ARTICLES

Partial Suppression of SOS-Inducing Activity of Furylfuramide by Dibasic Acids from *Ipomoea nil* in the *Salmonella typhimurium* TA1535/pSK1002 *umu* Test

Mitsuo Miyazawa,*,† Hideo Shimamura,† Sei-ichi Nakamura,‡ and Hiromu Kameoka†

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higashiosaka-shi, Osaka 577, Japan, and Osaka Prefectural Institute of Public Health, Nakamichi-1, Higashinari-ku, Osaka 537, Japan

An acidic fraction from *Ipomoea nil* showed suppression of SOS-inducing activity of the mutagen 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) in the *Salmonella typhimurium* TA1535/pSK1002 *umu* test. The suppressive compounds in *I. nil* were fractionated by SiO_2 column chromatography. The active compounds in the suppressing fraction were identified as butanedioic acid, pentanedioic acid, hexanedioic acid, heptanedioic acid, octanedioic acid, nonanedioic acid, decanedioic acid, undecanedioic acid, dodecanedioic acid, tridecanedioic acid, tetradecanedioic acid, pentadecanedioic acid, hexadecanedioic acid, 3,4-dimethoxycinnamic acid, and linoleic acid by GC and GC–MS. These dibasic acids partially suppressed the SOS-inducing activity of AF-2. The dose–response values of all dibasic acids were in the range 0.3–1.5 μmol/mL. Tetradecanedioic acid suppressed 70% of the SOS-inducing activity of AF-2 under 1.5 μmol/mL. In addition tetradecanedioic acid was assayed with other mutagens (3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]indole (Glu-P-1)), and it also showed a suppressive effect on each mutagen. The ID_{50} (50% inhibitory dose) values of tetradecanedioic acid for AF-2, Trp-P-1, and Glu-P-1 were estimated as 0.44, 0.80, and 0.65 μmol/mL, respectively.

Keywords: *Ipomoea nil; dibasic acid; tetradecanedioic acid; SOS-inducing activity; umu test; Salmonella typhimurium TA1535/pSK1002*

INTRODUCTION

Mutagens and antimutagens have been found in several crude drugs, and some of these structures have been elucidated (dictamnine, γ-fagarin, Mizuta et al., 1985; eugenol, hydroxychavicol, Amonkar et al., 1986; dioscin, gracillin, Kim et al., 1989). The *umu* test is a short-term assay to detect potential environmental carcinogens and mutagens using the inducibile SOS genes *umuC* in *Salmonella typhimurium* TA1535/pSK1002 (Oda et al., 1985; Nakamura et al., 1987).

In a search for new naturally occurring antimutagenic compounds in plants with a history as medicines in China, we found that the dichloromethane extract of *Ipomoea nil* (Kengoshi in Japanese) partially suppressed SOS-inducing activity. The acidic fraction of the dichloromethane extract suppressed SOS-inducing activity of AF-2 more than the dichloromethane extract. In this paper, we report the identification and structure–activity relationship of dibasic acids as suppressive compounds from *I. nil*.

MATERIALS AND METHODS

Materials. Commercially available air-dried seeds of *I. nil* (Nippon Funmastu Ltd. Japan) were used. The air-dried seeds for use as crude drug were collected in 1992 from plants cultivated in Nara prefecture in Japan. The dibasic acids (C_{11} and C_{16}) were purchased from Aldrich Chemical Co. The dibasic acids (C_{13}, C_{15}, and C_{20}), 3,4-dimethoxycinnamic acid, and linoleic acid were from Tokyo Kasei Chemical Co. Other dibasic acids were from Nakarai Tesque, Inc.

***Umu* Assay.** The *umu* test in *S. typhimurium* TA1535/pSK1002 for detecting SOS-inducing activity was carried out according to Oda et al., 1985. Briefly, an overnight culture of the tester bacterial strain in Luria broth (1% Bactotryptone, 0.5% NaCl, and 0.5% yeast extract) was diluted 50-fold with fresh 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 at 600 nm reached 0.25–0.30. The culture was divided into 2.3 mL portions in test tubes. The test compound (50 μL), 0.1 M phosphate buffer (290 μL, pH 7.4), and AF-2 (60 μL, 1 μg/mL in DMSO) were added to each tube. In the case of Trp-P-1 (50 μL, 40 μg/mL in DMSO) and Glu-P-1 (50 μL, 100 μg/mL in DMSO), 300 μL of S9 metabolizing enzyme instead of 0.1 M phosphate buffer mixture was added. After 2 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 cell density was read at 600 nm with one portion (1.0 mL) of the suspension. Using the other portion (0.25 mL), the level of β-galactosidase activity in the cell was assayed by the method of Miller (1972).

Extraction of the Suppressive Compounds from *I. nil*. The sample of the dry powder (2.29 kg) was refluxed with dichloromethane for 12 h to give the dichloromethane extract (143 g) (Figure 1).

Fractionation of the Suppressive Compounds. The dichloromethane extract 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 (0.67 g) (fraction 2). The acidic fraction showed suppression of SOS-inducing activity of AF-2. This fraction was then treated with CH_2N_2 and fractionated to fractions 3–6

* Author to whom correspondence should be addressed.
† Kinki University.
‡ Osaka Prefectural Institute of Public Health.

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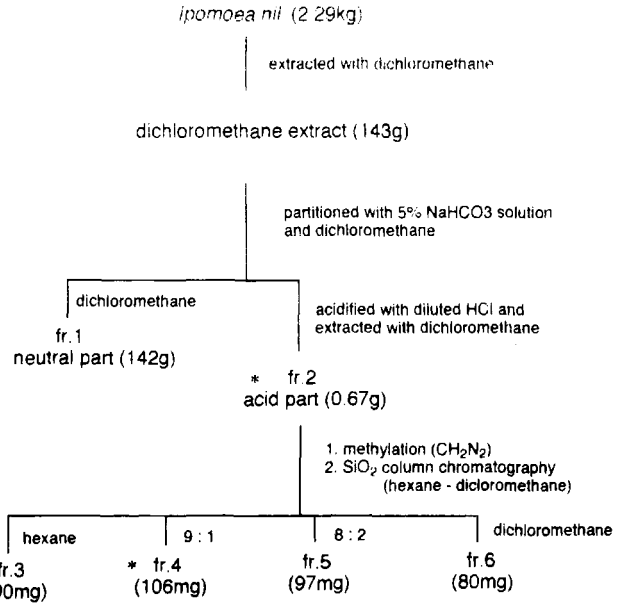

Figure 1. Purification procedure for suppressive compounds from *I. nil:* (*) active fraction.

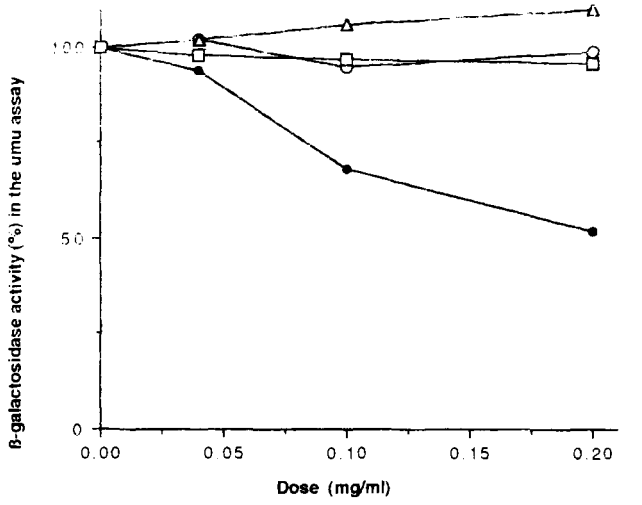

Figure 2. Effect of fractions 3–6 on the suppression of SOS-inducing activity of AF-2 in *S. typhimurium* TA1535/pSK1002 without S9 mix: (○) fraction 3, (●) fraction 4, (△) fraction 5, (□) fraction 6.

by SiO_2 column chromatography using hexane–dichloromethane as eluent (Figure 1). For the *umu* assay, each fracton was hydrolyzed with NaOH. Fractions 3–6 were assayed by the *umu* test. The suppressing fraction (106 mg) (fraction 4) was obtained with hexane–dichloromethane (9:1).

GC and GC–MS of Suppressing Substances in Fraction 4. The methyl esters of active fraction were identified by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) on a column of OV-1 (25 m × 0.25 mm). In the gas-chromatographic analysis, the column temperature progressed at 4 °C/min from 140 to 240 °C, and the port of injection was 240 °C. A stream of nitrogen with a flow rate of 1 mL/min was used as carrier.

RESULTS

The acidic fraction of the dichloromethane extract exhibited suppression of SOS-inducing activity of AF-2. The result of the *umu* test from these fractions (fractions 3–6) is shown in Figure 2. The suppressing fraction 4 was obtained with hexane–dichloromethane (9:1). The components of the active fraction were analyzed by GC and GC–MS. The methyl esters of

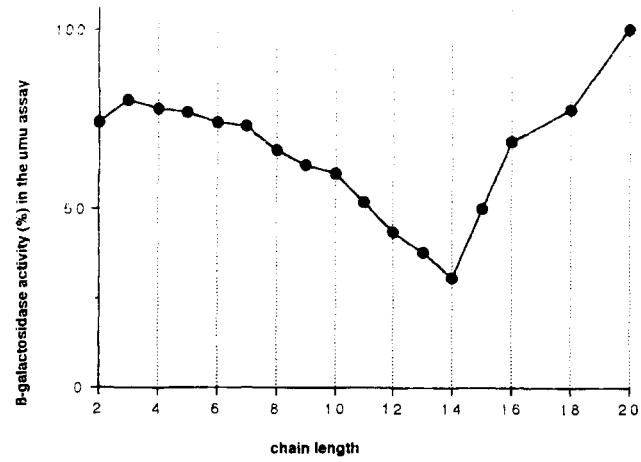

Figure 3. Effect of dibasic acids of various chain lengths on the suppression of SOS-inducing activity of AF-2 in *S. typhimurium* TA1535/pSK1002 without S9 mix. The concentration of dibasic acids was 1.5 μmol/mL. Eicosanedioic acid was toxic under the 1.5 μmol/mL.

octadioic acid (C_8), nonanedioic acid (C_9), decanedioic acid (C_{10}), undecanedioic acid (C_{11}), dodecanedioic acid (C_{12}), tridecanedioic acid (C_{13}), tetradecanedioic acid (C_{14}), hexadecanedioic acid (C_{16}), 3,4-dimethoxycinnamic acid, and linoleic acid were identified as prominent peaks of the active fraction. Minor components of the active fraction were C_4–C_7 and C_{15} dibasic acids. The quantitative amounts of these acids were butanedioic acid (0.2%: by peak area of GC), pentanedioic acid (0.1%), hexanedioic acid (0.8%), heptanedioic acid (0.3%), octanedioic acid (3.9%), nonanedioic acid (52.7%), decanedioic acid (6.3%), undecanedioic acid (2.7%), dodecanedioic acid (3.3%), tridecanedioic acid (2.8%), tetradecanedioic acid (4.8%), pentadecanedioic acid (0.8%), hexadecanedioic acid (3.2%), 3,4-dimethoxycinnamic acid (4.7%), and linoleic acid (4.3%). In the quantitative analysis of the active fraction by peak area of GC, the dibasic acids were 81.5%.

The dibasic acids of major components in suppressing fraction were assayed by the *umu* test using standard acids (Table 1). Eight of the dibasic acids in Table 1 exhibited suppression of the SOS-inducing activity of AF-2. Tetradecanedioic acid was the most effective suppressor of the SOS-inducing activity of AF-2. The level of the β-galactosidase activity was varied with chain lengths. Octanedioic acid (C_8) and hexadecanedioic acid (C_{16}) showed only 25% suppression of SOS-inducing activity at a concentration 1.5 μmol/mL. To specify the chain length–activity relationship of dibasic acids, saturated dibasic acids of C_2–C_{16}, C_{18}, and C_{20} were examined for their ability to suppress the SOS-inducing activity of AF-2 at a concentration 1.5 μmol/mL. As shown in Figure 3, all of the dibasic acids (C_2–C_{20}) exhibited suppression of SOS-inducing activity, and tetradecanedioic acid (C_{14}) exhibited the greatest suppression. Suppression of SOS-inducing activity increased in proportion to the carbon number, but in the C_{14}–C_{20} dibasic acids suppression decreased inversely with carbon number. Eicosanedioic acid (1.5 μmol/mL) was toxic; at concentrations of 0.1, 0.2, and 0.4 μmol/mL the suppression of the SOS-inducing activity of AF-2 was not observed (data not shown).

Tetradecanedioic acid (C_{14}) was also tested by the mutagens Trp-P-1 (0.8 μg/mL) and Glu-P-1 (2.0 μg/mL), which requires S9 is activation. The result of the *umu* test with Trp-P-1 and Glu-P-1 is shown in Figure 4. The

Table 1. Effect of Dibasic Acids of Identified Major Components in Suppressing Fraction 4 on Suppression of SOS-Inducing Activity of AF-2 in *S. typhimurium* TA1535/pSK1002 without S9 Mix

component	dose–response (β-galactosidase activity (units)), μmol/mL			
	0	0.30	0.75	1.50
octanedioic acid		640 ± 19	558 ± 18	533 ± 9
nonanedioic acid		621 ± 9	564 ± 15	510 ± 7
decanedioic acid		575 ± 11	540 ± 11	498 ± 13
undecanedioic acid		547 ± 7	523 ± 12	454 ± 15
dodecanedioic acid		507 ± 3	475 ± 6	410 ± 11
tridecanedioic acid		484 ± 6	432 ± 13	380 ± 12
tetradecanedioic acid		466 ± 18	410 ± 10	342 ± 5
hexadecanedioic acid		662 ± 14	609 ± 9	545 ± 18
control	177 ± 8			
AF-2 (0.024 μg/mL)	711 ± 15			

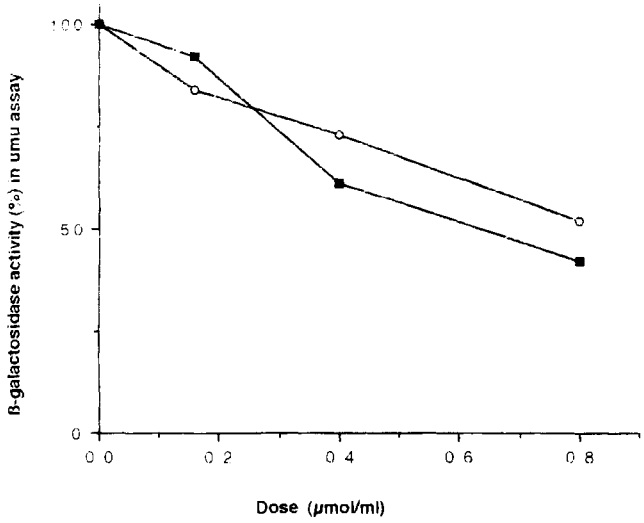

Figure 4. Effect of tetradecanedioic acid on the suppression of SOS-inducing activity of Trp-P-1 and Glu-P-1 with S9 mix for *S. typhimurium* TA1535/pSK1002 with S9 mix: (○) Trp-P-1, (■) Glu-P-1.

tetradecanedioic acid (C_{14}) decreased SOS-inducing activity linearly with increasing dose.

3,4-Dimethoxycinnamic acid and linoleic acid were identified as the compounds in the suppressing fraction 4 except for the dibasic acids. 3,4-Dimethoxycinnamic acid showed suppression of SOS-inducing activity of AF-2. Linoleic acid did not show suppression of SOS-inducing activity of AF-2 (Figure 5). In contrast, linoleic acid had suppressed the SOS-inducing activity (53% inhibition) of Trp-P-1 with S9 under the 0.15 μmol/mL (data not shown).

DISCUSSION

Dogasaki et al. (1992) showed that fatty acids (oleic acid, linoleic acid, and linolenic acid) in medicinal plant (*Prunus mume*) exhibited antimutagenic activity in the Ames test. Antimutagenicity of fatty acids against various mutagens was reported by many workers (Hayatsu et al., 1981; Yamaguchi, 1989). The antimutagenic effect of oleic acid was observe for Trp-P-1 with S9 mix, but not for AF-2 without S9 mix (Stich, 1983); Stich reported that oleic acid showed inhibition on the enzymic demethylation of *N*-nitrosodimethylamine (NDMA) and that the inhibition was parallel with that of the mutagenic activity. The dibasic acids of major components in suppressing fraction 4 exhibited suppression of the SOS-inducing activity of AF-2 (Table 1). As shown in Table 1, suppression of SOS-inducing activity

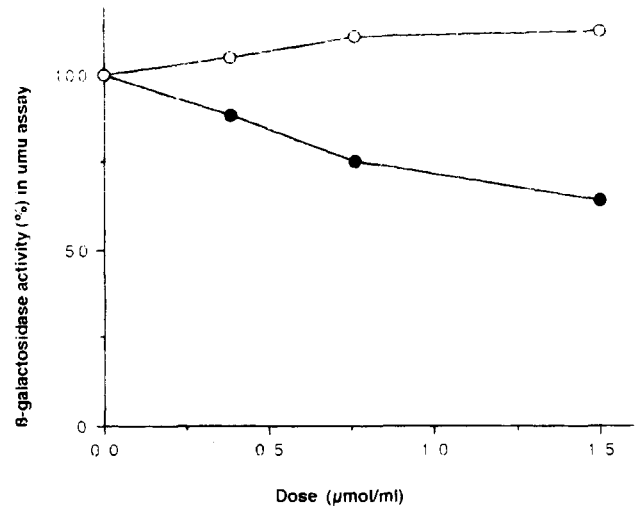

Figure 5. Effect of 3,4-dimethoxycinnamic acid and linoleic acid on the suppression of SOS-inducing activity of AF-2 in *S. typhimurium* TA1535/pSK1002 without S9 mix: (●) 3,4-dimethoxycinnamic acid, (○) linoleic acid.

was affected by chain lengths of dibasic acids. As shown in Figure 3, the chain length–activity relationship of the dibasic acids suggested that suppression of SOS-inducing activity increased in proportion to the carbon number, but in C_{14}–C_{20} dibasic acids, suppression decreased inversely with carbon number and tetradecanedioic acid exhibited the greatest suppression. Negishi et al. (1984) reported that upon modulating the activity and mechanism of saturated fatty acids having carbon numbers 2–12 upon nitrosamine-mediated mutagenesis, in the saturated acids C_5–C_{12}, stronger inhibition was observed as the chain length became longer.

3,4-Dimethoxycinnamic acid and linoleic acid were identified as the components in the active fraction (fraction 4) except for dibasic acids. 3,4-Dimethoxycinnamic acid exhibited suppression of the SOS-inducing activity of AF-2 (Figure 5). Linoleic acid exhibited suppression of the SOS-inducing activity of Trp-P-1 (data not shown).

Tetradecanedioic acid also exhibited suppression of the SOS-inducing activity of Trp-P-1 and Glu-P-1 (Figure 4). The ID_{50} values of tetradecanedioic acid for AF-2, Trp-P-1, and Glu-P-1 were 0.44, 0.80, and 0.65 μmol/mL, respectively. In this experiment, suppressing compounds in *I. nil* were primarily dibasic acids, 3,4-dimethoxycinnamic acid, and linoleic acid.

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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.

Amonkar, A. J.; Nagabhushan, M.; D'Souza, A. V.; Bhide, V. Hydroxychavicol: A new phenolic antimutagen from betel leaf. *Food Chem. Toxicol.* **1986**, *24*, 1321–1324.

Dogasaki, C.; Murakami, H.; Nishijima, M.; Yamamoto, K.; Miyazaki, T. Antimutagenic activities of hexane extracts and

the kernels of Prunus mume Sieb. et Zucc. *Yakugaku Zasshi* **1992**, *112*, 577–584.

Hayatsu, S.; Arimoto, S.; Togawa, K.; Makita, M. Inhibitory effect of the ether extract of human feces on activities of mutagens: Inhibition by oleic acid and linoleic acid. *Mutat. Res.* **1981**, *81*, 287–293.

Kim, S. W.; Son, K. H.; Chung, K. C. Mutagenic effect of steroidal saponins from Smilax china rhizomes. *Yakhak Hoechi.* **1989**, *33*, 285–289.

Miller, J. H. *Experiments in Molecular Genetics*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1972; pp 352–355.

Mizuta, M.; Kanamori, H. Mutagenic activities of dictamnine and γ-fagarine from Dictamin Radicis Cortex (Rutaceae). *Mutat. Res.* **1985**, *144*, 221–225.

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

Negishi, T.; Hayatsu, H. Inhibitory effect of saturated fatty acids on the mutagenicity of N-nitrosodimethylamine. *Mutat. Res.* **1984**, *135*, 87–96.

Oda, Y.; Nakamura, S.; Oki, I.; Kato, T.; H. Shinagawa, H. Evaluation of the new system (umu-test) for the detection of environmental mutagens and carcinogens. *Mutat. Res.* **1985**, *147*, 219–229.

Stich, H. F. *Carcinogens and mutagens in the environment*; CRC Press: Boca Raton, FL, 1983; pp 89–99.

Yahagi, T.; Nagao, M.; Seino, Y.; Masushima, T.; Sugimura, T.; Okada, M. Mutagenicities of N-nitrosoamines on Salmonella. *Mutat. Res.* **1977**, *48*, 121–130.

Yamaguchi, T. Mutagenic activity of various kinds of cheese on the Ames, *rec* and *umu* assays. *Mutat. Res.* **1989**, *224*, 493–502.

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