Influence of Pyrolytic and Aqueous-Phase Reactions on the Mechanism of Formation of Maillard Products

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The influence of the reaction phase on the mechanism of formation of Maillard products was studied by comparison of ^{13}C-label incorporation patterns of the common products formed in model systems consisting of labeled glycine and D-glucoses subjected to both pyrolysis and heating in aqueous solutions. Pyrolysis experiments were performed at 250 °C for 20 s, and aqueous model systems were heated in sealed vials for 3 h at 120 °C followed by GC/MS analysis. Label incorporation patterns of the following compounds were analyzed: cyclotene, furanmethanol, acetylpyrrole, 5-methyl-pyrrole, trimethylpyrazine, acetic acid, 3-hydroxy-2-butanone, 2,3-butanedione, and 2-methyl-4,5-dihydro-3(2*H*)-furanone. Although pyrolysis reaction produced higher number of products, however, the major pathways of formation of variety of important Maillard products followed the same mechanism under both pyrolytic and aqueous systems. Furthermore, contrary to literature speculations, 2-methyl-4,5-dihydro-3(2*H*)-furanone was shown to be formed by ring contraction of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one, through benzilic acid rearrangement, followed by decarboxylation.

Keywords: *Maillard reaction mechanism; D-[^{13}C]glucoses; [^{13}C-2]glycine; cyclotene; furans; pyrroles; trimethylpyrazine; 2,3-butanedione; 3-hydroxy-2-butanone; 2-methyl-4,5-dihydro-3(2H)-furanone*

INTRODUCTION

Maillard reaction can occur in liquid and solid phases during various thermal treatments of foods or manufacture of "reaction flavors". During roasting or baking, Maillard reaction products are mainly formed through solid-phase interactions in the presence of moisture. This process can be mimicked through pyrolysis experiments (Baltes and Mevissen, 1988). Aqueous-phase Maillard reactions can be encountered during domestic cooking and manufacture of "reaction flavors" that in turn can be mimicked through aqueous model system studies (Tressl et al., 1993a). Thermal processing under pressure or closed containers can also cause some interactions of the volatile intermediates to occur in the gas phase. The influence of the reaction phase on the course of Maillard reaction is manifested in the differences of the perceived flavors of roasted versus boiled food products.

In general, solid-phase reactions are faster than their liquid-phase counterparts due mainly to the more efficient energy transfer and the concentration effect and partially due to substantial loss of energy to the solvent in the liquid-phase reactions. Furthermore, in solid phase, the energy absorbed is directly expended through chemical transformations and degradations. However, in aqueous-phase reactions, solvent-assisted isomerizations and attainment of equilibrium conditions are more pronounced than in solid-phase reactions. In addition, chemical transformations with polar transition states are more favored in liquid phase than in solid phase due to solvent stabilization effects. It is expected therefore that solid-phase reactions produce a higher number of products in a shorter period of time compared with aqueous reactions under comparable reaction conditions. In our laboratories, a pyrolysis−gas chromatography/mass spectrometry (Py−GC/MS) system has been extensively employed as a microscale reactor to study the mechanism of aroma formation from mixtures of ^{13}C or ^{15}N-labeled amino acids and ^{13}C-labeled reducing sugars through Maillard reaction (Yaylayan and Keyhani, 1996). Although the mechanistic pathways identified through pyrolysis experiments are relevant to roasting conditions, questions could be raised regarding its relevance to aqueous reaction conditions. Preliminary analysis of literature data (Yaylayan and Keyhani, 1999) has already indicated that mechanistic pathways operating under pyrolytic conditions are similar to that of aqueous reactions. Confirmation of the relevance of pyrolytic mechanisms to that of aqueous conditions is of practical importance since carrying out the reaction in the pyrolysis probe can substantially reduce the analysis time (from hours to minutes) and eliminate the need for solvent extraction. In addition, the amount of reactants required to perform the experiments is on the order of a few milligrams. This property can facilitate studies with expensive isotopically labeled reactants. One of the advantages of using labeled reactants is that all of the atoms of a reaction product can be traced back to their origin in the starting material (if properly labeled reactants are available). This fact not only facilitates elucidation of their mechanism of formation, but also the assignment of their mass spectral fragments (Yaylayan and Huyghues-Despointes, 1996). Huyghues-Despointes et al. (1994) demonstrated that the pyrolysis of the proline Amadori compound in the quartz tube for 20 s at 250 °C is comparable to autoclaving of a proline/glucose mixture at 150 °C for 1.5 h in water. Huyghues-Despointes and Yaylayan (1996)

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using ^{13}C-labeled glucoses elucidated several mechanistic pathways of formation of proline-specific products through retro-aldol reactions using Py−GC/MS. Due to the convenience of carrying out such experiments to obtain mechanistic information regarding the Maillard reaction, it is informative to explore similarities and differences, if any, between the aqueous-phase and pyrolytic reactions. In this paper, we compare label incorporation patterns of selected Maillard reaction products generated under pyrolytic and aqueous reaction conditions.

EXPERIMENTAL PROCEDURES

D-[1-^{13}C]Glucose, D-[3-^{13}C]glucose, D-[4-^{13}C]glucose, D-[5-^{13}C]-glucose, D-[6-^{13}C]glucose, and [2-^{13}C]glycine were purchased from Cambridge Isotope Laboratories (Andover, MA); all other chemicals and D-[2-^{13}C]glucose were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Aqueous Reactions. Labeled ([^{13}C-2]glycine) or unlabeled glycine (6.0×10^{-4} M) and labeled (at [^{13}C-1]-, [^{13}C-2]-, [^{13}C-3]-, [^{13}C-4]-, [^{13}C-5]-, or [^{13}C-6]-) or unlabeled D-glucose (2.0×10^{-4} M) in 100 μL of water was placed in a 0.1 mL crimp-cap vial (Labcor, Anjou, QC). The samples were then heated in an oil bath (Fisher Scientific, Canada) at a temperature of 120 °C for 3 h and immediately quenched by placing the vials into an ice bath. The samples were subsequently extracted with ethyl ether (3×100 μL) and concentrated before analysis.

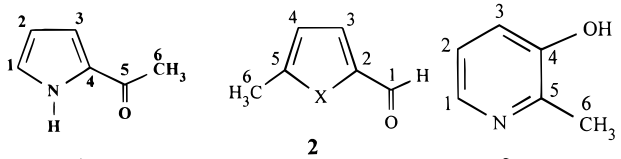
GC/MS Analysis of Aqueous Extracts. The analysis was performed using a Hewlett-Packard GC/mass selective detector (5890 series II GC/5971B MSD). Samples (1 μL) were injected using a cool on-column injector and a fused silica capillary needle. An HP PLOT-Q capillary column (30m × 0.32 mm × 0.20 μm, Hewlett-Packard, Mississagua, Canada) was used. The 3 mL/min constant flow of helium was maintained by an electronic pressure controller (Hewlett-Packard). The column initial temperature (30 °C) was held for 2 min, then increased to 100 °C at a rate of 30 °C/min, then further increased to 250 °C at a rate of 7 °C/min, and held for 35 min. Capillary direct MS interface temperature was 280 °C, and the ion source temperature was 180 °C. The ionization voltage was 70 eV, and the electron multiplier was 2047 V. The mass range analyzed was 28−200 amu, with 3.7 scans/s.

Pyrolysis GC/MS Analysis. A Hewlett-Packard GC/mass-selective detector (5890 series II GC/5971B MSD) interfaced to a CDS Pyroprobe 2000 unit, through a valved interface (CDS 1500), was used for Py−GC/MS analysis. In all experiments, solid samples of a mixture of labeled or unlabeled D-glucose−glycine (1:3 ratio, 3.5 mg) were introduced inside the quartz tube (0.3 mm thickness), plugged with quartz wool and inserted inside the coil probe. The pyroprobe was set at 250 °C at a heating rate of 50 °C/ms with a total heating time of 20 s. The pyroprobe interface temperature was set at 250 °C. The samples were introduced under splitless mode. The 3 mL/min constant flow was maintained by an electronic pressure controller (Hewlett-Packard). Capillary direct MS interface temperature was 180 °C; the ion source temperature was 280 °C. The ionization voltage was 70 eV, and the electron multiplier was 2047 V. The mass range analyzed was 17−200 amu. The column was an HP PLOT-Q (30 m × 0.32 mm × 0.20 μm (Hewlett-Packard, Mississagua, ON). The column initial temperature (30 °C) was held for 2 min, then increased to 100 °C at a rate of 30 °C/min, then further increased to 250 °C at a rate of 7 °C/min, and held for 35 min. The reported percent label incorporation values are the average of triplicate analyses.

RESULTS AND DISCUSSION

The influence of the reaction phase on the mechanism of formation of Maillard products was studied by comparison of ^{13}C-label incorporation pattern of the common products formed in model systems consisting of labeled glycine and D-glucose subjected to both pyrolysis and heating in aqueous solutions. Pyrolysis experiments were performed under conditions previously optimized to carry out Maillard reactions (250 °C for 20 s). On the other hand, aqueous solutions were heated in sealed vials for 3 h at 120 °C, and the reaction mixtures were extracted with ethyl ether and analyzed by GC/MS after concentration under a stream of nitrogen. Both samples were separated on a PLOT-Q column able to retain water and other volatile and low-molecular-weight compounds in addition to neutral gases. Analysis of the two samples have indicated that around 40 compounds from pyrolysis and 11 compounds from the extract of the aqueous sample were retained by the column. Although the pyrolysis reactions were shorter in duration relative to aqueous-phase reactions (20 s vs 3 h), and yet produced more reaction products. Such a difference is not surprising and is consistent with the above discussion on the differences between the two phases. In addition, pyrolysis produced most of the compounds observed in the aqueous extract. Utilization of PLOT-Q column during pyrolysis allowed the detection of carbon dioxide, water, methylamine, and acetic acid, indicating facile decarboxylation and deamination of amino acid and dehydration of sugar under pyrolytic conditions.

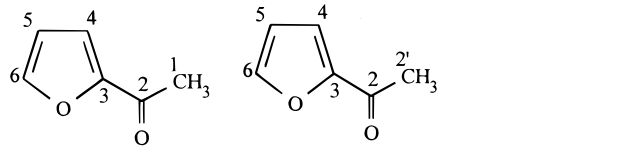
Table 1. Position[a] and Percent Label Distribution of D-Glucose Carbon Atoms in Pyrrole and Pyridine Derivatives



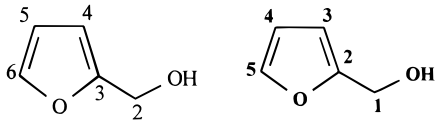
1	2	3
	X = NH **a**, O **b**	
pyrolysis (100%)	pyrolysis (100%)	pyrolysis (100%)
aqueous (100%)	aqueous (100%)	aqueous (100%)

[a] Numbers indicate original D-glucose carbon atoms.

Table 2. Position[a] and Percent Label Distribution of D-Glucose Carbon Atoms in Furan Derivatives



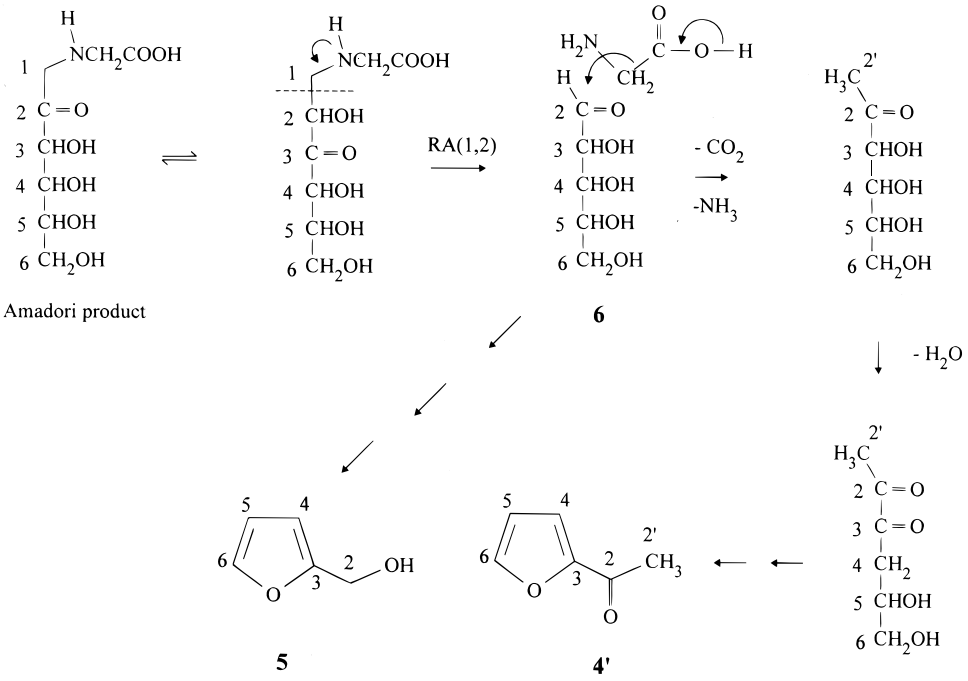
4	4'
major	*minor*
pyrolysis (100%)	pyrolysis (0%)
aqueous (70%)	aqueous (30%)



5	5'
major	*minor*
pyrolysis (90%)	pyrolysis (10%)
aqueous (100%)	aqueous (0%)

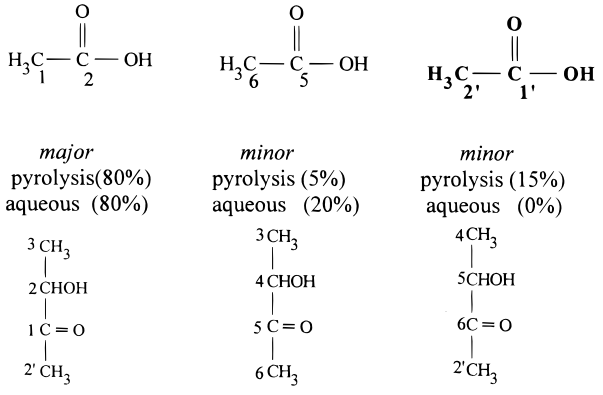
[a] Numbers indicate original D-glucose and glycine (primed) carbon atoms.

Scheme 1. Proposed Mechanism of Formation of Furanmethanol (5) and 2-acetylfuran (4′)[a]



[a] RA (*x*,*y*) = retro aldol cleavage between C-*x* and C-*y* atoms of D-glucose. Numbers indicate original D-glucose carbon locations.

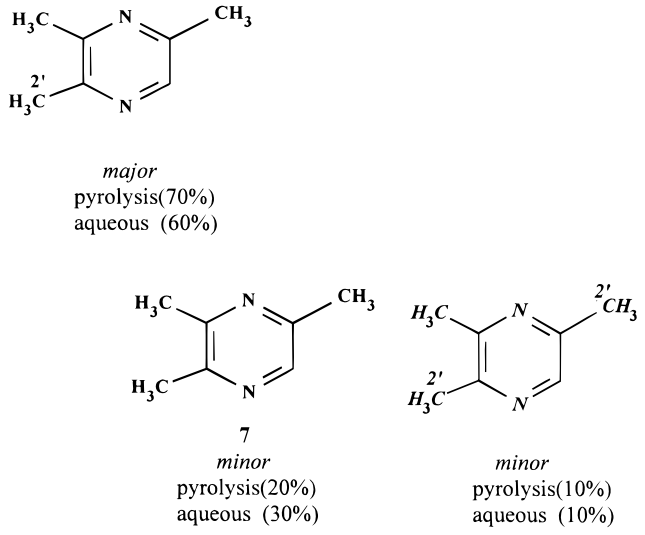
Table 3. Position[a] and Percent Label Distribution of D-Glucose and Glycine Carbon Atoms in Acyclic Derivatives



major	*minor*	*minor*
pyrolysis(80%)	pyrolysis (5%)	pyrolysis (15%)
aqueous (80%)	aqueous (20%)	aqueous (0%)
pyrolysis (30%)	pyrolysis (30%)	pyrolysis (40%)
aqueous (35%)	aqueous (30%)	aqueous (35%)

[a] Numbers indicate original D-glucose and glycine (primed) carbons.

Table 4. Position and Percent Label Distribution of [^{13}C-2]Glycine in Trimethylpyrazine



major
pyrolysis(70%)
aqueous (60%)

7

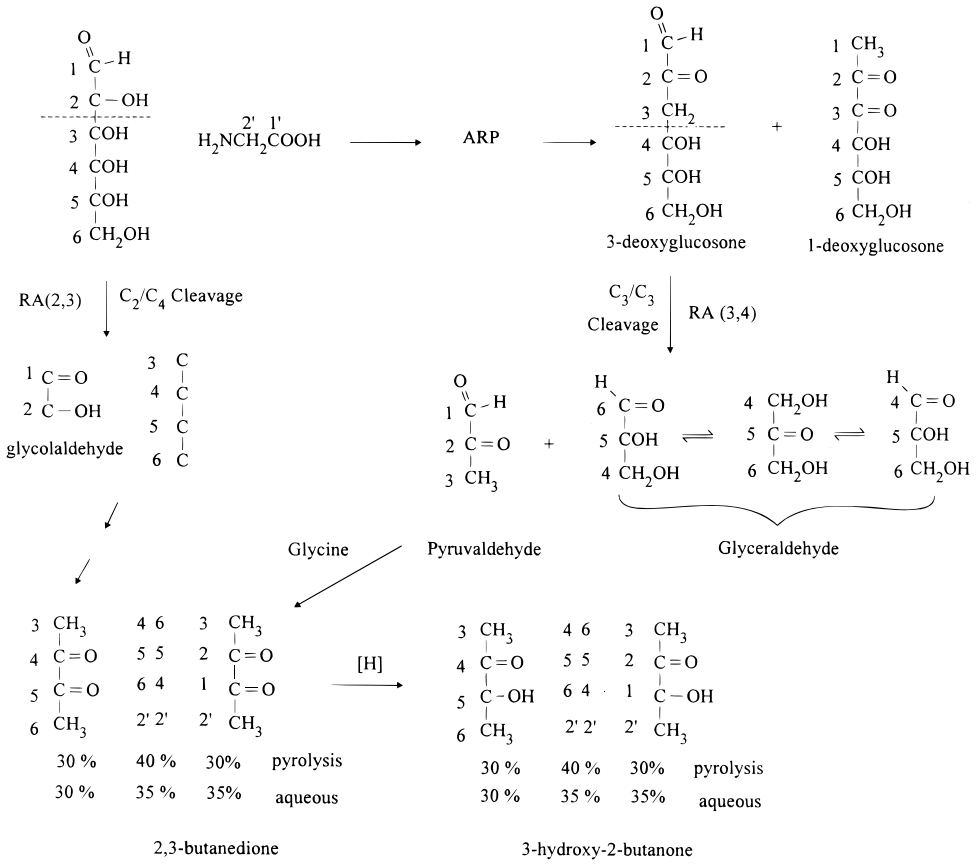
minor
pyrolysis(20%)
aqueous (30%)

minor
pyrolysis(10%)
aqueous (10%)

Formation of Pyrrole and Pyridine Derivatives. One pyridine and two pyrrole derivatives were identified in both pyrolysis and aqueous systems (see Table 1). All three products, 1-(1*H*-pyrrol-2-yl)ethanone (**1**), 2-formyl-5-methylpyrrole (**2a**), and 2-methyl-3-pyridinol (**3**) showed 100% incorporation of all six carbon atoms of D-glucose in both pyrolysis and aqueous systems. Analysis of their mass spectral fragmentation patterns indicated that the sequence of D-glucose carbon atoms incorporated in these structures did not change and that intact hexose skeletons were involved in their formation. The positions of D-glucose carbon atoms in these structures are indicated in Table 1. The mechanism proposed by Tressl et al. (1994) for the formation of compounds **1**, **2a**, and **3** from 3-deoxyglucosone is consistent with this observation. In addition, the position of the labeled [^{13}C-1]-glucose atom in these products also corresponds to that observed by Tressl et al. (1994).

Formation of Furan Derivatives. The following three furan derivatives (Table 2) were identified in both systems: 2-formyl-5-methylfuran (**2b**), 2-acetylfuran (**4**), and furanmethanol (**5**). Similar to its nitrogen counterpart, 2-formyl-5-methylfuran (**2b**) showed 100% incorporation of all six carbon atoms of D-glucose in both pyrolysis and aqueous systems. Analysis of the mass spectral fragmentation pattern indicated that the sequence of D-glucose carbon atoms incorporated did not change and that intact glucose skeletons were involved in their formation. The positions of incorporated glucose carbon atoms are indicated in Table 1. The position of labeled [^{13}C-1]glucose atom also corresponded to that observed by Tressl et al. (1993a). The obvious precursor of furan **2b** is 3-deoxyglucosone. Analysis of label incorporation in 2-acetylfuran (**4**) indicated the existence of two pathways of formation in the aqueous system

Scheme 2. Proposed Fragmentation and Formation of Important Intermediates in D-glucose/Glycine Model System[a]



[a] RA (x,y) = retro aldol cleavage between C-x and C-y atoms of D-glucose; aldol (x,y) = aldol condensation between C-x and C-y atoms of D-glucose; [H] = reduction; numbers indicate original D-glucose carbon positions.

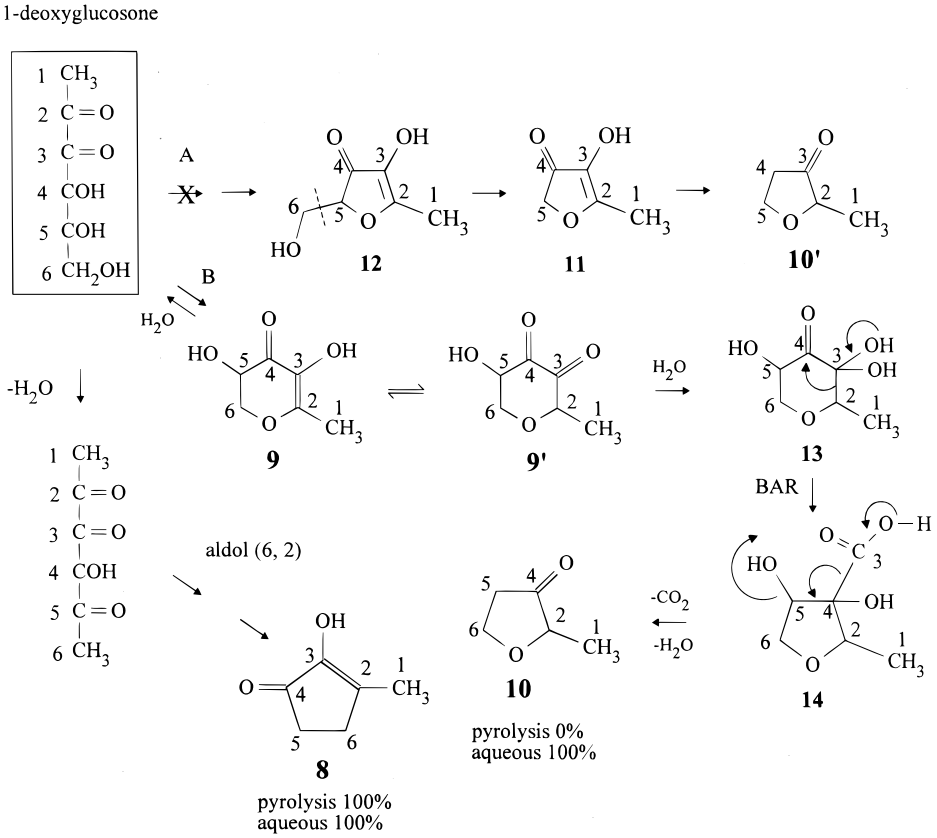
(Table 2). A major pathway (70%) involved incorporation of all six carbon atoms of D-glucose with the retention of the original sequence of the carbon atoms and a minor pathway (30%) involving the C-2′ atom of glycine and the formation of isotopomer **4′** (see Table 2). Pyrolysis mixture on the other hand, generated only **4**. Tressl et al. (1993a) identified furanmethanol (**5**) from [^{13}C-1]-glucose model systems with no label incorporation, which is consistent with the data shown in Table 2. However, the pyrolysis mixture also generated **5′** through a minor pathway (10%) incorporating glucose carbon atoms one through five. Scheme 1 proposes the formation of **4′** and **5** through a common pentose intermediate (**6**) originating from the Amadori product through a retro-aldol reaction. The aldehyde **6** can either cyclize and eventually form furanmethanol (**5**) or react with glycine to form **4′** via 1-deoxy-2-ketohexose. Although the important reaction of amino acids as C-nucleophiles with aldehydes has been demonstrated earlier during pyrolytic reactions (Keyhani and Yaylayan, 1996, Yaylayan and Keyhani, 1999), this is the first observation of this transformation in an aqueous medium.

Formation of Acetic Acid. 2,3-Butanedione and 3-Hydroxy-2-butanone. The major pathway (80%) of formation of acetic acid in both systems involved C-1 and C-2 atoms of glucose (see Table 3). A minor pathway comprising 20% in the case of aqueous reaction and 5% in the case of pyrolytic reaction involved C-5 and C-6 atoms of glucose. In the special case of glycine model system, deamination of the amino acid under pyrolysis can also generate acetic acid (15%). In a previous study on the origin of α-dicarbonyl compounds in glucose model systems (Yaylayan and Keyhani, 1999), three pathways of formation of 2,3-butanedione have been identified during pyrolysis (see Scheme 2). Interestingly, similar label distribution as in 2,3-butanedione was also found in 3-hydroxy-2-butanone, indicating direct reduction of 2,3-butanedione into 3-hydroxy-2-butanone, possibly through disproportionation with α-hydroxycarbonyl compounds (Huyghues-Despointes and Yaylayan, 1996). Similar label distribution patterns were also found in the aqueous system (see Scheme 2 and Table 3).

Formation of Trimethylpyrazine. Comparison of the incorporation of C-2′ glycine atom into trimethylpyrazine (**7** in Table 4) in both aqueous and pyrolytic systems indicated that the major pathway (60−70%) in both systems produced singly labeled pyrazine and the two minor pathways produced unlabeled (20−30%) and doubly labeled (10%) trimethylpyrazines (see Table 4). The label distribution is consistent with the incorporation pattern of C-2′ atoms of glycine in 2,3-butanedione (see Scheme 2). Strecker reaction of glycine with pyruvaldehyde and 2,3-butanedione (Keyhani and Yaylayan, 1996) to eventually produce trimethylpyrazine can justify the C-2′ label distribution observed in **7**, based on the origin of 2,3-butanedione and pyruvaldehyde (see Scheme 2).

Formation of 2-Hydroxy-3-methyl-2-cyclopenten-1-one (8, cyclotene). In both model systems, all six carbon atoms of D-glucose were 100% incorporated into the cyclotene structure (see Scheme 3), indicating involvement of intact hexose chain in its formation. Tressl et al. (1993b) also observed 100% incorporation

Scheme 3. Proposed Mechanism of Formation of Cyclotene (8) and 2-Methyl-4,5-dihydro-3(2*H*)-furanone (10)[a]

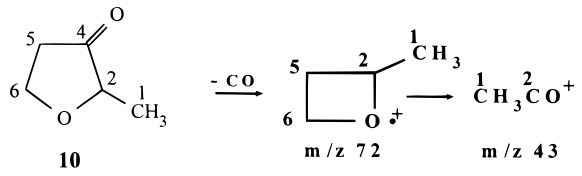


[a] Aldol (*x*,*y*) = aldol condensation between C-*x* and C-*y* atoms of D-glucose; BAR = benzilic acid rearrangement; numbers indicate original D-glucose carbon positions.

of labeled [^{13}C-1]glucose atom into cyclotene generated by autoclaving proline- and hydroxyproline-containing model systems. However, the position of the label varied with the amino acid. Proline generated cyclotene with label incorporation at the methyl carbon, similar to the cyclotene (**8**) generated by both pyrolysis and aqueous model systems, whereas, in the case of hydroxyproline, a [^{13}C-1]glucose label was incorporated at the position C-4 of cyclotene. Tressl et al. (1993b) proposed 1-deoxyglucosone as a precursor of cyclotene (see Scheme 3), and Kim and Baltes (1996) identified cyclotene in the degradation mixture of pure 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (**9** in Scheme 3). The fact that hydrolysis of **9** regenerates 1-deoxyglucosone, is a further confirmation of the above proposition.

Formation of 2-Methyl-4,5-dihydro-3(2H)-furanone (10). On the basis of labeling studies using only [^{13}C-1]glucose, Rewicki et al. (1994), suggested the formation of **10** (Table 5 and Scheme 3) from 4-hydroxy-5-methyl-3(2*H*)-furanone (**11**) via 4-hydroxy-2-(hydroxymethyl)-5-methyl-3(2*H*)-furanone (**12**) through cleavage of terminal hydroxymethyl group (see Scheme 3, pathway A). Mass spectral analysis also indicated the incorporation of the [^{13}C-1]glucose label at the methyl carbons in both **11** and **10′**. According to this pathway, the C-6 atom of D-glucose should not be incorporated into the 2-methyl-4,5-dihydro-3(2*H*)-furanone (**10**) structure. However, studies performed with variously labeled D-glucoses (see Tables 5 and 6), indicated 100% incorporation of the C-6 atom of D-glucose and no incorporation of the C-3 atom of D-glucose in **10**. Furthermore, analysis of mass spectral fragmentation pattern of **10** (see Tables 5 and 6) indicated the C-4 atom of D-glucose as the carbonyl carbon and the presence of two intact

Table 5. Mass Spectral Fragmentation and Percent Label Distribution in 2-Methyl-dihydro-3(2*H*)-furanone (10)



10

pyrolysis (0%)
aqueous (100%)

Table 6. Percent Label Distribution[a] in Selected Mass Spectral Fragments of 2-Methyl-dihydro-3(2*H*)-furanone (10)

	100	101	72	73	43	44
model system (*m*/*z*)	M	M + 1	M	M + 1	M	M + 1
D-glucose/glycine	100	0	100	0	100	0
D- [1-^{13}C]glucose/glycine	0	100	0	100	0	100
D- [2-^{13}C]glucose/glycine	0	100	0	100	0	100
D- [3-^{13}C]glucose/glycine	100	0	100	0	100	0
D- [4-^{13}C]glucose/glycine	0	100	100	0	100	0
D- [5-^{13}C]glucose/glycine	0	100	0	100	100	0
D- [6-^{13}C]glucose/glycine	0	100	0	100	100	0
D-glucose/L-[^{13}C-2]glycine	100	0	100	0	100	0

[a] Percentages are corrected for natural abundance and for less than 100% enrichment.

hexose chains: C-6, C-5, C-4 and C-1, C-2. This fact and the lack of label scrambling in **10** strongly suggest a rearrangement mechanism for the formation of **10** starting from an intact hexose chain or ring. According to Kim and Baltes (1996), **10** was one of the main

products formed when pure **9** (see Scheme 3) was degraded at 150 °C in aqueous solution. Sugars containing α-dicarbonyl moiety are known to undergo benzilic acid rearrangement under basic conditions to form α-hydroxy carboxylic acid derivatives (saccharinic acids) through alkyl group migration (Sowden, 1957). Similar rearrangement is also known to occur with cyclic α-diketones such as **9′** (see Scheme 3) through ring carbon migration that leads to ring contraction and formation of geminal hydroxy carboxylic acids such as **14** (Vollhardt and Schore, 1994). Decarboxylative dehydration of **14** can yield structure **10** that is consistent with the observed label incorporation pattern.

CONCLUSION

Comparison of the label incorporation patterns of the common products formed in aqueous and pyrolytic Maillard model systems have indicated that the major pathways of formation of a variety of important Maillard products follow the same mechanisms and that mechanistic information gained through pyrolysis can be applied to aqueous reactions.

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