Specific Desmutagens (Antimutagens) in Oregano against a Dietary Carcinogen, Trp-P-2, Are Galangin and Quercetin

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This study found two compounds which strongly suppressed the mutagenicity of one of the dietary carcinogens, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2), in oregano (*Origanum vulgare*). The compounds were purified chromatographically monitoring the suppressing activity with *Salmonella typhimurium* TA98. An instrumental elucidation identified two flavonoids as 3,5,7-trihydroxy-2-phenyl-4*H*-1-benzopyran-4-one (galangin) and 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one (quercetin). Galangin and quercetin are strong desmutagens that neutralized Trp-P-2 before mutating the bacteria. The amounts of galangin and quercetin required for 50% inhibition against the mutagenicity of 20 ng of Trp-P-2 (IC_{50}) were 0.12 and 0.81 μg, respectively, while the IC_{50} of chlorophyll in oregano was 230 μg. Toward the other mutagens, benzo[*a*]pyrene, 1-nitropyrene, 2-(acetylamino)fluorene, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, they were not effective. Galangin and quercetin are the specific desmutagens against the dietary carcinogen Trp-P-2.

Keywords: *Desmutagen; galangin; quercetin; Trp-P-2; cancer prevention; dietary antimutagen*

INTRODUCTION

In humans, 35% of cancer is considered to be caused by diet (Doll and Peto, 1981). The dietary carcinogens include pyrolysates of amino acids and proteins (Wakabayashi et al., 1992), micotoxins such as aflatoxin B_1 (Tazima, 1982), pyrrolizine alkaloids in some edible plants (Schoental, 1982), and nitrosoamines produced in fried bacon (Hotchkiss and Vecchio, 1985) and in the digestive tract (Tannenbaum et al., 1979; Danno et al., 1993). We can avoid most of the dietary carcinogens by taking care of our daily foods, but it is rather difficult to avoid the pyrolysates formed during the cooking process. Pyrolysates may therefore contribute substantially to the development of human cancer.

Of the 10 pyrolysates identified as the carcinogens, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) is a liver-specific carcinogen (Wakabayashi et al., 1992; Knasmüller et al., 1992), is one of the strongest mutagens (Sugimura, 1985), and occurs frequently in our diet (Yamaizumi et al., 1980). It is very important to mitigate the cancer risk from Trp-P-2 in our daily life, and therefore we have an interest in dietary compounds which can neutralize Trp-P-2. The toxicity of Trp-P-2 can be evaluated with the mutagenicity as the initiator in carcinogenesis, using *Salmonella typhimurium* TA98 strain in the presence of S9 mix (Kato and Yamazoe, 1987). We previously found that a small amount of herb extracts (water extract from 50 mg of fresh herbs) strongly suppressed the mutagenicity of 20 ng of Trp-P-2 (Natake et al., 1989) and thought that herbs must contain some compounds to detoxify Trp-P-2.

The present study examined the active compounds in oregano and found that two flavonoids, galangin and quercetin, acted as a desmutagen to neutralize Trp-P-2 before expressing the mutagenicity (Kada and Shimoi, 1987).

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MATERIALS AND METHODS

Materials. Fresh oregano (*Origanum vulgare*) grown in our garden or dry oregano powder purchased from McCormick & Co. Inc. was used. Trp-P-2 was obtained from Wako Pure Chemical Ind., Ltd., and the other mutagens were from Aldrich Chemical Co., Ltd. Galangin was purchased from Funakoshi Co. Ltd., and quercetin and naringenin were from Wako Pure Chemical Ind., Ltd. Agar and extracts from beef and yeast (nutrient broth) for the cultivation of bacteria were purchased from Difco Laboratory. Organic solvent and water were distilled twice. All other chemicals were commercially available in high grade.

Extract from Oregano. Fresh oregano leaf or dry oregano powder was washed with water and homogenized by a Waring blender in water or methanol for 5 min. The extracts were dried, weighed, and submitted to the following bioassay. For the separation of active compounds, the dry oregano powder was sonicated in 10 volumes of a solvent for 20 min and the extract was recovered by filtration through Whatman 1PS filter paper. The solvents were used in order of the 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 then bioassayed.

Determination of the Suppressing Activity against the Mutagenicity of Trp-P-2. The suppressing activity of oregano extracts against the mutagenicity of 20 ng of Trp-P-2 was tested essentially by the method of Ames et al. (1975) with some minor modifications and using S9 mix as mentioned previously (Danno et al., 1993). The S9 mix was prepared by the S9 fraction obtained from the liver of Sprague–Dawley rats given 500 mg/kg of body weight polychlorinated biphenyl 5 days before the sacrifice (Mizuno et al., 1987). *S. typhimurium* TA98 was grown overnight in liquid broth medium at 37 °C. The extracts from oregano were dried, dissolved in 0.1 mL of dimethyl sulfoxide (DMSO), and then incubated with 20 ng of 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 bacterial suspension (0.1 mL), the mixtures were incubated again at 37 °C for 20 min. They were mixed with 2 mL of molten top agar and then poured onto an agar medium of minimal glucose. After culturing for 2 days, the His^+ revertant colony number was counted.

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To detect cytotoxicity of the oregano extracts, the surviving bacterial number was measured simultaneously. The above incubation mixture of bacteria with the extract and Trp-P-2 was washed with 0.4 mL of 0.1 mM sodium phosphate buffer (pH 7.4) by centrifugation at 3000 rpm for 10 min twice. The bacteria were resuspended in the buffer and diluted to 10^6-fold with saline solution. The 0.1 mL portion was cultured on the medium containing a 5 mM excess histidine and nutrient broth. After the culture, surviving colonies (His^-) were counted.

Calculation of the Suppressing Activity. The suppressing activity on the mutagenicity of 20 ng of Trp-P-2 was tested by three separate experiments with three plates per experimental point, and the data were calculated as follows: $[(A - B) - (C - D)]/(A - B) \times 100$; A, revertant number given by 20 ng of Trp-P-2 (mean ± SD; 2379 ± 93, $n = 64$); B, spontaneous revertants (22 ± 2, $n = 64$); C, revertants by both the extract and 20 ng of Trp-P-2; and D, revertants by each extract.

When dose–response curves were constructed with six different doses of the extracts, their IC_{50} values were determined. The IC_{50} is the amount required for 50% inhibition of the mutagenicity of 20 ng of Trp-P-2. The values were determined by plotting the suppressing activity versus log of the dosed amount.

Bio-antimutagenicity Test. The bio-antimutagenicity of flavonoids was examined by the method mentioned previously (Mizuno et al., 1989) with some minor modifications. Trp-P-2 (20 ng in 0.1 mL of water) was activated by the incubation with 0.5 mL of S9 mix for 5 min at 37 °C and then put in boiling water for 20 s to inactivate the S9 enzymes. After cooling, the solution was added to 0.1 mL of the bacterial suspension for 15 min at 37 °C. The bacterial solution was then incubated with flavonoids in 0.1 mL of DMSO for 20 min at 37 °C and cultured with the same method as the above suppressing activity test. The bio-antimutagenicity of flavonoids was evaluated with a decrease in the His^+ colony number by flavonoids.

Chromatography. The ethyl acetate extract from oregano was dried, dissolved in methanol, and then gel-filtrated through a Sephadex LH-20 column (2.5 × 50 cm o.d.) with methanol. The elution rate was 2.5 mL/min, and 5 mL each was fractionated monitoring the absorbance at 280 and 360 nm. The obtained fractions were dried under a nitrogen stream and weighed, and then their suppressing activity toward the mutagenicity of 20 ng of Trp-P-2 was compared. The active fraction was chromatographed on a silica gel column (Wakogel C-100, 1.4 × 30 cm o.d.; immobile phase, ethyl acetate) eluting stepwise with 100 mL each of mixed solvents of benzene/ethyl acetate (9:1, 8:2, and 7:3). The elution rate was 0.5 mL/min, and 3 mL each was fractionated monitoring the absorbance at 360 nm. Then, the activity of obtained fractions was determined.

Instrumental Analysis. The active compounds purified by recrystallization were analyzed by ultraviolet, infrared (with a Shimadzu IR-408), nuclear magnetic resonance (NMR) (Bruker AC-250), and electron ionization mass (EI/MS) (JEOL DX-500) spectra. For the EI/MS, the direct inlet probe was used elevating the temperature from 70 °C at 16 °C/min, and the analysis conditions were an ion source temperature of 220 °C and an ionizing voltage of 70 eV.

RESULTS

Purification of the Active Compounds from Oregano. The suppressing activity of extracts from oregano against the mutagenicity of 20 ng of Trp-P-2 was measured because Yamaizumi et al. (1980) reported that 1 g of grilled sardine contained 13.1 ng of Trp-P-2, 13.3 ng of Trp-P-1 which is a related and weaker carcinogen as compared with Trp-P-2, and small amount of other pyrolysates, and therefore the sum of the mutagenicity of these pyrolysates was estimated to be equivalent to 20 ng of Trp-P-2. Both extracts from fresh and dry oregano suppressed the mutagenicity in a dose-dependent manner, and the methanol extracts showed stronger activity than the water extracts (Table 1). Also, both extracts from fresh and dry oregano did not decrease the surviving bacterial number, and they were considered not to have the cytotoxicity. In the following experiments to obtain the active compounds, the commercial dry oregano powder was used.

Table 1. Suppression of Mutagenicity of 20 ng of Trp-P-2 by Fresh and Dry Oregano

	colony number [a]			
	water extract		methanol extract	
dosed (μg)[b]	revertant	survival (×10⁶)	revertant	survival (×10⁶)
	Fresh Oregano			
control	2438 ± 106	229 ± 12	2349 ± 96	226 ± 11
10	1782 ± 53	243 ± 14	1592 ± 129	235 ± 10
50	1419 ± 54	236 ± 12	326 ± 57	230 ± 9
100	729 ± 42	266 ± 13	101 ± 9	233 ± 13
	Dry Oregano			
10	2165 ± 43	219 ± 11	1461 ± 54	232 ± 10
50	1543 ± 77	221 ± 10	218 ± 11	230 ± 12
100	907 ± 74	220 ± 13	61 ± 8	225 ± 10

[a] Mean ± SD minus the spontaneous revertants. [b] By dry weight.

Table 2. Suppressing Activity of the Extracts from Dry Oregano against the Mutagenicity of Trp-P-2

extract[a]	activity (%)[b]	yield (g)[c]
hexane	68	2.55
methylene chloride	71	3.48
ethyl acetate	72	1.31
acetone	17	3.40
methanol	11	2.21
water	3	3.89

[a] Oregano was extracted with these solvents in order of the polarity, one after another. [b] Suppressing activity of 10 μg (by dry weight) each of the extracts against the mutagenicity of 20 ng of Trp-P-2. [c] Yield from 100 g of dry oregano.

Dry oregano was extracted with six solvents in order of the polarity, one after another, and the activity and yield in each extract were determined (Table 2). The fractions of hexane, methylene chloride, and ethyl acetate showed strong activity to suppress the mutagenicity of Trp-P-2, and the latter two fractions had a dose-dependent activity. Methylene chloride gave larger yield than ethyl acetate, but the methylene chloride extract was composed mainly of chlorophyll. Chlorophyll is known to suppress the mutagenicity by adsorption of the mutagen (Negishi et al., 1990; Arimoto et al., 1993). Then, this study purified the active compounds in ethyl acetate extract.

Figure 1 shows the purification process and the activity and yield of the fractions obtained. The ethyl acetate extract was dried and dissolved in methanol. Most of the active compounds were soluble in methanol, and then the methanol-soluble compounds were separated into three fractions on the Sephadex LH-20 column. Fraction 3 had the greatest activity and was further chromatographed on the silica gel column. Activity was detected in fractions 4, 5, and 8, and after allowing to stand on ice, crystals appeared in fractions 5 and 8. The crystals were recovered and recrystallized in methanol three times to purify the active compounds.

These active fractions showed the dose-dependent activity without decrease in the surviving cell numbers. Then, to determine their IC_{50} values, the suppressing activity against the mutagenicity of 20 ng of Trp-P-2 was plotted versus log of the dosed amount. For brevity

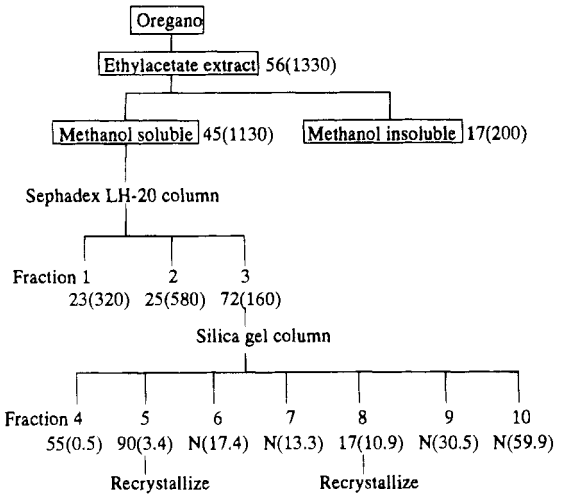

Figure 1. Separation of the active compounds from oregano. Methanol soluble in the ethyl acetate extract from dry oregano (Table 2) was gel-filtrated through a Sephadex LH-20 column. Fraction 3 was further chromatographed on a silica gel column, and the obtained fractions 5 and 8 were purified by recrystallization. Figures outside the parentheses are suppressing activities (%) against the mutagenicity of 20 ng of Trp-P-2 ("N" means nonactive), and the figures in parentheses are yields (mg) from 100 g of oregano. The suppressing activities were evaluated with 5 μg (by dry weight) each of the ethyl acetate extract, methanol soluble, and fractions 1–3 and with 1 μg (by dry weight) each of fractions 4–10.

only data obtained with recrystallized compounds from fractions 5 and 8 are shown in Figure 2. The IC_{50}'s of fractions 4, 5, and 8 were 18-, 70-, and 9-fold lower than that of the ethyl acetate extract, respectively (Table 3). Also, comparing with chlorophyll, the recrystallized compounds in fractions 5 and 8 had the 1920- and 280-fold stronger activity, respectively. The yields of fractions 5 and 8 were 3.4 and 10.9 mg from 100 g of oregano, while the yield of fraction 4 was 0.5 mg (Figure 1). Therefore, both of the compounds in fractions 5 and 8 were considered to contribute greatly to the suppressing activity against the mutagenicity of Trp-P-2 in oregano. Then, the crystals from 5 and 8 were elucidated on their chemical structures.

Identification of the Active Compounds. The crystal from fraction 5 had a melting point at 220–222 °C. The ultraviolet spectrum in methanol gave λ_{max} = 265, 288, 305, and 358 nm, and an addition of aluminum chloride shifted the λ_{max} to longer wavelength. The infrared spectrum in KBr showed an aromatic ketone (ν_{max} = 1620 cm^{-1}). These indicated that this crystal had a flavonoid skeleton (Mabry et al., 1970). The EI/MS spectrum of the crystal gave a molecular ion peak at *m/z* 270 (M^+) as a base ion peak and fragment ion peaks at *m/z* 242 (−CO) as the secondary peak and at *m/z* 77 ($[C_6H_5]^+$) as the tertiary peak. The spectrum of ^{1}H-NMR in DMSO-d_6 gave signals at δ 6.25 (1H, d, J = 2.0 Hz), 6.49 (1H, d, J = 1.9 Hz), 7.55 (3H, m), 8.18 (2H, m), 9.70 (1H, OH), 10.9 (1H, OH), 12.4 (1H, perie OH). These signals were assigned to protons on C-6, C-8, C-3′, -4′, -5′, C-2′, -6′, C-7, C-3, and C-5 on the flavonol ring, respectively. These instrumental analysis data on the fraction 5 crystal completely coincided with those of authentic galangin. Therefore, the active compound in fraction 5 was identified as 3,5,7-trihydroxy-2-phenyl-4*H*-1-benzopyran-4-one.

The crystal from fraction 8 had a melting point at 313–315 °C. Since the ultraviolet spectrum in methanol (λ_{max} = 257, 300, and 375 nm) shifted to longer

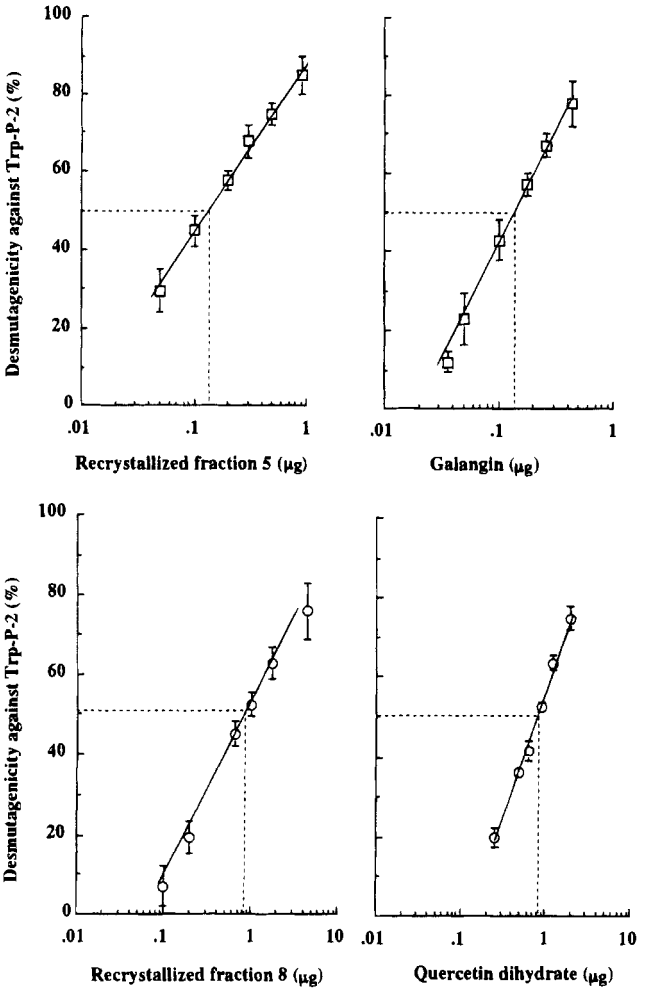

Figure 2. Determination of IC_{50} for the desmutagenicity against 20 ng of Trp-P-2. The desmutagenicities (%) were plotted versus log of the dosed amount as mentioned in the text, and the amounts required for 50% desmutagenicity were determined. All figures are mean ± SD minus the spontaneous revertants.

Table 3. IC_{50} of the Purified Oregano Compounds toward 20 ng of Trp-P-2

separation	IC_{50} (μg)[a]
ethyl acetate extract	9.8
fraction 3	3.8
fraction 4	0.54
fraction 5	0.14
fraction 8	1.1
recrystallized fraction 5	0.12
recrystallized fraction 8	0.83
chlorophyll in methylene chloride extract[b]	230

[a] IC_{50} is the amount required for the 50% inhibition of mutagenicity of 20 ng of Trp-P-2. [b] Chlorophyll was obtained from a methylene chloride extract of fresh oregano by thin-layer chromatography (Merck Kieselgel 60 PF_{254}) developing with toluene/diethyl ether (7:3).

wavelength with aluminum chloride and the infrared spectrum in KBr showed the aromatic ketone (ν_{max} = 1620 cm^{-1}), this crystal was also suggested to be a flavonoid. The EI/MS spectrum gave a molecular ion peak at *m/z* 302 (M^+) as a base ion peak and fragment ion peaks at *m/z* 137 ($[C_7H_5O_3]^+$) as the secondary peak and at *m/z* 273 (−CO) as the tertiary peak. The spectrum of ^{1}H-NMR in DMSO-d_6 gave signals at δ 6.19 (1H, d, J = 2.0 Hz), 6.41 (1H, d, J = 2.1 Hz), 6.89 (1H, d, J = 8.5 Hz), 7.54 (1H, m), 7.68 (1H, d, J = 2.2 Hz), 9.34 (4H, OH), 12.5 (1H, perie OH). These signals were

Table 4. Desmutagenicity of Galangin and Quercetin against Trp-P-2[a]

flavonoid (μg)		revertant number[b] or IC_{50} (μg)
	Bio-antimutagenicity	
control		3156 ± 156
galangin	1.0	2874 ± 116
	5.0	2954 ± 129
quercetin	5.0	2413 ± 94
	20.0	2722 ± 96
	Against Activated Trp-P-2[c]	
none		1147 ± 79
galangin	0.5	981 ± 17
	2.0	1123 ± 59
	10.0	1143 ± 61
quercetin	2.0	911 ± 50
	10.0	1053 ± 39
	50.0	927 ± 46
	Desmutagenicity[d]	
galangin		0.12
quercetin dihydrate		0.81

[a] Commercial flavonoids were used. [b] Mean ± SD minus the spontaneous revertants. [c] The activated Trp-P-2 prepared as shown in the text was incubated with flavonoids for 15 min at 37 °C and then added to the TA98 cells. The revertants were detected with the same method as the suppressing activity test. [d] The suppressing activity of flavonoids against the mutagenicity of Trp-P-2 was referred here to the desmutagenicity.

assigned to protons on C-6, C-8, C-5′, C-6′, C-2′, C-3, -7, -3′, -4′, and C-5 on the flavonol ring, respectively. These data completely coincided with those of authentic quercetin. The active compound in fraction 8 was identified as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one.

Specific Desmutagenicity of Galangin and Quercetin against Trp-P-2. The antimutagen is classified into desmutagen and bio-antimutagen according to modes of action (Kada and Shimoi, 1987). The classification is based on a comparison between the desmutagenicity and bio-antimutagenicity. The desmutagenicity is evaluated with the same test as the antimutagenicity. The bio-antimutagenicity means suppressing activity at the process of mutagenesis (mutation fixation) after DNA has been damaged by a mutagen. The fixed His^+ revertant, which has been induced by activated Trp-P-2 (Kato and Yamazoe, 1987), can be evaluated by counting the number of His^+ revertant colonies. Then, it was determined whether the suppressing activity of galangin and quercetin against the mutagenicity of Trp-P-2 was bio-antimutagenic or desmutagenic using the commercial compounds (Table 4). Both galangin and quercetin decreased little the His^+ revertant number even though a 40 or 24 times larger amount as their IC_{50}, shown in Table 3, was dosed. Also, the effects of both flavonoids on the activated Trp-P-2 were determined. When flavonoids were incubated with the activated Trp-P-2 and then added to the cell, little decrease in the revertant number was detected with their 83- or 60-fold larger amounts than their IC_{50}. Therefore, the strong suppressing activity of galangin and quercetin against the mutagenicity of Trp-P-2 was referred to a desmutagenicity which neutralized Trp-P-2 during or before the activation (Kada and Shimoi, 1987).

Commercial galangin and quercetin dihydrate exhibited the dose-dependent desmutagenicity against 20 ng of Trp-P-2. The desmutagenicity was plotted versus log of the dosed amounts (Figure 2). The IC_{50} values for the desmutagenicity of commercial galangin and quercetin dihydrate were 0.12 and 0.81 μg, respectively

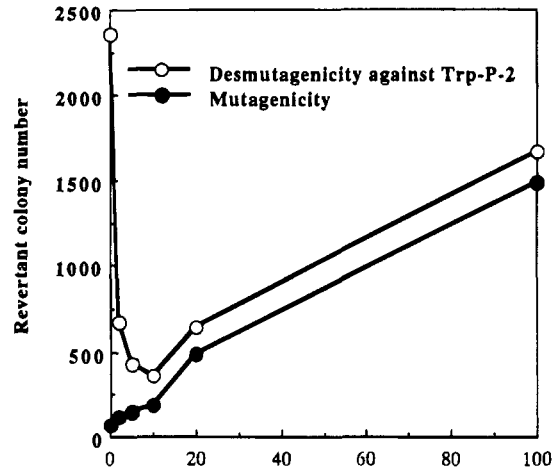

Figure 3. Mutagenicity and desmutagenicity of quercetin. The mutagenicity of quercetin was compared with the desmutagenicity against 20 ng of Trp-P-2 at concentrations of 0, 2, 5, 10, 20, and 100 μg/plate in the presence of S9 mix using the TA98 cell. The mutagenicity was determined by the method of Ames et al. (1975).

(Table 4). These values almost coincided with those of the corresponding compounds purified from oregano in Table 3.

On the other hand, Macgregor and Jurd (1978) have described that quercetin had a mutagenicity. Therefore, we compared the mutagenicity with the desmutagenicity against Trp-P-2 of quercetin (Figure 3). More than 10 μg/plate of quercetin in the presence of S9 mix increased the revertants of the TA98 cell, but the less than 10 μg/plate markedly suppressed the revertants given by 20 ng of Trp-P-2. Thus, quercetin at the high concentration was a mutagen but at the lower concentration was a desmutagen.

Galangin and quercetin may also express desmutagenicity toward the other mutagens. Table 5 examined the desmutagenicity against four different types of mutagens: benzo[*a*]pyrene (B[*a*]P), 1-nitropyrene (1-NP), 2-(acetylamino)fluorene (AAF), and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG). The mutagenicity of B[*a*]P and 1-NP can be detected as a flame-shift type with the TA98 strain. That of AAF and MNNG can be detected as a base-pair-change type with the TA100 strain. B[*a*]P and AAF require the activation by S9 mix for expressing the mutagenicity. Both galangin and quercetin little decreased the revertant number given by every mutagen, even with such large amounts as 5 and 50 μg/plate, respectively. Galangin rather enhanced the mutagenicity of AAF as shown with an increase of 4642 revertants by AAF to 7876. Quercetin exhibited the mutagenicity in the presence of S9 on cells of both TA98 and TA100, whereas the spontaneous revertant numbers of TA98 (48) and TA100 (32) increased to 661 and 709, respectively, by the addition of quercetin. Thus, the results of Tables 4 and 5 reveal that galangin and quercetin are the specific desmutagens against Trp-P-2.

DISCUSSION

This study demonstrated that the strong and specific desmutagens against the dietary carcinogen Trp-P-2 in oregano are the flavonoids galangin and quercetin. Galangin and quercetin were the major components in fractions 5 and 8 whose dry weights, prepared from 100 g of dry oregano, were 3.4 and 10.9 mg, respectively (Figure 1). Chlorophyll was in the methylene chloride

Table 5. Effect of Galangin and Quercetin toward the Other Mutagens[a]

	revertant number[c]							
	TA98 cell				TA100 cell			
	B[a]P + S9[b]		1-NP		AAF + S9[b]		MNNG	
mutagen (μg)	0	500	0	2	0	100	0	5
control	48 ± 4	349 ± 14	58 ± 5	8523 ± 467	32 ± 3	4642 ± 395	186 ± 16	751 ± 41
galangin (5 μg)	55 ± 4	259 ± 35	53 ± 5	6654 ± 693	67 ± 5	7876 ± 1006	180 ± 16	662 ± 44
quercetin (50 μg)	661 ± 36	757 ± 42	153 ± 13	8206 ± 923	709 ± 18	4113 ± 447	173 ± 34	502 ± 38

[a] The effects of flavonoids toward B[a]P, benzo[a]pyrene; 1-NP, 1-nitropyrene; AAF, 2-(acetylamino)fluorene; and MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were examined with the same method as the test against Trp-P-2. [b] B[a]P and AAF were tested in the presence of S9 mix. [c] Mean ± SD minus the spontaneous revertants.

extract whose dry weight was 3.48 g (Table 2). The content of these flavonoids was lower than that of chlorophyll in oregano. However, the IC_{50}'s for the desmutagenicity of galangin and quercetin were 1920 and 280 times stronger than that of chlorophyll, respectively (Table 3). So, flavonoids were considered to contribute greatly to the desmutagenicity in oregano.

Quercetin exhibited mutagenicity on the bacteria at the larger amount of more than 10 μg/plate (Figure 3 and Table 2). However, in the animal body, 97% of the dosed quercetin has been reported to be inactivated by 3'-*O*-methylation (Zhu et al., 1994). The small amount of quercetin evading the methylation may express the desmutagenicity in our body.

Galangin and quercetin were such specific desmutagens against Trp-P-2 that they neutralized Trp-P-2 before damaging DNA (Tables 4 and 5). Trp-P-2 is one of the dietary carcinogens formed by pyrolysis of tryptophan during cooking. Edenharder et al. (1993) have also reported that flavonoids suppressed the mutagenicity of other carcinogenic pyrolysates. Flavonoids are the common phytochemicals contained in various vegetables, fruits, and tea but are not an unique compound. We think such specific desmutagenicity of flavonoids against the dietary carcinogen plays an important part in cancer prevention in our daily life, as exemplified with the data of daily flavonoid intake in the Netherlands (Hertog et al., 1993).

In addition, many reports show that the dietary flavonoids have various biofunctions including the anticarcinogenic (Verman et al., 1988; Bon et al., 1992), antioxidative (Cholbi et al., 1991; Tournaire et al., 1993), anti-inflammatoric (Abad et al., 1993), and gastroprotecting (Lastra et al., 1993). We need to have more understanding on the biofunction of flavonoids. Alldrick et al. (1986) have described that the inhibitory effect of quercetin depended on the enzyme source to activate Trp-P-2. Quercetin inhibited the mutagenicity of Trp-P-2 when activated with S9 mix derived from Swiss Webster mice but did not inhibit mutagenicity when activated with S9 mix derived from Syrian hamsters. They suggested that quercetin exerted the effect by inhibiting enzyme/substrate interactions. This hypothesis is supported by the work of Sousa and Marletta (1985) who showed that quercetin inhibited cytochrome P450 functions. Sousa and Marletta also showed that the inhibitory effect on P450 enzymes of galangin was greater than that of quercetin. This result coincided with the present results in Table 4 which showed that the IC_{50} for the desmutagenicity against Trp-P-2 of galangin was lower than that of quercetin. This indicates that the activation of Trp-P-2 by P450 enzymes has different sensitivity to the inhibitory effect of quercetin depending on the enzyme sources. Alldrick et al. obtained 11.1 for the IC_{50} of quercetin as a molar ratio of quercetin:Trp-P-2 with S9 derived from mouse. We obtained 0.81 μg for the IC_{50} against 20 ng of Trp-p-2 with S9 derived from rat (Table 4) which was calculated to 25 as the molar ratio. It is therefore very important to know the sensitivity to inhibitory effects of flavonoids on human P450 enzymes. In our laboratory, the study using a recombinant yeast strain expressing the human P450 enzymes is ongoing.

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