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Maillard Reaction Products Formed from D-Glucose-Glycine, System and Their Formation Mechanism

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Equimolar aqueous solutions of D-glucose and glycine were heated at 50°C and 95°C at pH 6.7. The headspace volatiles and the ether extracts from the reaction mixture were analyzed by gas chromatography and gas chromatography-mass spectrometry using a fused silica capillary column.

The major components formed were identified as diacetyl, three furfurals, two pyrroles, one furanone, two pyranones and two amides. In order to elucidate the formation mechanisms of the amides formed from amino-carbonyl reaction, two model systems were adopted. N-butylacetamide were formed as major components from diacetyl-butylamine and glyoxal-butylamine systems, respectively. The results obtained suggest that such α-dicarbonyls as 3-deoxy-D-erythro-2,3-hexodiulose and diacetyl generated in the amino-carbonyl reaction react with amino compounds, amides then being formed by cleavage of the C-C bond in the α-dicarbonyls.

Introduction

Low molecular weight compounds formed by the Maillard reaction of sugar-amino acids or amines have been identified as aldehydes, ketones, furans, pyrroles, pyridines, pyrazines and so on (Fors, 1983; Shibamoto, 1983; Hodge, 1967). These compounds have been long studied in connection with flavor chemistry and play an important role as cooked or roasted flavor of various foodstuffs as well as sea-food products. Hayase and Kato (1985) also identified some Maillard reaction products containing amides formed from a D-glucose and butylamine system at various pH values. With the advance of the amino-carbonyl reaction, melanoidins are formed as final major products.

Recently, the nitrogen in the melanoidins has been reported to be mainly in the secondary amide type (Benzing-Purdie et al., 1983). The authors also postulated that glycine was extensively incorporated into nondialyzable melanoidin, as the amide form (Hayase et al., 1984; Kim et al., 1985). In the present paper, we report an investigation of the reaction products from a glucose and glycine system, verifying the formation of amides and discussing the formation mechanisms using model systems.

Materials and Methods

(1) Heating glucose with glycine.

Thirty six grams of D-glucose, 15 *g* of glycine and 3.36 *g* of sodium bicarbonate were dissolved in 100 *ml* of water (pH 6.7) and refluxed at 95°C. The reaction mixture was extracted with ethyl ether and the ether extracts were concentrated at 36°C. The aqueous fraction was adjusted to pH 1 using hydrogen chloride and extracted sufficiently with ethyl acetate. The extracts were concentrated

and methylated by diazomethane.

(2) Analysis of headspace volatiles formed from the glucose-glycine reaction system.

0.1 M glucose, 0.1 M glycine and 0.01 M sodium bicarbonate as starting materials were dissolved in 100 *ml* of water in a two-necked egg-plant-shaped flask. Tenax GC trapping of the headspace volatiles with a stream (30 *ml*/min) of air or nitrogen was performed as described in the previous paper (Hayase et al., 1985). The apparatus was placed for various times and refluxed in a water bath heated to 95°C, 28 minutes being required until the temperature of the solution reached at 95°C. The volatile components were continuously adsorbed to the Tenax GC by the circulation system. The headspace voltiles were analyzed by direct injection to a gas chromatograph(GC), according to the method of Tsugita et al. (1979). In the GC -MS analysis, desorption of the volatile components being directly injected from an injection port.

(3) Heating diacetyl and glyoxal with butylamine.

Butylamine (7.3 *g*) was dissolved in water(50 *ml*) and the solution was adjusted to pH 6.7 with phosphoric acid. To the resulting solution, 8.6 *g* of diacetyl (Tokyo Kasei Kogyo Co. Ltd.) or 19.3 *g* of glyoxal (Merck, 30% of aqueous solution) was added during cooling in an ice bath. These mixtures were refluxed in a water bath heated at 95°C and periodical sampling was performed immediately after setting the reaction flask in the bath. Each reaction mixture was extracted with ether. The ether extracts were concentrated at 36°C and 200 μg of n-tridecane was added to the concentrates as an internal standard (diacetyl-butylamine system: D-B fraction; glyoxal-butylamine system: G-B fraction).

These D-B and G-B fractions were analyzed by GC and GC-MS, respectively.

(4) Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS)

The experimental conditions were performed according to the method of Hayase et al., (1984); a fused silica WCOT capillary column (50 *m*×0.25 *mm i.d.*) coated with PEG 20 M was used. Measurement of the gas chromatographic peak area was determined by a Shimadzu Model Chromatopac E1—B integrator connected to the gas chromatograph. GC-CI MS analysis using isobutane as the CI gas was also tried.

Results

(1) Components formed by heating glucose with glycine.

Figure 1 shows the gas chromatogram of ether extracts obtained from the reaction of glucose with

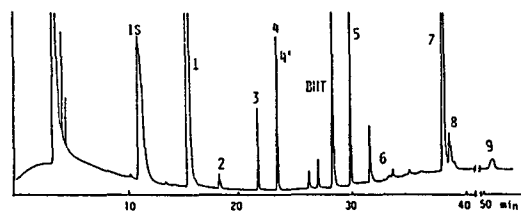

Fig. 1. Gas chromatogram of ether extracts obtained from the reaction between glucose and glycine at 95°C.
1, acetic acid;2, propionic acid; 3, furfuryl alcohol; 4, 2,5-dimethylpyrrole; 4', 5-methyl-2-furfuryl alcohol; 5, 2-acetylpyrrole; 6, 4-hydroxy-2,5-dimethyl-3(2H) furanone; 7, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; 8, 2-methyl-3,5-dihydroxy-4H-pyran-4-one; 9, 5-(hydroxymethyl)-2-furfural. Butylated hydroxytoluene (BHT) was a contaminant originating from ether used as the solvent.

glycine at 95°C for 4 hr. These ether extracts were subjected to GC-MS analyses, and acetic acid, furfuryl alcohol, 2,5-dimethylpyrrole, 2-acetylpyrrole and 2,3-dihydro-6-methyl-4H-pyran-4-one were identified as the major components (Table 1). Five minor compounds were identified as propionic acid

Table 1. The relative amounts* of each component in ether extracts obtained from reaction mixtures of glucose-glycine system

Peak No	Compounds	10 min	30 min	4 hr
1	Acetic acid	0.002	0.079	352
3	Furfuryl alcohol	—	—	0.157
4	2,5-Dimethylpyrrole	—	0.005	0.323
5	2-Acetylpyrrole	—	0.001	0.462
7	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	0.005	1.65	2.420

* Peak area of each component/each area of internal standard (n-tridecane).

(peak 2), 5-methyl-2-furfuryl alcohol (peak 4'), 4-hydroxy-2, 5-dimethyl-3(2H)furanone (peak 6), 2-methyl-3, 5-dihydroxy-4H-pyran-4-one (peak 8) and 5-(hydroxymethyl)-furfural (peak 9). The acids in the acidic fraction of the ethyl acetate extracts obtained from the same reaction mixture were methylated with diazomethane. Figure 2 shows the gas chromatograms of the acidic fraction. N-

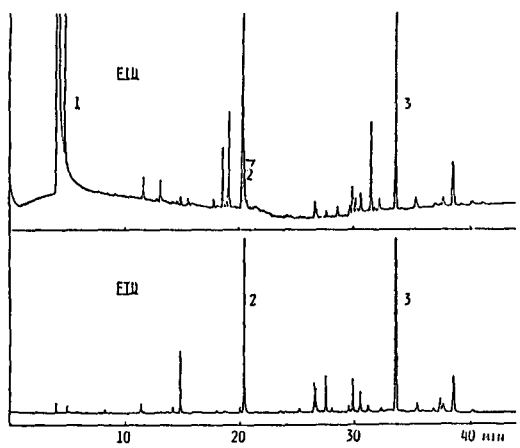

Fig. 2. Gas chromatograms of the acidic fraction of ethyl acetate extracts obtained from the reaction between glucose and glycine at 95°C for 4 hr at pH 6.7.
1, acetic acid; 2, N-methylacetamide; 3, N-acetylglycine.

methylacetamide and N-acetylglycine were identified as major components by GC analyses (FID; FTD, flame thermionic detector) and GC-MS analyses (EI, electron impact ionization; CI, chemical ionization).

In addition, the headspace volatiles formed from the glucose-glycine system were investigated by the Tenax GC trapping method. Figure 3 shows

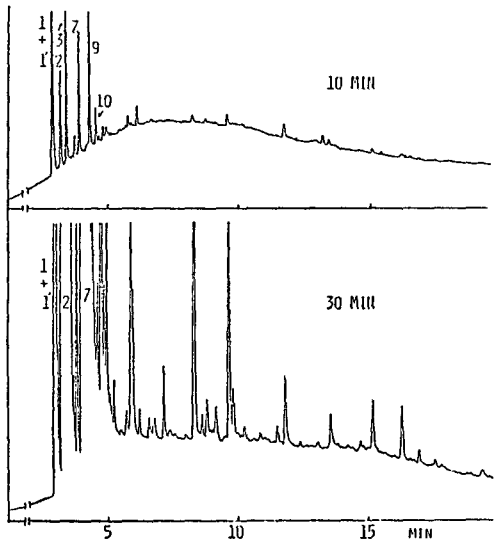

Fig. 3. Gas chromatograms of headspace volatiles formed by the reaction of glucose-glycine system at 95°C by Tenax GC trapping.

typical gas chromatograms of the volatile components formed by the reaction of glucose with glycine at 95°C for 10 min and 30 min. Some peaks were observed after 10 min and their relative amounts increased at 30 min.

The relative amounts of major volatiles which were identified by GC-MS are summarized in Table 2. Diacetyl (Fig. 4) was most abundant and occupied

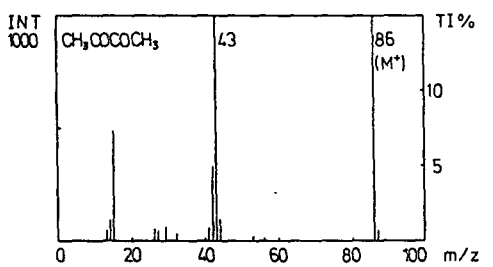

Fig. 4. Mass spectrum of diacetyl formed from glucose-glycine system obtained by the Tenax GC trapping method.

70% of the total peak area on heating for 2 hr. The low boiling point components were markedly

Table 2. The relative amount* of volatile components formed by the reaction of glucose-glycine system at pH 6.7 after various times**

Peak No.	Compound	In a water bath (95°C)					At 50°C
		10min	10min(N_2)	30 min	60 min	120 min	4 hr
1′	Acetaldehyde	t***	—	t	t	t	t
2	Furan+Acetone	2.1	—	232	432	2262	5.9
3	2-Methylfuran	3.6	—	84	200	303	14.0
4	2-Butanone	—	—	43	122	723	5.3
6	2,5-Dimethylfuran	0.4	—	10	34	71	3.8
7	Diacetyl	1.9	0.1	1863	4121	14572	6.1
11	2,3-Pentanedione	—	—	6	127	1176	—

* Peak area of each component measured with an integrator×1/100.

** The temperature of the reaction mixture at 10 min and after 28 min had reached 65°C and 95°C, respectively.

*** Compounds detected as trace amounts.

suppressed under bubbling of nitrogen than under air. On heating at 50°C for 4 hr, similar components to those already described were formed, but with some differences in the gas chromatographic pattern.

(2) Volatile components formed by heating diacetyl or glyoxal with butylamine.

The formation of amides as major components by an amino-carbonyl reaction was clarified. In addition, diacetyl was the main compound in the headspace volatiles from the result described here. Hayashi and Namiki (1980) reported that glyoxal was found at an early stage of the reaction of sugar with amine. Therefore, both reaction systems of diacetyl or glyoxal and butylamine (D-B or G-B system) were used as models of the amino-carbonyl reaction. Components of the D-B or G-B fractions were analyzed by GC and GC-MS, N-butylacetamide and N-butylformamide being identified as major components in these respective fractions (Fig. 5). Table 3 shows the relative amounts of the two amides with each sampling time.

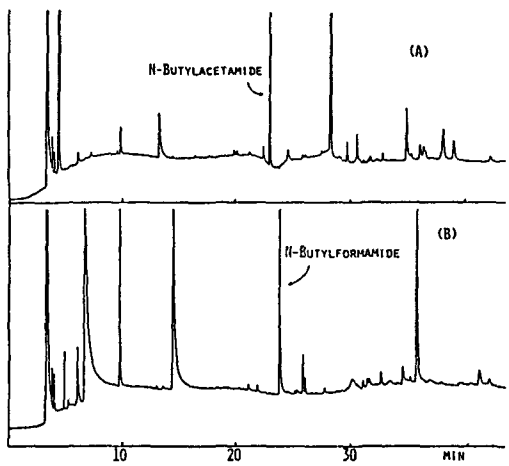

Fig. 5. Gas chromatograms of ether extracts obtained from reaction mixture of diacetyl (A) and glyoxal (B) with butylamine at 95°C for 5 min.

Table 3. The relative amount* of two amides detected by GC of ether extracts obtained from reaction mixtures of diacetyl (D) or glyoxal (G) with butylamine (B) at pH 6.7 after various times**

Compound	G-B				D-B			
	2 min	5 min	10 min	30 min	3 min	5 min	20 min	60 min
N-Butylacetamide	—	—	—	—	6.4	8.0	7.4	6.6
N-Butylformamide	1.7	0.9	0.4	0.4	—	—	—	—

* Peak area of each component/peak area of internal standard (n-tridecane).

** The temperature of the reaction mixture at 2 min, 5 min, 10 min, 20 min and after 28 min had reached 37°C, 57°C, 65°C, 80°C and 95°C respectively.

N-butylacetamide was formed at the early stage of the reaction, the relative amount of N-butylformamide being a maximum at 2 min and gradually decreasing over the next 2 min.

Discussion

The results of headspace analyses in the glucose and glycine systems indicate that fission compounds of C_2 to C_5 of glucose molecule were formed at 50°C and at an early stage after reaching 95°C. The formation of these low boiling point compounds was conspicuously suppressed under bubbling of nitrogen(Table 2). This result suggests that oxidative cleavage of glucose occurs in the early stage of the browning reaction. Diacetyl was identified as a major low boiling point compound in the headspace volatiles from the glucose-glycine system. Lento et al. (1960) have reported that diacetyl was produced from glucose in strong alkaline boiling solutions, but not formed from glucose at pH 8-9. In the present investigation, it became clear that larger amounts of diacetyl were formed as headspace volatiles in neutral pH ranges in the amino-carbonyl reaction.

The major components of ether extracts from the glucose-glycine system had been already identified in many amino-carbonyl reaction systems except for 2,5-dimethylpyrrole (Hayase and Kato, 1985; Olsson et al., 1978). Nyhammar et al. (1983) have reported that the methyl group of 2-acetylpyrrole is derived from the C-6 position of the glucose, and proposed a new route to 2-acetylpyrrole through an enamine derived from 2-deoxy-D-arabino-hexose.

On the other hand, 1-deoxy-D-erythro-2, 3-hexodiulose is known to be an important intermediate, having a methyl group at the C terminal, in the dehydration of hexoses and the Maillard reaction. Consequently, diacetyl is assumed to be formed by fission of the C_2-C_3 bond or C_4-C_5 bond of the glucose.

Acetic acid, N-methylacetamide and N-acetylglycine are similarly considered to be formed from cleavage of the C_2-C_3 bond or C_4-C_5 bond of the sugar moiety. 5-(Hydroxymethyl)-2-furfural (HMF) were deduced to be formed from 3-deoxy-glucosone via 1,2-enolization and is well known as a major compound formed on the Maillard reaction (Hodge, 1967), dehydration of carbohydrates (Feather and Harris, 1973), acid-catalyzed degradation of D-fructose (Shaw et al., 1967) and so on. 4-Hydroxy-2,5-dimethyl-3(2H)furanone and 2-methyl-3, 5-dihydroxy-4H-pyran-4-one, which were identified as minor components, have a caramel aroma and are supposed to be important to the aromatic flavor generated by an amino-carbonyl reaction. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one were postulated to be formed via 2,3-enolization and is important as a precursor of maltol and 2-methyl-3,5-dihydroxy-4H-pyran-4-one.

In the present paper, the formation of amides as fission compounds observed in a glucose and butylamine system (Hayase and Kato, 1985) was also verified in a glucose and glycine system. Recently, it has been speculated that the nitrogen in the melanoidins is mainly in the amide form (Benzing-Purdie et al., 1983; Hayase et al., 1984). Therefore, the formation mechanisms of these amides were investigated by using model systems. As shown in Table 3, N-butylacetamide from the diacetyl-butylamine system and N-butylformamide from the glyoxal-butylamine system were formed as major compounds, respectively. These findings suggest that Schiff's bases are formed by diacetyl or glyoxal with butylamine, and amides are subsequently formed by oxidative cleavage of the C-C bond of α-dicarbonyls (Fig. 6).

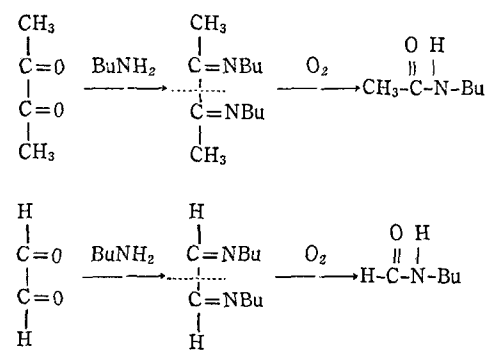

Fig. 6. Possible schemes for the formation of amides by the reaction of diacetyl or glyoxal with butylamine ($BuNH_2$).

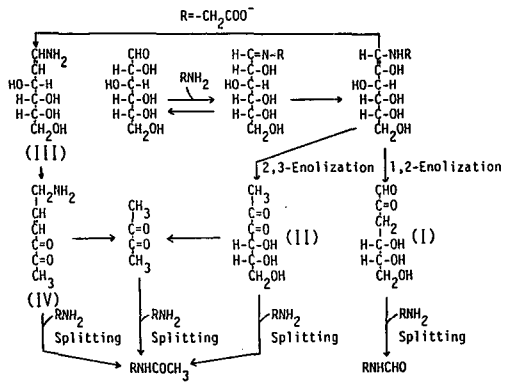

Fig. 7. Possible schemes for the formation of diacetyl and amides by the reaction of sugar with amino compounds.

Accordingly, as shown in Figure 7, the formamides and acetamides formed by the Maillard reaction are considered to be formed through the cleavage of the C-C bond in α-dicarbonyl groups after the addition of amino compounds to 3-deoxyosone (I) via 1, 2-enolization, and to 1-deoxy-D-erythro-2, 3-hexodiulose (II) via 2, 3-enolization as well as to diacetyl, respectively. N-methylacetamide is considered to be formed from the decarboxylation of N-acetylglycine. However, since formamide was not detected in the glucose-glycine system, cleavage of the C_1-C_2 bond via 3-deoxy-D-erythro-hexosulose in the glucose-amino acid system is presumed to hardly occur.

α-Dicarbonyls are known to be active intermediate compounds in the browning reaction and have been reported to have mutagenicity (Bjeldanes and Chew, 1979). The mechanisms involved in the conversion to amides, as described above, is considered to be the oxidative inactivation of active carbonyls by amino groups.

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D-Glucose-Glycine 系의 Maillard 反應生成物 및 그 生成機構

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D-glucose-glycine 系를 使用하여 Maillard 反應에 의하여 生成되는 低分子 揮發性成分을 비롯하여 amide 化合物의 生成 및 그 機構를 檢討하였다. 그 結果, 同定된 低分子 揮發性成分 中에서, headspace gas 中의 揮發性成分은 furan, acetone, 2-methylfuran, 2,5-dimethylfuran 2-butanone 2,3-pentanedione, diacetyl 등이었다, 이 中에서 diacetyl의 生成量이 가장 많아, 全 peak 면적의 약 70%를 차지하였다. 또한, 에테르 抽出物中의 主要反應生成物은 초산, furfuryl alcohol 2,5-dimethylpyrrole 2-acetylpyrrole 2,3-dihydro-3,5-dihydroxy-6-methyl-4H- pyran-4-one 등이었고, ethyl acetate로 抽出한 酸性劃分中에는 N-acetylglycine와 N-methylacetamide 등 2種類의 amide 化合物의 生成이 밝혀졌다. 이들 amide 化合物의 生成機構를 밝히기 위하여, Maillard 反應初期生成物인 diacetyl 및 glyoxal을 각각 butylamine과 反應시킨 結果, Schiff 鹽基의 酸化的 分解로 N-butylacetamide 및 N-butylformamide의 生成이 인정되었다.

따라서 N-acetylglycine 및 N-methylacetamide는 glucosylamine의 2,3-enol 化 및 β-elimination에 의한 脫水의 進行으로 生成된 dicarbonyl 化合物이 glycine과 反應하여 Schiff 鹽基를 形成하고, 이 Schiff 鹽基가 酸化的分解를 받아서 N-acetylglycine이 生成되고, N-methylacetamide는 N-acetylglycine의 脫炭酸에 의해서 生成된다고 생각한다.