Formation of 2-Pentylpyridine from the Thermal Interaction of Amino Acids and 2,4-Decadienal

Young-Suk Kim,† Thomas G. Hartman,‡ and Chi-Tang Ho*,†

Department of Food Science and Center for Advanced Food Technology, New Jersey Agricultural Experiment Station, Cook College, Rutgers University, New Brunswick, New Jersey 08903

To study the mechanism of 2-pentylpyridine formation in model systems, 2,4-decadienal was reacted with five amino acids (glycine, aspartic acid, asparagine, glutamic acid, and glutamine) at 180 °C for 1 h (pH 7.5). In addition to 2-pentylpyridine, 3-pentylpyridine was also tentatively identified from the thermal reactions. The relative yields of alkylpyridine formation from the reactions were asparagine > glutamine > aspartic acid > glutamic acid > glycine. When amide-^{15}N-labeled glutamine and asparagine were heated with 2,4-decadienal, the relative contribution of amide nitrogens to the formation of alkylpyridine was determined. Approximately half of nitrogen atoms in 2-pentylpyridine formed were contributed by the amide nitrogens of asparagine, whereas almost all of them came from the amide nitrogens in glutamine. The results above may indicate that both free ammonia and α-amino groups bound in amino acids can contribute to the formation of alkylpyridines, but free ammonia does so more effectively.

Keywords: *Amino acid−lipid interaction; 2-pentylpyridine; 2,4-decadienal; deamidation and deamination; ammonia generation*

INTRODUCTION

The interaction of primary amines in proteins or amino acids with carbonyls in lipids may produce N-containing heterocyclic volatile compounds such as alkylpyridines. The importance of heterocyclic compounds in food flavors are well-known, but relatively little attention has been given to pyridine derivatives even though many of them have been found in a large number of foods (Maga, 1981; Vernin, 1982; Ishihara et al., 1992; Thomas and Bassole, 1992). Among the pyridine derivatives, 2-pentylpyridine deserves special attention due to its strong fatty and tallow-like flavor in diluted solution (Buttery et al., 1977) and its low odor threshold (0.2 ng/L of air) (Schieberle, 1993). 2-Pentylpyridine has also been identified as the major odor-active component in mutton meat (Buttery et al., 1977; Cramer, 1983) and was a major product when valine was heated with linoleic acid or its esters (Henderson and Nawar, 1981).

It was proposed that 2-pentylpyridine is formed in the amino acid−linoleic acid system as a result of the interaction of ammonia with 2,4-decadienal, one of the major degradation products of linoleic acid (Henderson et al., 1980; Kimoto and Gaddis, 1969; Patton et al., 1959). In this mechanism, ammonia condensed with 2,4-decadienal to form a Schiff base intermediate, followed by ring closure, leading to the formation of a dihydropyridine which can then be oxidized to 2-pentylpyridine. Therefore, the free ammonia is needed for the formation of 2-pentylpyridine. This is further supported by a recent study of Schieberle (1993) who showed that the amount of 2-pentylpyridine was increased by the addition of ammonium sulfate or grinding before roasting in a commercial roasted sesame oil. On the other hand, when Zhang et al. (1989) reacted 2,4-decadienal with glutathione, 2-pentylpyridine was identified as one of the major volatiles even though they could not identify dithiazine or thiadiazine which requires free ammonia for their formation. It might indicate that free ammonia was not available in those systems and 2-pentylpyridine was formed without the free ammonia. An alternative mechanism in the formation of 2-pentylpyridine as a result of the direct condensation of the amino group of amino acids or peptides with the aldehydic group of 2,4-decadienal is proposed.

In the present study, the mechanism of alkylpyridines formation was investigated from the thermal interaction of 2,4-decadienal and each of the five amino acids (glycine, aspartic acid, asparagine, glutamic acid, and glutamine), separately. Aspartic acid, asparagine, and glutamine can produce free ammonia readily, but glutamic acid and glycine cannot under the conditions used. The effects of amino acids and their relative reactivities on the formation of alkylpyridines were also studied. In addition, we examined the relative contribution of free ammonia to the formation of alkylpyridines compared with that of α-amino groups bound in amino acids. The free ammonia can be derived from both amino groups and amide side chains in asparagine and glutamine. The contribution of amide nitrogens as well as amino nitrogens to the formation of N-containing flavor compounds has been reported previously (Hwang et al., 1993; Izzo and Ho, 1992). We used asparagine and glutamine labeled with ^{15}N at the sites of amide side chains to study the effects of amide nitrogens on the formation of alkylpyridines.

EXPERIMENTAL PROCEDURES

Sample Preparation. Mixtures of *trans,trans*-2,4-decadienal (0.001 mol) (Sigma Chemical Co., St. Louis, MO) and five amino acids, i.e. glycine, L-aspartic acid, L-asparagine, L-glutamic acid, and L-glutamine (0.005 mol) (Sigma), were dissolved in 100 mL of distilled water, and the pH was adjusted to 7.5 with 0.1 N NaOH or 0.1 N HCl. Each sample solution was transferred into a 0.3 L Hoke SS-Dot sample cylinder and then heated at 180 °C in an oil bath for 1 h. After the reaction, undecane (100 ppm or 1000 ppm) (Polyscience, Niles, IL) was added as an internal standard into each sample product which

* Author to whom correspondence should be addressed [fax (908) 932-6776].

† Department of Food Science.

‡ Center for Advanced Food Technology.

S0021-8561(95)00623-6 CCC: $12.00

was then extracted with methylene chloride in a separatory funnel by multiple extraction (3 × 100 mL). The solvent extract was washed with distilled water (100 mL) three times and dried over anhydrous sodium sulfate. The extract was then concentrated with a Kuderna-Danish apparatus to a volume of 5 mL and further concentrated with nitrogen gas to a final volume of 0.1 mL. L-Asparagine-*amide*-^{15}N and L-glutamine-*amide*-^{15}N (Isotech, Inc., Miamisburg, OH) were used for the determination of derived ammonia to pentylpyridine formation.

Volatile Separation by Gas Chromatography. A Varian 3400 gas chromatograph with an FID and a nonpolar fused silica capillary column [60 m × 0.25 nm (i.d.), 0.25-μm thickness, DB-1; J&W] was employed to analyze the volatile compounds produced from the thermal reactions. The GC equipment was operated with an injector temperature of 270 °C, a detector temperature of 300 °C, and a helium carrier gas flow rate of 1.0 mL/min. The temperature of the GC column was increased from 40 to 150 °C at a rate of 2 °C/min, from 150 to 190 °C at a rate of 1 °C/min, and from 190 to 280 °C at a rate of 2 °C/min, holding at 280 °C for 30 min. For each sample, 0.2 μL of solution was injected with a split ratio of 50:1.

After the GC was run, volatiles were quantified by comparing their relative peak areas to the area of the internal standard. Linear retention indices for the volatiles were determined by comparing their retention times with those of *n*-paraffin standard (C_6−C_{19}; PolyScience, Niles, IL), according to the method of Majlat et al. (1974).

GC/MS Analysis. The samples were analyzed by GC/MS comprising a Varian 3400 gas chromatograph coupled with a Finnigan MAT 8230 high-resolution, double-focusing magnetic sector mass spectrometer (TD-GC−MS). Mass spectra were obtained by using electron ionization of 70 eV and ion source temperature of 250 °C. All mass spectra were identified by an on-line computer library (NIST) or published literature.

Calculations for the Relative Contribution of ^{15}N in Amide Side Chains to Alkylpyridines Formation. If one ^{15}N in an amide side chain of asparagine or glutamine is transferred into a pyridine ring, the molecular weight of the pyridine will be increased by one mass unit. There will, therefore, be two kinds of pyridines which have different molecular weights. The relative ratios to the overall alkylpyridine of the two different pyridines produced can be designated by W_1 and W_2. W_1 is the relative ratio of pyridines which have no ^{15}N in the pyridine ring to the overall alkylpyridines, and W_2 is the relative ratio of pyridines which have a ^{15}N in the pyridine ring to the overall alkylpyridines, respectively.

We compared *m*/*z* 92 (due to loss of one hydrogen from 93), 93, and 94 (due to natural isotope) in the mass spectra obtained from the normal asparagine and glutamine with *m*/*z* 93, 94, and 95 increased by one unit from ^{15}N-labeled ones. The relative abundance of these peaks were selected to reduce errors in calculation because *m*/*z* 93 was the ion which had the maximum abundance and retained nitrogen in its ring structure from the reaction of normal asparagine and glutamine with 2,4-decadienal. When it was assumed that M_{L93} and M_{L94} were the experimental relative abundances of the ion peaks in the reaction of ^{15}N-labeled asparagine or glutamine with 2,4-decadienal and M_{92}, M_{93}, and M_{94} were the experimental relative abundances of the ion peaks in the reaction of unlabeled asparagine or glutamine with 2,4-decadienal, the following equations could be used to obtain W_1 and W_2. The experimental data are given in Table 1.

$$M_{L93} = M_{93}W_1 + M_{92}W_2$$

$$M_{L94} = M_{94}W_1 + M_{93}W_2$$

The percentage contribution of amide nitrogens to the formation of alkylpyridines were calculated by using W_1 and W_2 as follows: % contribution = $(W_2/(W_1 + W_2)) \times 100$

RESULTS AND DISCUSSION

2-Pentylpyridine was identified in the mixtures when 2,4-decadienal was reacted with glycine, aspartic acid, asparagine, glutamic acid, and glutamine separately. The identification of 2-pentylpyridine was accomplished by comparing its mass spectral and retention index data with those of the NIST computer library and published literature data (Zhang and Ho, 1989). In addition to 2-pentylpyridine, 3-pentylpyridine was tentatively identified from the mass spectral data. The mechanism of 3-pentylpyridine is not understood at the present time. The mass spectral data of two alkylpyridines are shown in Table 2.

Figure 1 shows the amount of 2-pentylpyridine and 3-pentylpyridine formed from the interaction of 2,4-decadienal with each of the five amino acids. As expected asparagine and glutamine showed the most activity toward the formation of two pentylpyridines. It is well-established that asparagine and glutamine are susceptible to nonenzymatic deamidation in the side chain amide group and lead to aspartic acid and glutamic acid with the release of a molecule of ammonia (Wright, 1991). In asparagine and glutamine the rate

Table 1. Relative Ion Abundances (Percent) of Pentylpyridines Formed from the Reaction of Asparagine or Glutamine with 2,4-Decadienal

amino acid	alkylpyridine	M_{92}[a]	M_{93}[a]	M_{94}[a]	M_{L93}[b]	M_{L94}[b]
asparagine	2-pentylpyridine	7.37	100	7.19	100	89.27
	3-pentylpyridine	74.51	100	9.70	100	59.77
glutamine	2-pentylpyridine	9.32	100	7.58	12.35	100
	3-pentylpyridine	71.02	100	11.02	71.27	100

[a] M_{92}, M_{93}, and M_{94} are the relative abundances of the ion peaks from the reaction of unlabeled asparagine or glutamine with 2,4-decadienal. [b] M_{L93} and M_{L94} are the relative abundances of the ion peaks from the reaction of asparagine-*amide*-^{15}N or glutamine-*amide*-^{15}N with 2,4-decadienal.

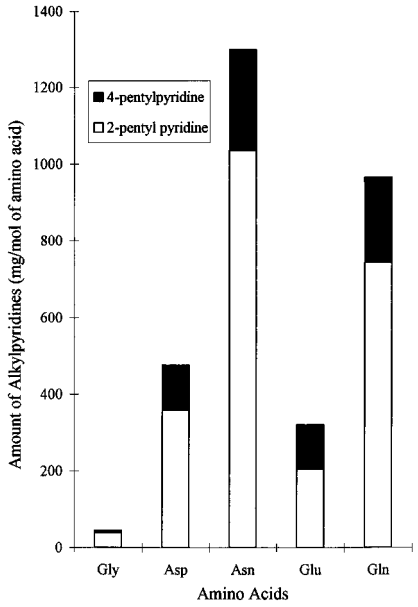

Figure 1. Amount of alkylpyridines formed from the interactions of 2,4-decadienal and amino acids at 180 °C for 1 h (pH 7.5).

Table 2. Mass Spectral Data of Pentylpyridines Identified in the Reactions of Amino Acids with 2,4-Decadienal

pentylpyridine	mass spectral data, *m*/*z* (rel intensity)
2-pentylpyridine	93 (100), 106 (24), 120 (22), 65 (9), 78 (8), 92 (8), 94 (7), 51 (6), 107 (5); MW 149
3-pentylpyridine	93 (100), 92 (73), 39 (49), 149 (42), 65 (41), 41 (36), 106 (31), 51 (14), 57 (14), 78 (10); MW 149

Table 3. Relative Contribution of Amide Nitrogens to Pentylpyridine Formation from the Reaction of Asparagine-*amide*-^{15}N or Glutamine-*amide*-^{15}N with 2,4-Decadienal

amino acid	pentylpyridine	% contribution
asparagine	2-pentylpyridine	46.77
	3-pentylpyridine	47.46
glutamine	2-pentylpyridine	97.00
	3-pentylpyridine	99.50

of deamidation is faster than the rate of thermal deamination of the α-amino groups (Sohn and Ho, 1995). It was further demonstrated that free ammonia generated from the deamidation and the deamination of amino acids may participate in the formation of nitrogen-containing flavor compounds such as pyrazines, pyridines, and pyrroles (Izzo and Ho, 1992; Hwang et al., 1993). In a study reported by Sohn and Ho (1995), it has been shown that asparagine released more ammonia than glutamine under the same conditions. The asparagine released ammonia from both amide and α-amino groups, whereas the ammonia released from glutamine was mainly due to the deamidation of the amide group. The fact that the interaction of 2,4-decadienal with asparagine and glutamine generated more 2-pentylpyridines than other amino acids may suggest that free ammonia is essential to the formation of 2-pentylpyridines.

As shown in Figure 1, both aspartic acid and glutamic acid formed significant amounts of pentylpyridines when they reacted with 2,4-decadienal. Sohn and Ho (1995) reported that aspartic acid produced more than 60% of free ammonia by deamination when heated at 180 °C in an aqueous solution. On the other hand, glutamic acid was shown to be quite stable and released only 1.3% of ammonia from its α-amino groups. The difference in the reactivity of asparatic acid and glutamic acid in releasing free ammonia and in forming pentylpyridine may suggest that pentylpyridines can indeed be formed from the interaction of 2,4-decadienal with the α-amino groups of amino acids through the mechanism suggested by Zhang and Ho (1989).

Glycine generated very small amounts of pentylpyridine as shown in Figure 1. The difference in the amount of pentylpyridine formed between glycine and glutamic acid might be due to their difference in the reactivities of side chains. The larger side chain of glutamic acids may make it more reactive than glycine.

To study the effects of amide nitrogens on the formation of pentylpyridines, asparagine and glutamine labeled with ^{15}N at the amide side chains were used. Table 3 shows the relative contribution of amide nitrogens to pentylpyridine formation from the reaction of 2,4-decadienal with asparagine-*amide*-^{15}N and glutamine-*amide*-^{15}N. Approximately half of the nitrogen atoms in pentylpyridines came from the amide side chain in asparagine whereas almost all of the pyridine nitrogens came from the amide side chain in glutamine. It is also interesting to note that there was no difference between the relative ratios of 2-pentylpyridine and 3-pentylpyridine formation by amide nitrogens in both asparagine and glutamine. These labeling studies indicated that in the cases of asparagine and glutamine the major route for the formation of pentylpyridine was through the formation of free ammonia by either deamidation or deamination.

In conclusion, 2-pentylpyridine may be formed through the interaction of 2,4-decadienal with either free ammonia or the α-amino group of amino acids or peptides. However, the reactivity of free ammonia is probably much higher than that of the α-amino group.

LITERATURE CITED

Buttery, R. G.; Ling, L. C.; Teranishi, R.; Mon, T. R. Roasted fat: basic volatile components. *J. Agric. Food Chem.* **1977**, *25*, 1227−1229.

Cramer, D. A. Chemical compounds implicated in lamb flavor. *Food Technol.* **1983**, *37* (5), 249−260.

Henderson, S. K.; Nawar, W. W. Thermal interaction of linoleic acid and its esters with valine. *J. Am. Oil Chem. Soc.* **1981**, May, 632−635.

Henderson, S. K.; Witchwoot, A.; Nawar, W. W. The autoxidation of linoleates at elevated temperature. *J. Am. Oil Chem. Soc.* **1980**, December, 409−413.

Hwang, H. I.; Hartman, T. G.; Rosen, R. T.; Ho, C. T. Formation of pyrazine from the Maillard reaction of glucose and glutamine-amide-^{15}N. *J. Agric. Food Chem.* **1993**, *41*, 2112−2115.

Ishihara, M.; Tsuneya, T.; Shiga, M.; Kawashima, S.; Yamagishi, K.; Yoshida, F.; Sato, H.; Uneyama, K. New pyridine derivatives and basic components in spearmint oil (*Mentha gentilis* f. *cardiaca*) and peppermint oil (*Mentha piperita*). *J. Agric. Food Chem.* **1992**, *40*, 1647−1655.

Izzo, H. V.; Ho, C. T. Ammonia affects Maillard chemistry of an extruded autolyzed yeast extract: pyrazine aroma generation and brown color formation. *J. Food Sci.* **1992**, *57*, 657−674.

Kimoto, W. I.; Gaddis, A. M. Precursors of alk-2,4-dienals in autoxidized lard. *J. Am. Oil Chem. Soc.* **1969**, *46*, 403−408.

Majlat, P.; Erdos, Z.; Takacs, J. Calculation and application of retention indices in programmed temperature gas chromatography. *J. Chromatogr.* **1974**, *91*, 89−103.

Maga, J. A. Pyridines in foods. *J. Agric. Food Chem.* **1981**, *29*, 895−898.

Patton, S.; Barnes, I. J.; Evans, L. E. n-Deca-2,4-dienal, its origin from linoleate and flavor significance in fats. *J. Am. Oil Chem.* **1959**, *36*, 280−283.

Schieberle, P. Studies on the flavor of roasted white sesame seeds. In *Progress in Flavor Precursor Studies*; Schreier, P., Winterhalter, P., Eds.; Allured Publishing: Carol Stream, IL, 1993; pp 343−360.

Schieberele, P.; Grosch, W. Model experiments about the formation of volatile carbonyl compounds. *J. Am. Oil Chem. Soc.* **1981**, May, 602−607.

Sohn, M. G.; Ho, C. T. Ammonia generation during thermal degradation of amino acids. *J. Agric. Food Chem.* **1995**, *43*, 3001−3003.

Thomas, A. F.; Bassole, F. Occurrence of pyridines and other bases in orange oil. *J. Agric. Food Chem.* **1992**, *40*, 2236−2243.

Vernin, G. Heterocyclic compounds in flavors and fragrances. Part III. Pyridine and derivatives. *Perfum. Flavor.* **1982**, *7*, 23−35.

Wright, H. T. Nonenzymatic deamidation of asparaginyl and glutaminyl residues in proteins. *Crit. Rev. Biochem. Mol. Biol.* **1991**, *26*, 1−51.

Zhang, Y.; Ho, C. T. Volatile compounds formed from thermal interaction of 2,4-decadienal with cysteine and glutathione. *J. Agric. Food Chem.* **1989**, *37*, 1016−1020.

Received for review September 18, 1995. Revised manuscript received April 15, 1996. Accepted September 16, 1996.[⊗] New Jersey Agricultural Experiment Station Publication D-10205-1-95 supported by State Funds and Regional NE-116 Project.

JF950623+

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1996.