Communications to the Editor

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The Formation of the Mutagenic Substances in the Reaction between L-Ascorbic Acid and L-Tryptophan

The reaction between L-ascorbic acid and L-tryptophan in phosphate buffer (pH 7.0) at 37° for 2 months gave several reaction products of which 2 were mutagenic on *S. typhimurium* TA 100 in the absence of S-9 Mix. The structure of these 2 compounds were determined to be 1-(2-furyl)-pyrido[3,4-*b*]indole and 1-(2-furyl)pyrido[3,4-*b*]indole-3-carboxylic acid respectively by their mass spectral and ^{13}C NMR analysis.

Keywords——L-ascorbic acid; L-tryptophan; DNA damaging potency, mutagenicity; *S. typhimurium* TA 100; 1-(2-furyl)-pyrido[3,4-*b*]indole; 1-(2-furyl)-pyrido[3,4-*b*]indole-3-carboxylic acid

We have previously reported that L-ascorbic acid could undergo C_2–C_3, C_3–C_4 and C_4–C_5 cleavage reaction in the presence of amines or amino acids and gave various amino-carbonyl reaction products including amino derivatives of 2,3-dehydroascorbic acid, 2-deoxyascorbic acid, oxalic acid, mesoxalic acid, urea and isatin.[1)]

In the extended study of this browning reaction, we examined mutagenic activities of reaction products and found that the products from L-ascorbic acid with L-tryptophan showed not only DNA damaging activity on *Bacillus subtilis* but mutagenic activity in the strain TA 100 of *Salmonella typhimurium*.

In this paper, we report the isolation and identification of the mutagenic substances from the reaction mixture of L-ascorbic acid and L-tryptophan.

A mixture of L-ascorbic acid (2 mol) and L-tryptophan (0.5 mol) in 0.1 M phosphate buffer (pH 7.0, 3 l) was kept at 37° for 2 months. The reaction mixture was then extracted by benzene (500 ml ×3) and the extract after evaporation was applied to a silica-gel (Kieselgel 60) column (4×30 cm). By eluting the column with benzene and ethylacetate (9: 1) mixture, eight fractions (fr. 1—fr. 8) were obtained. Fraction 3 which showed highest mutagenic activity among them was purified through a silica-gel (Kieselgel 60, F-254) TLC developed by mixture of benzene and ethylacetate (3: 1). A single spot of *Rf* 0.45 with fluorescence was detected under UV light and colorless crystals of mp 179—180° (substance A) was obtained by extracting the spot with benzene.

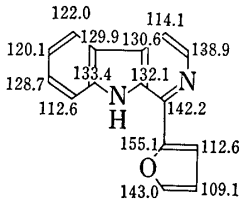
The chemical formula of $C_{15}H_{10}N_2O$ was assigned (a molecular ion peak appeared at *m/e* 234) to this substance and the final structural elucidation as 1-(2-furyl)-pyrido[3,4-*b*]indole was done by comparing its IR, UV, NMR and mass spectral data with those of authentic compound which was synthesized from L-tryptophan and furfural by the method of Kermack *et al.*[2)]

Another mutagenic substance (substance B) was obtained as white crystals of mp 263—264° from fr. 8 in a similar purification technique by the use of column chromatography and TLC as shown for substance A. The chemical formula of substance B was determined to be $C_{16}H_{10}N_2O_3$ from its mass spectrum with *m/e* 278. Substance B was characterilized as 1-(2-furyl)-pyrido[3,4-*b*]indole-3-carboxylic acid from ^{13}C-NMR analysis. The chemical shift of these two compounds on ^{13}C-NMR are shown in Fig. 1 a and b.

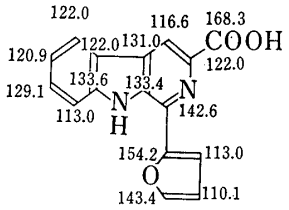
The yield of substance A and B from L-tryptophan were 0.057% and 0.18% respectively.

As shown in Fig. 2, the substance A showed dose-responded mutagenicity on *S. typhimurium* TA 100 in the presence or absence of S-9 Mix on the soft agar method of Ames

1) T. Ozawa, Y. Nakamura, and N. Kinae, *Yakugaku Zasshi*, **96**, 608 (1976); *ibid.*, **96**, 932 (1976).
2) W.O. Kermack, W.H. Perkin, and R. Robinson, *J. Chem. Soc.*, **119**, 1602 (1921).



a) substance A



b) substance B

Fig. 1. Structures and ^{13}C-NMR Spectra of Substance A and B

Numbers indicate chemical shift (ppm).

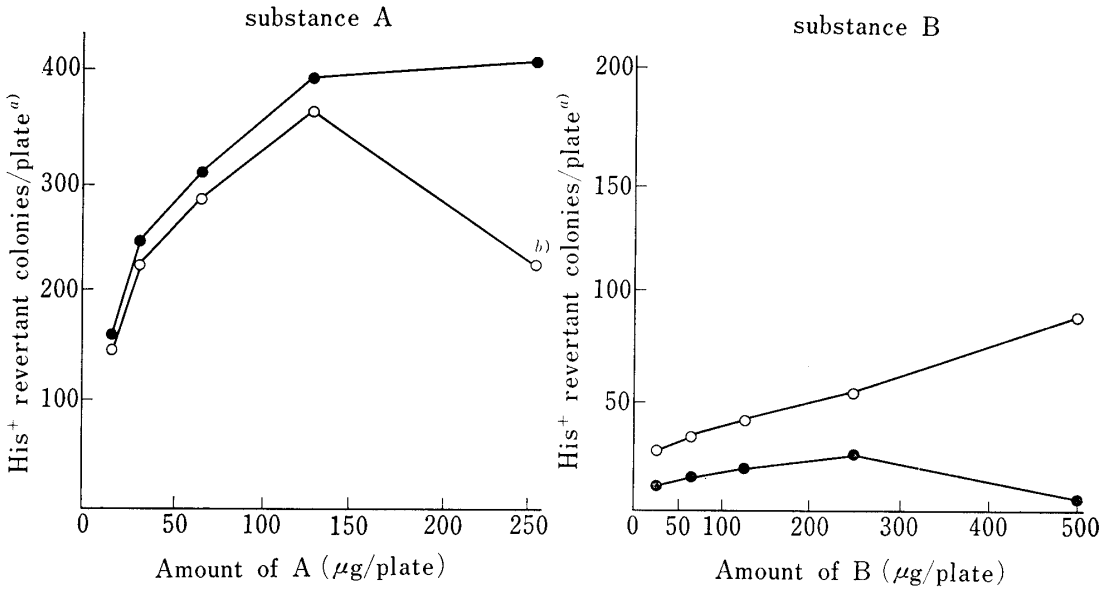


Fig. 2. Mutagenic Activities of Substance A and B on *S. typhimurium* TA 100

Substance A and B were tested for mutagenic activity using *S. typhimurium* TA 100 in the presence (●) or absence (○) of S-9 Mix.
a) Spontanous revertant colonies (106 with S-9 Mix, 113 without S-9 Mix) are subtracted.
b) Killing of bacteria was observed.

et al.,[3] while the substance B exhibited relatively weak mutagenicity which decreased in the presence of S-9 Mix. Both compounds A and B showed no mutagenic activities on *S. typhimurium* TA 98.

The present study would suggest that the foods containing L-ascorbic acid and L-tryptophan may form mutagenic compounds during their storage and cooking.

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3) B.N. Ames, J. McCann, and E. Yamasaki, *Mutation Res.*, **31**, 347 (1975).