Note

Isolation and Identification of 5-Hydroxymaltol, a Mutagenic Substance in Glucose Pyrolysate

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We have previously demonstrated the mutagenicities of the charred parts of fish and meat and pyrolysates of amino acids and proteins.[1～5] Subsequently, many mutagenic compounds have been isolated from the pyrolysates of amino acids and protein and from cooked foods and their structures have been determined.[5～12] Most of these mutagenic compounds were heterocyclic amines and they showed higher activity on *Salmonella typhimurium* strain TA98 than strain TA100, in the presence of a metabolic activation system, S9 mix.[13～15] Recently, some of them have been proved to be carcinogenic.[14～16]

Pyrolysates of sugars and starch have also been found to be mutagenic.[4,17] These mutagenicities were noticeable on TA100 in the absence of S9 mix and were decreased by the addition of S9 mix. On TA98, very weak mutagenicity was observed with S9 mix, and none without S9 mix. These results suggest that mutagenic compounds formed by the pyrolysis of sugars and starch differ in nature from those formed by the pyrolysis of amino acids, proteins and protein foods. This paper describes the isolation and identification of a mutagenic compound produced by the pyrolysis of glucose.

The preincubation method for the *Salmonella* mutation test previously reported by Sugimura and Nagao[18] was adopted.

Glucose (1.5 kg) in a flask was heated directly over a gas burner and the resulting distillate of tar (1.0 kg) was condensed in a cooled flask on ice. When the tar was distilled under 20 mmHg at 50°C, almost all the mutagenic activity was recovered in the residual fraction (480 g). The residue was separated by counter-current distribution with *n*-butanol–water (1 : 1, v/v), as the solvent. When the lower phase was transferred ten times ($n=10$), mutagenic activity was detected in the third to sixth fractions ($r=2\sim5$). The pooled active fraction (52 g) was further purified by silica gel column chromatography, eluting with benzene followed by benzene–chloroform (1 : 1, v/v). A mutagenic compound (24 mg) was obtained in a crystalline form from the benzene–chloroform (1 : 1, v/v) eluate. The compound was recrystallized from methanol to colorless plates with an mp of 183～185°C. The molecular formula was determined to be $C_6H_6O_4$ by elemental analysis and examination of the mass spectrum (*m/z*: 142 (M^+)). The ^{1}H-NMR spectrum of this compound shown in Fig. 1 is identical with that of 5-hydroxymaltol. The UV and IR spectra of this isolated compound were also the same as those of 5-hydroxymaltol.

The mutagenic activity of 5-hydroxymaltol on TA100 is

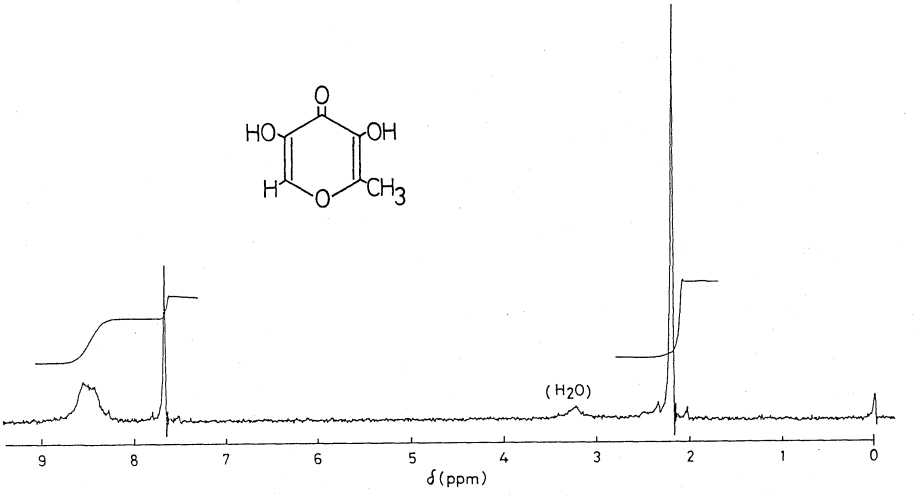

FIG. 1. NMR Spectrum of the Mutagenic Compound Isolated from a Glucose Pyrolysate.
$(CD_3)_2SO$ was used as the solvent.

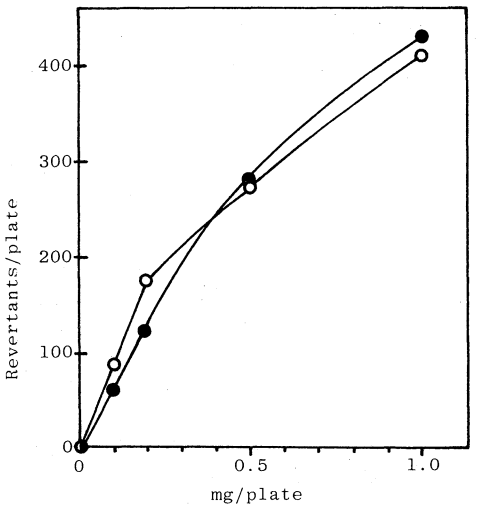

FIG. 2. Mutagenicity of 5-Hydroxymaltol with (●) or without (○) S9 Mix to TA100.

S9 mix, in a total of 500 μl, contained 100 μl of S9.

shown in Fig. 2. It gave 279 and 275 revertants of TA100 per 0.5 mg with and without S9 mix, respectively. However, at 1 mg it did not give more than twice the spontaneous number of revertants of TA98 with or without S9 mix and higher doses had a lethal effect. The mutagenicity of 5-hydroxymaltol was similar to that of maltol, ethylmaltol and kojic acid, which are also γ-pyrone derivatives.[19]

The mutagenicity of 5-hydroxymaltol to *Escherichia coli* WP-2 *uvrA*/pkM101 was also tested by the preincubation method,[18] in which minimal glucose-agar plates containing tryptophan in place of histidine were used. This gave 360 and 346 revertants per mg with and without S9 mix, respectively. However, 5-hydroxymaltol at concentrations of up to 50 mg per plate without S9 mix did not induce prophage λ in lysogenic *Escherichia coli* K12 strain GY5027 in the Inductest III of Moreau *et al.*[20]

One milligram of glucose tar induced 432 and 5,180 revertants of TA100 with and without S9 mix, respectively. Therefore, the mutagenic activity of 5-hydroxymaltol is too weak to explain the mutagenic activity of the glucose pyrolysate. We are now attempting to identify other mutagens besides 5-hydroxymaltol in the glucose pyrolysate.

Like the glucose pyrolysate, coffee, tea and alcoholic beverages are mutagenic to TA100 without S9 mix.[21,22] However, the mutagenic components of these beverages have not yet been elucidated. It is also important to identify these direct-acting mutagens in daily foods.

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