JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of 2-Acetylfuran Formation between Ribose and Glucose in the Maillard Reaction

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Sugar type is a major factor regulating the reaction rates and pathways in Maillard reaction. Ribose and glucose were used to compare their reactivities and pathways of 2-acetylfuran formation. A stable isotope labeling method was used to study their reactivity. A 1:1 mixture of [$^{13}C_6$]glucose and unlabeled ribose (or other unlabeled sugar) was reacted with proline at 145 °C for 40 min. The reactivity of each sugar was revealed by the ratio of isotopomers. The reactivity of sugars in 2-acetylfuran formation decreased in the order ribose, fructose, glucose, rhamnose, and sucrose. This method simplified the reaction system and the calculation process and gave a direct comparison of reactivity as seen via mass spectrum. The difference between glucose and ribose in 2-acetylfuran formation was that glucose could form 2-acetylfuran directly from cyclization of its intact carbon skeleton, whereas ribose first underwent degradation into fragments before forming a six-carbon unit leading to 2-acetylfuran. In the presence of cysteine, ribose could not generate 2-acetylfuran at a detectable level. When ribose was reacted with glycine, formaldehyde generated from glycine combined with ribose to form 2-acetylfuran. In other amino acids, a symmetric structure of the ribose intermediate was formed, making fragmentation more complicated.

KEYWORDS: 2-Acetylfuran; reactivity; labeling study; ribose; glucose

INTRODUCTION

The Maillard reaction has been extensively studied for the development of flavor and color in processed foods. In recent years some studies have also reported the potential toxicological effects and health problems of Maillard reaction products (*1, 2*). Formation of these Maillard compounds depends on the types of sugars and amino acids as well as reaction conditions that may alter reaction kinetics and pathways. For example, fructose shows a higher reactivity in 5-hydroxymethyl-2-furaldehyde (HMF) generation compared to glucose and sucrose (*3, 4*), and the formation pathways of 2-acetylfuran vary with different amino acids (*5*).

2-Acetylfuran is an important sweet balsamic−cinnamic note in natural or processed foods. It has been detected in sweet corn products, fruits, flowers, wine, beer, and cola (*6*−*8*). In some model system studies, such as serine/threonine/glutamine with ribose/glucose/fructose, 2-acetylfuran was also determined to be an important flavor compound (*9*).

The formation pathways of 2-acetylfuran have not been systematically studied. Yaylayan et al. (*10*) proposed that 2-acetylfuran was generated from 1-deoxyglucosone-6-phosphate during the thermal degradation of glucose-6-phosphate. In our previous study we showed that the formation pathways for 2-acetylfuran could be either from glucose or from glucose and glycine (*5*). In the presence of phenylalanine, cysteine, or serine, glucose can undergo cyclization and form 2-acetylfuran via 1,4-dideoxyosone. However, when glucose reacted with lysine, alanine, proline, or arginine, it degraded into different fragments, which then recombined to form 2-acetylfuran. Glycine, which is involved in 2-acetylfuran formation, degraded into formaldehyde that then connected with a [C-5] unit of glucose (*5*).

Formation pathways of 2-acetylfuran from glucose based on selected amino acids have been studied recently (*5*), but the influence of sugars on 2-acetylfuran generation, particularly the reactivity and mechanistic pathways from pentose, has not been evaluated. Therefore, this study first aimed at elucidating the reactivity of sugars in 2-acetylfuran formation and their generation pathways from ribose. Another aim of this study was to develop a fast and visual method to compare the reactivity of sugars. Labeled and unlabeled sugars were used together in the same system, and reactivity order of sugars was obtained by calculating the ratio of different isotopomers using GC-MS.

MATERIALS AND METHODS

Materials. L-Phenylalanine, L-glycine, L-cysteine, L-proline, L-lysine, formaldehyde (37 wt % in water), phosphate buffer (pH 7.4, 0.01 M), sodium hydroxide, anhydrous sodium sulfate, 2-acetylfuran, 2,5-dimethyl-4-hydroxy-3(^{2}H)-furanone, [$^{13}C_6$]-D-glucose, D-glucose, D-ribose, D-fructose, L-rhamnose, and sucrose were purchased from Sigma Chemical Co. (St. Louis, MO). [2-^{13}C]-L-Glycine, [$^{13}C_5$]ribose, and

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10.1021/jf802683a CCC: $40.75
Published on Web 11/19/2008

[5-^{13}C]ribose were obtained from Cambridge Isotope Laboratories (Andover, MA). HPLC grade dichloromethane was purchased from Fisher Scientific (Springfield, NJ).

Preparation of Model Systems. Labeled or unlabeled sugars and different amino acids were dissolved separately in the phosphate buffer (0.01 M, pH 7.4). The pH was adjusted with 1 M sodium hydroxide. The concentrations were 0.6 and 0.2 M for sugars and amino acids, respectively. An aliquot (1 mL) of sugar (1:1 mixture of labeled and unlabeled sugars or only labeled sugars) was mixed separately with 1 mL of different amino acids. All samples were prepared in sealed glass tubes, heated at 145 °C for 40 min, and then cooled in an ice bath. The reaction mixture was extracted three times with 10 mL of dichloromethane. The organic phase was separated, dried over anhydrous sodium sulfate, and concentrated under nitrogen gas for the GC-MS analysis. All of the models were analyzed in duplicate.

GC-MS System. The analyses of volatiles were performed with a HP6890 gas chromatograph. An Agilent gas chromatograph (6890 series) is equipped with an autosampler (7673 series injector) and an Agilent 5973 mass spectrometric detector (EI, 70 eV). The column was an HP-1701 [14% (cyanopropyl-phenyl)methylpolysiloxane capillary (60 m × 0.25 mm i.d., film thickness = 0.25 μm]. The injector was in 1:1 split mode. The constant carrier gas (helium) flow rate was set at 1.0 mL/min. The GC oven temperature was programmed as follows: the initial oven temperature of 40 °C was set and increased to 280 °C at a rate of 5 °C/min and then held at 280 °C for 12 min. The total run time was 60 min. The injector and detector temperatures were both 250 °C.

Calculation of Labeling Percentage. The percentage of labeling distribution for 2-acetylfuran was calculated by subtracting the natural abundance of ^{13}C (1.1%). All percentages below 1% were taken as 0%.

Comparison of Reactivity. Comparison of reactivity between glucose and other sugars was based on mass spectral data. [$^{13}C_6$]Glucose and other unlabeled sugars were used in a ratio of 1:1, and values of the reactivity were calculated using the following equations:

$$R = \mathrm{PIO}/\mathrm{PIG}$$

$$\mathrm{PIO} = P[^{12}C_n] + (1/n) \times P[^{12}C_1] + (2/n) \times P[^{12}C_2] + ... + ((n-1)/n) \times P[^{12}C_{n-1}]$$

$$\mathrm{PIG} = P[^{13}C_6] + (1/6) \times P[^{13}C_1] + (2/6) \times P[^{13}C_2] + ... + (5/6) \times P[^{13}C_5]$$

R = reactivity comparison, PIO = percentage of isotopomers from other sugars, PIG = percentage of isotopomers from glucose, and P = percentage of n-number of carbons in a specific sugar.

RESULTS AND DISCUSSION

Measuring Reactivity of Sugars on 2-Acetylfuran Formation. Maillard reaction is a complicated reaction consisting of many parallel and consecutive reactions. Many factors such as types of reactants and reaction conditions (temperature ranges, pH, and water activity) play key roles in controlling the reaction rates and mechanisms. Reactants generally regulate the type of Maillard products and formation pathways, and reaction conditions influence the kinetics (*11*). However, for some specific compounds, type of sugar or amino acid can also affect the reaction rate. For example, fructose browned more quickly than glucose and sucrose (*12*). Therefore, in the current study different sugars were used to study their reactivity in 2-acetylfuran formation. A novel method was developed to compare reactivity by reacting a 1:1 mixture of [$^{13}C_6$]glucose and another unlabeled sugar and then comparing the ratio of labeled and unlabeled isotopomers. **Figure 1** shows a comparison of reactivity between glucose and other sugars in 2-acetylfuran formation in the presence of proline. At 145 °C and pH 7.4, the reactivity of ribose was 6.5-fold higher than that of glucose,

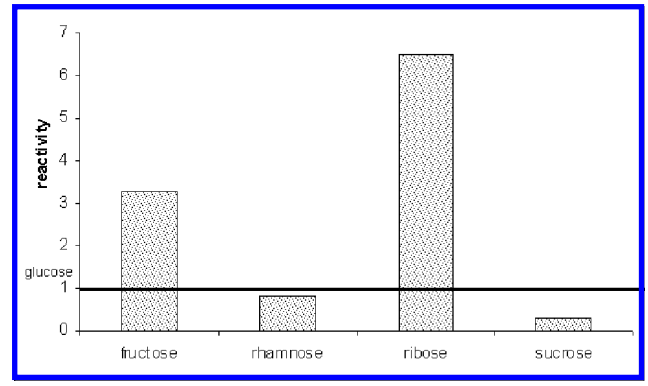

Figure 1. Comparison of reactivity between glucose and other sugars in 2-acetylfuran formation in the presence of proline: black line at 1 represents glucose reactivity. All models were analyzed in duplicate and heated at 145 °C for 40 min. The calculation was based on mass spectral data and equations provided under Materials and Methods.

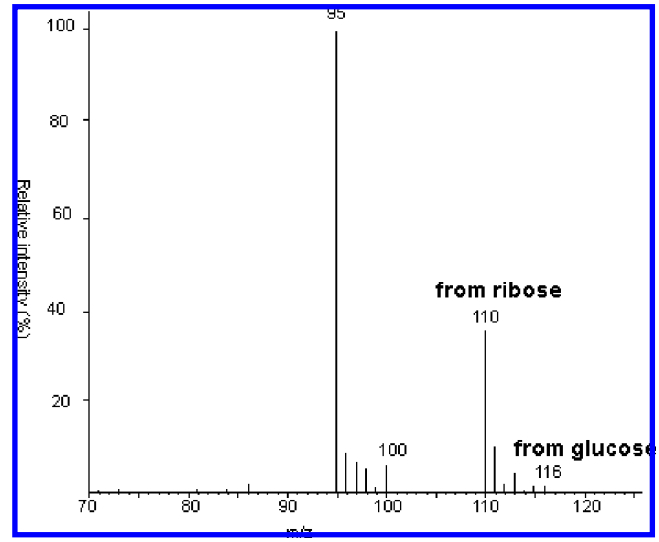

Figure 2. Mass spectra showing the comparison of reactivity between ribose and glucose in 2-acetylfuran formation.

followed by fructose at 3.3-fold, and sucrose was only 0.3-fold of glucose. The reactivity of sugars in 2-acetylfuran formation declined in the order ribose, fructose, glucose, rhamnose, and sucrose. It is generally accepted that the higher the concentration of the open-chain form of the sugar, the higher the reactivity (*13*). In other words, compared to glucose, ribose and fructose are more able to retain the open-chain form, which can then influence the reactivity of 2-acetylfuran formation.

The uniqueness of this developed method for measuring the reactivity of sugars in Maillard compound formation gave a direct visual comparison via the mass spectrum of the compound. A higher activity of ribose compared to glucose in 2-acetylfuran generation is clearly shown in **Figure 2**. Another example of this is 6-deoxysugars, such as rhamnose, which are more effective in forming 2,5-dimethyl-4-hydroxy-3(^2H)-furanone (DMHF) as compared to hexoses (*14*). This is in accordance with the spectrum shown in **Figure 3a**, where a 1:1 mixture of labeled glucose and unlabeled rhamnose was used. In the presence of proline, the intensity of *m/z* 128 ([$^{12}C_6$]DMHF) generated from [$^{12}C_6$]rhamnose was significantly higher than that of *m/z* 134 ([$^{13}C_6$]DMHF) formed from cyclization of [$^{13}C_6$]glucose. In addition, this method can also be used to study the formation mechanism of a Maillard compound. It can differentiate precursors of compounds from different sugars. For instance, acetylformoin is considered to

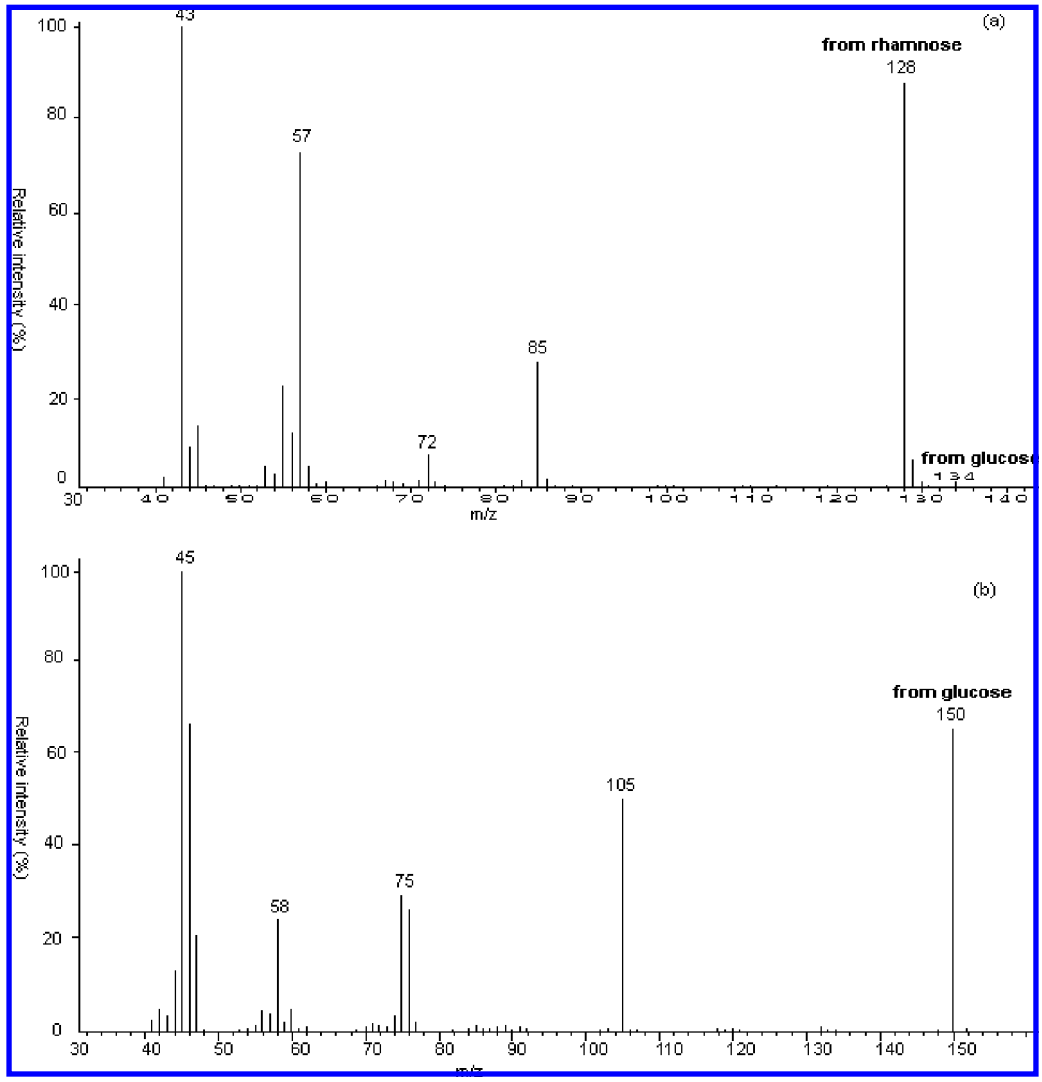

Figure 3. GC-MS spectra of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF) (**a**) and acetylformoin (**b**) from a 1:1 mixture of [$^{13}C_6$]glucose and [$^{12}C_6$]rhamnose in the presence of proline.

be a precursor of DMHF from the degradation of hexoses (*15*). In the same model of a 1:1 mixture of labeled glucose and unlabeled rhamnose, the [M + 6] isotopomer of acetylformoin from glucose was observed, whereas the [M] isotopomer from rhamnose was not identified. Thus, DMHF is formed from rhamnose through a different precursor (**Figure 3b**). The same method was used in the determination of the DMHF formation pathway from methylglyoxal and 2-methylfuranthiol formation from norfuraneol (*16, 17*).

Formation of 2-Acetylfuran via Ribose. It is a challenge to study 2-acetylfuran formation pathways from ribose, because pentose must degrade into different fragments and form six-carbon 2-acetylfuran via a complicated recombination reaction. Glucose has been reported to have three pathways that form 2-acetylfuran on the basis of the type of amino acid. In the presence of glycine, the [C-5] unit of glucose combines with formaldehyde from glycine, leading to 2-acetylfuran. For other amino acids, either cyclization of intact glucose or recombination of glucose fragments can lead to 2-acetylfuran formation (*5*).

In the presence of cysteine, a 1:1 mixture of [$^{13}C_6$]-2-acetylfuran and [$^{12}C_6$]-2-acetylfuran is observed from a 1:1 mixture of [$^{13}C_6$]glucose and [$^{12}C_6$]glucose, suggesting no fragmentation of glucose during 2-acetylfuran formation (*5*). When a 1:1 mixture of [$^{13}C_6$]glucose and unlabeled ribose was reacted with cysteine, the reactivity of ribose was higher than that of glucose, and besides the [$^{13}C_6$] isotopomer, all other isotopomers formed in this reaction system could be attributed to ribose. However, it was very interesting to observe that the only isotopomer to form is the [$^{13}C_6$] isotopomer (**Table 1**), indicating cysteine acts as a fragmentation inhibitor or a scavenger. To prove the inhibition or scavenging ability of cysteine, unlabeled ribose was reacted with cysteine, and no 2-acetylfuran was detected. The difference between glucose and ribose in 2-acetylfuran formation was that glucose could undergo cyclization via its intact carbon skeleton to form 2-acetylfuran, whereas ribose must degrade into fragments before it can form 2-acetylfuran with a six-carbon unit. Thus, 2-acetylfuran could not be formed from ribose in the presence of a fragmentation inhibitor or scavenger.

Table 1. Percent Labeling Distribution of 2-Acetylfuran Generated in Different Models

model	M	M + 1	M + 2	M + 3	M + 4	M + 5	M + 6
[$^{13}C_6$]Glc + Rib + Cys	0	0	0	0	0	0	100
[5-^{13}C]Rib + Rib + Cys	0	0	0	0	0	0	0
[$^{13}C_6$]Glc + Rib + Gly	71	7	4	6	0	9	4
[$^{13}C_6$]Glc + Rib + [2-^{13}C]Gly	45	31	4	7	0	0	13
[5-^{13}C]Rib + Rib + Pro	41	45	14	0	0	0	0
[5-^{13}C]Rib + Pro	6	47	44	3	0	0	0

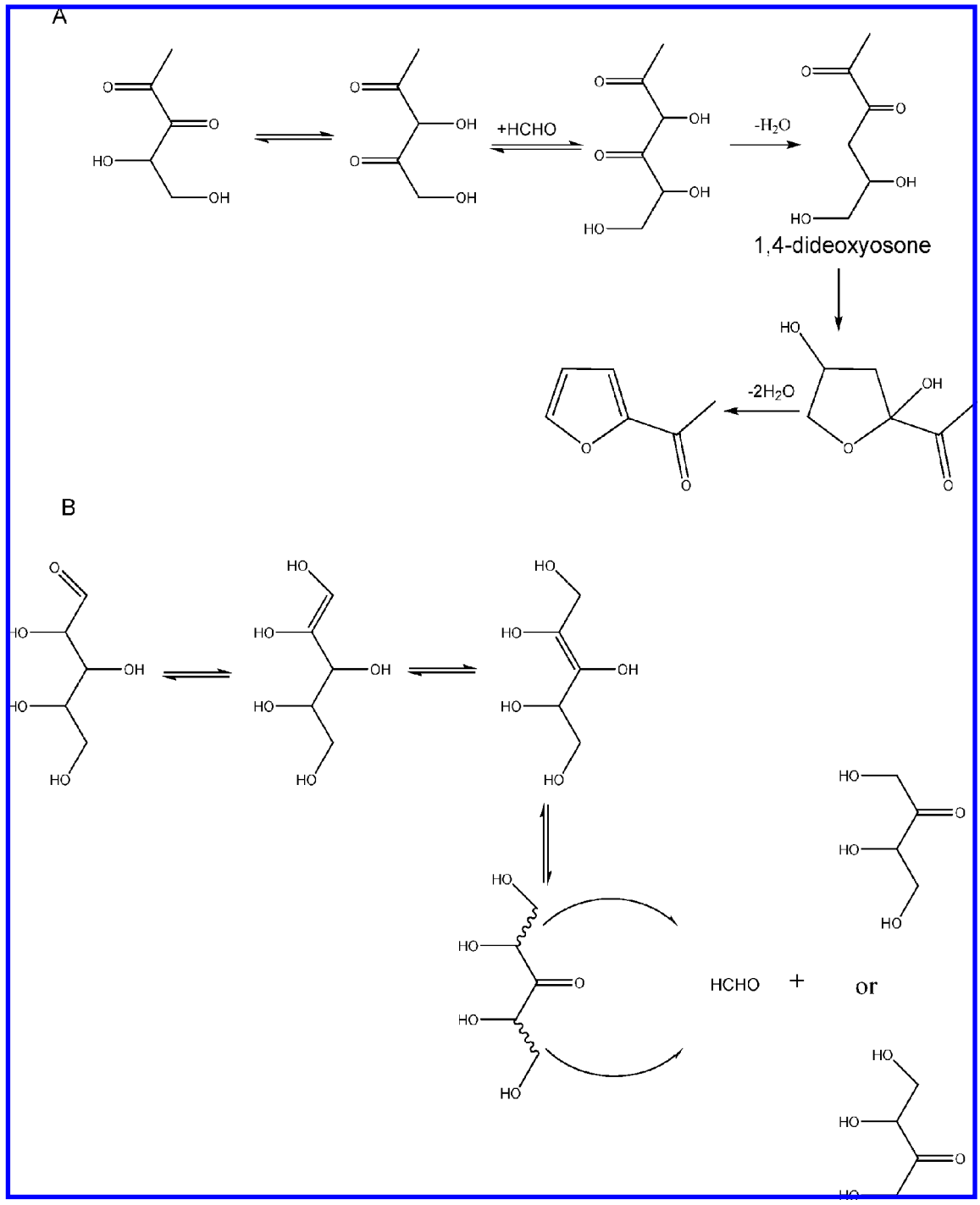

Figure 4. Proposed formation of 2-acetylfuran from ribose and formaldehyde (**A**) and formation of formaldehyde from a symmetric structure of ribose (**B**).

When glucose reacts with glycine, 2-acetylfuran is formed from the degradation of the [C-5] unit of glucose and the formaldehyde of glycine together. Glycine can be degraded into formaldehyde via Strecker degradation. Further fragmentation of the 1-deoxyglucosone can lead to the five-carbon hydroxyl−carbonyl intermediate, which can then react with formaldehyde via enolization, aldol condensation, and cyclization leading to 2-acetylfuran (*5*). Two models containing a 1:1 mixture of [$^{13}C_6$]glucose and unlabeled ribose with glycine or [2-^{13}C]glycine were set up to study the formation of 2-acetylfuran from ribose and glycine to determine their similarity to glucose. The percentage of [$^{12}C_6$] isotopomer was higher than that of [$^{13}C_6$] isotopomer, suggesting that in the presence of glycine, ribose still had a higher reactivity. When [2-^{13}C]glycine was used instead of unlabeled glycine, the percentage of [$^{13}C_6$] isotopomer increased 9%, whereas the percentage of [$^{13}C_5$] decreased 9% (**Table 1**). This result indicates the involvement of one carbon atom from glycine in the 2-acetylfuran formation from glucose, in accordance with our previous data (*5*). The same result was observed in ribose. The percentage of [$^{12}C_6$] isotopomer decreased 26%, whereas [$^{12}C_5$] isotopomer increased 24%. To elucidate formaldehyde reacting with ribose to form 2-acetylfuran, a [$^{13}C_5$]ribose was reacted with formaldehyde. The [M + 5] isotopomer of 2-acetylfuran was obtained, suggesting one possible formation pathway of 2-acetylfuran from ribose and formaldehyde (data not shown). After dehydration, 1-deoxypentosone could react with formaldehyde from glycine to form 2-acetylfuran via enolization and aldol condensation, which is

similar to the proposed pathway for the formation of 2-acetylfuran from glucose (**Figure 4A**).

The third type of 2-acetylfuran formation from ribose was fragmentation and recombination. A 1:1 mixture of [5-^{13}C]ribose and unlabeled ribose was reacted with proline. A 1:1 mixture of [M] and [M + 1] isotopomers was observed, and the percentage of [M + 2] isotopomer was found to be only 14% (**Table 1**). These data were not enough to speculate on the formation pathways. First, a 1:1 mixture of [5-^{13}C]ribose and unlabeled ribose was reacted with either phenylalanine or lysine; the percentages of isotopomers distribution were the same as when reacted with proline, suggesting that, with the exception of cysteine and glycine, other amino acids do not have a great influence on the fragmentation of ribose (data not shown). Therefore, proline was used as a representative. Second, only [5-^{13}C]ribose mixed with proline. A 1:1 mixture of [M + 1] and [M + 2] isotopomers is shown (**Table 1**) and compared with the model of a 1:1 mixture of [5-^{13}C]ribose and unlabeled ribose (**Table 1**), indicating that the possibility of breakages of ribose from C1−C2 or C2−C3 was equal to that from C3−C4 or C4−C5, respectively. In other words, a symmetric structure occurred from ribose during 2-acetylfuran formation. One possible pathway is proposed in **Figure 4B**. Ribose was rearranged into 3-pentulose through keto−enol tautomerism. By retro-aldol condensation, the carbon at either position 1 or position 5 can be cleaved into formaldehyde, which could react with ribose to form 2-acetylfuran.

In conclusion, the results of this study showed the difference between ribose and glucose in 2-acetylfuran formation from reaction reactivity and mechanism pathways. The reactivity of ribose in 2-acetylfuran formation was higher than that of glucose. In the presence of cysteine, 2-acetylfuran could not be generated from ribose. When ribose was reacted with glycine, formaldehyde generated from glycine was involved in the formation of 2-acetylfuran. For other amino acids, a symmetric structure of ribose formed.

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Received for review August 30, 2008. Revised manuscript received November 8, 2008. Accepted November 9, 2008.

JF802683A