Antimutagenic Activity of (+)-β-Eudesmol and Paeonol from *Dioscorea japonica*

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A methanol extract from *Dioscorea japonica* showed a suppressive effect on *umu* gene expression of the SOS response in *Salmonella typhimurium* TA1535/pSK1002 against the mutagen 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (furylfuramide). The methanol extract from *D. japonica* was re-extracted with dichloromethane, *n*-butanol, and water, respectively. The suppressive compounds in the dichloromethane extract fraction were isolated by SiO_2 column chromatography and identified as (+)-β-eudesmol and 2-hydroxy-4-methoxyacetophenone (paeonol) by EI-MS and ^1H and ^{13}C NMR spectroscopy. (+)-β-Eudesmol suppressed the SOS-inducing activity of furylfuramide in the *umu* test. Gene expression was suppressed 80% at <0.18 μmol/mL, and the ID_{50} value was 0.09 μmol/mL. Paeonol also suppressed the SOS-inducing activity of furylfuramide in the *umu* test. Gene expression was suppressed 60% at <1.2 μmol/mL, and the ID_{50} value was 0.99 μmol/mL. (+)-β-Eudesmol and paeonol were assayed with the mutagen 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), which requires liver metabolizing enzymes, and showed suppressive effects on the SOS induction of the mutagen. (+)-β-Eudesmol suppressed gene expression 48% at <0.18 μmol/mL. Paeonol suppressed gene expression 76% at <1.2 μmol/mL, and the ID_{50} was 0.41 μmol/mL. The antimutagenic activities of these compounds against furylfuramide and Trp-P-1 were tested by an Ames test using *S. typhimurium* TA100, which indicated that these compounds showed antimutagenic activity.

Keywords: *Dioscorea japonica; Dioscoreaceae; Chinese yam; (+)-β-eudesmol; paeonol; antimutagenic activity; umu test; Ames test*

INTRODUCTION

Chinese yam (Dioscoreaceae) occurs naturally in Japan, where it is cultivated and its storage rhizome is used mainly for treatment of diarrhea, asthma, polyuria, and diabetes. Several phenanthrenes, dihydrophenanthrenes, and bibenzyls have been isolated from *Dioscorea* species (Takasugi et al., 1987; Sunder et al., 1978), and antifungal compounds were isolated from *Dioscorea batatas* (Takasugi et al., 1987). Hikino et al. (1986) reported on the isolation of hypoglycemic active compounds (dioscorans A−F) from *Dioscorea japonica*.

In the evaluation of the carcinogenicity or mutagenicity of environmental chemicals, it is quite important to determine factors present in our environment that may affect these activities. With the development of techniques for detecting possible environmental carcinogens and mutagens (Ames et al., 1975), it has been shown that ordinary diets contain many kinds of mutagens and antimutagens, and as one example Kakinuma et al. (1984a) reported the identification of an antimutagen (cinnamaldehyde) from *Cinnamomum cassia* using *Escherichia coli* WP2 B/r *uvr*A$^-$ *trp*E$^-$.

The *umu* test system was developed to evaluate the genotoxic activities of a wide variety of environmental carcinogens and mutagens, using the expression of one of the SOS genes to detect DNA-damaging agents (Oda et al., 1985; Nakamura et al., 1987). In our search for new naturally occurring antimutagenic compounds in plants that have a history of safe use as Chinese crude drugs (Miyazawa et al., 1995a−c), we found that the methanol extract of *D. japonica* (sanyaku in Japanese) exhibited suppression of the SOS-inducing activity of 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (furylfuramide). In this paper, we report the isolation and identification of the antimutagenic compounds contained in *D. japonica*.

MATERIALS AND METHODS

General Procedure. Electron-impact mass spectra (EI-MS) were obtained on a Hewlett-Packard 5972A mass spectrometer. IR spectra were determined with a Perkin-Elmer 1760-x infrared Fourier transform spectrometer. Nuclear magnetic resonance (NMR) spectra (δ, *J* in hertz) were recorded on a JOEL GSX 270 NMR spectrometer. Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^1H NMR spectra measured in $CDCl_3$. This solvent was used for ^{13}C NMR spectra. Specific rotation was determined with a Jasco DIP-140 digital polarimeter.

Materials. Commercially available air-dried rhizome of *D. japonica* was purchased from Takasago Yakugiyo Co. (Osaka, Japan). The rhizomes for use as crude drug were collected in 1994 from plants cultivated in Nagano prefecture in Japan. Furylfuramide and 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) were purchased from Wako Pure Chemical Co. S9 metabolizing enzyme was purchased from Oriental Yeast Co.

***Umu* Test.** The *umu* test for detecting the SOS-inducing activity of chemicals was carried out essentially as described by Oda et al. (1985) using *Salmonella typhimurium* TA1535/pSK1002, having the plasmid pSK1002 which carries a *umu*C′-*lac*Z′ fused gene. The SOS-inducing potency is estimated by the measurement of the level of *umu* operon expression in terms of cellular β-galactosidase activity.

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Table 1. Suppressive Effect of *D. japonica* Fractions on Furylfuramide[a] Using *S. typhimurium* TA1535/pSK1002

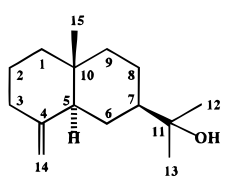
		dose response[c]			
sample	control[b]	200 μg/mL	100 μg/mL	50 μg/mL	0 μg/mL
MeOH extract	173.6	815.9	912.3	956.2	972.5
CH_2Cl_2 fraction	183.9	648.6	746.8	785.4	830.3
n-BuOH fraction	183.9	763.2	790.5	850.9	830.3
water fraction	183.9	792.7	809.6	824.3	830.3
fraction 1	176.7	592.3	634.2	792.2	945.6
fraction 2	176.7	882.9	960.2	953.1	945.6
fraction 3	203.5	812.2	923.0	921.7	994.7
fraction 4	203.5	432.5	587.9	860.7	994.7
fraction 5	203.5	867.8	1011.9	962.4	994.7
fraction 6	203.5	1025.3	968.6	982.5	994.7

[a] Furylfuramide (1 μg/mL in DMSO) was added at 60 μL. [b] Control was a treatment without furylfuramide. [c] β-Galactosidase activity (units).

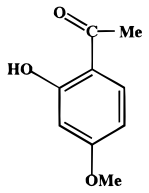
Ames Test. The mutation test was carried out according to the preincubation method (Yahagi et al., 1977), which is a modification of Ames method (Ames et al., 1975).

Purification of the Suppressive Compounds 1 and 2. The dry powder of rhizome (2 kg) of *D. japonica* was refluxed with methanol for 12 h to give a methanol extract (21.7 g). This extract was suspended in water (1 L) and partitioned between dichloromethane (1 L) and water and then *n*-butanol (1 L) and water, successively. Each soluble fraction was concentrated under reduced pressure to give dichloromethane (6.7 g), *n*-butanol (3.4 g), and water (11.6 g) fractions, and each fraction was tested for suppression of the SOS-inducing activity. As shown in Table 1, the dichloromethane extract fraction had positive activity, whereas the *n*-butanol and water extract fractions did not show activity. The suppressive fraction (dichloromethane extract fraction) was dissolved with dichloromethane and partitioned with 5% $NaHCO_3$ solution. The organic layer was given as a neutral fraction (fraction 1). The aqueous layer was acidified with diluted HCl and then extracted with dichloromethane to yield the acidic fraction (fraction 2). The results of the *umu* test showed that fraction 1 had positive activity, whereas fraction 2 did not show activity. Fractionation of fraction 1 was then carried out as described in Figure 1. Finally, suppressive compounds **1** (45 mg) and **2** (7 mg) were isolated.

Suppressive Compound **1**: crystalline; mp 69.8−72.5 °C; $[\alpha]^{20}_D$ +37.5 ($CHCl_3$; *c* 1.0); MS, *m/z* (%) 222 (M^+, 1.7), 204 (5.0), 189 (10.0), 164 (32.5), 149 (70.8), 59 (100%); $IR\gamma_{max}^{KBr}$ 3500, 2972, 2931, 2866, 1646, 1441, 1378, 886 cm^{-1}. The ^1H NMR spectrum of **1** confirmed the presence of a methyl group at δ 0.70 (C-15) and 1.20 (C-12 and C-13), and the signals at δ 4.49 and 4.73 could be assigned as the two exocyclic vinyl hydrogens (C-14). The ^{13}C NMR agreed with those of (+)-β-eudesmol (Cordano et al., 1978). Suppressive compound **1** was identified as (+)-β-eudesmol from these spectral data and physical properties.



Compound 1



Compound 2

Suppressive Compound **2**: crystalline; mp 48.8−50.3 °C; $[\alpha]^{20}_D$ −0.71 ($CHCl_3$; *c* 0.25); MS, *m/z* (%) 166 (M^+, 3.9), 151 (100), 108 (5.8), 95 (9.2), 43 (6.7); $IR\gamma_{max}^{KBr}$ 2921, 1620, 1505, 1464, 1369, 1334, 1258, 1209, 1141, 1071, 1022, 978, 950, 859, 816 cm^{-1}. The ^1H NMR spectrum of **2** indicated the appearance of a δ 12.75 for the strong intramolecular hydrogen bonding. The EI-MS of **2** agreed with that of 2-hydroxy-4-methoxyacetophenone (paeonol) (Fukuhara et al., 1987). Compound **2** was identified as paeonol from these spectral data and physical properties.

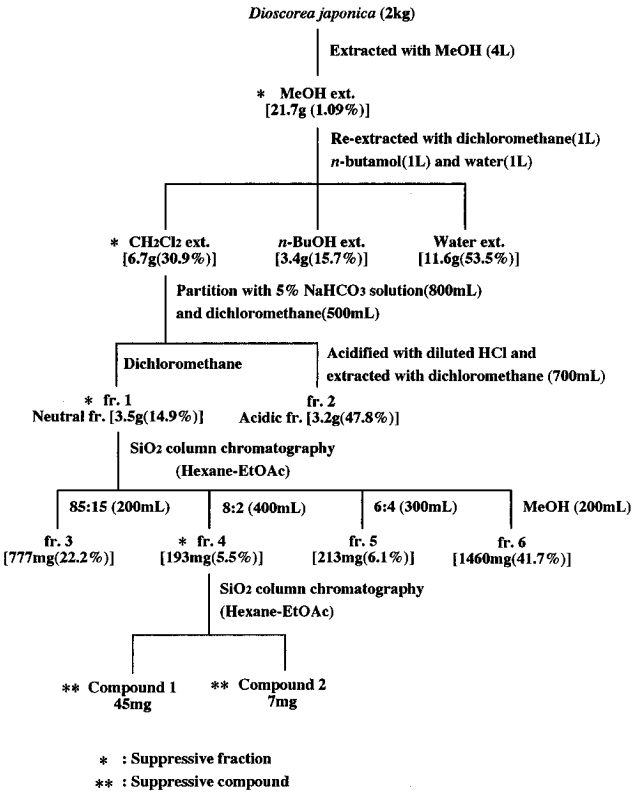


Figure 1. Isolation scheme for the suppressive compounds from *D. japonica*.

Acetylation of 1. The acetylate of **1** (compound **1A**) was obtained by the reaction with acetic anhydride and pyridine. Compound **1A** was an oil; MS, *m/z* (%) 204 ($M^+ - CH_3COOH$, 42.5), 189 (25.8), 161 (50.8), 59 (23.3), 43 (100); $IR\gamma_{max}^{KBr}$ 3080, 2940, 2873, 1733, 1646, 1456, 1368, 1257, 1210, 1134, 1018, 886 cm^{-1}. The ^1H NMR spectrum of **1A** confirmed the presence of an acetate methyl group at δ 1.98 and agreed with that of the monoacetate of β-eudesmol (El-Sayed et al., 1989). Compound **1A** was identified as the monoacetate of β-eudesmol from these spectral data.

Dehydration of 1. The dehydrated derivative of **1** (compound **1D**) was obtained by the reaction of **1** with thionyl chloride and pyridine (Iwamoto et al., 1982). Compound **1D** was an oil; MS, *m/z* (%) 204 (M^+, 61.7), 189 (50.8), 175 (25.8), 161 (57.5), 147 (50.8), 133 (55.0), 121 (63.3), 107 (88.3), 105 (100), 93 (96.7), 91 (84.2), 79 (95.8), 67 (72.5), 55 (50.0), 41 (86.7). The ^1H NMR spectrum of **1D** confirmed the presence of a methyl group at δ 0.73 (*s*, 3H) and 1.75 (*s*, 3H) and vinyl hydrogens at δ 4.44 (*dd*, *J* = 2.0, 3.1, 1H) and 4.72 (*m*, 3H). Compound **1D** was identified as β-selinene (Lionel et al., 1983).

RESULTS

Fractionation of the Extract from *D. japonica* and Isolation of 1 and 2. The methanol extract was fractionated to search for suppressive compounds using the *umu* test as a guide. To obtain dose−response data, test samples were evaluated at dose levels of 0.2, 0.1, and 0.04 mg/mL. As shown in Table 1, the methanol extract from *D. japonica* showed a suppressive effect on *umu* gene expression of the SOS responses in *S. typhimurium* TA1535/pSK1002 against furylfuramide. To prepare the suppressive fraction, fractionation of the methanol extract was carried out as described in Figure 1 and Table 1. Finally, suppressive compounds **1** (45 mg) and **2** (7 mg) were isolated.

Table 2. Suppressive Effect of 1 and 2 on Furylfuramide[a] and Trp-P-1[b] Using *S. typhimurium* TA1535/pSK1002

				dose response[d]			
chemical	furylfuramide	Trp-P-1	control[c]	0.18 μmol/mL	0.11 μmol/mL	0.07 μmol/mL	0.04 μmol/mL
1	930.1		252.9	382.9	451.7	645.5	787.7
		567.6	210.0	395.9	411.0	448.7	477.8

				dose response[d]		
chemical	furylfuramide	Trp-P-1	control[c]	1.20 μmol/mL	0.60 μmol/mL	0.24 μmol/mL
2	930.1		252.9	529.2	668.0	782.5
		567.6	210.0	295.1	346.6	420.3

[a] Furylfuramide (1 μg/mL in DMSO) was added at 60 μL. [b] Trp-P-1 (40 μg/mL in DMSO) was added at 50 μL. [c] Control was treatment without mutagens. [d] β-Galactosidase activity (units).

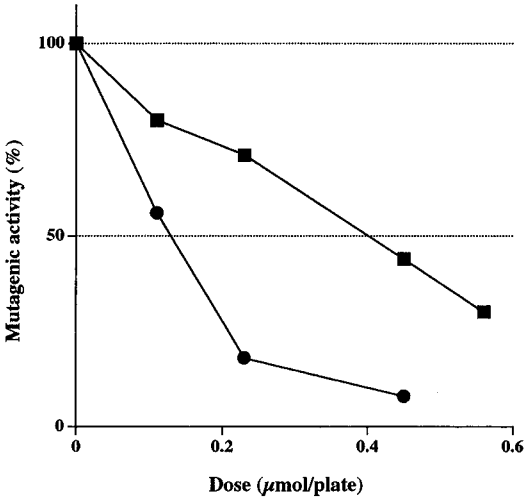


Figure 2. Effect of **1** on the mutagenicity of furylfuramide (●) and Trp-P-1 (■) in *S. typhimurium* TA100. Furylfuramide (0.5 μg/mL in DMSO) was added at 20 μL/plate. Trp-P-1 (40 μg/mL in DMSO) was added at 50 μL/plate. In the case of the effect of **1** on the mutagenicity of furylfuramide, **1** was toxic at under 0.56 μmol/plate.

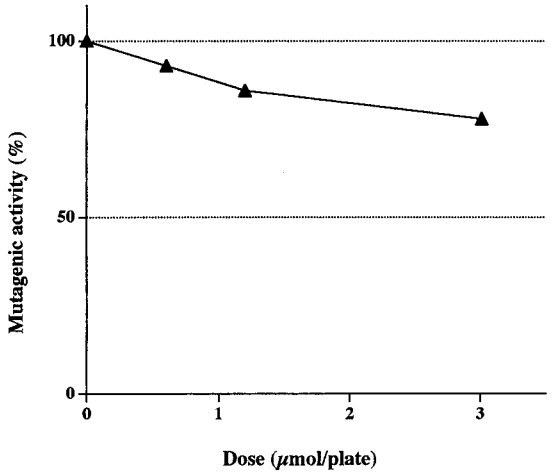


Figure 3. Suppressive effect of **2** on the mutagenicity of furylfuramide using *S. typhimurium* TA100. Furylfuramide (0.5 μg/mL in DMSO) was added at 20 μL/plate.

Structure Determination of 1 and 2. The identities of compounds **1** and **2** as β-eudesmol and paeonol were established by comparison of spectra (^{1}H NMR, ^{13}C NMR, MS) and physical constants (mp, $[\alpha]_D$) with literature data.

Inhibition of the SOS-Inducing Activity of 1 and 2. The suppressive effects of **1** and **2** were determined in the *umu* test. As shown in Table 2, **1** and **2** exhibited inhibition of the SOS induction of furylfuramide. Compound **1** suppressed 80% of the SOS-inducing activity at concentrations <0.18 μmol/mL, and the ID_{50} value was 0.09 μmol/mL. Compound **2** suppressed 60% of the SOS-inducing activity at concentrations <1.2 μmol/mL, and the ID_{50} value was 0.99 μmol/mL. Compounds **1** and **2** also showed suppression of the SOS-inducing activity of Trp-P-1, which requires metabolic activation. The former suppressed 48% of the SOS-inducing activity at concentrations <0.18 μmol/mL. The latter suppressed 76% of the SOS-inducing activity at a concentration <1.2 μmol/mL, and the ID_{50} value was 0.41 μmol/mL.

Antimutagenic Activity of 1 and 2. The antimutagenic activities of these compounds against furylfuramide and Trp-P-1 were also demonstrated by the Ames test using *S. typhimurium* TA100. As shown in Figure 2, compound **1**, which was an authentic sample, suppressed 93% of the mutagenicity of furylfuramide at 0.45 μmol/plate, and the ID_{50} value was 0.13 μmol/plate. Compound **1** also suppressed 70% of the mutagenicity of Trp-P-1 at 0.56 μmol/plate, with an ID_{50} of 0.39 μmol/plate. **1** showed toxicity at a concentration of 0.56 μmol/plate. As shown in Figure 3, compound **2**, which was also an authentic sample, suppressed only 20% of the mutagenicity of furylfuramide.

Relationship of the Structure and Activity of 1. To explore the structure−activity relationship of **1**, compounds **1A** and **1D** were examined for their ability to suppress the SOS-inducing activity of furylfuramide. Neither of them suppressed the SOS-inducing activity of furylfuramide at all, even at higher concentrations than those of **1** (data not shown). This result suggests that a hydroxyl group at C-11 is a necessary feature for β-eudesmol to show a suppressive effect.

DISCUSSION

The antimutagenic compounds in *D. japonica* were clearly identified as **1** and **2**. These compounds had a suppressive effect on *umu* gene expression of the SOS response in *S. typhimurium* TA1535/pSK1002 against furylfuramide and Trp-P-1. As shown in Table 2, **1** suppressed the SOS-inducing activity of both mutagens at lower concentrations than did **2**. In the Ames test using *S. typhimurium* TA100, **1** similarly inhibited the mutagenicity of furylfuramide and Trp-P-1 and **2** showed a low suppressive effect of the mutagenicity against furylfuramide (Figures 2 and 3).

Compounds **1A** and **1D** did not show any suppressive effects on the SOS-inducing activity of furylfuramide, which indicated that a hydroxyl group at C-11 is important for the suppressive effect. Compound **1** has other biological activities; for example, it has a preventive activity against experimental ulcerations (Nogami et al., 1986) and antianoxic activity (Yamahara et al., 1990). Further, **1** has an inhibitory activity on Epstein−

Barr virus early antigen (EVB-EA) activation. EVB-EA activation is an assay to detect tumor promoters, so it indicated that **1** might be an antitumor promoter (Konoshima et al., 1991). This seems interesting in relation to the antimutagenic activities shown in the present study.

Compound **1** also showed toxicity for *S. typhimurium* TA1535/pSK1002 at more than 0.18 μmol/mL with each mutagen (furylfuramide and Trp-P-1). On the other hand, it showed toxicity for TA100 at more than 0.45 μmol/plate with furylfuramide and at more than 0.56 μmol/plate with Trp-P-1. Similarly, Kakinuma et al. (1984b) reported antimutagenic diterpenoids (enmein, nodosin, and oridonin) from *Isodon japonicus* using *E. coli* B/r WP2 *trp*$^-$. Oridorin showed bactericidal activity at a higher dose. In this matter, it is necessary to test the antimutagens for their toxicity. Previously, Fukuhara et al. (1987) reported on a bioantimutagen (paeonol) from *Paeonia suffruticosa* using *E. coli* B/r WP2 *trp*$^-$, *uvr*A$^-$, and paeonol has a bioantimutagenic effect on 4NQO-induced mutation.

Compound **1** is one of the components of the essential oils of *Chenopodium botrys* (El-Sayed et al., 1989) and *Humulus lupulus* (Hop) (Tressl et al., 1983). Compound **2** is a component of the essential oils of *Paean mouton* and *Paean lactiflora* (Miyazawa et al., 1983, 1984). Osawa et al. (1986) reported on the desmutagenic action of food compounds (ascorbic acid, cysteine) on mutagens formed by the sorbic acid/nitrite reaction, and yam juice exhibited a desmutagenic action. In this paper, **1** and **2** were principal components of antimutagenic activity in *D. japonica*, though these were minor components in this crude drug.

LITERATURE CITED

Ames, B. N.; McCann, J.; Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.* **1975**, *31*, 347−363.

Cordano, G.; Merrien, M. A.; Polonsky, J. Balanitol, a new sesqiterpene from *Balanites roxburghii*. Carbon-13 NMR analysis of eudesmane sesquiterpenoids. *J. Indian Chem. Soc.* **1978**, 1148−1151.

El-Sayed, A. M.; Al-Yahya, M. A. Chemical composition and antimicrobial activity of the essential oil of *Chenopodium botrys* growing in Saudi Arabia. *Int. J. Crude Drug Res.* **1989**, *27* (4), 185−188.

Fukuhara, Y.; Yoshida, D. Paeonol: a bioantimutagen isolated from a crude drug, moutan cortex. *Agric. Biol. Chem.* **1978**, *51* (5), 1441−1442.

Hikino, H.; Konno, C.; Takahashi, M. Isolation and hypoglycemic activity of dioscorans A, B, C, D, E, and F; glycans of *Dioscorea japonica* rhizophors. *Planta Med.* **1986**, 1968−1971.

Iwamoto, H.; Kaneko, M.; Matsubara, Y. Hydration of eight kinds of sesquiterpene hydrocarbons with chloroacetic acids. *Nippon Nogeikagaku Kaishi* **1982**, *56* (8), 641−647.

Kakinuma, K.; Koike, J.; Kotani, K. Cinnamaldehyde; identification of an antimutagen from a crude drug, cinnamoni cortex. *Agric. Biol. Chem.* **1984a**, *48* (7), 1905−1906.

Kakinuma, K.; Okada, Y.; Ikekawa, N. Antimutagenic diterpenoids from a crude drug isodonis harba (enmei-so). *Agric. Biol. Chem.* **1984b**, *48* (6), 1647−1648.

Konoshima, T.; Kozuka, M.; Tokuda, H. Studies on inhibitors of skin tumor promotion, IX. Neolignans from *Magnolia officinalis*. *J. Nat. Prod.* **1991**, *54* (3), 861−822.

Lionel, M.; David, G.; Joseph, W. Ene-ene-retroene conversion of (−)-β-pinene to (+)-β-selinene. *J. Org. Chem.* **1983**, *48*, 3754−3759.

Miyazawa, M.; Maruyama, H.; Kameoka, H. Essential oil constituents of "moutan radicis cortex" *Paeonia moutain* SIMS (=*P. suffruticosa* Andrews). *Agric. Biol. Chem.* **1983**, *47* (12), 2925−2927.

Miyazawa, M.; Maruyama, H.; Kameoka, H. Essential oil constituents of "paeoniae radix" *Paeonia lactiflora* Pall. (*P. albilora* Pall.). *Agric. Biol. Chem.* **1984**, *48* (11), 2847−2849.

Miyazawa, M.; Shimamura, H.; Nakamura, S. Partial suppression of SOS-inducing activity of furylfuramide by dibasic acids from *Ipomoea nil* in the *Salmonella typhimurium* TA1535/pSK1002 *umu* test. *J. Agric. Food Chem.* **1995a**, *43* (2), 284−287.

Miyazawa, M.; Shimamura, H.; Nakamura, S. Antimutagenic activity of isofraxinellone from *Dictamnus dasycarpus*. *J. Agric. Food Chem.* **1995b**, *43* (6), 1428−1431.

Miyazawa, M.; Shimamura, H.; Nakamura, S. Antimutagenic activity of (+)-polyalthic acid from *Vitex rotundiforia*. *J. Agric. Food Chem.* **1995c**, *43* (12), 3012−3015.

Nakamura, S.; Oda, Y.; Shimada, T. SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chamicals. *Mutat. Res.* **1987**, *192*, 239−246.

Nogami, M.; Moriura, T.; Kubo, M. Studies on the origin, processing and quality of crude drugs. II. Pharmacological evaluation of the Chinese crude drug "zhu" in experimental stomach ulcer. (2). Inhibitory effect of extract of *Atractylodes lancea* on gastric secretion. *Chem. Pharm. Bull.* **1986**, *34* (9), 3854−3860.

Oda, Y.; Nakamura, S.; Oki, I. Evalution of the new system (umu-test) for the detection of environmental mutagens and carcinogens. *Mutat. Res.* **1985**, *147*, 219−229.

Osawa, T.; Ishibashi, H.; Namiki, M. Desmutagenic action of food compounds on mutagens formed by the sorbic acid/nitrite reaction. *Agric. Biol. Chem.* **1986**, *50* (8), 1971−1977.

Sunder, R.; Rangaswami, S.; Reddy, G. C. S. A new dihydrophenanthrene from *Dioscorea decipiens*. *Phytochemistry* **1978**, *17*, 1067.

Takasugi, M.; Kawashima, S.; Monde, K. Antifungal compounds from *Dioscorea batatas* inoculated with *Pseudomonas cichorii*. *Phytochemistry* **1987**, *26* (2), 371−375.

Tressl, R.; Engel, K.; Kossa, M. Characterization of tricyclic sesquiterpenes in hop (*Humulus lupulus*, var. Hersbrucker Spat). *J. Agric. Food Chem.* **1983**, *31*, 892−897.

Yahagi, T.; Nagao, M.; Seino, T. Mutagenicity of *N*-nitrosamines on *Salmonella*. *Mutat. Res.* **1977**, *48*, 121−130.

Yamahara, J.; Matsuda, H.; Naitoh, Y. Antianoxic action and active constituents of *Atractylodis lanceae* rhizoma. *Chem. Pharm. Bull.* **1990**, *38* (7), 2033−2034.

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