

Communications to the Editor

[Chem. Pharm. Bull.]
28(10):3143--3144(1980)

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 ¹³C 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 C₂-C₃, C₃-C₄ and C₄-C₅ 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 *R_f* 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 C₁₅H₁₀N₂O 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 C₁₆H₁₀N₂O₃ from its mass spectrum with *m/e* 278. Substance B was characterized as 1-(2-furyl)-pyrido[3,4-*b*]indole-3-carboxylic acid from ¹³C-NMR analysis. The chemical shift of these two compounds on ¹³C-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

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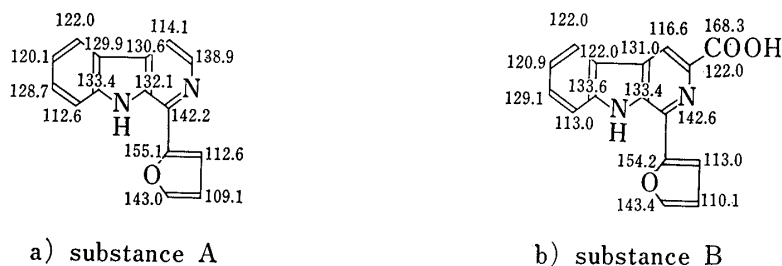


Fig. 1. Structures and ^{13}C -NMR Spectra of Substance A and B
Numbers indicate chemical shift (ppm).

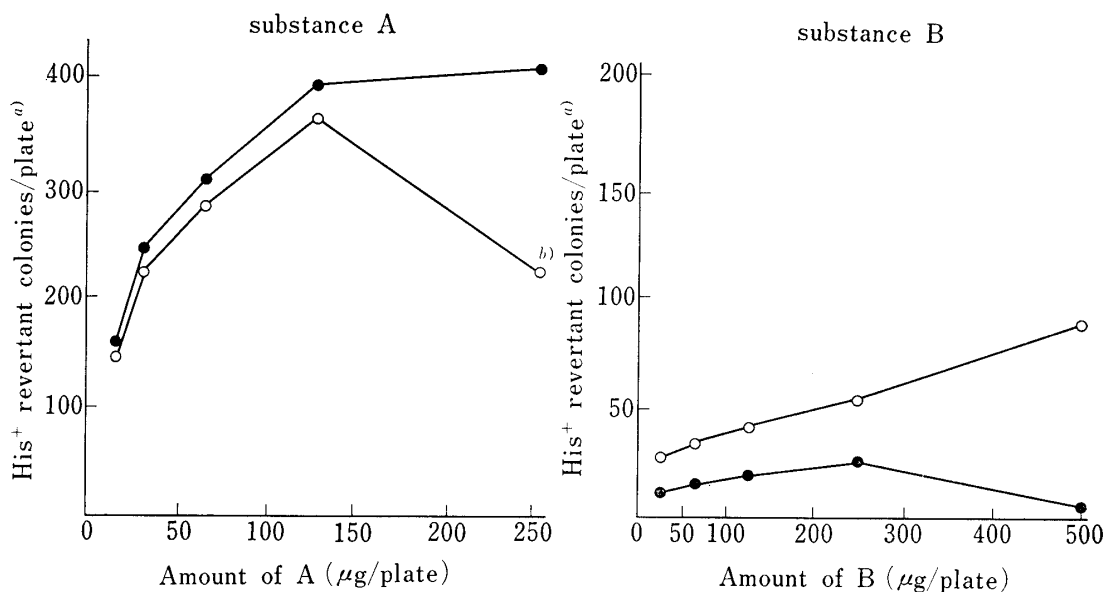


Fig. 2. Mutagenic Activities of Substance A and B on *S. typhimurium* TA 100

Substance A and B were tested for mutagenic activity using *S. typhimurium* TA 100 in the presence (●) or absence (○) of S-9 Mix.

a) Spontaneous 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.

Acknowledgement The authors are greatly indebted to Dr. K. Yamasaki, Institute of Pharmaceutical Sciences, School of Medicine, Hiroshima University, for the measurement of ^{13}C -NMR spectra.

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Received May 29, 1980

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