Mutagenicity of the L-Rhamnose-Ammonia-Hydrogen Sulfide Browning Reaction Mixture

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The browning reaction mixture obtained from the reaction of L-rhamnose, NH_3, and H_2S was separated into two layers with an extraction of dichloromethane. The aqueous fraction did not show any positive response toward *Salmonella typhimurium*/microsomal mutagenicity tests with TA 98 and TA 100. The dichloromethane extract, on the other hand, exhibited strong mutagenicity. The dichloromethane fraction, the gas chromatogram of which indicates the presence of over 300 chemicals, was then fractionated into 10 fractions by silica gel column chromatography. Fraction 10 (consisted mainly of alkylimidazoles) exhibited strongest mutagenicity. Authentic samples of alkylimidazoles and any mixtures of those did not, however, respond to either *S. typhimurium* strain TA 98 or *S. typhimurium* strain TA 100. The two new fractions obtained from fraction 10 by high-performance liquid chromatography showed considerable loss of mutagenicity.

Hodge (1953, 1967) had reviewed the chemistry of browning reactions and pointed out that simple model reaction systems could be used to learn about the more complex food systems. Since then the formation of browning products has been investigated intensively by using model systems of a sugar and an amine (Heyns et al., 1974; Hodge et al., 1972; Koehler et al., 1969). Most of the studies have been concerned with the flavor chemistry of the browning reaction (Rizzi, 1972; Shibamoto and Bernhard, 1978; Sakaguchi and Shibamoto, 1978).

In recents years, mutagenicity studies of browning products have begun to receive considerable attention, especially since a bacterial short-term assay for mutagenicity was developed by Ames and Durston (1973).

Nagao et al. (1977), using the Ames assay, found strong mutagenic activity in the tars obtained from various heated foods, e.g., egg white lysozyme, serum albumin, and calf thymus histone. They reported that these protein pyrolysate products required metabolic activation by S-9 mix and were more mutagenic toward *Salmonella typhimurium* strain TA 98 than toward strain TA 100 (base-pair substitution). Spingarn and Garvie (1979) tested the mutagenicity of reaction products from ammonia–sugar model systems (including glucose and rhamnose) and found that all products showed some mutagenicity toward TA 98. Their most active sample was the dichloromethane extract of the reaction mixture from rhamnose and ammonia. They observed increased mutagenicity of a sugar–ammonia reaction mixture with increased formation of pyrazines, which are typical browning reaction products (Hodge et al., 1972).

The fractions obtained from the dichloromethane extracts of the D-glucose–cysteamine reaction mixture exhibited some mutagenicity toward *S. typhimurium* TA 98 and TA 100 (Mihara and Shibamoto, 1980). The reaction of cysteamine, D-glucose, and $NaNO_2$ produced strong mutagens, which are identified as *N*-nitroso-2-alkylthiazolidines. Among them, *N*-nitrosoisopropylthiazolidine showed the strongest mutagenicity toward TA 100 without metabolic activation (Sekizawa and Shibamoto, 1980).

Given this evidence, one can predict that browning reactions produce mutagenic materials. A rhamnose–NH_3–H_2S model system was chosen to study the genetic toxicity of the browning reaction mixture because this system produces a large number of products and their reproducibility is excellent (Shibamoto and Bernhard, 1978; Yamaguchi et al., 1979). This study is a first step toward the isolation and characterization of these materials from browning reaction model systems.

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EXPERIMENTAL SECTION

Reaction of L-Rhamnose, Hydrogen Sulfide, and Ammonia. Hydrogen sulfide gas was bubbled through an aqueous solution (120 mL) containing 32 g (0.2 mol) of L-rhamnose for 20 min at 0 °C in a 300-mL Kjeldahl flask. Six milliliters of a 30% ammonium hydroxide solution (0.1 mol as NH_3) was then added to the solution. The neck of the flask was flame sealed and the flask was placed in an oven at 90 °C for 5 h.

Sample Preparation of the Volatile Fraction (Dichloromethane-Soluble Fraction). The reaction mixture obtained from the above experiment was extracted with 200 mL of dichloromethane by using a liquid–liquid continuous extractor for 16 h. Approximately 5.3 g of an oily brown material was obtained after the solvent was removed. The material obtained was stored for silica gel column chromatography and the mutagenicity test. The volatile constituents was identified following the GC/MS technique described by Yamaguchi et al. (1979).

Sample Preparation of the Less-Volatile Fraction (Water-Soluble Fraction). After the volatile fraction was removed from the above reaction mixture, the residual aqueous fraction was concentrated (to syrup form) by distillation under pressure (40 °C; 15 mmHg). The resulting brown syrup (~4.1 g) was stored for the mutagenicity test.

Silica Gel Column Chromatography. Seventy grams of silica gel (100–200 mesh) was slurry packed into a glass column (25 × 3 cm i.d.) with dichloromethane. The volatile sample (5 g) was dissolved in 10 mL of dichloromethane poured onto the column and drawn into the absorbent. The material was eluted successively with dichloromethane, dichloromethane–acetone (90:10), ethyl acetate, and methanol. The amounts of solvents used and products obtained after the removal of solvents are presented in Table I.

High-Performance Liquid Chromatography (LC). Fraction 10 was further fractioned on a Hitachi HPLC (System) Model 635A equipped with an UV absorbance detector (230 nm). The high-performance LC column (25

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Table I. Amounts of Solvent Used and Products Obtained for and in the Column Chromatography[a]

fraction	solvent	amount of solvent, mL	amount of products, mg
1	dichloromethane	50	19
2	dichloromethane	50	12
3	dichloromethane	50	4
4	dichloromethane	50	3
5	dichloromethane–acetone	120	87
6	dichloromethane–acetone	120	1159
7	dichloromethane–acetone	60	148
8	ethyl acetate	60	35
9	ethyl acetate	60	316
10	methanol	200	2520

[a] All fractions were stored for the mutagenicity test.

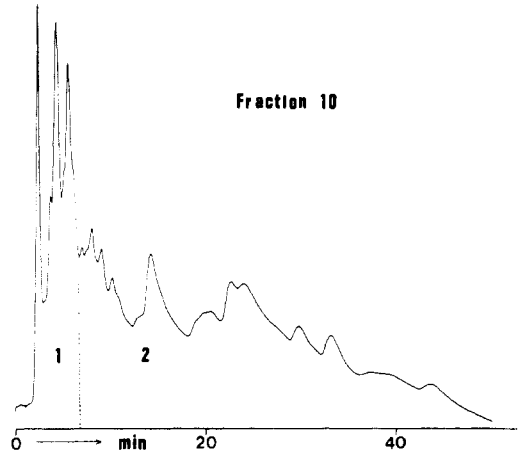

Figure 1. High-performance liquid chromatogram of fraction 10.

cm × 4 mm i.d.) was slurry packed with Merck Lichrosorb RP-18. Methanol–water (2:3) was used for the mobile phase. Fraction 10 (19 mg) was dissolved into 200 μL of a methanol–water (2:3) mixture. Twenty separate 10 μL portions of this solution were injected into the high-performance LC. The fractionation was conducted as shown in Figure 1. The new fractions 10-1 (1 mg) and 10-2 (17 mg) were freeze-dried and then stored for mutation tests.

Mutagenicity Test. The mutagenicity of the various samples was tested following the method of Ames et al. (1975) with minor modifications. Liver homogenate (S-9) was prepared from male Sprague–Dawley rats, which were treated with polychlorinated biphenyl to activate liver enzymes. *S. typhimurium* strains TA 98 and TA 100 were used throughout the experiments. The samples were dissolved in dimethyl sulfoxide and preincubated with a strain at 30 °C for 30 min (prior to plating) with or without S-9 mix. A buffer was substituted when S-9 was not used. After addition of the test samples, the plates were incubated at 37 °C for 2 or 3 days. The mutagenic activity is expressed as the slope value of a linear dose–response curve (Figure 2).

Gas–Liquid Chromatography/Mass Spectrometry (GC/MS). The 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 under the following conditions: ionization volate, 70 eV; ion source temperature, 200 °C; emission current, 80 μA; ion acceleration voltage, 3100 V.

Identification of products obtained in this study was made by comparison of their mass spectra and Kovâts Indices to those of authentic compounds.

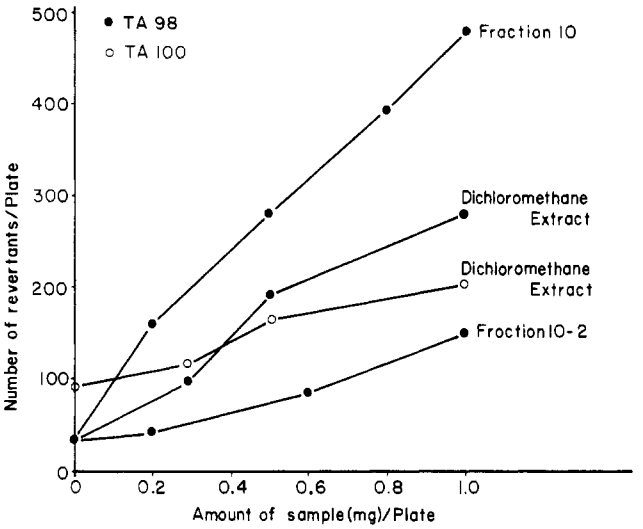

Figure 2. Mutagen dose–response curve of the fractions obtained from the reaction of L-rhamnose, NH_3, and H_2S on strains TA 98 and TA 100 with S-9 mix. Each point is the average of at least triplicate runs.

Table II. Results of the Preliminary Mutagenicity Test on the Fractions Obtained from the Reaction Mixture of L-Rhamnose, NH_3, and H_2S (All Results Are an Average of Triplicate Runs)

	X[a]			
	TA 98		TA 100	
sample, 1 mg/plate	+S-9[b]	−S-9	+S-9[b]	−S-9
aqueous fraction	−	−	−	−
dichloromethane fraction	++	−	+	−
fraction 1	−	−	−	−
fraction 2	−	−	−	−
fraction 3	−	−	−	−
fraction 4	−	−	−	−
fraction 5	−	−	−	−
fraction 6	−	−	−	−
fraction 7	−	−	−	−
fraction 8	−	−	−	−
fraction 9	−	−	−	−
fraction 10	++	−	−	−
fraction 10-1	−	−	−	−
fraction 10-2	++	−	−	−

[a] X = (number of revertants per plate of sample)/(number of revertants per plate of control): (++) $X \geq 5$; (+) $5 > X > 2$; (−) $X < 2$. Spontaneous rates: TA 98 (+S-9/−S-9) = 25-35; TA 100 (+S-9/−S-9) = 90-110. [b] 50 μL/plate.

RESULTS AND DISCUSSION

Comprehensive chemical analyses of the volatile fraction from the reaction mixture of L-rhamnose, ammonia, and hydrogen sulfide have been reported previously (Yamaguchi et al., 1979). This reaction system produces a tremendous number of heterocyclic compounds: thiophenes, pyrazines, thiazolines, thiazoles, pyrroles, furans, and imidazoles with pyrazines and imidazoles as the main constituents.

Recently, Shibamoto (1980) isolated a mutagenic polycyclic pyrazine from a cyclotene–NH_3 browning model system. The possible formation of mutagens in the L-rhamnose–NH_3–H_2S browning model system was further investigated in this study.

Table II shows the results of preliminary mutagenicity tests on all fractions obtained from the experiment. Those fractions showing positive responses toward either TA 98

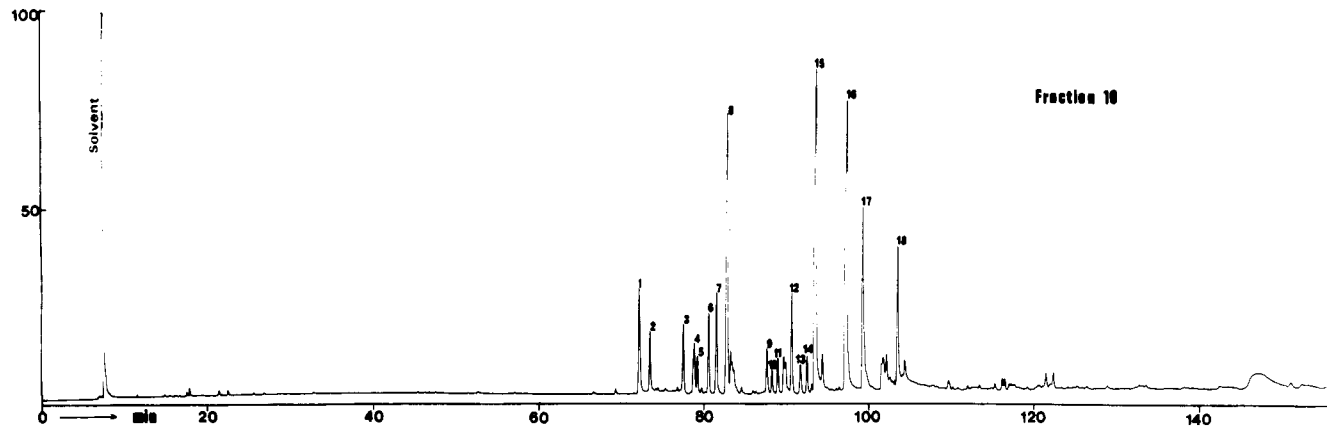

Figure 3. Gas chromatogram of fraction 10. A Hewlett-Packard Model 5710A gas chromatograph equipped with a flame ionization detector and a 40 m × 0.28 mm i.d. glass capillary column coated with Carbowax 20M was used. The oven temperature was programmed from 80 to 200 °C at 1 °C/min. The nitrogen carrier gas flow rate was 13 cm/s (~0.68 mL/min). The injector and detector temperatures were 250 °C. The injector split ratio was 1:100. For peak identification see Table III.

Table III. Compounds Identified in Fraction 10

peak (Figure 3)	compd	MS ref
1	imidazole derivative (M^+ = 138)	
2	imidazole derivative (M^+ = 138)	
3	unknown	
4	imidazole derivative (M^+ = 152)	
5	imidazole derivative (M^+ = 152)	
6	imidazole derivative (M^+ = 122)	
7	imidazole derivative (M^+ = 108)	
8	imidazole derivative (M^+ = 124)	
9	imidazole derivative (M^+ = 122)	
10	imidazole derivative (M^+ = 138)	
11	imidazole derivative (M^+ = 138)	
12	imidazole derivative (M^+ = 152)	
13	imidazole derivative (M^+ = 138)	
14	imidazole derivative (M^+ = 152)	
15	2,4-dimethyl-imidazole	Stenhagen et al. (1974)
16	2-methylimidazole	Bowie et al. (1967)
17	2-ethyl-4-methyl-imidazole (tentative)	110 (95), 109 (94), 95 (100), 82 (10), 68 (28), 54 (28), 42 (10)
18	2-ethylimidazole	Stenhagen et al. (1974)

or TA 100 were further tested to obtain their dose–response curves (Figure 2). Figure 2 shows the dose–response curve of the fractions obtained by using strains TA 98 and TA 100 with S-9 mix. All samples required metabolic activation by the S-9 mix.

The less-volatile fraction (aqueous fraction) obtained from the reaction of L-rhamnose, NH_3, and H_2S was mutagenic neither to the frame shift type mutant TA 98 nor to base pair substitution type mutant TA 100. The volatile fraction showed more activity in strain TA 98 than in strain TA 100. On the other hand, carbonyl compounds like glyoxal or diacetyl, which are typical precursors of browning products (Hodge, 1953), have shown (dose-related) activity toward strain TA 100 but not TA 98 (Bjeldanes and Chew, 1979). The frame shift type strain seems to be more sensitive in detecting volatile mutagens from browning reactions than the base pair substitution type strain (Spingarn and Garvie, 1979; Shibamoto, 1980). Mutagenicity tests of the volatile fraction indicated that both frame shift type and base pair substitution type mutagens were present. Among the 10 fractions obtained from the column chromatography only fraction 10 showed, however, appreciable activity toward strain TA 98. Fraction 10 was more mutagenic than the total volatile fraction but showed the same pattern of activity (more active in TA 98 than in TA 100). The gas chromatogram of fraction 10 is shown in Figure 3. Fractions 10-1 and 10-2 obtained from high-performance LC on fraction 10 showed considerable loss of mutagenicity. It is not, however, clear whether this is due to the fractionation or the loss of actual mutagens from the sample during fractionation. The gas chromatographic/mass spectrometric analysis of fraction 10 suggests that this fraction consisted for the most part of alkylimidazoles (Table III). The authentic alkylimidazoles and any mixtures of those did not, however, exhibit mutagenic activity. Voogd et al. (1979) also reported that alkylimidazoles were not mutagenic.

The results indicate that browning reactions do produce some mutagenic materials. It is, however, not yet clear whether the mutagenicity of the browning reaction mixture is due to the presence of individual mutagens or to any multiple effects of the components reacting together. Further studies will be necessary to identify the mutagenic determinants of browning reaction mixtures.

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Effect of Magnesium Fertilization on the Quality of Potatoes. Yield, Discoloration, Phenols, and Lipids

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The effect of magnesium fertilization on yield, discoloration, phenols, and lipid content of potatoes was examined during a 2-year study. Magnesium sulfate was applied at rates of 0, 20, 40, and 100 lb/acre. Maximum yield was obtained at 20 lb/acre. Tubers receiving 40 lb/acre $MgSO_4$ discolored significantly less ($p < 0.05$), were significantly lower ($p < 0.05$) in total phenolic content, and were significantly higher ($p < 0.01$) in crude lipid and phospholipid content than the controls.

Potatoes are more sensitive to magnesium deficiency than many other crops (Bolton, 1977), yet such deficiences may be corrected through the use of magnesium sulfate in fertilizers (Houghland and Strong, 1941; Houghland, 1964). In acid peat and sandy soils magnesium deficiency is the principal cause of poor growth (Mulder, 1950). Response to magnesium is particularly apparent on acid and sandy loams which also contain less than 76 lb of exchangeable magnesium/acre and have a pH below 5.5 (Doll and Thurlow, 1965). Sawyer and Dallyn (1966) found that plots of potatoes fertilized with over 40 lb/acre magnesium sulfate (oxide equivalent) tended to depress yield and that 40–60 lb/acre was adequate in building up and maintaining the lower soil levels found on Long Island. Laughlin (1966) observed that soil or spray applications of magnesium sulfate had no significant effect on yield, yet magnesium sulfate was applied to the soil at high rates of 0, 250, and 500 lb/acre. In addition, soil analyses prior to fertilization suggested already high fertility levels ranging from 119 to 350 lb/acre available magnesium. Adams et al. (1978a) observed that yields of tomatoes grown in peat demonstrated an overall increase in yield of 8.6% with added magnesium but neither the quality nor the composition was affected. Adams et al. (1978b) also found, however, that lettuce grown in peat did not respond to added magnesium. These authors suggest that such results are consistent with earlier trials which demonstrate that lettuce is not severely affected by magnesium deficiency, whereas tomato crops are highly susceptible.

Crop fertilization may also affect the quality and chemical composition of the product. Magnesium is essential for the translocation of sugars in potato plants (Lewin and Lewin, 1956). Magnesium sulfate fertilization increases anaerobic respiration, decreases O_2 consumption, increases CO_2 evolution, and increases chlorophyll formation by tubers in the light (Vermes et al., 1974). Magnesium does not appear to affect tuber discoloration consistently, however. Length of time prior to analysis may result in an increase, decrease, of insignificant effect on black spot production in potatoes (Jacob, 1959). Mueller (1976) observed that magnesium-fertilized tubers discolored more than control tubers 1 month following harvest yet discoloration was significantly less than that for controls after 10 months of storage. Supplementation of magnesium with potassium decreased discoloration, whereas magnesium fertilization on its own increased discoloration (Massey, 1952).

Since crop fertilization may affect the chemical composition and quality of the product and thus the ultimate nutritive value and economic return, it is important that the outcome of such agricultural practices be as clearly delineated as possible. This study was therefore undertaken in order to establish the effect of magnesium fertilization on yield, discoloration, and phenolic and lipid contents of Katahdin potatoes grown during 2 successive years.

MATERIALS AND METHODS

Katahdin potatoes grown at the Cornell Vegetable Research Farm in Riverhead, Long Island, during the 1978 (year 1) and 1979 (year 2) growing seasons were used in the studies. Soil type was Riverhead fine sandy loam. Magnesium in the form of magnesium sulfate was banded at planting at rates of 0, 20, 40, and 100 lb/acre. Different plots were used each of the 2 years in order to avoid a cummulative effect. Available magnesium levels on these plots averaged 70 lb/acre. Soil organic matter averaged 2.9%, and soil pH was ~6.1 during both years. The randomized block design contained two replicated plots per treatment, and all plots were irrigated in the same manner during both seasons.

Tubers were harvested 24 weeks after planting and stored at 5 °C for 5 months prior to analysis. Uniform tubers of medium size were sliced longitudinally from bud

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