Origin of Carbohydrate Degradation Products in L-Alanine/D-[^{13}C]Glucose Model Systems

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Maillard model systems consisting of labeled D-[^{13}C]glucoses and L-[^{13}C]alanines have been utilized to identify the origin of carbon atoms in glycolaldehyde, pyruvaldehyde, 1-hydroxy-2-propanone (acetol), 2,3-butanedione, 3-hydroxy-2-butanone, 2,3-pentanedione, and compounds containing C_5 and C_6 intact glucose carbon chains. The origin of carbon atoms in glycolaldehyde and pyruvaldehyde was inferred from the analysis of label incorporation pattern of methyl and dimethylpyrazines. The origin of carbon atoms in the remaining compounds was determined by direct analysis. The data indicated that glycolaldehyde incorporated intact C5−C6 and C1−C2 carbon chains of glucose. Acetol and pyruvaldehyde incorporated intact C1−C2−C3 and C4−C5−C6 carbon chains of glucose. On the other hand, 2,3-butanedione and 3-hydroxy-2-butanone incorporated intact C3−C4−C5−C6 carbon chain of glucose. In addition, analysis of compounds containing intact glucose C_5 carbon chains have indicated that glucose in the presence of L-alanine can lose either C-1 atom to produce a pentitol moiety responsible for the formation of furanmethanol or it can lose the C-6 atom to produce a pentose moiety responsible for the formation of furfural. Plausible mechanisms, consistent with the observed label incorporation, were proposed for the formation of sugar degradation products.

Keywords: *Sugar degradation mechanisms; Maillard reaction; acetol; dicarbonyl compounds; ^{13}C-labeled glucoses and ^{13}C-alanines; Py-GC/MS*

INTRODUCTION

Carbohydrate degradations during Maillard reaction play a crucial role in determining product distribution of various sugar-derived Maillard products, especially in model systems containing amino acids with alkyl side chains. Maillard reaction pathways occurring in the so-called "sugar fragmentation pool" provide the reactive α-dicarbonyl compounds and carbon skeletons for various precursors essential for the formation of different heterocyclic aroma compounds (Yaylayan, 1997). The main feature of the degradative reactions in the "sugar fragmentation pool" is the formation of C_2, C_3, C_4, C_5, and C_6 sugar-derived building blocks of Maillard products. The cleavage of carbon skeleton of the parent hexose-derived C_6 intermediates (1-deoxy-, 3-deoxy-, and 1-amino acid-1,4-dideoxyglucosones) leads to the formation of smaller C_2, C_3, C_4, and C_5 fragments. Alternatively, the reactive C_6 intermediates can undergo dehydration, isomerization, and cyclizations (Weenen, 1998) to produce compounds containing intact C_6 glucose carbon chains such as 5-methylfurfural and 2-acetylpyrrole. Weenen and Apeldoorn (1996) investigated carbohydrate degradations in different L-alanine model systems and quantified various dicarbonyl compounds through reaction with *o*-diaminobenzene. The amounts of glyoxal, pyruvaldehyde, 2,3-butanedione, and 2,3-pentanedione were higher in the presence of amines than in the presence of amino acids but were not detected in the absence of both compounds. They attributed the origin of these compounds to the fragmentation of deoxyglucosones and Amadori products. Recently, a new pathway was identified involving amino acid participation in the formation of reactive C_3, C_4, and C_5 fragments (Keyhani and Yaylayan, 1996; Yaylayan and Keyhani, 1998, 1999). To verify the origin of these reactive intermediates, it is essential to perform ^{13}C-labeling studies with selectively enriched sugars and amino acids. The use of a single-labeled sugar to identify the origin of fragments smaller than C_6 will generate inconclusive data. A cost-effective and convenient approach to perform multiple experiments with labeled reactants is through utilization of the quartz tube of a pyrolysis/gas chromatography/mass spectrometry (Py-GC/MS) system as a microreactor (Yaylayan, 1999). Although under pyrolytic conditions, a higher number of products are formed compared with aqueous reactions; however, most of the products identified in aqueous systems are also formed under pyrolytic conditions albeit in different amounts. In addition, experimental evidence was provided that the position and label distribution in the common products observed in the same model systems, between aqueous and pyrolytic reactions, are identical (Yaylayan and Wnorowski, 1999). 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 relevance to the aqueous reactions. In this study, Py-GC/MS system was used to trace the origin of the carbon atoms in C_2, C_3, C_4, and C_5 fragments formed in the D-glucose/L-alanine system using independently labeled D-[^{13}C]glucoses and L-[^{13}C]alanines.

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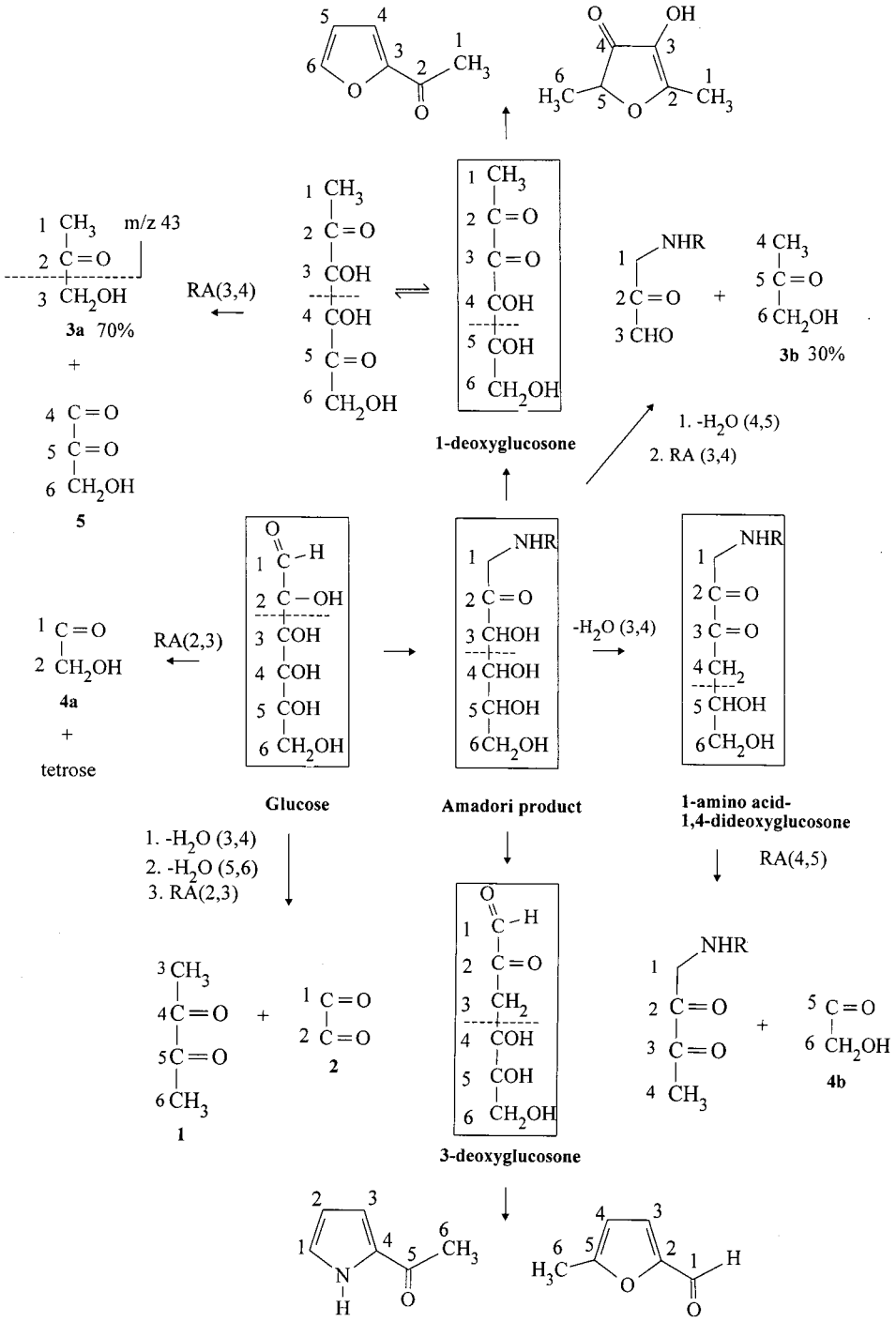

Figure 1. Origin of C_2, C_3, and C_4 reactive intermediates and compounds containing intact C_6 glucose carbon chains formed in L-alanine/D-glucose model system based on labeling studies. Carbon numbers indicate original D-glucose carbon positions. RA-[*x*,*y*] = retro-aldol cleavage at C_x−C_y; −H_2O(*x*,*y*) = dehydration from C_x−C_y.

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 (99%), D-[4-^{13}C]glucose 99%), D-[5-^{13}C]glucose (99%), 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 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 210 °C at a heating rate of 50 °C/ms and with 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. 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 (60 m length × 0.25 mm i.d. × 25 um film thickness; Supelco, Inc.). Unless otherwise specified, the column initial temperature was −5 °C for 3 min. and was 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.

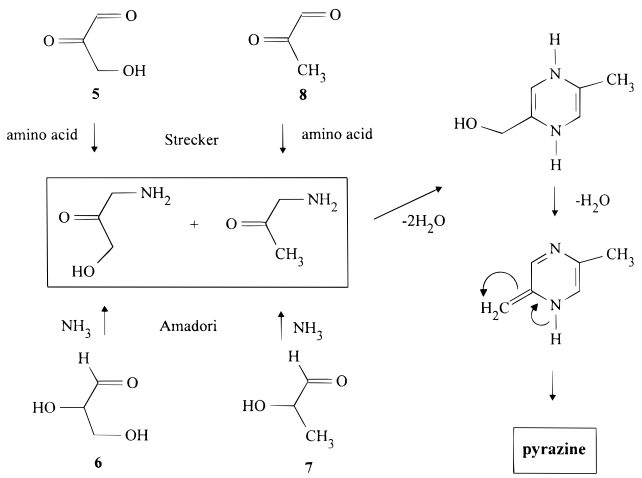

Figure 2. Carbonyl precursors and proposed pathway of formation of dimethylpyrazine.

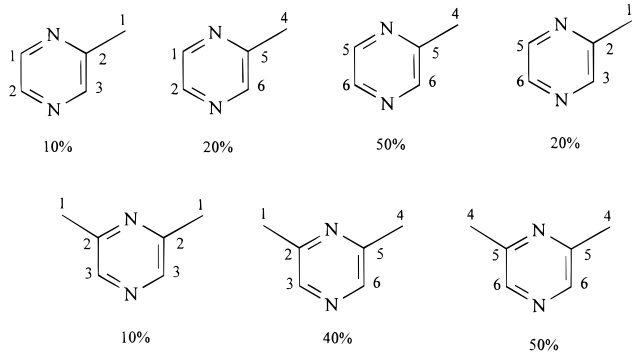

Figure 3. Percent distribution of glucose carbon atoms in methyl and dimethylpyrazines. Carbon numbers indicate original D-glucose carbon positions.

RESULTS AND DISCUSSION

Analysis of D-glucose/L-alanine model systems by Py-GC/MS indicated the formation of different pyrazine, pyrrole, pyridine, pyran, and furan derivatives in addition to acyclic intermediates such as 2,3-butanedione (**1**), 2,3-pentanedione (**9**), 3-hydroxy-2-butanone, and 1-hydroxy-2-propanone (**3** acetol). The origin of carbon atoms in these compounds was determined by analysis of their mass spectra generated by pyrolysis of different model systems containing specifically labeled D-[^{13}C] glucoses and L-alanines. On the other hand, the origin of carbon atoms in glycolaldehyde (**4**) and pyruvaldehyde (**8**) or its equivalents (**3**, **5**, **6**, and **7**) was inferred from the analysis of label incorporation pattern in methyl- and 2,5-dimethylpyrazines. The major C_6 reactive intermediates (1-deoxy-, 3-deoxy-, and 1-amino acid-1,4-dideoxyglucosones) that further cleave to produce smaller sugar fragmentation products are shown in Figure 1.

Origin of Glycolaldehyde (4) and Pyruvaldehyde (8). Incorporation of reactive carbonyl compounds into stable end-products can be utilized to investigate their origin in Maillard model systems (Yaylayan et al., 1998). Useful and stable end-products that form abundantly during Maillard reaction, are the different pyrazines. They can be formed by the dimerization of α-aminocarbonyl compounds followed by oxidation, except when one of the reactants is an α-amino-α′-hydroxyl derivative, then a dehydration step instead of oxidation can generate pyrazines (see Figure 2). α-Aminocarbonyl compounds in turn can be formed either by Strecker reaction of **5** and **8** or by Amadori rearrangement of a α-hydroxycarbonyl compounds (**6** and **7**) with ammonia (see Figure 2). According to Figure 2, the C_3 fragments involved in the formation of dimethylpyrazine could be glyceraldehyde (**6**), pyruvaldehyde (**8**), 2-hydroxybutanal (**7**), and 3-hydroxypyruvaldehyde (**5**). Both glyceraldehyde and pyruvaldehyde produced dimethylpyrazines upon reaction with alanine. Using [^{13}C]glucoses labeled at the different carbon atoms and analyzing the label incorporation in methyl- and dimethylpyrazines, the origin of C_2 and C_3 carbonyl precursors were identified based on the above discussion. Figure 3 summarizes the result of mass spectral analysis of the two pyrazines. The data indicate that C_3 and C_2 carbonyl fragments involved in the formation of the two pyrazines incorporating the intact sequences of C1−C2−C3 (40%), C4−C5−C6 (60%) and C1−C2 (30%), C5−C6 (70%) atoms of glucose, respectively. As mentioned, the C_3 fragment may represent glyceraldehyde, pyruvaldehyde, 2-hydroxybutanal or 3-hydroxypyruvaldehyde and C_2 glycolaldehyde (**4**) or glyoxal (**2**). The analysis of percent distribution of the observed isotopomers shown in Figure 3 can be used to estimate the percentage of different isotopomers of reactive C_2 and C_3 fragments. Figures1 and 4 propose retro aldol cleavages of 3-dexoyglucosone, 1-dexoyglucosone, and Amadori product as possible mechanisms of formation of different C_3 reactive carbonyl intermediates (**3**, **5**, **6**, and **8**) and similar retro aldol cleavages of glucose and 1-amino acid-1,4-dideoxyglucosone as possible pathways of formation of C_2 reactive fragments (**2** and **4**). The indicated retro aldol cleavages are consistent with the observed label incorporation patterns.

Origin of Acetol (3). The position and label distribution of glucose carbon atoms in acetol (**3**) is shown in Figure 1. Analysis of label incorporation in fragment *m*/*z* 43 of acetol allowed accurate identification of the exact positions of label incorporation. The major pathway (70%) for the formation of acetol (**3a**) involves a retro aldol cleavage of isomerized 1-deoxyglucosone to generate acetol with a C1−C2−C3 sequence and a reactive C_3 fragment (**5**) as a possible pyrazine precursor with a carbon atom sequence of C4−C5−C6. The minor pathway (30%) also involves a retro aldol cleavage of dehydrated Amadori product to generate acetol (**3b**) with a C4−C5−C6 sequence of glucose atoms as indicated in Figure 1.

Origin of 2,3-Butanedione (1), 3-Hydroxy-2-butanone, and 2,3-Pentanedione (9). The mechanism of formation of 2,3-pentanedione (**9**) in alanine model system has been published previously (Yaylayan and Keyhani, 1999). According to this study 2,3-pentanedione is formed by two pathways, one involving only glucose carbon atoms (10%) and the other (90%) involves the participation of C2′-C3′ atoms of L-alanine and a C_3 fragment pyruvaldehyde (**8**) from D-glucose (see Figure 4). Analysis of label incorporation into selected mass spectral fragments of 2,3-pentanedione have also indicated that pyruvaldehyde originates either from C1−C2−C3 or from C4−C5−C6 fragments of D-glucose. On the other hand, analysis of label incorporation into 2,3-butanedione (**1**) have indicated its formation by a single pathway involving only glucose carbon atoms having an intact sequence of C3−C4−C5−C6 atoms. Figure 1 proposes dehydrations of glucose followed by a retro-aldol cleavage as a possible pathway of formation of 2,3-

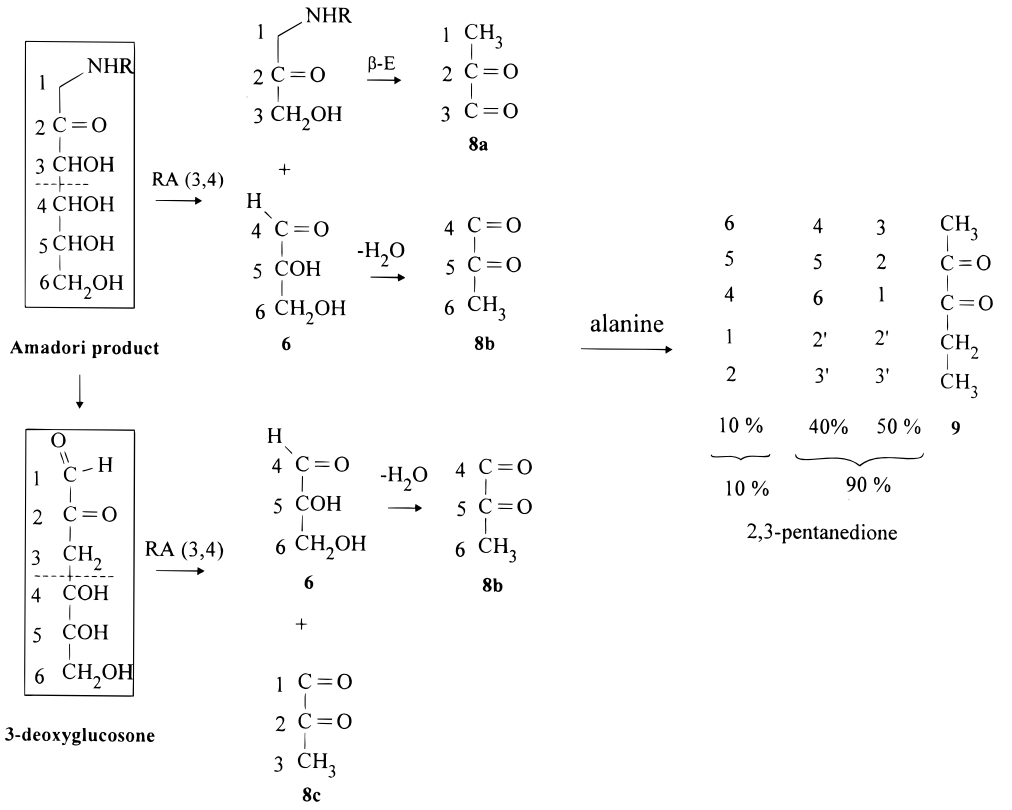

Figure 4. Origin of C_3 reactive intermediates involved in the formation of 2,3-pentanedione. Carbon numbers indicate original D-glucose carbon positions. Primed numbers indicate alanine carbon positions. RA[*x*,*y*] = retro-aldol cleavage at C_x-C_y; *β*-E = *β*-elimination.

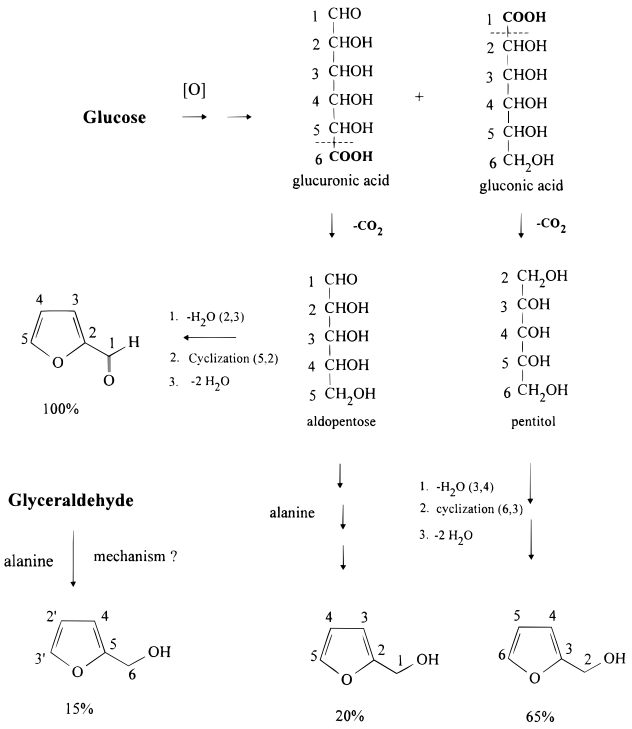

Figure 5. Origin of C_5 reactive intermediates and compounds containing intact C_5 glucose carbon chains formed in L-alanine/D-glucose model system based on labeling studies. Carbon numbers indicate original D-glucose carbon positions. Primed numbers indicate alanine carbon positions. $-H_2O(x,y)$ = dehydration from C_x-C_y; cyclization(*x*,*y*) = cyclization occurring between hydroxyl group at carbon *x* and carbonyl group at carbon *y*.

butanedione. This cleavage will also generate glyoxal (**2**) with a C1−C2 sequence of glucose carbons consistent with label incorporation observed in C_2 reactive fragments involved in pyrazine formation. Similar to 2,3-butanedione, 3-hydroxy-2-butanone also showed the same sequence of glucose carbon atoms indicating its formation through reduction of 2,3-butanedione.

Origin of Compounds Containing Intact C_5 and C_6 Glucose Chains. Analysis of ^{13}C-glucose label distribution in furfural and furanmethanol (see Figure 5) indicated that the glucose moiety loses either C-1 or C-6 carbon atoms to produce intact C_5 fragments incorporated into their structures. A plausible mechanism of formation of intact C1−C2−C3−C4−C5 and C2−C3−C4−C5−C6 glucose carbon atom chains that can serve as precursors of furfural and furanmethanol is shown in Figure 5. According to this figure, glucose can undergo oxidation at either C-1 or C-6 positions to produce gluconic and glucuronic acids respectively, both are well-known glucose oxidation products and detected in Maillard model systems (Yaylayan and Huyghues-Despointe, 1996). These acids can decarboxylate to produce aldopentose and pentitol moieties as shown in Figure 5. The aldopentose can undergo dehydrations and cyclization to produce furfural with observed label distribution. When ribose was pyrolyzed in the presence and absence of alanine, the major product obtained in both cases was furfural, confirming the suggested pathway. On the other hand, the pentitol can also undergo dehydrations and cyclization to produce furanmethanol. However, analysis of label incorporation indicated that only 65% of furanmethanol is formed through this pathway and 20% through the conversion of the aldopentose, incorporating this time, intact C1−C2−C3−C4−C5 carbon chain of glucose. The latter pathway could be considered specific to pyrolytic conditions (Yaylayan and Wnorowski, 1999). Similarly, two

pathways were also observed in the case of glycine. Furthermore, a minor pathway (15%) that also generates furanmethanol, incorporating only C4−C5−C6 glucose carbon atoms and C2′-C3′ alanine carbon atoms, was also observed. When pyruvaldehyde and glyceraldehyde, probable C_3 fragments involved in this transformation, were pyrolyzed with alanine, only glyceraldehyde produced furanmethanol. The significance of this observation is the indication that glyceraldehyde produced in the glucose/alanine system contained only C4−C5−C6 glucose carbon chain as predicted in Figure 4.

Figure 1 shows the origin of 5-methylfurfural, 2-acetylpyrrole, 2-acetylfuran, and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol, a registered trademark of Firmenich S. A., Geneva, Switzerland) containing intact C_6 glucose carbon atoms. These assignments are consistent with other studies performed in aqueous systems with labeled sugars (Tressl et al., 1993, 1995).

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