Suppression of SOS-Inducing Activity of Chemical Mutagens by Cinnamic Acid Derivatives from *Scrophulia ningpoensis* in the *Salmonella typhimurium* TA1535/pSK1002 *umu* Test

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A methanol extract from *Scrophulia ningpoensis* showed suppressive effect of the SOS-inducing activity of the mutagen 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (furylfuramide) in the *Salmonella typhimurium* TA1535/pSK1002 *umu* test. The methanol extract was re-extracted with hexane, dichloromethane, butanol, and water. An acidic fraction of the dichloromethane fraction showed a suppressive effect. Suppressive compounds in the acidic fraction were isolated by SiO_2 column chromatography and identified as *trans*-cinnamic acid (**1**), *p*-methoxycinnamic acid (**2**), 3,4-dimethoxycinnamic acid (**3**), and 4-hydroxy-3-methoxycinnamic acid (**4**) by GC, GC/MS, and ^{1}H NMR spectroscopy. Compounds **1**−**4** suppressed the SOS-inducing activity of furylfuramide in the *umu* test. Compounds **1**−**4** suppressed 44, 31, 37, and 36% of the SOS-inducing activity at a concentration of 1.4 μmol/mL. These compounds were assayed with other mutagens, 4-nitroquinoline 1-oxide (4NQO) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). In addition, compounds **1**−**4** were assayed with 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and aflatoxin B_1 (AfB1), which requires liver metabolizing enzymes. These compounds showed suppresssive effects of SOS-inducing activity against all mutagens. Methyl esters of compounds **1**−**4** also showed a suppressive effect of the SOS-inducing activity against furylfuramide and Trp-P-1.

Keywords: *Scrophulariaceae; Scrophulia ningpoensis; cinnamic acid derivatives; SOS response; umu test*

INTRODUCTION

It has been known that carcinogenicity and mutagenicity are caused by environmental chemicals, and it is important to determine materials that provide activity or inhibition against these actions. With the development of techniques for detecting possible environmental carcinogens and mutagens, it has been shown that ordinary diets contain many kinds of mutagens and antimutagens (Ames et al., 1975).

The *umu* test system was developed to evaluate the genotoxic activities of a wide variety of environmental carcinogens and mutagens, using the expression of the SOS genes to detect DNA-damaging agents in *Salmonella typhimurium* (Oda et al., 1985; Nakamura et al., 1987). The system is based upon the abilities of carcinogens and mutagens to induce expression of an *umu* gene in *S. typhimurium* TA1535/pSK1002 in which a plasmid pSK1002 carrying a fused gene *umuC'*-'lacZ has been introduced; the *umu* gene seems to be involved in mutagenesis more directly than other known SOS genes (Kato et al., 1982; Shinagawa et al., 1983). The results of this test are also in agreement with the results of the Ames test and may be more useful with respect to simplicity, sensitivity, and rapidity (Reifferscheid et al., 1996).

The SOS response appears to be induced by an alteration in DNA synthesis, either directly by DNA damage blocking to the replication fork or indirectly by antibiotics, such as novobiocin, that inhibit DNA synthesis. The SOS regulatory system is controlled in part by the interplay of two proteins—the lexA protein, which represses asset of unlinked genes during normal cell growth, and the recA protein, which is required in vivo for inactivation of lexA protein after treatments that derepress the system by DNA damaging its metabolism (Little et al., 1982, 1984; Kato et al., 1982).

Scrophulia ningpoensis is the root of *S. ningpoensis* HEMSL and is cultivated as a medicinal plant in China. The plant has been used for treatment of fever, swelling, constipation, pharyngitis, neuritis, and laryngitis in traditional Chinese medicine. Various iridoid glycoside and iridoid-related aglycons were isolated and identified from *S. ningpoensis* (Kitagawa et al., 1967; Kajimoto et al., 1989; Qian et al., 1992). In our search for new naturally occurring antimutagenic compounds in plants, with a history of safe use as Chinese crude drugs (Miyazawa et al., 1995a−c, 1996), we found that the methanol extract of *S. ningpoensis* ("Genzin" in Japanese) exhibited a suppression of the SOS-inducing activity of furylfuramide. In this paper, we report the isolation and identification of the suppressive compounds on SOS response against mutagens in *S. ningpoensis*.

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MATERIALS AND METHODS

General Procedure. Gas chromatography (GC) was performed on a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector (FID). GC/MS was performed on a Hewlett-Packard 5972 series mass spectrometer interfaced with a Hewlett-Packard 5890 gas chromatograph fitted with a column (HP-5MS, 30 m $\times$ 0.25 mm i.d.). 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 JEOL GSX 270 NMR spectrometer. Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^{1}H NMR spectra measured in $CDCl_3$.

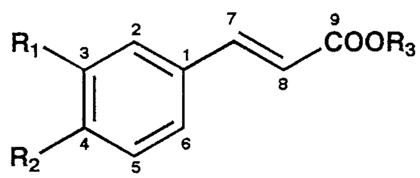
Materials. Commercially available air-dried tips of *S. ningpoensis* (Genzin) were obtained from Nippon Funmatsu Yakuhin & Co., Ltd. Furylfuramide, 4-nitroquinoline 1-oxide (4NQO), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), and aflatoxin B_1 (AfB_1) were purchased from Wako Pure Chemical Co. S9 (supernatant of 9000*g*) and coenzyme, NADPH, NADH, and G-6-P were purchased from Oriental Yeast Co.

***umu* Test.** The *umu* test for detecting the SOS-inducing activity of chemicals was carried out according to the method of Oda et al. (1985) using *S. typhimurium* TA1535/pSK1002, whose plasmid pSK1002 carries an *umuC'*-*'*lacZ fused gene. The overnight culture of bacterial strain was diluted 50-fold into TGA medium (1% Bactotryptone, 0.5% NaCl, and 0.2% glucose; supplemented with 20 mg/L of ampicllin) and incubated at 37 °C until the bacterial density reached 0.25−0.30 in OD600. The bacterial culture was subdivided into 2.1 mL portions in test tubes, and the test compound (50 mL), 0.1 M phosphate buffer (300 mL, pH 7.4), and mutagens(50 mL, in DMSO) were added to each tube. For Trp-P-1 and AfB1, S9 mix was added in each tube instead of phosphate buffer. After 2 h of incubation at 37 °C with shaking, the culture was centrifuged (3000 rpm) to collect cells, which were resuspended in 2.5 mL of PBS. The level of β-galactosidase activity was measured according to a slight modification of Miller's method (Miller, 1972). Fractions (0.25 mL) of the culture were diluted with 2.25 mL of Z buffer, and 0.1% SDS solution (50 mL) and chloroform (10 mL) were added to each fraction. The enzyme reaction was initiated by the addition of 0.25 mL of 2-nitrophenyl β-D-galactopyranoside solution (OMNG; 4 mg/mL in 0.1 M phosphate buffer, pH 7.4) at 28 °C. After 15 min, the reaction was stopped by 0.1 M Na_2CO_3, and the absorbance at OD_{420} and OD_{550} was measured. Using the remainder of culture, the bacterial density was measured at OD_{600}. The unit of β-galactosidase activity was calculated according to the method of Miller (1972).

UV Irradiation. The overnight cultured cells (*S. typhimurium* TA1535/pSK1002) were diluted 50-fold with fresh TGA medium and incubated at 37 °C until the bacterial density at 600 nm reached 0.25−0.30. The cultured cells were centrifuged to collect them and then suspended with 5 mL of 0.1 M phosphate buffer. They were removed into a Petri dish (4 cm) and UV irradiated for 20 s (4.0 J/m^2) with a germicidal lamp at room temperature.

Effects of Suppressive Compounds on mRNA Synthesis Enzymic Activity by IPTG. *Escherichia coli* was a gift from Dr. Oda, Osaka Prefectural Institute of Public Health. The strain produces a β-galactosidase by adding IPTG. The tester bacterial overnight culture was diluted 50-fold into TG medium (1% Bactotryptone, 0.5% NaCl, and 0.2% glucose) and incubated at 37 °C until the bacterial density reached 0.25−0.30 at OD_{600}. The bacterial culture was subdivided into 1.9 mL portions in test tubes, and the test compound (50 mL), 0.1 M phosphate buffer (300 mL, pH 7.4), and 10^{-2} M isopropyl β-D-thiogalactopyranoside (IPTG) (in 0.1 M phosphate buffer, 250 mL) were added to each tube. After 1 h of incubation at 37 °C with shaking, the culture was centrifuged to collect cells, which were resuspended in 2.5 mL of PBS. The level of β-galactosidase activity was measured by a slight modification of Miller's method (Miller, 1972).

Purification and Identification of the Suppressive Compounds. As shown in Figure 1, the dry powder (4 kg) of *S. ningpoensis* was refluxed with methanol for 12 h to give a methanol extract (895.3 g). This extract was suspended in water (3 L) and partitioned between hexane (3 L) and water, dichloromethane (3 L) and water, and then butanol (3 L) and water, successively. Each soluble fraction was concentrated under reduced pressure to give hexane (19.4 g), dichloromethane (9.3 g), butanol (69.7 g), and water (796.9 g) fractions. To purify the compound responsible for suppression of the SOS-inducing activity, these fractions were subjected to the *umu* test. The dichloromethane fraction showed a suppressive effect. The dichloromethane fraction was fractionated to fractions 1 and 2 by SiO_2 column chromatography with chloroform and methanol as eluents. Fraction 1 showed suppression of SOS-inducing activity of furylfuramide in the *umu* test, and this fraction was partitioned with 5% $NaHCO_3$ solution. The aqueous layer was acidified with diluted HCl and then extracted with dichloromethane to yield the acidic fraction (2.6 g). The acidic fraction 4 showed suppression of SOS-inducing activity of furylfuramide in the *umu* test. This fraction was refractionated by SiO_2 column chromatography using the *umu* test as a guide, and suppressive compounds **1** (212 mg), **2** (360 mg), **3** (113 mg), and **4**



1: R_1=R_2=R_3=H **1Me**: R_1=R_2=H, R_3=Me
2: R_1=R_3=H, R_2=OMe **2Me**: R_1=H, R_2=OMe, R_3=Me
3: R_1= R_2=OMe, R_3=H **3Me**: R_1=R_2=OMe, R_3=Me
4: R_1=OMe, R_2=O'H, R_3=H **4Me**: R_1=OMe, R_2=OH, R_3=Me

(22 mg) were isolated. Compounds **1**−**4** were identified as *trans*-cinnamic acid, *p*-methoxycinnamic acid, 3,4-dimethoxycinnamic acid, and 4-hydroxy-3-methoxycinnamic acid by GC, GC/MS, and ^{1}H NMR, respectively.

Preparation of Activated Trp-P-1 (Act.-Trp-P-1). Preparation of Act.-Trp-P-1 was carried out according to the method of Arimoto et al. (1980).

Methyl Esters of Compounds 1−4 (1Me−4Me). Methyl esters of **1**−**4** were obtained by reaction with diazomethane. These structures were identified by GC, GC/MS, and IR.

Suppressive Compounds 1−4 and Methyl Esters 1Me−4Me. *Compound 1*. Compound **1** was a white crystal: mp 131−134 °C; MS, *m/z* 148 (M^+, 74%), 147 (100%), 131 (21%), 103 (53%), 77 (47%), 51 (38%); IR γ_{max} Kbr (cm^{-1}) 3216, 1689, 1630, 1578, 1498, 980; ^{1}H NMR ($CDCl_3$) δ 6.46 (1H, d, J = 16 Hz, H-7), 7.41 (3H, m, J = 9, 3 Hz, H-3, 4, 5), 7.55 (2H, m, J = 9, 3 Hz, H-2, 6), 7.81 (1H, d, J = 16 Hz, H-8). Methyl ester **1Me** was a clear oil: MS, *m/z* 162 (M^+, 47%), 131 (100%), 103 (68%), 77 (51%); IR γ_{max} Kbr (cm^{-1}) 1718, 1264, 1172.

Compound 2. Compound **2** was a white crystal: mp 173−175 °C; MS, *m/z* 178 (M^+, 100%), 161 (32%), 147 (2%), 133 (15%), 118 (6%), 77 (15%), 63 (13%); IR γ_{max} Kbr (cm^{-1}) 3216, 1687, 1600, 1514, 1458, 1431, 1257; ^{1}H NMR ($CDCl_3$) δ 3.85 (3H, s, OMe-9), 6.32 (1H, d, J = 16 Hz, H-7), 6.92 (2H, dt, J = 9, 3, 3 Hz, H-3, 5), 7.51 (2H, dt, J = 9, 3, 3 Hz, H-2, 6), 7.75 (1H, d, J = 16 Hz, H-8). Methyl ester **2Me** was a white crystal: MS *m/z* 192 (M^+, 65%), 161 (100%), 133 (30%), 118 (15%), 89 (21%), 77 (13%); IR γ_{max} Kbr (cm^{-1}) 1720, 1290, 1178.

Compound 3. Compound **3** was a white crystal: mp 181−183 °C; MS, *m/z* 208 (M^+, 100%), 193 (20%), 161 (6%), 147 (10%), 133 (12%), 119 (10%), 103 (7%), 91 (17%), 71 (19%); IR γ_{max} Kbr (cm^{-1}) 2940, 1683 1597, 1516, 1459, 1341, 1264; ^{1}H NMR ($CDCl_3$) δ 3.97 (6H, s, OME-3, 4), 6.33 (1H, d, J = 16 Hz, H-7), 6.89 (1H, d, J = 9 Hz, H-5), 7.08 (1H, d, J = 3 Hz, H-2), 7.14 (1H, dd, J = 9, 3 Hz, H-6), 7.74 (1H, d, J = 16 Hz, H-8). Methyl ester **3Me** was a white crystal: MS, *m/z* 222

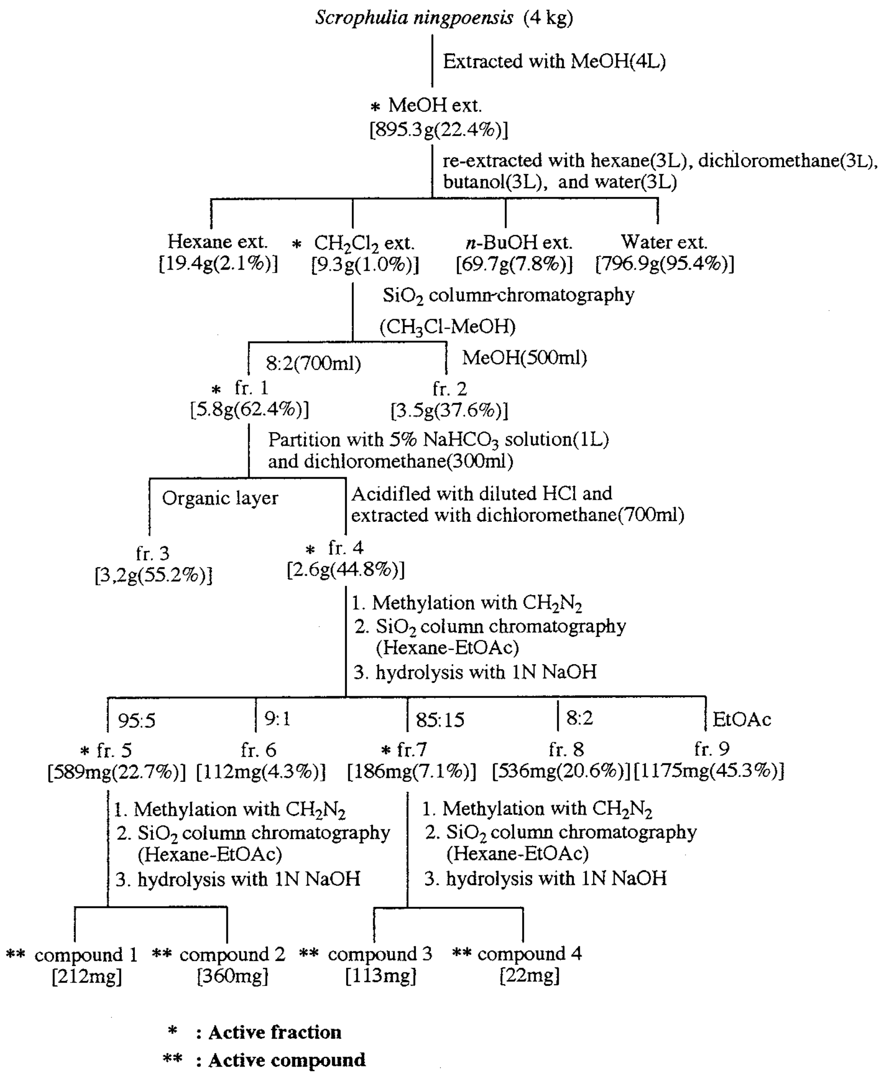


Figure 1. Isolation scheme for the suppressive compounds from *S. ningpoensis.*

(M^+, 100%), 191 (30%), 164 (11%), 133 (6%), 77 (14%); IR γ_{max} Kbr (cm^{-1}) 1712, 1259, 1160.

*Compound **4**.* Compound **4** was a white crystal: mp 167–169 °C; MS, *m/z* 194 (M^+, 100%), 179 (24%), 151 (78%), 133 (22%), 77 (19%); IR γ_{max} Kbr (cm^{-1}) 3087, 1620, 1595, 1516, 1467, 1325, 1277, 1205. Methyl ester **4Me** was a white crystal: MS, *m/z* 208 (M^+, 100%), 177 (60%), 145 (36%), 133 (13%), 117 (14%), 77 (13%); IR γ_{max} Kbr (cm^{-1}) 1702, 1272, 1161; ^1H NMR($CDCl_3$) δ 3.79 (3H, s, H-10), 3.92 (3H, s, OMe-4), 5.91 (1H, s, OH-4), 6.29 (1H, d, $J = 16$ Hz, H-7), 6.91 (1H, d, $J = 16$ Hz, H-5), 7.02 (1H, d, $J = 3$, H-2), 7.07 (2H, dd, $J = 9, 3$ Hz, H-6), 7.62 (1H, d, $J = 16$, H-8).

RESULTS

Fractionation of the Extract from *S. ningpoensis* and Isolation of Suppressive Compounds 1–4. The methanol extract of *S. ningpoensis* was fractionated to search for suppressive compounds using the *umu* test as a guide (Figure 1). 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 acidic fraction (fraction 4) of the dichloromethane fraction exhibited a suppressive effect of SOS-inducing activity of furylfuramide in *S. typhimurium* TA1535/pSK1002. This fraction was further fractionated by SiO_2 column chromatography; the suppressive fraction 5 eluted with 95:5 and fraction 7 eluted with 85:15 hexanes/ethyl acetate as eluents were, respectively, obtained. Finally, suppressive compounds **1** and **2** were isolated from the suppressing fraction 5, and compounds **3** and **4** were isolated from fraction 7. Compound **1**, **2**, **3**, and **4** were identified as *trans*-cinnamic acid, *p*-methoxycinnamic acid, 3,4-dimethoxycinnamic acid, and 4-hydroxy-3-methoxycinnamic acid by GC, GC/MS, ^1H NMR, respectively.

Inhibition of SOS–Inducing Activity by Compounds 1–4. The suppressive effects of compounds **1**–**4** were determined in the *umu* test. As shown in Table 2, compounds **1**–**4** exhibited inhibition of SOS induction of furylfuramide. Compounds **1**–**4** suppressed 44, 31, 37, and 36% of SOS-inducing activity at a concentration of 1.4 μmol/mL, respectively (Figure 2). Compounds **1**–**4** were also assayed with other mutagens, which do not require a liver-metabolizing enzymes mixture. The suppressive effect of **1**–**4** on 4NQO and MNNG is shown in Table 2 and is similar to the suppressive effects observed in the case of furylfuramide. These compounds were also assayed with Trp-P-1 and AfB_1, which require liver metabolic activation. As shown in Table 3, compounds **1**–**4** are more suppressive on the SOS induction of AfB_1 than of Trp-P-1.

Table 1. Suppressive Effects of *S. ningpoensis* Fractions on Furylfuramide[a] Using *S. typhimurium* TA1535/pSK1002

sample	control[b]	dose response[c]			
		200 μg/mL	100 μg/mL	50 μg/mL	0 μg/mL
MeOH extract	196 (±6.3)	*653.4 (±6.3)	*707.6 (±5.0)	*734.4 (±6.3)	777.1 (± 11.3)
haxane fraction	148.9 (±4.2)	*326.2 (±8.4)	*331.1 (±8.9)	*357.9 (±10.2)	403.2 (±4.8)
CH_2Cl_2 fraction	148.9 (±4.2)	*306.2 (±0.9)	*309.1 (±11.6)	*311.6 (±5.8)	403.2 (±4.8)
n-BuOH fraction	148.9 (±4.2)	372.1 (±13.3)	356.9 (±11.2)	345.7 (±10.5)	403.2 (±4.8)
water fraction	148.9 (±4.2)	397.4 (±15.0)	388.7 (±11.8)	359.8 (±14.9)	403.2 (±4.8)
fraction 1	75.6 (±3.9)	*158.0 (±12.5)	*175.2 (±0.4)	199.3 (±9.9)	201.7 (±2.9)
fraction 2	75.6 (±3.9)	187.3 (±5.3)	204.4 (±1.0)	187.1 (±5.4)	201.7 (±2.9)
fraction 3	261.3 (±6.8)	*517.2 (±13.8)	*553.9 (±14.5)	*610.4 (±14.4)	733.3 (±9.5)
fraction 4	261.3 (±6.8)	*570.8 (±15.5)	*630.9 (±14.6)	665.6 (±14.9)	733.3 (±9.5)
fraction 5	353.7 (±4.1)	*539.1 (±4.2)	*571.3 (±4.3)	574.8 (±6.0)	612.0 (±11.7)
fraction 6	353.7 (±4.1)	607.2 (±5.0)	609.2 (±6.2)	610.0 (± 6.6)	612.0 (±11.7)
fraction 7	353.7 (±4.1)	*540.5 (±5.8)	585.3 (±7.2)	601.3 (±9.7)	612.0 (±11.7)
fraction 8	353.7 (±4.1)	604.3 (±13.7)	611.3 (±3.3)	612.8 (±14.7)	612.0 (±11.7)
fraction 9	353.7 (±4.1)	585.8 (±8.3)	604.8 (±5.6)	610.5 (±7.5)	612.0 (±11.7)

[a] Furylfuramide (1 mg/mL in DMSO) was added at 50 mL. [b] Control was a treatment without furylfuramide. [c] β-Galactosidase activity. * $p < 0.05$ when compared with controls.

Table 2. Suppression of Furylfuramide,[a] 4NQO,[b] and MNNG[c]-Induced SOS Response by Compounds 1−4 Using *S. typhimurium* TA1535/pSK1002

mutagen	compd	control	dose response[d]			
			0.14 μmol/mL	0.07 μmol/mL	0.03 μmol/mL	0 μmol/mL
furylfuramide	**1**	136.0 (±6.3)	*248.8 (±14.0)	300.1 (±14.7)	325.4 (±8.6)	336.0 (±12.0)
	2	136.0 (±6.3)	*274.4 (±6.2)	285.9 (±15.5)	322.7 (±12.6)	336.0 (±12.0)
	3	136.0 (±6.3)	*272.6 (±11.1)	292.6 (±6.9)	324.4 (±12.7)	336.0 (±12.0)
	4	136.0 (±6.3)	*264.2 (±10.8)	295.4 (±11.4)	302.0 (±10.6)	336.0 (±12.0)
4NQO	**1**	169.9 (±7.5)	*272.0 (±10.9)	*360.6 (±6.9)	*411.4 (±10.6)	493.3 (±8.9)
	2	169.9 (±7.5)	*316.5 (±8.6)	*416.3 (±8.7)	484.9 (±6.2)	493.3 (±8.9)
	3	169.9 (±7.5)	*389.2 (±11.7)	446.4 (±12.0)	464.2 (±8.6)	493.3 (±8.9)
	4	169.9 (±7.5)	*465.2 (±2.7)	474.4 (±9.6)	492.9 (±3.1)	493.3 (±8.9)
MNNG	**1**	250.3 (±6.5)	*442.2 (±12.1)	*483.3 (±10.5)	*554.1 (±8.6)	654.1 (±10.7)
	2	250.3 (±6.5)	*499.0 (±11.4)	*522.9 (±9.7)	*579.1 (±11.1)	654.1 (±10.7)
	3	250.3 (±6.5)	*505.6 (±10.6)	*591.5 (±11.8)	*6059.1 (±7.4)	654.1 (±10.7)
	4	250.3 (±6.5)	*559.8 (±10.9)	*604.5 (±5.8)	652.3 (±3.4)	654.1 (±10.7)

[a] Furylfuramide (1 μg/mL in DMSO) was added at 50 μL. [b] 4NQO (20 μg/mL in DMSO) was added at 50 μL. [c] MNNG (200 μg/mL in DMSO) was added at 50 μL. [d] β-Galactosidase activity (units). *Significant at $p < 0.05$.

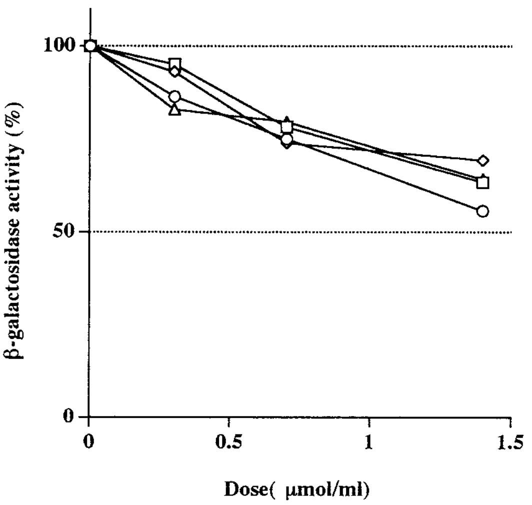


Figure 2. Suppression of furylfuramide-induced SOS response by compounds **1**−**4**: (○) effect of **1** on furylfuramide; (◇) effect of **2** on furylfuramide; (□) effect of **3** on furylfuramide; (△) effect of **4** on furylfuramide. Furylfuramide (1 μg/mL in DMSO) was added at 50 μL.

Compound **1** suppressed 94.3% of SOS-inducing activity on AfB_1 at a concentration of 1.4 μmol/mL, and the ID_{50} (50% inhibitory dose) value was 0.20 μmol/mL. Compound **2** suppressed 91.0% of SOS induction at a concentration of 1.4 μmol/mL, and the ID_{50} value was 0.40 μmol/mL. From these results of the *umu* test, compounds **1** and **2** had greater suppressive effect of the induction of the SOS genes against mutagens, 4NQO and AfB_1, than furylfuramide, MNNG, and Trp-P-1. Compounds **3** and **4** showed weakly suppressive effect of all mutagens.

Suppressive Effect of Methyl Esters (1−4Me) of Compounds 1−4. Methyl esters (**1**−**4Me**) of **1**−**4** were examined for their ability to suppress the SOS-inducing activity of furylfuramide (Table 4; Figure 3). These methyl esters showed greater suppressive effect than **1**−**4**. Especially, the methyl ester of *p*-methoxycinnamic acid suppressed 65.7% of the SOS-inducing activity by furylfuramide at a concentration of 0.70 μmol/mL, and the ID_{50} value was 0.20 μmol/mL. These methyl esters were also assayed with the mutagen Trp-P-1. As shown in Figure 4, **1**−**4Me** showed a suppressive effect on the SOS induction by each mutagen, and the ID_{50} values were 0.7, 0.66, 0.21, and 0.23 μmol/mL, respectively.

Suppressive Effects of Methyl Esters (1−4Me) on Metabolic Activation of Trp-P-1. The suppressive effects of **1**−**4Me** on metabolic activation of Trp-P-1 were determined by the *umu* test. The value of β-galactosidase activity observed in the absence of these compounds was for Act.-Trp-P-1. As shown in Figure 4, suppressive effect of methyl esters **1**−**4Me** on Act.-

Table 3. Suppression of Trp-P-1[a] and AfB_1[b]-Induced SOS Response by Compounds 1−4 Using *S. typhimurium* TA1535/pSK1002

			dose response[c]			
mutagen	compd	control	0.14 μmol/mL	0.07 μmol/mL	0.03 μmol/mL	0 μmol/mL
Trp-P-1	**1**	83.5 (±3.0)	*264.6 (±7.2)	278.4 (±5.8)	296.9 (±2.6)	318.7 (±12.7)
	2	83.5 (±3.0)	*269.1 (±0.1)	295.2 (±7.2)	309.7 (±7.7)	318.7 (±12.7)
	3	83.5 (±3.0)	*242.7 (±13.0)	*248.8 (±10.8)	*266.8 (±3.3)	318.7 (±12.7)
	4	83.5 (±3.0)	294.7 (±8.7)	307.7 (±8.9)	312.6 (±6.8)	318.7 (±12.7)
AfB_1	**1**	220.2 (±6.6)	*232.0 (±6.5)	*276.5 (±11.8)	*299.6 (±14.2)	430.1 (±3.1)
	2	220.2 (±6.6)	*238.6 (±12.5)	*295.6 (±8.9)	*346.9 (±7.3)	430.1 (±3.1)
	3	220.2 (±6.6)	*311.6 (±0.4)	*367.2 (±8.4)	379.9 (±11.7)	430.1 (±3.1)
	4	220.2 (±6.6)	*333.8 (±3.3)	*339.1 (±15.1)	*371.9 (±11.7)	430.1 (±3.1)

[a] Trp-P-1 (40 μg/mL in DMSO) was added at 50 μL. [b] AfB_1 (10 μg/mL in DMSO) was added at 50 μL. [c] β-Galactosidase activity (units). *Significant at $p < 0.05$.

Table 4. Suppression of Furylfuramide,[a] Trp-P-1,[b] and Act.-Trp-P-1[c]-Induced SOS Response by Compounds 1Me−4Me Using *S. typhimurium* TA1535/pSK1002

			dose response[d]				
mutagen	compd	control	0.7 μmol/mL	0.5 μmol/mL	0.3 μmol/mL	0.1 μmol/mL	0 μmol/mL
furylfuramide	**1Me**	116.8 (±7.1)	*433.2 (±8.7)	*446.0 (±11.5)	*470.2 (±8.5)		556.9 (±6.3)
	2Me	116.8 (±7.1)	*267.8 (±7.8)	*293.7 (±6.5)	*371.6 (±12.1)		556.9 (±6.3)
	3Me	116.8 (±7.1)	*427.1 (±10.1)	*501.0 (±8.5)	519.9 (±16.7)		556.9 (±6.3)
	4Me	116.8 (±7.1)	*467.6 (±7.4)	*480.3 (±8.4)	484.3 (±7.9)		556.9 (±6.3)
Trp-P-1	**1Me**	273.3 (±6.1)	*426.4 (±5.1)	*519.5 (±4.3)	556.8 (±3.1)	561.8 (±2.8)	579.0 (±10.3)
	2Me	273.3 (±6.1)	*415.1 (±6.3)	*466.0 (±6.4)	*481.7 (±6.8)	529.9 (±5.6)	579.0 (±10.3)
	3Me	273.3 (±6.1)	*345.6 (±7.8)	*352.9 (±6.2)	*370.6 (±7.4)	*484.0 (±5.6)	579.0 (±10.3)
	4Me	273.3 (±6.1)	*358.2 (6.9)	*360.9 (±7.5)	397.1 (±5.6)	*469.1 (±8.1)	579.0 (±10.3)
Act.-Trp-P-1	**1Me**	139.4 (±11.7)	*425.5 (±10.1)	*498.3 (±10.3)	562.5 (±8.5)	542.4 (±9.7)	572.1 (8.9)
	2Me	139.4 (±11.7)	*398.0 (±9.9)	*434.8 (±10.8)	*503.8 (±8.2)	511.1 (±9.0)	572.1 (8.9)
	3Me	139.4 (±11.7)	*539.3 (±5.4)	550.0 (±5.0)	540.2 (±8.6)	528.6 (±8.9)	572.1 (8.9)
	4Me	139.4 (±11.7)	*488.0 (±10.7)	*494.7 (±8.1)	563.3 (±11.2)	550.7 (±12.1)	572.1 (8.9)

[a] Furylfuramide (1 μg/mL in DMSO) was added at 50 μL. [b] Trp-P-1 (40 μg/mL in DMSO) was added at 50 μL. [c] Act.-Trp-P-1 (10 μg/mL in DMSO) was added at 100 μL. [d] β-Galactosidase activity (units). *Significant at $p < 0.05$.

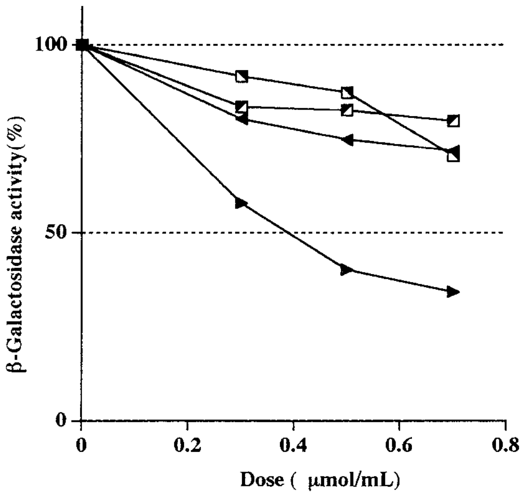


Figure 3. Suppression of furylfuramide-induced SOS response by compounds **1Me**−**4Me**: (solid triangle pointing left) effect of **1** on furylfuramide; (solid triangle pointing right) effect of **2** on furylfuramide; (◪) Effect of **3** on furylfuramide; (◩) effect of **4** on furylfuramide. Furylfuramide (1 μg/mL in DMSO) was added at 50 μL.

Trp-P-1 was decreased compared with Trp-P-1. This result suggests that the inhibition of SOS-inducing activity of Trp-P-1, which was caused by **1**−**4Me**, is due to the inhibition of metabolic activation.

DISCUSSION

The suppressive compounds of SOS-inducing activity in *S. ningpoensis* were identified as due to **1**−**4**. These compounds showed a suppressive effect on *umu* gene expression of the SOS response in *S. typhimurium* TA1535/pSK1002 against furylfuramide, 4NQO, MNNG, Trp-P-1, and AfB_1. As shown in Tables 2 and 3, **1** had greater suppressive potency against all mutagens than **2**−**4**. The principle of the *umu* test is based on the ability of DNA-damaging agents, most of which are potential mutagens and carcinogens, to induce the *umu* operon. The expression of the *umu* operon is known to be regulated by the *recA* gene and *lexA* gene products (Shinagawa et al., 1983; Walker, 1984). In mechanisms for the inhibition of SOS-inducing activity by **1**−**4**, it is necessary to exclude the following possibilities: (i) inhibition of inactivation of the LexA repressor by the RecA protease, (ii) inhibition of transcription of the *recA* gene, and (iii) inhibition of RecA protein synthesis. Effects of suppressive compounds on mRNA synthesis enzymic activity were determined by the *umu* test using *E. coli* CSH 26T/*lac*$^+$, which produced β-galactosidase by IPTG. These compounds did not suppress SOS-inducing activity (Figure 5). This result can exclude the possibility that **1**−**4** inhibit the *lax*A−*rec*A regulation of the *umu* operon. On the other hand, these compounds also did not show suppressive effects on UV irradiation induced SOS response using *S. typhimurium* TA1535/pSK1002 *umu* test (data not shown). In addition, compounds **1Me**−**4Me** exhibited greater suppression of the mutagenicity of furylfuramide and Trp-P-1 than did **1**−**4** and were examined for their ability to suppress the metabolic activation of Trp-P-1 by S9 (Table 4; Figure 4).

Recently, the antimutagenic activity of structurally related cinnamic acid derivatives was reported fre-

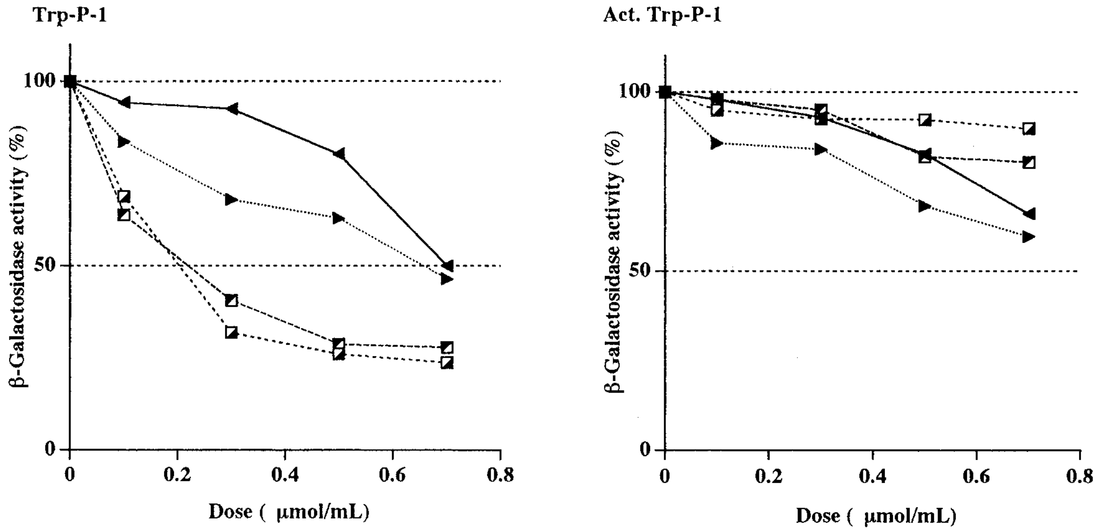


Figure 4. Suppression of Trp-P-1- and Act.-Trp-P-1-induced SOS response by compounds **1Me**−**4Me**: (solid triangle pointing left) effect of **1Me** on Trp-P-1 and Act.-Trp-P-1; (solid triangle pointing right) effect of **2** on Trp-P-1 and Act.-Trp-P-1; (◪) effect of **3** on Trp-P-1 and Act.-Trp-P-1; (◪) effect of **4** on Trp-P-1 and Act.-Trp-P-1. Trp-P-1 (40 μg/mL in DMSO) was added at 50 μL. Act.-Trp-P-1 was added at 100 μL.

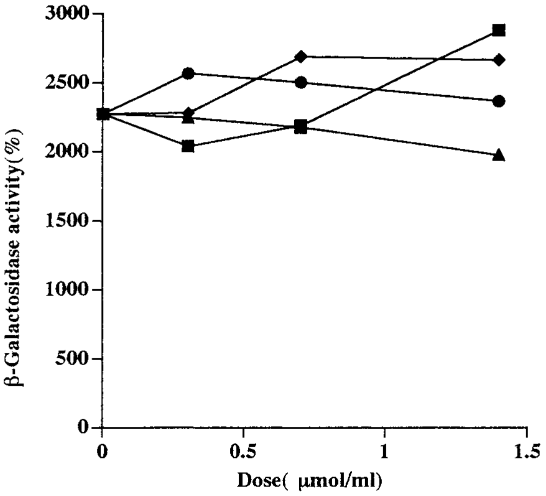


Figure 5. Effect of **1**−**4** on mRNA synthesis induced by IPTG in *E. coli* CSH26T/Flac+: (●) effect of **1** on mRNA synthesis induced; (◆) effect of **2** on mRNA synthesis induced; (■) effect of **3** on mRNA synthesis induced; (▲) effect of **4** on mRNA synthesis induced. IPTG (10^{-2} M) was added at 200 μL.

quently. Ohta et al. reported the antimutagenic effect of cinnamaldehyde on the mutagenesis induced by 4NQO in *E. coli* WP2s, and it might act by interfering with an inducible error-prone DNA repair pathway. It is reported that 4-hydroxy-3-methoxycinnamic acid can inhibit the mutagenicity of the ultimate carcinogenic metabolite of B[a]P (Wood et al., 1982) and inhibit the tongue carcinogenesis induced by 4NQO (Tanaka et al., 1993). Methyl cinnamate derivatives were reported to enhance UV-induced mutagenesis in *E. coli B/r WP2* (Shimoi et al., 1985). This effect was not seen in the DNA excision-repair-deficient strain, *E. coli* WP2S *vur*A, which is SOS-repair-proficient. In addition, cinnamic acid derivatives were reported to have other biological activities, for example, antifungal activity (Lattanzio et al., 1994) and anti-inflammatory action (Chawla et al., 1987). The isolation and identification of cinnamic acid derivatives from, for example, *Balanophra tobiracola* (Balanophpraceae) and *Gaillaradia pulchella* (Compositae) have been reported (Ito et al., 1980; Inayama et al., 1984). In this paper, we suggest that suppressive compounds in *S. ningpoensis* were primarily *trans*-cinnamic acid, *p*-methoxycinnamic acid, 3,4-dimethoxycinnamic acid, and 4-hydroxy-3-methoxycinnamic acid.

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