Glycine Specific Novel Maillard Reaction Products: 5-Hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone and Related Compounds

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A major product of the reaction of D-glucose with excess glycine was detected by Py/GC/MS analysis and subsequently synthesized and isolated using focused microwave irradiation at atmospheric pressure conditions. Spectroscopic analyses by NMR, FTIR, MS, and UV in conjunction with labeling studies have indicated the unknown compound to be 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone. Analysis of the mass spectra of the unknown compound obtained by reacting separately labeled [^{13}C]-D-glucoses with unlabeled glycines and reacting separately labeled [^{15}N]- and [^{13}C]glycines with unlabeled D-glucoses indicated the incorporation of 10 carbon atoms (six from sugar, one C-1 atom of glycine, and three C-2 atoms of glycine) and two nitrogens. In addition, other derivatives of quinoxalinone formed in D-glucose/glycine and D-glucose/L-alanine mixtures were also tentatively identified based on similar amino acid incorporation patterns and mass spectroscopic analysis.

Keywords: *Amadori; decomposition mechanisms; Maillard reaction; ^{13}C-labeled D-glucose; [^{15}N]- and [^{13}C]glycine and L-alanine; pyrazinones; quinoxalinone; Py/GC/MS; microwave-assisted synthesis*

INTRODUCTION

The complexity of the Maillard reaction mixtures precludes their complete analysis through classical organic chemistry and requires the use of isotopically labeled starting materials to establish the origin and fate of multitudes of reactive intermediates that form during the process. Identification of these intermediates can help to predict the formation of certain end products and eventually can lead to the classification of Maillard reaction into its underlying elementary processes. Such approaches have been successfully applied on a microscale level by the use of pyrolysis/gas chromatography/mass spectrometry (Py/GC/MS) as an integrated reaction, separation, and identification system to elucidate the mechanism of formation of alkyl-substituted pyrazinones from D-glucose/glycine model systems (Yaylayan and Keyhani, 1996; Keyhani and Yaylayan, 1996a) and pyridines and naphthalenes from D-glucose/L-phenylalanine (Keyhani and Yaylayan, 1996b). However, many of the major products formed and detected by GC/MS in the D-glucose/glycine model system could not be identified and required the production of analytically pure samples for spectroscopic identification.

The ability of focused microwave irradiation under atmospheric pressure conditions to generate and extract chemical reaction products, using a two-stage microwave-assisted process (MAP) (Paré et al., 1991, 1994), was successfully applied (Yaylayan et al., 1997) to synthesize and isolate some of the major unknown products formed when D-glucose was pyrolyzed in the presence of excess glycine. MAP has been applied successfully to various liquid-phase and gas-phase extractions and is currently used extensively as a sample preparation tool (Bèlanger et al., 1996). Spectroscopic analysis (Yaylayan et al., 1996) of one of the major products that has been isolated using the microwave extraction has indicated that glycine/D-glucose model systems can generate also fused benzopyrazinone derivatives (quinoxalinones) in addition to alkyl-substituted pyrazinones, by a related mechanism. Alkyl- and tetrahydroquinoxaline derivatives have been identified in different food products such as brewed coffee (Sasaki et al., 1987), pork liver (Mussinan and Walradt, 1974), and roasted filberts (Kinlin et al., 1972). Imidazoquinoxalines, specific products of a creatinine/glucose/glycine mixture, have been identified in fried beef (Nagao et al., 1983).

In this paper, we report the isolation and mechanism of formation of 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone in a glycine/D-glucose model system. Details of the microwave-assisted synthesis (Yaylayan et al., 1997) and the spectroscopic characterization (Yaylayan et al., 1996) have been reported elsewhere.

MATERIALS AND METHODS

All reagents, chemicals, [1-^{13}C]-D-glucose (99% enriched), [2-^{13}C]-D-glucose (99% enriched), [6-^{13}C]-D-glucose (99% enriched), and [^{15}N]glycine (98% enriched) were purchased from Aldrich Chemical Co. (Milwaukee, WI). [3-^{13}C]-D-Glucose (99% enriched), [4-^{13}C]-D-glucose (99% enriched), [5-^{13}C]-D-glucose (99% enriched), [1-^{13}C]glycine (99% enriched), [2-^{13}C]glycine (90% enriched), [1,2-^{13}C]glycine (99% enriched), [3-^{13}C]-DL-alanine (99% enriched), and [1-^{13}C]-L-alanine (99% enriched) were purchased from ICON Services Inc. (Summit, NJ). [^{15}N]-L-Alanine (99% enriched) was purchased from CIL, Inc. (Andover, MA). [2-^{13}C]-DL-Alanine (92.1% enriched) was purchased from C/D/N Isotopes Inc. (Pointe-Claire, Quebec). Precoated preparative silica gel plates were obtained from Merck (Germany). The synthesis of Amadori glycine was performed according to Sosnovsky et al. (1993). ^{13}C- and ^{1}H-NMR spectra were recorded in $CDCl_3$ at 300 MHz using TMS as internal standard on a Varian Unity Inova 300 instrument. Infrared spectra were recorded in CaF_2 IR cells with a 25 μm Teflon spacer, on a Nicolet 8210 Fourier-transform spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector. The Soxwave100 focused microwave extraction system was obtained from Prolabo (Fontenay-Sous-Bois, France), operating at an emission frequency of 2450 MHz and a 300 W full power, equipped with at 250 mL quartz vessel and a Graham type refrigerant column (400 mm length).

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S0021-8561(96)00528-6 CCC: $14.00

Table 1. Mass Spectrometric Data of Quinoxalinones and Pyrazinones

5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone (from glycine/D-glucose)	191(11), 190(100), 162(32), 161(23), 134(18), 133(42), 119(23), 106(6), 93(12), 92(19), 56(27)
5-hydroxy-1,3,7-trimethyl-2(1*H*)-quinoxalinone (from glycine/D-glucose)	205(12), 204(100), 176(21), 175(36), 161(11), 148(17), 147(48), 133(11),92(14), 56(28)
5-hydroxy-1-methyl-2(1*H*)-quinoxalinone (from glycine/D-glucose)	177(10), 176(100), 148(30), 120(21), 119(23), 105(24), 92(11), 79(15), 51(15)
1,7-dimethylcyclopenta-2(1*H*)-pyrazinone (from glycine/D-glucose or glycine/cyclotene)	165(11), 164(100), 149(34), 135(37), 121(21), 107(24), 106(26), 94(15), 92(10), 79(10), 66(9)
5-hydroxy-1-ethyl-3-methyl-2(1*H*)-quinoxalinone (from L-alanine/D-glucose)	205(13), 204(100), 176(34), 148(24), 147(30), 120(22), 119(14), 106(15), 92(17), 79(14), 65(13)
1-ethyl-3,7-dimethylcyclopenta-2(1*H*)-pyrazinone (from L-alanine/D-glucose or L-alanine/cyclotene)	193(13), 192(100), 191(91), 177(69), 163(11), 149(10), 121(19), 120(47), 108(29), 106(20), 94(25)

Py/GC/MS Analysis. A Hewlett-Packard GC/mass selective detector (5890 GC/5971B MSD) interfaced to a CDS pyroprobe 2000 unit was used for the Py/GC/MS analysis. Solid samples (1−4 mg) of amino acid/glucose in different ratios were introduced inside a quartz tube (0.3 mm thickness), plugged with quartz wool, and inserted inside the coil probe. The pyroprobe was set at the desired temperature (250 °C) at a heating rate of 50 °C/ms and with a THT (total heating time) of 20 s. The GC column flow rate was 0.8 mL/min for a split ratio of 92:1 and a septum purge of 3 mL/min. The pyroprobe interface temperature was set at 250 °C. Capillary direct MS interface temperature was 180 °C; ion source temperature was 280 °C. The ionization voltage was 70 eV, and the electron multiplier was 1682 V. The mass range analyzed was 30−300 amu. The column was a fused silica DB-5 column (30 m length × 0.25 mm i.d. × 25 μm film thickness; Supelco, Inc.). Unless otherwise specified, the column's initial temperature was −5 °C for 2 min and was increased to 50 °C at a rate of 30 °C/min; immediately the temperature was further increased to 250 °C at a rate of 8 °C/min and kept at 250 °C for 5 min. Products that were not found in the mass spectral libraries were identified by generating them from their proposed precursors and comparing mass spectra and chromatographic retention times.

Microwave-Assisted Synthesis and Extraction of 5-Hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone. A D-glucose (1.00 g, 0.005 mol) and glycine (1.25 g, 0.016 mol) mixture was transferred into the 250 mL quartz extraction vessel of the Soxwave 100 microwave extraction system; 2 mL of water was then added. The vessel was inserted inside the extraction cavity and fitted with a condenser. The irradiation was carried out in the following sequence at full power (300 W): 2 min on, 30 s off, 2 min on, 30 s off, 2 min on, 30 s off, and 2 min on, for a total of 8 min of irradiation. At the end of the irradiation sequence a dark brown and dry slurry was obtained. The extraction step was carried out with 40 mL of hexane using the following sequence of irradiation: 40 s on, 30 s off, and 90 s on. The solvent was decanted, dried over sodium sulfate, and evaporated under vacuum. The resulting oil was further purified by thick layer chromatography on silica gel using ethyl acetate as the solvent. The title compound was the only fluorescent band visible under UV at 365 nm: ν_{max} (ethyl ether) (absorbance units) 350 nm (0.68), 279 (2.58), 207 (2.47); FTIR ($CDCl_3$) 3158 cm^{-1} (Ar−OH), 2979 (−CH_3), 2894 (−CH_3), 1668 (N−C═O), 1653 (C═N), 1600, 1560, and 1539 (ArH), 1469 and 1382 (CH_3), 1095 (C−O); ^{1}H-NMR ($CDCl_3$) δ 7.49 (ArH, dd, 1H), 6.66 (ArH, dd, 1H), 6.51 (ArH, q, 1H), 2.62 (N═C−CH_3, s, 3H), 3.60 (N−CH_3, s, 3H), 2.20 (OH, br, H/D exch); EIMS *m*/*z* (rel intensity) 191 (12), 190 (100), 162 (29), 161 (22), 134 (20), 133 (51), 120 (6), 119 (28), 106 (7), 93 (14), 92 (22), 81 (7), 79 (5), 78 (6), 77 (11), 76 (5), 68 (5), 67 (5), 66 (9), 64 (14), 63 (5), 56 (44), 55 (5), 54 (5), 52 (7), 51 (23), 50 (9), 42 (12), 39 (14), 38 (5).

RESULTS AND DISCUSSION

Multiple addition reactions of amino acids to sugar dicarbonyl fragments have not been studied extensively, despite their importance in elucidating cross-linking of proteins. Model studies (Keyhani and Yaylayan, 1996a) using [^{13}C]-D-glucoses and a series of C_2, C_3, and C_4 dicarbonyl compounds with labeled [^{15}N]- or [^{13}C]glycines have indicated that methyl-substituted pyrazinones in these model systems are formed through such addition reactions. These studies have also identified a novel aldol type reaction between the C-2 atom of glycine and α-keto aldehydes followed by deamination and decarboxylation, resulting in the conversion of the aldehyde moiety into a methyl ketone, such as the conversion of pyruvaldehyde into 2,3-butanedione and glyoxal into pyruvaldehyde. This property of glycine as a methylating agent, during Maillard reaction, was also demonstrated in the present study. In addition, it has been observed that carbon atoms of sugar, especially C-1, can also be incorporated to a certain extent, as methyl groups, to form methylated quinoxalinones.

Prediction, Confirmation, and Identification of Products Arising from Multiple Additions of Glycine during Maillard Reaction. Products arising from the incorporation of more than one amino acid moiety into a sugar fragment could be identified by a method based on Py/GC/MS utilized as an integrated reaction, separation, and identification system and by the use of labeled sugars and amino acids as reactants (Yaylayan and Keyhani, 1996). In this approach, sugars are reacted in the pyrolysis probe with increasing concentrations of the amino acid relative to the sugar; consequently, chromatographic peaks arising from multiple additions of the amino acid will increase and thus could be identified and further confirmed by reacting the sugar with ^{15}N- and ^{13}C-labeled amino acids and observing the incorporation of multiple labels into the product by Py/GC/MS analysis. Performing such experiments with D-glucose/glycine revealed the participation of three glycine molecules in the formation of alkyl-substituted pyrazinones (Keyhani and Yaylayan, 1996a).

In addition, other chromatographic peaks (Table 1) such as *m*/*z* 176, 190, and 204 also showed increased intensity by the addition of excess amino acid. Experiments performed with ^{13}C-labeled D-glucoses (independently labeled at each carbon atom) and ^{15}N- and ^{13}C-labeled glycines indicated the incorporation of all six carbon atoms of the sugar and two nitrogens, one C-1 and three C-2 atoms of glycine into *m*/*z* 190 (see Scheme 1 and Table 2). However, *m*/*z* 176 contained only two C-2 atoms of glycine, and *m*/*z* 204 contained up to four C-2 atoms of glycine (see Table 3). Comparison of their mass spectra (see Figure 1) indicated that they are structurally related compounds, differing only in the number of methyl group substituents, arising mainly from the C-2 atom of glycine. The chromatographic peak related to *m*/*z* 190 was the most abundant followed by *m*/*z* 204 and 176, respectively. When excess glycine was reacted with synthetic Amadori glycine, the peak due to *m*/*z* 190 was the most intense in the pyrogram.

In addition, due to the similarity of the glycine substitution pattern to that of alkylpyrazinones (Keyhani and Yaylayan, 1996a) and intense molecular ions in their mass spectra, it was predicted that they should possess aromatic pyrazinone structures and as such

Scheme 1. Origin of Carbon Atoms in Quinoxalinones

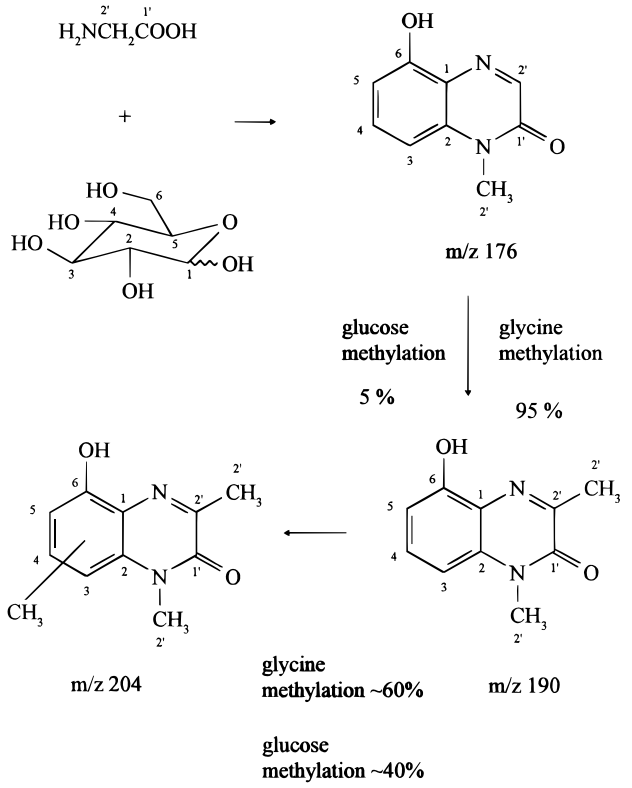


Table 2. Percent Distribution of Molecular Ion *m/z* 190 Generated from Labeled D-Glucoses or Excess Labeled Glycines[a]

	m/z 190	*m/z* 191	*m/z* 192	*m/z* 193
D-Glucose/Excess Glycine				
D-glucose/glycine	99	1	0	0
[1-^{13}C]-D-glucose/glycine	0	100	0	0
[2-^{13}C]-D-glucose/glycine	0	100	0	0
[3-^{13}C]-D-glucose/glycine	0	100	0	0
[4-^{13}C]-D-glucose/glycine	0	100	0	0
[5-^{13}C]-D-glucose/glycine	0	100	0	0
[6-^{13}C]-D-glucose/glycine	0	95	5	0
D-glucose/[1-^{13}C]glycine	0	100	0	0
D-glucose/[2-^{13}C]glycine (90% enriched)	0	0	16	84
D-glucose/[^{15}N]glycine (98% enriched)	0	2	98	0
ARP Glycine/Excess Glycine				
ARP glycine/[1-^{13}C]glycine	63	37	0	0
ARP glycine/[2-^{13}C]glycine (90% enriched)	64	28	8	0
ARP glycine/[^{15}N]glycine (98% enriched)	35	52	15	0

[a] Values are adjusted for natural abundance; compounds less than 99% enriched are indicated. ARP = Amadori rearrangement product.

could be extracted into nonpolar solvents such as hexane. All attempts to extract these compounds from heated D-glucose/glycine systems failed to produce enough quantities for spectroscopic analysis; however, when the synthesis and extraction by hexane were performed by focused microwave irradiation under atmospheric pressure conditions (Yaylayan et al., 1997), a simple mixture was obtained containing the compound having the ion *m/z* 190 as the major component. Further purification by preparative chromatography yielded the pure compound which was assigned the structure of 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone (Scheme 1), based on spectroscopic analysis.

Spectroscopic Characterization of 5-Hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone. The mass spectra of compound *m/z* 190 obtained by reacting separately

Table 3. Percent Distribution of Molecular Ion *m/z* 204 Generated from Labeled D-Glucoses or Excess Labeled Glycines[a]

	m/z 204	*m/z* 205	*m/z* 206	*m/z* 207	*m/z* 208
D-Glucose/Excess Glycine					
D-glucose/glycine	98	2	0	0	0
[1-^{13}C]-D-glucose/glycine	0	85	15	0	0
[2-^{13}C]-D-glucose/glycine	0	97	3	0	0
[3-^{13}C]-D-glucose/glycine	0	92	8	0	0
[4-^{13}C]-D-glucose/glycine	0	100	0	0	0
[5-^{13}C]-D-glucose/glycine	0	97	3	0	0
[6-^{13}C]-D-glucose/glycine	0	92	8	0	0
D-glucose/[1-^{13}C]glycine	11	91	0	0	0
D-glucose/[2-^{13}C]glycine (90% enriched)	0	3	11	34	52
D-glucose/[^{15}N]glycine (98% enriched)	0	2	98	0	0
ARP Glycine/Excess Glycine					
ARP glycine/[1-^{13}C]glycine	67	33	0	0	0
ARP glycine/[2-^{13}C]glycine (90% enriched)	0	42	41	17	0
ARP glycine/[^{15}N]glycine (98% enriched)	0	0	97	3	0

[a] Values are adjusted for natural abundance; compounds less than 99% enriched are indicated. ARP = Amadori rearrangement product.

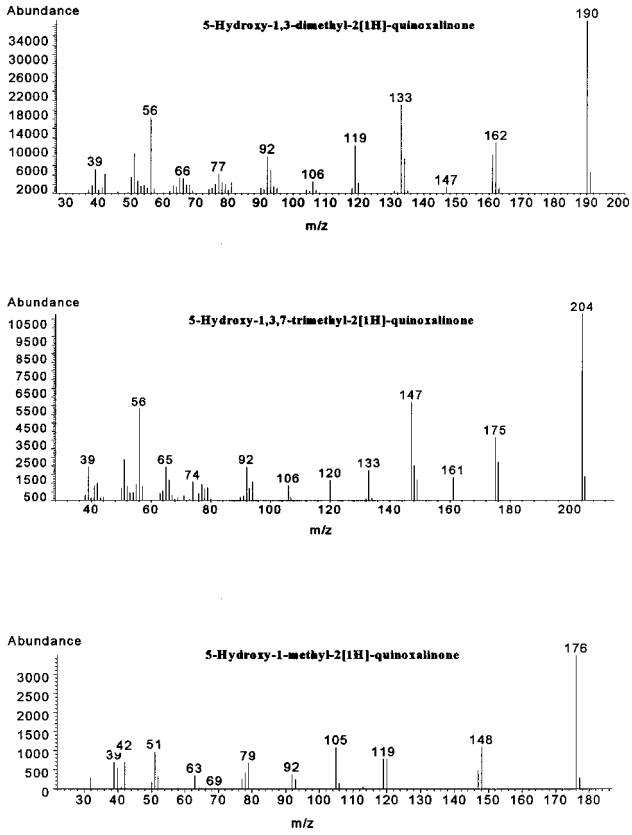
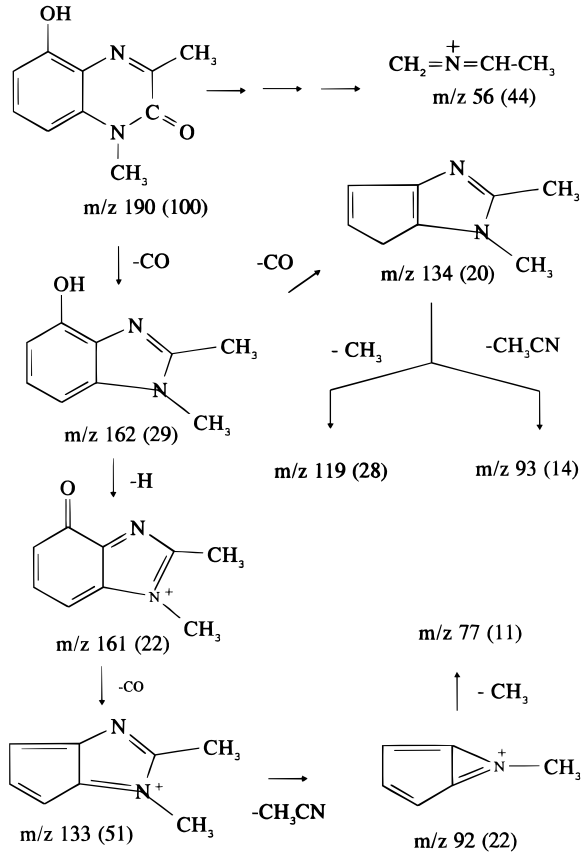


Figure 1. Mass spectra of selected quinoxalinone derivatives formed in the D-glucose/glycine mixture.

labeled [^{13}C]-D-glucoses at each carbon atom with unlabeled glycines and reacting separately labeled [^{15}N]- and [^{13}C]glycines with unlabeled D-glucose revealed the incorporation of 10 carbon atoms (six from sugar, one C-1 atom of glycine, and three C-2 atoms of glycine) and two nitrogens into the structure of *m/z* 190. This combination of carbons and nitrogens can produce only the following elemental formula: $C_{10}H_{10}N_2O_2$. Analysis by 2-D NMR (COSY) and ^{1}H-NMR confirmed the presence of three mutually coupled aromatic protons, one phenolic proton and two methyl groups. FTIR analysis indicated the presence of phenolic, alkyl, carbonyl, and aromatic functional groups. The parent 2(1*H*)-quinoxalinone shows carbonyl-stretching absorption in the region of 1660−1690 cm^{-1} and UV maxima

Scheme 2. Electron Impact Fragmentation Pathways of 5-Hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone, Based on Labeling Studies



(H_2O) at 343, 287, 254, and 228 nm (Cheeseman and Cookson, 1979). These values are consistent with those reported in Materials Methods. In addition, the parent 2(1*H*)-quinoxalinone fragments under electron impact conditions by successive losses of carbon monoxide and hydrogen cyanide from the molecular ion (Kovacik et al., 1973). According to Scheme 2, the compound *m/z* 190 shows similar fragmentations. Since position C-3 is substituted with a methyl group, a loss of methyl cyanide (41 amu) is observed rather than that of a hydrogen cyanide.

One of the advantages of using labeled reactants is that all the atoms of a product can be traced back to their origin in the starting material. This fact facilitates not only the elucidation of their mechanism of formation but also the assignment of their mass spectral fragments. The molecular ion *m/z* 190 (100%) loses carbon monoxide (originating from C-1 of glycine) to produce *m/z* 162 (29%), which in turn loses another carbon monoxide (originating from C-6 of glucose) to produce *m/z* 134 (20%), which either loses a methyl or methyl cyanide group to produce *m/z* 119 (28%) and 93 (14%), respectively. Ion at *m/z* 162 (29%) can also lose a hydrogen atom to produce *m/z* 161 (22%), which in turn can lose carbon monoxide to produce *m/z* 133 (51%) that undergoes successive losses of methyl cyanide and a methyl group to produce *m/z* 92 (22%) and 77 (11%) successively, as illustrated in Scheme 2 (see also Table 4). Labeling experiments indicate that fragment *m/z* 56 (44%) incorporates one C-1 atom of D-glucose, one nitrogen atom, and two C-2 atoms of glycine. The importance of this fragment lies in the fact that it establishes the connectivity of nitrogen atom to C-1 atom of D-glucose, indicating the possibility of 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone formation directly from the Amadori product.

Table 4. Incorporation of Labels in Selected Mass Spectral Fragments of 5-Hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone[a]

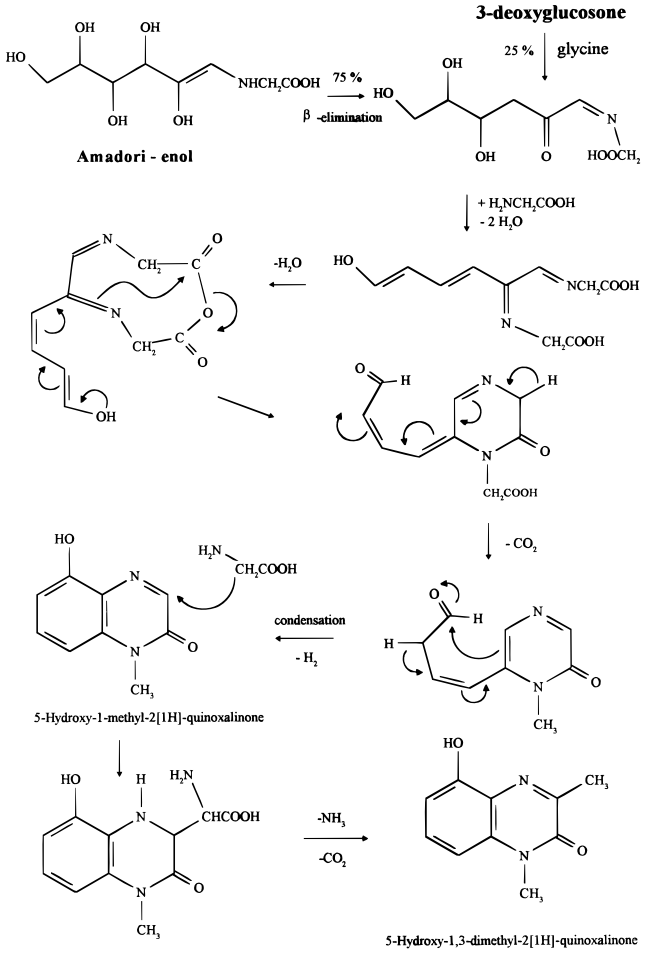
	m/z 190	*m/z* 162	*m/z* 161	*m/z* 133	*m/z* 56
D-Glucose/Excess Glycine					
D-glucose/glycine	190	162	161	133	56
[1-^{13}C]-D-glucose/glycine	191	163	162	134	57
[2-^{13}C]-D-glucose/glycine	191	163	162	134	56
[3-^{13}C]-D-glucose/glycine	191	163	162	134	56
[4-^{13}C]-D-glucose/glycine	191	163	162	134	56
[5-^{13}C]-D-glucose/glycine	191	163	162	134	56
[6-^{13}C]-D-glucose/glycine	191	163	162	133	56
D-glucose/[1-^{13}C]glycine	191	162	161	133	56
D-glucose/[2-^{13}C]glycine (90% enriched)	193	165	164	136	58
D-glucose/[^{15}N]glycine (98% enriched)	192	164	163	135	57

[a] Compounds less than 99% enriched are indicated.

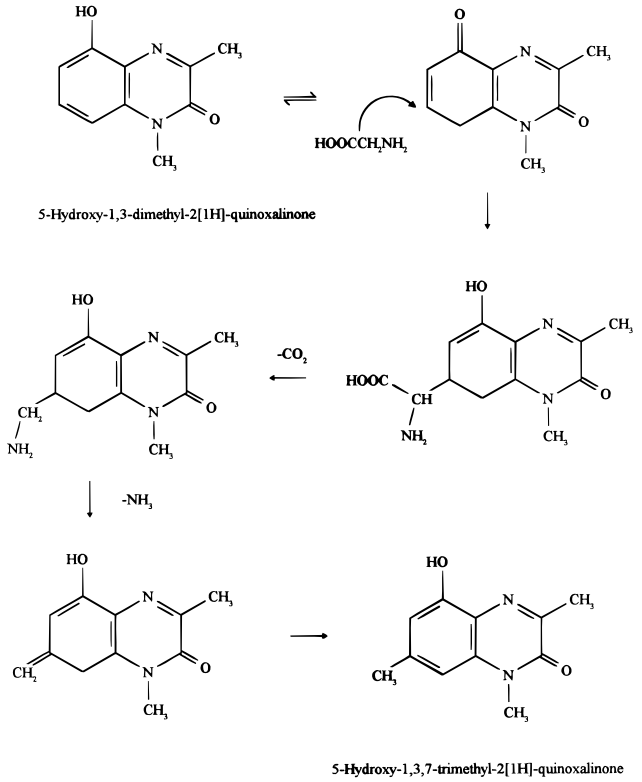
Mechanism of Formation of 5-Hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone. To confirm the above assertion that 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone could be formed directly from the glycine Amadori compound, 2-fold molar excess of [^{15}N]glycine was reacted with unlabeled glycine Amadori product (ARP), and label distribution was analyzed for the parent ion *m/z* 190 and fragment *m/z* 56. If the compound is formed only through the 3-deoxyglucosone (3DG) pathway, by reacting with 3 mol of free glycine, it is expected to observe a high percentage of ^{15}N incorporation into the product due to the presence of excess [^{15}N]glycine, with the formation of ions at *m/z* 192 and 57. For example, in the same reaction mixture, trimethylpyrazinone (*m/z* 138), which is formed by the reaction of 3 mol of glycine with 2,3-butanedione (Keyhani and Yaylayan, 1996a), through a similar pathway, generates 60% of doubly labeled parent ion (*m/z* 140), 30% singly labeled, and 10% unlabeled. However according to Table 2, only 15% of the parent ion of 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone was doubly labeled (*m/z* 192, 3DG + 2 × [^{15}N]glycine), 52% was singly labeled (*m/z* 191, ARP + [^{15}N]glycine or 3DG + [^{15}N]glycine + glycine), and 35% was unlabeled (*m/z* 190, ARP + glycine). In addition, only 25% of *m/z* 56 was labeled (*m/z* 57), indicating 75% of the 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone was formed by direct addition of glycine (either released from ARP or added) to the Amadori product when it was pyrolyzed in the presence of excess [^{15}N]glycine.

The proposed mechanism of formation of 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone is illustrated in Scheme 3. The enol form of Amadori glycine undergoes a β-elimination reaction to produce an α-keto imine derivative which could be generated also from 3DG through reaction with glycine. Reaction with a second mole of glycine and subsequent dehydrations produce a conjugated diimine which undergoes a dehydration reaction between the two carboxylic acid groups of the glycine to generate a cyclic anhydride. The cyclic anhydride then undergoes an intramolecular cyclization followed by decarboxylation to form a 6-substituted *N*-methylpyrazinone. The latter can undergo an aldol type intramolecular condensation and aromatization to produce 5-hydroxy-1-methyl-2(1*H*)-quinoxalinone, which has been detected in the same reaction mixture (Table 1). The imine functionality in 2(1*H*)-quinoxalinones is known to react with carbon nucleophiles (Cheeseman and Cookson, 1979); the C-2 carbon of glycine or acetic acid generated from either glycine or glucose can

Scheme 3. Proposed Mechanism of Formation of 5-Hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone from D-Glucose and Glycine, Based on Labeling Studies



Scheme 4. Proposed Mechanism of Formation of 5-Hydroxy-1,3,7-trimethyl-2(1*H*)-quinoxalinone from D-Glucose and Glycine, Based on Labeling Studies



methylate (Keyhani and Yaylayan, 1996a) this position to form 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone.

Other Quinoxalinone and Pyrazinone Derivatives Detected in Glycine or L-Alanine Model Systems. Pyrazinones and quinoxalinones, in general, are formed during Maillard reaction, by multiple additions of amino acids to an α-dicarbonyl compound. This interaction can lead to the formation of different derivatives, depending on the structure of the dicarbonyl fragment. It has been demonstrated that pyruvaldehyde and 2,3-butanedione can generate alkyl-substituted pyrazinones, and Amadori products or 3-DG can generate alkyl-substituted quinoxalinones. In addition, the initially formed 5-hydroxy-1-methyl-2(1*H*)-quinoxalinone can undergo methylation at the imine site to produce 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone, which in turn can produce a 5-hydroxy-1,3,7-trimethyl-2(1*H*)-quinoxalinone by methylation, through, for example, Michael type addition, as proposed in Scheme 4. However, the position of the methylation site is not confirmed. To demonstrate the generality of this reaction with other amino acids containing alkyl side chains, the L-alanine reaction was investigated, similar to that of glycine, with variously labeled starting materials. The main quinoxalinone structure, tentatively identified by labeling studies, was 5-hydroxy-1-ethyl-3-methyl-2(1*H*)-quinoxalinone (*m*/*z* 204, Tables 1 and 5), the equivalent product to that of 5-hydroxy-1-methyl-2(1*H*)-quinoxalinone from glycine (Scheme 1, *m*/*z* 176). It is worth mentioning that when D-glucose was replaced with a *keto* sugar, such as D-fructose or D-tagatose, the peaks

Table 5. Percent Distribution of Molecular Ion *m*/*z* 204 Generated from Labeled D-Glucoses or Excess Labeled L-Alanine[a]

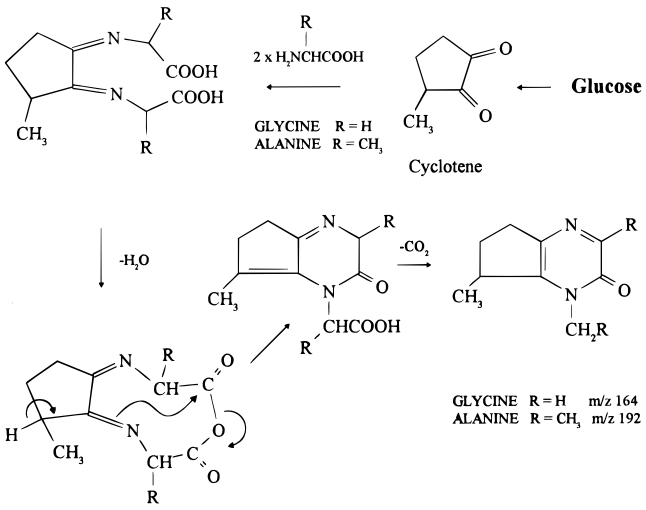
D-glucose/L-alanine	*m*/*z* 204	*m*/*z* 205	*m*/*z* 206
D-glucose/L-alanine	98	2	0
[1-^{13}C]-D-glucose/L-alanine	0	98	2
[2-^{13}C]-D-glucose/L-alanine	0	98	2
[3-^{13}C]-D-glucose/L-alanine	0	98	2
[4-^{13}C]-D-glucose/L-alanine	0	100	0
[5-^{13}C]-D-glucose/L-alanine	0	100	0
[6-^{13}C]-D-glucose/L-alanine	0	100	0
D-glucose/[1-^{13}C]-L-alanine	12	88	0
D-glucose/[2-^{13}C]-DL-alanine (92% enriched)	1	18	81
D-glucose/[3-^{13}C]-DL-alanine	0	5	95
D-glucose/[^{15}N]-L-alanine	0	1	99

[a] Values are adjusted for natural abundance; compounds less than 99% enriched are indicated.

associated with quinoxalinone structures increased significantly, in both glycine and L-alanine systems. This observation could be related to the fact that Heyn's product is more reactive toward the second amino acid attack, considering the carbonyl group being reacted is an aldehyde.

Cyclic α-dicarbonyls, such as cyclotene, are also known to be formed in Maillard systems. Since cyclotene was detected in D-glucose/glycine and D-glucose/L-alanine systems, its ability to undergo similar reactions was also investigated. Commercial cyclotene was reacted in the pyrolysis probe, with excess glycine or L-alanine in separate experiments, and the mass spectra of the products formed were compared to that of glycine or L-alanine reaction products with glucose (Table 1). The main product formed in both systems had a molecular ion at *m*/*z* 188 which could arise from dimerization of cyclotene followed by the loss of 2 mol of water. However, the glycine/cyclotene reaction also produced

Scheme 5. Proposed Mechanism of Formation of Cyclopenta-2(1*H*)-pyrazinones from D-Glucose or Cyclopentene and Glycine or L-Alanine, Based on Labeling Studies



a product with a molecular ion at *m*/*z* 164 and the cyclotene/L-alanine mixture produced a product with a molecular ion at *m*/*z* 192. Both of these compounds were also detected in the respective mixtures of amino acids with D-glucose and exhibited similar retention times and amino acid incorporation patterns as that of quinoxalinones and pyrazinones. Based on these findings, they were tentatively assigned 1,7-dimethylcyclopenta-2(1*H*)-pyrazinone and 1-ethyl-3,7-dimethylcyclopenta-2(1*H*)-pyrazinone structures, respectively. Scheme 5 depicts the proposed mechanism of formation of cyclopentapyrazinones from glycine and L-alanine model systems.

CONCLUSION

Quinoxalinones and pyrazinones are formed in Maillard systems by multiple additions of amino acids to different dicarbonyls; as such, they could be viewed as *in situ* indicators of formation of dicarbonyl compounds in Maillard model systems. Elucidiation of their mechanism of formation was possible through the use of labeled sugars and amino acids utilizing Py/GC/MS.

ACKNOWLEDGMENT

V.Y. acknowledges funding for this research by the Natural Sciences and Engineering Research Council (NSERC) of Canada.

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Received for review July 12, 1996. Revised manuscript received December 6, 1996. Accepted December 27, 1996.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts*, February 15, 1997.