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ARTICLE

Formation of Pent-4-en-1-amine, the Counterpart of Acrylamide from Lysine and Its Conversion into Piperidine in Lysine/Glucose Reaction Mixtures

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Isotope labeling studies performed using lysine/glucose model systems have indicated that lysine can generate piperidine, a reactive amine capable of undergoing Maillard type interactions. Two possible mechanisms were identified for the formation of piperidine: one arising through decarboxylation of lysine alone to generate cadaverine (1,5-diaminopentane) followed by deamination to form pent-4-en-1-amine which in turn can cyclize into piperidine where both Nε and Nα atoms of lysine can be equally involved in its generation due to the symmetrical nature of the precursor diamine. On the other hand, in the presence of sugars, lysine, similarly to asparagine and phenylalanine, can undergo carbonyl-assisted decarboxylative deamination reaction to generate pent-4-en-1-amine, the counterpart of acrylamide. The pent-4-en-1-amine can then cyclize to form piperidine through the Nε atom of lysine. To confirm the formation of pent-4-en-1-amine in the lysine/glucose model system, a useful strategy based on Py-GC/MS analysis was developed using isotope labeling technique to identify sugar adducts of pent-4-en-1-amine. Products simultaneously possessing five lysine carbon atoms (C2′−C6′) and the Nε-amino group from lysine in addition to glucose carbon atoms were targeted using specifically labeled precursors such as [^{15}Nα]lysine·2HCl, [^{15}Nε]lysine·2HCl, [U-$^{13}C_6$]lysine·2HCl, [^{13}C-6]lysine·2HCl and [U-$^{13}C_6$]glucose. The complete labeling studies along with structural analysis using synthetic and other available precursors have shown the presence of a peak that satisfies the above criteria, and the peak was tentatively identified as *N*-(5-methylfuran-2-yl)methylidene]penta-1,3-dien-1-amine incorporating pent-4-en-1-amine in its structure.

KEYWORDS: Acrylamide; cadaverine; pent-4-en-1-amine; piperidine; lysine; glucose

INTRODUCTION

The Maillard reaction products that are formed in a similar fashion to that of acrylamide have been termed as "vinylogous compounds" (*1*). Only two such compounds have been studied so far, the acrylamide itself and styrene (*2*) (**Figure 1**). The terminology used to describe this category of Maillard reaction products could be misleading since the term "vinylogous" implies an analogy in reactivity behavior due to conjugation rather than similarity in structure i.e. possessing a double bond. It is preferable that this type of Maillard-generated compound be rather referred to as amino acid derived "vinylic compounds". All amino acids except glycine are capable of undergoing such decarboxylative deamination reactions in the presence of carbonyls and generating amino acid-specific vinylic compounds such as acrylic acid from aspartic acid (*1*) and 3-butenamide from glutamine (*3*). However, the most studied amino acids in this respect are asparagine (*4*) and phenylalanine (*2*) due to the potential safety concerns of the resulting products in heated foods. Studies performed in asparagine model systems (*4*) have indicated that the initially formed decarboxylated Schiff base can generate the vinylic compound either directly (pathway D in **Figure 2**) or indirectly through hydrolysis (pathway C) and formation of decarboxylated amino acid (*5*). The relative importance of these pathways (D or C in **Figure 2**) will very much depend on the reaction matrix and conditions and the type of amino acid involved (*4*). Lysine, on the other hand, is considered to be one of the important amino acids in the Maillard reaction, and its ability to undergo such decarboxylative deamination reactions to generate pent-4-en-1-amine, the counterpart of acrylamide, has not been explored. In this study we demonstrate that pent-4-en-1-amine can form in lysine model systems and can exist as piperidine, a reactive secondary amine capable of interacting with furfural to form yellow pigments as demonstrated by Hofmann (*6*). Related structures such as tetrahydropyridine have been also identified in glucose/lysine mixtures (*7*) and presumed to be formed through intramolecular cyclization of the Strecker aldehyde of lysine (**Figure 1**). Piperidinium cation at *m*/*z* 84 was found to constitute the base peak in the mass spectrum of lysine Amadori product (*8*). Mills et al. (*9*) have synthesized a piperidine Amadori product and studied its degradations. Piperidine is classified as toxic and can also be formed enzymatically from lysine in biological systems and has been detected in human urine and in various animal organs (*10*). Due to its reactivity, piperidine may also be involved in the generation of dietary advanced glycation end-products. The purpose of this study was to demonstrate that lysine, similarly

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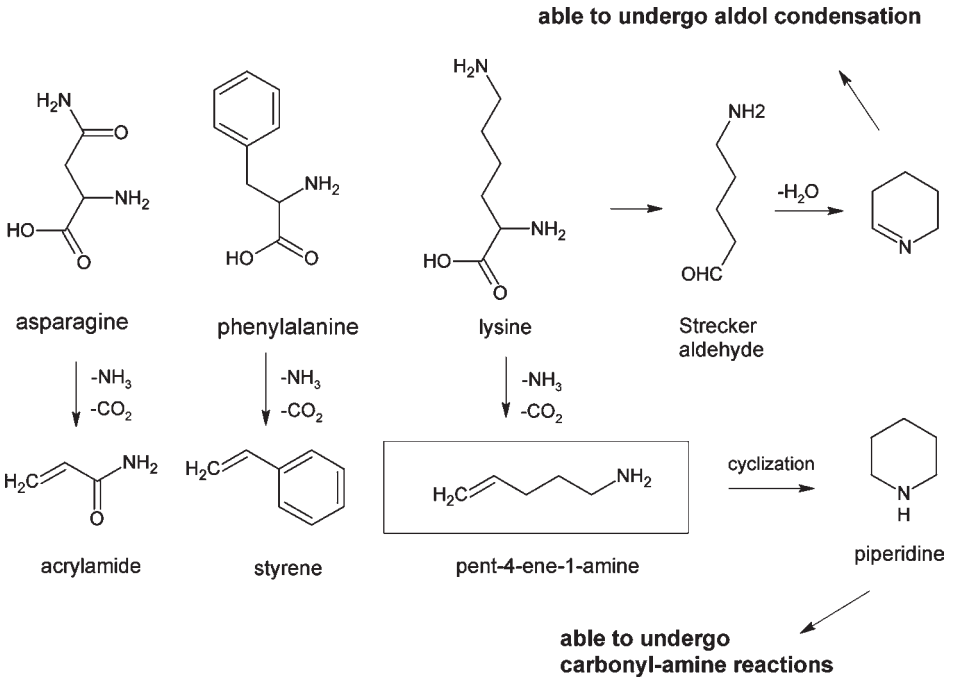


Figure 1. Formation of amino acid-derived vinylic compounds acrylamide, styrene and pent-4-en-1-amine from asparagine, phenylalanine and lysine respectively.

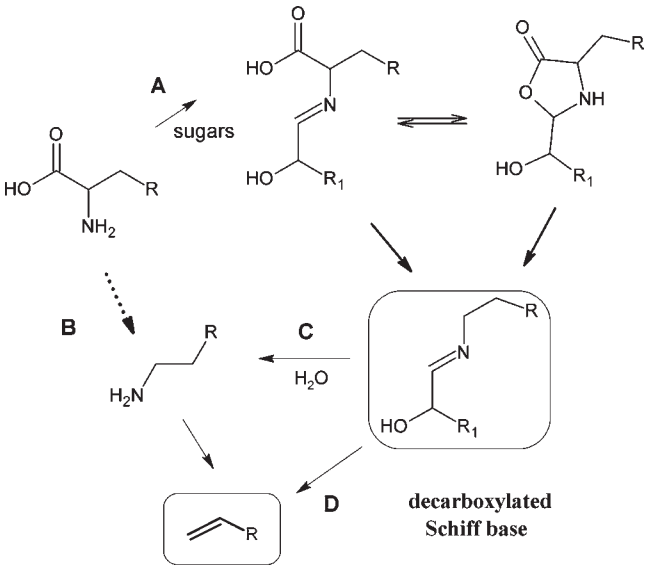


Figure 2. Pathways of formation of amino acid-derived vinylic compounds based on refs *4* and *5*. Pathway A in the presence of reducing sugars, pathway B in the absence of reducing sugars. R = amino acid side chain; R_1 = sugar moiety.

to asparagine, can undergo decarboxylative deamination reaction and generate pent-4-en-1-amine, the counterpart of acrylamide.

MATERIALS AND METHODS

Materials. DL-Lysine (98%), 5-methylfurfural (99%), glycolaldehyde dimer, 5-hydroxymethylfurfural, glyceraldehydes, 2,3-butanedione and pyruvic acid were purchased from Aldrich Chemical Co. (Milwaukee, WI). [^{15}Nα]lysine·2HCl, [^{15}Nε]lysine·2HCl, [U-$^{13}C_6$]lysine·2HCl, [^{13}C-6]lysine·2HCl, [U-$^{13}C_6$]glucose, [^{13}C-1]glucose, [^{13}C-2]glucose, [^{13}C-3]glucose, [^{13}C-4]glucose, [^{13}C-5]glucose and [^{13}C-6]glucose were all 98%+ and purchased from CIL (Andover, MA); DL-lysine·2HCl (99%, Fluka, Buchs, Switzerland), D-glucose (99%, BDH, Toronto, Canada), piperidine (99%, BDH, Poole, U.K.). Melting points were determined on an OptiMelt automated melting point system (Sunnyvale, CA). The ^{13}C and ^{1}H NMR spectra were acquired in CD_3OD on a 300 MHz Varian Unity spectrometer. Infrared spectra were recorded on a Bruker Alpha-P spectrometer (Bruker Optic GmbH, Ettlingen, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector, a temperature controlled single bounce diamond attenuated total reflectance (ATR) crystal and a pressure application device for solid samples.

Sample Preparation. The dihydrochloride salts of the commercially available isotopically labeled lysines were unreactive when pyrolyzed as such, however, mixing the salts with an equimolar amounts of unlabeled free lysine resulted in increased reactivity when pyrolyzed. Consequently, equimolar amounts of unlabeled DL-lysine and specifically labeled DL-lysine·2HCl were mixed and homogenized before mixing with equimolar amount of D-glucose. For isotope labeling studies [^{15}Nα]lysine·2HCl, [^{15}Nε]lysine·2HCl, [U-$^{13}C_6$]lysine·2HCl, [^{13}C-6]lysine·2HCl, [U-$^{13}C_6$]-glucose, [^{13}C-1]glucose, [^{13}C-2]glucose, [^{13}C-3]glucose, [^{13}C-4]glucose, [^{13}C-5]glucose and [^{13}C-6]glucose were used. Similar experiments with glycolaldehyde dimer, glyceraldehydes, 2,3-butanedione and pyruvic acid were conducted only to confirm the formation of piperdine in these systems.

ESI-TOF MS Samples. Glucose (10 mg) and lysine·2HCl (16 mg) were dissolved in distilled water (0.8 mL) and heated in an open vial at 110 °C for 45 min or until dryness, generating a brown powder. The experiments were repeated with [U-$^{13}C_6$]glucose and [^{15}Nα]lysine. The pyrolysis of this powder generated a similar profile to that of lysine glucose samples pyrolyzed without prior heating as described above.

Pyrolysis-GC/MS. Analyses were conducted using a Varian CP-3800 GC coupled with a Saturn 2000 Ion Trap Mass Spectrometer (Varian, Walnut Creek, CA). The pyrolysis unit included a CDS Pyroprobe 2000 and a CDS 1500 valved interface (CDS Analytical, Oxford, PA) installed onto the GC injection port. About 2.5 mg of sample mixture was packed inside a quartz tubes (0.3 mm thickness), plugged with quartz wool, and inserted inside the coil probe and pyrolyzed for 20 s at a temperature of 250 °C. The sample separation was carried out on a DB-5MS (5% diphenyl, 95% dimehtyl polysiloxane) capillary column with dimensions of 50 m length by 0.2 mm internal diameter and 0.33 μm film thickness (J&W Scientific, ON, Canada), using helium as the carrier gas. The GC column flow rate was regulated by an electronic flow controller (EFC) and set at a pressure pulse of 70 psi for the first 4 min and later maintained with a constant flow of 1.5 mL/min for the remainder of the run. The GC oven temperature was set at −5 °C for 5 min using CO_2 as the cryogenic cooling source. The temperature was increased to 50 °C at a rate of 50 °C/minute and then to 270 °C at a rate of 8 °C/minute, and kept at 270 °C for 5 min. The samples were detected by using an ion-trap mass spectrometer with a

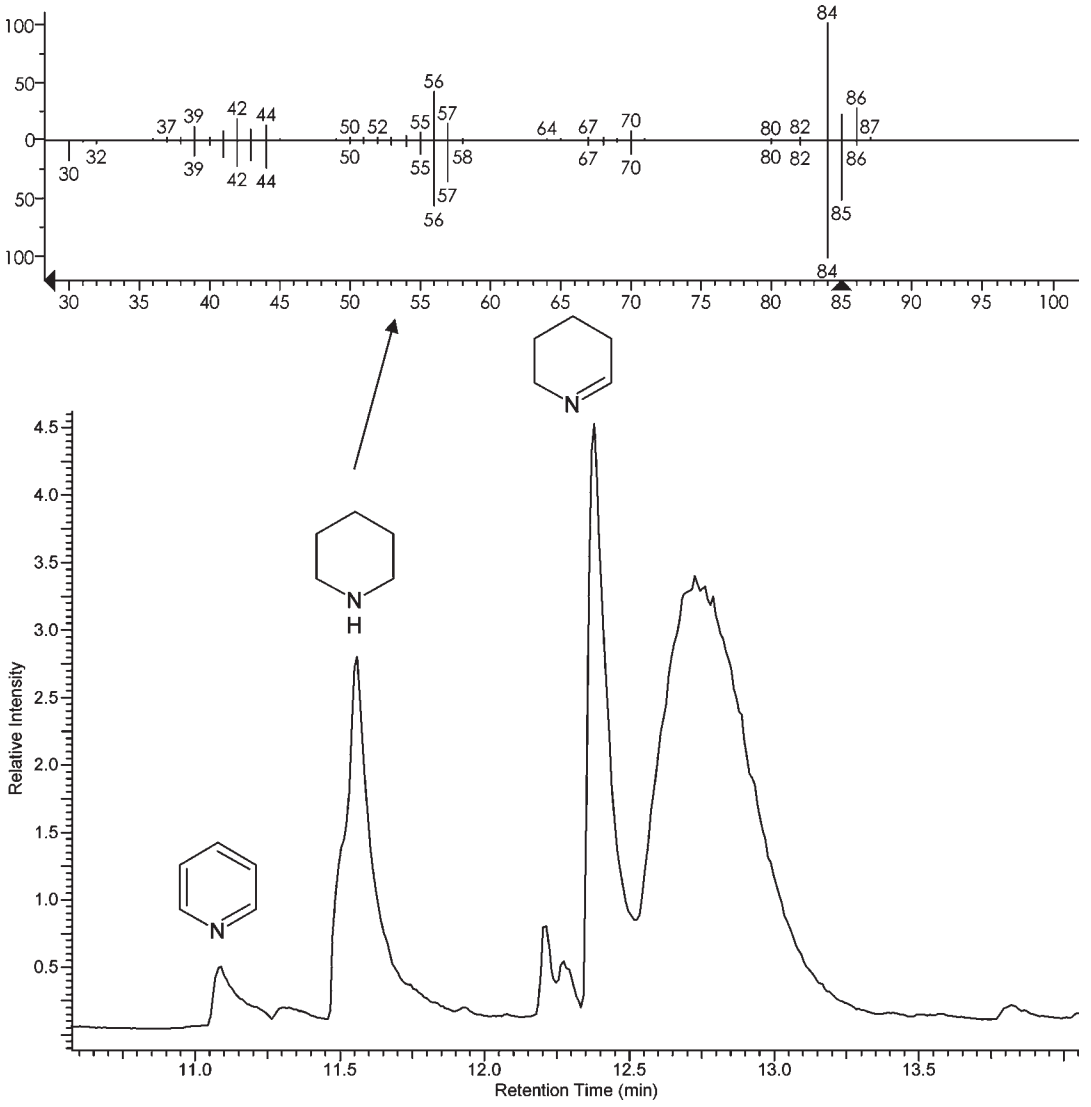


Figure 3. Partial GC chromatogram of lysine generated through pyrolysis at 250 °C showing the peak of piperidine at 11.5 min and its mass spectrum compared with authentic NIST library spectrum in a head-to-tail fashion.

scan range of 20−650 *m*/*z*. The MS transfer-line temperature was set at 250 °C, the manifold temperature was set at 50 °C, and the ion-trap temperature was set at 175 °C. An ionization voltage of 70 eV was used, and the EMV was set at 1700 V. The number of compounds was calculated using the peak area of analytes integrated by Varian MS Data Review software with the following parameters: peak width 2.0 s; slope sensitivity 4 (SN); tangent 10%; and peak size reject 10000 counts. Compound identification was performed using AMDIS (ver 2.65) and NIST Standard Reference Databases (data version 05 and software ver 2.0d) to compare the target compounds with the existing mass spectral libraries or by injecting commercially available standards. The reported percent label incorporation values (corrected for natural abundance and for percent enrichment) are the average of duplicate analyses and are rounded off to the nearest multiple of 5%.

ESI-TOF MS Analysis. Samples were diluted in 1 mL of water and then again 1/100 with 50% methanol and 0.1% formic acid. Each sample (5 μL injections) was directly analyzed by liquid chromatography−mass spectrometry (LC−MS), on a 1200 series Agilent rapid resolution LC system coupled to an Agilent 6210 time-of-flight (ESI-TOF) instrument. The mobile phase consisted of 50% methanol, 0.1% formic acid at a flow rate of 0.3 mL/min. Data were acquired in positive electrospray mode with an acquisition mass range of *m*/*z* 100−1000 and internal calibration using *m*/*z* 121.050873 and *m*/*z* 922.009798 (Agilent ESI tuning mix) for accurate mass measurements with a dual sprayer ESI source and constant infusion of calibrant ions. Source conditions were as follows: gas temperature 350 °C, ESI voltage 4000 V, dry gas flow (nitrogen) 12 L/min, nebulizer gas pressure 35 psig, fragmentor and skimmer voltages of 100 and 60 V, respectively.

Synthesis of 1,1′-[(5-Methylfuran-2-yl)methanediyl]dipiperidine (2). The 5-methylfurfural (50 mg) was added to an excess piperidine solution in the absence of any solvent and stirred at room temperature for 10 min or until heavy precipitate formed. The resulting solid was filtered and crystallized from acetonitrile to give white needles in almost quantitative yield, mp 61.2 °C. ^{1}H NMR: δ 1.45−1.56 (m, 12H, H-3′ to H-5′ and H-3″ to H-5″), 2.27 (s, 3H, H-1), 2.55 (t, 2H, H-2′), 2.65 (t, 2H, H-2″), 2.76 (t, 4H, H-6′,6″), 4.64 (s, 1H, H-6), 5.98 (s, 1H, H-4), 6.20 (s, 1H, H-3). ^{13}C NMR: δ 151.7 (C-2), 149.3 (C-5), 109.2 (C-3), 105.5 (C-4), 92.6 (C-6), 48.5 (C-6′,6″), 46.2 (C-2′,2″), 25.9 (C-3′,3″) 25.3 (C-5′,5″) 24.4 (C-4″) 24.3 (C-4′) 12.0 (C-1). FTIR (solid): 2965−2700 cm^{-1} (alkyl), 1000 cm^{-1} (C−N stretch), 1108 cm^{-1} (C−N stretch), 790 cm^{-1} (C═C*H*). MS *m*/*z* (% abundance): 39 (13.2), 41 (11.5), 43 (1.35), 84 (16.4), 95 (100), 96 (16.9), 97 (27.8), 98 (18.9), 136 (15.9), 178 (42.9), 179 (48.5) 180 (38.4).

RESULTS AND DISCUSSION

Analyses of the reaction mixtures generated through pyrolysis experiments from lysine and various sugars (glucose, glyceraldehydes and glycolaldehyde) and carbonyl compounds (2,3-butanedione, pyruvic acid) have indicated that under the experimental conditions all the model systems produced piperidine including lysine alone (see **Figure 3**). The identity of the piperidine was confirmed through mass spectral library searches and by

comparison of its retention time to an authentic sample (11.6 vs 11.5 min) and through spiking experiments where piperidine was copyrolyzed with lysine generating a more intense peak relative to the unspiked sample at the same retention time. In addition, labeling experiments (see subsequent sections) have also confirmed the exact origin of all the atoms originating from lysine. Furthermore, under the experimental conditions piperidine was the major product of lysine/glycolaldehyde mixture indicating its possible importance as an intermediate in lysine containing foods during roasting, baking, toasting, grilling and other processes associated with pyrolysis (*11*).

The Origin and Mechanism of Formation of Piperidine in Lysine/Glucose Model Systems. To investigate the origin of piperidine in the lysine/glucose model system, labeling studies were carried out using [^{15}Nα]lysine, [^{15}Nε]lysine, [U-$^{13}C_6$]lysine, [^{13}C-6]lysine and [U-$^{13}C_6$]glucose. When lysine/glucose model systems were analyzed piperidine was shown to incorporate predominantly

Table 1. Percent and Number of Labeled Atom Incorporation in Piperidine[a] Generated through the Interaction of Labeled Lysine in the Presence of Glucose

	[^{15}Nα]lysine	[^{15}Nε]lysine	[U-$^{13}C_6$]lysine	[^{13}C-6]lysine
% label incorporation	10%	90%	100%	100%
no. of labeled atoms	1	1	5	1

[a] Retention time 11.56 min (11.55 min standard).

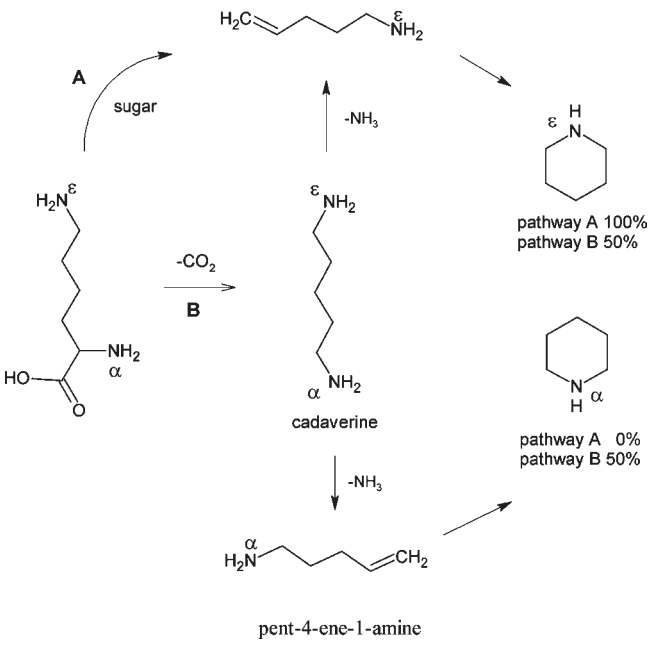


Figure 4. Proposed formation pathways of piperidine from lysine. Pathway A in the presence of sugars (to form decarboxylated Schiff base followed by pathway D) as shown in **Figure 2** where R = $-CH_2CH_2CH_2CH_2CH_2NH_2$ and pathway B in the absence of sugar through formation of cadaverine the decarboxylated lysine.

(>90%) Nε atom of lysine and the five carbon atoms of piperidine originated solely from the C2−C3−C4−C5−C6 carbon chain of lysine as shown in **Table 1**. Accordingly, no sugar carbon atom incorporation was observed eliminating the possibility of sugar carbon contribution to the structure of piperidine. The predominant formation of Nε-piperidine on the expense of Nα-piperidine in the glucose/lysine model system is significant in terms of determining its mechanistic origin. As shown in **Figure 4**, piperidine can be formed by the cyclization of pent-4-en-1-amine generated from lysine. In fact, pent-4-en-1-amine intermediate can be considered as the only logical precursor of piperidine that allows cyclization through a nucleophilic addition reaction (*12*) to form piperidine. The formation of piperidine from lysine alone can be rationalized by proposing decarboxylation of lysine to generate the symmetrical 1,5-diaminopentane or cadaverine (pathway B in **Figure 4**). This diamine can first deaminate into a 50% mixture of Nε- and Nα-pent-4-en-1-amines and then cyclize into piperidine in turn generating a 50% mixture of nitrogen isotopomers due to the symmetrical nature of the precursor. In fact, the addition of commercially available cadaverine to lysine enhanced the formation of piperidine by 15-fold implicating cadaverine as a precursor of piperidine in the lysine model system. If piperidine originated only from cadaverine (decarboxylated lysine) in the glucose/lysine system, then the observed incorporation of Nε atom would have been 50% and not 90%. To justify the predominant formation of Nε-piperidine in the lysine/glucose system, a pathway (**Figure 4** pathway A) that favors the exclusive deamination of Nα nitrogen over the Nε of lysine should be identified. One such pathway is shown in **Figures 2** and **5** (pathway A followed by D). This pathway, based on the mechanism of acrylamide formation (*4*, *5*, *13*), ensures deamination of only Nα nitrogen and exclusive generation of Nε-piperidine from the intermediate pent-4-en-1-amine (see **Figure 5**). Such intramolecular hydroamination reactions of alkenylamines similar to pent-4-en-1-amines have already been shown to be catalyzed by different agents including Brønsted acids at 100 °C in toluene to generate pyrrolidines and piperidines (*12*). In lysine/glucose model systems the formation of ~10% Nα-piperidine can be attributed to its direct formation from free lysine.

Isotope Labeling Studies for the Identification of Pent-4-en-1-amine Adducts. Only indirect evidence is presented above for the formation of pent-4-en-1-amine in the glucose/lysine system, through detection of its possible cyclization product the piperidine. However, due to the known reactivity of amines with aldehydes its further reaction products were also sought in glucose/lysine reaction mixtures using specifically labeled precursors such as [^{15}Nα]lysine·2HCl, [^{15}Nε]lysine·2HCl, [U-$^{13}C_6$]-lysine·2HCl, [^{13}C-6]lysine·2HCl and [U-$^{13}C_6$]glucose. Products simultaneously possessing Nε nitrogen atom and five carbon atoms from lysine (C2′ to C6′) in addition to glucose carbon atoms were specifically investigated to identify pent-4-en-1-amine related sugar adducts.

Analysis of lysine/glucose model systems yielded a total of 87 potential compounds. **Table 2** summarizes the percent distribution

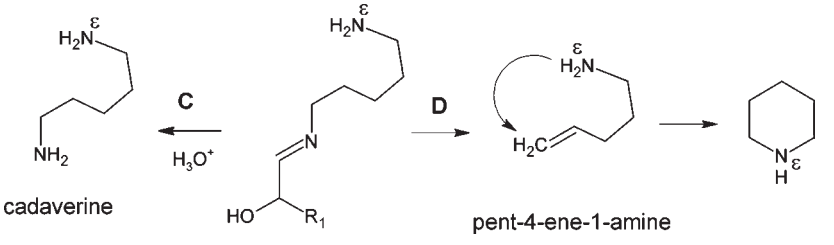


Figure 5. Proposed formation pathway of piperidine from the decraboxylated Schiff base shown in **Figure 2** (pathway D).

of Maillard reaction products possessing specific atoms from each reactant in the system. The labeling studies confirmed the presence of eleven compounds containing Nα atom of lysine and fifteen compounds possessing Nε atom, and three compounds were identified containing both nitrogens. Although the Nα atom incorporated into fewer peaks, however the total area of these peaks represented a much higher percentage contribution to the total abundance relative to compounds incorporating the Nε atom, which is in agreement with previous observations (*14*). **Table 2** also summarizes the percent distribution of Maillard reaction products in lysine/glucose system containing glucose and lysine carbon atoms.

Three compounds containing Nε and five carbon atoms of lysine (C2′ to C6′) in addition to glucose carbon atoms were detected. The label distribution is shown in **Table 3**. Such interaction products of lysine and glucose listed in **Table 3** can lead to the detection of pent-4-en-1-amine or piperidine-related adducts. It was observed that in these products glucose contributed either three or six carbons. In addition no product was identified possessing all six carbon atoms from lysine and the loss was always the C-1 atom most probably through decarboxylation. The incorporation of C-6′ of lysine was verified due to labeling experiments with [^{13}C-6]lysine.

Confirmation of Pent-4-en-1-amine Generation: Formation of Proposed *N*-(5-Methylfuran-2-yl)methylidene]penta-1,3-dien-1-amine (1). One of the reaction products possessing simultaneously one Nε nitrogen atom (mainly) and five carbon atoms (C2′−C5′) from lysine in addition to six glucose carbon atoms was observed at the retention time of 24.317 min with nominal molecular weight of *m*/*z* 175. The molecular weight and the labeling data (**Table 3**) suggested that the compound could arise from the interaction of a six carbon glucose moiety such as 5-methylfurfural (5-MF) or 5-hydroxymethylfurfural (HMF) with piperidine to form the structure **1′** shown in **Figure 6**. Furthermore, a peak possessing similar properties was generated from the LC−TOF-MS analysis

Table 2. Number and Percent Distribution of Peak Areas Incorporating Nα and Nε Nitrogens and Carbon Atoms in the Pyrolysis Products of the Lysine/Glucose Model System

	no. of peaks	percentage of total area
^{15}Nα + [^{13}C]glucose	11	18.1%
^{15}Nε + [^{13}C]glucose	15	10.6%
^{15}Nα + ^{15}Nε + [^{13}C]glucose	3	1.9%
[^{13}C]glucose atoms only	41	68.5%
undetermined		0.9%

Table 3. Percent Label Distribution[a] in Selected Products Generated from the Lysine/Glucose Model System

	M	M + 1	M + 2	M + 3	M + 4	M + 5	M + 6
Retention Time 19.435 min, MW 141							
[^{15}Nα]lysine	0	10	0	0	0	0	0
[^{15}Nε]lysine	0	90	0	0	0	0	0
[^{13}C-6]lysine	0	100	0	0	0	0	0
[^{13}U$_6$]lysine	0	0	0	0	0	100	0
[^{13}U$_6$]glucose	0	0	0	100	0	0	0
Retention Time 24.03 min, MW 137							
[^{15}Nα]lysine	0	10	0	0	0	0	0
[^{15}Nε]lysine	0	90	0	0	0	0	0
[^{13}C-6]lysine	0	100	0	0	0	0	0
[^{13}U$_6$]lysine	0	0	0	0	0	100	0
[^{13}U$_6$]glucose	0	0	0	100	0	0	0
Retention Time 24.317, MW 175							
[^{15}Nα]lysine	0	10	0	0	0	0	0
[^{15}Nε]lysine	0	90	0	0	0	0	0
[^{13}C-6]lysine	0	100	0	0	0	0	0
[^{13}U$_6$]lysine	0	0	0	0	0	100	0
[^{13}U$_6$]glucose	0	0	0	0	0	0	100

[a] Corrected and adjusted for the 50% mix.

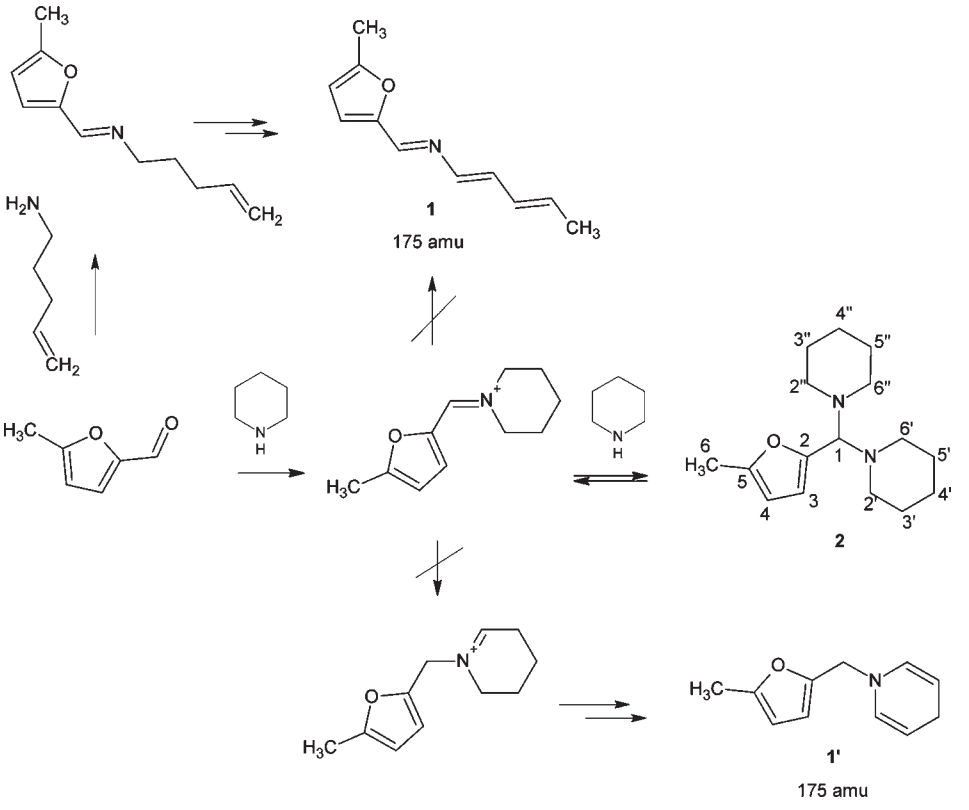
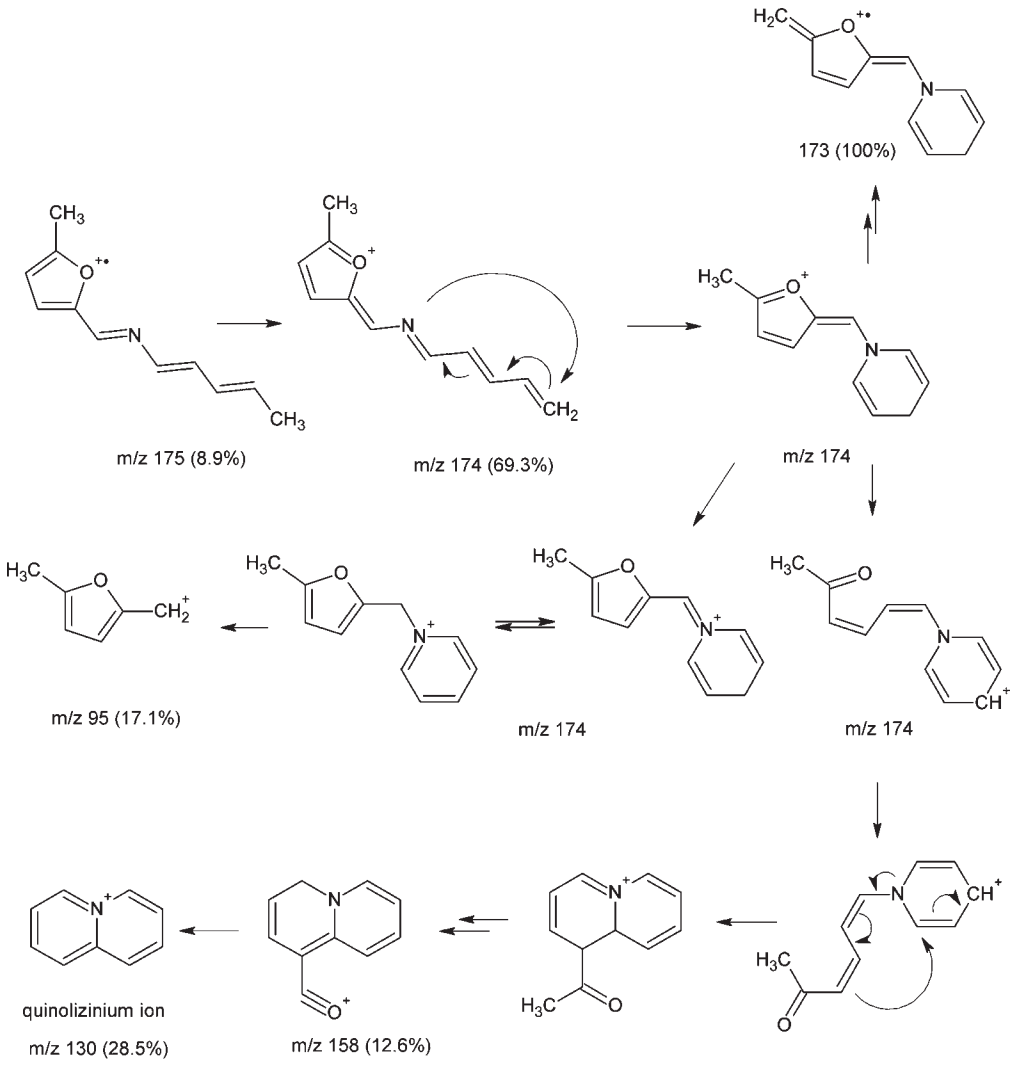


Figure 6. Proposed reaction of 5-methylfurfural with pent-4-en-1-amine and piperidine.

Table 4. Summary of Structural Information for the Proposed Structure *N*-(5-Methylfuran-2-yl)methylidene]penta-1,3-dien-1-amine (**1**)[a]

MW $[M + H]^+$	176.1068 amu	TOF-MS
elemental formula	$C_{11}H_{13}NO$	TOF-MS
no. of C atoms from glucose	C-1, C-2, C-3, C-4, C-5, C-6	labeling studies with MS
no. and identity of lysine atoms	Nε, C-2′, C-3′, C-4′, C-5′, C-6′ (90%); Nα, C-2′, C-3′, C-4′, C-5′, C-6′ (10%)	labeling studies with Py-GC/MS
partial elemental formula	$C_{11}N\varepsilon$ (90%), $C_{11}N\alpha$ (10%)	labeling studies with Py-GC/MS
precursor studies		
enhanced signal	glucose, 5-MF, lysine	Py-GC/MS
no change in signal	HMF, piperidine, 1,1′-[(5-methylfuran-2-yl)methanediyl]dipiperidine (**2**)	Py-GC/MS, synthesis
mass spectrum	Labeled mass spectral fragments consistent with proposed structure	Py-GC/MS (see **Figure** 7)
necessary precursor moieties	5-MF and pent-4-en-1-amine	labeling studies with MS, precursor studies, elemental formula and MW

[a] 5-MF = 5-methylfurfural, HMF = hydroxymethylfurfural.



m/z	175	174	173	158	130	95
[$^{13}U_6$]Glucose	+6	+6	+6	+5	+4	+6
[$^{13}U_6$]Lysine	+5	+5	+5	+5	+5	0
[^{13}C-6]lysine	+1	+1	+1	+1	+1	0
[^{15}Nε] Lysine	+1	+1	+1	+1	+1	0
[^{15}Nα] Lysine	+0	+0	+0	+0	+0	0

Figure 7. Mass spectral fragmentation pattern of compound **1** and the number of labeled atoms incorporated into the major mass spectral fragments.

of an aqueous model system consisting of glucose and lysine heated to dryness at 110 °C for 45 min in an open vial. The $[M + H]^+$ ion had a molecular weight of 176.1068 amu corresponding to elemental composition of $C_{11}H_{13}NO$ for the unprotonated species. This was consistent with the label incorporation data of 6 (glucose) + 5 (lysine) carbons and a nitrogen atom generated from Py-GC/MS analysis. The spiking experiments with 5-MF yielded a significant increase in the abundance of the peak at

24.317 min in the lysine/glucose model system. Similar spiking experiments with HMF did not change significantly the intensity of the peak at 24.317 min. On the other hand, if this unknown peak was indeed the result of piperidine reaction with 5-MF, the interaction of the two should generate the same compound. In an attempt to synthesize this adduct we obtained instead a solid product (see Materials and Methods) that matched the structural properties of 1,1′-[(5-methylfuran-2-yl)methanediyl]dipiperidine (**2**) based on ^{13}C and ^{1}H NMR analysis (see **Figure 6**). However, the pyrolysis of **2** did not generate compound **1** or any peak with *m*/*z* value of 175. In addition, spiking of glucose/lysine model system with excess piperidine also did not enhance the intensity of the peak in question. Similarly, model systems of piperidine/HMF or piperidine/5-MF did not generate the peak in question. Based on the elemental composition of compound **1** and the number and origin of carbon and nitrogen atoms from glucose and lysine (see **Table 4**) and based on the fact that 5-MF significantly enhances the intensity of this peak in the presence of glucose, we propose that the Schiff base adduct is formed between 5-MF and pent-4-en-1-amine instead of piperidine and undergoes oxidation to generate the extensively conjugated compound **1** as depicted in **Figure 6**. This proposed structure is consistent not only with the isotope labeling data incorporating a non-piperidine moiety possessing C2′−C′6 atoms including Nε of lysine and six carbons of glucose in the form of 5-MF but also with the spiking experiments with piperidine and 5-MF. Furthermore, **Figure 7** shows the mass spectral fragmentation of compound **1** consistent with the labeling data. In addition, experiments carried out with specifically labeled glucoses have also indicated that the fragment at *m*/*z* 158 showed no incorporation of C-6 atom of glucose and the fragment at *m*/*z* 130 showed no incorporation of both C-5 and C-6 atoms of glucose as expected from the proposed fragmentation pattern shown in **Figure 7**.

Complete isotope mapping and precursor studies of the unknown compound **1** together with other relevant information shown in **Table 4** have indicated that the compound in question is composed of both glucose and lysine moieties and that the lysine moiety can only arise from pent-4-en-1-amine or one of its noncyclic derivatives such as penta-1,3-dien-1-amine and not from piperidine, confirming the ability of lysine to undergo a similar chemical transformation to that of asparagine in generating amino acid derived vinylic compounds.

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