Bay Laurel Contains Antimutagenic Kaempferyl Coumarate Acting against the Dietary Carcinogen 3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2)

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A novel antimutagen in bay laurel (*Laurus nobilis* L.) acts against the dietary carcinogen 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2). The antimutagen was purified chromatographically from ethyl acetate extract of bay leaf and identified instrumentally to be 3-kaempferyl *p*-coumarate. The yield was 20 mg from 100 g of bay, and its IC_{50} value, the amount required for 50% inhibition of the mutagenicity of 20 ng of Trp-P-2, was 1.9 μg. This value is close to those for strong antimutagens such as flavones and flavonols. The antimutagenicity was due to a desmutagenic action that inhibited the metabolic activation of Trp-P-2 to its ultimate carcinogenic form. The kaempferyl moiety contributed to the activity. Interestingly, this compound had weak bioantimutagenicity and could also suppress the mutagenicity of direct mutagens.

Keywords: *Kaempferyl coumarate; antimutagen; flavonoid; dietary carcinogen; cancer prevention*

INTRODUCTION

A close correlation is found epidemiologically between human cancer and diet (Dole and Peto, 1981). Human carcinogenesis depends on intakes of both food-derived carcinogens and anticarcinogens. The most abundant carcinogens in the human diet are heterocyclic amines, estimated to be consumed at 0.4−16 μg per day per capita (Knasmüller et al., 1992; Wakabayashi et al., 1992). One is unable to avoid heterocyclic amines because they form during cooking (Yamaizumi et al., 1980). However, some vegetables may prevent carcinogenesis (Cannon et al., 1997). Thus, a better understanding of which vegetables provide anticarcinogenic factors against heterocyclic amines is needed.

All heterocyclic amines exert genotoxicity via the same mechanism after undergoing metabolic activation (Kato et al., 1983). For example, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) is metabolized to 3-hydroxyamino-Trp-P-2 (*N*-hydroxy-Trp-P-2) and then to *N*-acetoxy-Trp-P-2 (Kato and Yamazoe, 1987; Davis et al., 1993). Both metabolites easily make DNA adducts and can induce a frameshift mutation (Wakata et al., 1985; Frandsen et al., 1990; Turesky et al., 1991; Synderwine et al., 1993). The mutation is an initiation step of carcinogenesis and can be evaluated by *Salmonella* tests in the presence of an S9 mix (Kato and Yamazoe, 1987). There have been several attempts using this method to find vegetables with antimutagenic activity against heterocyclic amines (Alldrick et al., 1986; Edenharder et al., 1995). In particular, herbs manifest strong antimutagenicity and contain the active compounds flavones and flavonols (Kanazawa et al., 1995; Samejima et al., 1995). Their antimutagenic action is a specific inhibition of the activation of heterocyclic amines by hepatic cytochrome P450 1A enzymes (Kanazawa et al., 1998).

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We have found that bay laurel (*Laurus nobilis* L.) contains strong antimutagens against Trp-P-2 (Natake et al., 1989). Bay, unlike most other popular herbs, is a tree leaf rather than a grass. Its active compounds may be novel. In the present study, we examined these active compounds and found a novel desmutagen, 3-kaempferyl *p*-coumarate.

MATERIALS AND METHODS

Materials. Fresh leaves of bay laurel were collected in our garden. Trp-P-2 was used from Wako Pure Chemical Industries Ltd., and the other carcinogens were from Aldrich Chemical Co. Ltd. Standard chemicals, kaempferol, *p*-coumaric acid, and kaempferol 3-*O*-glycosides, were obtained from Funakoshi Co. Ltd., Tokyo Kasei Co. Ltd. and Extrasynthèse S.A. Co., respectively. Agar and extracts of beef and yeast (nutrient broth) for the bacterial cultivation were purchased from Difco Laboratory. Organic solvents and water were distilled twice. All other chemicals were commercially available in high grade.

Extract from Bay. The fresh leaves were washed with water, homogenized in liquid nitrogen with a Waring blender, and lyophilized. The freeze-dried bay powder was sonicated in 10 volumes of a solvent for 20 min, and the extract was recovered by filtration through a Whatman 1PS filter paper. The solvents were used in order of polarity: hexane, methylene chloride, ethyl acetate, acetone, methanol, and water. After extraction with one solvent three times, the residue was extracted with another solvent. These extracts were recovered quantitatively, dried, weighed, and evaluated for antimutagenicity.

Evaluation of Antimutagenicity against Trp-P-2. Each of the extracts was evaluated as to the antimutagenicity against 20 ng of Trp-P-2 essentially according to the method of Ames et al. (1975) with some minor modifications as mentioned previously (Kanazawa et al., 1995). The S9 mix was prepared with the S9 fraction obtained from the liver of Sprague−Dawley rats given 500 mg/kg of body weight polychlorinated biphenyl 5 days before sacrifice (Mizuno et al., 1987). *Salmonella typhimurium* TA98 was grown overnight in liquid broth medium at 37 °C. The bay extracts were dried

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and dissolved in 0.1 mL of dimethyl sulfoxide and then incubated with Trp-P-2, in 0.1 mL of water at 37 °C for 30 min. After the additions of 0.5 mL of S9 mix and 0.1 mL of bacterial suspension, the mixture was incubated again at 37 °C for 20 min. It was then mixed with 2 mL of molten top agar and poured onto an agar medium of minimal glucose. After a 2-day culture, the His^+-revertant colonies were enumerated.

These tests were carried out independently three times with three plates each time, and the results were calculated as follows: $[(A - B) - (C - D)]/(A - B) \times 100$, where A is the number of revertants obtained with Trp-P-2, B is the number of spontaneous revertants, C is the number of revertants obtained with both the extract and Trp-P-2, and D is the number of revertants obtained with the extract. Dose−response curves were constructed with six different doses of the extracts, and IC_{50} values were determined by plotting the antimutagenicity versus the log of the dosed amounts (Kanazawa et al., 1995). The IC_{50} is the amount required for 50% inhibition of the mutagenicity of 20 ng of Trp-P-2, and the lower the value, the greater the activity.

Bioantimutagenicity Test. The bioantimutagenicity of bay compound was examined as described by Mizuno et al. (1989) with some minor modifications. Trp-P-2 (20 ng in 0.1 mL of water) was metabolically activated with 0.5 mL of S9 mix for 5 min at 37 °C and then placed in boiling water for 20 s to inactivate the S9 enzymes. After cooling to room temperature, the solution was incubated with 0.1 mL of bacterial suspension for 15 min at 37 °C. Following this, the bay compound in 0.1 mL of dimethyl sulfoxide was added and the incubation continued for another 20 min. The His^+-revertants were enumerated as above.

Chromatography. The ethyl acetate extract from bay was dried and dissolved in methanol and subjected to gel filtration chromatography on a Sephadex LH-20 column (∅ 2.5 × 55 cm) with methanol at a flow rate of 2.5 mL/min. The absorbance of the effluents was monitored at 280 and 360 nm. Different fractions obtained were dried under a nitrogen stream, weighed, and assayed for antimutagenicity. The most active fraction was chromatographed with 0.5 mL/min of chloroform/methanol (9:1) on a silica gel column (Wakogel C-100, ∅ 14 × 30 cm). Finally, the active fraction obtained was purified on the same silica gel column with chloroform/ethyl acetate (1:1) and by recrystallization from chloroform/hexane (1:1).

Instrumental Analyses. The chemical structure of the purified compound was elucidated with spectral analyses of ultraviolet nuclear magnetic resonance (NMR) (Bruker AC-250) and mass (JEOL DX-500) with gas chromatography−mass spectrometry (GC/MS), field desorption (FD/MS), and electron ionization (EI/MS). In the EI/MS, a direct inlet probe elevating the temperature from 70 °C at 16 °C/min, an ion source temperature of 200 °C, and an ionizing voltage of 70 eV were used.

RESULTS

Separation of the Antimutagenic Compounds in Bay. Table 1 compares the antimutagenicity among extracts when freeze-dried bay leaf was extracted successively with hexane, methylene chloride, ethyl acetate, acetone, methanol, and water. Every extract showed a dose-dependent curve except the water extract. The ethyl acetate extract showed the most potent activity, and hence it was chosen for further study. Gel filtration chromatography of this extract on Sephadex LH-20 produced an active fraction with an IC_{50} value of 10 μg. The fraction was passed through a silica gel column with chloroform/methanol to obtain a more active fraction (IC_{50} = 5 μg). This product was further chromatographed on the same column with chloroform/ethyl acetate and recrystallized from chloroform/hexane. The pure antimutagen showed an IC_{50} value of 1.9 μg. Its yield was 20 mg from 100 g of the freeze-dried bay leaf. This IC_{50} value was close to the values that had been given by the strong antimutagens quercetin (IC_{50} = 0.81 μg), galangin (IC_{50} = 0.12 μg), and luteolin (IC_{50} = 0.14 μg) (Kanazawa et al., 1995; Samejima et al., 1995). In addition, the present compound did not decrease the number of surviving bacteria, indicating no cytotoxicity (data not shown). The isolated compound was submitted to the following analysis of chemical structure.

Table 1. Antimutagenicity of the Extracts from Bay Leaf and of the Purified Compound toward Trp-P-2

extract and separated fraction	IC_{50}[a] (μg)	yield[b] (g)
extract		
hexane	80	1.9
methylene chloride	33	3.2
ethyl acetate	25	1.7
acetone	>105[c]	1.4
methanol	>120[c]	19.2
water	not active	5.9
purification in the ethyl acetate extract[d]		
fraction from Sephadex LH-20	10	0.10
fraction from 1st silica gel column	5.0	0.05
purified compound	1.9	0.02

[a] The IC_{50} values (the amount required for 50% inhibition of the mutagenicity of 20 ng of Trp-P-2) were determined with the dose-dependent curves of antimutagenicity as mentioned under Materials and Methods. [b] Yields from 100 g of the freeze-dried bay leaf. [c] These values were estimated with an exploration from the dose-dependent curves using <100 μg because larger amounts were refractory to dissolution in the assay solution. [d] The purification procedures are outlined in the text.

Identification of the Antimutagen in Bay. The purified antimutagen was positive to ferric chloride, 2,4-dinitrophenylhydrazine, and hydroxylamine reagents, indicating the presence of phenolic hydroxyls, carbonyls, and ester bonds, respectively. The compound has λ_{max} at 210, 225, 270, and 315 nm in methanol. The FD/MS spectrum showed m/z 432 (M^+) as a molecular ion peak and two major fragment ion peaks: m/z 286 ($[C_{15}H_{10}O_6]^+$) as a base peak and m/z 164 ($[C_9H_8O_3]^+$). Next, this compound was subjected to methanolysis with 5% HCl in methanol and directly injected to GC/MS. The spectrum showed a molecular ion at m/z 178 (M^+) and fragment ions at m/z 147 ($-OCH_3$) as a base peak, m/z 119 ($-OCH_3$ and $-CO$), and m/z 91 ($C_7H_7^+$). This fragmentation coincided with that of standard methyl *p*-coumarate. Also, a co-injection of the methanolysis product with methyl *p*-coumarate gave a single peak on gas chromatography. These results indicated that the isolated antimutagen contained a *p*-coumaryl moiety.

On the other hand, the antimutagen turned yellow after the methanolysis. The yellow product was purified by thin-layer chromatography with chloroform/methanol (9:1). The product has λ_{max} at 210, 225, 305, and 358 nm in methanol. The spectrum shifted to a longer wavelength upon addition of aluminum chloride, indicating a flavonoid skeleton (Mabry et al., 1970). The EI/MS spectrum of the yellow product gave a molecular ion at m/z 286 (M^+) as a base peak and fragment ions at m/z 258 ($-CO$), 153 ($[C_7H_5O_4]^+$), and 121 ($[C_7H_5O_2]^+$). The latter two fragment ion peaks appeared to be from the A ring and B ring of the flavonoid skeleton, respectively, and the fragmentation coincided with that of standard kaempferol. These results indicated that one moiety in the bay antimutagen was kaempferol.

An analysis with ^{13}C NMR was employed for the bay antimutagen in dimethyl sulfoxide-d_6, comparing it to the yellow product and standard *p*-coumaric acid and

Table 2. Pertinent Carbon NMR Absorptions of Bay Antimutagen, Standard Kaempferol, and *p*-Coumaric Acid

	chemical shifts[b] (ppm)		
carbon[a]	bay antimutagen	kaempferol	*p*-coumaric acid
2	155.3	147.4	
3	136.7	135.8	
4	176.7	176.1	
5	156.6	156.6	
6	98.4	98.7	
7	164.1	163.9	
8	93.7	94.1	
9	161.1	160.8	
10	103.7	103.5	
1′	122.4	122.2	
2′	130.2	130.1	
3′	115.6	115.9	
4′	159.2	159.1	
5′	115.6	115.9	
6′	130.2	130.1	
1″	125.9		125.7
2″	130.9		130.4
3″	113.9		114.0
4″	161.0		161.0
5″	113.9		114.0
6″	130.9		130.4
7″	133.8		144.2
8″	127.4		115.4
9″	160.6		166.2

[a] See the carbon number in Figure 1. [b] The internal standard is tetramethylsilane.

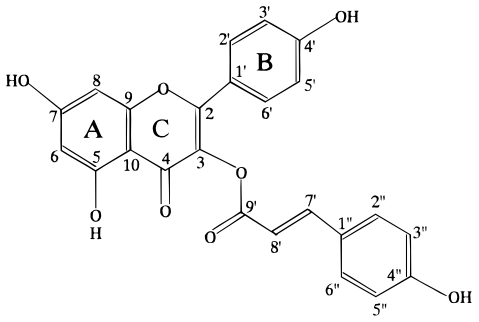

Figure 1. Antimutagen in bay laurel leaf, 3-kaempferyl *p*-coumarate.

kaempferol (Table 2). The spectrum of yellow product was identical with that of standard kaempferol. The chemical shifts from C-3 to C-6′ of bay antimutagen coincided with those of standard kaempferol, and the shifts of C-1″ to C-6″ coincided with those of standard *p*-coumaric acid. With regard to the binding site of the *p*-coumaryl moiety, the chemical shift for C-2 of the bay antimutagen was at a large downfield shift (+7.9 ppm) compared to that of kaempferol. It is shown that an acylation of OH on C-3 causes the downfield shift of C-2 by analyses of kaempferol 3-*O*-glycosides (Wollenweber and Dietz, 1981). We further analyzed kaempferol 3-*O*-glucoside and 3-*O*-rutinoside and confirmed the shift of C-2 (data not shown). Thus, the antimutagen in bay laurel was identified as 3-kaempferyl *p*-coumarate [5,7-dihydroxy-2-(4-hydroxyphenyl)-3-(4′-hydroxyphenyl)-2-propenoyl-4*H*-1-benzopyran-4-one] as shown in Figure 1.

Desmutagenicity of Kaempferyl Coumarate. Antimutagenicity is classified into desmutagenicity and bioantimutagenicity according to mode of action (Kada and Shimoi, 1987). The former is an inhibiting effect on the metabolic activation of mutagens and/or a neutralizing effect on the activated mutagens before the attack on DNA. The latter suppresses activity in the process of mutagenesis (mutation fixation) after DNA has been damaged by the mutagen. Kaempferyl coumarate isolated in the present study was examined for its bioactivity (Figure 2). This compound manifested bioantimutagenicity at levels between 10 and 100 μg/plate dose-dependently. However, the activity was different from the IC_{50} value of 1.9 μg in Table 1. When the compound was added to previously activated Trp-P-2 (*N*-hydroxy-Trp-P-2), the revertant number remained almost unchanged. In contrast, addition during the activation of Trp-P-2 by S9 mix suppressed the mutagenicity at levels between 1 and 10 μg/plate dose-dependently. The suppressing effect was very close to the IC_{50} value of 1.9 μg. Thus, the kaempferyl coumarate was a desmutagen inhibiting the metabolic activation of Trp-P-2 by the S9 enzymes.

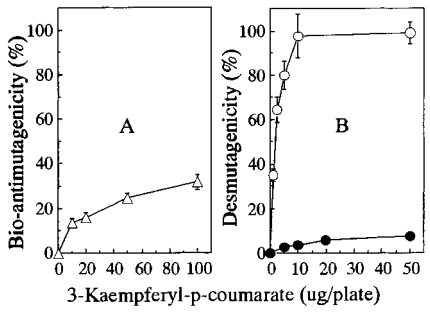

Figure 2. Bioantimutagenicity (A) and desmutagenicity (B) of the bay kaempferyl coumarate. The bioantimutagenicity (△) against mutated bacteria was tested as mentioned under Materials and Methods. In panel B, the effect on the activation of Trp-P-2 (○) was determined according to the method used for the antimutagenicity test under Materials and Methods. To examine the effect on the activated Trp-P-2 (*N*-hydroxy-Trp-P-2) (●), 20 ng of Trp-P-2 in 0.1 mL of water was metabolically activated with 0.5 mL of S9 mix for 5 min at 37 °C, and then the S9 enzymes were inactivated in boiling water for 20 s. After cooling, the solution was incubated with kaempferyl coumarate and bacterial suspension and assayed as to His^+-revertant number as for the antimutagenicity test.

Table 3. Comparison of Desmutagenicities between Kaempferyl Coumarate and Its Constituent Chemicals

chemical[a]	desmutagenicity against 20 ng of Trp-P-2, IC_{50}[b] (μg)
3-kaempferyl *p*-coumarate	1.9
kaempferol	0.24
p-coumaric acid	36
methyl *p*-coumarate	32

[a] Kaempferyl coumarate was purified from bay leaf, and the others were commercial chemicals. [b] See Table 1.

So which moiety, kaempferol or coumaric acid, contributes to the desmutagenicity? Table 3 compares the desmutagenicities among kaempferol, *p*-coumaric acid, and methyl *p*-coumarate. Kaempferol showed a lower IC_{50} value (0.24 μg) than kaempferyl coumarate, but both coumaric acid and methyl coumarate gave much higher values. These results indicated that the activity of kaempferyl coumarate was mostly due to the kaempferyl moiety.

Another point of interest is whether kaempferyl coumarate is effective against other mutagenic carcinogens. Table 4 shows the effects of kaempferyl coumarate on the four different types of carcinogens. Benzo[*a*]pyrene (B[*a*]P) and 1-nitropyrene (1-NP) are frameshift type mutagens on *S. typhimurium* TA98 strain, and 2-(acetylamino)fluorene (AAF) and *N*-methyl-*N*″-nitro-*N*-nitrosoguanidine (MNNG) are base-pair-change type

Table 4. Effects of Kaempferyl Coumarate on the Mutagenicity of Four Different Types of Carcinogens[a]

kaempferyl coumarate (μg)	revertant number[b]			
	TA98 cell		TA100 cell	
	B[*a*]P + S9	1 − NP	AAF + S9	MNNG
0	301 ± 12	8470 ± 455	4610 ± 401	545 ± 53
5	287 ± 70	7026 ± 988	7837 ± 1016	315 ± 61
10	182 ± 55	4215 ± 522	8536 ± 1159	178 ± 24

[a] The effects were determined on 500 μg of B[*a*]P; 2 μg of 1-NP; 100 μg of AAF; and 5 μg of MNNG ″ with the method in Table 1, except 1-NP and MNNG were tested in the absence of S9 mix and *S. typhimurium* TA100 strain was used for AAF and MNNG. [b] Mean ± SD minus the spontaneous revertants.

mutagens. B[*a*]P and AAF are indirect mutagens as they require metabolic activation by S9 mix to express mutagenicity, whereas 1-NP and MNNG are direct mutagens and do not require the activation. Kaempferyl coumarate reduced slightly the number of revertants obtained with B[*a*]P but markedly enhanced the mutagenicity of AAF. Interestingly, this compound at the concentration of 10 μg/plate suppressed the mutagenicity of indirect mutagens, 1-NP and MNNG, by 50% or more.

DISCUSSION

This study identified a novel desmutagen, 3-kaempferyl *p*-coumarate, in bay laurel leaf. The moiety, kaempferol, is a flavonol and is widespread in plants, commonly occurring as *O*-glycosides (Herrmann, 1976; Harborne, 1986). Acylation occasionally takes place at the sugar moiety (Fiorini et al., 1998) but rarely at the flavonol nucleus itself (Wollenweber, 1985). The present compound was a unique phytochemical because it was acylated directly with an acid, coumaric acid (Table 2 and Figure 1). A compound, named platanetin, in the bud of platanus (*Platanus acerifolia* Willd.) had been speculated to be kaempferyl coumarate (Kaouadji, 1986). However, the present study is the first to identify chemically 3-kaempferyl *p*-coumarate as a bioactive compound in bay leaf.

The IC_{50} value of kaempferyl coumarate to suppress the mutagenicity of 20 ng of Trp-P-2 was 1.9 μg (Table 1), which converts to 4.4 nmol with a molecular weight of 432 (Figure 1). This value is much lower than those of other phytochemicals such as chlorophylls, vitamins, carotenoids, xanthophylls, sterols, and saponins (Samejima, 1995). Kaempferyl coumarate is a strong desmutagen. Other strong desmutagens include flavonoids: flavones, flavonols, isoflavones, and their glycosides (Kanazawa et al., 1998). The IC_{50} values of flavones and flavonols are in the range of 0.23 (flavone) to 3.8 nmol (fisetin). The values of isoflavones lie between 13 (genistein) and 79 nmol (daidzein), and those of glycosides between 4.1 (quercitrin) and 529 nmol (apigetrin). The desmutagenicity of kaempferyl coumarate was in the same range as that of flavones and flavonols, the strongest desmutagens.

The activity of kaempferyl coumarate was due to a desmutagenic action that inhibited the metabolic activation of Trp-P-2 to its ultimate carcinogenic form, *N*-hydroxy-Trp-P-2 (Figure 2). The activation is mediated by cytochrome P450 1A family enzymes (Funae and Imaoka, 1993; Minamoto and Kanazawa, 1995), and thus kaempferyl coumarate inhibits the enzymes as flavones and flavonols (Lee et al., 1994; Tsyrlov et al., 1994; Siess et al., 1995; Kanazawa et al., 1998). The inhibitory effect was mainly due to the flavonol moiety (kaempferol) rather than the coumaric moiety (Table 3). Flavonols contain a region of affinity for the enzymes considered to be constructed of 7-, 8-carbons, 1-pyran, and 2′-, 3′-carbons (Kanazawa et al., 1998). Kaempferyl coumarate has this region without any large side chains. Interestingly, the 3-acylation of kaempferol with coumarate did not influence the activity, whereas the 3-glycosidation of flavonols decreased the activity. The coumarylation of kaempferol increased the IC_{50} value from 0.84 to 4.4 nmol (Table 3), but the glycosidation of quercetin to rutin raised the value from 2.4 to 31 nmol (Kanazawa et al., 1998). Coumarate, more hydrophobic than glucoside, appears not to interfere with the approach to the enzyme.

Kaempferyl coumarate suppressed the mutagenicity of B[*a*]P but enhanced that of AAF (Table 4), although both are activated by the same P450 1A family as Trp-P-2 (Aoyama et al., 1989; Funae and Imaoka, 1993; Doehmer, 1995). On the other hand, Propst et al. (1997) reported that P450 1A family enzymes mediated only partly the activation of AAF. The effect of kaempferyl coumarate on AAF may implicate the involvement of other metabolic enzymes. The kaempferyl coumarate suppressed the mutagenicity of direct mutagens (Table 4) and further showed weak bioantimutagenicity in a dose-dependent manner (Figure 2). This compound is expected to have activity other than cytochrome P450 inhibition, such as a direct interference with the DNA attack of mutagens, but this contention requires further examination.

The strong desmutagenicity of kaempferyl coumarate against Trp-P-2 indicates that it should be effective against other carcinogenic heterocyclic amines, because all heterocyclic amines undergo metabolic activation via the same mechanism (Kato et al., 1983). Heterocyclic amines are produced by heating meat. Grilled or broiled meats contain an appreciable amount of carcinogenic heterocyclic amines (Yamaizumi et al., 1980), and the sum of the mutagenicity is estimated to be equivalent to 20 ng of Trp-P-2/g of meat (Sugimura, 1985). Bay leaf is often used in soup or stews with meat. Bay leaf contains 200 μg/g of kaempferyl coumarate: 1.9 μg suppressed the toxicity of 20 ng Trp-P-2 by 50% (Table 1). Thus, adding 2 g of bay leaf for every 100 g of meat should mitigate heterocyclic amine toxicity.

LITERATURE CITED

Alldrick, A. J.; Flynn, J.; Rowland, I. R. Effects of plant-derived flavonoids and polyphenolic acids on the activity of mutagens from cooked food. *Mutat. Res.* **1986**, *163*, 225−232.

Ames, B. N.; McCann, J.; Yamasaki, E. Method for detecting carcinogens and mutagens with *Salmonella*/Mammalian microsomes mutagenicity test. *Mutat. Res.* **1975**, *31*, 347−364.

Aoyama, T.; Gonzalez, F. J.; Gelboin, H. V. Human cDNA-expressed cytochrome P450 1A2: mutagen activation and substrate specificity. *Mol. Carcinog.* **1989**, *2*, 192−198.

Cannon, G. *Food, Nutrition and the Prevention of Cancer: A Global Perspective*; World Cancer Research Fund/American Institute for Cancer Research: Washington, DC, 1997.

Davis, C. D.; Schut, H. A. J.; Snyderwine, E. G. Enzymatic phase II activation of the N-hydroxylamines of IQ, MeIQx, and PhIP by various organs of monkeys and rats. *Carcinogenesis* **1993**, *14*, 2091−2096.

Doehmer, J.; Holtkamp, D.; Soballa, V.; Raab, G.; Schmalix, W.; Seidel, A.; Greim, H.; Jacob, J. Cytochrome P450 mediated reactions studied in genetically engineered V79 Chinese hamster cells. *Pharmacogenetics* **1995**, *5*, S91−S96.

Doll, R.; Peto, R. *The Cause of Cancer*; Oxford University Press: Oxford, U.K., 1981.

Edenharder, R.; Leopold, C.; Kries, M. Modifying actions of solvent extracts from fruit and vegetable residues on 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) induced mutagenesis in *Salmonella typhimurium* TA98. *Mutat. Res.* **1995**, *341*, 303−318.

Fiorini, C.; David, B.; Fourasté, I.; Vercauteren, J. Acylated kaempferol glycosides from *Laurus nobilis* leaves. *Phytochemistry* **1998**, *47*, 821−824.

Frandsen, H.; Rasmussen, E. S.; Nielsen, P. A.; Farmer, P.; Dragsted, L.; Larsen, J. C. Metabolic formation, synthesis and genotoxicity of the N-hydroxy derivative of the food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Mutagenesis* **1990**, *6*, 93−98.

Funae, Y.; Imaoka, S. Cytochrome P450 in rodents. In *Handbook of Experimental Pharmacology*; Schenkman, J. B., Greim, H., Eds.; Springer-Verlag: Berlin, Germany, 1993; Vol. 105, pp 221−238.

Harborne, J. B. Nature, distribution, and function of plant flavonoids. In *Plant Flavonoids in Biology and Medicine*; Cody, V., Middleton, E., Harborne, J. B., Eds.; Alan R. Liss: New York, 1985; pp 15−24.

Herrmann, K. Flavonols and flavones in food plants: a review. *J. Food Technol.* **1976**, *11*, 433−448.

Kada, T.; Shimoi, K. Desmutagens and bio-antimutagens—Their modes of action. *BioEssays* **1987**, *7*, 113−116.

Kanazawa, K.; Kawasaki, H.; Samejima, K.; Ashida, H.; Danno, G. The specific desmutagens (antimutagens) in oregano against a dietary carcinogen, Trp-P-2, are galangin and quercetin. *J. Agric. Food Chem.* **1995**, *43*, 404−409.

Kanazawa, K.; Ashida, H.; Danno, G. Antimutagenic mechanism of flavonoids against a food-derived carcinogen, Trp-P-2, elucidated with the structure−activity relationships. In *Functional Foods for Disease Prevention II: Medicinal Plants and Other Foods*; Osawa, T., Shibamoto, T., Terao, J., Eds.; Symposium Series 702; American Chemical Society: Washington, DC, 1998; in press.

Kaouadji, M. Les bourgeons de platane, une source de flavonoides originaux. *Bull. Liaison: Groupe Polyphenols* **1986**, *13*, 586−589.

Kato, R.; Yamazoe, Y. Metabolic activation and covalent binding to nucleic acids of carcinogenic heterocyclic amines from cooked foods and amino acid pyrolysates. *Jpn. J. Cancer Res.* **1987**, *78*, 297−311.

Kato, R.; Kamataki, T.; Yamazoe, Y. *N*-Hydroxylation of carcinogenic and mutagenic aromatic amines. *Environ. Health Perspect.* **1983**, *49*, 21−25.

Knasmüller, S.; Kienzl, H.; Huber, W.; Herman, R. S. Organ-Specific Distribution of Genotoxic Effects in Mice Exposed to Cooked Food Mutagens. *Mutagenesis* **1992**, *7*, 235−241.

Lee, H.; Wang, H.-W.; Su, H.-Y.; Hao, N. J. The structure−activity relationships of flavonoids as inhibitors of cytochrome P-450 enzymes in rat liver microsomes and the mutagenicity of 2-amino-3-methyl-imidazo[4,5-*f*]quinoline. *Mutagenesis* **1994**, *9*, 101−106.

Mabry, T. J.; Markham, K. R.; Thomas, M. B. The Ultraviolet Spectra of Isoflavones, Flavanones and Dihydroflavonols. In *Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970; pp 165−226.

Minamoto, S.; Kanazawa, K. Electrochemical determination for enzymic production of ultimate carcinogen from tryptophan pyrolysate by rat hepatic microsomes. *Anal. Biochem.* **1995**, *225*, 143−148.

Mizuno, M.; Ohara, A.; Danno, G.; Kanazawa, K.; Natake, M. Mutagens formed from butylated hydroxyanisole treated with nitrite under acidic conditions. *Mutat. Res.* **1987**, *176*, 179−184.

Mizuno, M.; Toda, M.; Ueno, N.; Danno, G.; Kanazawa, K.; Natake, M. Desmutagenicity of a dibenzofuran-quinone derivative toward the mutagenicity of Trp-P-2. *Agric. Biol. Chem.* **1989**, *53*, 959−964.

Natake, M.; Kanazawa, K.; Mizuno, M.; Ueno, N.; Kobayashi, T.; Danno, G.; Minamoto, S. Herb water-extracts markedly suppress the mutagenicity of Trp-P-2. *Agric. Biol. Chem.* **1989**, *53*, 1423−1425.

Propst, T. L.; Fort, D. J.; Stover, E. L.; Schrock, B.; Bantle, J. A. Evaluation of the developmental toxicity of benzo[*a*]-pyrene and 2-acetylaminofluorene using Xenopus: modes of biotransformation. *Drug Chem. Toxicol.* **1997**, *20*, 45−61.

Samejima, K.; Kanazawa, K.; Ashida, H.; Danno, G. A strong antimutagen against dietary carcinogen, Trp-P-2, in peppermint, sage and thyme is luteolin. *J. Agric. Food Chem.* **1995**, *43*, 410−414.

Siess, M.-H.; Leclerc, J.; Canivenc-Lavier, M.-C.; Rat, P.; Suschetet, M. Heterogeneous effects of natural flavonoids on monooxygenase activities in human and rat liver microsomes. *Toxicol. Appl. Pharmacol.* **1995**, *130*, 73−78.

Snyderwine, E. G.; Davis, C. D.; Nouso, K.; Roller, P. P.; Schut, H. A. J. ^{32}P-Postlabeling analysis of IQ, MeIQx and PhIP adducts formed *in vitro* in DNA and polynucleotides and found *in vivo* in hepatic DNA from IQ-, MeIQx- and PhIP-treated monkeys. *Carcinogenesis* **1993**, *14*, 1389−1395.

Sugimura, T. Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. *Mutat. Res.* **1985**, *150*, 33−41.

Tsyrlov, I. B.; Mikhailenko, V. M.; Gelboin, H. V. Isozyme- and species-specific susceptibility of cDNA-expressed CYP1A *P*-450s to different flavonoids. *Biochim. Biophys. Acta* **1994**, *1205*, 325−335.

Turesky, R. J.; Lang, N. P.; Butler, M. A.; Teitel, C. H.; Kadlubar, F. F. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. *Carcinogenesis* **1991**, *12*, 1839−1845.

Wakabayashi, K.; Nagao, M.; Esumi, H.; Sugimura, T. Food-Derived Mutagens and Carcinogens. *Cancer Res.* **1992**, *52*, 2092s−2098s.

Wakata, A.; Oka, N.; Hiramoto, K.; Yoshioka, A.; Negishi, K.; Wataya, Y.; Hayatsu, H. DNA strand cleavage in vitro by 3-hydroxyamino-1-methyl-5*H*-pyrido[4,3-*b*]-indole, a direct-acting mutagen formed in the metabolism of carcinogenic 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole. *Cancer Res.* **1985**, *45*, 5867−5871.

Wollenweber, E. On the occurrence of acylated flavonoid aglycones. *Phytochemistry* **1985**, *24*, 1493−1494.

Wollenweber, E.; Dietz, V. H. Occurrence and distribution of free flavonoid aglycones in plants. *Phytochemistry* **1981**, *20*, 869−932.

Yamaizumi, Z.; Shiomi, T.; Kasai, H.; Nishimura, S.; Takahashi, Y.; Nagao, M.; Sugimura, T. Detection of potent mutagens, Trp-P-1 and Trp-P-2, in broiled fish. *Cancer Lett.* **1980**, *9*, 75−83.

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