Origin of 2,3-Pentanedione and 2,3-Butanedione in D-Glucose/L-Alanine Maillard Model Systems

Varoujan A. Yaylayan* and Anahita Keyhani

McGill University, Department of Food Science and Agricultural Chemistry, 21111 Lakeshore,
Ste. Anne de Bellevue, Quebec, Canada H9X 3V9

Model studies using independently labeled D-[^{13}C]glucoses and L-[^{13}C]alanines have indicated that 2,3-butanedione is formed by a single pathway involving only glucose carbon atoms, whereas 2,3-pentanedione is formed by two pathways, one involving glucose carbon atoms (10%) and the other (90%) through the participation of C2′–C3′ atoms of L-alanine and a C_3 carbon unit from D-glucose. Analysis of label incorporation into selected mass spectral fragments of 2,3-pentanedione have indicated that the C_3 carbon unit originates either from C1–C2–C3 or from C4–C5–C6 fragments of D-glucose. In addition, model studies with pyruvaldehyde and glyceraldehyde have implicated these intermediates as plausible C_3 glucose carbon units capable of producing 2,3-pentanedione upon reaction with L-alanine. The labeling studies have also confirmed a previously identified chemical transformation of α-keto aldehydes affected by the amino acid that leads to the addition of the C-2 atom of the amino acid to the aldehydic carbon atom of α-keto aldehydes.

INTRODUCTION

Reactive α-dicarbonyl intermediates generated during the Maillard reaction play an important role in the formation of aroma compounds. The origin of these reactive C_2, C_3, C_4, and C_5 α-dicarbonyl intermediates detected in Maillard reaction mixtures are mainly attributed to the fragmentation of C_6 α-dicarbonyls originating from the reducing sugars or to the intact sugars themselves through retro aldol reactions. Glyoxal, pyruvaldehyde, 2,3-butanedione, and 2,3-pentanedione have been detected in different Maillard model systems and in many cases have been trapped with suitable reagents. However, the origin of these intermediates cannot be verified conclusively without ^{13}C-labeling experiments with selectively enriched sugars and amino acids. A convenient approach to perform such experiments is through utilization of the quartz tube of a pyrolysis/gas chromatography/mass spectrometry (Py/GC/MS) system as a microreactor (Keyhani and Yaylayan, 1996). Although under pyrolytic conditions a higher number of products are formed compared with aqueous reactions, most of the products identified in aqueous systems are also formed under pyrolytic conditions. In addition, there are indications that the position and label distribution in the common products observed in the same model systems, between aqueous and pyrolytic reactions, are identical (see Table 1). This indicates the similarity of mechanisms of formation of these common products under both conditions. Consequently, mechanistic conclusions derived from label incorporation in the products observed under pyrolytic conditions that are common to both systems have significant relevance to the aqueous reactions. In this study, the Py/GC/MS system was used to trace the origin of the carbon atoms of 2,3-pentanedione formed in the D-glucose/L-alanine system using independently labeled D-[^{13}C]glucoses and L-[^{15}N/^{13}C]alanines. It has been reported (Weenen and Apeldoorm, 1996) that L-alanine enhances the formation of this α-dicarbonyl intermediate, and it has been speculated that the Strecker aldehyde (acetaldehyde) is involved in the process of formation of 2,3-pentanedione.

MATERIALS AND METHODS

All reagents, chemicals, and D-[1-^{13}C]glucose (99%), D-[2-^{13}C]glucose (99%), and D-[6-^{13}C]glucose were purchased from Aldrich Chemical Co. (Milwaukee, WI). D-[3-^{13}C]glucose, D-[4-^{13}C]glucose, D-[5-^{13}C]glucose, L-[^{15}N]alanine, L-[1-^{13}C]alanine, DL-[2-^{13}C]alanine (92%), and L-[3-^{13}C]alanine (98%) were purchased from ICON Services Inc. (Summit, NJ).

Pyrolysis/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 glycine/glucose or alanine/glucose were introduced inside a quartz tube (0.3 mm thickness), which was plugged with quartz wool and inserted inside the coil probe. The pyroprobe was set at the desired temperature (250 °C for glycine model systems and 210 °C for L-alanine model systems) at a heating rate of 50 °C/ms and a THT (total heating time) of 20 s. The pyroprobe interface temperature was set at 250 °C. 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 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 1682 V. The mass range analyzed was 30–300 amu. The column was a fused silica DB-5 column (60 m length × 0.25 mm i.d. × 25 μm film thickness; Supelco, Inc.). Unless otherwise specified, the column initial temperature was −5 °C for 3 min and increased to 50 °C at a rate of 30 °C/min; immediately, the temperature was further increased to 270 °C at a rate of 8 °C/min and kept at 270 °C for 5 min.

RESULTS AND DISCUSSION

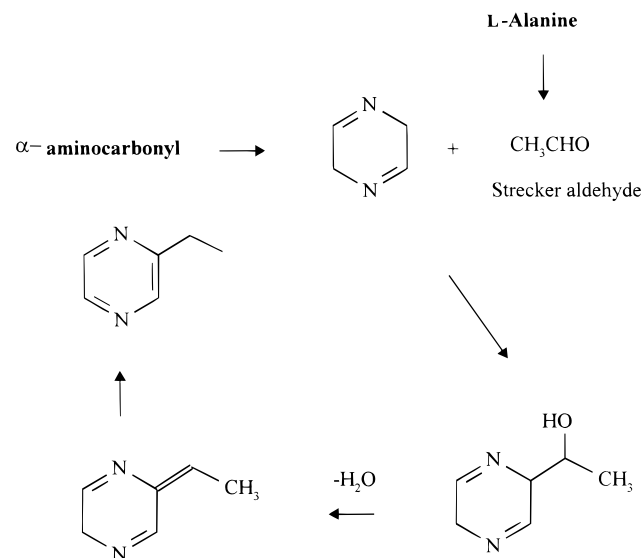The origin of α-dicarbonyl intermediates formed during the Maillard reaction has not been investigated in

---

* To whom all correspondence should be addressed.

Origin of 2,3-Pentanedione and 2,3-Butanedione

J. Agric. Food Chem., Vol. 47, No. 8, 1999 **3281**

Table 1. Comparison of Percent Incorporation and Position of ^{13}C-Atoms in Selected Model Systems during Pyrolytic and Aqueous Reaction Studies

| compound	pyrolysis[a]	aqueous[b,c]
methylpyrazine[b]	0%	0%
2,5-dimethylpyrazine[b]	0%	0%
2,6-dimethylpyrazine[b]	0%	0%
2-ethyl-5-methylpyrazine[b]	72% at C-2 of ethyl	70% at C-2 of ethyl
3,5-diethyl-2-methylpyrazine[b]	65% s, 23% d at C-2 of ethyl(s)	70% s, 20% d at C-2 of ethyl(s)
1-(1′-pyrrolidinyl)-2-propanone[c]	45% at C-3	40% at C-3
2-acetylpyrrole[d]	97% at C-5	98% at C-5
1-formyl-5-methylpyrrole[d]	98% at CHO	98% at CHO

[a] 250 °C, 20 s. [b] Incorporation of C-3 atom of L-alanine in 3-[^{13}C]alanine/glucose model system heated at 180 °C, pH 5.6, for 7 min (Amrani-Hemaimi et al., 1995). [c] Incorporation of C-1 atom of glucose during autoclaving of proline/glucose system at 150 °C, 1.5 h, in water (Tressl et al., 1993). [d] Incorporation of C-1 atom of glucose during autoclaving of glycine/glucose system at 150 °C, 1.5 h, in water (Tressl et al., 1995).

Scheme 1. Mechansim of Incorporation of L-Alanine Carbon Atoms in Pyrazines As Proposed by Amrani-Hemaimi et al. (1995)

Scheme 2. Proposed Mechanisms of Formation of 2,3-Pentanedione through the Interaction of L-Alanine with Glyceraldehyde and Pyruvaldehyde

detail by labeling studies, although there are indications that amino acid carbon atoms are also involved in their formation through chain elongation of smaller α-dicarbonyl units originating from the sugar. One such example is 2,3-pentanedione in the L-alanine model system (Weenen and Apeldoorm, 1996). Indirect evidence has been provided for the amino acid involvement in the formation of pyruvaldehyde from glyoxal and 2,3-butanedione from pyruvaldehyde in glycine model systems (Keyhani and Yaylayan, 1996). The formation of pyruvaldehyde and 2,3-butanedione in the above model systems was inferred by observing the incorporation of the ^{13}C-2 atom of glycine as methyl substituents into the resulting pyrazines, through a Strecker pathway. Incorporation of L-[^{13}C-3]alanine carbon atoms as ethyl substituents in pyrazines was also observed by Amrani-Hemaimi et al. (1995). They attributed the incorporation of ^{13}C-3 atoms of L-alanine to the reaction of Strecker aldehyde (acetaldehyde) with dihydropyrazine intermediate (Scheme 1). Although this mechanism explains the incorporation of a single ethyl group into pyrazine, it cannot explain the formation of pyrazines with more than one ethyl group originating from L-alanine, such as in 3,5-diethyl-2-methylpyrazine (see Table 1). It is, therefore, more likely that incorporation of such amino acid carbon atoms occurs prior to dihydropyrazine formation and at the dicarbonyl stage through a chain elongation reaction, as suggested by Keyhani and Yaylayan (1996) and illustrated in Scheme 2. Furthermore,
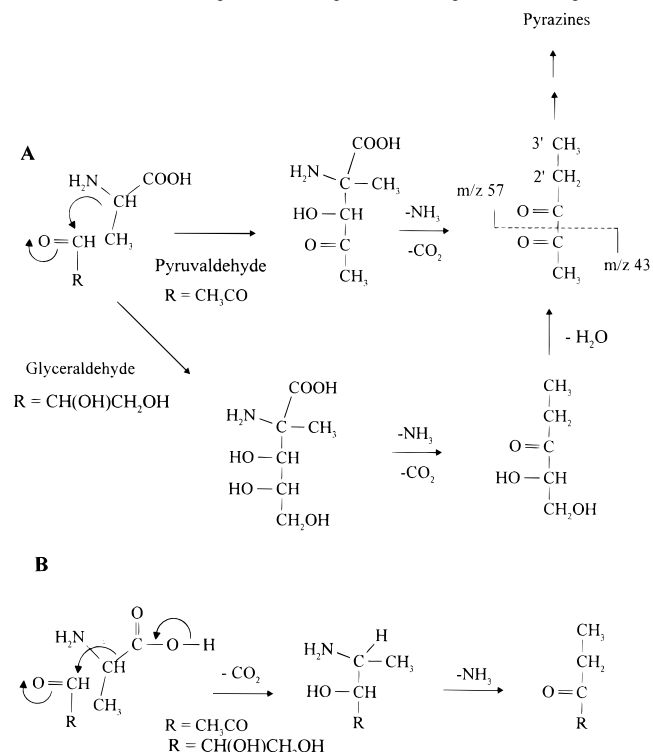
dicarbonyl compounds containing carbon atoms from amino acids have already been identified (Yaylayan and Keyhani, 1998) in [^{13}C-2] glycine/glucose model systems. In this system, 70% of the 2,3-butanedione generated was singly labeled and 30% was unlabeled (containing C-3, C-4, C-5, and C-6 atoms of glucose) as shown in Scheme 3. In the [^{13}C-2] glycine/glyoxal system, 100% of 2,3-butanedione was doubly labeled, and in the [^{13}C-2] glycine/pyruvaldehyde system, 100% was singly labeled (Yaylayan and Keyhani, 1998). These model studies with glyoxal and pyruvaldehyde clearly indicate that the incorporation of amino acid carbon atoms occurs by direct interaction between amino acid and smaller dicarbonyl compounds. In addition, it also indicates that the C-2 atom of glycine acts as a nucleophile during chain elongation reactions and not as an electrophile in the form of a Strecker aldehyde as suggested by others (Weenen and Apeldoorm, 1996; Amrani-Hemaimi et al., 1995).

Origin of 2,3-Butanedione and 2,3-Pentanedione in the L-Alanine Model System. Studies performed with labeled D-glucoses and glycines (Keyhani and

Scheme 3. D-Glucose Cleavage Reactions and Percent Formation of 2,3-Butanedione and 2,3-Pentanedione through Different Pathways in L-Alanine/D-Glucose and Glycine/D-Glucose Model Systems, Based on Labeling Studies[a]
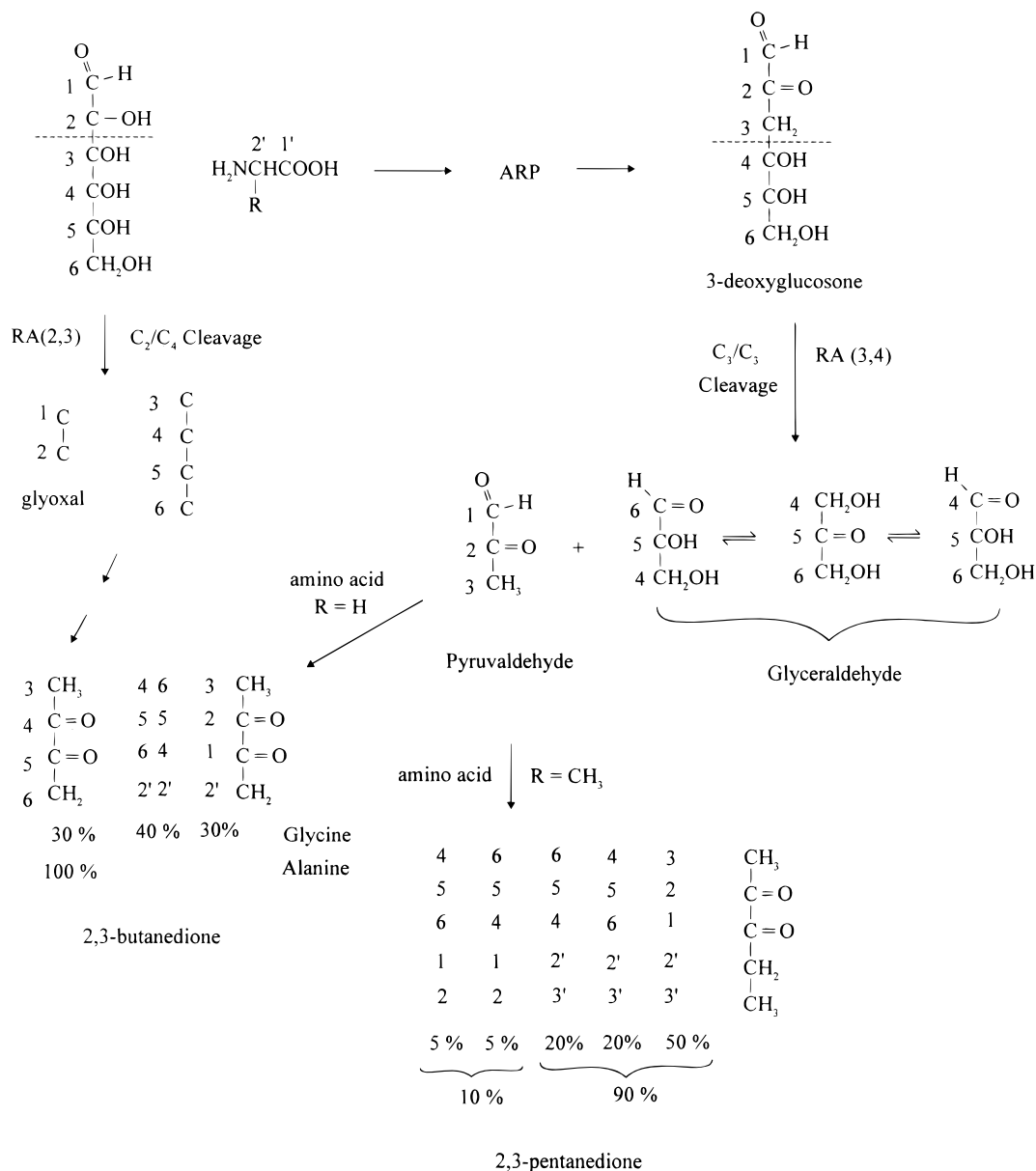
[a] Carbon numbers indicate original D-glucose carbon locations. RA[x,y] = retro-aldol cleavage at Cx−Cy.

Yaylayan, 1996; Yaylayan and Keyhani, 1998) have indicated that 2,3-butanedione is formed by two pathways. One is through a C_2/C_4 cleavage of the glucose moiety, and the other through a C_3/C_3 cleavage of 3-deoxyglucosone to produce pyruvladehyde that subsequently undergoes amino-acid-mediated chain elongation to form 2,3-butanedione (see Schemes 2 and 3). To elucidate the origin of 2,3-butanedione and 2,3-pentanedione in L-alanine model systems, similar studies were conducted using variously labeled D-glucoses and L-alanines. As expected, analysis of label incorporation in 2,3-butanedione indicated the existence of only one pathway of formation through a C_2/C_4 cleavage of the D-glucose moiety, similar to the glycine system with 100% incorporation of C-3, C-4, C-5, and C-6 atoms of D-glucose, as indicated in Scheme 3. The label incorporation in 2,3-pentanedione and 2-butenal (aldol self-condensation product of acetaldehyde) from all model systems studied is summarized in Table 2. The label

incorporation in 2-butenal indicates that acetaldehyde produced under the experimental conditions arises only from Strecker degradation of L-alanine. On the other hand, 10% of 2,3-pentanedione is produced by the incorporation of D-glucose carbon atoms only and 90% through the participation of two carbon atoms from L-alanine and three carbon atoms of glucose. This is consistent with literature observation (Weenen and Apeldoorn, 1996) that L-alanine enhances the formation of 2,3-pentanedione. To identify the nature of C_3 glucose fragment(s) involved in the formation of 2,3-pentanedione, L-[^{13}C-2]alanine was separately reacted with pyruvaldehyde and glyceraldehyde. Both model systems generated 2,3-pentanedione with 100% label incorporation, implicating both C_3 glucose fragments as possible precursors. To test the possibility of acetaldehyde (Strecker aldehyde) reacting with acetol to produce 2,3-pentanedione, the model system was reacted similarly and analyzed. No 2,3-pentanedione was detected in the

Origin of 2,3-Pentanedione and 2,3-Butanedione

J. Agric. Food Chem., Vol. 47, No. 8, 1999 **3283**

Table 2. Percent Label Distribution[a] in 2,3-Pentanedione and 2-Butenal Formed in Different Model Systems

model system	2,3-pentanedione		2-butenal		
	100 (*M*)	101 (*M* + 1)	70 (*M*)	71 (*M* + 1)	72 (*M* + 2)
D-glucose/L-alanine	100	0	100	0	0
D-[1-^{13}C]glucose/L-alanine	40	60	100	0	0
D-[2-^{13}C]glucose/L-alanine	40	60	100	0	0
D-[3-^{13}C]glucose/L-alanine	50	50	100	0	0
D-[4-^{13}C]glucose/L-alanine	50	50	100	0	0
D-[5-^{13}C]glucose/L-alanine	50	50	100	0	0
D-[6-^{13}C]glucose/L-alanine	50	50	100	0	0
D-glucose/L-[^{15}N]alanine	100	0	100	0	0
D-glucose/L-[1-^{13}C]alanine	100	0	100	0	0
D-glucose/DL-[2-^{13}C]alanine	10	90	0	0	100
D-glucose/L-[3-^{13}C]alanine	10	90	0	0	100
D-glyceraldehyde/DL-[2-^{13}C]alanine	0	100	nd[b]	nd[b]	nd[b]
pyruvaldeyde/DL-[2-^{13}C]alanine	0	100	0	0	100

[a] Percentages are corrected for natural abundance and for less than 100% enrichment. [b] nd = not detected.

Table 3. Percent Label Distribution[a] in Selected Mass Spectral Fragments of 2,3-Pentanedione Formed in Different Model Systems

model system	*m/z* 43 (*M*)	*m/z* 44 (*M* + 1)	*m/z* 57 (*M*)	*m/z* 58 (M + 1)
D-glucose/L-alanine	100	0	100	0
D-[1-^{13}C]glucose/L-alanine	50	50	90	10
D-[2-^{13}C]glucose/L-alanine	50	50	90	10
D-[3-^{13}C]glucose/L-alanine	100	0	50	50
D-[4-^{13}C]glucose/L-alanine	80	20	75	25
D-[5-^{13}C]glucose/L-alanine	50	50	100	0
D-[6-^{13}C]glucose/L-alanine	70	30	75	25
D-glucose/L-[^{15}N]alanine	100	0	100	0
D-glucose/L-[1-^{13}C]alanine	100	0	100	0
D-glucose/ DL-[2-^{13}C]alanine (92%)	100	0	10	90
D-glucose/L-[3-^{13}C]alanine	100	0	10	90

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

mixture, but the three main products identified were 2-butenal (20%), 3-hydroxybutanal (40%), and furaneol (23%).

Position of Carbon Atoms Originating from D-Glucose and L-Alanine in 2,3-Pentanedione. The data in Table 2 indicate that of the total detected 2,3-pentanedione, only 10% is formed from sugar carbon atoms and 90% formed with the involvement of C2′−C3′ atoms of L-alanine. More detailed information regarding the position of different atoms originating from the sugar and the amino acid requires analysis of label incorporation in the mass spectral fragments *m/z* 43 and 57, shown in Scheme 2. An increase in the mass of *m/z* 43 will indicate the presence of label in either the C-1 or C-2 position of 2,3-pentanedione. Similarly, the presence of label in any of the C-3, C-4, and C-5 positions of 2,3-pentanedione could be identified by the examination of the ion at *m/z* 57. On the basis of the analysis of the data listed in Table 3, it could be concluded that 50% of 2,3-pentanedione is formed through reaction of L-alanine with a C1−C2−C3 sugar unit and 40% through reaction of L-alanine with a C4−C5−C6 sugar unit. As predicted above, the remaining 10% is formed through reaction of the C1−C2 fragment of the glucose with a C4−C5−C6 sugar unit (see Scheme 3). In addition, the data also indicate that C2′−C3′ atoms of L-alanine are connected to C-1 (50%), C-4 (20%), and C-6 (20%) atoms of D-glucose. A retro aldol cleavage initiated from 3-deoxyglucosone can justify the observed distribution and positions of carbon atoms based on the proposed mechanisms shown in Schemes 2 and 3. A C_3/C_3 cleavage through retro aldol reaction

can produce pyruvaldehyde with a fixed electrophilic carbon atom at C-1 of D-glucose and a glyceraldehyde molecule able to enolize and furnish electrophilic carbon atoms at C-4 and C-6 positions of D-glucose, through the intermediate dihydroxyacetone structure, as shown in Scheme 3. Recently, spectroscopic evidence was presented for the above-mentioned interconversion of glyceraldehyde and dihydroxyacetone (Yaylayan et al., 1999). This mechanism assumes that the C_2 unit of glucose (probably glycolaldehyde) can react and produce 2,3-pentanedione only with the glyceraldehyde moiety, since no doubly labeled product was identified.

Proposed Mechanism of Interaction of Amino Acids with Aldehydes. Amino acids are known to react as C-nucleophiles through their α-carbons, in addition to their reactivity as N-nucleophiles. Under basic conditions, free glycine or its cobalt or copper complexes can react with acetaldehyde or benzaldehyde through aldol condensation to generate threonine (Kaneko et al., 1974) or β-phenylserine (Barton and Ollis, 1979), respectively. This reaction is used industrially for large-scale production of threonine. Scheme 2 illustrates two similar mechanisms of nucleophilic interaction of α-carbons of amino acids with pyruvaldehyde and glyceraldehyde to produce 2,3-pentanedione. In mechansim A (Barton and Ollis, 1979), the amino acid decarboxylates after aldol condensation, whereas in mechansim B, decarboxylation occurs simultaneously with aldol additon followed by deamination to produce condensation product. It seems such interactions play a major role in Maillard reactions as an alkylation mechanism involving α-keto aldehydes or α-hydroxyaldehydes and amino acids (Yaylayan and Keyhani, 1998).

CONCLUSION

Evidence from labeling studies suggests that α-dicarbonyl intermediates produced during Maillard reaction have mixed origin. They could be formed either from a sugar degradation pathway or through further interaction of sugar degradation products with amino acids.

LITERATURE CITED

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

Barton, D. H.; Ollis, W. D. (Eds.) *Comprehensive Organic Chemistry*; Pergamon Press: Oxford, 1979; Vol. 2, p 825.

Huyghues-Despointes, A.; Yaylayan, V. Retro-aldol and redox reactions of Amadori compounds: mechanistic studies with

3284 *J. Agric. Food Chem.,* Vol. 47, No. 8, 1999

Yaylayan and Keyhani

variously labeled D-[^{13}C]glucose. *J. Agric. Food Chem.* **1996**, *44*, 672−681.

Huygues-Despointes, A.; Yaylayan, V., Keyhani, A. Pyrolysis/GC/MS analysis of 1-[(2′-carboxyl)pyrrolidinyl]-1-deoxy-D-fructose (Amadori Proline). *J. Agric. Food Chem*. **1994**, *42*, 2519−2524.

Kaneko, T.; Izumi, Y.; Chibata I.; Itoh, T. (Eds.) *Synthetic production and utilization of amino acids*; Wiley: New York, 1974; p 197.

Keyhani, A.; Yaylayan, V. Elucidation of the mechanism of pyrazinone formation in glycine model systems using labeled sugars and amino acid. *J. Agric. Food Chem.* **1996**, *44*, 2511−2516.

Tressl, R.; Helak, B.; Kersten, E.; Rewicki, D. Formation of proline and hydroxyproline-specific Maillard products from [1-^{13}C]-D-glucose. *J. Agric. Food Chem.* **1993**, *41*, 547−553.

Tressl, R.; Nittka, C.; Kersten, E. Formation of isoleucine-specific Maillard products from [1-^{13}C]-D-glucose and [1-^{13}C]-D-fructose. *J. Agric. Food Chem.* **1995**, *43*, 1163−1169.

Yaylayan, V.; Keyhani, A. The origin and fate of α-dicarbonyls formed in Maillard model systems: mechanistic studies using ^{13}C- and ^{15}N-labeled amino acids. In *The Maillard reaction in Foods and Medicine*. O'Brien, J., Nursten, H. E.,

Crabbe, M. J., Ames, J., Eds.; Royal Society of Chemistry: Cambridge, U.K., 1998; pp 51−56.

Yaylayan, V.; Keyhani, A.; Huyghues-Despointe, A. Generation and the fate of C$_2$, C$_3$, and C$_4$ reactive fragments formed in Maillard model systems of [^{13}C]glucose and [^{13}C]glycine or proline. In *Process induced chemical changes in food*; Shahidi, F., Ho, C.-T., Nguyan, C. V., Eds.; Plenum Press: New York, 1998; pp 237−244.

Yaylayan, V.; Harty-Majors, S.; Ismail, A. Investigation of DL-glyceraldehyde−dihydroxyacetone interconversion by FTIR spectroscopy. *Carbohydr. Res.* **1999**, in press.

Weenen, H.; Apeldoorn, W. Carbohydrate cleavage in the Maillard reaction. In *Flavour Science recent developments*; Taylor, A. J., Mottram, D. S., Eds.; The Royal Society of Chemistry; Cambridge, 1996; pp 211−216.