Communications to the Editor

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INHIBITORY EFFECT OF TANNINS ON DIRECT-ACTING MUTAGENS

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Remarkably strong inhibition of mutagenicity of 3-hydroxyamino-1-methyl-5H-pyrido[4,3-*b*]indole due to direct action of tannins on the mutagen was found. Strong inhibition of mutagenicity of 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene was observed for the extract of *Geranium thunbergii*.

KEYWORDS —— inhibition of mutagenicity; Trp-P-1; Trp-P-2; *N*-OH-Trp-P-2; benzopyrene diol epoxide; tannin; geraniin; penta-*O*-galloyl-β-D-glucopyranose; epigallocatechin gallate; *Geranium thunbergii*

We have examined the influence of tannins on mutagens, and previously found significant inhibition of the mutagenicity of Trp-P-1 (+S9) and MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine) by the extracts of tea, and also by (-)-epigallocatechin gallate and (-)-epicatechin gallate which were isolated from green tea.[1] Strong inhibition of these mutagens by other tannins, geraniin,[2] mallotusinic acid,[3] pedunculagin,[4] and agrimoniin,[5] which were isolated from several medicinal plants, was also observed. These tannins and related polyphenols and the extracts containing these compounds did not show any mutagenicity by themselves.[1] Association of tannins with S9 was provisionally regarded as one of the main actions of inhibiting Trp-P-1, based on the generally known affinity of tannins to proteins. Since the metabolically activated form of Trp-P-2, *i.e.* 3-hydroxyamino-1-methyl-5H-pyrido[4,3-*b*]indole (*N*-OH-Trp-P-2),[6] is now available, we have now explored the possibility that tannins might interfere with the activity of this directly acting mutagen. The inhibition of (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (B[*a*]p diol epoxide), another direct mutagen, by the extracts of *Geranium thunbergii* SIEB. *et* ZUCC., which is one of the most frequently used medicinal plants in Japan, was also examined.

The experiments were carried out by the preincubation method[7] using *Salmonella typhimurium* TA98 and TA100. The bacterial culture (0.1 ml) and S9 mix (0.5 ml) or 0.1 M HEPES buffer (pH 7.4) were added to the test compound and mutagen in 0.1 ml of dimethylsulfoxide or water. The mixture was poured onto a plate of minimal glucose agar containing 0.1 μmol each of histidine and bioton. The S9 mix contained 0.02 ml S9 which was prepared from the livers of rats

treated with polychlorinated biphenyls (PCB), 2 μmol NADPH, 2 μmol NADH, 2.5 μmol ATP and 2.5 μmol G6P as co-factors in a total volume of 0.5 ml. Colonies were counted after incubation for two days. The amounts of each tannin as the test compound were 0-0.3 mg/plate for Trp-P-2 (0.30 nmol), 0-1.0 mg/plate for B[*a*]p diol epoxide (0.1 nmol), and 0-3.0 μg/plate for *N*-OH-Trp-P-2 (0.035 nmol). The herb (6 g) of *G. thunbergii*, which had been cut into pieces of about 5 mm in length, was heated in water (100 ml). Samples (3 ml portions) of this aqueous extract were taken at desired intervals and freeze-dried to prepare materials for assay.

The strong inhibition of mutagenicity of Trp-P-2 (+S9) and *N*-OH-Trp-P-2 (-S9) by the tannins and related polyphenols is shown in Fig. 1. The ratios of the amounts of tannins and polyphenols to *N*-OH-Trp-P-2 required for the inhibition of *N*-OH-Trp-P-2 were mostly less than one-tenth of the ratios required for the inhibition of Trp-P-2.

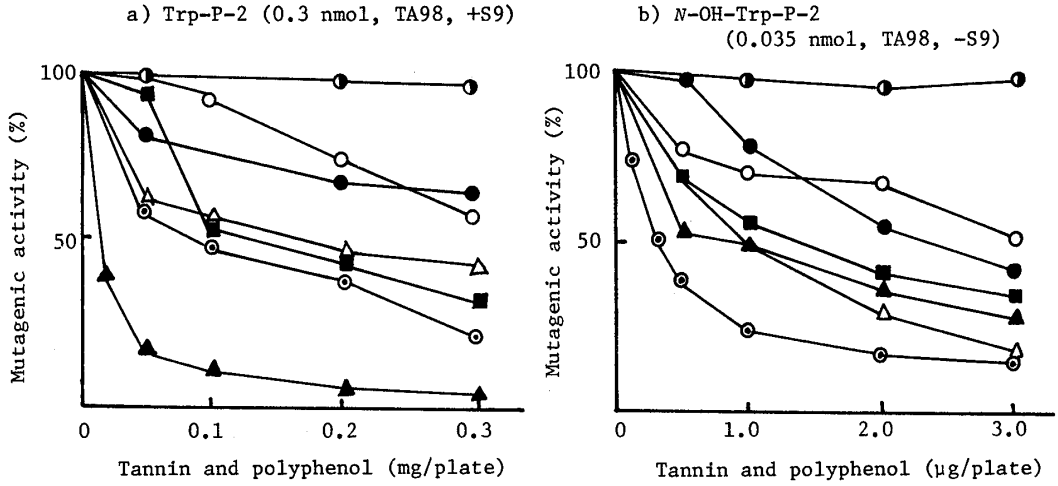


Fig. 1. Effects of Tannins on the Mutagenicity of Trp-P-2 and *N*-OH-Trp-P-2

-●- gallic acid -○- (-)-epicatechin gallate -●- (-)-epigallocatechin gallate
-△- ellagic acid -■- pedunculagin -⊙- penta-*O*-galloylglucose
-▲- geraniin

The His^+ revertant colonies observed in the absence of inhibitors were 3330/plate for *N*-OH-Trp-P-2 and 3890/plate for Trp-P-2. The mutagenic activity was calculated subtracting the revertant numbers from the observed results. The background found in solvent controls was 24±5.

The mutagenicity of B[*a*]p diol epoxide was reported to be exceptionally susceptible to the inhibition by ellagic acid.[8] Ellagic acid is abundantly produced in the extract of *G. thunbergii* as the result of hydrolysis of geraniin, the main tannin of this herb (see Chart 1).[9] The hydrolysis can be traced by high-performance liquid chromatography (HPLC).[10] We have examined the inhibition of mutagenicity of B[*a*]p diol epoxide by several isolated tannins, and also the change of the inhibition induced by difference of the conditions from extraction and hydrolysis of geraniin.

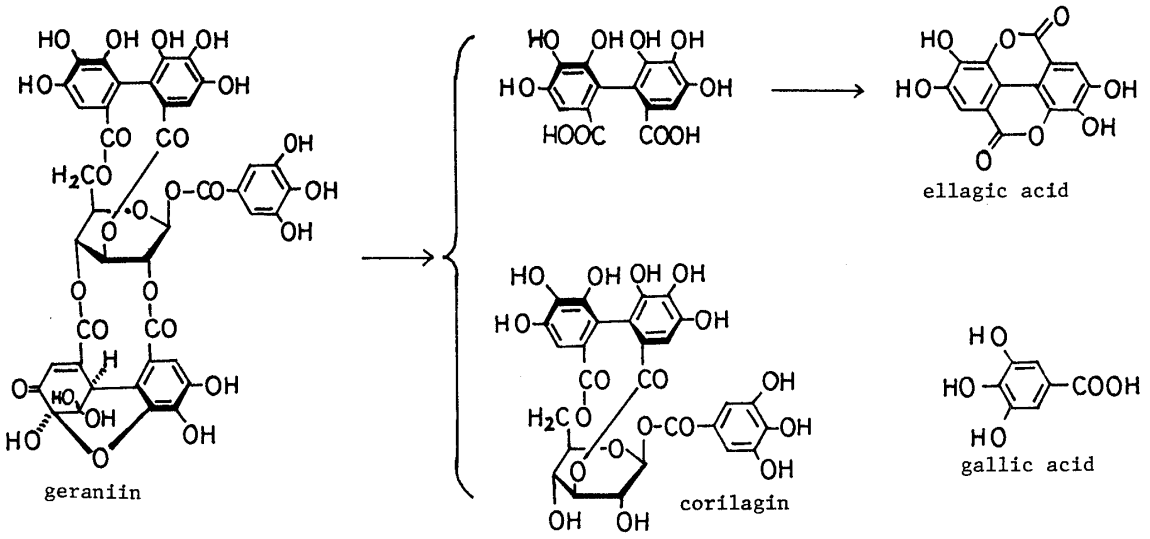
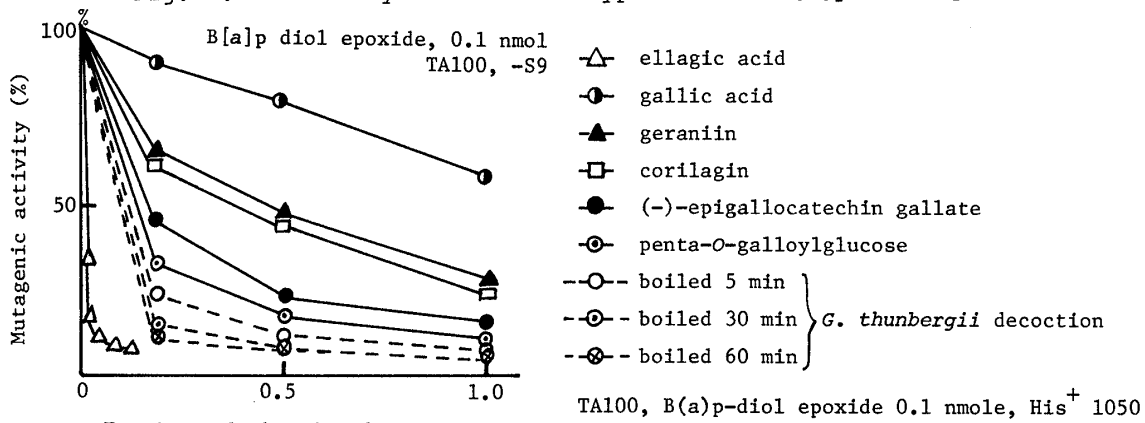


Chart 1

Fig. 2. Inhibitory Effect of Polyphenols on B[*a*]p Diol Epoxide



TA100, B(a)p-diol epoxide 0.1 nmole, His^+ 1050

The His^+ revertants found in the absence of the inhibitors were 1050/plate. The revertant numbers in solvent controls were 120±20.

As shown in Fig. 2, the inhibitory effect by the extract obtained by boiling for a short time (5 min), which contains geraniin as the main component, was significantly enhanced upon continuation of boiling for 30 minutes. Increase of ellagic acid in the extract was concurrently exhibited by HPLC. However, when boiling was continued over 30 minutes, both the amount of ellagic acid and the inhibitory effect did not show any further prominent increase.

This observation is in accord with the intensity of inhibition of B[*a*]p diol epoxide by each of the tannins and polyphenols among which ellagic acid showed the strongest inhibition.

The difference between this result and the inhibition of Trp-P-1 by the decoction of *G. thunbergii*, upon which the maximum of inhibition was observed at an earlier time when the amount of geraniin in the solution was largest,[2] is supported by the fact that the inhibition of Trp-P-1 by tannins is stronger than that by their hydrolysis products.[2]

These results indicate that the inhibition of Trp-P-1 and Trp-P-2 is most pronounced when the tannins having strong affinity to the basic compounds and also to proteins, as indicated by the high RMB[11,13] and RA[12,13] values, are not decomposed, while the specifically strong inhibition of B[*a*]p diol epoxide is exhibited by ellagic acid, which is one of the hydrolysis products from ellagitannins. It is noteworthy that significant inhibition of B[*a*]p diol epoxide, Trp-P-1 and Trp-P-2 was observed for (-)-epigallocatechin gallate; this compound is a main polyphenol in the tea leaf, and in spite of its comparatively small molecular weight, it shows fairly large RMB and RA values.

It is also notable that the extent of the inhibitory effect by penta-*O*-galloyl-β-D-glucopyranose, which is one of the components of tannic acid extracted from Chinese gall and Turkish gall, on *N*-OH-Trp-P-2 is almost comparable with the "exceptionally strong"[8] inhibition of B[*a*]p diol epoxide by ellagic acid.

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13) RMB (relative affinity for methylene blue) and RA (relative astringency) are the values for determination of tannin content in plant extracts and also of activity of polyphenols as tannin. They are shown relative to the value of tannic acid JP.

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