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Characterization of a New Maillard Type Reaction Product Generated by Heating 1-Deoxymaltulosyl-glycine in the Presence of Cysteine

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The reaction between Amadori compounds and cysteine was investigated. When 1-deoxymaltulosyl-glycine (glycyl-fructosyl-glucose) was heated at 100 °C with cysteine in a neutral aqueous solution, a novel intermediate composed of 1-deoxyosone and cysteine was detected. NMR and mass spectrometry studies revealed the structure of the isolated intermediate to be 7,8a-dihydroxy-4a-methyl-8-(α-D-glucopyranosyloxy)hexahydro-5-oxa-4-thia-1-azanaphthalene-2-carboxylic acid. This intermediate easily generated isomaltol and acetylfuran as volatile compounds in 1 mol/L HCl at 100 °C.

KEYWORDS: Maillard reaction; maltose; cysteine; Amadori compound; flavor; 1-deoxyosone; acetylfuran; isomaltol

INTRODUCTION

The Maillard reaction plays an important role in flavor generation during food processing. Cysteine (Cys) is known to be one of the most important precursors of meatlike flavor compounds. Many volatiles have been identified in model experiments on reducing sugars/Cys mixtures. Model systems composed of D-glucose and Cys have long been used to study the thermal generation of flavor compounds (*1*−*5*). D-Ribose/Cys model systems have also been used (*6*−*8*). The reaction pathways of these compounds are studied using intermediate compounds such as Cys/2,3-butanedione (*6*), cysteamine/2,3-butanedione (*7*, *9*), cysteamine/2-oxopropanal (*10*), and Cys/4-hydroxy-5-methyl-3(2*H*)furanone (*11*). Amadori compounds are known to be the main products of the initial stage of the Maillard reaction (*12*). Amadori compounds are degraded to dicarbonyl intermediates such as 1-deoxyosone during heat processing. However, the reactions among Cys and Amadori compounds, especially derived from disaccharides, have not been reported. The reaction pathway of Amadori compounds derived from disaccharide and monosaccharide in the presence of Cys may differ, because Maillard reactions of disaccharides had been revealed to be different from that of monosaccharides (*13*, *14*). To elucidate the mechanism of these reactions, it is necessary to isolate and identify nonvolatile flavor intermediates. In this paper, we mainly focus on the initial stage of the reaction, and characterization of the Cys-containing intermediate from a reaction mixture of an Amadori compound derived from disaccharide, 1-deoxymaltulosyl-glycine (Mal-Gly), and Cys are discussed.

MATERIALS AND METHODS

Chemicals. Glycine (Gly), maltose, Cys, ^{15}N-Cys, and CH_3CN were purchased from Nakarai Tesque Co. (Japan) and used without further purification. $H_2{}^{18}O$ (97%) was purchased from Shoko Tsusho Co. (Japan). Acetylfuran was purchased from Tokyo Kasei Co. (Japan).

Analysis. HR-fast atom bombardment (FAB)-MS was recorded at JEOL Co. (Japan). A JMS-700 (double focusing magnetic sector analyzer) was used.

LC-[Electrospray Ionization (ESI)]MS Analysis. These were run on an HP1100 (Hewlett-Packard) high-performance liquid chromatography (HPLC) system coupled to a Navigator (Thermoquest) quadrupole mass spectrometer equipped with an ESI interface. The column (Capcell pak, NH_2, 5 μm, 2.0 mm × 250 mm, Shiseido, Japan) used a flow rate of 0.2 mL/min, an injection volume of 10 μL, and a solvent of 0.1 mol/L ammonium acetate buffer, pH 8:CH_3CN (1:1, v/v). Samples were dissolved in water. The ESI capillary voltage was 3.5 keV, and the cone voltage was 100 V.

NMR Spectroscopy. ^{1}H NMR (400 MHz), ^{13}C NMR (100.6 MHz), distortionless enhancement polarization transfer (DEPT), ^{1}H−^{1}H correlation spectroscopy (COSY), ^{1}H−^{13}C COSY, ^{1}H−^{1}H total correlated spectroscopy (TOCSY), and heteronuclear multiple bond correlation (HMBC) spectra were recorded on a Bruker AMX400 spectrometer in D_2O, and 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt (TSP-d_4) was used as an internal standard.

Preparative HPLC. The preparation of the HPLC−recycling technique system (Shimadzu Co., Japan) consisted of an LC6A gradient pump system combined with a refractive index (RI) detector and a column (Capcell pak, NH_2, 5 μm, 20 mm × 250 mm, Shiseido, Japan); flow rate, 10 mL/min; injection volume, 4.5 mL; solvent, 0.1 mol/L ammonium formate buffer, pH 8/CH_3CN (1:1 or 2:3, v/v). A sample was loaded on a column with the HPLC system, and an eluted fraction

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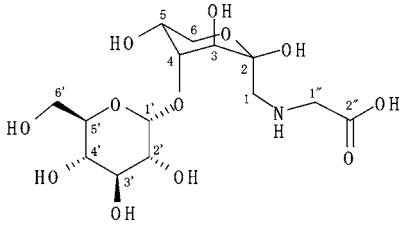

Figure 1. Structure of Mal-Gly.

containing a desired compound from the column back was reintroduced into the column inlet several times until the target compound was purified.

Solid Phase Microextraction (SPME) and Gas Chromatography−Mass Spectrometry (GC-MS) Analysis of the Volatiles. The sample headspace was analyzed by SPME (FOCUS COMBI-PAL SPME System, GL Science Inc., Japan) in combination with a HP6890 (Hewlett-Packard) gas chromatograph coupled to a BU-20 (JEOL) mass spectrometer [headspace (HS)-SPME-GC-MS]. After 1 h of equilibration at room temperature, a fiber [poly(dimethylsiloxane)-divinylbenzene (PDMS-DVB); film thickness, 65 μm (Spelco)] was exposed for 15 min at 35 °C to the headspace of the sample in the glass vials with agitation. After sampling, the SPME device was placed for 2 min in an injector equipped with a 0.75 mm i.d. liner (Spelco) and heated at 200 °C.

GC-MS analysis was performed on an HP6890 (Hewlett-Packard) gas chromatograph coupled to a BU-20 (JEOL) mass spectrometer using an HP-5 MS column (0.25 mm × 30 m × 0.25 μm, Hewlett-Packard). After insertion of the SPME device into the injector, the oven temperature program was started and the temperature was maintained for 5 min at 50 °C, then raised at 5 °C/min from 50 to 100 °C, 50 °C/min from 100 to 220 °C, and maintained for 5 min at 220 °C. Mass spectra in the electron impact mode (EI) were generated at 70 eV and at a scan range from *m*/*z* 39 to 338.

Reactions. *Synthesis of Mal-Gly (1).* The Amadori product of Gly with maltose was synthesized according to the procedure of Mossine et al. (*15*); 1.5 g of Gly (0.02 mol), 18.0 g of maltose·H_2O (0.05 mol), and 0.5 g of $NaHSO_3$ (0.005 mol) were dissolved in a mixture of 15 mL of ethylene glycol and 30 mL of methanol and reacted for 0.5 h at 90 °C with stirring. To the solution, 2 mL of glacial acetic acid was added, and it was reacted for 4 h at 90 °C. The reaction mixture was cooled to ice temperature, 50 mL of water was added, and it was subjected to ion-exchange column chromatography (Dowex 50 W × 8 H^+ form, 200 mesh, 50 g). After it was washed with water, Mal-Gly was eluted from the column with 1.5% aqueous ammonium and then lyophilized; 3.48 g of white powder was obtained (44% yield). LC-(ESI$^+$)MS: *m*/*z* 400 (M + H^+). ^{1}H and ^{13}C NMR for Mal-Gly [400 MHz; D_2O; COSY, heteronuclear multiple quantum coherence (HMQC), DEPT; arbitrary numbering of carbon atoms refers to structure **1** in **Figure 1**]: δ_H 3.35 {m, H_2−C(1)}, 3.43 {m, H−C(4′)}, 3.59 {m, H−C(2′)}, 3.67−4.20 {m, H_2−C(1″), H−C(3), H−C(4), H−C(5), H_2−C(6), H−C(3′), H−C(5′), H_2−C(6′)}, 5.24 {d, *J* = 3.9 Hz, H−C(1′)}. δ_C 52.6 {CH_2, C(1″)}, 56.0 {CH_2, C(1)}, 63.3 {CH_2, C(6′)}, 66.8 {CH_2, C(6)}, 71.7 {CH, C(5)}, 72.2 {CH, C(3)}, 72.2 {CH, C(4′)}, 74.6 {CH, C(2′)}, 75.5 {CH, C(5′)}, 75.6 {CH, C(3′)}, 80.5 {CH, C(4)}, 98.2 {C, C(2)}, 103.4 {CH, C(1′)}, 174.0 {C, C(2″)}.

Preparation of 7,8a-Dihydroxy-4a-methyl-8-(α-D-glucopyranosyloxy)hexahydro-5-oxa-4-thia-1-aza-naphthalene-2-carboxylic Acid (Compound 2). Mal-Gly (**1**, 0.28 g, 0.70 mmol) and Cys (0.17 g, 1.4 mmol) were dissolved in water (10 mL), and the pH was adjusted to 7.4 with 27% aqueous NaOH. The mixture was heated at 100 °C for 3 h. Next, the mixture was cooled to room temperature and subjected to a preparative HPLC (solvent; 0.1 M $HCOONH_4$:CH_3CN 1:1, v/v). The fraction with t_R = 18−20 min was lyophilized. This crude product was dissolved in water (4 mL) and subjected to the preparation HPLC−recycling technique system (solvent; 0.1 M $HCOONH_4$:CH_3CN 2:3, v/v). The t_R = 23−26 min fraction was injected into the column. The eluted fraction was lyophilized, and compound **2** was obtained (24 mg, 8.0%). LC-(ESI$^+$)MS: *m*/*z* 428 (M + H^+). HR-FAB-MS: *m*/*z* 426.1082 (M − H^-) (426.1070, calculated for $C_{15}H_{24}O_{11}NS$); for NMR data, see **Table 1**.

Table 1. NMR Spectral Data for Compound **2**

	chemical shifts		coupling constant	correlation pattern		
position	δ ^{1}H (ppm)	δ ^{13}C (ppm)	JHH (Hz)	COSY	TOCSY	HMBC
2	3.8–3.9	70.6		3		2-*C*OOH
2–*C*OOH		179.8				
3	2.87 (t)	41.2	10.5	2		2,2-*C*OOH
	3.30 (dd)		10.5, 5.7	2		2, 4a
4a		89.2				
4a–CH_3	1.45 (s)	22.8				4a, 8a
6	3.67 (dd)	65.9	12.0, 6.0	7	7, 8	8
	3.8–3.9					
7	4.00	82.8		6, 8	6, 8	8, 8a
8	4.68 (d)	82.7	7.8	7	6, 7	7, 4a, 1′
8a		109.7				
1′	5.43 (d)	102.5	3.6	2′		8
2′	3.53 (dd)	74.1	10.2, 3.6	1′, 3′	3′, 4′	3′, 4′
3′	3.8–3.9	75.4		2′	2′, 4′	2′
4′	3.44 (t)	70.6	9.6	5′	2′, 3′	5′, 6′
5′	3.72	75.6		4′		
6′	3.84	63.3				

Preparation of ^{15}N-Containing 2. Mal-Gly (**1**, 0.10 g, 0.25 mmol) and ^{15}N-Cys (0.12 g, 0.98 mmol) were dissolved in water (2 mL). The mixture was heated at 100 °C for 1 h, then cooled to room temperature, and subjected to preparative HPLC as described for the synthesis of **2**. The recycled fraction was lyophilized, and ^{15}N-containing **2** was obtained (6.0 mg, 5.6%). LC-(ESI$^+$)MS: *m*/*z* 429 (M + H^+). ^{1}H NMR (D_2O): δ_H 1.46 {s, 3, H_3−C(4a)}, 2.88 {t, 1, *J* = 10.5 Hz, H_2−C(3)}, 3.31 {dd, 1, *J* = 10.5 Hz, 5.7 Hz, H_2−C(3)}, 3.44 {t, 1, *J* = 9.6 Hz, H−C(4′)}, 3.53 {dd, 1, *J* = 10.2 Hz, 3.6 Hz, H−C(2′)}, 3.69 {dd, 1, *J* = 12.0 Hz, 6.0 Hz, H_2−C(6)}, 3.72 {m,1, H−C(5′)}, 3.8−3.9 (m,5), 4.00 {m,1, H−C(7)}, 4.69 {d, 1, *J* = 7.8 Hz, H−C(8)}, 5.44 {d, 1, *J* = 3.6 Hz, H−C(1′)}.

Preparation of ^{18}O-Containing 2. Mal-Gly (**1**, 0.039 g, 0.098 mmol) and Cys (0.024 g, 0.20 mmol) were dissolved in $H_2{}^{18}O$ (2 mL). The mixture was heated at 100 °C for 1 h. The mixture was cooled to room temperature and subjected to preparative HPLC as described for the synthesis of **2**. The recycled fraction was lyophilized, and ^{18}O-containing **2** was obtained (2.3 mg, 5.5%). LC-(ESI$^+$)MS: *m*/*z* 430 (M + H^+). ^{1}H NMR (D_2O): δ_H 1.46 {s, 3, H_3−C(4a)}, 2.88 {t, 1, *J* = 10.5 Hz, H_2−C(3)}, 3.30 {dd, 1, *J* = 10.5 Hz, 5.7 Hz, H_2−C(3)}, 3.45 {t, 1, *J* = 9.6 Hz, H−C(4′)}, 3.54 {dd, 1, *J* = 10.2 Hz, 3.6 Hz, H−C(2′)}, 3.69 {dd, 1, *J* = 12.0 Hz, 6.0 Hz, H_2−C(6)}, 3.72 {m, 1, H−C(5′)}, 3.8−3.9 (m,5), 4.01 {m,1, H−C(7)}, 4.69 {d, 1, *J* = 7.8 Hz, H−C(8)}, 5.44 {d, 1, *J* = 3.6 Hz, H−C(1′)}.

Degradation of 2 and GC-MS Analysis of the Volatiles. To 60 mg of **2**, 0.6 mL of 1 mol/L HCl was added. The mixture was heated at 100 °C for 1 h and cooled to room temperature, and 0.6 mL of 1 mol/L NaOH was added. Then, 0.6 mL of the solution was put into a septum-sealed vial and kept standing for 3 h at room temperature. The sample headspace was analyzed by SPME-GC-MS.

The MS data of isomaltol [*m*/*z* (%): 111 (100), 126 (60), 43 (47), and 55 (27)] were in agreement with a spectrum published by Ito (*16*). The MS data of 2-acetylfuran [*m*/*z* (%): 95 (100), 110 (38), 43 (28), and 67 (5)] were in agreement with those of an authentic sample.

Preparation of 1-[3-(α-D-Glucopyranosyloxy)-2-furanyl]-1-ethanone (isomaltol-glucoside) from Maltose and Proline (17). To 100 mL of ethanol were added maltose·H_2O (5.4 g, 0.015 mol), proline (3.45 g, 0.03 mol), and triethylamine (7.5 mL), and the mixture was refluxed for 27 h. The mixture was concentrated in vacuo, 100 mL of acetone was added, and it was stirred for 12 h at room temperature followed by filtering. The filtrate was concentrated in vacuo, 10 mL of water was added, and it was subjected to ion-exchange column chromatography (Dowex 50 W × 8 H^+ form, 200 mesh, 50 g), eluted with water, and then lyophilized. The crude product was dissolved with 2 mL of water and subjected to ODS-HPLC {Inertsil-ODS, GL Science Inc., 20 mm × 250 mm, solvent A:water, solvent B:CH_3CN, A:B 95:5 ∼ 70:30 (20 min) ∼ 70:30 (25 min) ∼ 95:5 (30 min) 8 mL/min, 254 nm}. The t_R = 11−12 min fraction was lyophilized and recrystallized

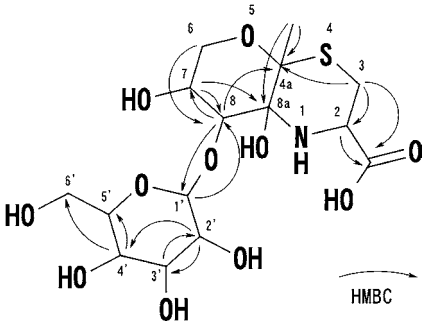

Figure 2. Planar structure of compound **2**.

from ethanol, and isomaltol-glucoside was obtained (23 mg, 0.5%). LC-(ESI$^+$)MS: *m*/*z* (%), 594 (2M + NH_4^+, 20), 306 (45), 289 (M + H^+, 100).

^{1}H and ^{13}C NMR for isomaltol-glucoside (D_2O); arbitrary numbering of carbon atoms refers to the structure in **Figure 5**. δ_H 2.43 {s, 3, H_3−C(1)}, 3.46 {dd, 1, J = 9.1 Hz, 10.0 Hz, H−C(4′)}, 3.58 {m, 1, H−C(5′)}, 3.67 {d, 2, J = 4.0 Hz, H_2−C(6′)}, 3.72 {dd, 1, J = 3.5 Hz, 9.8 Hz, H−C(2′)}, 3.84 {dd, 1, J = 9.1 Hz, 9.8 Hz, H−C(3′)}, 5.68 {d, 1, J = 3.5 Hz, H−C(1′)}, 6.66 {d, 1, J = 2.1 Hz, H−C(5)}, 7.62 {d, 1, J = 2.1 Hz, H−C(6)}. δ_C 26.6 {CH_3, C(1)}, 60.5 {CH_2, C(6′)}, 69.4 {CH, C(4′)}, 71.1 {CH, C(2′)}, 73.3 {CH, C(3′)}, 73.8 {CH, C(5′)}, 99.3 {CH, C(1′)}, 104.7 {CH, C(5)}, 138.1 {C, C(3)}, 149.2 {CH, C(6)}, 154.0 {C, C(4)}, 189.1 {C, C(2)}. The assignments were supported by DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC.

Preparation of Isomaltol-glucoside from 2. To 82 mg (0.19 mmol) of **2**, 0.5 mL of 1 mol/L HCl was added. The mixture was heated at 100 °C for 1 h. The mixture was cooled to room temperature and subjected to ODS-HPLC {inertsil-ODS3, GL Science Inc., 4.6 mm × 250 mm, solvent A:water, solvent B:CH_3CN, A:B 98:2 ∼ 75:25 (20 min) ∼ 75:25 (25 min) ∼ 98:2 (30 min) 1 mL/min, 254 nm}. The t_R = 10.0−10.4 min fraction was lyophilized, and isomaltol-glucoside was obtained (3.2 mg, 5.8%). LC-(ESI$^+$)MS: *m*/*z* (%), 594 (2M + NH_4^+, 29), 306 (60), 289 (M + H^+, 100).

RESULTS AND DISCUSSION

Incubation of the mixture of Mal-Gly (isolated) and Cys led to formation of **2**, which was detected with LC-(ESI$^+$)-MS as a molecular ion $[M + H]^+$ at *m*/*z* 428, and thiazolidine derivative was detected (*m*/*z* 446).

Compound **2** was separated and isolated with an HPLC−recycling technique using an NH_2 column with an RI detector. The HR-FAB-MS (*m*/*z* 426.1082, M − H^-) gave the molecular formula $C_{15}H_{25}O_{11}NS$.

The chemical structure of **2** was investigated by ^{1}H and ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, TOCSY, ^{1}H−^{13}C COSY, and HMBC. A summary of the ^{1}H and ^{13}C NMR data for **2** is shown in **Table 1**.

Fifteen carbons were observed in ^{13}C NMR and DEPT spectra of **2** in D_2O comprising a methyl (δc 22.8), three methylenes (δc 41.2, 63.3, and 65.9), eight sp3 methines (δc 70.6, 70.6, 74.1, 75.4, 75.6, 82.7, 82.8, 102.5), a carbonyl (δc 179.8), and two quaternary carbons (δc 89.2, 109.7). Furthermore, the result of ^{1}H−^{13}C COSY and HMBC experiments correlated each proton with the vicinal carbons of **2**. As shown in **Figure 2**, the analysis of the HMBC spectrum of **2** resulted in the determination of most of the structure. NMR data revealed the structure of an α-glucosyl moiety in **2**.

In the HMBC spectrum of **2** (**Figure 3**), 3-H (δ_H 2.87) showed a correlation with C-2 (δc 70.6) and C-2-COOH (δc 179.8), while 2-H (δ_H 3.8−3.9) showed a cross-peak with C-2-COOH (δc 179.8). Judging from the chemical shifts and the molecular formula ($C_{15}H_{25}O_{11}NS$), C-2 and C-3 were Cys residue carbon atoms.

In the HMBC spectrum (**Figure 3**), 4a-methyl-H (δ_H 1.45) showed a correlation with two quaternary carbons, C-4a (δc 89.2) and C-8a (δc 109.7). These data indicate the partial structure of a 1-deoxyosone derivative.

HMBC cross-peaks between 3-H (δ_H 3.30) and C-4a, between 6-H (δ_H3.67) and C-8 (δc 82.7), between 7-H (δ_H 4.00) and C-8 (δc 82.7), C-8a (δc 109.7), and between 8-H (δ_H 4.68) and C-4a (δc 89.2), C-7 (δc 82.8), and C-1′(δc 102.5) were observed. However, no cross-peaks neither between 2-H (δ_H 3.8−3.9) and C-8a (δc 109.7), nor between 6-H (δ_H 3.67) and C-4a (δc 89.2) were observed (**Figure 3**).

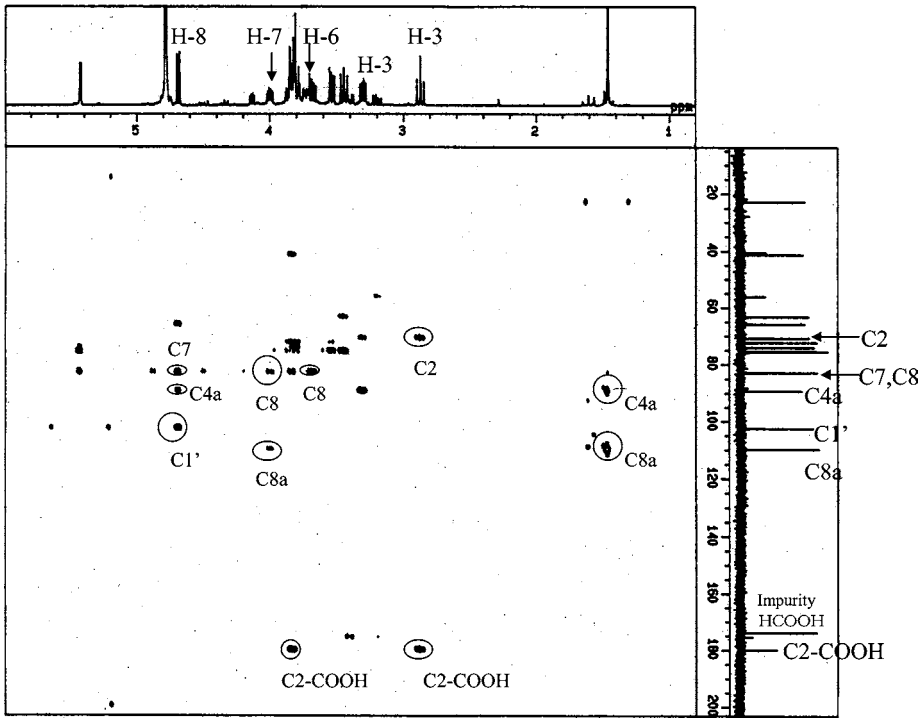

Figure 3. HMBC of compound **2**.

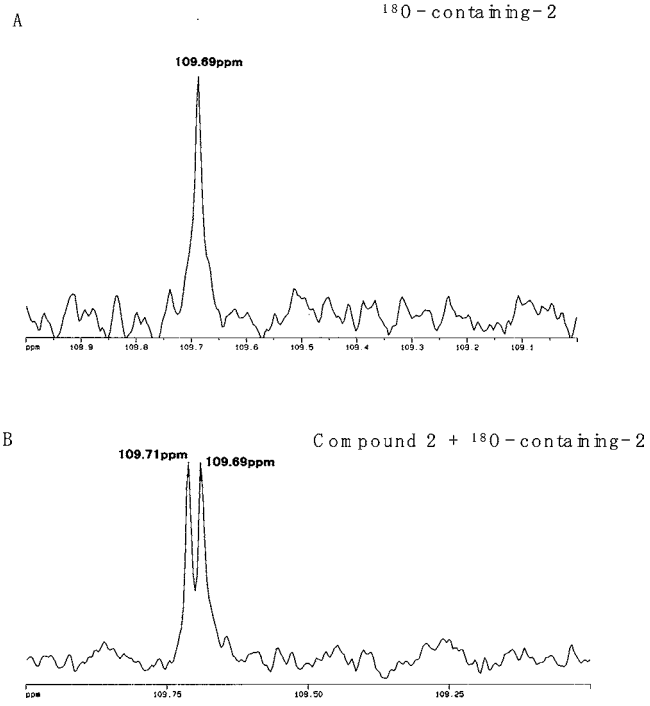

Figure 4. ^{13}C NMR spectrum of **2** and ^{18}O-containing **2**, focused on C-8a. (**A**) ^{18}O-containing **2** (δc 109.69) and (**B**) mixture of compound **2** (δc 109.71) and ^{18}O-containing **2**.

To unambiguously determine the carbons connected to the NH group of the Cys residue and the hemiacetal-OH group, we performed two experiments as follows. First, ^{15}N-Cys was reacted with Mal-Gly, and ^{15}N-containing **2** was obtained. The ESI$^+$-mass spectrum of ^{15}N-containing **2** gave *m*/*z* 429 as a $(M + H)^+$ ion peak. In the ^{13}C NMR spectrum, C-8a (δc 109.7) was a doublet. This suggested that ^{15}N of the Cys residue was adjacent to C-8a. Second, to substitute hemiacetal-^{16}OH for ^{18}OH, Cys and Mal-Gly were reacted in $H_2{}^{18}O$ according to a method reported by Risley and Van Etten (*18*). ^{18}O-containing **2** was obtained. The ESI$^+$-mass spectrum of ^{18}O-containing **2** gave *m*/*z* 430 as an $(M + H)^+$ ion peak. In the ^{13}C NMR spectrum, only C-8a was shifted by the isotope effect of ^{18}OH group (δc 109.69, **Figure 4**). This suggested that the hemiacetal-OH group was adjacent to C-8a. The planar structure of **2**, 7,-8a-dihydroxy-4a-methyl-8-(α-D-glucopyranosyloxy)hexahydro-5-oxa-4-thia-1-aza-naphthalene-2-carboxylic acid was constructed as shown in **Figure 2**. Although C-4a and C-8a are asymmetric carbon atoms, only one of four diastereomers was obtained (absolute configuration not determined). It revealed that **2** had a semiaminal group and was stable in an aqueous solution.

The Maillard reaction of maltose was studied by Kramhöller et al. (*13*). They reported that 1-deoxyhexosulose was formed as a hemiacetal when the Amadori compound was degraded. Recently, 1-deoxy-D-*erythro*-hexo-2,3-diulose (1-DG) was synthesized by Glomb et al. (*19*). They found that the major structure of 1-DG was a six-member ring hemiacetal (1-deoxy-D-*erythro*-hexo-2,3-diulo-2,6-pyranose) in CD_3OD from an NMR study. Taking their results into consideration, we postulate that **2** formed by coupling 1-deoxyosone of which the structure was a six-member ring hemiacetal and Cys. We also examined a glucose-containing Amadori compound (1-deoxyfructosylglycine)/Cys system with the same procedure as for the Mal-Gly/Cys system. However, 1-DG-Cys conjugate could not be purified because of its small amount (data not shown). Kramhöller et al. reported that when monosaccharide is reacted with amino acids, elimination of the OH group at C2 from cyclic hemiacetal intermediate leads to the formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one.

In disaccharide-derived intermediates elimination of the OH group at C5 leads to β-pyranone (*13*). It was assumed that the elimination of water from the monosaccharide-derived intermediate might be faster than that of disaccharide intermediate, so that it might be difficult to trap 1-DG with Cys.

Degradation of 2. When **2** was dissolved in 1 mol/L HCl and heated at 100 °C for 1 h, the reaction mixture had a sweet odor. From the reaction mixture, isomaltol-glucoside was obtained (5.8% yield) and the structure was confirmed by ^{1}H NMR and ESI$^+$-MS with an authentic sample synthesized from proline and maltose, according to the method reported by Goodwin (*17*). Furthermore, HS-SPME-GC-MS analysis of the reaction mixture revealed that isomaltol and acetylfuran were

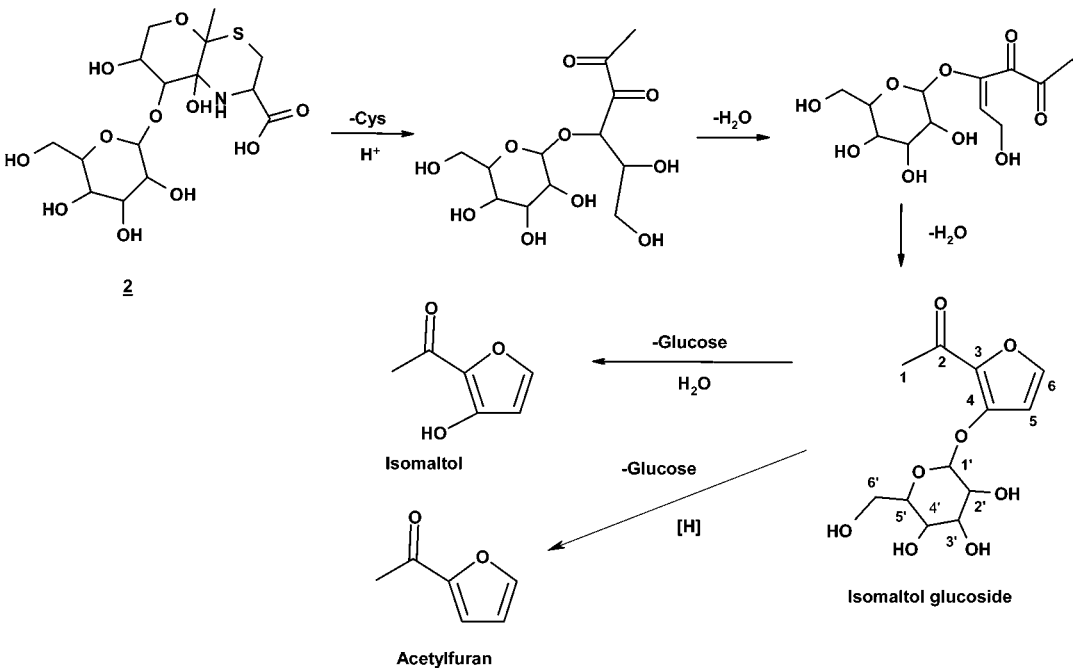

Figure 5. Simplified reaction pathway of the formation of isomaltol and acetylfuran from **2**.

produced as volatile compounds (these were not quantified). It is known that isomaltol has a sweet, burnt sugar odor (*20*) and that acetylfuran has a smoky odor (*21*). These data suggest that **2** is a precursor of isomaltol and acetylfuran by way of isomaltol-glucoside, as shown in **Figure 5**. Kramhöller et al. reported that isomaltol-glucoside was obtained by way of 1-deoxyhexosulose and β-pyranone when an Amadori compound (1-deoxy-1-piperidinomaltulose) was heated (*13*). It is assumed that **2** degraded to isomaltol-glucoside in the same pathway mentioned by Kramhöller et al.

α-Dicarbonyl compounds are major intermediates of the Maillard reaction in the initial stage. However, they cannot be isolated from reaction mixtures due to their high reactivity. They can be trapped with the reagents such as *o*-phenylenediamine (*14*, *23*) or aminoguanidine (*22*, *23*), and they are detected as stable quinoxaline or triazine derivatives, respectively. However, these derivatives are too stable to degrade to the next step in the Maillard reaction pathway. Compound **2** is an α-dicarbonyl compound, which is stable enough to be isolated by coupling with Cys but still has reactivity to be degraded to become several compounds.

ABBREVIATIONS USED

COSY, correlation spectroscopy; Cys, cysteine; DEPT, distortionless enhancement polarization transfer; 1-DG, 1-deoxy-D-*erythro*-hexo-2,3-diulose; ESI, electrospray ionization; FAB, fast atom bombardment; GC-MS, gas chromatography−mass spectrometry; Gly, glycine; Mal-Gly, 1-deoxymaltulosyl-glycine; TOCSY, total correlated spectroscopy; HMBC, heteronuclear multiple bond correlation; HMQC, heteronuclear multiple quantum coherence; HPLC, high-performance liquid chromatography; HR-MS, high-resolution mass spectrum; HS-SPME, headspace solid phase microextraction; RI, refractive index; TSP, 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid sodium salt.

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