

## Notes

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**Kôiti Kimura,\*<sup>1</sup> Kazuko Yamauchi,\*<sup>2</sup> and Shigeaki Kuwano\*<sup>2</sup> : Studies on Tannins. IX.<sup>1)</sup> Effect of Tannins and Related Compounds on Tryptophanase Activity of *Escherichia coli*.**

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Tryptophan is decomposed in the large intestine to form indole, pyruvic acid, and ammonia by the action of the enzyme tryptophanase present in *Escherichia coli*. This reaction, first observed by Hopkins and Cole, occurs aerobically and has been studied in detail by a number of investigators.<sup>2)</sup> Wood, *et al.*<sup>3)</sup> extracted the enzyme from *E. coli* and demonstrated that pyridoxal phosphate functions as a coenzyme in this reaction. The formation of indole which, together with skatole, is mainly responsible for the odor of feces is a phenomenon characteristic to putrefaction in the intestine. It is also well known that degradation products of indole are frequently found in normal human urine.

In earlier papers of this series<sup>1,4)</sup> it was reported that tannins and related compounds exert rather contradictory actions towards two pyridoxal phosphate-dependent enzyme systems of *E. coli*, i. e., amino acid decarboxylase and cysteine desulfhydrase. Effects of polyphenolic compounds, especially gallic acid, on the activity of cysteine desulfhydrase under aerobic conditions were also described. The elucidation of this inhibition mechanism was, however, accompanied by difficulties because of the facts that the substrate protects gallic acid from oxidation and that the substrate itself is apt to undergo spontaneous oxidation even in neutral media.

In the present investigation, effects of tannins and related compounds on the action of another pyridoxal phosphate enzyme, tryptophanase of *E. coli*, were examined. The substrate of this enzyme, tryptophan, is resistant to aeration in slightly alkaline solution. It was thus found that most of the tannins and related compounds tested effectively inhibit the tryptophanase activity as it was the case with amino acid decarboxylase and cysteine desulfhydrase activities. It seems quite probable, therefore, that these compounds are effective inhibitors of putrefaction in the intestine.

## Materials and Methods

Preparation of cell suspensions (*E. coli* UKT-B strain) and assay of the tryptophanase activity (based on the determination of indole formed) were carried out according to a modification of the methods described by Murti, *et al.*<sup>5)</sup> and DeMoss, *et al.*<sup>6)</sup> The compounds tested in the present study were similar to those described in the previous paper.<sup>1)</sup> After the inhibitor was preincubated with cells\*<sup>4</sup> for 15 min. in the absence of substrate, the enzyme reaction was run at pH 7.5 and 37° for 30 min. under continuous aeration.

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\*<sup>4</sup> Dry weight approximately 0.7 mg.

1) Part VIII : This Bulletin, **7**, 426(1959).

2) F. C. Happold : *Advances in Enzymol.*, **10**, 51(1950).

3) W. A. Wood, I. C. Gunsalus, W. W. Umbreit : *J. Biol. Chem.*, **170**, 313(1947).

4) K. Kimura, S. Kuwano, H. Hikino : *Yakugaku Zasshi*, **78**, 236(1958).

5) K. L. Arora, C. R. K. Murti : *J. Sci. Ind. Research (India)*, **14C**, 6(1955).

6) L. H. Frank, R. D. DeMoss : *Arch. Biochem. Biophys.*, **67**, 387(1957).

### Results and Discussions

**Survey of Inhibitory Effects:** The inhibition tendency observed with tannins and related compounds towards the tryptophanase activity of *E. coli* was essentially the same as that reported for the cysteine desulphydrase system. As shown in Fig. 1, the inhibi-

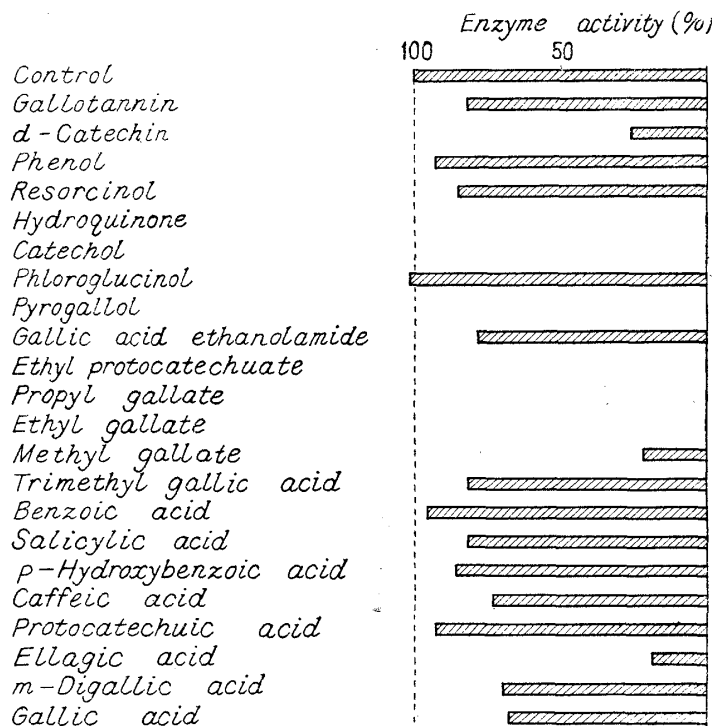


Fig. 1. Effect of Various Inhibitors at 0.0125M

tion-reducing effect of free carboxyl groups in the inhibitor molecule was more pronounced in the present system as compared with the latter system. Thus, even with salicylic, trimethylgallic, and caffeic acid only a small inhibition was observable. Ellagic acid which exerted only a slight inhibition towards cysteine desulphydrase was found to be a considerably powerful inhibitor in the present system. This acid (I) is formed when gallic acid is oxidized in alkaline media under aeration. This inhibition is of special interest in view of the fact that ellagic acid is the basic constituent of naturally

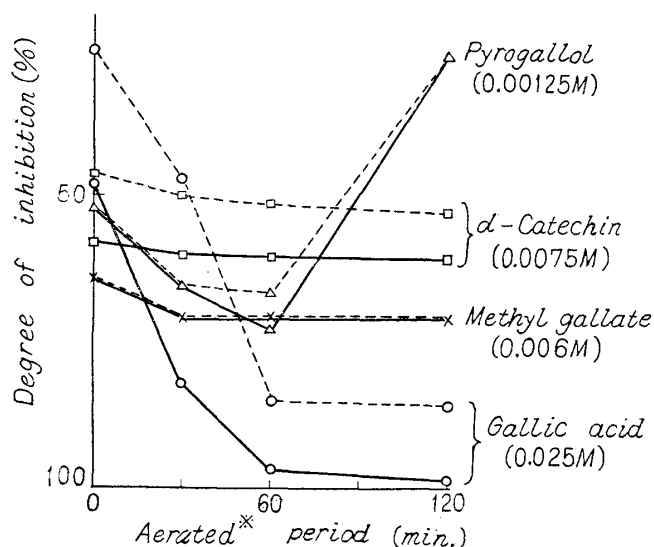
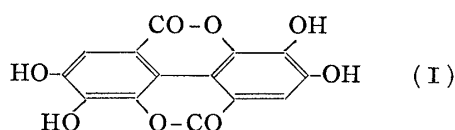


Fig. 2.

Effect of Aeration of Inhibitor Solution

\* Aerated in 0.1M phosphate buffer. After the inhibitor solution was preincubated with cells for 15 min., enzyme reaction is started.

Condition of enzyme reaction  
 — aerobic  
 - - - anaerobic (N<sub>2</sub> bubbling)



occurring ellag-tannins and that it possesses a very limited solubility. As is shown in Fig. 2, the inhibition caused by gallic acid increased progressively with aeration. This may be due, at least partly, to the formation of ellagic acid by oxidation of gallic acid. Since oxidation of methyl gallate and *d*-catechin by air did not result in any increase in their inhibitory action (Fig. 2), it seems untenable that the increase in inhibition by aeration of gallic acid solution is solely due to oxidation of the acid to quinone-type compounds.

As can be seen from Fig. 2, pyrogallol behaved in a very peculiar way during aeration. The inhibitory effect of this compound increased considerably with aeration, but on further extensive aeration its inhibition was completely reversed. The reason for this rather peculiar phenomenon is not yet clear.

When the effect of gallic acid and its three alkyl esters, e.g. methyl, ethyl, and propyl gallates, was compared, the inhibitory action was found to increase in the order of gallic acid, methyl gallate, ethyl gallate, and propyl gallate. The effect of propyl gallate was particularly predominant at higher concentrations.

The maximum inhibition by gallic acid, methyl gallate, pyrogallol, and *d*-catechin could only be attained after prolonged preincubation of cells with the inhibitor. Furthermore, when the inhibitory effect was examined at different pH's ranging from 6.5 to 8.0, the inhibition by gallic acid and pyrogallol became stronger with increased alkalinity probably due to the accelerated oxidation at higher pH's. On the contrary, the inhibition by methyl gallate and *d*-catechin was unaffected by increasing pH values. The inhibition at a given pH value was not different in either phosphate or citrate buffer. The inhibitory actions were, however, found to increase when the concentration of phosphate was lowered from 0.075*M* to 0.0125*M*.

**Mechanism of Inhibition**— These inhibitions could not be essentially antagonized by the coenzyme. They are non-competitive with the substrate and are not generally protected by the substrate.\*<sup>5</sup> Although the inhibition by gallic acid or pyrogallol is partially reversed by cysteine, glutathione, sodium thioglycollate, or glucose, these compounds may probably act as protectors of gallic acid and pyrogallol from oxidation. The evidence that the degree of inhibition by gallic acid or *d*-catechin was invariable with varying cell concentrations is quite in contrast with the results obtained with the cysteine desulfhydrase system.

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\*<sup>5</sup> Methyl gallate and pyrogallol, slightly, are protective.