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Role of the Ribose-Specific Marker Furfuryl-amine in the Formation of Aroma Active 1-(Furan-2-ylmethyl)-1*H*-pyrrole (or Furfuryl-pyrrole) Derivatives

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ABSTRACT: Furfuryl-pyrroles possess a diverse range of organoleptic properties described as roasted, chocolaty, green, horseradish-like, and mushroom-like and are detected in various foods such as coffee, chocolate, popcorn, and roasted chicken. Although their origin in food was attributed to furfuryl-amine, the latter has not been detected so far in Maillard model systems or in foods. In this study, furfuryl-amine was shown to be formed specifically from ribose through nitrogen atom transfer from the α-amino group of any amino acid. Such a transfer can be achieved through decarboxylation of the Schiff base adduct and isomerization followed by hydrolysis. Through the use of $^{15}N\alpha$-lysine it was revealed that only the $^{15}N\alpha$ nitrogen atom was incorporated into its structure, indicating a specific role for the carboxylate moiety in the mechanism of its formation. Furthermore, isotope labeling studies have indicated that furfuryl-pyrrole derivatives can be formed by the interaction of 2 mol of furfuryl-amine with 3-deoxyribosone followed by dehydration and cyclization to form 1-(furan-2-yl)-*N*-{[1-(furan-2-ylmethyl)-1*H*-pyrrol-2-yl]methylidene}methanamine. After hydrolysis, this intermediate can generate furfuryl-formyl-pyrrole, furfuryl-pyrrole carboxylic acid, and furfuryl-pyrrole. In this study, the furfuryl-amine derivatives were also detected in different coffee beans after pyrolysis and analysis by GC-MS. The potential of these compounds to form in aqueous model systems at a temperature of 120 °C was also demonstrated.

KEYWORDS: *furfuryl-amine, ribose, furfuryl-pyrroles, isotope labeling, formation mechanism*

INTRODUCTION

Although furfuryl-amine (**1** in Figure 1) has not been detected in food or in model systems, it has been postulated to be the precursor[1] of various pyrrole-containing furan derivatives[2,3] such as furfuryl-pyrrole (**2**) shown in Figure 1. The latter has been identified in many amino acid model systems including cysteine,[4] tryptophan,[1] serine, and threonine[5] and in foods including coffee,[6] dark chocolate,[2] chicory aroma,[7] popcorn,[8] and roasted peanuts[3] and has been found as an aroma constituent in bread, roasted chicken, and sandalwood oil.[9] Furfuryl-pyrrole has been shown to possess important organoleptic properties described as roasted, chocolatey, green,[2] horseradish-like, mustard seed, and mushroom-like.[10] Another proposed furfuryl-amine derivative, furfuryl-formyl-pyrrole (**3**), has been detected in model systems including tryptophan,[1] serine, and glutamine and as one of the main volatile products in a ribose/threonine system.[5] In foods, compound **3** has been identified in coffee,[6] roasted peanuts,[3] popcorn,[8] and chicory aroma.[7] The origin of the pyrrole moiety in furfuryl-pyrrole derivatives (**2** and **3** in Figure 1) has not been confirmed; however, it was first postulated[1] to be formed in a manner similar to the nucleophilic attack by amino groups to the carbon 5 position of the furan moiety[11] (Figure 1). Tressl et al.,[12] on the other hand, suggested that the source of the pyrrole moiety in furfuryl-pyrrole was due to not only the furfural but also the presence of hydroxyproline (for details see section 3.3). This hypothesis was further supported when both furfuryl-pyrrole and furfuryl-formyl-pyrrole were observed to experience 130- and 20-fold increases in peak areas, respectively, when dent corn was spiked with hydroxyproline.[8] Due to the importance and widespread occurrence of furfuryl-pyrrole derivatives, their origin and mechanism of formation were investigated in both dry and aqueous Maillard model systems utilizing ribose, arginine, and lysine as precursors. Both lysine and arginine models generated similar furfuryl-pyrrole derivatives.

MATERIALS AND METHODS

Materials. Arginine (>98%), arginine-HCl (>98.5), ornithine-HCl (>99%), citrulline (>98), lysine (98%), lysine-HCl (>99%), ribose (>99), furfural (99%), 5-methylfurfural (99%), furfuryl alcohol (99%), furfuryl-amine (98%), and 1-furfurylpyrrole (98%) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). [$^{15}N\alpha$]Lysine-2HCl, [U-$^{13}C_5$]ribose, and [^{13}C-1]ribose were all >98% enriched and purchased from CIL (Andover, MA, USA). D-Glucose (99%) was from BDH (Toronto, Canada). Coffee samples were purchased from local markets.

Pyrolysis-GC-MS Analysis. Analyses were conducted using a Varian CP-3800 GC coupled with a Saturn 2000 ion trap mass spectrometer (Varian, Walnut Creek, CA, USA). The pyrolysis unit included a CDS Pyroprobe 2000 and a CDS 1500 valved interface (CDS Analytical, Oxford, PA, USA) installed onto the GC injection port. Between 0.5 and 1.5 mg of a sample mixture (see Table 1) was packed inside a quartz tube (0.3 mm thickness), plugged with quartz wool, inserted inside the coil probe, and pyrolyzed for 20 s at a temperature of 250 °C. The separation was carried out on a DB-5MS

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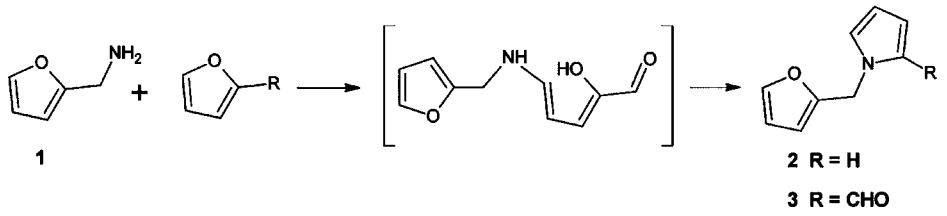

Figure 1. Proposed formation pathway of furfuryl-pyrrole derivatives according to Baltes et al.[1]

(5% diphenyl, 95% dimethylpolysiloxane) 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/min and then to 270 °C at a rate of 8 °C/min and kept at 270 °C for 5 min. The samples were detected by using an ion-trap mass spectrometer with a scan range of m/z 20−650. 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 EMV was set at 1700 V. Structural identification was performed using AMDIS (ver. 2.65) and NIST Standard Reference Databases (data version 05 and software ver. 2.0d) and by comparison of the retention times and mass spectra to those of commercially available standards in addition to isotope labeling data. 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%.

Sample Preparation. The dihydrochloride salts of the commercially available isotopically labeled lysines were unreactive when pyrolzed as such; however, mixing the salts with an equimolar amount of unlabeled free lysine resulted in increased reactivity when pyrolzyed. Consequently, equimolar amounts of unlabeled DL-lysine and specifically labeled DL-lysine-2HCl were mixed and homogenized before mixing with an equimolar amount of D-glucose. Coffee samples (1 mg) were pyrolyzed according to the above-described procedure.

Aqueous Samples. An equimolar (0.1 M) ratio of ribose and arginine-HCl was dissolved in distilled water (1 mL), and the sample was placed in a sealed Q-tube reactor (Q Labtech LLC) and heated at 120 °C for 20 min. The sample was dried under a fume hood, and the residue was dissolved in methanol and directly injected into the above-mentioned GC-MS system using the same parameters for analysis except the oven temperature was set at 41 °C and the temperature was increased to 50 °C at a rate of 10 °C/min and then to 270 °C at a rate of 8 °C/min and kept at 270 °C for 5 min.

RESULTS AND DISCUSSION

The postulated furfuryl-amine derivatives mentioned above have been shown to form independently of the type of amino acid used in the model systems.[1,4,5] In contrast to the lack of specificity of the precursor amino acids, Chen et al.[5] have demonstrated that furfuryl-amine derivatives were formed exclusively from ribose-containing model systems and that glucose or fructose is unable to generate such derivatives. Rizzi[11] attributed the formation of furfuryl-amine derivatives from ribose models to ribose's ability to form furfural; similarly, Baltes et al.[1] suggested that furfuryl-amine can be formed during the hydrolysis of the imine bond of a decarboxylated amino acid-furfural adduct. On the other hand, Shibamota et al.[13] have proposed ammonia as a potential source of nitrogen in the formation of furfuryl-amine from furfural. To investigate the mechanism of formation of furfuryl-amine, model systems listed in Table 1 were analyzed, and here for the first time we report the detection of furfuryl-amine (**1**) in these model systems.

Mechanism of Formation of Furfuryl-amine in Ribose/Amino Acid Model Systems. So far, furfuryl-amine has been assumed to be the precursor of furfuryl-pyrrole derivatives without being detected or identified in the same model systems. When the ribose/arginine model system was analyzed as described under Materials and Methods, a prominent peak was detected at the retention time of 12.796 min, matching the mass spectral fragmentation pattern and the retention time of a commercial standard of furfuryl-amine (see Table 2) and that of the NIST library. Spiking experiments with the standard sample further confirmed the identity of the peak. The same peak was also generated when arginine was replaced with lysine and with various other amino acids (see Table 1); however, when ribose was replaced with glucose or fructose, the furfuryl-amine peak was not observed. Furthermore, isotope labeling experiments with $^{13}U_5$-ribose and $^{15}N\alpha$-lysine confirmed the presence of five carbon atoms from ribose and one Nα nitrogen atom (100% incorporation) from lysine in the furfuryl-amine structure; no Nε nitrogen was incorporated in the structure of furfuryl-amine (Table 2). To examine the role of furfural in the formation of furfuryl-amine, furfural was also reacted with ammonium chloride and with several amino acids; however, none of the models studied generated furfuryl-amine, including glucose/ammonium chloride and ribose/ammonium chloride model systems, thus excluding furfural and ammonia as major precursors of furfuryl-amine.

Clearly, furfuryl-amine is formed by the interaction of any amino acid with ribose, through Nα atom transfer at the carbon 1 position of ribose as indicated by the isotope labeling data in Table 2. Because ammonia was excluded as the source of nitrogen, one mechanism that is capable of rationalizing such Nα transfer from amino acids into carbonyl compounds is shown in Figure 2; this proposed mechanism is based on the formation of a 5-oxazolidinone intermediate followed by a decarboxylation step[14,15] to eventually form decarboxylated and isomeric Schiff bases of which one isomer can hydrolyze to transfer the nitrogen atom at the C-1 position of ribose and release the Strecker aldehyde (see Figure 2). Amino groups that are not located at the α-position of the carboxylic acid are not able to undergo such transformation due to their inability to form 5-oxazolidinone intermediates. This fact can rationalize the observation that only the Nα atom was transferred into the furfuryl-amine structure from lysine. As shown in Figure 2 the initial intermediate can undergo dehydration and cyclization reactions leading to furfuryl-amine.

Proposed Mechanism of Formation of Furfuryl-pyrrole (FP) and Other Derivatives of Furfuryl-amine. As speculated in the literature, furfuryl-amine derivatives furfuryl-pyrrole and furfuryl-formyl-pyrrole (FFP) were also detected in the same model systems studied in which furfuryl-amine was observed. Furfuryl-pyrrole (**2**) eluted at the

Table 1. Composition of Model Systems Analyzed by Py-GC-MS[a]

compound/target ion	model
furfuryl-amine (**1**) *m/z* 97	ribose + arginine-HCl
	ribose + lysine-HCl
	ribose + ornithine-HCl
	ribose + citrulline
	ribose + furfuryl-amine
furfuryl-pyrrole (**2**) *m/z* 147	ribose + arginine-HCl
	ribose + arginine-HCl (aqueous)
	ribose + lysine-HCl
	ribose + ornithine-HCl
	ribose + citrulline
	ribose + hydroxyproline
	ribose + furfuryl-amine
	furfural + arginine-HCl
	furfural + hydroxyproline
	glucose + arginine-HCl + furfuryl-amine
	1-furfuryl-pyrrole
furfuryl-formyl-pyrrole (**3**) *m/z* 175	ribose + arginine-HCl
	ribose + arginine-HCl (aqueous)
	ribose + lysine-HCl
	ribose + ornithine-HCl
	ribose + citrulline
	ribose + furfuryl-amine
	glucose + arginine-HCl + furfuryl-amine
furfurylidene-furfuryl-amine (**4**) *m/z* 175	ribose + arginine-HCl
	ribose + lysine-HCl
	ribose + ornithine-HCl
	ribose + citrulline
	ribose + furfuryl-amine
	furfural + arginine-HCl
	furfural + furfuryl-amine
	glucose + arginine-HCl + furfuryl-amine
1-(furan-2-yl)-*N*-{[1-(furan-2-ylmethyl)-1*H*-pyrrol-2-yl] methylidene}methanamine (**5**) *m/z* 254	ribose + arginine-HCl
	ribose + lysine-HCl
	ribose + ornithine-HCl
	ribose + citrulline
	ribose + arginine-HCl + furfuryl-amine
di-(2,2′-furfuryl)amine (**7**) *m/z* 177	ribose + arginine-HCl
	ribose + lysine-HCl
	ribose + ornithine-HCl
	furfuryl alcohol + furfuryl-amine
1-(furan-2-yl)-*N*,*N*-bis(furan-2-ylmethyl)methanamine (**8**) *m/z* 257	ribose + arginine-HCl
	ribose + lysine-HCl
	ribose + ornithine-HCl
	ribose + furfuryl-amine
	furfuryl-amine

[a]Model systems containing ribose or lysine were also analyzed using their labeled counterparts ([$^{13}U_5$]ribose or [^{13}C-1]ribose and [$^{15}N\alpha$]-lysine).

retention time of 19.8 min, and its identity was confirmed by matching the mass spectral fragmentation pattern and the retention time of a commercial standard (see Table 3) and through the NIST library searchers. Spiking experiments with

Table 2. Number of Isotopically Labeled Atoms Incorporated in Furfuryl-amine[a] (1) Generated in Ribose/Lysine Models[a]

	m/z				
	97	81	69	54	39
[$^{13}U_5$]ribose	5	5	3	3	3
[^{13}C-1]ribose	1	1	0	0	0
[$^{15}N\alpha$]lysine	1	0	1	0	0

[a]t_R = 12.796 min, standard t_R = 12.779 min, mw 97. Model: *m/z* (%) 39 (40.9), 53 (16.2), **69 (100)**, 81 (56.2), 96 (45.1), 97 (39.6). Commercial standard: *m/z* (%) 39 (50.2), 53 (38.7), **69 (100)**, 81 (21.7), 96 (57.2), 97 (78.4). NIST: *m/z* (%) 39 (37.0), 53 (46.2), **69 (100)**, 81 (27.5), 96 (26.8), 97 (53.9).

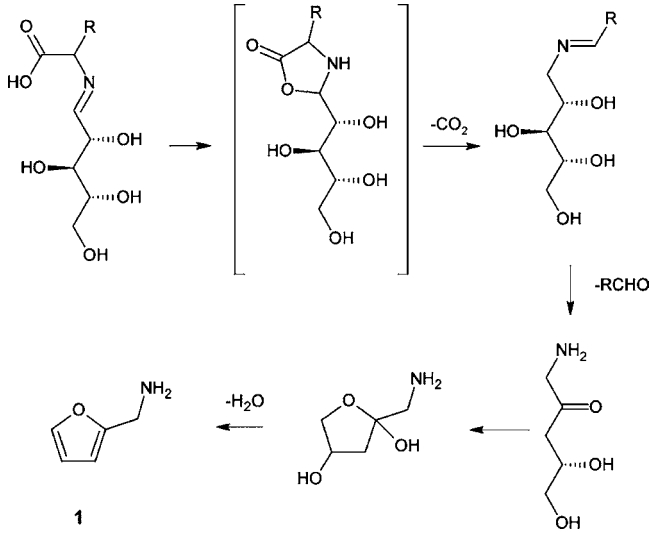

Figure 2. Proposed mechanism of formation of furfuryl-amine (**1**) from Schiff base adducts of ribose with any α-amino acid.

Table 3. Number of Isotopically Labeled Atoms Incorporated in Furfuryl-pyrrole[a] (2) Generated in Ribose/Lysine-HCl Models

	m/z			
	147	81	53	39
[$^{13}U_5$]ribose	9	5	4	3
[^{13}C-1]ribose	1	1	1	0
[$^{15}N\alpha$]lysine	1	0	0	0

[a]t_R = 19.813, standard t_R =19.792, mw 147. Model: *m/z* (%) 39 (9.40), 53 (33.6), **81(100)**, 147 (77.2). Commercial standard: *m/z* (%) 39 (10.7), 53 (33.8), **81 (100)**, 147 (83.9). NIST: *m/z* (%) 39 (9.0), 53 (38.3), **81(100)**, 147 (47.0).

the standard sample further confirmed the identity of the peak. Isotope labeling studies have indicated that all nine carbon atoms of FP originated from ribose with the loss of one carbon atom from the C-1 position and only one nitrogen atom originated from Nα of lysine as in the case of furfuryl-amine (Table 3). Furthermore, reacting ribose with a 50:50 mixture of [$^{15}N\alpha$]lysine and ammonium chloride generated the furfuryl-pyrrole peak with 100% $^{15}N\alpha$-lysine incorporation, thus ruling out ammonia as a source of nitrogen. Interestingly, a model system consisting of only ribose and furfuryl-amine also generated furfuryl-pyrrole without the need of an amino acid, indicating furfuryl-pyrrole can result through the interaction of ribose with furfuryl-amine. A proposed mechanism consistent

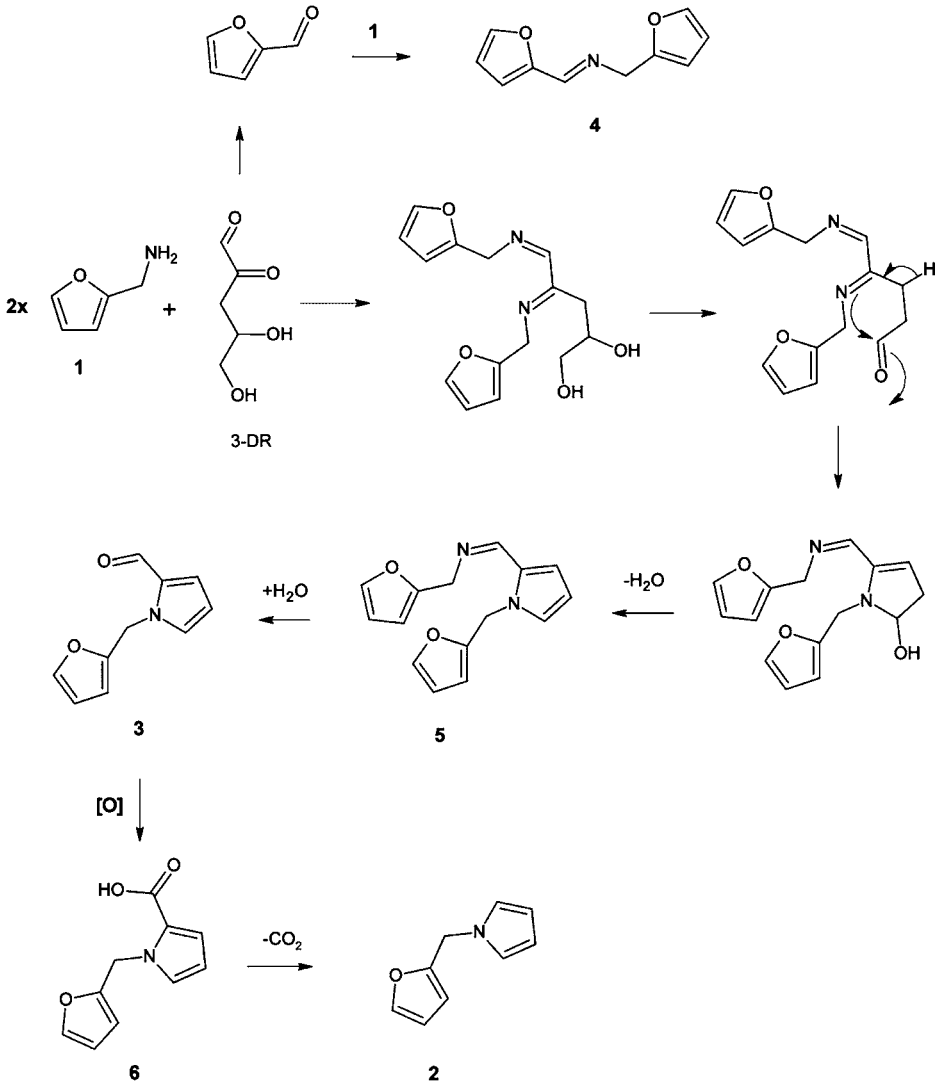

Figure 3. Proposed mechanism of formation of furfuryl-pyrole (**2**) and other furfuryl-amine derivatives from the interaction of furfuryl-amine (**1**) with 3-deoxyribosone (3-DR).

with the above observations is shown in Figure 3, which postulates double Schiff base formation between 3-deoxyribosone and 2 mol of furfuryl-amine. Intramolecular cyclization of this intermediate followed by dehydration can generate furfuryl-pyrrole derivative **5**. Hydrolysis of **5** can form furfuryl-formyl-pyrrole (**3**), which in turn after oxidation into a carboxylic acid functionality can undergo decarboxylation with the loss of the C-1 atom from ribose to form furfuryl-pyrrole (**2**) as confirmed above through isotope labeling experiments.

The proposed intermediate **3** was observed as the highest peak in the chromatogram at a retention time of 24.12 min (see Table 4) and was tentatively identified as furfuryl-formyl-pyrrole on the basis of the matching of its reported mass spectrum[1] and the complementary isotope labeling data that confirmed incorporation of 10 carbon atoms from ribose and only 1 Nα atom from lysine (see Table 4). Consistent with the proposed mechanism, furfuryl-formyl-pyrrole is often detected in tandem with furfuryl-pyrrole in food samples such as coffee,[6] popcorn,[8] roasted peanut,[3] and chicory aroma,[7] and in different model systems including serine/threonine with ribose[5] and tryptophan.[1] We were also able to detect both compounds when commercially obtained roasted coffee beans were

Table 4. Number of Isotopically Labeled Atoms Incorporated in Furfuryl-formyl-pyrrole[a] (3) Generated in Ribose/Lysine-HCl Models

	m/z			
	175	147	81	53
[$^{13}U_5$]ribose	10	9	5	4
[^{13}C-1]ribose	2	1	1	1
[^{15}Nα]lysine	1	1	0	0

[a] t_R = 24.122 min, mw 175. Model: *m/z* (%) 39 (6.60), 53 (21.8), **81** (**100**), 146 (7.6), 175 (63.5). Literature:[1] *m/z* (%) 39 (12.0), 53 (29.0), **81** (**100**), 146 (2.0), 175 (28.0).

pyrolyzed at 250 °C. On the other hand, intermediate **6** was not observed in this investigation, although a compound consistent with the structure of **6** has been detected and reported in ribose/tryptophan[1] model systems together with **2** and **3**, further confirming the sequence of steps proposed in Figure 3. Furthermore, a peak at a retention time of 31.442 min consistent with the proposed structure of intermediate **5** was also detected (see Table 5). As expected, isotope labeling data (Table 5) indicated the incorporation of 15 carbon atoms from

Table 5. Number of Isotopically Labeled Atoms Incorporated in the Proposed Structure 1-(Furan-2-yl)-*N*-{[1-(furan-2-ylmethyl)-1*H*-pyrrol-2-yl]methylidene}methanamine[a] (5) Generated in Ribose/Lysine-HCl Models

	m/z						
	254	225	173	146	118	81	53
[$^{13}U_5$]ribose	15	14	10	9	8	5	4
[^{13}C-1]ribose	3	3	2	1	1	1	1
[^{15}Nα]lysine	2	2	2	1	1	0	0

[a] t_R = 31.448 min, mw 254, *m/z* (%):53 (41.4), 81 (56.4), 118 (23.5), 146 (33.1), **173** (**100**), 225 (34.4), 254 (77.7). The mass spectral fragments shown and label incorporation pattern indicated in the table are consistent with the proposed structure of **5**.

ribose and 2 Nα atoms from lysine. In addition, spiking the ribose/arginine model system with furfuryl-amine resulted in a nearly 260-fold increase in intensity of intermediate **5**, whereas the peak was not detected at all in furfural/lysine or arginine models.

In general, ribose-containing model systems are known to generate furfural as a major product,which may react with furfuryl-amine and form a Schiff base adduct similar to amino acids;[16,17] such a predicted product (structure **4**) was reported by Baltes et al.[1] in ribose-containing model systems, and a peak having a retention time at 23.8 min matched its mass spectral fragmentation pattern and that reported in the NIST library (see Table 6). In fact, a peak having the same retention time

Table 6. Number of Isotopically Labeled Atoms Incorporated in Furfurylidene-furfuryl-amine[a] (4) Generated in Ribose/Lysine-HCl Models

	m/z			
	175	147	81	53
[$^{13}U_5$]ribose	10	9	5	4
[^{13}C-1]ribose	2	2	1	1
[^{15}Nα]lysine	1	1	0	0

[a] t_R = 23.749 min, mw 175. Model: *m/z* (%) 39 (11.0), 53 (32.4), **81** (**100**), 147 (19.6), 175 (24.2). Literature:[1] *m/z* (%) 39 (7.0), 53 (20.0), **81** (**100**), 175 (17.0). NIST: *m/z* (%) 39 (14.6), 53 (29.8), **81** (**100**), 147 (5.0), 175 (21.3).

and mass spectral fragmentation pattern as those of **4** was generated as the major product from furfural and furfuryl-amine reaction, confirming its proposed precursors. The peak experienced an approximately 25-fold increase in intensity when the ribose/arginine model was spiked with furfuryl-amine. Isotope labeling studies reported in Table 6 further confirm the presence of 10 carbon atoms from ribose and 1 Nα from lysine.

Hydroxyproline-Specific Pathway for Furfuryl-pyrrole (2). One of the most commonly detected furfuryl-amine derivatives, furfuryl-pyrrole (**2**) has been also postulated to be formed as the result of the interaction between furfural and hydroxyproline.[12] To confirm this specific pathway, hydroxyproline reaction with ribose was investigated, and indeed the model generated a 600-fold excess of furfuryl-pyrrole relative to the ribose/arginine model. Although this pathway is more efficient, it is only specific to a rare[18] amino acid in food, hydroxyproline, and generates only one furfuryl-amine derivative. The proposed mechanism shown in Figure 4 is based on the reported reaction of furfural with secondary amines.[16]

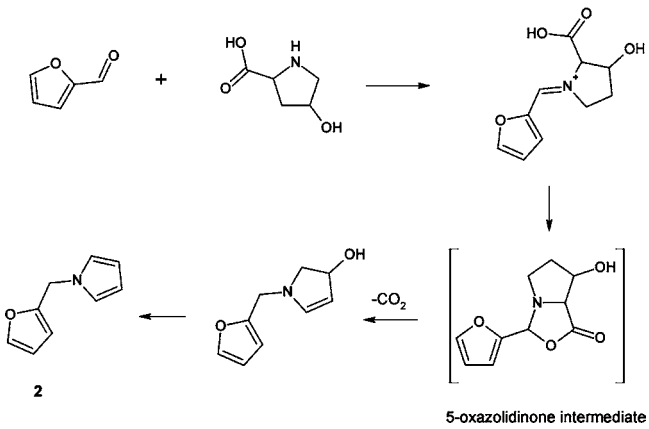

Figure 4. Alternate formation pathway of furfuryl-pyrrole (**2**) through hydroxyproline-specific reaction with furfural.

Other Reactions of Furfuryl-amine. In addition to the above-mentioned furfuryl-amine derivatives, other non-pyrrole-containing adducts of furfuryl-amine were also identified in the same model systems studied. A peak detected at 22.98 min in a ribose/arginine model was tentatively characterized as di-(2,2′-furfuryl)amine (**7**) on the basis of mass spectral evidence[1] and NIST library searches in addition to the data based on isotope labeling experiments (Table 7). The data indicated the

Table 7. Number of Isotopically Labeled Atoms Incorporated in Di-(2,2′-furfuryl)amine[a] (7) Generated in Ribose/Lysine-HCl Models

	m/z				
	177	109	96	81	53
[$^{13}U_5$]ribose	10	6	5	4	4
[^{13}C-1]ribose	2	2	1	1	1
[^{15}Nα]lysine	1	1	1	0	0

[a] t_R = 22.982 min, mw 177. Model: *m/z* (%) 39 (8.2), 53 (30.2), **81**(**100**), 96 (21.1), 109 (40.8), 177 (3.5). Literature:[1] *m/z* (%) 39 (5.0), 53 (21.0), **81**(**100**), 96 (25.0), 109 (27.0), 177 (3). The mass spectral fragments shown and label incorporation pattern indicated in the table are consistent with the proposed structure of **7**.

incorporation of 10 carbon atoms from ribose and 1 Nα from lysine. The same peak was also generated from furfuryl alcohol reaction with furfuryl-amine or only from furfuryl-amine, prompting us to propose 2-methylidene-2*H*-furanium ion as the key intermediate in the mechanism of formation of **7**; the furanium intermediate then can react with furfuryl-amine to generate **7** (see Figure 5). If this assumption is correct, the resulting new amine (**7**) should similarly interact with the furanium ion to generate the trimeric derivative 1-(furan-2-yl)-*N*,*N*-bis(furan-2-ylmethyl)methanamine (**8**) shown in Figure 5. Indeed, a peak at the retention time of 29.211 min in a ribose/lysine model system and possessing 15 carbons atoms from ribose and 1 nitrogen atom from lysine was detected matching the elemental formula and the mass fragmentation profile of the proposed structure (see Table 8). As shown in Figure 5, the furanium ion can be generated from either furfuryl alcohol or furfuryl-amine. Furfuryl alcohol has been previously detected in model systems containing ribose,[19] and in the present study it

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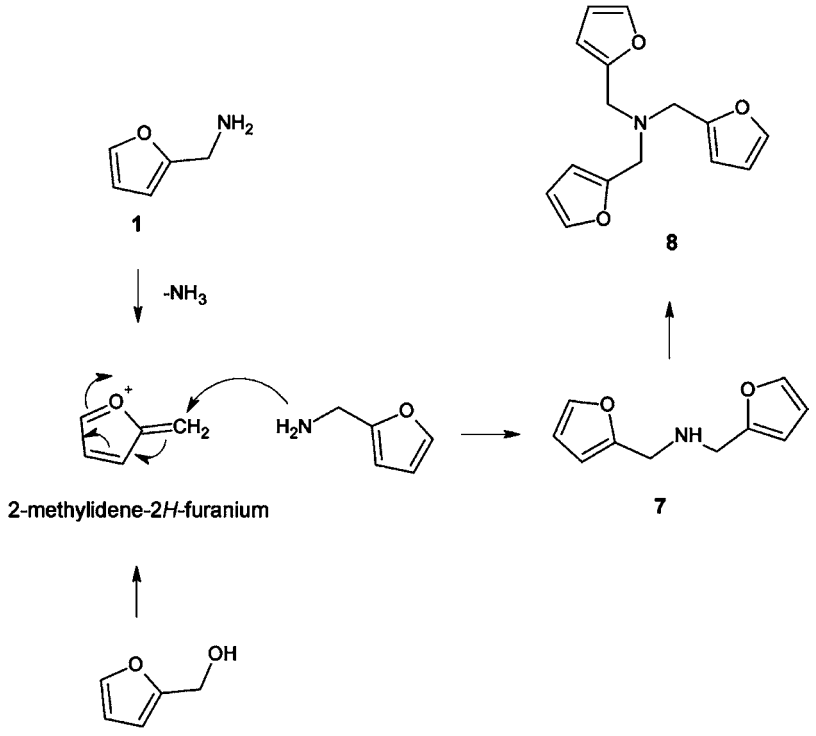

Figure 5. Chemical activation of furfuryl-amine and furfuryl-methanol into 2-methylidene-2*H*-furanium ion and formation of proposed structures **7** and **8**.

Table 8. Number of Isotopically Labeled Atoms Incorporated in the Proposed Structure 1-(Furan-2-yl)-*N*,*N*-bis(furan-2-ylmethyl)methanamine[a] (8) Generated in Ribose/Lysine-HCl Models

	m/*z*						
	257	229	176	148	108	81	53
[$^{13}U_5$]ribose	15	14	10	9	6	5	4
[^{13}C-1]ribose	3	3	2	2	2	1	1
[$^{15}N\alpha$]lysine	1	1	1	1	1	0	0

[a] t_R = 29.211 min, mw 257, *m*/*z* (%) **53** (29.1), **81** (100), **108** (24.9), **148** (10.0), **176** (9.9), **229** (7.7), **257** (7.5). The mass spectral fragments shown and label incorporation pattern indicated in the table are consistent with the proposed structure of **8**.

was observed at the retention time of 13.237 min confirmed through comparison of its retention time and mass spectral fragmentation pattern with those of a commercial standard in addition to the label incorporation data from a $^{13}U_5$-ribose/arginine model. Theoretically, other furfuryl alcohol derivatives such as 5-methylfurfuryl alcohol that can form from glucose should undergo similar activation and form the corresponding 5-methylmethylidene furanium ion, which can similarly interact with various amines including furfuryl-amine.

Formation of Furfuryl-amine Derivatives in Aqueous Medium. Model systems mentioned so far have been studied under pyrolytic conditions in a molten state at 250 °C for the purpose of performing isotope labeling studies. To verify the ability of the corresponding aqueous solutions at lower temperatures to generate furfuryl-amine derivatives, ribose/arginine or ribose/lysine aqueous models were heated in a sealed reactor at 120 °C for 20 min and analyzed by direct injection into a GC, bypassing the pyrolysis interface. This sample also generated all of the derivatives of furfuryl-amine, demonstrating the ability of ribose/amino acid model systems to undergo similar pathways under aqueous conditions. Furfuryl-amine therefore can be considered an important reactive intermediate in ribose/amino acid model systems and a characteristic marker for the presence of ribose in food products. Glucose, on the other hand, is unable to generate the corresponding 5-hydroxymethyl-1-furfuryl-amine structure in detectable levels due to the relative stability of the resulting glucosyl amino acid ring system versus 5-oxazolidinone, the required intermediate for its transformation into the furfuryl-amine derivative.

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