile	131	+	+	−	−	−	−
phenylacetaldehyde	120	−	+	−	+	+	−
N-(2-phenethyl)acetamide	163	−	−	−	+	+	+
benzenepropionic acid	150	−	+	+	−	+	−
styrene	104	+	+	+	+	+	−
N-(2-phenethyl)formamide	149	−	+	+	+	−	+
benzeneethanol	122	−	+	+	+	+	+
trans,*trans*-1,4-diphenyl-1,3-butadiene	206	+	+	+	+	+	−
methylbenzene	92	+	+	+	+	+	+
ethylbenzene	106	+	+	+	+	+	+
1,1-diphenylpropene	194	+	+	+	+	+	+
pyridines							
3-phenylpyridine	155	+	+	+	+	+	−
4-(phenylmethyl)pyridine	169	−	+	+	+	+	−
3,5-diphenylpyridine (**X**)	231	+	+	+	+	+	+
naphthalenes							
2′-phenylpyrollo[4,5-*a*]-3,4-dihydronaphthalene	245	−	+	+	+	−	−
1(2)-naphthaleneamine	143	+	+	+	+	+	−
N-methylnaphthaleneamine	157	+	+	+	+	−	+
aminoanthracene	193	+	+	+	+	−	−
N-phenethyl-1(2)-naphthaleneamine (**Y**)	247	+	+	+	+	+	+
N-phenethyl-*N*-methyl-1(2)-naphthaleneamine (**Z**)	261	+	+	+	+	+	+
miscellaneous compounds							
1,2-dimethyl-1*H*-imidazole	96	−	−	−	−	−	+
lactone*	255	−	+	−	+	−	−
pyranone*	144	−	+	−	+	−	−
acetic acid	60	−	+	−	+	−	−
1-hydroxy-2-propanone	74	−	+	−	−	+	+

[a] Phe, phenylalanine; Glu, glucose; Gly, glyceraldehyde; Rib, ribose; ARPP, Amadori phenylalanine. [b] +, present; −, absent. [c] Lactone, 2-[5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl]-3-phenylpropionic acid lactone. [d] Pyranone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one.

was set at the desired temperature at a heating rate of 50 °C/ms. Capillary direct MS interface temperature was 180 °C; ion source temperature was 280 °C. The ionization voltage was 70 eV, and the electron multiplier was 1494 V. The mass range analyzed was 35−350 amu. The column was a fused silica DB-5 column (30 m length × 0.25 mm i.d. × 25 μm film thickness; Supelco, Inc.). The column initial temperature was −5 °C for 3 min and was increased to 50 °C at a rate of 30 °C/min; immediately the temperature was further increased to 270 °C at a rate of 8 °C/min and kept at 270 °C for 5 min. Products that were not found in the mass spectral libraries were identified by comparison with literature mass spectral data or by generating the products from their proposed precursors and comparing mass spectra and chromatographic retention times.

Confirmation of the Precursors of Pyridine and Naphthalene Derivatives. Equimolar concentrations of the postulated reactants were mixed with or without a solvent (ether) and heated at 130 °C for 15 h and analyzed by GC/MS. The details of each reaction are elaborated in the text.

RESULTS AND DISCUSSION

To investigate the mechanism of formation of phenylalanine specific products during Maillard reaction, phenylalanine, Amadori phenylalanine (ARPP), and equimolar mixtures of glucose/phenylalanine, ribose/phenylalanine, glyceraldehyde/phenylalanine, and Amadori phenylalanine in the presence of phenylalanine were pyrolyzed using Py/GC/MS as described under Materials and Methods. Ribbon pyrolysis was used to study the effect of temperature (150, 200, 250 °C) on the efficiency of formation of initial pyrolysis products from phenylalanine and Amadori phenylalanine. Quartz tube pyrolysis was used at 250 °C to enhance the secondary reaction products. The products identified in all of the model systems studied using the quartz tube at 250 °C are listed in Table 1. Thermal degradation of phenylalanine alone at temperatures above 150 °C

Table 2. Mass Spectrometric Data

3-phenylfuran	145 (12), **144 (100)**, 116 (11), 115 (86), 89 (14), 87 (3), 86 (3), 63 (12), 62 (5)
	146 (10), **145 (100)**, 144 (38), 117 (10), 116 (82), 115 (36), 90 (10), 89 (10), 64 (7), 63 (10), 63 (12), 62 (5)[a]
3,5-diphenylpyridine (**X**)	232 (19), **231 (100)**, 230 (24), 203 (5), 202 (12), 115 (3), 102 (10), 101 (6), 77 (4), 76 (6)
3,4-diphenylpyridine	232 (19), **231 (100),** 230 (70), 216 (14), 202 (21), 115 (9), 114 (9), 102 (8), 101 (13), 88 (8), 76 (7)
2,6-diphenylpyridine	232 (18), **231 (100),** 230 (61), 228 (6), 204 (4), 203 (3), 202 (9), 154 (6), 127 (10), 116 (4), 114 (8), 102 (20), 101 (7), 77 (15), 76 (10)
4-(phenylmethyl)pyridine	170 (13), 169 (98), **168 (100)**, 167 (38), 166 (6), 155 (3), 154 (18), 143 (1), 142 (8), 141 (13), 139 (9), 115 (20), 63 (10), 62 (3)
	171 (11), 170 (84), **169 (100)**, 168 (53), 167 (13), 166 (1), 155 (14), 154 (7), 143 (5), 142 (11), 141 (21), 140 (8), 139 (7), 115 (19), 63 (9), 62 (4)[a]
3-phenylpyridine	156 (12), **155 (100)**, 154 (49), 128 (11), 127 (13), 102 (10), 77 (5), 51 (7)
	157 (4), 156 (26), **155 (100)**, 154 (46), 128 (12), 127 (13), 102 (11), 77 (10), 51 (12)
2-[5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl]-3-phenylpropionic acid lactone	256 (11), 255 (27), 211 (4), 210 (5), 193 (2), 182 (2), 180 (2), 167 (2), 148 (5), 147 (5), 131 (11),120 (9), 108 (12), 104 (5), 103 (4), 92 (11), **91 (100)**, 78 (7), 77 (7), 63 (5), 51 (8)
N-(phenethyl)-1(2)-naphthaleneamine (**Y**)	248 (7), 247 (31), 157 (10), **156 (100)**, 143 (9), 128 (6), 127 (3), 78 (9), 77 (4)
N-(phenethyl)-*N*-methyl-1(2)-aminonaphthalene (**Z**)	262 (7), 261 (34), 171 (13), **170 (100)**,157 (3), 155 (3), 156 (3), 128 (7), 92 (4)
N-(phenylmethylene)phenethylamine	210 (1), 209 (5), 208 (3), 132 (7), 119 (9), **118 (100)**, 117 (6), 103 (4), 92 (7), 91 (95), 90 (6), 89 (7), 78 (4), 77 (11), 65 (11), 63 (4), 51 (7)

[a] With [1-^{13}C]glucose.

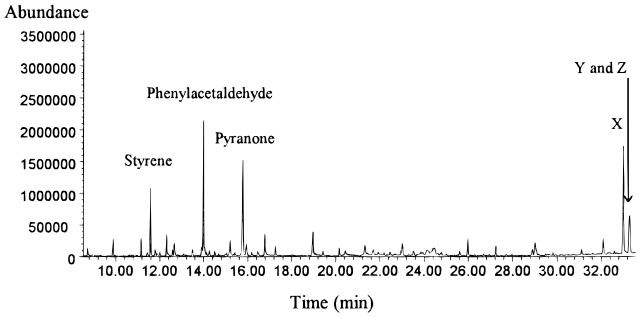

Figure 1. Pyrogram of phenylalanine Amadori product generated from the ribbon probe at 250 °C for 20 s. Pyranone = 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one.

leads to the formation of components with benzyl and phenyl residues as characteristic structural features (Kato et al., 1971). Py/GC/MS of phenylalanine on the ribbon probe and in the quartz tube yields phenethylamine as the main degradation product. No thermal degradation products of phenylalanine were observed below 200 °C. However, ARPP degraded at 150 °C and exhibited a more complex product profile. The main reaction products were phenylacetaldehyde (Strecker aldehyde), styrene, 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (pyranone), and three unknown compounds designated **X**, **Y**, and **Z** (see Figure 1). In addition, pyrazines were identified only in the ARPP model systems. The identification of the Strecker aldehyde and the pyrazines in the glucose/phenylalanine model system and the similarity of the pyrograms of ARPP and the glucose/phenylalanine mixture are indicative of the efficiency of Amadori rearrangement under pyrolysis conditions similar to the proline/glucose system (Huyghues-Despointes et al., 1994). Formation of phenylacetaldehyde from phenylalanine has been reported at trace levels (Kato et al., 1971). Due to its acidic α-hydrogens, phenylacetaldehyde is capable of condensation reactions with other aldehydes such as glycoladehyde to form 3-phenylfuran, as reported by Baltes et al. (1988), and was also detected in our model systems containing glucose, ribose, and glyceraldehyde. Labeling experiments with [1-^{13}C]glucose indicated that 70% of the glycoaldehyde moiety in 3-phenylfuran originates from C1-C2 of the sugar (see Table 2). 2-[5′-(Hydroxymethyl)-2′-formylpyrrol-1′-yl)-3-phenylpropionic acid lactone reported by Kunert-Kirchhoff et al. (1990) was similarly observed in the model systems containing glucose as the carbohydrate source.

A. Pyridine Derivatives. Pyridines as a group have exhibited mutagenic activity based on the Ames and Williams tests (Sasaki et al., 1987). Two pyridine derivatives, 3-phenylpyridine and 4-(phenylmethyl)-pyridine, were identified in the model systems on the basis of a spectral library search. 3-Phenylpyridine has also been identified by Baltes and Mevissen (1988). In addition, one of the major chromatographic peaks designated X (molecular ion at *m*/*z* 231) in the pyrogram of ARPP (see Figure 1) showed close similarity with 2,6-diphenyl- and 3,4-diphenylpyridine mass spectra (see Table 2). However, one of the common major peaks (*m*/*z* 230) differed in relative intensity when compared with the known diphenylpyridine spectra, indicating a different isomer that was tentatively assigned to 3,5-diphenylpyridine structure. The two pyridine derivatives, 3-phenylpyridine and 4-(phenylmethyl)pyridine, and compound **X** were formed during pyrolysis of ARPP on the ribbon probe at 250 °C, while at 150 °C only compound **X** was formed (Table 3) and it increased in intensity with increasing temperature (Table 4). Pyrolysis of phenylalanine on the ribbon probe produced compound **X** at all temperatures studied, whereas the other two pyridine derivatives were formed only at 250 °C. These observations indicate that the precursors of compound **X** are readily formed from phenylalanine compared to the precursors of the two other pyridine derivatives.

*Proposed Mechanism of Formation of 3,5-Diphenylpyridine (Compound **X**) and 3-Phenylpyridine.* Phenethylamine can be formed by the decarboxylation of phenylalanine. Phenylacetaldehyde, on the other hand, can be formed through Strecker degradation, although it has been detected in trace amounts in the absence of sugars (Kato et al., 1971). These two compounds are known to react to form an imine which rearranges into enamine due to conjugation with the benzene ring (Figure 2). The corresponding benzaldehyde adduct [*N*-(phenylmethylene)phenethylamine] was detected in the heated (10 min) methanolic solution of ARPP in 10% water (Table 2). If the above enamine reacts with another mole of phenylacetaldehyde as an N-nucleophile, it eventually leads to the formation of *N*-(2-phenethyl)-2,4-diphenylpyrrole (Papadopoulou and Ames, 1994). However, enamines can act as C-nucleophiles and undergo condensation reactions with other carbonyls such as phenylacetaldehyde or acetaldehyde to produce **1** (Figure 2). Intermediate **1** can lose a styrene molecule as shown in Figure 2 to produce the triene **2**, which can undergo

Table 3. Effect of Temperature on the Formation of Pyrolysis Products from Phenylalanine Amadori Compound Using the Ribbon Probe

system	MW	150 °C	200 °C	250 °C
aromatics				
benzaldehyde	106	−[a]	−	+[a]
trans,trans-1,4-diphenyl-1,3-butadiene	206	−	+	+
methylbenzene	92	−	+	+
ethylbenzene	106	−	+	+
styrene	104	−	+	+
benzenepropionic acid	150	−	+	+
benzeneethanol	122	−	+	+
1,1-diphenylpropene	194	+	+	+
phenylacetaldehyde	120	+	+	+
furans				
2-furanmethanol	98	−	−	+
1-(2-furanyl)ethanone	110	−	+	+
5-methyl-2-furancarboxaldehyde	110	−	+	+
3-phenylfuran	144	−	+	+
pyridines				
4-(phenylmethyl)pyridine	169	−	−	+
3-phenylpyridine	155	−	+	+
3,5-diphenylpyridine (**X**)	231	+	+	+
naphthalenes				
2′-phenylpyrollo[4,5-*a*]-3,4-dihydronaphthalene	245	−	−	+
anthracenamine	193	−	+	+
N-phenylethyl-1(2)-naphthaleneamine (**Y**)	247	−	+	+
N-phenylethyl-*N*-methyl-1(2)-naphthaleneamine **Z**)	261	−	+	+
1(2)-naphthaleneamine	143	+	+	+
N-methyl-1(2)-naphthaleneamine	157	+	+	+
miscellaneous compounds				
acetic acid	60	−	+	+
2-hydroxypropanone	74	−	+	+
pyranone[b]	144	+	+	+
lactone[c]	255	+	+	+

[a] +, present; −, absent. [b] 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one. [c] 2-[5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl]-3-phenylpropionic acid lactone.

Table 4. Effect of Temperature on the Efficiency of Formation ($\times 10^{11}$) in Area/Mole of Molecular Ions of Pyridine and Naphthalene Derivatives and Their Precursors Generated from Ribbon Pyrolysis for 20 s of Phenylalanine and Amadori Phenylalanine

		150 °C		200 °C		250 °C	
compound	MW	Phe[a]	ARPP[a]	Phe	ARPP	Phe	ARPP
phenylacetaldehyde	120	0	3	0	18	0	25
phenethylamine	121	0	0	6	0	135	0
benzenemethanamine	107	0	0	0	0	0	0
benzaldehyde	106	0	0	0	0	0	tr[b]
benzeneethanol	122	tr	0	0	tr	0	tr
N-methyl-1(2)-naphthaleneamine	157	0	tr	0	tr	0	tr
1(2)-naphthaleneamine	143	0	2	0	7	0	9
1-(*N*-benzyl)naphthaleneamine	233	0	0	0	0	0	0
1(2)-(*N*-phenethyl)naphthaleneamine (**Y**)	247	0	0	0	4	tr	10
1(2)-(*N*-phenethyl-*N*-methyl)naphthaleneamine (**Z**)	261	0	0	0	2	0	5
3-phenylpyridine	155	0	0	0	2	tr	4
4-(phenylmethyl)pyridine	169	0	0	0	0	0.5	2
3,5-diphenylpyridine (**X**)	231	tr	9	17	41	9	113

[a] Phe, phenylalanine; ARPP, Amadori phenylalanine; systems containing two components are equimolar. [b] tr, trace.

thermally allowed electrocyclic ring closure to produce either 3,5-diphenylpyridine or 3-phenylpyridine after an aromatization step. To test the validity of this assumption, equimolar concentrations of phenylacetaldehyde and phenethylamine were dissolved in ether solution and mixed at room temperature for 15 h. The solution was injected through the pyrolysis interface and also through the injection port and analyzed by GC/MS under the same conditions as mentioned under Materials and Methods. The resulting chromatogram had a peak at the same retention time with a mass spectrum identical to that of compound **X**. When ARPP was dissolved in methanol and heated for 1 h at 90 °C and analyzed by GC/MS, 3,5-diphenylpyridine was observed as the predominant reaction product in the pyrogram.

Proposed Mechanism of Formation of 4-Substituted Pyridine Derivatives. The 4-substituted pyridine derivatives observed in the model systems can be envisaged to be formed by a mechanism similar to that depicted in Figure 2. Replacing phenylacetaldehyde with acetaldehyde, which can be formed from phenylalanine in trace amounts (Kato et al., 1971) and more efficiently from sugars, a similar condensation product to that shown in Figure 2 can be formed as shown in Figure 3. The enamine (**3**) formed can react with either phenylacetaldehyde or any other aldehyde such as formaldehyde or acetaldehyde to form a triene (**4**) after losing a benzene molecule. The phenylacetaldehyde adduct, for example, can form 4-(phenylmethyl)pyridine after an electrocyclic ring closure and aromatization steps. When an equimolar mixture of acetaldehyde and phenethylamine was stirred at room temperature for 15 h and subsequently heated with phenylacetaldehyde and injected through the pyrolysis interface and also

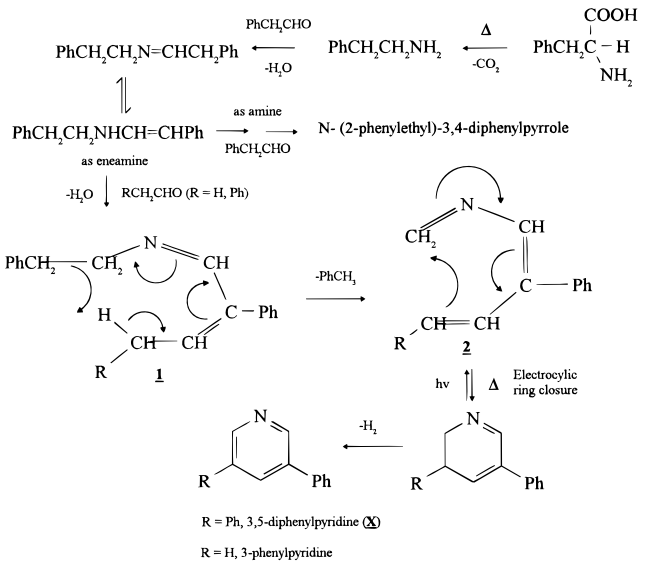

Figure 2. Proposed mechanism of formation of 3- and/or 5-substituted pyridine derivatives.

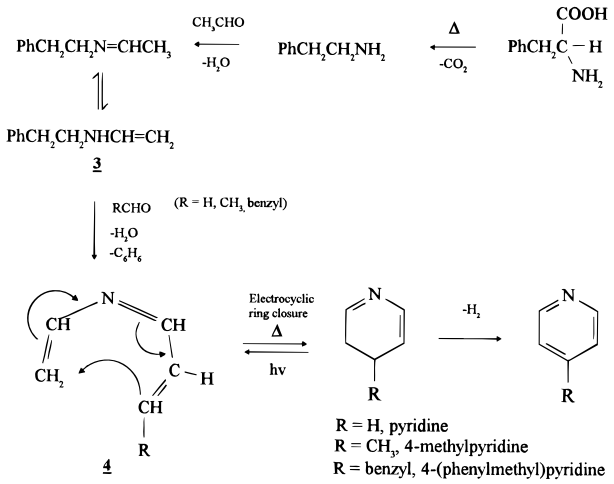

Figure 3. Proposed mechanism of formation of 4-substituted pyridine derivatives.

through the injection port and analyzed by GC/MS under the same conditions as the pyrolysis of ARPP, the resulting chromatogram had a peak at the same retention time with a mass spectrum identical to that of 4-(phenylmethyl)pyridine. In addition, experiments with [1-^{13}C]glucose (Table 2) show a large percentage of label incorporation in 4-(phenylmethyl)pyridine, indicating involvement of an acetaldehyde fragment coming from the sugar moiety, whereas 3,5-diphenylpyridine did not show any label incorporation.

B. Naphthalene Derivatives. Naphthalene derivatives are suspected carcinogens (Orzechowski, 1992). Aminoanthracene is a recognized promutagen (Phillipson, 1983). Thermal degradation of ARPP at 250 °C, both in the quartz tube and on the ribbon probe, resulted in the formation of several naphthalene derivatives such as 1(2)-naphthaleneamine, *N*-methyl-1(2)-aminonaphthalene, 1-aminoanthracene, and 2′-phenylpyrrolo[4,5-*a*]dihydronaphthalene (Tables 1 and 3). At 200 °C on the ribbon probe only the pyrrolo derivative did not form, and at 150 °C only naphthaleneamine and its *N*-methyl derivative were formed from the ARPP. On the other hand, phenylalanine alone on the ribbon probe did not produce any of the naphthalene derivatives mentioned above. However, in the quartz tube at 250 °C all, except the pyrrolo derivative, were formed.

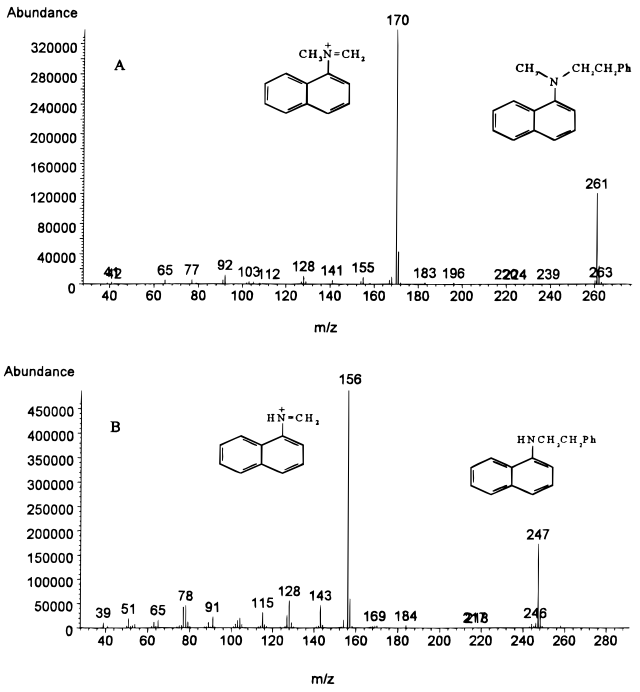

Figure 4. (A) Mass spectrum of compound **Z**, tentatively assigned the structure of *N*-methyl-*N*-phenethyl-1(2)-aminonaphthalene. (B) Mass spectrum of compound **Y**, tentatively assigned the structure of *N*-phenethyl-1(2)-aminonaphthalene.

In addition to the above naphthalene derivatives, two peaks in the chromatogram of various model systems containing phenylalanine and sugar or ARPP were observed, one at a retention time of 31.5 min with a parent ion at *m*/*z* 247 (compound **Y**) and the other at 31.7 min with a parent ion at *m*/*z* 261 (compound **Z**). Both compounds exhibited characteristic fragmentations of a naphthalene derivative (see Figure 4 and Table 2) such as a fragment ion at *m*/*z* 128 (parent naphthalene) and the presence of multiply charged ions. These two compounds were produced at trace levels in phenylalanine pyrolysis products. On the ribbon probe phenylalanine produced only compound **Y** (*m*/*z* 247) at 250 °C, whereas in the quartz tube both compounds were produced at 250 °C (Tables 4 and 5). On the basis of their molecular weights and mass spectral fragmentation patterns (Table 2 and Figure 4), naphthalene derivatives containing phenethyl groups were postulated for the structures of the two unknown compounds as shown in Figure 4. In general, the number of naphthalene derivatives and the efficiency of their formation (area/mole) increased for phenylalanine when pyrolysis was conducted in the quartz tube at 250 °C (Table 4). At 250 °C the naphthalene derivatives produced by ARPP and phenylalanine in the quartz tube were similar; however, the efficiency of their formation increased by 8-fold on the basis of area/mole in the case of the Amadori compound in the presence of phenylalanine.

Proposed Mechanism of Formation of N-Substituted 1- and 2-Aminonaphthalene Derivatives. N-Substituted 1- or 2-aminonaphthalene derivatives could be formed from corresponding aminonaphthalenes by nucleophilic substitution reaction with proper electrophiles. Protonated benzeneethanol could be one such reactant. When commercially available 2-naphthaleneamine and benzeneethanol were mixed in a reaction vial in the presence of trace amounts of phenylalanine as the proton source and then incubated at 130 °C for 15 h,

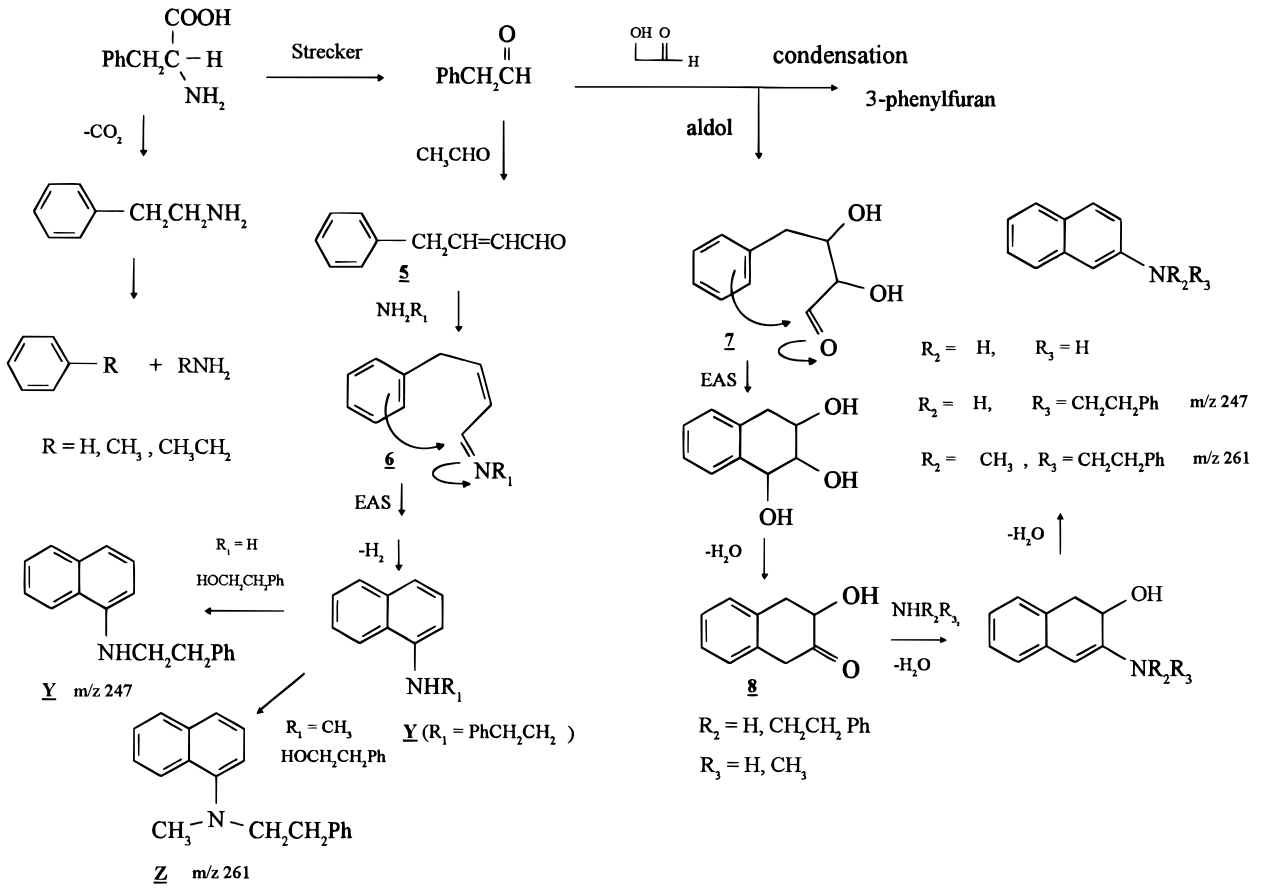

Figure 5. Proposed mechanisms of formation of N-substituted 1- and 2-aminonaphthalenes.

Table 5. Efficiency of Formation ($\times 10^{11}$) in Area/Mole of Molecular Ions of Pyridine and Naphthalene Derivatives and Their Precursors Generated from Pyrolysis at 250 °C for 20 s in the Quartz Tube of Model Systems Containing Phenylalanine

compound	MW	Phe[a]	Glu/Phe[a]	ARPP[a]	ARPP/Phe[a]
phenylacetadehyde	120	0	34	27	0
phenethylamine	121	187	0	0	340
benzenemethanamine	107	tr[a]	0	0	tr
benzaldehyde	106	0	0	0	0
benzeneethanol	122	0	3	4	6
N-methyl-1(2)-naphthaleneamine	157	tr	tr	tr	tr
1(2)-naphthaleneamine	143	3	8	4	7
1-(*N*-benzyl)naphthaleneamine	233	tr	tr	0	0
1(2)-(*N*-phenethyl)naphthaleneamine (**Y**)	247	4	17	6	35
1(2)-(*N*-phenethyl-*N*-methyl)naphthaleneamine (**Z**)	261	3	9	4	20
3-phenylpyridine	155	tr	3	4	6
4-(phenylmethyl)pyridine	169	0.5	1	3	4
3,5-diphenylpyridine (**X**)	231	14	30	88	156

[a] Phe, phenylalanine; Glu, glucose; ARPP Amadori phenylalanine; systems containing two components are equimolar. [b] tr, trace.

the GC/MS analysis of the reaction mixture yielded a compound with a parent ion of *m/z* 247 and with the same mass spectrum and retention time as compound **Y**. 1-Aminonaphthalenes and N-substituted 1-aminonaphthalenes could also be formed in phenylalanine model systems by the aldol condensation of phenylacetaldehyde and acetaldehyde as illustrated in Figure 5. The resulting α,*β*-unsaturated aldehyde (**5**) can react with any primary amine to produce an α,*β*-unsaturated imine (**6**), and this adduct can undergo an intramolecular electrophilic aromatic substitution reaction (EAS) and can aromatize to produce the target compounds. An equimolar mixture of phenylacetaldehyde and acetaldehyde was refluxed for 30 min, at the end of which time phenethylamine was added and refluxing was continued for an additional 15 min. Analysis of the mixture indicated the presence of compound **Y**. Alternatively, phenylacetaldehyde can condense with glycoaldehyde to produce N-substituted 2-aminonaphthalene derivatives. The initial α,*β*-dihydroxyaldehyde intermediate (**7**) can undergo intramolecular electrophilic aromatic substitution reaction (EAS) and after dehydration can produce hydroxy *β*-tetralone [3,4-dihydro-3-hydroxy-2(1*H*)-naphthalenone] (**8**), which can react with different primary and secondary amines to form N-substituted 2-aminonaphthalene derivatives. When phenylacetaldehyde was reacted with glycoladehyde dimer for 15 h at 120 °C, the reaction mixture contained 3-phenylfuran as the major product. Subsequently, further reaction with phenethylamine in the presence of a catalytic amount of phenylalanine produced compound **Y** (*m/z* 247). The aminonaphthalene derivatives, therefore, could be a mixture of 1- and/or 2-substituted isomers.

Conclusion. Thermal degradation of phenylalanine containing model systems produced naphthalene and pyridine derivatives, some of which have already been classified as mutagenic (Orzechowski, 1992). Pyridines as a group are heterocyclic compounds which produce positive results with the Ames and Williams test (Sasaki et al., 1987). Py/GC/MS was demonstrated to be a useful and rapid technique that can be employed in understanding the degradation pathways of Amadori compounds. The use of isotopically enriched compounds to determine reaction mechanisms is facilitated since Py/GC/MS analyses can be conducted on a microscale.

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