Water-Soluble Thiazolidines Formed in a Cysteamine–D-Glucose Browning Model System

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Cysteamine and D-glucose were heated in an aqueous solution under simulated cooking conditions. The volatile fraction of the reaction mixture was removed by methylene chloride extraction and the residual aqueous phase was subjected to chemical analysis. 2-(1,2,3,4-Tetrahydroxybutyl)thiazolidine and 2-(1,2,3,4,5-pentahydroxypentyl)thiazolidine were identified by gas chromatography–mass spectrometry following the methylation of the hydroxyl groups of each compound with trimethylchlorosilane and hexamethyldisilazane. Authentic samples of 2-(hydroxyalkyl)thiazolidines were synthesized from cysteamine and corresponding sugars to confirm the structures and flavor descriptions. The authentic thiazolidines exhibited no mutagenic activity toward histidine-requiring *Salmonella typhimurium* strains TA 98 and TA 100.

Model systems consisting of sugar and an amine are frequently used to investigate browning reactions. A large number of volatile chemicals has been identified in the organic solvent extracts of various sugar–amine browning model systems (van Praag et al., 1968; Rizzi, 1974; Shibamoto and Bernhard, 1978).

Some water-soluble browning reaction products have been also isolated and identified. Anet (1958) observed the formation of *N*-(carboxymethyl)-1-amino-1-deoxy-D-fructose and *N,N*-di-(D-*arabino*-3,4,5,6-tetrahydroxy-2-oxohexyl)glycine in the reaction mixture of glycine and D-glucose. Fujii et al. (1966) isolated 4(5)-(DL-*glycero*-2,3-dihydroxypropyl)imidazole from the reaction mixture of DL-glucose and ammonia using thin-layer chromatography. Tsuchida et al. (1976) identified 2-(D-*arabino*-1,2,3,4-tetrahydroxybutyl)-5-(D-*erythro*-2,3,4-trihydroxybutyl)pyrazine from the browning reaction mixture of fructose and ammonium formate. Bonner and Meyer zu Reckendorf (1961) isolated 2-(D-*gluco*-pentahydroxy-1-pentyl)thiazolidine from the reaction mixture of D-glucose and cysteamine in a methanol solution. In this study, D-glucose and cysteamine were heated in an aqueous solution, and the products remaining in the aqueous phase after methylene chloride extraction were isolated and identified. The authentic samples of identified chemicals were tested for mutagenicity by using the Ames assay.

EXPERIMENTAL SECTION

Materials. D-Glucose, D-arabinose, and glyceraldehyde were purchased from Mallinckrodt, Inc. (St. Louis, MO). Cysteamine hydrochloride and glycolaldehyde were obtained from Sigma Chemical Co. (St. Louis, MO). The authentic (polyhydroxyalkyl)thiazolidines, 2-(hydroxymethyl)thiazolidine (I), 2-(1,2-dihydroxyethyl)thiazolidine (II), 2-(1,2,3,4-tetrahydroxybutyl)thiazolidine (III), and [2-(1,2,3,4,5-pentahydroxypentyl)]thiazolidine (IV) were prepared from the reaction of cysteamine with glycolaldehyde, glyceraldehyde, arabinose, and glucose, respectively, by using the method described by Bonner and Meyer zu Reckendorf (1961).

Purification of Compounds I–IV. The compounds obtained by the method of Bonner and Meyer zu Reckendorf were further purified with recrystallization and thin-layer chromatography. Each compound was recrystallized from hot ethanol. The crystallization was repeated twice. The crystals obtained from recrystallization were further purified with TLC (Merk preparative silica gel 60). The recrystallized samples were dissolved in methanol–water (99:1) solution and applied to a TLC plate. The plate was developed with ethyl acetate–methanol (2:1) solution. The thiazolidines were not moved with this solvent so that they were recovered from the base-line area with hot methanol. TLC purification was also repeated twice. Each purified thiazolidine was recovered from the methanol solution with removing methanol by a rotary flash evaporator. The structures of the samples were confirmed by IR and NMR.

Reaction of D-Glucose and Cysteamine. D-Glucose (0.2 mol) and 0.1 mol of cysteamine, which had previously been converted from cysteamine hydrochloride by the addition of sodium hydroxide, were dissolved in 100 mL of deionized water. The solution was heated at 90 °C for 5 h in a pressurized bottle.

Sample Preparation of the Water-Soluble Fraction. The above reaction mixture (100 mL) was extracted with 200 mL of methylene chloride by using a liquid–liquid continuous extractor to remove the volatile fraction. The aqueous phase was concentrated to syrup form by distillation under reduced pressure (50 °C/10 mmHg). Approximately 10 g of a brown syrup was obtained, which was subsequently subjected to ion-exchange column chromatography.

Ion-Exchange Column Chromatography. For isolation of the (hydroxyalkyl)thiazolidine derivatives, the brown syrup was diluted with 15 mL of deionized water and added to a column (35 × 2.5 cm i.d.) packed with Amberlite IR-120B (H^+ form, 500 mL). Five hundred milliliters of deionized water was drawn through the column to wash off D-glucose residue and brown pigments. The development was continued with 1 N ammonium hydroxide solution (4.5 L). The ammonia eluate was concentrated to ~30 mL in volume by vacuum distillation. The concentrate was passed through a second ion-exchange column (Amberlite IRA-410, OH^- form, 500 mL) with 20 L of deionized water. The water eluate was concentrated to syrup form by distillation under reduced pressure (40 °C/10 mmHg), resulting in a brown syrup (7 g).

Methylsilylation of Hydroxyl Groups of Products. The compounds in the above syrup were expected to possess polyhydroxy groups coming from D-glucose. To test this hypothesis, we reacted the syrup with trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) to form trimethylsilyl ether, which can be de-

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Table I. Taste Descriptions of Thiazolidines and Mass Spectral Data of Their Trimethylsilyl Derivatives

compound	MS fragment, m/e (%)	taste descriptions[a]
I, 2-(hydroxymethyl)thiazolidine	73 (5), 75 (7), 88 (100), 116 (5), 176 (3), M = 191	bitter
II, 2-(1,2-dihydroxyethyl)thiazolidine	73 (40), 75 (19), 88 (100), 101 (49), 103 (11), 116 (72), 147 (20), 160 (25), 188 (8), 204 (19), 278 (4), M = 293	bitter
III, 2-(1,2,3,4-tetrahydroxybutyl)thiazolidine	74 (27), 75 (24), 88 (89), 103 (43), 126 (12), 160 (11), 202 (11), 217 (100), 292 (15), 305 (43), 307 (17), 392 (12), 482 (5), M = 497	bitter, mild sweet
IV, 2-(1,2,3,4,5-pentahydroxypentyl)thiazolidine	73 (15), 75 (12), 88 (77), 103 (12), 147 (11), 191 (13), 205 (25), 217 (100), 232 (12), 292 (26), 307 (21), 319 (11), 404 (10), 406 (10), 584 (10), M = 599	bitter

[a] An aqueous solution (0.1%) was tasted by five trained flavorists.

tected by GC. The syrup (10 mg) was dissolved into 0.5 mL of *N,N*-dimethylformamide in a test tube, and then TMCS (0.03 mL) and HMDS (0.05 mL) were added. The neck of the test tube was flame-sealed and placed in boiling water for 2 min. The solvent (*N,N*-dimethylformamide) in the reaction mixture was removed in a vacuum desiccator, leavng a brown liquid. A small amount of anhydrous *n*-hexane was added before the liquid was analyzed by GC–MS.

Analysis of Reaction Products. GC peaks of reaction mixtures were identified by comparing their MS and GC retention indexes to those of authentic compounds. A Hewlett-Packard Model 5880 A gas chromatograph, equipped with a flame ionization detector and 20 m × 0.25 mm i.d. fused silica capillary column coated with Carbowax 20M, was used. The column temperature was programmed from 80 to 200 °C at 5 °C/min. The temperature of injector and detector was 250 °C. A Hitachi Model RMU-6M combination mass spectrometer–gas chromatograph (Hitachi Model M-5201) equipped with Hitachi Model M-6010 and 10 II/A data system was used for GC–MS analysis under the following conditions: ionization voltage, 70 eV; emission current, 80 μA; ion acceleration voltage, 3.1 KV; ion source temperature, 200 °C.

Mutagenicity Test. A sterile aqueous solution of compounds II–IV (50 mg/mL each) and an aqueous dimethyl sulfoxide (10% Me_2SO) solution of compound I (44.8 mg/mL) were filtered through 0.45-μm Millipore Millex filters (Bedford, MA and then serially diluted to 10-, 100-, and 1000-with sterile doubling distilled water.

The modified method of the Ames *Salmonella*/microsome assay (Ames et al., 1975; Yahagi et al., 1975) was applied to test the mutagenic potentials of these chemical solutions. In a tube containing 0.1 mL of the solution was added 0.5 mL of S-9 mix (which contains per milliliter 0.1 mL of the rat liver S-9, 8 μmol of KCl, 5 μmol of glucose 6-phosphate, 4 μmol of NADP, and 100 μmol of sodium phosphate buffer, pH 7.4) or Dulbecco's phosphate buffer saline (Grand Island Biological Co., Grand Island, NY) and 0.1 mL of a culture of *Salmonella typhimurium* tester strain TA 98 (3×10^8 cells) or TA 100 (6×10^7 cells). The mixture was preincubated at 37 °C for 20 min, mixed with 2 mL of top agar at 45 °C, and poured onto a bottom agar plate. After incubation for 2 days at 37 °C, the colonies on the plates were counted by using an Automated Colony Counter (New Brunswick Scientific Co., Edison, NJ). All experiments were triplicated and the results were interpreted from the average of the triplicate runs.

RESULTS AND DISCUSSION

The volatile compounds generated in cysteamine–D-glucose browning model system have been isolated and identified by Sakaguchi and Shibamoto (1978). The volatile thiazolidines identified include 2-methyl-, 2-ethyl-, 2-*n*-propyl-, and *N*-methylthiazolidine. In this study, we attempted to isolate water-soluble (polyhydroxyalkyl)-thiazolidines, which possess weak mutagenic activities toward *S. typhimurium* strains in TA 98 and TA 100 (Mihara and Shibamoto, 1980).

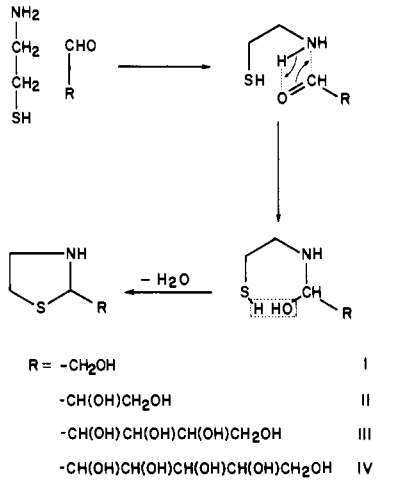

Figure 1. Proposed formation pathway of the (polyhydroxyalkyl)thiazolidines.

The water-soluble thiazolidines identified in the cysteamine–D-glucose browning model system were 2-(1,2,3,4-tetrahydroxybutyl)thiazolidine and 2-(1,2,3,4,5-pentahydroxypentyl)thiazolidine. 2-(Hydroxymethyl)thiazolidine and 2-(1,2-dihydroxyethyl)thiazolidine were identified tentatively. The gas chromatographic peaks of these two thiazolidines were quite small. The mass fragmentation obtained from GC–MS showed only the thiazolidine ring, but the retention indexes of the compounds were identical with those of the authentic samples.

The proposed formation pathways of (polyhydroxyalkyl)thiazolidines are shown in Figure 1. Table I gives the taste descriptions of authentic thiazolidines and the MS data of their trimethylsilyl derivatives. None of the silyl ether derivatives shown exhibited the molecular ion, but they did show a fairly strong M – 15 ($\cdot CH_3$) fragment peak due to the α fission of methyl ether. Figure 2 shows a typical mass fragmentation of 2-(1,2,3,4,5-pentahydroxypentyl)thiazolidine trimethyl ether. The major fragment ions are formed by the fragmentation of the trimethylsilyl ethers of the polyhydroxyalkyl chain (Budzikiewicz et al., 1979). The fragment at m/e = 217 (base peak) for compounds III and IV are typical for trimethylsilyl derivatives of carbohydrates (De Jongh et al., 1969). Thiazolidine derivatives comprise the major m/e = 88 fragment, which is a stable thiazolidine ring ion (base peak for I and II).

The mutagenicity tests on compounds I–IV indicated that these (hydroxyalkyl)thiazolidines are not mutagenic to *S. typhimurium* tester strains TA 98 and TA 100. Although incorporation of liver activation systems to the

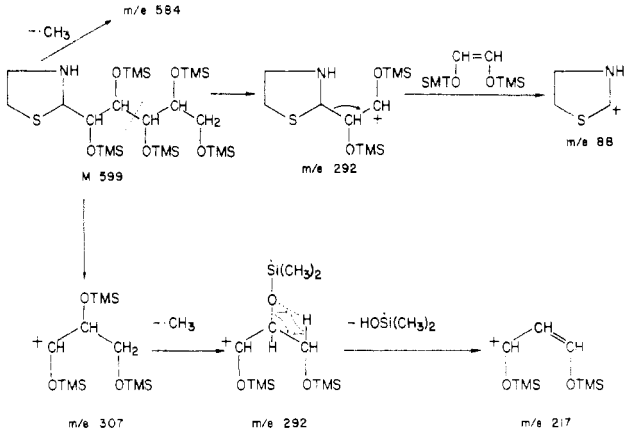

Figure 2. MS fragmentation of 2-(1,2,3,4,5-pentahydroxypentyl)thiazolidine.

assay system slightly increased and decreased the number of revertant colonies of TA 98 and TA 100, respectively, the compounds exhibited no appreciable mutagenic activity to those tester strains (the standard derivations of number of colonies from triplicate runs were varied between 1 and 16).

Compound IV, 2-(1,2,3,4,5-pentahydroxypentyl)thiazolidine, prepared from cysteamine and D-glucose showed some mutagenicity toward TA 98 and TA 100 in our previously study (Mihara and Shibamoto, 1980). The loss of the mutagenic activity of this compound is due to purification. The compound was purified more completely in this study. The same kind of phenomenon is observed in the other browning reaction products (Toda et al., 1981). It is necessary to investigate further to know whether the loss of mutagenicity is due to loss of the actual mutagenic contaminants or to some multiple reactions between the compound and contaminants.

These water-soluble thiazolidine compounds have not been found in natural food yet. Volatile thiazoles and thiazolines, however, which have been isolated from many foods (Wilson et al., 1973; Tonsbeek et al., 1968; Watanabe and Sato, 1971; Stoll et al., 1967), could be formed from these water-soluble (polyhydroxyalkyl)thiazolidines by fragmentation of the sugar moiety and dehydrogenation of the thiazolidine ring (Sakaguchi and Shibamoto, 1978).

The aqueous fraction of browning reaction products has not been as well studied as the volatile fraction, probably because it is difficult to analyze less-volatile compounds with gas chromatographic techniques. Our study, however, showed the aqueous fraction of browning reaction mixtures also contains compounds important to the overall composition and the taste of the foods.

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