Antimutagenic Activity of Phenylpropanoids from Clove (*Syzygium aromaticum*)

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Phenylpropanoids that possess antimutagenic activity were isolated from the buds of clove (*Syzygium aromaticum*). The isolated compounds suppressed the expression of the *umu* gene following the induction of SOS response in the *Salmonella typhimurium* TA1535/pSK1002 that have been treated with various mutagens. The suppressive compounds were mainly localized in the ethyl acetate extract fraction of the processed clove. This ethyl acetate fraction was further fractionated by silica gel column chromatography, which resulted in the purification and subsequent identification of the suppressive compounds. Electron impact mass spectrometry, IR, and ^{1}H and ^{13}C NMR spectroscopy were then used to delineate the structures of the compounds that confer the observed antimutagenic activity. The secondary suppressive compounds were identified as dehydrodieugenol (**1**) and *trans*-coniferyl aldehyde (**2**). When using 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (furylfuramide) as the mutagen, compound **1** suppressed 58% of the *umu* gene expression as compared to the controls at a concentration of 0.60 μmol/mL, with an ID_{50} (50% inhibitory dose) value of 0.48 μmol/mL, and compound **2** suppressed 63% of the *umu* gene expression as compared to the controls at a concentration of 1.20 μmol/mL, with an ID_{50} value of 0.76 μmol/mL. Additionally, compounds **1** and **2** were tested for their ability to suppress the mutagenic activity of other well-known mutagens such as 4-nitroquinolin 1-oxide (4NQO) and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), which do not require liver metabolizing enzymes, and aflatoxin B$_1$ (AfB$_1$) and 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), which require liver metabolizing enzymes and activated Trp-P-1 and UV irradiation. Compounds **1** and **2** showed dramatic reductions in their mutagenic potential of all of the aforementioned chemicals or treatment. For the search of the structure−activity relationship, the derivatives of **1** and **2** (**1a** and **2a**−**c**) were also assayed with all mutagens. Finally, the antimutagenic activities of compounds **1**, **1a**, **2**, and **2a**−**c** against furylfuramide, Trp-P-1, and activated Trp-P-1 were assayed by the Ames test using the *S. typhimurium* TA100 strain.

KEYWORDS: Clove; *Syzygium aromaticum*; dehydrodieugenol; *trans*-coniferyl aldehyde; antimutagenic activity

INTRODUCTION

Cancer is currently one of the most dreaded diseases, although methods for its detection at early stages and therapeutic remedies have greatly advanced. In Japan, cancer has been the leading cause of mortality since 1981 (*1*). Kee (*2*) reported that the causes of cancer exist in the environment, especially in the diet and tobacco. Cancer can be initiated by DNA damage, which is caused by natural and man-made chemical substances (mutagens) in the environment (*3*).

Efforts in cancer chemotherapy have intensified over the past several decades, but many cancers still remain difficult to cure; cancer prevention could become an increasingly useful strategy in our fight against cancer. Human epidemiology and animal studies have indicated that cancer risk may be modified by changes in dietary habits or dietary supplements. Humans ingest large numbers of naturally occurring antimutagens, and anticarcinogens may inhibit one or more stages of the carcinogenic process and prevent or delay the formation of cancer. Recent studies indicate that compounds with antioxidant or antiinflammatory properties, as well as certain phytochemicals, can inhibit tumor initiation, promotion, and progression in experimental animal models. Epidemiological studies indicate that dietary factors play an important role in the development of human cancer. Attempts to identify naturally occurring dietary antimutagens and anticarcinogens may lead to new strategies for cancer prevention.

In the evaluation of the carcinogencity or mutagenicity of environmental chemicals, it is quite important to determine factors present in the environment that may affect these activities. With the development of laboratory techniques for

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the detection of possible environmental carcinogens and mutagens (*4*), it has been shown that ordinary human diets contain several mutagens and antimutagens. In particular, the *umu* test system was developed as a simple but sensitive tool 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 (*5, 6*). The results of this test are in agreement with the results of the Ames test and may be more useful with respect to simplicity and rapidity (*7*). The *umu* test detects the induction of the SOS response following treatment of *Salmonella typhimurium* TA1535 with test compounds. This strain carries the plasmid pSK1002 in which the *umu*C′ gene is fused inframe to the *lac*Z′ gene. The SOS-inducing potency of test compounds would therefore be estimated by the measurement of induction of the level of *umu* operon in terms of intracellular β-galactosidase activity.

Furylfuramide is one of the nitrofuran derivatives that has been widely used as a food preservative in Japan. Its genetic effects were reported by many researchers, which led to its classification as a potent mutagen (*8, 9*). Likewise, 4-nitroquinolin 1-oxide (4NQO), a nitroheterocyclic compound, and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), a direct-acting alkylating agent, were classified as potent mutagens (*10−13*). Alternatively, aflatoxin B_1 (AfB$_1$), a difurofuran ring fused to a substituted coumarin moiety, and 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), a heterocyclic amine derived from protein pyrolysis, were shown to be highly mutagenic following their activation by the enzymes contained in the liver S9 fraction (*14−16*).

Antimutagenic effects of some naturally occurring compounds against mutagens have been investigated, including *S*-(*N*,*N*-diethyldithiocarbamoyl)-*N*-acetyl-L-cysteine (*17*), α-pinene-7β-*O*-β-D-2,6-diacetylglucopyranoside (*18*), cinnamic acid (*19*), and palmitic acid (*20*). The resveratrol, a stilbene derivative, was shown to suppress Trp-P-1-induced SOS as measured by the *umu* test and also to suppress its mutagenic potential as measured by the Ames test (*21*); Jang et al. (*22*) have also shown its ability to act as a potent cancer chemopreventive agent.

Clove (*Syzygium aromaticum*, Myrtaceae) is a plant that is cultivated as a spice in many tropical countries. The most important clove-exporting countries are Tanzania, Indonesia, Sri Lanka, and the Malagasy Republic. For oil production, the clove buds are brought to European and American distilleries. Clove oil is frequently used in perfumery and medicine, but the largest part by far is used in flavorings. The dried flower buds of clove ("Tyouji" in Japanese) are an oriental drug, which has been used as vermifuge, an antibacterial agent, and to treat toothaches (*23*). The clove species have been demonstrated to produce a wide variety of potentially useful chemical compounds that include sesquiterpenes (*24*), tannins (*25*), and triterpenoids (*26*). It is well-known that clove possesses a phenolic compound, 4-allyl-2-methoxyphenol, commonly called eugenol. Eugenol acts as an antioxidant on oleogenous foods, as an anticarminative, antispasmodic, antiseptic in pharmacy, and also as an antimicrobial agent (*27−29*).

In our search for novel naturally occurring antimutagenic compounds derived from plants that have a history of safe use as Chinese crude drugs (*30, 31*), we reported the suppression of chemical mutagen-induced SOS response by alkylphenols from the hexane extract fraction of clove (*S. aromaticum*) (*32*). These compounds suppressed furylfuramide, 4NQO, AfB$_1$, and Trp-P-1-induced SOS responses in the *umu* test.

Additional investigations demonstrated that the ethyl acetate extract fraction of clove had secondary suppressive properties toward the furylfuramide-induced SOS response. We thought that this plant might be useful as a cancer chemopreventive agent, and we were interested in the yet unidentified antimutagenic compounds in clove. In this paper, we report the isolation by chemical extraction and silica gel column chromatography and the identification by mass spectrometry, IR spectrometry, and NMR of these secondary antimutagenic compounds contained in clove.

MATERIAL 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 FT/IR-470 Plus Fourier Transform Infrared Spectrometer. NMR spectra (δ, *J* in Hertz) were recorded on a JEOL FX-500 NMR spectrometer. Tetramethylsilane (TMS) was used as the internal reference (δ 0.00) for ^{1}H NMR spectra measured in CDCl$_3$. This solvent was also used for ^{13}C NMR spectra.

Materials. Commercially available air-dried tips of clove (Tyouji) were obtained from Yamada Yakken Co., Ltd. Furylfuramide, 4NQO, MNNG, Trp-P-1, and 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 chemical-induced SOS response was carried out according to the method of Oda et al. (*5*) using *S. typhimurium* TA1535/pSK1002, in which a plasmid (pSK1002) carrying a fused gene (*umu*C′−*lac*Z) had been introduced. 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 ampicillin) and incubated at 37 °C until the bacterial density reached 0.25−0.30 in OD$_{600}$. The bacterial culture was subdivided into 2.1 mL portions in test tubes, and the test compound (50 μL, diluted in dimethyl sulfoxide (DMSO)), 0.1 M phosphate buffer (300 μL, pH 7.4), and mutagens furylfuramide (50 μL, 2 μg/mL in DMSO), 4NQO (50 μL, 20 μg/mL in DMSO), MNNG (50 μL, 200 μg/mL in DMSO), and activated Trp-P-1 (50 μL, 10 μg/mL in DMSO) were added to each tube. In the case of AfB$_1$ (50 μL, 20 μg/mL in DMSO) and Trp-P-1 (50 μL, 40 μg/mL in DMSO), 300 μL of S9-metablizing enzyme mixture including the cofactors was added instead of the phosphate buffer. As a positive control, an equivalent volume of DMSO was added instead of the test compound, whereas with negative control an equivalent volume of DMSO was added instead of both the test compound and the mutagen. After 2 h of incubation at 37 °C with shaking, the culture was centrifuged (3000 rpm) to collect cells, which were centrifuged in 2.5 mL of phosphate-buffered saline (PBS). The level of β-galactosidase activity was measured according to a slight modification of Miller's method (*33*). Fractions (0.25 mL) of the culture were diluted with 2.25 mL of Z buffer, and 0.1% sodium dodecyl sulfate solution (50 μL) and chloroform (10 μL) were added to each fraction. The enzyme reaction was initiated by the addition of 0.25 mL of 2-nitrophenyl-β-D-galactopyranoside solution (ONPG; 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$_2$CO$_3$, 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 (*33*).

Preparation of Activated Trp-P-1. Preparation of activated Trp-P-1 was carried out according to the method of Arimoto et al. (*34*).

UV Irradiation. An overnight culture of the tester bacterial strain (*S. typhimurium* TA1535/pSK1002) incubated at 37 °C in Luria broth was diluted 50-fold with fresh TGA medium and incubated at 37 °C until the optical density at 600 nm of 0.25−0.30 was reached. The cultures were then collected by centrifugation and suspended in 5 mL of 0.1 M phosphate buffer. The cell suspensions were then poured into Petri dishes and exposed to UV light (2.0 J/m²) for 10 s using a germicidical lamp at room temperature.

Effect of Suppressive Compounds on mRNA Synthesis Induced by IPTG. The test strain *Escherichia coli* CSH 26T/F*lac*$^+$, which was kindly supplied by Dr. Yoshimitsu Oda (Osaka Prefectural Institute of Public Health, Japan), was used to investigate the effect on mRNA
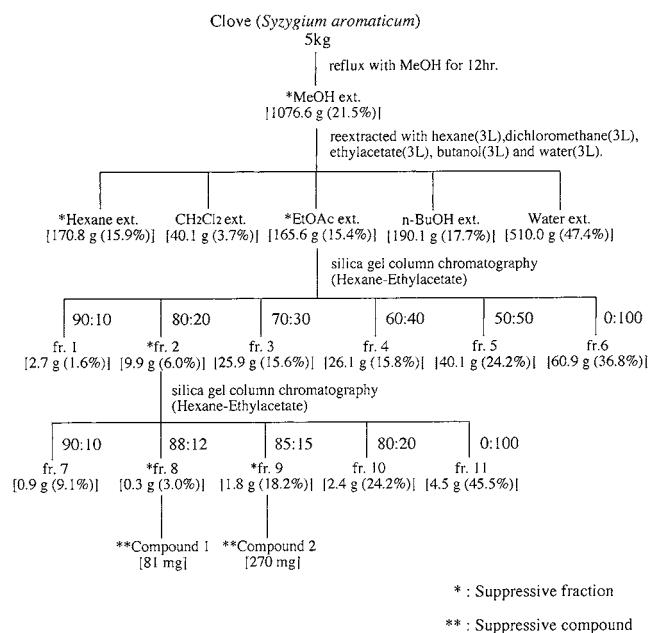
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Figure 1. Isolation scheme for the suppressive compounds from Clove (*S. aromaticum*).

synthesis in the *umu* test; this strain induces β-galactosidase activity by addition of IPTG instead of mutagens. The affect on β-galactosidase synthesis by suppressive compound was assayed as follows: an overnight culture of the tester bacterial strain (*E. coli* CSH 26T/F*lac*$^+$) incubated at 37 °C and diluted 50-fold with TGA medium (1% Bactotryptone, 0.5% NaCl, and 0.2% glucose) was incubated at 37 °C until the bacterial density at 600 nm reached 0.25−0.30. The culture was divided into 1.9 mL portions in the test tubes. The test compound (50 μL, diluted in DMSO), 0.1 M phosphate buffer (300 μL, pH 7.4), and 0.01 M IPTG (200 μL) were added to each tube. After 60 min of incubation at 37 °C with shaking, the culture was centrifuged to collect cells, which were resuspended in 2.5 mL of PBS; the cell density was read at 600 nm with one portion (1.0 mL) of the suspension. Using the other portion (50 μL), the level of β-galactosidase activity was assayed according to the method of Miller (*33*).

Ames Test. The mutation test was carried out according to the preincubation method (*35*), which is a modification of the Ames method (*4*). The test compound (50 μL), 0.1 M phosphate buffer (500 μL), and mutagens frylfuramide (50 μL, 0.5 μg/mL in DMSO), activated Trp-P-1 (50 μL, 10 μg/mL in DMSO) were added to each test tube. In the case of Trp-P-1 (50 μL, 20 μg/mL in DMSO), 500 μL of S9-metabolizing enzyme mixture instead of 0.1 M phosphate buffer was added. The culture of the tester bacterial strain (*S. typhimurium* TA100) was divided into 100 μL portions into the test tube. The mixture was preincubationed at 37 °C for 20 min, mixed with 2.0 mL of top agar at 45 °C, and poured onto a minimal glucose agar plate. After incubation for 2 days at 37 °C, the colonies on the plate were counted.

Purification and Identification of the Suppressive Compounds 1 and 2. As shown in **Figure 1**, the dry powder (5 kg) of clove was refluxed with methanol for 12 h to give a methanol extract (1076.6 g). This extract was suspended in water and reextracted with hexane, dichloromethane, ethyl acetate, butanol, and water, respectively. Each soluble fraction was concentrated under reduced pressure to give hexane (170. 8 g), dichloromethane (40.1 g), ethyl acetate (165.6 g), butanol (190.1 g), and water (510.0 g) fractions. To purify the compound responsible for suppression of the furylfuramide-induced SOS response, these fractions were eveluted to the *umu* test. The ethyl acetate fraction showed a secondary suppressive effect and was fractionated to fractions 1−6 by silica gel column chromatography with hexane and ethyl acetate as eluents. Fraction 2 showed a suppression of the furylfuramide-induced SOS response in the *umu* test, and this fraction was repeatedly fractionated to fractions 7−11 by silica gel column chromatography with hexane and ethyl acetate as eluents. Fractions 8 and 9 showed a suppression of the furylfurmaide-induced SOS response in the *umu* test.

Finally, suppressive compound **1** (81 mg) was isolated from fraction 8, and compound **2** (270 mg) was isolated from fraction 9. Compounds **1** and **2** were identified as dehydrodieugenol (**1**) and *trans*-coniferyl aldehyde (**2**) by EI-MS, IR, and ^{1}H and ^{13}C NMR, respectively.
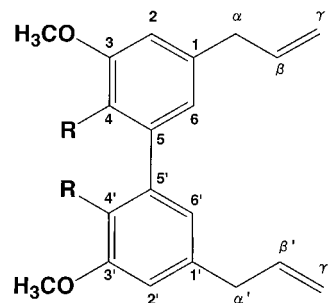
dehydrodieugenol (**1**): **R=OH**

di-*O*-methyldehydrodieugenol (**1a**): **R=OCH₃**
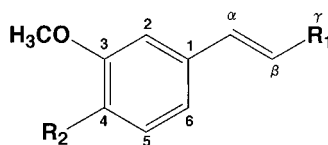
trans-coniferyl aldehyde (**2**): **R₁=CHO, R₂=OH**

trans-3,4-dimethoxy cinnamaldehyde (**2a**): **R₁=CHO, R₂=OCH₃**

trans-coniferyl alcohol (**2b**): **R₁=CH₂OH, R₂=OH**

trans-3,4-dimethoxy cinnamylalcohol (**2c**): **R₁=CH₂OH, R₂=OCH₃**

Compound 1. Compound **1** was a yellow crystalline solid; mp 106−108 °C. MS: *m/z* 326 (M$^+$, 100%), 297 (18.6%), 284 (7.2%), 253 (16.0%), 244 (9.3%), 229 (4.1%), 221 (5.2%). IR γ_{max} KBr (cm^{-1}): 3242, 1599, 1490, 1454, 1257. ^{1}H NMR (CDCl$_3$): δ 3.36 (4H, d, J = 6.6, CH$_2$-α,α'), 3.91 (6H, s, OCH$_3$), 5.06 (2H, dd, J = 1.5, 10.0, CH$_2$−H$_a$-γ,γ'), 5.11 (2H, dd, J = 1.5, 17.2, CH$_2$−H$_b$-γ,γ'), 5.98 (2H, ddt, J = 6.6, 10.0, 17.2, CH-β,β'), 6.72 (2H, d, J = 1.8, H-6,6'), 6.75 (2H, d, J = 1.8, H-2,2'). ^{13}C NMR (CDCl$_3$): δ 147.2 (C- 3,3'), 140.9 (C-4,4'), 137.6 (C-β,β'), 131.9 (C-1,1'), 124.4 (C-5,5'), 123.1 (C-6,6'), 115.7 (C-γ,γ'), 110.7 (C-2,2'), 56.1 (OCH$_3$), 39.9 (C-α,α'). Compound **1** was identified as dehydrodieugenol 1,1'-di-2-propenyl-4,4'-dihydroxy-3,3'-dimethoxy-5,5'-biphenyl from these spectral data.

Compound 2. Compound **2** was a yellowish crystalline solid; mp 75−76 °C. MS: *m/z* 178 (M$^+$, 100%), 147 (48.5%), 135 (72.1%), 107 (81.5%), 77 (85.0%), 51 (70.0%), 40 (48.3%). IR γ_{max} KBr (cm^{-1}): 3239, 1661, 1587, 1514, 1134. ^{1}H NMR (CDCl$_3$): δ 3.93 (3H, s, OCH$_3$), 6.59 (1H, dd, J = 7.7, 15.8, CH-β), 6.95 (1H, d, J = 8.2, H-5), 7.06 (1H, d, J = 2.1, H-2), 7.11 (1H, dd, J = 2.1, 8.2, H-6), 7.40 (1H, d, J = 15.8, CH-α), 9.64 (1H, d, J = 7.7, CHO-γ). ^{13}C NMR (CDCl$_3$): δ 193.4 (C-γ), 153.0 (C- α), 148.9 (C-3), 146.9 (C-4), 126.5 (C-1), 126.2 (C- β), 123.9 (C-6), 114.9 (C-5), 109.5 (C-2), 56.0 (OCH$_3$). Compound **2** was identified as *trans*-coniferyl aldehyde [*trans*-3-(4-hydroxy-3-methoxy phenyl)-2-propenaldehyde] from these spectral data.

Methyl Ethers of Compounds 1 and 2. Methyl ethers of compounds **1** and **2** (**1a** and **2a**) were obtained by reaction with diazomethane. These structures were identified by EI-MS and IR.

Methyl Ether of Compound 1 (1a). MS: *m/z* 354 (M$^+$, 100%), 353 (8.1%), 298 (36.7%), 283 (24.1%), 282 (21.2%), 115 (10.0%), 41 (28.3%). IR v_{max} KBr (cm^{-1}): 2944, 2838, 1645, 1588, 1488. The methyl ether of compound **1** (**1a**) was identified as di-*O*-methyldehydrodieugenol [1,1'-di-2-propenyl-3,4,3',4'-tetramethoxy-5,5'-biphenyl] from these spectral data.

Methyl Ether of Compound 2 (2a). MS: *m/z* 192 (M$^+$, 100%), 177 (18.8%), 161 (66.7%), 149 (19.8%), 121 (16.7%), 91 (17.7%), 77 (25.0%). IR v_{max} KBr (cm^{-1}): 1671, 1620, 1597, 1514, 1270. The methyl ether of compound **2** (**2a**) was identified as *trans*-3,4-dimethoxy cinnamaldehyde [*trans*-3-(3,4-dimethoxy phenyl)-2-propenaldehyde] from these spectral data.

Table 1. Suppression of Furylfuramide-Induced[a] SOS Response by Clove Fractions in *S. typhimurium* TA1535/pSK1002

| sample | control[b] | dose response[c] (g/mL) | | | |
		200	100	40	0
MeOH extract[d]	141.8 (±4.6)	341.3 (±3.6)	364.4 (±2.8)	389.9 (±4.4)	407.4 (±6.6)
hexane fraction[d]	141.8 (±4.6)	260.6 (±4.5)	338.7 (±1.9)	374.2 (±5.6)	407.4 (±6.6)
CHCl₃ fraction	141.8 (±4.6)	386.9 (±7.1)	381.0 (±2.9)	393.4 (±8.9)	407.4 (±6.6)
EtOAc fraction[d]	141.8 (±4.6)	303.1 (±3.8)	365.9 (±5.8)	388.7 (±1.5)	407.4 (±6.6)
BuOH fraction	141.8 (±4.6)	379.2 (±6.8)	393.8 (±5.7)	398.5 (±8.9)	407.4 (±6.6)
water fraction	141.8 (±4.6)	366.2 (±9.9)	384.1 (±5.2)	394.6 (±11.4)	407.4 (±6.6)
fraction 1	107.2 (±3.9)	651.2 (±4.2)	723.6 (±6.1)	754.3 (±2.2)	794.1 (±5.8)
fraction 2[d]	107.2 (±3.9)	449.0 (±2.8)	532.8 (±4.0)	641.4 (±3.9)	794.1 (±5.8)
fraction 3	107.2 (±3.9)	586.3 (±7.3)	664.3 (±8.1)	723.1 (±2.0)	794.1 (±5.8)
fraction 4	107.2 (±3.9)	542.0 (±3.0)	639.9 (±11.8)	709.0 (±4.8)	794.1 (±5.8)
fraction 5	107.2 (±3.9)	668.2 (±6.7)	713.4 (±3.3)	760.7 (±9.7)	794.1 (±5.8)
fraction 6	107.2 (±3.9)	727.3 (±9.3)	740.0 (±2.7)	759.0 (±5.5)	794.1 (±5.8)
fraction 7	94.4 (±5.5)	612.9 (±4.6)	678.8 (±10.6)	704.2 (±2.3)	774.7 (±7.6)
fraction 8[d]	94.4 (±5.5)	344.7 (±1.9)	459.2 (±4.8)	600.0 (±6.3)	774.7 (±7.6)
fraction 9[d]	94.4 (±5.5)	397.9 (±4.2)	497.4 (±5.8)	616.3 (±5.0)	774.7 (±7.6)
fraction 10	94.4 (±5.5)	552.2 (±6.0)	618.1 (±8.3)	679.7 (±1.8)	774.7 (±7.6)
fraction 11	94.4 (±5.5)	659.0 (±9.2)	694.2 (±7.3)	715.2 (±3.5)	774.7 (±7.6)

[a] Furylfuramide (2 μg/mL in DMSO) was added at 50 μL. [b] Control was exposed to DMSO. [c] β-Galactosidase activity (units). [d] Suppressive fraction.

Table 2. Suppressive Effect of Compounds **1** and **1a** on Furylfuramide,[a] 4NQO,[b] and MNNG[c] Using *S. typhimurium* TA1535/pSK1002

| mutagen | compd | control | dose response[d] (μmol/mL) | | | | | ID₅₀[e] |
			0.60	0.48	0.36	0.24	0	
furylfuramide	**1**	97.2 (±1.8)	370.2 (±2.4)	422.7 (±5.3)	490.9 (±4.1)	585.3 (±7.2)	748.8 (±9.3)	0.48
	1a	97.2 (±1.8)	590.8 (±5.0)	607.7 (±6.9)	636.2 (±9.9)	670.0 (±6.7)	748.8 (±9.3)	
4NQO	**1**	130.7 (±4.2)	288.2 (±1.7)	345.7 (±3.3)	426.6 (±5.9)	486.0 (±1.1)	639.2 (±5.8)	0.42
	1a	130.7 (±4.2)	472.8 (±4.9)	500.9 (±2.6)	517.0 (±6.9)	517.2 (±7.8)	639.2 (±5.8)	
MNNG	**1**	106.6 (±5.1)	458.9 (±0.4)	491.8 (±3.2)	528.8 (±5.0)	529.2 (±6.3)	687.0 (±5.7)	
	1a	106.6 (±5.1)	273.9 (±8.3)	318.8 (±1.9)	382.0 (±4.0)	465.2 (±8.2)	687.0 (±5.7)	0.33

[a] Furylfuramide (2 μg/mL in DMSO) was added at 50 μL. [b] 4NQO (40 μg/mL in DMSO) was added at 50 μL. [c] MNNG (200 μg/mL in DMSO) was added at 50 μL. [d] β-Galactocidase activity (units). [e] 50% inhibition dose.

Reduction of Compounds 2 and 2a (2b,c). Alcohols of compounds **2** and **2a** were obtained by reaction with sodium borohydride. These structures were identified by EI-MS and IR.

Alcohol of Compound 2 (2b). MS: *m/z* 180 (M⁺, 54.9%), 137 (100%), 124 (57.0%), 119 (30.5%), 91 (42.3%), 77 (42.9%). IR v_{max} KBr (cm⁻¹): 3595, 3525, 3000, 1600, 1510. The alcohol of compound **2** (**2b**) was identified as *trans*-coniferyl alcohol [*trans*-3-(4-hydroxy-3-methoxy phenyl)-2-propenol] from these spectral data.

Alcohol of Compound 2a (2c). MS: *m/z* 194 (M⁺, 100%), 165 (52.0%), 151 (38.8%), 139 (29.6%), 124 (6.1%). IR v_{max} KBr (cm⁻¹): 2925, 1516, 1263, 1139, 1026. The alcohol of compound **2a** (**2c**) was identified as *trans*-3,4-dimethoxy cinnamyl alcohol [*trans*-3-(3,4-dimethoxy phenyl)-2-propenol] from these spectral data.

RESULTS

Fractionation of the Extract from Clove and Isolation of Suppressive Compounds 1 and 2. The initial methanol extract of clove was further fractionated to identify a suppressive compound using the *umu* test as a guide (**Figure 1**). To obtain dose−response data for purification of the suppressive compound, test samples were evaluated at dose levels of 0.2, 0.1, and 0.04 mg/mL. As shown in **Table 1**, the ethyl acetate fraction exhibited a secondary suppressive effect of the furylfuramide-induced SOS response in *S. typhimurium* TA1535/pSK1002. After fractionating the ethyl acetate fraction, only the suppressive fraction 2 eluted with 80:20 hexane/ethyl acetate as eluents had a clear-cut dose−response effect in the first fractionation (fractions 1−6). The fraction 2 was repeatedly fractionated to fractions 7−11 by silica gel column chromatography with

hexane and ethyl acetate as eluents. Fraction 8 eluted with 88:12 hexane/ethyl acetate as eluents and fraction 9 eluted with 85:15 hexane/ethyl acetate as eluents showed a suppression of the furylfuramide-induced SOS response in the *umu* test. Finally, suppressive compound **1** (81 mg) was isolated from fraction 8, and suppressive compound **2** (270 mg) was isolated from fraction 9.

Suppression of Chemical Mutagen-Induced Responses by Compounds 1 and 1a. Compounds **1** and **1a** were examined for their ability to suppress the SOS-inducing activity on furylfuramide, 4NQO, and MNNG, which do not require a liver-metabolizing enzyme mixture (**Table 2**). Compounds **1** and **1a** suppressed 58 and 24% of the SOS-inducing activity on furylfuramide at a concentration of 0.60 μmol/mL, and the ID₅₀ value of **1** was 0.48 μmol/mL, respectively. Compounds **1** and **1a** suppressed 69 and 33% of the SOS-inducing activity on 4NQO at a concentration of 0.60 μmol/mL, and the ID₅₀ value of **1** was 0.42 μmol/mL, respectively. Compounds **1** and **1a** suppressed 39 and 71% of the SOS-inducing activity on MNNG at a concentration of 0.60 μmol/mL, and the ID₅₀ value of **1a** was 0.33 μmol/mL, respectively. Compounds **1** and **1a** were also assayed with AfB₁ and Trp-P-1, which require liver metabolic activation (**Table 3**). Compounds **1** and **1a** suppressed 93 and 50% of the SOS-inducing activity on AfB₁ at a concentration of 0.60 μmol/mL, and the ID₅₀ value of **1** was 0.26 μmol/mL. Compounds **1** and **1a** suppressed 86 and 41% of the SOS-inducing activity on Trp-P-1 at a concentration of 0.60 μmol/mL, and the ID₅₀ value of **1** was 0.31 μmol/mL. As

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Table 3. Suppressive Effect of Compounds **1** and **1a** on AfB$_1$,[a] Trp-P-1,[b] and Activated Trp-P-1[c] Using *S. typhimurium* TA1535/pSK1002

| mutagen | compd | control | dose response[d] (mol/mL) | | | | | ID$_{50}$[e] |
			0.60	0.48	0.36	0.24	0	
AfB$_1$	**1**	92.3 (±3.1)	127.3 (±0.9)	176.6 (±3.0)	261.1 (±1.7)	363.9 (±5.8)	605.9 (±10.5)	0.26
	1a	92.3 (±3.1)	349.9 (±4.3)	398.7 (±6.8)	428.0 (±2.9)	504.2 (±8.4)	605.9 (±10.5)	0.60
Trp-P-1	**1**	109.7 (±5.5)	179.9 (±1.9)	222.8 (±5.3)	316.0 (±3.2)	392.5 (±7.9)	590.0 (±7.3)	0.31
	1a	109.7 (±5.5)	390.9 (±0.7)	429.5 (±3.9)	470.4 (±8.3)	523.7 (±4.1)	590.0 (±7.3)	
activated Trp-P-1	**1**	94.9 (±4.8)	365.5 (±6.6)	391.1 (±2.3)	422.5 (±7.4)	477.0 (±1.5)	633.3 (±5.8)	0.60
	1a	94.9 (±4.8)	510.6 (±8.5)	539.0 (±5.2)	561.8 (±6.9)	590.0 (±2.0)	633.3 (±5.8)	

[a] AfB$_1$ (20 µg/mL in DMSO) was added at 50 µL. [b] Trp-P-1 (40 µg/mL in DMSO) was added at 50 µL. [c] Activated Trp-P-1 (10 µg/mL in DMSO) was added at 50 µL. [d] β-Galactocidase activity (units). [e] 50% inhibition dose.

Table 4. Suppressive Effect of Compounds **2** and **2a**–**c** on Furylfuramide,[a] 4NQO,[b] and MNNG[c] Using *S. typhimurium* TA1535/pSK1002

| mutagen | compd | control | dose response[d] (mol/mL) | | | | | ID$_{50}$[e] (mol/mL) |
			1.20	0.84	0.60	0.36	0	
furylfuramide	**2**	102.8 (±4.1)	333.9 (±3.1)	397.4 (±2.7)	454.2 (±5.1)	493.8 (±4.2)	727.3 (±6.9)	0.76
	2a	102.8 (±4.1)	457.4 (±0.9)	507.7 (±4.2)	552.0 (±3.6)	604.1 (±8.3)	727.3 (±6.9)	
	2b	102.8 (±4.1)	470.0 (±4.7)	522.5 (±2.5)	571.7 (±7.2)	633.4 (±3.8)	727.3 (±6.9)	
	2c	102.8 (±4.1)	530.8 (±3.3)	567.0 (±10.1)	621.9 (±2.3)	658.7 (±7.0)	727.3 (±6.9)	
4NQO	**2**	112.1 (±3.4)	258.6 (±3.7)	303.5 (±1.6)	354.0 (±7.2)	420.5 (±4.3)	605.8 (±9.1)	0.58
	2a	112.1 (±3.4)	366.5 (±6.6)	408.1 (±7.2)	454.4 (±2.0)	509.0 (±6.2)	605.8 (±9.1)	
	2b	112.1 (±3.4)	417.7 (±3.7)	455.9 (±3.8)	473.2 (±8.3)	521.1 (±10.0)	605.8 (±9.1)	
	2c	112.1 (±3.4)	472.0 (±4.7)	497.8 (±6.7)	533.1 (±2.2)	549.3 (±5.5)	605.8 (±9.1)	
MNNG	**2**	133.0 (±1.9)	447.7 (±1.6)	480.9 (±7.3)	536.3 (±5.0)	604.0 (±8.7)	711.4 (±10.3)	
	2a	133.0 (±1.9)	373.9 (±3.6)	422.2 (±4.9)	484.0 (±5.9)	538.7 (±2.8)	711.4 (±10.3)	0.84
	2b	133.0 (±1.9)	534.1 (±5.2)	564.8 (±10.3)	606.2 (±3.5)	651.9 (±4.9)	711.4 (±10.3)	
	2c	133.0 (±1.9)	483.0 (±7.9)	514.7 (±4.7)	573.0 (±6.3)	628.2 (±3.2)	711.4 (±10.3)	

[a] Furylfuramide (2 µg/mL in DMSO) was added at 50 µL. [b] 4NQO (40 µg/mL in DMSO) was added at 50 µL. [c] MNNG (200 µg/mL in DMSO) was added at 50 µL. [d] β-Galactocidase activity (units). [e] 50% inhibition dose.

Table 5. Suppressive Effect of Compounds **2** and **2a**–**c** on AfB$_1$,[a] Trp-P-1,[b] and Activated Trp-P-1[c] Using *S. typhimurium* TA1535/pSK1002

| mutagen | compd | control | dose response[d] (mol/mL) | | | | | ID$_{50}$[e] (mol/mL) |
			1.20	0.84	0.60	0.36	0	
AfB$_1$	**2**	80.8 (±4.5)	122.0 (±4.1)	160.8 (±2.2)	220.3 (±6.3)	309.8 (±4.8)	524.4 (±9.9)	0.38
	2a	80.8 (±4.5)	210.7 (±3.9)	249.0 (±4.1)	310.3 (±5.4)	382.5 (±6.6)	524.4 (±9.9)	0.63
	2b	80.8 (±4.5)	185.6 (±4.3)	214.4 (±7.5)	282.0 (±11.2)	363.7 (±4.5)	524.4 (±9.9)	0.54
	2c	80.8 (±4.5)	243.1 (±1.9)	277.0 (±3.8)	359.9 (±4.1)	431.2 (±8.6)	524.4 (±9.9)	0.76
Trp-P-1	**2**	139.9 (±5.1)	219.5 (±4.3)	268.3 (±7.2)	338.1 (±6.1)	427.6 (±9.0)	625.7 (±11.4)	0.48
	2a	139.9 (±5.1)	304.6 (±6.2)	362.5 (±3.7)	423.8 (±10.0)	496.8 (±5.1)	625.7 (±11.4)	0.76
	2b	139.9 (±5.1)	279.7 (±4.0)	330.0 (±8.3)	393.6 (±3.1)	394.2 (±6.9)	625.7 (±11.4)	0.64
	2c	139.9 (±5.1)	362.0 (±5.0)	420.6 (±3.5)	497.2 (±7.5)	551.3 (±6.8)	625.7 (±11.4)	1.07
activated Trp-P-1	**2**	107.3 (±3.6)	365.7 (±2.8)	398.0 (±4.3)	452.6 (±5.1)	520.7 (±4.2)	625.0 (±5.7)	1.20
	2a	107.3 (±3.6)	421.2 (±1.9)	448.8 (±3.3)	486.5 (±2.2)	487.3 (±6.9)	625.0 (±5.7)	
	2b	107.3 (±3.6)	485.5 (±2.9)	510.1 (±1.4)	551.7 (±5.6)	590.0 (±3.3)	625.0 (±5.7)	
	2c	107.3 (±3.6)	540.5 (±4.5)	561.2 (±4.7)	579.2 (±5.7)	602.0 (±3.0)	625.0 (±5.7)	

[a] AfB$_1$ (20 µg/mL in DMSO) was added at 50 µL. [b] Trp-P-1 (40 µg/mL in DMSO) was added at 50 µL. [c] Activated Trp-P-1 (10 µg/mL in DMSO) was added at 50 µL. [d] β-Galactocidase activity (units). [e] 50% inhibition dose.

these results of the *umu* test, the suppressive effects of **1** and **1a** on 4NQO are similar to the suppressive effects observed in the case of furylfuramide, and the suppressive effects of **1** and **1a** on AfB$_1$ are similar to the suppressive effects observed in the case of Trp-P-1. Compound **1** had stronger suppressive effects on chemical mutagens except MNNG than compound **1a** did.

Suppression of Chemical Mutagen-Induced Responses by Compounds 2 and 2a−c. Compounds **2** and **2a−c** were examined for their ability to suppress the SOS-inducing activity on furylfuramide, 4NQO, and MNNG, which do not require a liver-metabolizing enzyme mixture (**Table 4**). Compounds **2** and **2a−c** suppressed 63, 43, 41, and 31% of the SOS-inducing activity on furylfuramide at a concentration of 1.20 µmol/mL,

and the ID$_{50}$ value of **2** was 0.76 µmol/mL, respectively. Compounds **2** and **2a−c** suppressed 70, 48, 38, and 27% of the SOS-inducing activity on 4NQO at a concentration of 1.20 µmol/mL, and the ID$_{50}$ value of **2** was 0.58 µmol/mL, respectively. Compounds **2** and **2a−c** suppressed 46, 58, 31, and 39% of the SOS-inducing activity on MNNG at a concentration of 1.20 µmol/mL, and the ID$_{50}$ value of **2a** was 0.84 µmol/mL, respectively. Compounds **2** and **2a−c** were also assayed with AfB$_1$ and Trp-P-1, which require liver metabolic activation (**Table 5**). Compounds **2** and **2a−c** suppressed 91, 71, 76, and 63% of the SOS-inducing activity on AfB$_1$ at a concentration of 1.20 µmol/mL, and the ID$_{50}$ values of **2** and **2a−c** were 0.38, 0.63, 0.54, and 0.76 µmol/mL. Compounds **2** and **2a−c** suppressed 84, 66, 71, and 54% of the SOS-inducing

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Table 6. Suppressive Effect of Compounds **1**, **2**, **1a**, and **2a**−**c** on UV Irradiation[a] Using *S. typhimurium* TA1535/pSK1002

compd	control	dose response[b] (μmol/mL)				
		0.60	0.48	0.36	0.24	0
1	103.3 (±5.8)	662.6 (±4.4)	463.0 (±5.2)	512.2 (±4.2)	585.7 (±7.9)	731.2 (±10.9)
1a	103.3 (±5.8)	431.8 (±6.4)	677.2 (±5.8)	693.5 (±4.9)	715.3 (±11.2)	731.2 (±10.9)

compd	control	1.20	0.84	0.60	0.36	0
2	90.9 (±3.6)	412.6 (±3.3)	454.0 (±2.9)	524.8 (±1.4)	643.2 (±6.4)	712.9 (±7.8)
2a	90.9 (±3.6)	494.2 (±5.1)	536.8 (±3.0)	577.4 (±8.8)	653.5 (±7.6)	712.9 (±7.8)
2b	90.9 (±3.6)	636.3 (±8.4)	656.9 (±6.4)	684.0 (±9.0)	704.7 (±3.8)	712.9 (±7.8)
2c	90.9 (±3.6)	650.0 (±6.3)	663.3 (±5.7)	686.6 (±7.9)	706.2 (±3.4)	712.9 (±7.8)

[a] The cells were exposed to UV light (2.0 J/m²) with a germicidal lamp at room temperature. [b] β-Galactocidase activity (units).

activity on Trp-P-1 at a concentration of 1.20 μmol/mL, and the ID$_{50}$ values of **2** and **2a**−**c** were 0.48, 0.76, 0.64, and 1.07 μmol/mL. As these results of the *umu* test, the suppressive effects of **2** and **2a**−**c** on 4NQO are similar to the suppressive effects observed in the case of furylfuramide, and the suppressive effects of **2** and **2a**−**c** on AfB$_1$ are similar to the suppressive effects observed in the case of Trp-P-1. Compound **2** had stronger suppressive effects on chemical mutagens except MNNG than compounds **2a**−**c** did.

Suppressive Effect of Compounds 1, 2, 1a, and 2a−c on Metabolic Activation of Trp-P-1. The suppressive effect of compounds **1**, **2**, **1a**, and **2a**−**c** on metabolic activation of Trp-P-1 was determined by the *umu* test. The value of β-galactosidase activity observed in the absence of compounds **1**, **2**, **1a**, and **2a**−**c** was for activated Trp-P-1. As shown in **Table 3**, compounds **1** and **1a** suppressed 50 and 23% of the SOS-inducing activity on activated Trp-P-1 at a concentration of 0.60 μmol/mL. As shown in **Table 5**, compounds **2** and **2a**−**c** suppressed 50, 39, 27, and 16% of the SOS-inducing activity on activated Trp-P-1 at a concentration of 1.20 μmol/mL. The suppressive effect of compounds **1**, **2**, **1a**, and **2a**−**c** on activated Trp-P-1 was decreased as compared with that on Trp-P-1.

Suppressive Effects of Compounds 1, 2, 1a, and 2a−c on UV Irradiation. The suppressive effects of compounds **1**, **2**, **1a**, and **2a**−**c** on UV irradiation-induced SOS response were determined using the *umu* test. Compounds **1** and **1a** were evaluated at dose levels of 0.60, 0.48, 0.36, and 0.24 μmol/mL to obtain dose−response data, and compounds **2** and **2a**−**c** were evaluated at dose levels of 1.20, 0.84, 0.60, and 0.36 μmol/mL to obtain dose−response data. Compounds **1**, **2**, and **2a** exhibited inhibition on the UV irradiation-induced SOS response, and compounds **1a** and **2b,c** did not exhibit any inhibition (**Table 6**). Compound **1** suppressed 48% of the SOS-inducing activity due to UV irradiation at a concentration of 0.60 μmol/mL, respectively. Compounds **2** and **2a** suppressed 48 and 35% of the SOS-inducing activity due to UV irradiation at a concentration of 1.20 μmol/mL, respectively.

Effects of Compounds 1, 2, 1a, and 2a−c on mRNA Synthesis. In the mechanism for inhibition of mutagen-induced SOS response by compounds **1**, **2**, **1a**, and **2a**−**c**, there are some possibilities that compounds **1**, **2**, **1a**, and **2a**−**c** have an effect on the *lex*A−*rec*A regulation (mRNA synthesis) of the *umu* operon. Compounds **1**, **2**, **1a**, and **2a**−**c** were assayed for the effect on mRNA synthesis using *E. coli* CSH26T/F*lac*$^+$, which produces β-galactosidase without mutagens. This strain has a good characteristic function, which produces β-galactosidase activity, after treated with IPTG. As shown in **Figure 2**, compounds **1**, **2**, **1a**, and **2a**−**c** did not affect β-galactosidase activity caused by IPTG. The result can exclude the possibility
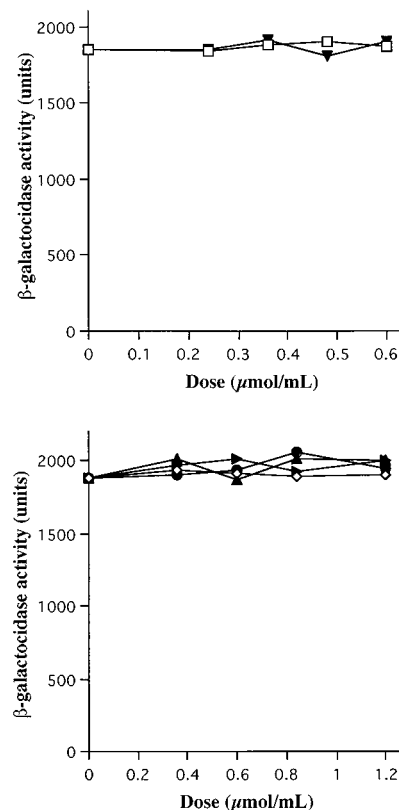

Figure 2. Effect of compounds **1**, **2**, **1a**, **2a**−**c** on mRNA synthesis induced by IPTG in *E. coli* CSH26T/F*lac*$^+$: effect of **1** (□), effect of **2** (◇), effect of **1a** (▼), effect of **2a** (●), effect of **2b** (▲), and effect of **2c** (right pointing solid triangle). IPTG (10^{-2} M) was added at 200 μL.

that compounds **1**, **2**, **1a**, and **2a**−**c** inhibit the *lex*A−*rec*A regulation of the *umu* operon.

Antimutagenic Activity of Compounds 1, 2, 1a, and 2a−c in the Ames Assay. The antimutagenic activity of compounds **1**, **2**, **1a**, and **2a**−**c** against furylfuramide, Trp-P-1, and activated Trp-P-1 were also demonstrated by the Ames test using *S. typhimurium* TA100. Compounds **1** and **1a** suppressed 52 and 36% of the mutagenicity of furylfuramide at a concentration of 0.60 μmol/plate, and the ID$_{50}$ value of **1** was 0.57 μmol/plate, respectively (**Figure 3**). Compounds **2** and **2a**−**c** suppressed 62, 54, 47, and 38% of the mutagenicity of furylfuramide at a concentration of 1.20 μmol/plate, and the ID$_{50}$ values of **2** and **2a** were 0.64 and 1.05 μmol/plate, respectively (**Figure 4**). As shown in **Figure 3**, compounds **1** and **1a** suppressed 91 and 78% of the mutagenicity of Trp-P-1 at a concentration of 0.60 μmol/plate, and the ID$_{50}$ values of **1** and **1a** were 0.14 and 0.27 μmol/plate. As shown in **Figure 4**, compounds **2** and **2a**−**c** suppressed 57, 47, 79, and 68% of the mutagenicity of Trp-

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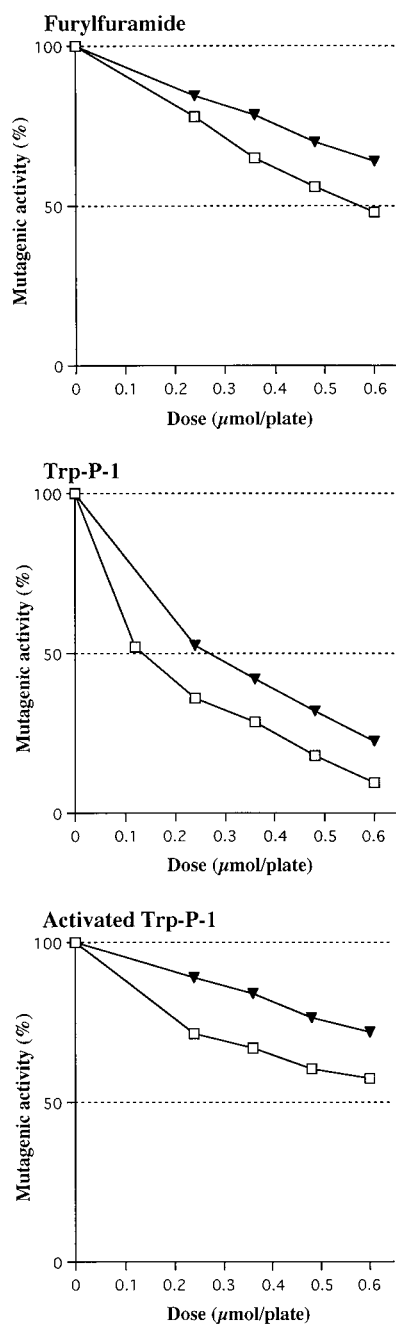*J. Agric. Food Chem.,* Vol. 51, No. 22, 2003 **6419**

Figure 3. Effect of compounds **1** and **1a** on the mutagenicity of furylfuramide, Trp-p-1, and activated Trp-p-1 in *S. typhimurium* TA100: effect of **1** (□) and effect of **1a** (▼). Furylfuramide (0.5 μg/mL in DMSO) was added at 50 μL/plate. Trp-p-1 (20 μg/mL in DMSO) was added at 50 μL/plate. Activated Trp-P-1 (10 μg/mL in DMSO) was added at 50 μL/plate.

Figure 4. Effect of compounds **2** and **2a–c** on the mutagenicity of furylfuramide, Trp-p-1, and activated Trp-p-1 in *S. typhimurium* TA100: effect of **2** (◇), effect of **2a** (●), effect of **2b** (▲), and effect of **2c** (right pointing solid triangle). Furylfuramide (0.5 μg/mL in DMSO) was added at 50 μL/plate. Trp-p-1 (20 μg/mL in DMSO) was added at 50 μL/plate. Activated Trp-P-1 (10 μg/mL in DMSO) was added at 50 μL/plate.

P-1 at a concentration of 1.20 μmol/plate, and the ID_{50} values of **2** and **2b,c** were 0.83, 0.36, and 0.66 μmol/plate. On the other hand, these antimutagenic activities against activated Trp-P-1 were remarkably decreased as compared with those on Trp-P-1. From this result, it is suggested that antimutagenic activity of these compounds on Trp-P-1 is due to the inhibition of metabolic activation of Trp-P-1 by S9.

DISCUSSION

The antimutagenic compounds in clove (*S. aromaticum*) were clearly identified as due to dehydrodieugenol (**1**) and *trans*-coniferyl aldehyde (**2**). These compounds showed suppressive
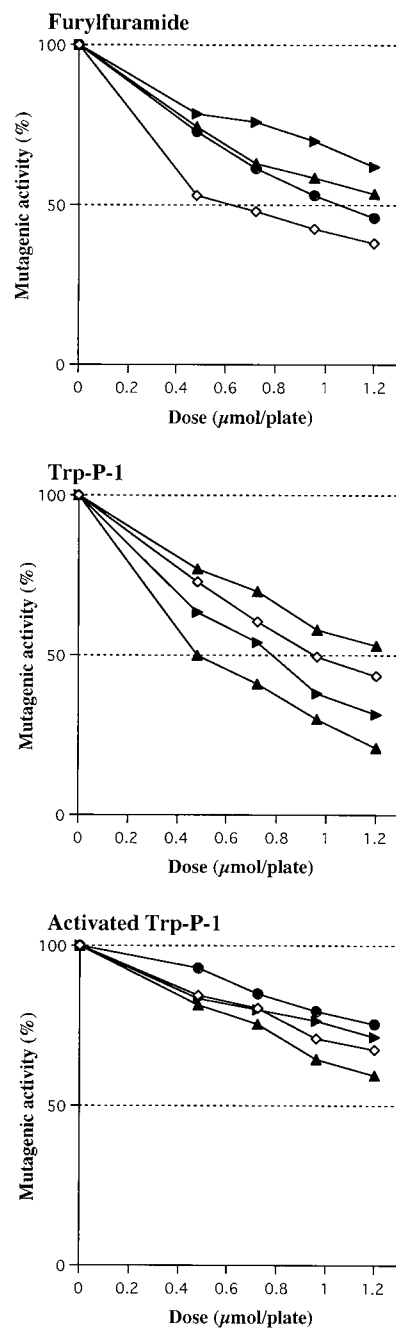
effects on *umu* gene expression of the SOS response in *S. typhimurium* TA1535/pSK1002 against furylfuramide, 4NQO, and MNNG, which do not require liver-metabolizing enzymes, and AfB_1 and Trp-P-1, which require liver-metabolizing enzymes and UV irradiation.

As shown in **Tables 2**, **3**, and **6**, compound **1** had stronger suppressive potencies than **1a** against mutagens all except MNNG. The differences in structure between **1** and **1a** are the presence of hydroxy groups at the C-4 and C-4′ positions in **1**, as opposed to methoxy groups in **1a**. These results indicated that hydroxy groups at the C-4 and C-4′ positions are important

factors for suppressing the SOS-inducing activity on mutagens except MNNG. On the other hand, compound **1a** had stronger suppressive potencies against MNNG than **1** did. These results indicated that methoxy groups at the C-4 and C-4′ positions are important factors for suppressing the SOS-inducing activity on MNNG.

As shown in **Tables 4−6**, compound **2** had stronger suppressive potencies against mutagens except MNNG than **2a** did, and compound **2b** had stronger suppressive potencies against mutagens except MNNG than **2c** did. The difference in structure between **2** and **2a** and between **2b** and **2c** is the presence of a hydroxy group at the C-4 position in **2** and **2b**, as opposed to a methoxy group in **2a** and **2c**. These results indicated that a hydroxy group at the C-4 position is an important factor for suppressing the SOS-inducing activity on mutagens except MNNG. On the other hand, compound **2a** had stronger suppressive potencies against MNNG than **2** did, and compound **2c** had stronger suppressive potencies against MNNG than **2b** did. These results indicated that a methoxy group at the C-4 position is an important factor for suppressing the SOS-inducing activity on MNNG.

Previously, we reported the suppression of chemical mutagen-induced SOS response by cinnamic acid derivatives from *Scrophulia ningpoensis* (*30*) and by alkylphenols from clove (*S. aromaticum*) (*31*). These compounds suppressed furylfuramide, 4NQO, MNNG, AfB_1, and Trp-P-1-induced SOS responses in the *umu* test. These results indicated the importance of a functional group at the C-γ position in *trans*-cinnamic derivatives on suppressive effects, changed by chemical mutagens and functional groups at C-3 and C-4 positions. The suppressive effects of furylfuramimde- and 4NQO-induced SOS response decreased in the order *trans*-isoeugenol $\simeq$ *trans*-coniferyl aldehyde > *trans*-methyl ferulate > *trans*-coniferyl alcohol > *trans*-ferulic acid in *trans*-4-hydroxy-3-methoxy cinnamic derivatives. These results indicated that the importance for suppressing the SOS-inducing activity on furylfuramide and 4NQO decreased in the order $CH_3 \simeq CHO > COOCH_3 > CH_2$-$OH > COOH$ on the functional group at the C-γ position in *trans*-4-hydroxy-3-methoxy cinnamic derivatives. The suppressive effects of MNNG-induced SOS response decreased in the order *trans*-coniferyl aldehyde > *trans*-methyl ferulate > *trans*-coniferyl alcohol > *trans*-isoeugenol $\simeq$ *trans*-ferulic acid in *trans*-4-hydroxy-3-methoxy cinnamic derivatives. These results indicated that the importance for suppressing the SOS-inducing activity on MNNG decreased in the order $CHO > COOCH_3 > CH_2OH > CH_3 \simeq COOH$ on the functional group at the C-γ position in *trans*-4-hydroxy-3-methoxy cinnamic derivatives. The suppressive effects of AfB_1- and Trp-P-1-induced SOS response decreased in the order *trans*-isoeugenol > *trans*-methyl ferulate > *trans*-coniferyl aldehyde > *trans*-coniferyl alcohol > *trans*-ferulic acid in *trans*-4-hydroxy-3-methoxy cinnamic derivatives. These results indicated that the importance for suppressing the SOS-inducing activity on AfB_1 and Trp-P-1 decreased in the order $CH_3 > COOCH_3 > CHO > CH_2OH > COOH$ on the functional group at the C-γ position in *trans*-4-hydroxy-3-methoxy cinnamic derivatives. On the other hand, the suppressive effects of furylfuramimde- and 4NQO-induced SOS response decreased in the order *trans*-3,4-dimethoxy cinnamaldehyde > *trans*-3,4-dimethoxy methyl cinnamate $\simeq$ *trans*-3,4-dimethoxy cinnamyl alcohol > *trans*-3,4-dimethoxy cinnamic acid > *trans*-methyl isoeugenol in *trans*-3,4-dimethoxy cinnamic derivatives. These results indicated that the importance for suppressing the SOS-inducing activity on furylfuramide and 4NQO decreased in the order $CHO > COOCH_3 \simeq CH_2OH >$

$COOH > CH_3$ on the functional group at the C-γ position in *trans*-3,4-dimethoxy cinnamic derivatives. The suppressive effects of MNNG-induced SOS response decreased in the order *trans*-methyl isoeugenol > *trans*-3,4-dimethoxy methyl cinnamate > *trans*-3,4-dimethoxy cinnamaldehyde > *trans*-3,4-dimethoxy cinnamyl alcohol > *trans*-3,4-dimethoxy cinnamic acid in *trans*-3,4-dimethoxy cinnamic derivatives. These results indicated that the importance for suppressing the SOS-inducing activity on MNNG decreased in the order $CH_3 > COOCH_3 > CHO > CH_2OH > COOH$ on the functional group at the C-γ position in *trans*-3,4-dimethoxy cinnamic derivatives. The suppressive effects of AfB_1- and Trp-P-1-induced SOS response decreased in the order *trans*-3,4-dimethoxy methyl cinnamate > *trans*-methyl isoeugenol $\simeq$ *trans*-3,4-dimethoxy cinnamaldehyde > *trans*-3,4-dimethoxy cinnamyl alcohol > *trans*-3,4-dimethoxy cinnamic acid in *trans*-3,4-dimethoxy cinnamic derivatives. These results indicated that the importance for suppressing the SOS-inducing activity on AfB_1 and Trp-P-1 decreased in the order $COOCH_3 > CH_3 \simeq CHO > CH_2OH > COOH$ on the functional group at the C-γ position in *trans*-3,4-dimethoxy cinnamic derivatives.

Compounds **1**, **2**, and **2a** had a suppressive effect on *umu* gene expression of SOS response in *S. typhimurium* TA1535/pSK1002 against UV irradiation, which is a physical mutagen (*36*). The antimutagenic factors are divided into two main classes: one type, desmutagen, inactivates or destroys mutagens directly or indirectly out of the cell, and the other type of factor is called bioantimutagen, which suppresses the process of mutagenesis itself in the cells. From this result, the mechanism for inhibition of the SOS-inducing activity by compounds **1**, **2**, and **2a** may involve not only acted action on the mutagens but also involvement with cellular repair systems, and so, compounds **1**, **2**, and **2a** might have the ability to be potent bioantimutagens.

Compounds **1**, **2**, **1a**, and **2a−c** were examined for the ability to suppress the metabolic activation of Trp-P-1 by S9. As shown in **Figure 3**, compounds **1**, **2**, **1a**, and **2a−c** suppressed the weaker SOS induction on activated Trp-P-1 than on Trp-P-1. This result suggested the possibility that the inhibition of the SOS-inducing activity on Trp-P-1, which was caused by compounds **1**, **2**, **1a**, and **2a−c**, was due to the inhibition of metabolic activation by S9.

The SOS regulatory system involves the action of two proteins: the LexA protein, which represses a set of unlinked genes, and the RecA protein, which is activated as a protease by an inducing signal and specifically inactivates the repressor. The SOS response is activation of the protease, and the later manifestations of the SOS response are a secondary consequence of these events (*37*). The mechanism for inhibition of mutagen-induced SOS response by compounds **1**, **2**, **1a**, and **2a−c** includes the following possibilities: (i) suppression of inactivation of the LexA repressor by the RecA protease, (ii) suppression of the transcription of the *rec*A gene, and (iii) suppression of RecA protein synthesis. Because the expression of the *umu*C gene is known to be regulated by the *rec*A and *lec*A gene products (*38, 39*), the present data with *E. coli* may exclude the possibility that the *lex*A−*rec*A regulation of the *umu* operon is suppressed. Compounds **1**, **2**, **1a**, and **2a−c** were assayed for its effect on mRNA synthesis using *E. coli* CSH26T/F*lac*⁺, which produces β-galactosidase without mutagens. Compounds **1**, **2**, **1a**, and **2a−c** did not affect β-galactosidase activity. Therefore, it suggested that compounds **1**, **2**, **1a**, and **2a−c** had a potent suppressive effect on the mutagen-induced SOS response.

Antimutagenic Activity of Phenylpropanoids

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Recently, the antimutagenic activity of cinnamaldehyde was reported frequently. Cinnamaldehyde reduced 4NQO- and UV-induced mutagenesis, as well as mutagenesis induced by furylfuramide (AF-2) in *E. coli* WP2s, and it might act by interfering with an inducible error prone DNA repair pathway (*40*). Cinnamaldehyde was shown to suppress Hprt mtations in UV- and X-ray-exposed V79 cells (*41*). In human-derived hepatoma cells, cinnamaldehyde suppressed the frequency of micronuclei induced by a variety of heterocyclic amines (*42*). Dehydrodieugenol has been isolated from *Litsea turfosa* (*43*), *Ocotea cymbarum* (*44*), *Virola carinata* (*45*), and *Nectandra polita* (*46*). Coniferyl aldehyde also has been isolated from some natural sources including *Salvia plebeia* (*47*) and *Balanophora latisepala* (*48*). Taira et al. reported that dehydrodieugenol and coniferyl aldehyde had hydroxyl radical scavenging abilities (*49*). However, inhibition of mutagen-induced SOS response by dehydrodieugenol and *trans*-coniferyl aldehyde has not been reported. In summary, this research suggests that suppressive compounds on SOS response against chemical and physical mutagens in clove (*S. aromaticum*) were primarily dehydrodieugenol (**1**) and *trans*-coniferyl aldehyde (**2**) and that compounds **1** and **2** and their derivatives showed potent suppressive effects of the SOS-inducing activity by chemical and physical mutagens and potent inhibition of the mutagenicity against chemical mutagens.

We expect that the antimutagenic compounds isolated from clove (*S. aromaticum*) will be useful cancer chemopreventive agents. However, these compounds may not exhibit their expected effects in vivo if they are adversely affected by factors such as absorption, biodisposition, and metabolism after they are incorporated into the human body. Further studies with mammalian cells in vitro or in vivo are needed to determine the efficacy of these compounds for the prevention of human cancer.

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