JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 12523–12529 12523
DOI:10.1021/jf103194k

Dimerization of Azomethine Ylides: An Alternate Route to Pyrazine Formation in the Maillard Reaction

Paula Vanessa Guerra and Varoujan A. Yaylayan*

Department of Food Science and Agricultural Chemistry, McGill University, 21,111 Lakeshore, Ste. Anne de Bellevue, Quebec, Canada H9X 3 V9

Recently, azomethine ylides have been implicated as reactive intermediates in the Maillard reaction. They are known to undergo 1,3-cycloaddition reactions with dipolarophiles to form pyrroles, and, more importantly, they can undergo dimerization reaction leading to the formation of a piperazine moiety. Although the reactivity of azomethine ylides toward dipolarophiles in Maillard model systems has been studied, their role as precursors of pyrazines remains unexplored. To study this possibility, a simple model system such as glyoxylic acid/glycine that is unable to generate α-dicarbonyl compounds but is able to form azomethine ylides was used to demonstrate pyrazine formation. The specific piperazine-2,5-dicarboxylic acid that is expected to form in this particular system can undergo oxidative decarboxylation to generate dihydropyrazine moieties similar to that of the dimerization product of the α-amino carbonyl compounds generated through the Strecker reaction. The model system when reacted under pyrolytic conditions at 200 °C indeed generated most of the theoretically expected pyrazines as major products, the structures of which were confirmed by comparison of their retention times with commercial standards and through NIST library matches in addition to isotope labeling data generated from labeled precursors such as [^{13}C-1]glycine, [^{13}C-2]glycine, and [^{15}N]glycine.

KEYWORDS: Glyoxylic acid; glycine; azomethine ylide; pyrazine formation; isotope labeling

INTRODUCTION

Pyrazine formation constitutes one of the main pathways of aroma generation during the Maillard reaction due to the characteristic sensory properties associated with pyrazines (*1*). The Strecker reaction plays a critical role in the transformation of α-dicarbonyl compounds that form abundantly during the Maillard reaction into pyrazine precursors, the α-amino carbonyl compounds (*2*). Essentially, the dimerization of such α-amino carbonyl compounds is the only known route to pyrazine moiety during the Maillard reaction. The resulting dihydropyrazines can either oxidize into pyrazine or interact with simple aldehydes to generate substituted pyrazines after a dehydration step without the need for oxidation. In addition to the above pathway, the interaction of α-dicarbonyl compounds with 1,2-diamino moieties can also lead to the formation of dihydropyrazines without the need to be transformed into α-amino carbonyl compounds; however, 1,2-diamino moieties are not very common in food systems, although 1,2-diaminobenzene is a well-known reagent trapping α-dicarbonyl compounds as their quinoxaline derivatives in many model and food systems (*3*). Recently, azomethine ylides (**Figure 1**) have been implicated as intermediates in the Maillard reaction (*2*, *4*−*7*) on the basis of evidence from spectroscopic (*8*, *9*) and isotope labeling studies (*8*, *10*) using different model systems at various temperatures. The azomethine ylides are known to undergo 1,3-cycloaddition reactions with dipolarophiles (*11*) to form pyrroles, and, more importantly, they can undergo dimerization reaction leading to the formation of a piperazine moiety (*11*, *12*) as shown in **Figure 1**. Although the addition of dipolarophiles to Maillard model systems has been shown to significantly reduce the intensity of UV absorption in the region between 400 and 500 nm (*9*), indicating the important role the azomethine ylides play as reactive intermediates, their role as precursors of pyrazines has not been studied yet. To explore this possibility, isotope labeling studies were performed using a simple model system that is unable to generate α-dicarbonyls but able to form azomethine ylides, such as the glyoxylic acid/glycine system.

*Corresponding author [phone (514) 398-7918; fax (514) 398-7977; e-mail varoujan.yaylayan@mcgill.ca].

MATERIALS AND METHODS

Reagents and Chemicals. Glycine (99%), glyoxylic acid monohydrate (95%), pyruvic acid, 2,3-butanedione, methylpyrazine (99%), 2,3-dimethylpyrazine (99%), 2,5-dimethylpyrazine (99%), 2,6-dimethylpyrazine (98%), 2,3,5-trimethylpyrazine (99%), 2-ethyl-3,6-dimethylpyrazine (>95%), and tetramethylpyrazine (98%) were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada). The labeled [^{13}C-1]-glycine (98%), [^{13}C-2]glycine (99%), and [^{15}N]glycine (98%) were purchased from Cambridge Isotope Laboratories (Andover, MI).

Pyrolysis−Gas Chromatography−Mass Spectrometry (Py-GC-MS). The Py-GC-MS analysis was performed according to the procedure described by Chu and Yaylayan (*13*) with some modifications. A Varian CP-3800 GC equipped with a sample preconcentration trap (SPT) filled with Tenax GR was coupled to a Varian Saturn 2000 mass spectrometry

Published on Web 11/03/2010

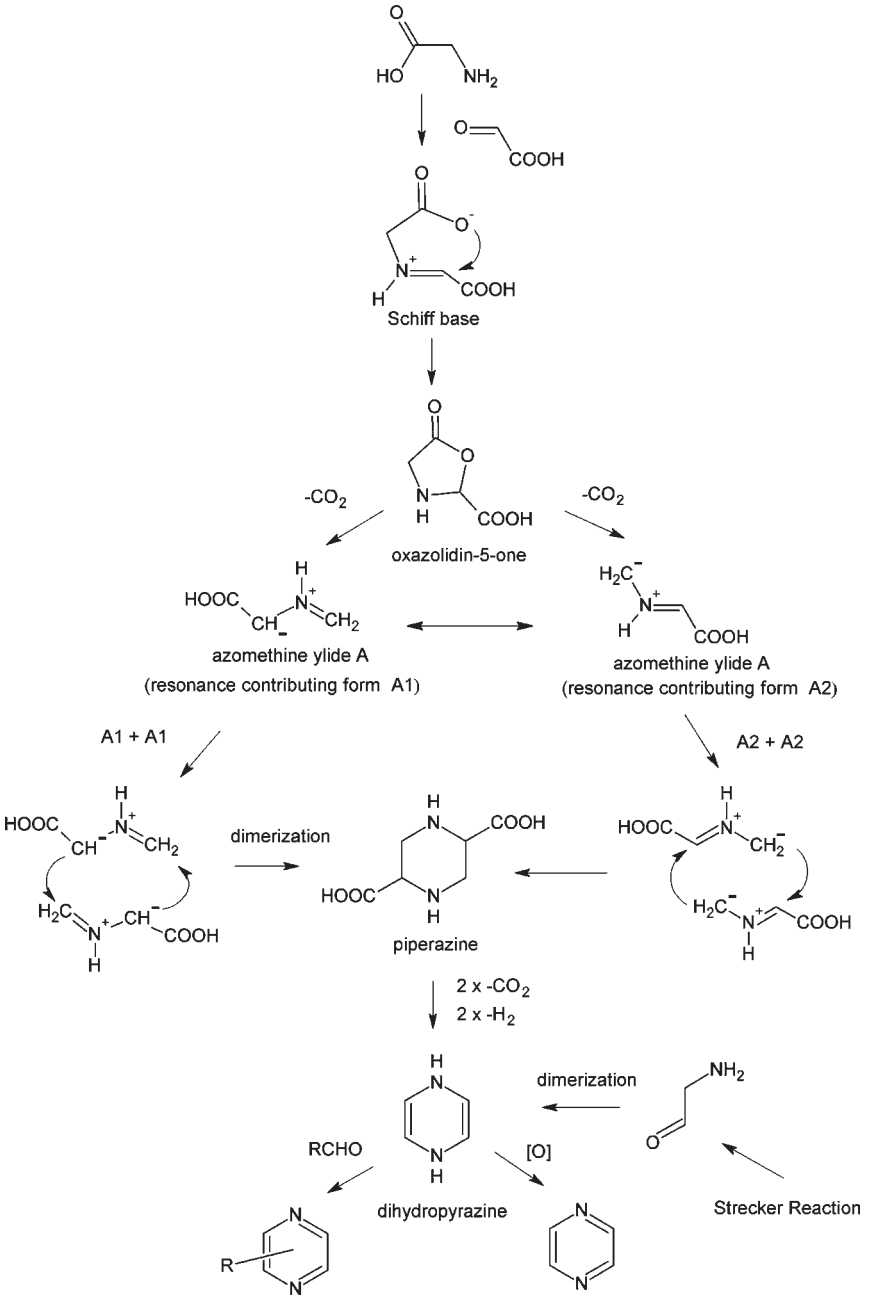


Figure 1. Generation and dimerization of azomethine ylides from glycine and glyoxylic acid.

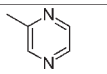
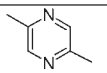
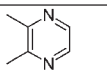
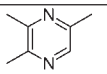
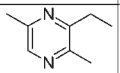
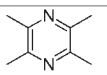
detector (Varian, Walnut Creek, CA). The pyrolysis unit included a valved interface (CDS 1500), which was installed onto the GC injection port and connected to a CDS Pyroprobe 2000 unit (CDS Analytical, Oxford, PA). The samples were analyzed on a DB-5MS column (5% diphenyl, 95% dimethylpolysiloxane) with column dimensions of 50 m length × 0.2 mm internal diameter × 33 μm film thickness (J&W Scientific, ON, Canada) using helium as the carrier gas. Two milligrams of a sample mixture containing a 1:3 ratio of glyoxylic acid to glycine, a 1:3:0.2 ratio of glyoxylic acid to glycine to pyruvic acid (spike), or a 1:3:1 ratio of glyoxylic acid to glycine to 2,3-butanedione model systems was packed inside a quartz tube (0.3 mm thickness), plugged with quartz wool, and inserted inside the coil probe and pyrolyzed at 200 °C for 20 s under He atmosphere. The volatiles after pyrolysis were concentrated on the SPT, trapped at 50 °C, and subsequently directed toward the GC column for separation. The GC column flow rate was regulated by an electronic flow controller (EFC) and set at a delayed (30 s) pressure pulse of 70 psi for the first 4 min and maintained with a constant flow of 1.5 mL/min for the rest of the run. The GC oven temperature was set at −5 °C for the first 5 min using CO_2 as the cryogenic cooling source and then increased to 50 °C at a rate of 50 °C/min. Then, the oven temperature was again increased 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. 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 1500 V.

Identification of Pyrazines. Pyrazines were identified by comparison of their retention times with commercial standards and through NIST library matches in addition to isotope labeling data (see **Tables 1** and **2**). The data reported in **Tables 1** and **2** are based on at least two replicate analyses with a percent standard deviation of < 15%.

RESULTS AND DISCUSSION

To investigate the possible pyrazine formation through the proposed azomethine ylide dimerization pathway shown in **Figure 1** as a distinct and new route to form pyrazines, a glyoxylic acid/glycine system was investigated as a model that is unable to

Table 1. Pyrazines and Their Retention Times Identified[a] in a Glycine/Glyoxylic Acid Model System

Pyrazines	Structure	Retention time (Standard) min	Relative amounts[b] in area per mole of glycine x 10^9	Origin[c]
2-methylpyrazine		10.78 (10.80)	1.38 (trace)	A + B A + A + CHO
2,5(6)-dimethylpyrazine[d]		11.80 (11.81)	4.04 (trace)	B2 + B2 A + B + CHO
2,3-dimethylpyrazine		11.90 (11.89)	2.39 (4.8)	B1 + B2 A + B + CHO
2,3,5-trimethylpyrazine		13.22 (13.21)	3.39 (14)	B + B + CHO
2-ethyl-3,6-dimethylpyrazine		14.58 (14.60)	0.18 (0)	B2 + B2 + CH_3CHO
Tetramethylpyrazine		14.78 (14.73)	0.09 (41)	C + C C + B + CHO

[a] Structures of all pyrazines were confirmed through NIST library searches, retention times using standards, partial elemental composition, and label incorporation in mass spectral fragments. [b 2] Based on the average of at least two replicates with a percent standard deviation of <15%,; values in parentheses are those produced in the presence of 2,3-butanedione. [3] Pairs of azomethine ylides (A, B1, and B2) needed for their oxidative formation in addition to an aldehyde in case of nonoxidative pathway (see also **Figures 4** and **5**). [4] Both standards have identical retention times (two isomers cannot be separated on DB-5).

Table 2. Percent Incorporation of [^{13}C-2]Glycine[a] into Pyrazines in a Glyoxylic Acid/Glycine Model

compound	M	M + 1	M + 2	M + 3	M + 4	M + 5
2-methylpyrazine	10	30	38	22	0	0
2,5(6)-dimethylpyrazine	5	12	28	32	22	0
2,3-dimethylpyrazine	5	14	28	32	21	0
2,3,5-trimethylpyrazine	0	7	18	31	28	16

[a] [^{15}N]Glycine incorporated two nitrogen atoms, and [^{13}C-1]glycine showed no incorporation in any of the listed pyrazines.

generate α-dicarbonyl precursors needed for the Strecker reaction, the only known route to pyrazines. Glycine was reacted with glyoxylic acid under pyrolytic conditions at 200 °C. Analysis of the chromatograms indicated that the above system generates mainly pyrazines in addition to a few unknown structures including some pyrazinones. The intensity of the pyrazine peaks dropped when the temperature was increased to 250 °C, and at 150 °C hardly any pyrazines were formed. The structures of all the pyrazines were confirmed through isotope labeling technique (see below) in addition to their retention times using commercially available standards and by NIST library searches as summarized in **Table 1**. The model system therefore generated different pyrazines as major products indicating the existence of a novel pathway of pyrazine formation in the absence of α-dicarbonyl compounds.

Formation and Dimerization of Azomethine Ylides in a Glyoxylic Acid/Glycine System. Glyoxylic acid, similar to other α-keto acids, is a known Maillard reaction product (*14*) and is capable of forming Schiff bases with amino acids. However, due to the inability of this Schiff base to stabilize itself through Amadori rearrangement, it prefers instead to undergo intramolecular cyclization and form oxazolidin-5-one moieties similar to other amino acid/carbonyl systems (*15*, *16*). Under Maillard reaction conditions, however, oxazolidin-5-ones are prone to undergo decarboxylation (*9*, *10*) followed by the formation of azomethine ylides (see **Figure 1**). One resonance-contributing form of the ylide has the negative charge on the α-carbon of the α-keto acid (form A1) and the other on the amino acid C-2 atom (form A2). In the absence of dipolarophiles, such nonstabilized acyclic azomethine ylides can either hydrolyze in the presence of water to generate the corresponding amine or dimerize to form piperazines (*11*, *12*) (see **Figure 1**). The specific piperazine-2,5-dicarboxylic acid that can be formed in this particular case can undergo oxidative decarboxylation (*17*) to generate dihydropyrazine moieties similar to that of the dimerization product of the α-amino carbonyl compounds generated through the Strecker reaction (see **Figures 1** and **2**). Such intermediates can be easily converted into pyrazines due to the oxidative and nonoxidative pathways available for their conversion, especially in the presence of simple aldehydes that can easily arise through decarboxylation of α-keto-acids such as glyoxylic acid (**Figure 3**). Furthermore, if the α-keto acid and the amino acids involved have substituents, then due to the regiochemistry of dimerization, two different isomers of pyrazines can result (see **Figure 2**). For example, if A1 and A2 forms dimerize in the system shown in **Figure 2**, then 2,3-disubstituted pyrazines are formed, and, on the other hand, dimerization of A2 and A2 or A1 and A1 can lead to the formation of 2,5-disubstituted pyrazines. To help elucidate and confirm the proposed new pathway of pyrazine formation, the reaction was studied using separately labeled glycine at N, C-1, and C-2 atoms. The structures of all the pyrazines detected in the model system were consistent with those predicted on the basis of the proposed mechanism in terms of not only label incorporation patterns but also patterns of their possible alkyl substituents.

Pyrazines from Glyoxylic Acid/Glycine Model System: Structural Evidence. According to the proposed mechanism shown in **Figure 4**, glycine can undergo two types of interactions with glyoxylic acid: one through carbonyl−amine reaction and formation of a Schiff base and the other through aldol-type chain

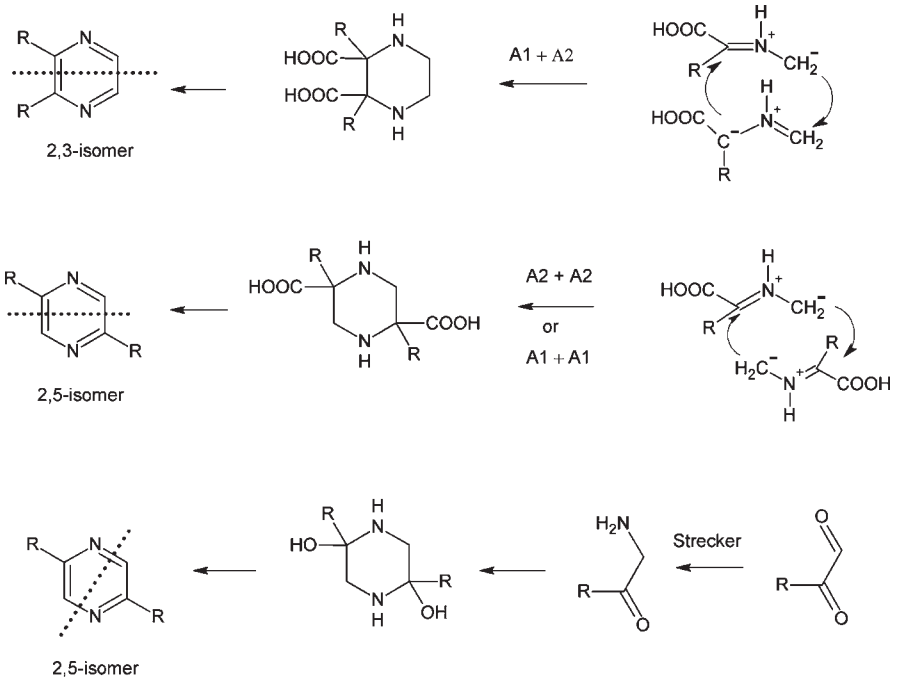


Figure 2. Dimerization of azomethine ylides and α-amino carbonyls and formation of pyrazine isomers.

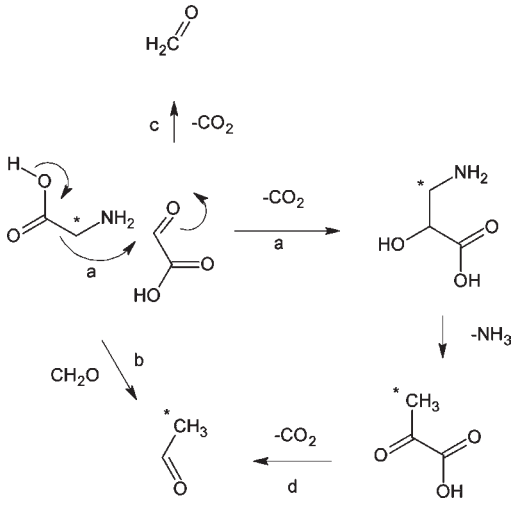


Figure 3. Formation of formaldehyde and acetaldehyde through chain elongation reactions (pathways a and b) and through decarboxylation of α-keto acids (pathways c and d) in a glycine/glyoxylic acid model system.

elongation reaction (*18*) (see **Figure 3**). The former can lead to the formation of azomethine ylide A (A1↔A2), and the latter can lead to chain elongation of glyoxylic acid and subsequent formation of pyruvic acid, which in turn can react further with glycine to generate new azomethine ylide B (B1↔ B2). Chain elongation reaction of glyoxal in the presence of alanine, for example, has been already demonstrated to generate 2-oxobutanal (*13*). **Figure 5** shows all four possible dihydropyrazine derivatives that theoretically could result from random dimerization of the azomethine ylides A1, B1, A2, and B2. Oxidation of these dihydropyrazines should generate the following pyrazines: the parent pyrazine, methylpyrazine, 2,5-dimethylpyrazine, and 2,3-dimethylpyrazine. According to **Table 1** all of these pyrazines were detected except the parent pyrazine (not easily retained on DB-5), and they constituted together >60% of the total pyrazine peak area. However, the methylpyrazine and all three isomers of dimethylpyrazine could also be formed through a nonoxidative pathway by the reaction of either dihydropyrazine or dihydromethylpyrazines with formaldehyde. Decarboxylation of glyoxylic acid (**Figure 3**) and hydrolysis of ylides A and B (**Figure 4**) could be efficient sources of formaldehyde in the system. The fact that a significant amount of 2,3,5-trimethylpyrazine was detected in the system confirms the importance of the nonoxidative pathway in this model system because the oxidative pathway to generate this pyrazine requires the presence of alanine, which is expected to form only in small amounts (labeling experiments below support this conclusion). The remaining two pyrazines, 2-ethyl-3,6-dimethyl- and tetramethylpyrazine, can be formed only through a nonoxidative pathway and are expected to be present in only small amounts, due to the requirement of acetaldehyde and alanine for their generation (see **Figure 4**).

Evidence from Labeling Studies. Label incorporation patterns in the most abundant pyrazines are shown in **Table 2**. As expected, all of the listed pyrazines incorporated two nitrogen atoms of glycine and none incorporated the C-1 atom, consistent with the proposed mechanism shown in **Figure 4**. This figure also traces the fate of the ^{13}C-2 atom of glycine as it reacts with glyoxylic acid to form ylides A and B. According to the proposed mechanism, ylide A should incorporate only one ^{13}C-label and ylide B should incorporate two ^{13}C-labels. Furthermore, hydrolysis of ylide A should generate unlabeled glycine from the unlabeled glyoxylic acid portion and labeled formaldehyde from the labeled glycine portion of ylide A. Similarly, hydrolysis of ylide B should generate labeled alanine and labeled formaldehyde. It is expected, however, that the reaction will proceed to generate mainly ylides A and B in addition to labeled formaldehyde and unlabeled glycine. Unlabeled formaldehyde, however, can arise from decarboxylation of glyoxylic acid (**Figure 3**). Consequently, the most intense pyrazines are expected to arise from random dimerization of ylides A and B and through formaldehyde participation in the nonoxidative pathway (**Figure 5**). On the other hand, pyrazines arising from acetaldehyde participation through a nonoxidative pathway are expected to be less intense due to the tendency of pyruvaldehyde to react with glycine

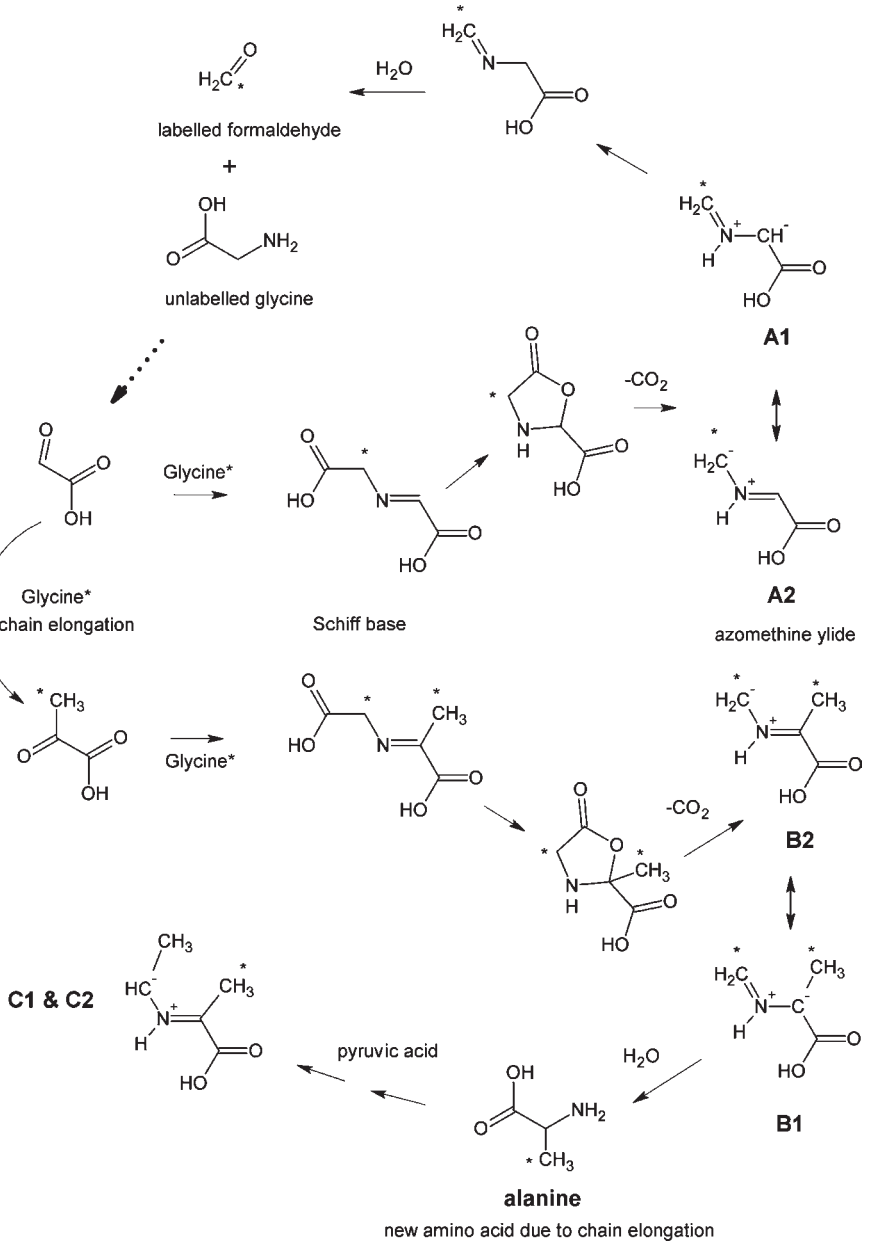


Figure 4. Proposed mechanism of interaction between [^{13}C-2]glycine and glyoxylic acid and generation of azomethine ylides A and B. Glycine* indicates [^{13}C-2]glycine.

rather than decarboxylate and generate acetaldehyde. Spiking the glycine/glyoxylic acid model with pyruvic acid enhanced the intensities of not only the peaks requiring ylide B but also those requiring acetaldehyde (results not shown). Therefore, on the basis of the proposed mechanism in **Figure 4**, of the 10 most probable pyrazine structures, the 6 most expected pyrazines (mainly requiring ylides A and B and formaldehyde) were detected in the system.

Label Incorporation in 2-Methylpyrazine. 2-Methylpyrazine can be formed through both oxidative (A + B) and nonoxidative pathways (A + A + CHO) (**Table 1**). According to **Figure 4**, the maximum number of label incorporation should not exceed three if it is formed through an oxidative pathway, and in the case of a nonoxidative pathway, the maximum number of label incorporation again should not exceed three if formaldehyde is labeled; otherwise, the maximum number of label incorporation will be two. Furthermore, as described above and shown in **Figure 5**, the hydrolysis of ylide A can generate unlabeled glycine, which in turn can recycle into the reaction system and generate unlabeled ylides A and B. As a consequence, 2-methylpyrazine can be generated with no label and with one, two, or three label incorporations as shown in **Table 2**. As expected, only 10% of the isotopomers had no label due to the small amount of water available in the system from glyoxylic acid monohydrate reagent.

Label Incorporation in 2,5(6)-Dimethylpyrazine and 2,3-Dimethylpyrazine. The dimethylpyrazines can be formed through oxidative (B + B) and nonoxidative pathways (A + B + CHO). Accordingly, they show very similar label incorporation patterns (see **Table 2**). In the oxidative pathway dimethylpyrazines are expected to incorporate a maximum of four labels and in the nonoxidative pathway, depending on the origin of formaldehyde, they can incorporate up to four labels. Again due to the generation of unlabeled glycine in the system, a pattern of unlabeled and partially labeled dimethylpyrazines as shown in **Table 2** is expected.

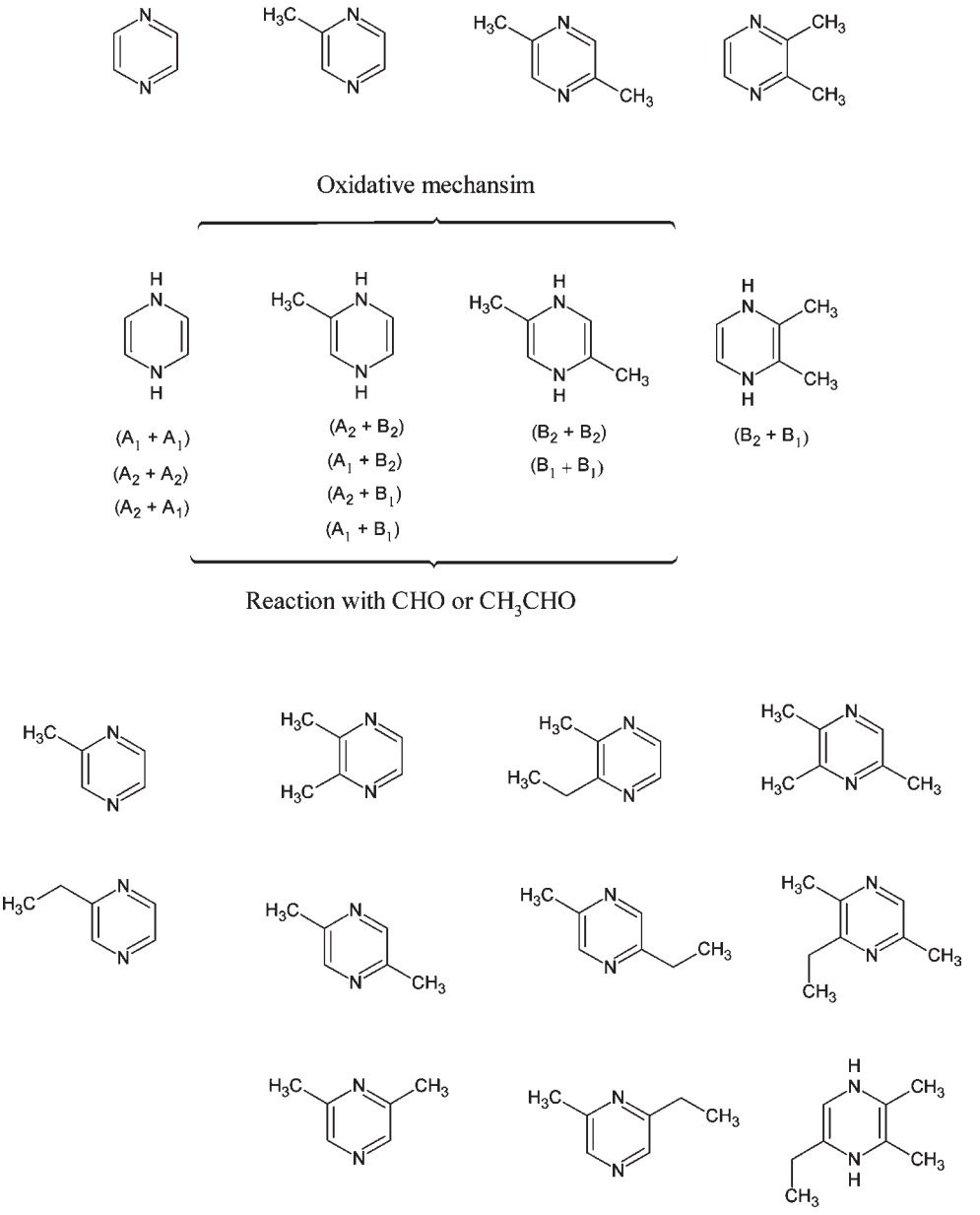


Figure 5. Possible dihydropyrazine structures that could be generated through random dimerization of azomethine ylides A and B and their subsequent conversion into various pyrazines through oxidative and nonoxidative pathways.

Label Incorporation in 2,3,5-Trimethylpyrazine. Trimethylpyrazine can be formed only through a nonoxidative pathway (B + B + CHO), and accordingly it is expected to exhibit incorporation of up to five labeled atoms depending on the origin of formaldehyde. In this particular case, there is statistically less chance to generate a completely unlabeled isotopomer; however, other partially labeled structures were detected as shown in **Table 2**.

Glycine Reaction with Glyoxylic Acid in the Presence of 2,3-Butanedione. To evaluate the importance of the azomethine ylide dimerization pathway relative to the Strecker reaction, glyoxylic acid/glycine reaction was performed in the presence of 2,3-butanedione in a 1:3:1 ratio and compared with the glyoxylic acid/glycine model in 1:3 ratio. In this model system, glycine is in competition to undergo both reactions generating tetramethylpyrazine only through the Strecker reaction and the other pyrazines only through azomethine ylide dimerization. The results have indicated that the presence of 2,3-butanedione did not prevent pyrazine formation through dimerization of azomethine ylide because, in addition to tetramethylpyrazine, comparable amounts of other pyrazines were also formed (see **Table 1**). When the ratio of glycine in the model system was increased, the amounts of all of the pyrazines were also increased.

Structural evidence and the observed isotope labeling patterns of the pyrazines identified in the glycine/glyoxylic acid model system point to a novel pathway of pyrazine generation in the Maillard reaction mixtures distinct from the Strecker reaction. Dimerization of azomethine ylides formed between the amino acid and the α-keto acid constitutes the only possible mechanism able to rationalize the formation of the observed pyrazines.

LITERATURE CITED

(1) Amrani-Hemaimi, M.; Cerny, C.; Fay, L. B. Mechanisms of formation of alkylpyrazines in the Maillard reaction. *J. Agric. Food Chem.* **1995**, *43*, 2818−2822.

(2) Rizzi, G. The Strecker degradation of amino acids. Newer avenues for flavour formation. *Food Rev. Int.* **2008**, *24*, 416−435.

(3) Hofmann, T. Quantitative studies on the role of browning precursors in the Maillard reaction of pentoses and hexoses with L-alanine. *Eur Food Res. Technol.* **1999**, *209*, 113−121.
(4) Yaylayan, V. A.; Wronowski, A.; Locas, C. P. Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753−1757.
(5) Zhang, Y.; Ren, Y; Zhang, Y. New research developments on acrylamide: analytical chemistry, formation mechanism, and mitigation recipes. *Chem. Rev.* **2009**, *109*, 4375−4397.
(6) Mottram, D. S.; Low, M. Y.; Elmore, J. S. The Maillard reaction and its role in the formation of acrylamide and other potentially hazardous compounds in foods. In *Acrylamide and Other Hazardous Compounds in Heat-Treated Foods*; Skog, K., Alexander, J., Eds.; Woodhead Publishing, CRC Press: Cambridge, U.K., 2006; pp 3−22.
(7) Blank, I.; Robert, F.; Goldmann, T.; Pollien, P.; Varga, N. Devaud, S.; Saudy, F.; Huynh-Ba, T.; Stadler, R. H. Mechanism of acrylamide formation: Maillard induced transformations of aspargine. In *Chemistry and Safety of Acrylamide in Food*; Friedman, M., Mottram, D., Eds.; Springer: New York, 2005; Vol. 561, pp 171−189.
(8) Chu, F. L.; Yaylayan, V. A. FTIR monitoring of oxazolidin-5-one formation and decomposition in glycolaldehyde/phenylalanine model system by isotope labeling techniques. *Carbohydr. Res.* **2009**, *344*, 229−236.
(9) Chu, F. L.; Yaylayan, V. A. Post-Schiff base chemistry of the Maillard reaction: mechanism of imine isomerization. *Ann. N.Y. Acad. Sci.* **2008**, *1126*, 30−37.
(10) Hidalgo, F. L.; Delgado, R. M.; Navarro, J. L.; Zamaro, R. Asparagine decarboxylation by lipid oxidation products in model systems. *J. Agric. Food Chem.* **2010**, *58*, 10512−10517.
(11) Tsuge, O.; Kanemasa, S. Recent advances in azomethine ylide chemistry. *Adv. Heterocycl. Chem.* **1989**, *45*, 231−349.
(12) Freeman, F.; Govindarajoo, G. Structures and dimerization reactions of acyclic azomethine ylides. *Rev. Heteroat. Chem.* **1995**, *13*, 123−147.
(13) Chu, F. L.; Yaylayan, V. Isotope labeling studies on the origin of 3,4-hexanedione and 1,2-butanedione in an alanine/glucose model system. *J. Agric. Food Chem.* **2009**, *57*, 9740−9746.
(14) Rössner, J.; Velisek, J.; Pudil, F.; Davidek, J. Strecker degradation products of aspartic and glutamic acids and their amides. *Czech. J. Food Sci.* **2001**, *19*, 41−45.
(15) Tsuge, O.; Kanemasa, S.; Ohe, M.; Takenaka, S. Simple generation of nonstabilized azomethine ylides through decarboxylative condensation of α-amino acids with carbonyl compounds via 5-oxazolidinone intermediate. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 4079−4089.
(16) Aurelio, L.; Box, J. S.; Brownlee, T. C.; Hughes, A. B.; Sleebs, M. M. An efficient synthesis of *N*-methyl amino acids by way of intermediate 5-oxazolidinone. *J. Org. Chem.* **2003**, *68*, 2652−2667.
(17) Murai, K.; Morishita, M.; Nakatani, R.; Fujioka, H.; Kita, Y. Oxidative decarboxylative synthesis of 2-*H*-imidazolines from glyoxylic acid and 1,2-diamines. *Chem. Commun.* **2008**, 4498−4500.
(18) Yaylayan, V. A.; Keyhani, A. Origin of 2,3-pentanedione and 2,3-butanedione in L-glucose/L-alanine Maillard model systems. *J. Agric. Food Chem.* **1999**, *47*, 3280−3284.

Received for review August 17, 2010. Revised manuscript received October 12, 2010. Accepted October 16, 2010. We acknowledge funding for this research by the Natural Sciences and Engineering Research Council of Canada (NSERC).