Photochemical Products of a Cysteine/D-Glucose Browning Model System

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Samples of the browning model system consisting of L-cysteine and D-glucose were irradiated by UV light at 253.7 or 310.0 nm under different conditions of time and temperature. A total of 22 compounds were identified in the sample irradiated at 253.7 nm and 25 °C for the longest period (72 h) by gas chromatography/mass spectrometry (GC/MS). They were sulfides, thiophenes, pyridines, pyrazines, thiazoles, thiazolines, an oxazole, pyrroles, and thiazolidines. The model system irradiated by the light of 253.7 nm produced more different chemicals than did the model system irradiated by the light of 310.0 nm. When samples of the model system were held at 7, 25, or 60 °C for 24 h in the dark, formation of only four compounds was observed. In contrast, when samples of the model system were irradiated at 7 °C for 24 h, 17 components were isolated and identified.

Maillard was the first to propose the reaction of an amino group of an amino acid with the carbonyl group of a sugar to account for the brown pigments and polymers produced by the reactions of amino acids and sugars (Maillard, 1912, 1916, 1917). Consequently, the reaction between amines and carbonyls has been named the Maillard browning reaction. Since Hodge (1953) summarized the occurrence of nonenzymatic browning reactions comprehensively, including studies using a browning model system consisting of an amino acid and a sugar, many chemical and biological studies have been done with a simple browning model system instead of with an actual, complex food. For example, a glycine/dextrose model system was used to study the effects of browning reaction on the cellular metabolism of some bacteria (Jemmali, 1969). The antioxidative activity of browning products was investigated on a glycine/D-xylose model system (Yamaguchi and Fujimaki, 1970), and isolation and identification of volatile browning reaction products were done with an L-rhamnose/ammonia model system (Shibamoto and Bernhard, 1978).

Nonenzymatic, volatile browning products are primarily responsible for the characteristic aromas of cooked foods. Many volatile chemicals have been reported as components responsible for flavors of cooked foods in the studies of heated sugar/amino acid browning model systems (Hodge, 1967; Kato et al., 1973; Scanlan et al., 1973). Certain foods are known to become brown when they are exposed to light, suggesting that light may promote a browning reaction in these foods. Therefore, we proposed that some volatile chemicals are formed by the action of light. In the present study, volatile chemicals formed in a cysteine/D-glucose model system with UV irradiation under various conditions were isolated and identified.

EXPERIMENTAL SECTION

Materials. L-Cysteine was purchased from Sigma Chemical Co., St. Louis, MO, D-glucose from MCB Manufacturing Chemists, Inc., Cincinnati, OH, cysteamine from Aldrich Chemical Co., Milwaukee, WI, and methylene chloride from J. T. Baker Chemical Co., Phillipsburg, NJ. All authentic chemicals were purchased from reliable commercial sources.

Sample Preparations. *Experiment I: Effect of Temperature on the Cysteine/D-Glucose Model System Irradiated at 253.7 nm.* Cysteine (0.05 mol) and D-glucose (0.05 mol) were dissolved in 200 mL of deionized water, and the solution was placed in a 500-mL quartz glass tube. Each such sample was irradiated with 253.7-nm UV light for 24 h on a Rayonet Model RPR-1000 photochemical reactor (Southern New England Ultraviolet Co., New Hamden, CT). While being irradiated, samples of the solution were held at constant temperature of 7, 25, or 60 °C with a Brinkman RM6 constant-temperature water circulator. After irradiation, the solution was adjusted to pH 8 with 6 N NaOH solution to improve the extraction of nitrogen-containing heterocyclic compounds. The photoreaction mixture was then extracted with 200 mL of dichloromethane by a liquid–liquid continuous extractor for 6 h, and the extract was dried over anhydrous sodium sulfate for 12 h. After removal of sodium sulfate, the extract was condensed to approximately 1 mL and 0.2 mL of internal standard (0.1 mg of undecane or pentadecane in 1 mL of dichloromethane) was added. Then the solution was condensed to exactly 1 mL under a purified nitrogen stream to obtain the relative yield of each product.

Experiment II: Effect of Temperature on the Cysteine/D-Glucose Model System Heated in the Absence of Light (Dark Reaction). Cysteine (0.05 mol) and D-glucose (0.05 mol) were dissolved in 200 mL of deionized water in an Erlenmeyer flask wrapped in aluminum foil to avoid any exposure of the sample to light. Contents of each flask were then held at a constant temperature of 7, 25, or 60 °C for 24 h. The samples were then treated in the same way as the samples in experiment I.

Experiment III: Effect of Irradiation Time on the Cysteine/D-Glucose Model System Irradiated at 253.7 or 310.0 nm. Cysteine (0.05 mol) and D-glucose (0.05 mol) were dissolved in 200 mL of deionized water. Each such sample was irradiated at 253.7 or 310.0 nm for 1, 12, 24, 48, or 72 h at 25 °C. After irradiation, the solutions were treated in the same way as the samples in experiment I.

Identification of the Products in the Cysteine/D-Glucose Model Systems. The samples prepared by the procedures just described were analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Identification of gas chromatographic peaks of the samples was made by comparison of their mass spectra and gas chromatographic retention indices (Kovats, 1965) to those of authentic compounds.

A Hewlett-Packard Model 5790 gas chromatograph equipped with a flame ionization detector (FID) and a 40 m × 0.21 mm (i.d.) fused silica capillary column coated with Carbowax 20M was used for routine analysis. Peak

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Table I. Products Identified in the Cysteine/D-Glucose Model System in the Dark or Irradiated at 253.7 nm and a Range of Temperatures for 24 h

	GC peak area ratio[a]					
	dark reactions			photoreactions		
product	7 °C	25 °C	60 °C	7 °C	25 °C	60 °C
dimethyl sulfide	*b*	*b*	*b*	*b*	*c*	0.05
ethylene sulfide	0.15	0.17	0.17	0.19	0.20	0.13
acetonitrile	0.05	0.06	0.06	0.32	0.50	0.38
thiophene	*b*	*b*	*b*	0.10	0.33	0.41
2-methylthiophene	*b*	*b*	*b*	*b*	*b*	0.02
2-methylpyridine	*c*	*c*	*c*	0.10	0.20	0.27
pyrazine	*b*	*b*	*b*	*c*	*c*	0.03
2-methylthiazole	*b*	*b*	*b*	2.46	4.04	2.98
thiazole	*b*	*b*	*b*	0.46	0.96	0.71
2-methylthiazoline	*b*	*b*	*b*	0.35	1.42	0.48
thiazoline	*b*	*b*	*b*	*c*	*c*	0.06
2,6-dimethylpyrazine	*b*	*b*	*b*	*c*	*b*	0.07
2,3-dimethylpyrazine	*b*	*b*	*b*	0.09	0.09	0.09
4-methylthiazole	*b*	*b*	*b*	*c*	0.10	0.12
2-methylthiazolidine	*b*	*b*	*b*	0.20	1.35	7.40
thiazolidine	*b*	*b*	*b*	0.35	0.78	1.00
pyrrole	0.08	0.10	0.07	0.10	0.11	0.09
2-methyl-4,5-dihydro-3(2*H*)-furanone	*b*	*b*	*b*	0.52	0.22	0.16
2-propionylthiazolidine	*b*	*b*	*b*	0.10	0.27	1.50

[a] GC peak area of product/GC peak area of internal standard. [b] Not detected. [c] GC peak area ratio less than 0.01.

Table II. Effect of Irradiation Time on the Yield of Products from a Cysteine/D-Glucose Model System Irradiated at 253.7 or 310 nm for Different Times at 25 °C

	GC peak area ratio[a]									
	1 h		12 h		24 h		48 h		72 h	
product	253.7	310.0	253.7	310.0	253.7	310.0	253.7	310.0	253.7	310.0
dimethyl sulfide	*b*	*b*	*c*	*b*	*c*	*b*	0.03	*b*	0.06	0.19
ethylene sulfide	0.09	0.09	0.12	0.07	0.20	0.08	0.09	0.04	0.07	0.01
acetonitrile	0.07	0.08	0.36	0.11	0.50	0.11	0.51	0.20	0.57	0.34
thiophene	*b*	*c*	0.22	*a*	0.33	*a*	0.45	0.04	0.49	0.04
2-methylthiophene	*b*	*b*	*b*	*b*	*c*	*b*	*c*	*b*	0.06	*b*
2,5-dimethyloxazole	*b*	*b*	*b*	*b*	*b*	*b*	*c*	*b*	0.07	*b*
2-methylpyridine	*c*	*b*	0.04	*b*	0.20	*b*	0.25	*b*	0.37	*b*
pyrazine	*b*	*b*	*b*	*b*	*c*	*b*	*c*	*b*	*c*	*b*
2-methylthiazole	0.27	*c*	2.26	1.36	4.04	2.41	5.30	4.62	5.55	5.09
thiazole	*c*	*b*	0.46	*c*	0.96	0.15	1.24	0.33	1.23	0.53
2-methylthiazoline	0.72	0.09	0.98	0.24	1.47	1.22	1.57	5.44	1.59	5.13
thiazoline	*c*	0.04	*c*	0.05	*c*	0.06	0.07	0.07	0.06	0.08
2,6-dimethylpyrazine	*b*	*b*	*c*	*b*	*c*	*c*	*c*	*c*	0.07	0.03
2,3-dimethylpyrazine	*b*	*b*	*c*	*b*	0.09	*b*	0.10	*c*	0.10	*c*
4-methylthiazoline	*b*	0.14	*b*	0.19	0.10	0.22	0.14	0.44	0.17	0.13
2-methylthiazolidine	0.15	*b*	0.80	0.57	0.78	2.31	0.51	7.05	0.33	4.90
thiazolidine	*c*	*b*	0.51	0.32	1.35	0.56	2.35	1.97	2.51	2.06
pyrrole	0.08	0.05	0.10	0.12	0.11	0.30	0.80	0.22	0.80	0.12
2-mercaptothiophene	*b*	*b*	*b*	*b*	*b*	*b*	*c*	*b*	0.13	*b*
2,5-dimethylpyrrole	*b*	*b*	*b*	*b*	*b*	*b*	*c*	*b*	0.02	*b*
2-methyl-4,5-dihydro-3(2*H*)-furanone	*c*	*b*	0.12	*b*	0.22	*b*	0.37	0.40	0.54	0.18
2-propionylthiazolidine	0.05	*b*	0.15	*b*	0.27	0.08	0.47	0.76	0.63	0.43

[a] Values are averages of three replications. GC peak area of product/GC peak area of internal standard. [b] Not detected. [c] GC peak area ratio less than 0.01.

areas were integrated on an HP 5880A series GC terminal. The GC oven was held at 60 °C for 10 min and then programmed at 2 °C/min to a final temperature of 180 °C which was held for 20 min. The temperature of both the injector and the detector was 300 °C. The helium carrier gas flow rate was 27 cm/s with a split ratio of 1:35. A ZAB-2F-HS high-resolution magnetic sector mass spectrometer combination gas chromatograph/mass spectrometer equipped with an INCOS MS data system was used for mass spectral identification of the GC components under the following conditions: filament current, 167 mA; multiplier voltage, −2.5 kV; electron energy, 70 eV.

RESULTS AND DISCUSSION

Table I shows both the products identified in the cysteine/D-glucose model system irradiated at 253.7 nm and 7, 25, °C, or 60 °C for 24 h and the products found in the dark reaction of the same model system under the same conditions of time and temperature. The products are listed in their GC elution order on Carbowax 20M. When the cysteine/D-glucose model system was held at 7, 25, or 60 °C for 24 h in the dark, the resulting solution was odorless and colorless. Only a few peaks were observed on a gas chromatogram of the extract obtained from the dark reactions. The relative yields of four compounds at three different temperatures in the dark reactions were quite similar except for 2-methylpyridine. The results from the dark reactions suggest that occurrence of browning reactions at temperatures lower than 60 °C without light was minimal. Therefore, studies on the effect of irradiation time were performed at 25 °C.

When the model system was irradiated at 253.7 nm and 7 °C for 24 h, the resulting solution was light yellow, with both a strong sulfurous odor and a strong popcornlike

aroma. When the temperature was raised to 25 and 60 °C, the solution became darker with strong odors like those of cooked meat and popcorn. The yield of 2-methylthiazole, which has a meaty aroma (Pittet and Hruza, 1974), was highest upon irradiation at 253.7 nm and explains the meaty smell of these irradiated samples.

As the temperature increased, the yield of 2-methylthiazolidine increased greatly. 2-Methylthiazolidine was not found in the dark reaction even when heated to 60 °C, and the same was true of thiazolidine. Formation of thiazolidine may be affected by thermal and photochemical energies. Thiazolidines have never been reported in a heated cysteine/glucose model system (Scanlan et al., 1973; Kato and Fujimaki, 1973). But many thiazolidines were found in a heated cysteamine/D-glucose model system (Sakaguchi and Shibamoto, 1978). Therefore, the presence of the intermediate, cysteamine, was suggested in the irradiated cysteine/D-glucose model system. Decarboxylation of cysteine occurred upon photoirradiation to give cysteamine, which then reacted with aldehydes from D-glucose to form thiazolidines. The second step, reaction between cysteamine and aldehydes, might be promoted by heat.

The yield of 2-methyl-4,5-dihydro-3(2*H*)-furanone decreased as the temperature increased. This type of furan derivative is a product of sugar caramelization (Hodge, 1967) and undergoes secondary reactions with amine compounds to give browning reaction products (Shibamoto, 1976; Nishimura et al., 1980).

Table II shows the products identified in the cysteine/D-glucose model system irradiated at 253.7 or 310.0 nm for various periods of time at 25 °C. The products are listed in their GC elution order on Carbowax 20M. Like the samples irradiated at 253.7 nm, the samples of the model system irradiated at 310.0 nm had meaty and popcornlike odors after 72-h irradiation.

Generally, the yield of products increased as irradiation time increased at both 253.7 and 310.0 nm. The number of products produced at 310.0 nm was fewer than that produced at 253.7 nm. 2-Methylthiophene, 2,5-dimethyloxazole, 2-methylpyridine, pyrazine, 2-mercaptothiophene, and 2,5-dimethylpyrrole were identified in the extract irradiated at 253.7 nm but were not found in the extract irradiated at 310.0 nm. This may be due to the difference in energy of light at 253.7 nm and at 310.0 nm. The products produced at 310.0 nm may be closer to those produced by natural sunlight because 310.0-nm light is of a similar energy level. Two heterocyclic compounds, 2-methylpyridine and pyrrole, were formed in the dark reactions because the formation of pyridines or pyrroles does not require a high sugar fragmentation (Shibamoto et al., 1979). Compounds requiring a high sugar fragmentation for formation, such as pyrazines or thiazoles, were therefore not found in the dark reaction. These compounds were formed when the system was irradiated for a longer time or at a higher temperature.

Formation of flavor chemicals such as pyrazines, thiazoles, or thiophenes by heat treatment has been known for many years. The formation of these products by the action of light has never been reported prior to this study. Foods are sometimes exposed to UV light, but such light can cause changes in both color and flavor. These possible changes should be considered in food treatment and storage with light.

Registry No. Cys, 52-90-4; ethylene sulfide, 420-12-2; dimethyl sulfide, 75-18-3; acetonitrile, 75-05-8; thiophene, 110-02-1; 2-methylthiophene, 554-14-3; 2-methylpyridine, 109-06-8; pyrazine, 290-37-9; 2-methylthiazole, 3581-87-1; thiazole, 288-47-1; 2-methylthiazoline, 2346-00-1; thiazoline, 31152-37-1; 2,6-dimethylpyrazine, 108-50-9; 2,3-dimethylpyrazine, 5910-89-4; 4-methylthiazole, 693-95-8; 2-methylthiazolidine, 24050-16-6; thiazolidine, 504-78-9; pyrrole, 109-97-7; 2-methyl-4,5-dihydro-3(2*H*)-furanone, 3188-00-9; 2-propionylthiazolidine, 113111-16-3; 2,5-dimethyloxazole, 23012-11-5; 2-mercaptothiophene, 7774-74-5; 2,5-dimethylpyrrole, 625-84-3; glucose, 50-99-7; IMP, 131-99-7; ATP, 56-65-5; ADP, 58-64-0; AMP, 61-19-8; Hx, 68-94-0; N_2, 7727-37-9; inosine, 58-63-9.

LITERATURE CITED

Hodge, J. E. *J. Agric. Food Chem.* **1953**, *1*, 928.
Hodge, J. E. *Symp. Foods: Chem. Physiol. Flavors*, [*Proc.*] *1965* **1967**, *15*, 476.
Jemmali, M. *J. Appl. Bacteriol.* **1969**, *32*, 151.
Kato, H.; Fujimaki, M. *Lebensm.-Wiss. Technol.* **1973**, *5*, 539.
Kato, H.; Kurata, T.; Fujimaki, M. *Agric. Biol. Chem.* **1973**, *37*, 537.
Kovats, E. *Adv. Chromatogr.* **1965**, *1*, 229.
Maillard, L. C. *C. R. Hebd. Seances Acad. Sci.* **1912**, *154*, 66.
Maillard, L. C. *C. R. Hebd. Seances Acad. Sci.* **1916**, *5*, 258.
Maillard, L. C. *Ann. Chim.* **1917**, *7*, 113.
Nishimura, O.; Mihara, S.; Shibamoto, T. *J. Agric. Food Chem.* **1980**, *28*, 39.
Pittet, A. O.; Hruza, D. E. *J. Agric. Food Chem.* **1974**, *22*, 264.
Sakaguchi, M.; Shibamoto, T. *J. Agric. Food Chem.* **1978**, *26*, 1179.
Scanlan, R. A.; Kayser, S. G.; Libbey, L. M.; Morgan, M. E. *J. Agric. Food Chem.* **1973**, *21*, 673.
Shibamoto, T. *J. Agric. Food Chem.* **1976**, *25*, 206.
Shibamoto, T.; Bernhard, R. A. *J. Agric. Food Chem.* **1978**, *26*, 138.
Shibamoto, T.; Akiyama, T.; Sakaguchi, M.; Enomoto, Y.; Masuda, H. *J. Agric. Food Chem.* **1979**, *27*, 1027.
Yamaguchi, N.; Fujimaki, M. *Nippon Shokuhin Kogyo Gakkaishi* **1970**, *4*, 136.

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