Note

Inhibitory Effects of Leaf Extracts from *Fagaceae* on Mutation in a Streptomycin-dependent Mutant of *Salmonella typhimurium* TA 98 under Assay Conditions without S-9 Mix

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Mutagens produced by chemical reactions of food constituents during storage, processing, and cooking are a

cause of great concern. *N*-Nitrosoamines are the best known of this kind,¹⁾ but recent finding of mutagenic pyrolytic products like 3-amino-1,4-dimethyl-5*H*-pyrido [4,3-*b*]indole (Trp-P1), 3-amino-1-methyl-5*H*-pyrido[4,3-*b*] indole (Trp-P2), and 2-amino-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P1)^{2~4)} found in broiled fish and meat, for example, have also been the cause of general alarm. Hence, many investigators have carried out detection and identification studies on desmutagens working on such powerful carcinogenic mutagens. We also reported that fermented milk and several strains of lactic acid bacteria used as starters for the manufacture of fermented milk have desmutagenic activities on such amino acid pyrolyzate mutagens.^{5~9)} Kada *et al.*¹⁰⁾ previously reported that extracts of several trees such as camellia, Japanese maple, beech, and oak had a considerable capacity to inactivate the mutagenicity of amino acid pyrolyzates. Ishii *et al.*¹¹⁾ checked the bio-antimutagenic capacities of extracts obtained from 303 different plants with criteria of suppressing UV-induced mutations in *Escherichia coli* B/r WP2 (trp) and the mutator activity of *Bacillus subtilis* NIG 1125 (his met), and found bio-antimutagenic activity in several trees like *Distylium racemonosus*, *Alnus sieboldiana*, and *Metasequoia glyptostroboides*. These studies promoted us to investigate inhibitory effects of

Table I. INHIBITORY ACTIVITIES OF THE LEAF EXTRACTS FROM *Fagaceae* AGAINST THE MUTAGENICITIES OF AMINO ACID PYROLYZATES

Sample	Extract ^a	Revertants/plate									
		Trp-P1			Trp-P2			Glu-P1			
		Control	Test	PI (%) ^b	Control	Test	PI (%) ^b	Control	Test	PI (%) ^b	
Quercus											
<i>Q. acutissima</i> Carr (<i>Kunugi</i>) ^c	55	608	43	92.9	1020	32	96.9	1160	96	91.7	
<i>Q. serrata</i> Thunb (<i>Konara</i>)	42	608	25	95.9	1020	42	95.9	1160	160	86.2	
<i>Q. crispula</i> Blume (<i>Mizunara</i>)	36	608	27	95.6	1020	34	96.7	1160	113	90.3	
<i>Q. variabilis</i> Blume (<i>Abemaki</i>)	36	608	17	97.2	1020	54	94.7	1160	65	94.4	
<i>Q. dentata</i> Thunb (<i>Kashiwa</i>)	52	608	66	89.2	1020	80	92.1	1160	126	89.1	
<i>Q. phylliraeoides</i> A. Gray (<i>Ubamekashi</i>)	75	608	95	84.4	1020	59	94.2	1160	184	84.1	
Cyclobalanopsis Oerst											
<i>C. glauca</i> Oerst (<i>Arakashi</i>)	46	608	145	76.1	1020	58	94.3	1160	189	83.7	
<i>C. myrsinaefolia</i> Oerst (<i>Shirakashi</i>)	63	608	134	78.0	1020	31	97.0	1160	158	86.4	
Castanea Mill											
<i>C. crenata</i> Sieb et Zucc (<i>Kuri</i>)	62	608	57	90.6	1020	35	96.6	1160	124	89.3	
Fagus Linn											
<i>F. crenata</i> Blume (<i>Buna</i>)	34	608	50	91.8	1020	102	90.0	1160	114	90.2	
<i>F. japonica</i> Maxim (<i>Inubuna</i>)	43	608	36	94.0	1020	40	96.0	1160	150	87.1	

^a Equal portions of each extract and distilled water were mixed and 40 μ l discharged on a sterile 8 mm paper disc as described in the text.

^b Percent inhibition (PI) was calculated by the following formula: PI = 100 - (Number of revertants of test plate)/(Number of revertants of control plates) $\times$ 100.

^c Words in the parentheses are Japanese common names.

various trees belonging to the family *Fagaceae*, which are largely grown in the central mountainous areas in Japan, on the mutagenicities of Trp-P1, Trp-P2, and Glu-P1 toward a streptomycin-dependent strain of *Salmonella typhimurium* TA 98.¹²⁾

Leaves of such trees as *Quercus acutissima* Carr, *Q. serrata* Thunb, *Q. crispula* Blume, *Q. variabilis* Blume, *Q. dentata* Thunb, *Q. phylliraeoides*, A. Gray, *Castanea crenata* Sieb et Zucc, *Cyclobalanopsis glauca* Oerst, *Cy. myrsinaefolia* Oerst, *Fagus crenata* Blume, and *F. japonica* Maxim were freshly harvested in the summer season in the Experimental Forest, Faculty of Agriculture, Shinshu University and their extracts were prepared. Fifty grams of the leaves from each tree were homogenized with 50 ml of ethanol for 3 min, and then centrifuged at 3000 rpm for 20 min. The whole volume of the supernatant was concentrated *in vacuo* to 10 ml, which was used as the extract. Inhibitory effect of the extracts on mutation in the streptomycin-dependent strain were estimated by measuring the decreases in mutations induced by Trp-P1, Trp-P2, or Glu-P1. A solution containing 100 μ l of either Trp-P1 (500 μ g/ml), Trp-P2 (500 μ g/ml), or Glu-P1 (1000 μ g/ml) in pure water was mixed with 100 μ l of each extract and incubated at $36 \pm 1^\circ\text{C}$ for 30 min with gentle shaking. Then, the mixture (40 μ l) was discharged on a sterile 8 mm paper disc placed in a plate containing Oxoid Nutrient Broth No.2 agar previously covered with 100 μ l of the *Salmonella* strain, which had been diluted 1000X with phosphate buffer, pH 6.98. All plates were incubated at $36 \pm 1^\circ\text{C}$ for 2 days, and revertant colonies per plate were counted after incubation. Distilled water was used instead of the extracts as a control. All tests were in triplicate.

All the extracts examined were weakly mutagenic for the *Salmonella* strain with a dose-related response. However, as will be seen from Table I, these extracts showed significantly high inhibitory effects of ~83% against Trp-P1, Trp-P2, and Glu-P1 with the only exception that the extracts of *Cy. glauca* and *Cy. myrsinaefolia* gave PI values of 76.1 and 78.0% against Trp-P1, respectively. In general, the majority of inhibitors of mutagenesis which naturally occurred in many plants were demonstrated to be aromatic isothiocyanates, coumarins, flavones, dithioglucosides, diterpenes, retinoid, ascorbic acid, α -tocopherol, selenium salts, and sterols, etc.¹³⁾ Furthermore, specific phenolics closely related to lignin biosynthesis in wood formation are also known to act as inhibitors of mutational and precarcinogenic events.¹⁴⁾ This has led to the suggestion that phenolics containing in the extracts examined could be, in part, inhibitors against mutation, although quantitative data on the actual contents of these compounds in the leaves have not yet been obtained. However, in fact, we

have recently isolated a fraction having strong inhibitory activity against the mutagenicities of tryptophane pyrolyzates from the ethanol extract of *Q. acutissima* Carr by high performance liquid chromatography, and found that ellagic and/or gallic acids are major constituents of the fraction (data not shown). Hence, extensive work on quantitative analysis and characterization of the inhibitors against mutation containing in the beech samples is envisaged. Chemical isolation studies are in progress.

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