scribed in eq 3 is accompanied by two competitive reactions: (a) the formation of MgO and the loss of citrate-soluble Mg_2PO_4Cl; (b) the formation of volatile HF and the loss of citrate-insoluble Mg_2PO_4F. Further studies will be necessary to find the optimum conditions leading to a phosphate fertilizer with maximum phosphate availability.

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Mutagenicity of 1,5(or 7)-Dimethyl-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine Obtained from a Cyclotene/NH_3 Browning Model System

1,5(or 7)-Dimethyl-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine (I), which was obtained from the browning reaction of 2-hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene) and ammonia under simulated cooking conditions, was tested for mutagenicity by using *Salmonella typhimurium* strain TA 1535, TA 100, TA 1537, TA 1538, and TA 98. I showed mutagenic activity with the frameshift mutation strains TA 98 and TA 1538.

Many nitrogen- and sulfur-containing heterocyclic compounds have been identified as the products of nonenzymatic browning model systems (Rizzi, 1972; Wilson and Katz, 1972; Shibamoto and Russell, 1977; Shibamoto and Bernhard, 1978). These chemicals include thiophenes, furans, oxazoles, thiazoles, pyrazines, pyrroles, and imidazoles. Mihara and Shibamoto (1980) observed mutagenicity of some fractions obtained from a D-glucose/cysteamine browning reaction mixture on Ames *S. typhimurium* strains TA 98 and TA 100. They also found that *N*-nitrosothiazolidine derivatives, which can be formed in a browning model system (Sakaguchi and Shibamoto, 1979), showed strong mutagenicity on *Salmonella typhimurium* strain TA 100 (base-pair substitution strain).

Recently, Bjeldanes and Chew (1979) reported that 1,2-dicarbonyl compounds (maltol, kojic acid, diacetyl, etc.) gave positive responses to the *Salmonella*/microsome mutagenicity assay. These dicarbonyls, which may be derived from degradation or caramelization of carbohydrates in foods (Hodge, 1967), have been shown to be precursors of pyrazines in browning reactions (Rizzi, 1972). Nishimura et al. (1980) obtained a polycyclic pyrazine (I) from a cyclotene (2-hydroxy-3-methyl-2-cyclopenten-1-one)/NH_3 browning model system. Cyclotene is known as a product of sugar degradation, as is maltol (Hodge, 1972). In the present study, the mutagenicity of the product of cyclotene and ammonia is investigated.

EXPERIMENTAL SECTION

Sample Preparation. 2-Hydroxy-3-methyl-2-cyclopenten-1-one (cyclotene, 0.1 mol) was dissolved in 50 mL of deionized water in a 100-mL Kjeldahl flask. Thirty milliliters of 30% ammonium hydroxide solution (0.5 mol as NH_3) was then added. The neck of the flask was flame-sealed, and the flask was placed in an oven at 90 °C for 2 h. The reaction mixture was extracted with 200 mL of methylene chloride, using a liquid–liquid continuous extractor for 16 h. The extract was dried over anhydrous magnesium sulfate. A brown oily liquid (ca. 0.5 g) was obtained after the solvent was removed. This brown material was dissolved in 10 mL of petroleum ether, and undissolved materials were filtered off. The petroleum ether filtrate was evaporated and the material obtained was purified by TLC [Merck silica gel; solvent, *n*-hexane/ethyl acetate 3:1; R_f = ca. 0.3]. The purified material (0.1 g) was identified as the mixture of four stereoisomers: *cis*-1,5-dimethyl- (*meso*), *trans*-1,5-dimethyl- (meso), *cis*-1,7-dimethyl- (meso), and *trans*-1,7-dimethyl-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine (*dl*). The structures of these isomers were confirmed by NMR, IR, and MS, using the methods described by Nishimura et al. (1980). Their possible structures are shown in Figure 1.

Mutagenicity Test. Mutagenicity tests were conducted as described by Umezawa et al. (1978), a modification of the method reported by Ames et al. (1975). Histidine-requiring *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 1538, and TA 98 were used as indicator organisms for mutagenic activity.

RESULTS AND DISCUSSION

The results obtained from the mutagenicity tests are shown in Figure 2. In high concentration, I exhibited considerable antibacterial activity. It was difficult, therefore, to find the optimum concentration for investi-

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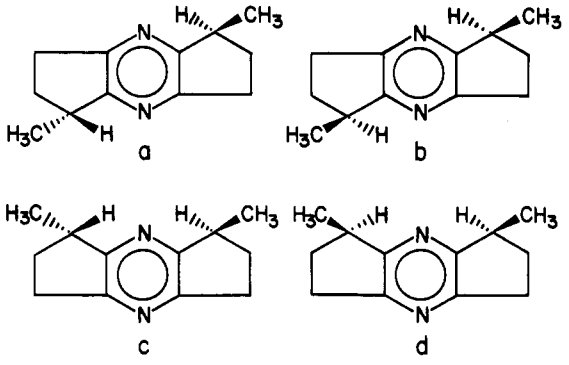

Figure 1. Possible structures of 1,5(or 7)-dimethyl-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine: (a) *trans*-1,5-dimethyl-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine, (b) *cis*-1,5-dimethyl-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine, (c) *trans*-1,7-dimethyl-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine, and (d) *cis*-1,7-dimethyl-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine.

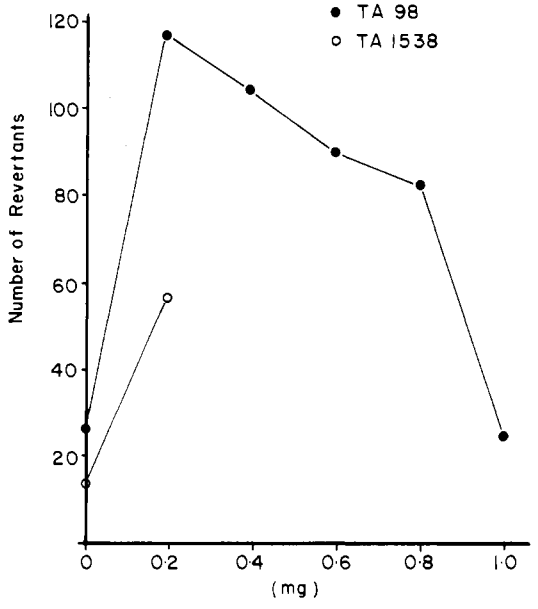

Figure 2. Mutagenicity of 1,5(or 7)-2,3,6,7-tetrahydro-1*H*,5*H*-biscyclopentapyrazine toward *S. typhimurium* strains TA 1535 and TA 98 without metabolic activation by S-9 mix.

gation of its mutagenicity. If the dose exceeded 1 mg, all bacteria were killed. I showed positive response only toward strains TA 1538 and TA 98 (frameshift mutants) without S-9 mix. No appreciable activity was observed with TA 1535, TA 100, or TA 1537 at any dose level of I. This lack of activity is of interest because other reported browning products were more mutagenic toward the base-pair mutation strain (TA 100) than toward the frameshift mutation strain (TA 98) (Mihara and Shibamoto, 1980). The dicarbonyl compounds, which are precursors of pyrazines, also responded positively toward TA 100, but not toward TA 98 (Bjeldanes and Chew, 1979).

It has been proposed that heat treatment causes the production of mutagens in food (Sugimura and Nagao, 1979). Sugimura et al. (1977) obtained some polycyclic nitrogen-containing compounds, which showed strong mutagenic activity toward TA 98 and TA 100, from a tar formed by pyrolysis of tryptophan. Their method was, however, much more vigorous than ordinary cooking conditions. Pyrazine I was obtained under much milder conditions, closer to normal cooking conditions.

Polycyclic pyrazines have not as yet been found in foods, but are formed in certain model systems (Rizzi, 1972; Nishimura et al., 1980). A large number of alkylpyrazines have been found in food products (Maga and Sizer, 1973). These pyrazines have, however, been reported as nonmutagenic (Spingarn and Garvie, 1979). More work is necessary to discover the formation mechanism of mutagenic polycyclic pyrazines in cooked food.

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Protein and Amino Acid Composition of Select Freshwater Fish

The protein content and amino acid composition of six species of freshwater fish [white sucker (*Catostomus commersoni*), burbot (*Lota lota*), black crappie (*Pomoxis nigromacultatus*), rainbow trout (*Salmo gairdneri*), walleye pike (*Stizostedion vitreum*), and yellow perch (*Perca flavescens*)] were determined. Little variation in composition was found among species.

Because of their abundance and their possible utilization as a source of food, freshwater fish species are being studied for the fabrication of new products (March et al., 1967; Lantz, 1966). Knowledge of the amino acid composition of freshwater fish is limited (March et al., 1967). In conjunction with product development we are concurrently determining the nutrient composition of several freshwater fish species (Kinsella et al., 1977a,b; Mai et al.,

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