# Formation of Novel 2(1H)-Pyrazinones as Peptide-Specific Maillard Reaction Products

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The flavor compounds generated from the reaction of dipeptides with glucose at 180 °C and a pH range of 5.3–5.5 derived mainly from two pathways: (1) the reaction of dipeptides with glucose degradation products to form pyrazinones or (2) the reaction of amino acids derived from peptide bond hydrolysis with glucose degradation products to form aldehydes via Strecker degradation.

## INTRODUCTION

Recent work on the generation of aroma compounds from the Maillard reaction was mostly concerned with simple model systems using amino acids (Baltes et al., 1989; Tressl et al., 1989). Although a wide range of peptides has been reported in considerable quantity in many food systems (Rizzi, 1989), the role of peptides as precursors in the generation of flavor compounds has not been investigated to an appreciable extent. Chuyen et al. (1973) studied the reaction of various dipeptides with glyoxal and reported the identification of 2-(3′-alkyl-2′-oxopyrazin-1′-yl)alkanoic acids as major products. Rizzi (1989) reported that model Maillard reactions of dipeptides and tripeptides with fructose generated Strecker degradation products, such as Strecker aldehydes and alkylpyrazines, from amino acids with blocked amino and carboxyl functionalities. Recently, we observed that the Maillard reaction of glucose with glycine and triglycine produced significantly greater amounts of pyrazines than did the reaction with either diglycine or tetraglycine (Oh et al., 1991). The similarity of the results of glycine with triglycine and diglycine with tetraglycine in the pyrazine formation suggested that tripeptides or tetrapeptides could be degraded through diketopiperazines.

The present paper reports the reaction of dipeptides, Gly-Leu and Leu-Gly, with glucose, with emphasis on the formation of novel 2(1H)-pyrazinones as the peptide's specific Maillard reaction products.

## EXPERIMENTAL PROCEDURES

Diglycine, triglycine, tetraglycine, Gly-Leu, Leu-Gly, and a mixture of glycine and leucine (Sigma Chemical Co., St. Louis, MO) (0.002 mol of each) were respectively dissolved with 0.002 mol of D-glucose in 50 mL of distilled water. The initial pH of each solution mixture was in the range 5.3–5.5. Each sample mixture was transferred into a 0.3-L Hoke SS-DOT sample cylinder and heated in an oil bath at 180 °C for 2 h. Each reaction mixture was adjusted to its pH >12 with aqueous NaOH and then extracted five times with 50 mL of methylene chloride containing an internal standard. Pentadecane ($C_{15}$) was used as an internal standard for the Gly-Leu and Leu-Gly model systems, while dodecane ($C_{12}$) was used as an internal standard for the mixture of glycine and leucine; *p*-cymene was used as the internal standard for diglycine, triglycine, and tetraglycine model systems. The methylene chloride extracts were dried over anhydrous sodium sulfate and concentrated by blowing with nitrogen gas to a final volume of 0.2 mL.

The concentrated samples were then analyzed by gas chromatography (Varian 3400) and gas chromatography–mass spectrometry; the instrument was equipped with an FID and a fused silica capillary column [50 m × 0.32 mm (i.d.), 1.05-μm thickness, HP-1; Hewlett-Packard]. The GC was operated with a split ratio of 50:1 and programmed as follows: injector temperature, 270 °C; detector temperature, 300 °C; helium carrier flow rate, 0.8 mL/min.; temperature program, 40–260 °C at 2 °C/min and held at 260 °C for 40 min. The samples were also analyzed by GC–MS using a Varian 3400 gas chromatograph coupled with a Finnigan MAT 8230. The same conditions and temperature program for gas chromatography were used in the GC–MS analysis. The mass spectrometer was set at 70 eV, a source temperature of 250 °C, and a filament emission current of 1 mA.

## RESULTS AND DISCUSSION

An equimolar amount of various peptides, i.e., Gly-Leu, Leu-Gly, diglycine, triglycine, tetraglycine, and a mixture of Gly and Leu, was separately heated with glucose at 180 °C for 2 h, pH 5.3–5.5. The volatile compounds produced were analyzed and identified by GC–MS. The relative concentration of the volatiles formed was quantified by comparison with an internal standard.

In our previous study of pyrazine formation from the reaction of glucose with glycine, diglycine, triglycine, and tetraglycine, we observed three structurally similar unknown compounds formed as major volatiles (Oh et al., 1991). After careful examination of their mass spectral data (Table I), these three unknown compounds were identified as novel pyrazinones. The pyrazinones identified were 1,6-dimethyl-2(1H)-pyrazinone, 1,5-dimethyl-2(1H)-pyrazinone, and 1,5,6-trimethyl-2(1H)-pyrazinone. Their retention indices and quantitation data are listed in Table II.

The structures, of the pyrazinones were confirmed by comparing their mass spectra and GC retention times with the authentic compounds synthesized by the reaction of diglycine with pyruvaldehyde. These three pyrazinones were formed in the reaction of diglycine with pyruvaldehyde with yields of 25.56%, 9.39%, and 20.16% for 1,6-dimethyl-2(1H)-pyrazinone, 1,5-dimethyl-2(1H)-pyrazinone, and 1,5,6-trimethyl-2(1H)-pyrazinone, respectively. The total pyrazinones amounted to 55.11%. The formation of 1,5,6-trimethyl-2(1H)-pyrazinone in this model system could be derived from the radical fragmentation of pyruvaldehyde into acetyl radical which recombined to form butanedione. The butanedione could react with diglycine to produce 1,5,6-trimethyl-2(1H)-pyrazinone as proposed from the mechanism in Figure 1. According to the mechanism proposed by Chuyen et al. (1972) for the

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**Table I. Mass Spectral Data of Identified Pyrazinones from Reaction between Dipeptides Gly-Gly, Gly-Leu, and Leu-Gly**

| pyrazinone | mass spectral data, *m/z* (rel intensity) |
|---|---|
| from Gly-Gly | |
| 1,6-dimethyl-2(1*H*)-pyrazinone | 124 ($M^+$, 80), 95 (100), 81 (22), 68 (34), 54 (19), 42 (47), 41 (46), 39 (42), 28 (25) |
| 1,5-dimethyl-2(1*H*)-pyrazinone | 124 ($M^+$, 95), 95 (100), 81 (14), 68 (27), 56 (48), 42 (31), 39 (31), 28 (30) |
| 1,5,6-trimethyl-2(1*H*)-pyrazinone | 138 ($M^+$, 75), 109 (100), 95 (33), 82 (8), 68 (20), 56 (42), 42 (30), 28 (25) |
| from Gly-Leu and Leu-Gly | |
| 1-methyl-3-isobutyl-2(1*H*)-pyrazinone | 166 ($M^+$, 24), 151 (56), 124 (100), 95 (48), 81 (16), 54 (20), 42 (28) |
| 1-isopentyl-2(1*H*)-pyrazinone | 166 ($M^+$, 44), 123 (12), 110 (100), 97 (48), 82 (56), 68 (40), 55 (56), 41 (48) |
| 1,6-dimethyl-3-isobutyl-2(1*H*)-pyrazinone | 180 ($M^+$, 24), 165 (48), 138 (100), 95 (12), 109 (60), 56 (40), 41 (20) |
| 1,5,6-trimethyl-3-isobutyl-2(1*H*)-pyrazinone | 194 ($M^+$, 28), 179 (60), 152 (100), 137 (32), 123 (40), 82 (8), 70 (12), 42 (24) |

**Table II. Amount of Pyrazinones Generated from the Reaction between Di-Gly, Tri-Gly, Tetra-Gly, and Glucose**

| | | | mg/mol of peptide | | |
|---|---|---|---|---|---|
| pyrazinone | MW | $I_k$[a] | tri-Gly | tri-Gly | tetra-Gly |
| 1,6-dimethyl-2(1*H*)-pyrazinone | 124 | 1315 | 323.00 | 119.35 | 422.35 |
| 1,5-dimethyl-2(1*H*)-pyrazinone | 124 | 1379 | t[b] | t | 1.37 |
| 1,5,6-trimethyl-2(1*H*)-pyrazinone | 138 | 1423 | 15.03 | 36.06 | 29.90 |

[a] Linear retention indices were calculated according to the method of Majlat et al. (1974) on an HP-1 column. [b] t, trace.

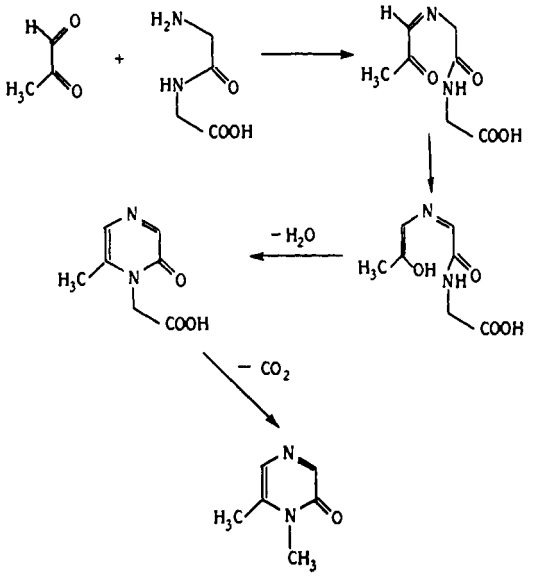



**Figure 1.** Mechanism for the formation of 1,6-dimethyl-2(1*H*)-pyrazinone from pyruvaldehyde and diglycine.

formation of pyrazinone derivatives, 2-(3′-alkyl-2′-oxopyrazin-1′-yl)alkanoic acids, in the reaction of various dipeptides with glyoxal, the α-dicarbonyl compounds formed by the degradation of glucose such as glyoxal, pyruvaldehyde, and diacetyl would react with the amino terminal of dipeptides. As shown in Figure 1, in the case of pyruvaldehyde, the amino end of the dipeptide should react with the less sterically hindered aldehydic carbonyl group more easily than the other ketone carbonyl carbon. In addition to steric factors, electronic factors also play a role in the reactivity of aldehydic carbonyl groups relative to ketone. After the tautomerization of the methyl ketone, the intermediate "dipeptide–pyruvaldehyde" was cyclized to form 2-(3′-alkyl-2′-oxopyrazin-1′-yl)alkanoic acid. At an elevated temperature (180 °C) as used in our model experiments, the 2-(3′-alkyl-2′-oxopyrazin-1′-yl)alkanoic acid would undergo decarboxylation to yield 2-pyrazinones. It is important to point out that the pyrazinones were only identified in the diglycine, triglycine, and tetraglycine systems but not in the free glycine system.

Peptides longer than two amino acids tend to undergo hydrolysis first to dipeptides and further to its constituent amino acids, and these dipeptides preferred to react with glucose degradation products and cyclize to form pyrazinones rather than to undergo a peptide bond cleavage. A peptide bond that is further away from the formal positive charge of the amino terminal is more easily hydrolyzed in the acidic condition (Hill, 1965). The positive charge at the amino terminal will repel the incoming $H^+$, because the close proximity of two positively charged van der Waal radii in the dipeptide is energetically unfavorable, and thus the dipeptide is not easily hydrolyzed.

The reaction between dipeptides, Gly-Leu and Leu-Gly, with glucose at 180 °C for 2 h produced various volatile compounds such as pyrazinones, pyrazines, furans, and 2-isopropyl-5-methyl-2-hexenal. Table III lists the identification and quantitation of volatile compounds generated. They are mainly derived from two pathways: (1) the reaction of dipeptides with glucose degradation products to form pyrazinones or (2) the reaction of amino acids, derived from the hydrolysis of peptide bonds, with glucose degradation products to form aldehydes and pyrazines via Strecker degradation. The dipeptides, Gly-Leu and Leu-Gly, that reacted with glucose produced four novel pyrazinones (Table III). From the mass spectral data (Table I), it is shown that two pyrazinones possess an identical molecular ion of *m/z* 166. From the formation mechanism, these two pyrazinones should be 1-methyl-3-isobutyl-2(1*H*)-pyrazinone and 1-isopentyl-2(1*H*)-pyrazinone. The assignment of these two pyrazinones was based on the fragmentation pattern from the mass spectral data. The base peak, *m/z* 124, in the mass spectrum of 1-methyl-3-isobutyl-2(1*H*)-pyrazinone resulted from the loss of 42 mass units from the molecular ion. This is the loss of the neutral molecule, propene, due to the well-established McLafferty rearrangement as shown in Scheme I. The second pyrazinone with a molecular weight of 166 possessed the base peak of *m/z* 110. This loss of 2-methylpropene from the molecular ion can also be explained by the McLafferty-type rearrangement as shown in Scheme II.

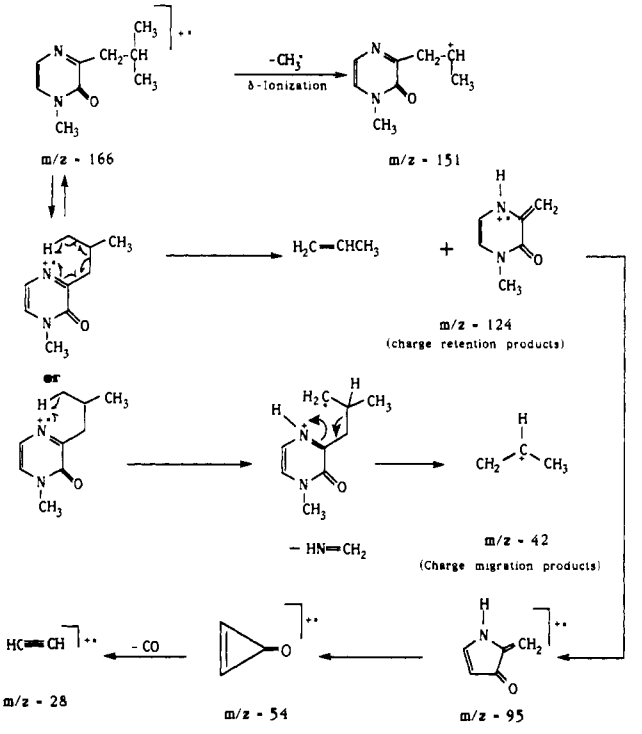
Table III shows that the dipeptides, Gly-Leu and Leu-Gly, produced the same type of pyrazinones. Quantitatively, the Gly-Leu dipeptide produced a slightly greater amount of pyrazinones than Leu-Gly. The only possible explanation for this phenomenon is that the Gly-Leu dipeptide is in equilibrium with the Leu-Gly dipeptide (Scheme III). In addition, the Gly-Leu dipeptide also produced about 2.5 times the amount of 2-isopropyl-5-methyl-2-hexenal, an aldol condensation product of 2-methylbutanal, as compared to the Leu-Gly dipeptide in this model system. The difference in the amount of total pyrazinones and 2-isopropyl-5-methyl-2-hexenal generated in this system indicated that a shift equilibrium from the

**Table III. Relative Concentration of Identified Volatiles with Its Retention Indices of the Reaction between Gly-Leu, Leu-Gly, and Gly + Leu with Glucose Model System Heated at 180 °C for 2 h**

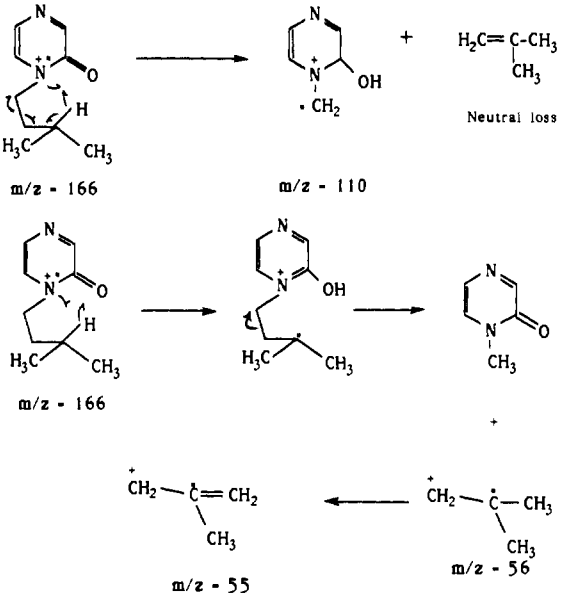
| compound | $I_k$[a] | Gly-Leu, ppm | Leu-Gly, ppm | Gly + Leu, ppm |
|---|---|---|---|---|
| 2-methylpyrazine | 799 | | | 785.31 |
| furfural | 815 | 7.63 | 13.76 | |
| 2,5-dimethylpyrazine | 891 | 42.81 | 43.00 | 128.67 |
| 5-methylfurfural | 948 | 39.42 | | |
| 2,3,5-trimethylpyrazine | 983 | 39.42 | 16.44 | 2016.54 |
| 2-isopropyl-5-methyl-2-hexenal | 1100 | 871.47 | 372.72 | 1830.34 |
| 1-methyl-3-isobutyl-2(1*H*)-pyrazinone | 1397 | 351.41 | 433.22 | |
| 1-isopentyl-2(1*H*)-pyrazinone | 1432 | 185.58 | 129.95 | |
| 1,6-dimethyl-3-isobutyl-2(1*H*)-pyrazinone | 1562 | 1629.69 | 1234.09 | |
| 1,5,6-trimethyl-3-isobutyl-2(1*H*)-pyrazinone | 1638 | 84.17 | 83.54 | |

[a] Linear retention indices were calculated according to the method of Majlat et al. (1974) on an HP-1 column.

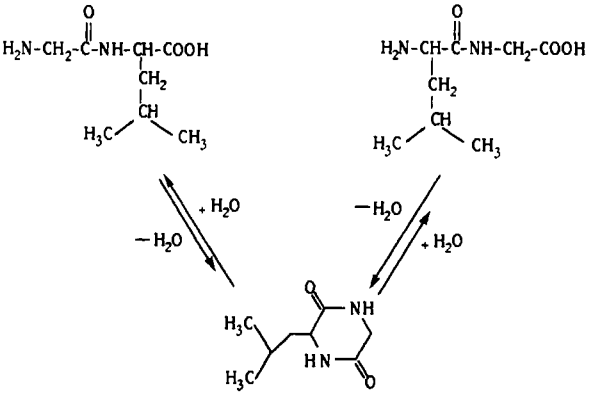
**Scheme I. Mass Fragmentation of 1-Methyl-3-isobutyl-2(1*H*)-pyrazinone**




**Scheme II. Mass Fragmentation of 1-Isopentyl-2(1*H*)-pyrazinone**




**Scheme III. Proposed Mechanism for the Equilibrium between Gly-Leu and Leu-Gly**



Leu-Gly to the Gly-Leu dipeptide has taken place. This interconversion of isomeric dipeptides has also been observed by heating dipeptides such as Gly-Val and Val-Gly in a diluted acid solution (Hill, 1965).

Other than the pyrazinones, the reaction of Gly-Leu and Leu-Gly with glucose produced Strecker aldehydes and pyrazines as major products. 2-Isopropyl-5-methyl-2-hexenal possessed a sharp cocoa-like aroma and was the aldol condensation product of 3-methylbutanal from leucine (Hartman, 1983). The reaction between glucose and a mixture of glycine and leucine generated larger amounts of 2-isopropyl-5-methyl-2-hexenal than the reaction of Gly-Leu or Leu-Gly with glucose. It is interesting to note that the Gly-Leu dipeptide produced a larger amount of 2-isopropyl-5-methyl-2-hexenal than the Leu-Gly dipeptide. This result suggests that the generation of 3-methylbutanal from Gly-Leu was more efficient than that from Leu-Gly. This phenomenon can be explained as follows: (1) As already mentioned in the above discussion, the equilibrium between Leu-Gly and Gly-Leu is a shift of equilibrium toward the Gly-Leu dipeptide. (2) Gly-Leu should, for steric reasons, be more susceptible to hydrolysis than Leu-Gly. Therefore, in a given time Gly-Leu could produce more leucine and 3-methylbutanal than Leu-Gly.

The other interesting compounds identified were pyrazines which are well-known for their roasted and nutty aromas. The reaction of the mixture of glycine and leucine with glucose produced the most abundant pyrazines (Table III). This result is similar to our previous report of the reaction of glucose with diglycine, triglycine, and tetraglycine (Oh et al., 1991).

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**Registry No.** Di-Gly, 556-50-3; tri-Gly, 556-33-2; tetra-Gly, 637-84-3; Gly-Leu, 869-19-2; Leu-Gly, 686-50-0; Gly, 56-40-6; Leu, 61-90-5; D-glucose, 50-99-7; 1,6-dimethyl-2(1*H*)-pyrazinone, 137232-62-3; 1,5-dimethyl-2(1*H*)-pyrazinone, 74879-12-2; 1,5,6-trimethyl-2(1*H*)-pyrazinone, 137232-63-4; 2-methylpyrazine, 109-08-0; 5-methylfurfural, 620-02-0; 2,5-dimethylpyrazine, 123-32-0; 2,3,5-trimethylpyrazine, 14667-55-1; 2-isopropyl-5-methyl-2-hexenal, 35158-25-9; furfural, 98-01-1; 1-methyl-3-isobutyl-2(1*H*)-pyrazinone, 137232-64-5; 1-isopentyl-2(1*H*)-pyrazinone, 137232-65-6; 1,6-dimethyl-3-isobutyl-2(1*H*)-pyrazinone, 137232-66-7; 1,5,6-trimethyl-3-isobutyl-2(1*H*)-pyrazinone, 137232-67-8.