Generation of Proline-Specific Maillard Compounds by the Reaction of 2-Deoxyglucose with Proline

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The proline-specific Maillard products including 2,3-dihydro-1*H*-pyrrolizines, hexahydro-7*H*-cyclopenta[*b*]pyridin-7-ones, and cyclopent[*b*]azepin-8(1*H*)-ones were generated by both 2-deoxyglucose/proline and glucose/proline model reactions at 180 °C for 2 h. The amounts of these products generated in 2-deoxyglucose/proline reaction were far less than (<12%) those generated in the glucose/proline model reaction at both pH 9.0 and 4.0. The pH effect on the formation of these products in the 2-deoxyglucose/proline model was not significant, while a lower pH favored the generation of these products in the glucose/proline reaction. The generation of the active precursors of these proline-specific Maillard products from 2-deoxyglucose is proposed.

Keywords: *Maillard reaction; proline, aroma generation; 2-deoxyglucose; pH effect*

INTRODUCTION

It is well-known that the Maillard reaction involves the interaction of amino acids with reducing sugars and is of widespread importance in foods. An Amadori intermediate is generated during this reaction and may be degraded to form 1-deoxyhexosone and 3-deoxyhexosone intermediates, which can also be generated by the catalysis of acid/base. The 1- and 3-deoxyhexosones can decompose to the precursors of the Strecker degradation products such as dicarbonyls, glyceraldehyde, and pyruvaldehyde (Whistler and Daniel, 1985). The proline-specific Maillard products, including 2,3-dihydro-1*H*-pyrrolizines, 7*H*-cyclopenta[*b*]pyridin-7-ones, cyclopent[*b*]-azepin-8(1*H*)-ones, 2-(1-pyrrolidinyl)-2-cyclopentanones, pyrrolidines, piperidine derivatives, and maltoxazine, can be generated when either the proline Amadori compound is rearranged or the deoxyhexosone intermediates or Strecker degradation precursors further react with proline (Birch et al., 1980; Helak et al., 1989a,b; Huyghues-Despointes et al., 1994; Mills and Hodge 1976; Shigematsu et al., 1975; Tressl et al., 1981, 1985a,c, 1993; Vernin et al., 1992a,b).

It has been demonstrated that the reaction of 2-deoxyglucose with selected primary amino acids cannot effectively produce pyrazines, pyrroles, pyridines, and other typical compounds that can otherwise be generated by the reaction of glucose and common primary amino acids (Lu et al., 1997). The 2-hydroxy group is important to the formation of Maillard products in the glucose/primary amino acids model system. In this paper we report the effect of the 2-hydroxy group on the generation of proline-specific Maillard products by comparing the amount of these products generated from glucose/proline and 2-deoxyglucose/proline model reactions.

EXPERIMENTAL PROCEDURES

Materials. 2-Deoxyglucose, glucose, and proline were obtained from Sigma Chemicals (St. Louis, MO).

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Sample Preparation. Equimolar amounts of proline and 2-deoxyglucose or glucose (0.0025 mol) were dissolved in 25 mL of distilled water. The pH of the solution was adjusted to 4.0 and 9.0 with 1 N HCl and 1 N NaOH. The solution was transferred into a 150 mL Hoke stainless steel cylinder, which was then capped and incubated in an oven at 180 °C for 2 h. After being cooled to room temperature with a stream of cool water, the reaction mixtures were spiked with 0.7 mL of dodecane (C_{12}, 1.75 mg/mL) as an internal standard and extracted twice with 25 mL of methylene chloride. The combined methylene chloride extracts were dried over anhydrous sodium sulfate and concentrated to 1 mL by a stream of nitrogen gas prior to gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS) analysis. All the reactions were carried out in duplicate.

Gas Chromatography (GC) and Gas Chromatography−Mass Spectrometry (GC−MS) Analysis. A Varian Model 3400 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (DB-1, J&W Scientific, 60 m × 0.32 mm i.d.) was used to analyze the volatiles from the reaction of 2-deoxyglucose or glucose with proline. The GC oven was temperature programmed from 50 to 280 °C at a rate of 4 °C/min. The carrier gas (He) flow rate was 2.0 mL/min. A split ratio 25:1 was used. The temperatures on the injection port and the detector were 270 and 300 °C, respectively. The GC−MS analysis was performed on an HP Model 5790 GC coupled with an HP 5970A mass-selective detector. The same column and temperature program were used. The compounds were identified by matching the mass spectra of samples with those reported in the literature (Tressl et al., 1985a,c; Helak et al., 1989b).

RESULTS AND DISCUSSION

Equimolar amounts of proline and glucose or 2-deoxyglucose in water (pH 9.0 or 4.0) were heated at 180 °C for 2 h. The pH of glucose/proline model reaction was changed from 9.0 and 4.0 to 5.2 and 5.3, respectively, after reaction, while the pH of 2-deoxyglucose/proline model reaction was changed from 9.0 and 4.0 to 6.9 and 5.1, respectively. The reaction mixtures were extracted with methylene chloride and analyzed by GC and GC−MS. Table 1 summarizes the identification and quantification results of this study. In total, 15 proline-specific Maillard products were found in these model reactions. Fourteen of them were generated by the reaction of glucose with proline, while only six of them were found in the reaction system containing 2-deoxy-

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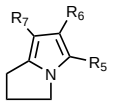
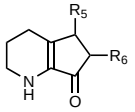
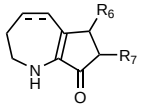
Table 1. Generation of Proline-Specific Maillard Products from the Reaction of Glucose or 2-Deoxyglucose with Proline

			amount (mg/mol of sugar)			
			Glc + proline		2-deoxy-Glc + proline	
peak no.	compound	RI[a]	pH 9.0	pH 4.0	pH 9.0	pH 4.0
	2,3-Dihydro-1*H*-pyrrolizine					
						
1	5-formyl	1319	170.9	252.7	21.0	38.0
2	5-acetyl	1382	1418.9	1947.1	61.7	71.6
3	5-propionyl	1468	15.9	25.1	–[b]	–
4	5-acetyl-7-methyl	1530	330.1	340.9	–	–
5	5-acetyl-6-methyl	1561	75.2	80.7	–	–
6	5-(3-hydroxypropionyl)	1723	64.1	73.2	–	–
		subtotal	2075.1	2719.7	82.7	109.6
	Hexahydro-7*H*-cyclopenta[*b*]pyridin-7-one					
						
7	1,2,3,4,5,6-	1401	842.7	1018.7	118.2	106.5
8	6-methyl-1,2,3,4,5,6-	1423	44.7	53.2	–	–
9	5-methyl-1,2,3,4,5,6-	1438	23.2	27.5	–	–
10	6-ethyl-1,2,3,4,5,6-	1521	36.0	47.8	–	–
11	5-(1-hydroxyethylidene)	1638	92.6	97.1	–	–
		subtotal	1039.2	1244.3	118.2	106.5
	Cyclopent[*b*]azepin-8(1*H*)-one					
						
12	2,3,4,5,6,7-hexahydro	1478	136.2	93.4	104.9	65.4
13	7-methyl-2,3,4,5,6,7-hexahydro	1495	135.4	144.7	–	–
14	2,3,6,7-tetrahydro	1513	29.6	39.2	29.8	22.8
15	6,7-dimethyl-2,3,6,7-tetrahydro	1725	–	–	50.8	74.8
		subtotal	301.2	277.3	185.5	163.0
		total	3415.5	4241.3	386.4	379.1

[a] RI = retention index. [b] Not dectected.

glucose and proline. The proline-specific Maillard products found in this study can be classified into three major groups as shown in Table 1. These are 2,3-dihydro-1*H*-pyrrolizines, hexahydro-7*H*-cyclopenta[*b*]-pyridin-7-ones, and cyclopent[*b*]azepin-8(1*H*)-ones, which were identified by comparing the mass spectra to the published data (Helak et al., 1989a; Tressl et al., 1985a,c). In terms of the quantification analysis, the amount of those proline-specific Maillard products generated in the model reaction of 2-deoxyglucose with proline was far less than that generated in the reaction of glucose with proline at both pH 9.0 and 4.0 as shown in Table 1. These results indicate that the formation of these compounds substantially involves the 2-hydroxy group in glucose. The amount of these products generated at pH 4.0 was higher than those at pH 9.0 in the reaction of glucose with proline; however, the pH effect on the generation of these compounds in the model system of 2-deoxyglucose/proline was not significant. These results suggest that the pathway for the generation of proline-specific Maillard products by the glucose/proline reaction is different than that of the 2-deoxyglucose/proline reaction.

The two most abundant products found in the 2-deoxyglucose/proline model reaction were 2-(1,2-dihydroxyethyl)furan (**16**) and catechol (**17**). The amount of 2-(1,2-dihydroxyethyl)furan (**16**) was 671 and 402 mg/mol of sugar at pH 9.0 and 4.0, respectively, and the amount of catechol (**17**) was 367 and 249 mg/mol of sugar at pH 9.0 and 4.0, respectively. A higher pH favored the formation of these two compounds. The pathway for the generation of compound **16** was proposed as shown in Figure 1. The hydroxy group at the C-4 position of 2-deoxyglucose attacks the carbonyl group at the C-1 position to form a five-membered ring intermediate, which is then transformed into compound **16** after losing two molecules of water. 2-Deoxyglucose may be dehydrated and keto−enol tautomerized to form a 2,4-dideoxy 5-ketone intermediate, which is then transformed into a six-membered ring derivative by aldol condensation and then dehydrated to generate the catechol (**17**) as shown in Figure 1.

Table 1 shows only two pyrrolizine compounds, 5-formyl- (**1**) and 5-acetyl-2,3-dihydro-1*H*-pyrrolizine (**2**), found from the 2-deoxyglucose/proline model reaction. The mechanisms for the formation of 5-acetyl-2,3-dihydro-1*H*-pyrrolizine (**2**) were proposed by Tressl et al. (1981, 1985a). One of these mechanisms involves the formation of an iminium ion by the reaction of proline with pyruvaldehyde. This iminium ion undergoes either an aldol condensation reaction with a hydroxyacetaldehyde or a nucleophilic attack by a hydroxyacetaldehyde followed by either a Michael addition or an aldol condensation. This proposed pathway demonstrates that two effective precursors for the formation of this proline-specific Maillard product are pyruvaldehyde and hydroxyacetaldehyde, which can possibly also be generated by retroaldol cleavage from

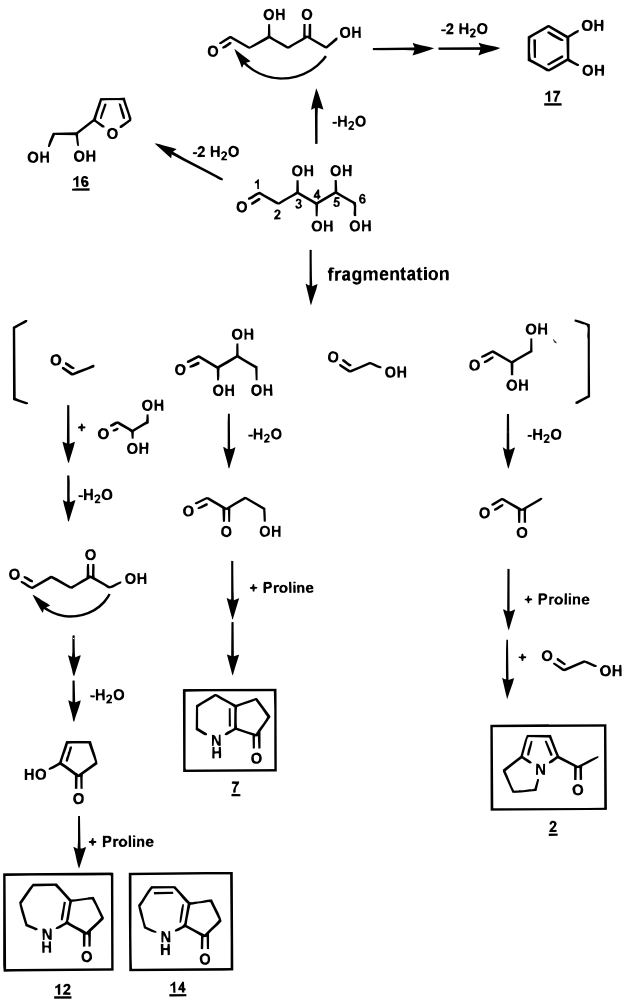


Figure 1. Formation pathways of proline specific Maillard compounds, 2-(1,2-dihydroxyethyl)furan, and catechol in 2-deoxyglucose/proline model reaction.

2-deoxyglucose as shown in Figure 1. Another possible mechanism for the formation of compound **2** is via a direct reaction of proline with 1-deoxypentosone, which can be generated from the proline/glucose (maltose) model reaction (Tressl et al., 1981). However, this pathway is less likely to be the one for the formation of this product by the 2-deoxyglucose/proline reaction because 1-deoxypentosone is less likely to be formed in the proline/2-deoxyglucose model reaction.

The 1,2,3,4,5,6-hexahydro-7*H*-cyclopenta[*b*]pyridin-7-one (**7**) was the only 7*H*-cyclopenta[*b*]pyridin-7-one compound found in the 2-deoxyglucose/proline reaction as demonstrated in Table 1. The precursor for the generation of this product was erythrose according to the erythrose/proline model reaction study (Tressl et al., 1985a; Helak et al., 1989a). Our study also showed that compound **7** was the major component found in the reaction of erythrose with proline by using our reaction system (data not shown). The pathways for the formation of this product were proposed by Tressl et al. (1985a). This product may be formed by the reaction of proline with 4-hydroxy-3-oxobutanal, a dehydration intermediate from erythrose, followed by ring enlargement, dehydration, and Michael addition reaction. The erythrose may be generated directly from 2-deoxyglucose via a retroaldol cleavage between C-2 and C-3 positions as presented in Figure 1.

As shown in Table 1, the total amount of cyclopent-[*b*]azepin-8(1*H*)-ones generated in the glucose/proline

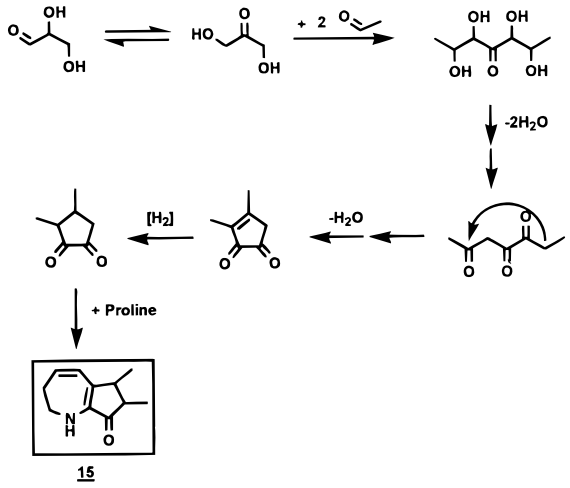


Figure 2. Formation pathway of 6,7-dimethyl-2,3,6,7-tetrahydrocyclopent[*b*]azepin-8(1*H*)-one in 2-deoxyglucose/proline model reaction.

model system at both pH 9.0 and 4.0 was significantly less than that of 2,3-dihydro-1*H*-pyrrolizines and hexahydro-7*H*-cyclopenta[*b*]pyridin-7-ones. These results indicate that this group of proline-specific Maillard products is less likely to be formed in this model reaction. The total amount of cyclopent[*b*]azepin-8(1*H*)-ones generated from the 2-deoxyglucose/proline reaction was about 60% of that generated from glucose/proline model reaction at both pH 9.0 and 4.0, while the total amount of the other two groups of product was less than 12%. These results suggest that the formation of cyclopent-[*b*]azepin-8(1*H*)-ones is less affected by the 2-hydroxy group in glucose. In comparison, the formation of 2,3-dihydro-1*H*-pyrrolizines and hexahydro-7*H*-cyclopenta-[*b*]pyridin-7-ones is more affected. As presented in Table 1, three cyclopent[*b*]azepin-8(1*H*)-one compounds, compounds **12**, **14**, and **15**, were found in the 2-deoxyglucose/proline model system. The pathway for the formation of compound **12** and **14** was reported by Tressl et al. (1985c, 1993). 2-Hydroxy-2-cyclopenten-1-one was demonstrated to be an effective precursor for the formation of these two compounds. This cyclic enolone compound can react with proline to form an iminium ion intermediate. Compounds **12** and **14** can be formed from this intermediate by hydration followed by ring enlargement, dehydration, and/or oxidation (Tressl et al., 1985c). The possible pathway for the formation of 2-hydroxy-2-cyclopenten-1-one from 2-deoxyglucose was proposed as shown in Figure 1. 2-Deoxyglucose is degraded into acetaldehyde and glyceraldehyde by a retroaldol mechanism. The aldol condensation reaction of both of these compounds leads to the 2,3-dideoxy-pentosone intermediate, which then undergoes intramolecular cyclization via an aldol condensation followed by loss of a water molecule to form the 2-hydroxy-2-cyclopenten-1-one.

Compound **15**, found in the 2-deoxyglucose/proline model reaction, was the only compound which was not generated in the glucose/proline model system. According to the report of Tressl et al. (1985c), this compound can be generated only in the 4,5-dimethylcyclopentanedione/proline model reaction. Compound **15** generated in the reaction of 2-deoxyglucose with proline suggests that there is a possible pathway to form the 4,5-dimethylcyclopentanedione from 2-deoxyglucose as presented in Figure 2. The glyceraldehyde undergoes aldol condensation with two molecules of acetaldehyde leading to a seven-membered intermediate, which then

loses two molecules of water to form a triketone intermediate. The 4,5-dimethylcyclopentanedione is formed after the intramolecular cyclization via an aldol condensation followed by dehydration and reduction from this triketone intermediate.

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