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Amino acid-dependent formation pathways of 2-acetylfuran and 2,5-dimethyl-4-hydroxy-3[2H]-furanone in the Maillard reaction

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ABSTRACT

The formation pathways of two furanoids, 2-acetylfuran and 2,5-dimethyl-4-hydroxy-3[2H]-furanone (DMHF) were studied by GC–MS in the Maillard-type model system based on glucose and selected amino acids. The reaction was performed in 0.01 M phosphate buffer by heating a 1:1 mixture of [^{13}C6] glucose and [^{12}C6] glucose with amino acid. There is only one major formation pathway for DMHF in which the glucose carbon skeleton stayed intact. Formation pathways for 2-acetylfuran were more complicated. They formed either from glucose or from glucose and glycine. In the presence of glycine, the [C-5] unit of glucose combined with formaldehyde from glycine leads to 2-acetylfuran. For other amino acids, either cyclisation of intact glucose or recombination of glucose fragments can lead to 2-acetylfuran formation. These results indicate a competitive trend in controlling Maillard reaction. Therefore, besides changing Miallard reaction impact factors (temperature, time, pH etc.), inhibiting or preventing the competitive reaction cascade may direct desired pathways of Maillard reaction.

1. Introduction

Maillard reaction, an interaction between a carbonyl group (i.e., reducing sugar) and an amine group (i.e., amino acids) is well-known for its importance in generation of flavours and colours of processed foods. Recently, Maillard reaction has also been demonstrated to cause toxicological effects or health problems (Baynes & Thorpe, 1999; Lo et al., 2008). For example, asparagine-mediated Maillard reaction is known to lead to the formation of neurotoxic acrylamide (Mottram, Wedzicha, & Dodson, 2002). Methylglyoxal (MG), a major flavour precursor in Maillard reaction, is a reactive carbonyl compound found in humans and modifies protein residues to form advanced glycation endproducts (AGEs) which are linked to hyperglycaemia and diabetes complications (Singh, Barden, Mori, & Beilin, 2001).

Maillard reaction is a very complex chemical reaction containing multiple reaction cascades, nevertheless, they all go through the classical initial, intermediate and final stages of reaction. The initial stage is the condensation of a reducing sugar with an amine, leading to Amadori products through *N*-glycosylamine. Because of limited stability of Amadori products, in the intermediate stage, they are degraded into deoxyosone (1-deoxyosone, 3-deoxyosone and others) as well as several derived fragmented α,β-dicarbonyl or α-hydroxy carbonyl compounds such as 1-hydroxy-2-propanone or methylglyoxal. In the intermediate stage, amino acids are acted only as catalysts, but in the final stage, particularly in Strecker degradation, amino acids participate in the generation of flavour and colour via dehydration, fragmentation, cyclisation and polymerisation reactions (Hodge, 1953; Namiki & Hayashi, 1982).

Furanoids, incorporating a furan ring into their molecular structure, such as furfural, 2-acetylfuran, 2,5-dimethyl-4-hydroxy-3[2H]-furanone (Fig. 1) and 5-hydroxymethyl-2-furfural (HMF), are important flavour compounds or intermediates in foods. 2-Acetylfuran that has a sweet balsamic-cinnamic note, widely occurs in essential oils, sweet corn products, fruits and flowers (Adams, 1995; Buttery, Stern, & Ling, 1994; Nishimura, Yamaguchi, Mihara, & Shibamoto, 1989; Yamaguchi & Shibamoto, 1979). 2,5-Dimethyl-4-hydroxy-3[2H]-furanone (DMHF, known as furaneol), with an intense caramel-like aroma, is found in various natural and processed foods such as pineapple, tomato and grape as well as roasted coffee, roasted almond and soy sauce (Buttery, Takeoka, Krammer, & Ling, 1994; Rapp, Knipser, Engel, Ullemeyer, & Heimann, 1980; Rodin, Himel, Silverstein, Leeper, & Gortner, 1965; Steinhaus & Schieberle, 2007; Tei & Yamanaishi, 1974; Tressl, Bahri, Köppler, & Jensen, 1978). At low pH, DMHF has been shown to react with cysteine or hydrogen sulphide in generating

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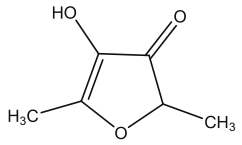

Fig. 1. Structure of 2,5-dimethyl-4-hydroxy-3[2H]-furanone.

meat-like aroma compounds (Shu & Ho, 1988; Zheng, Brown, Ledig, Mussinan, & Ho, 1997). Furfural is a precursor of 2-furfurylthiol, a key roast aroma in coffee (Cerny & Davidek, 2004).

Most furanoids are formed from deoxyosone in the presence or absence of amino acids during intermediate or final stage of Maillard reaction. HMF or furfural and DMHF are products obtained on the decomposition of 3-deoxyosone and 1-deoxyosone, respectively (Hodge, Mills, & Fisher, 1972). These furanoid compounds can be formed through cyclisation of an intact deoxyosone or the deoxyosone may be cleaved into fragments, such as MG or 1-hydroxy-2-propanone, which can recombine to form furanoids. Depending upon the reaction conditions, particularly the type of sugars and amino acids, the formation pathways for furanoid compounds may become more complicated. Systematic studies of possible pathways of furanoid formation have not been performed yet. In the current study, we aimed at understanding furanoid formation from glucose with different amino acids and evaluating relative importance of each pathway by using carbon module labelling (CAMOLA) (Schieberle, 2005).

2. Materials and methods

2.1. Materials

L-Phenylalanine, L-alanine, L-glycine, L-cysteine, L-proline, L-arginine, L-serine, L-lysine, methylglyoxal (40% wt in water), phosphate buffer (pH 7.4, 0.01 M), sodium hydroxide, anhydrous sodium sulphate and [^{13}C6] or [^{12}C6]D-glucose were purchased from Sigma Chemical Co. (St. Louis, MO). [2-^{13}C]-L-glycine was obtained from Cambridge Isotope Laboratories (Andover, MA). HPLC grade dichloromethane was purchased from Fisher Scientific (Springfield, NJ).

2.2. Preparation of model systems

[^{13}C6] or [^{12}C6] glucose 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 M and 0.2 M for glucose and amino acids, respectively. Carbon module labelling (CAMOLA) technique (Schieberle, 2005) was used to verify and evaluate the formation pathways of furanoids from glucose. An aliquot (1 mL) of 1:1 mixture of 0.6 M [^{13}C6] glucose and 0.6 M [^{12}C6] glucose was mixed separately with different amino acids. All these samples were prepared in sealed glass tubes and heated at 145 °C for 40 min, then cooled in an ice bath. The reaction mixture was extracted three times with 10 mL dichloromethane. The organic phase was separated, dried over anhydrous sodium sulphate, and concentrated under nitrogen gas for GC–MS analysis.

2.3. GC/MS system

The analyses of volatiles were performed with a HP6890 Gas Chromatograph. The 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 id, 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 held for 12 min. The total run time was 60 min. The injector temperature was 250 °C and detector temperature was 250 °C.

2.4. Calculation of labelling percentage

The percentage of labelling distribution for furanoids was calculated by subtracting the natural abundance of ^{13}C (1.1%). All the percentages below 1% were taken as 0%.

3. Results and discussion

Furanoids are a group of important Maillard derived volatile flavour compounds in processed foods. Most furanoid compounds are considered to derive from deoxyosones. They are formed either from the whole intact carbon skeleton of deoxyosones through cyclisation or from the cleavage of deoxyosones and recombination of their fragments. In order to understand these formation mechanisms, the carbon module labelling (CAMOLA) was used (Schieberle, 2005). An equal molar of [^{13}C6] labelled and [^{12}C6] unlabelled glucose were mixed with different amino acids, and the isotopomers of 2-acetylfuran and DMHF were analysed by GC–MS. If the glucose carbon skeleton remained intact during their formation, an equal molar of [^{13}C6] furanoids and [^{12}C6] furanoids should be obtained. On the other hand, if a fragmentation of glucose occurred before the formation of furanoids, partly labelled isotopomers would be observed.

3.1. Formation of 2-acetylfuran and DMHF via glucose and selected amino acids

Tables 1 and 2 demonstrate the labelling distribution of 2-acetylfuran and DMHF in different amino acid models with equal molars of [^{13}C6] labelled and [^{12}C6] unlabelled glucose. For the DMHF formation, except for lysine and arginine, other amino acids with glucose models showed a 1:1 mixture of [^{13}C6] DMHF and [^{12}C6]

Table 1
Percent labelling distribution of 2-acetylfuran generated in different models.

Sample model	*M*	*M*+1	*M*+2	*M*+3	*M*+4	*M*+5	*M*+6
Phe+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Lys+Glc (^{12}C6:^{13}C6)	27	4	4	26	4	4	31
Gly+Glc (^{12}C6:^{13}C6)	44	0	0	9	0	30	17
Ala+Glc (^{12}C6:^{13}C6)	38	0	0	27	0	0	35
Cys+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Pro+Glc (^{12}C6:^{13}C6)	24	9	5	25	6	9	22
Arg+Glc (^{12}C6:^{13}C6)	27	5	5	31	5	5	22
Ser+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Glu (^{12}C6)+glycine (2-^{13}C)	50	50	0	0	0	0	0

Table 2
Percent labelling distribution of DMHF generated in different models.

Sample model	*M*	*M*+1	*M*+2	*M*+3	*M*+4	*M*+5	*M*+6
Phe+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Lys+Glc (^{12}C6:^{13}C6)	44	4	1	2	1	4	44
Gly+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Ala+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Cys+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Pro+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Arg+Glc (^{12}C6:^{13}C6)	43	4	1	2	1	3	46
Ser+Glc (^{12}C6:^{13}C6)	50	0	0	0	0	0	50
Glu (^{12}C6)+glycine (2-^{13}C)	100	0	0	0	0	0	0

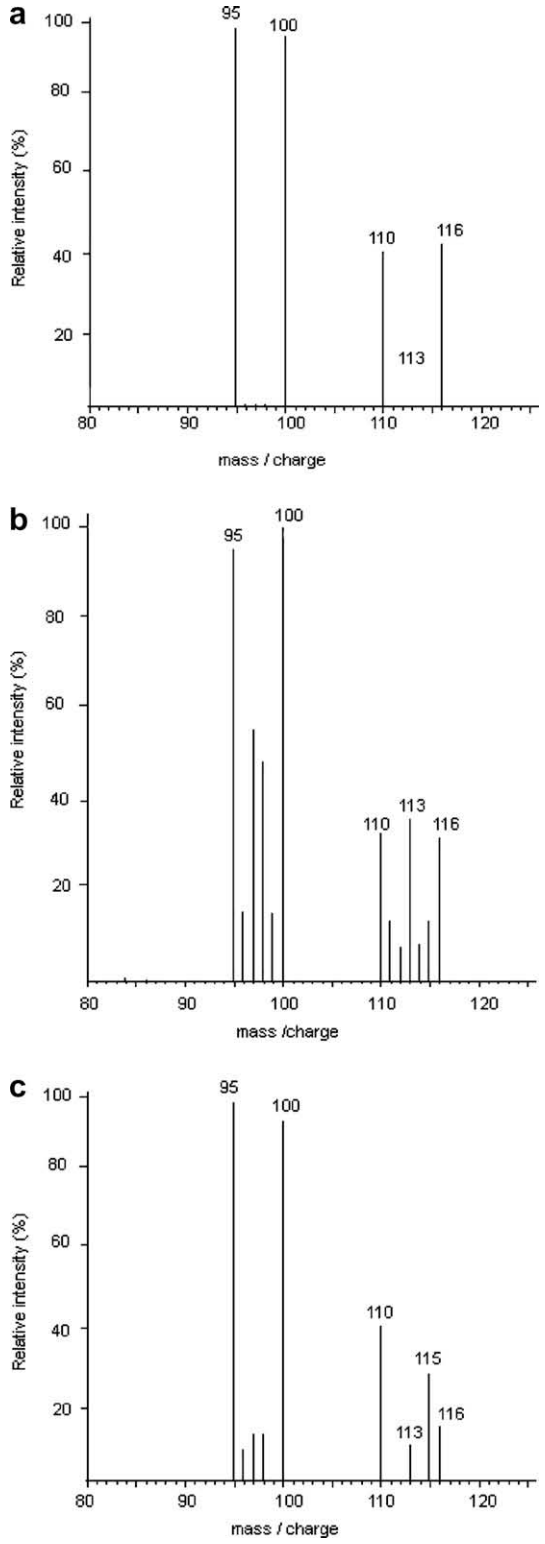

Fig. 2. GC–MS spectra of 2-acetylfuran from a 1:1 mixture of [^{13}C6] glucose and [^{12}C6] glucose in the presence of (a) phenylalanine, cysteine or serine (b) proline (c) glycine.

DMHF which is in accordance with our previous study of glycine or cysteine with glucose (Wang & Ho, 2008). Even though there were some fragments involved in DMHF formation from lysine or arginine models, the percentage of fragmentation (11–12%) was small. These results indicate that under our experimental conditions, most glucose carbon skeletons intact during DMHF formation, despite the type of amino acid used. The formation of 2-acetylfuran showed a different behaviour from DMHF. Firstly, a 1:1 mixture of [^{13}C6] 2-acetylfuran and [^{12}C6] 2-acetylfuran was observed in the models of phenylalanine, cysteine and serine suggesting no fragmentation (Fig. 2a). Secondly, models of lysine, alanine, proline and arginine showed about 50% of [^{13}C6] 2-acetylfuran and [^{12}C6] 2-acetylfuran, and the other half of 2-acetylfuran were due to fragmentation. It was calculated that about a quarter of 2-acetylfuran appeared as [^{13}C3] isotopomer in these models (Fig. 2b). Thirdly, glycine revealed a different characteristic from other amino acids in 2-acetylfuran formation. [^{12}C6] isotopomer showed in a higher level as compared to other [^{13}C6] isotopomer. The major fragmentation product was the [^{13}C5] 2-acetylfuran (Fig. 2c).

Generally, DMHF and 2-acetylfuran can be formed from glucose through 1-deoxyosone and 1,4-dideoxyosone as the intermediates, respectively. DMHF is generated from hexose via acetylformoin reduction which can be proceeded either by a disproportionation reaction, or in the case of amino acids, a Strecker reaction (Hofmann & Schieberle, 2001). The proposed formation pathway of 2-acetylfuran through 1,4-dideoxyosone from glucose is shown in Fig. 3A. 1-Amino-4-deoxyosone was formed from glucose and amino acids via 2,3-eneaminol. Then 1-amino-4-deoxyosone was transformed into 1,4-dideoxyosone through Strecker degradation (Cerny & Davidek, 2003). 2-Acetylfuran can be formed via cyclisation of 1,4-dideoxyosone by dehydration. In the intermediate stage of Maillard reaction, deoxyosones can be degraded into shorter fragments of carbonyl or hydroxyl carbonyl compounds which may recombine to form flavour compounds. A 1:1 mixture of [^{12}C6] and [^{13}C6] isotopomers was obtained indicating that no shorter chain fragments participated in 2-acetylfuran formation in the model system when phenylalanine, cysteine and serine were used. On the other hand, glycine showed a different mechanism from other amino acids in the formation of 2-acetylfuran. The major fragmentation product was the [^{13}C5] 2-acetylfuran, while the percentage of its corresponding isotopomer [^{13}C1] was zero. Moreover, the [^{12}C6] isotopomer showed a higher level than [^{13}C6] isotopomer. Therefore, based on the principals of CAMOLA, the glycine may take part in the reaction causing a significant difference in the distribution of corresponding isotopomers. In order to confirm this interpretation, [2-^{13}C]-L-glycine was reacted with [^{12}C6] glucose and the result indicated that half of 2-acetylfuran was from recombination of glucose and glycine fragments. Generally, flavour compounds can be generated either from sugars or from sugars and amino acids. In the presence of other amino acids, only glucose contributed to the carbons of 2-acetylfuran, whereas in the presence of glycine, one carbon of 2-acetylfuran was from glycine. A proposed formation pathway of 2-acetylfuran from glucose and glycine fragments is shown in Fig. 3B. Glycine can be degraded into formaldehyde via Strecker degradation. Further fragmentation of the 1-deoxyosone may lead to the five carbons aldehyde which could react with formaldehyde via enolization and aldol condensation.

3.2. Potential control of Maillard reaction

Significant difference was observed in this study between 2-acetylfuran and DMHF formation pathways. For the selected amino acids, high proportions of a 1:1 mixture of [^{12}C6] and [^{13}C6] DMHF demonstrated only one major pathway during DMHF formation in which the glucose carbon skeleton remained intact. However, in a 1:1 mixture of [^{12}C3] MG and [^{13}C6] glucose model in the presence of glycine and cysteine, [^{12}C6] and [^{13}C6] DMHF were both observed suggesting a pathway for DMHF from MG (Wang & Ho, 2008). MG may transform into 1-hydroxy-2-propanone and pyruvic acid through the Cannizzaro reaction, and subsequently lead to DMHF by reacting MG with 1-hydroxy-2-propanone (Wang &

Ho, 2008). In other words, DMHF can be formed from MG out of Maillard system, but in the Maillard reaction system, it can not be formed via the recombination of MG, because Maillard reaction is composed of various sub-reaction cascades which are competitive with each other in the generation of Maillard reaction product. For example, MG could participate in Strecker degradation, which is a major flavour generation reaction. Besides Strecker degradation, MG could also be involved in other flavour formation pathways. Observation of [^{13}C3] 2-acetylfuran indicated the participation of MG or other [C_3] fragments.

Flavour compound formation in the Maillard reaction depends on various factors such as the type of sugars and amino acids, reaction temperature, time, pH and water content. Generally, sugar and amino acid type may influence the flavour type, yield and formation pathway. In the presence of different amino acids, the yields of DMHF are different (Wang & Ho, 2008). Even for the same flavour compound such as 2-acetylfuran, the generation pathways vary with different amino acids. However, this is just one aspect in controlling Maillard reaction. If all these factors are fixed, for example, in our glucose and glycine model, glucose can be degraded into [C_1–C_5] fragments, and glycine can give rise to formaldehyde. All these molecules occur in the Maillard reaction and participate competitively in different reaction cascades. How the reaction proceeds depends on the competitive ability of each molecule. Therefore, although both DMHF and 2-acetylfuran contain a furan ring and can be formed from deoxyosone, in the glucose–gly-

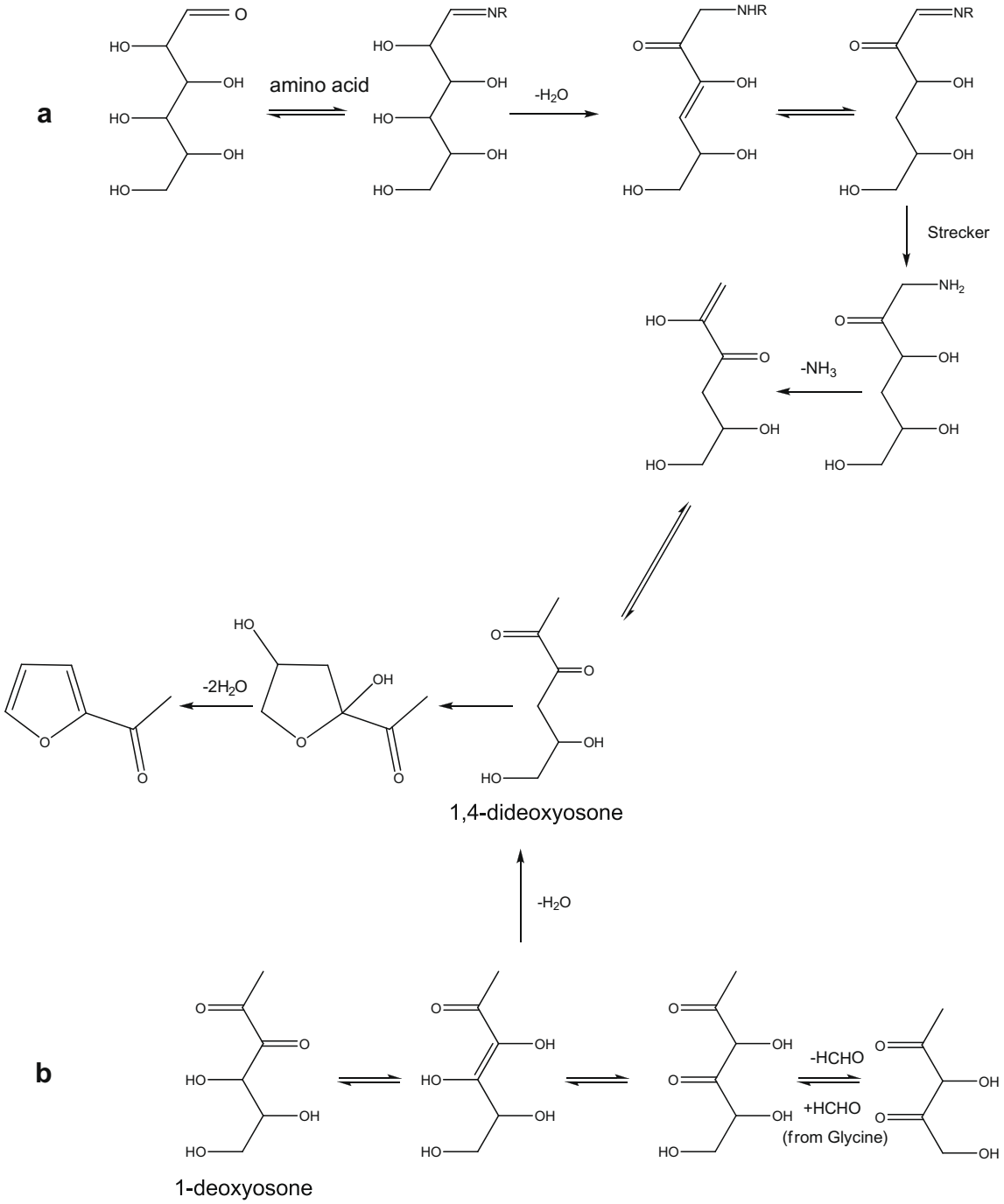

Fig. 3. Proposed formation of 2-acetylfuran from glucose and glycine (A) or glucose (B) via 1,4-dideoxyosone.

cine Maillard reaction, DMHF generated only from cyclisation of intact glucose, whereas 2-acetylfuran formed either from intact glucose or from [C_5] fragment and formaldehyde.

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