Suppression of Furylfuramide-Induced SOS Response by Acetophenones Using *Salmonella typhimurium* TA1535/pSK1002 *umu* Test

Mitsuo Miyazawa,*,† Hideo Shimamura,† Sei-ichi Nakamura,‡ and Wataru Sugiura‡

Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Kowakae, Higashiosaka-shi, Osaka 577-8502, Japan, and Osaka Prefectural Institute of Public Health, Nakamichi-1, Higashinari-ku, Osaka 537-0025, Japan

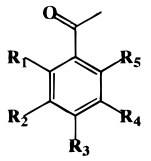
The recently isolated paeonol (2-hydroxy-4-methoxyacetophenone), as one of the antimutagenic compounds from *Discorea japonica*, was used as a lead compound for detailed structure−activity relationship studies. Nine acetophenones (2-hydroxy-4-methoxy, 2-hydroxy-5-methoxy, 2-hydroxy-6-methoxy, 4-hydroxy-3-methoxy, *o*-methoxy, *m*-methoxy, *p*-methoxy, and 2,5-dimethoxyacetophenone and acetophenone) were investigated for their ability of suppression of furylfuramide-induced SOS response using *Salmonella typhimurium* TA1535/pSK1002 in the *umu* test, against the mutagen, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (furylfuramide). The results showed that 2-hydroxy-6-methoxyacetophenone displayed the strongest activity ($EC_{50} = 0.6\ \mu$mol/mL), and a hydroxyl group at C-2 is necessary feature for acetophenone derivatives to show the suppressive effects of furylfuramide-induced SOS response.

Keywords: *Acetophenones; suppressive effects; SOS response; umu gene; Salmonella typhimurium TA1535/pSK1002; furylfuramide*

INTRODUCTION

Paeonol is a well-known compound of the essential oils of *Paeonia mouton* (Miyazawa et al., 1983), which is a component of prescriptions used as an analgesic, a sedative, an anti-inflammatory agent, a homeostatic, and a remedy for female diseases in traditional oriental medicine. This compound was isolated as a principal anticoagulative compound from *Paeonia mouton* (Ishida et al., 1987) and as a bio-antimutagenic compound from *Paeonia suffruiticosa* (Fukuhara et al., 1986). In our search for new naturally occurring antimutagenic compounds in plants that have a history of safe use as Chinese crude drugs (Miyazawa et al., 1995a, 1995b, 1996b, 1997), we previously reported about isolation of β-eudesmol and paeonol as antimutagenic compounds from *Dioscorea japonica* (Miyazawa et al., 1996a). The present paper deals with the structure−activity relationship of acetophenone derivatives. Paeonol (2-hydroxy-4-methoxyacetophenone, **1**), 2-hydroxy-5-methoxyacetophenone (**2**), 2-hydroxy-6-methoxyacetophenone (**3**), 4-hydroxy-3-methoxyacetophenone (apocynin, **4**), *o*-methoxyacetophenone (**5**), *m*-methoxyacetophenone (**6**), *p*-methoxyacetophenone (**7**), and 3,4-dimethoxyacetophenone (**8**), and acetophenone (**9**) were investigated for their abilities of a wide variety of the *S. typhimurium* strain TA1535 with test compounds (see Table 1). With the development of laboratory techniques for the detection of possible environmental carcinogens and mutagens (Ames et al., 1975), 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 (Oda et al., 1985; Nakamura et al., 1987). The *umu* test detects the induction of the SOS response following treatment of the *Salmonella typhimurium* strain TA1535 with test compounds. This strain carries the plasmid pSK1002 in which the *umuC′* gene is fused inflame to the *lacZ* 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 was one of the nitrofuran derivatives that had been widely used as a food preservative in Japan since 1965; however it was found to be mutagenic (McCann et al., 1975; Tazima et al., 1975; Ames, 1979). In 1974, its use was legally prohibited. The mutational specificity of furylfuramide in the *lacI* gene of *Escherichia coli* was investigated, and base substitu-

Table 1. Structures of Acetophenones Presented in This Paper



acetophenones	X_1	X_2	X_3	X_4	X_5
1	OH	H	OMe	H	H
2	OH	H	H	OMe	H
3	OH	H	H	H	OMe
4	H	OMe	OH	H	H
5	OMe	H	H	H	H
6	H	OMe	H	H	H
7	H	H	OMe	H	H
8	OMe	H	H	OMe	H
9	H	H	H	H	H

* Telephone: +81−6−6721−2332. Fax: +81-6-6727-4301. E-mail: miyazawa@apch.kindai.ac.jp.
† Kinki University.
‡ Osaka Prefectural Institute of Public Health.

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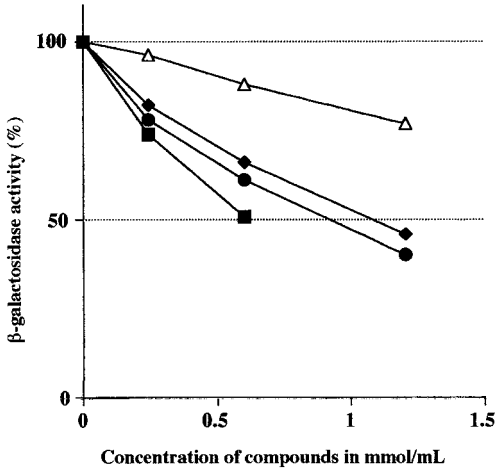

Figure 1. Suppression of furylfuramide-induced SOS responses by acetophenones which have hydroxy and methoxy substitutions (**1**−**4**). Key: (●) effect of **1**; (♦) effect of **2**; (■) effect of **3**; (△) effect of **4**. Furylfuramide (1 μg/mL in DMSO) was added at 60 μL.

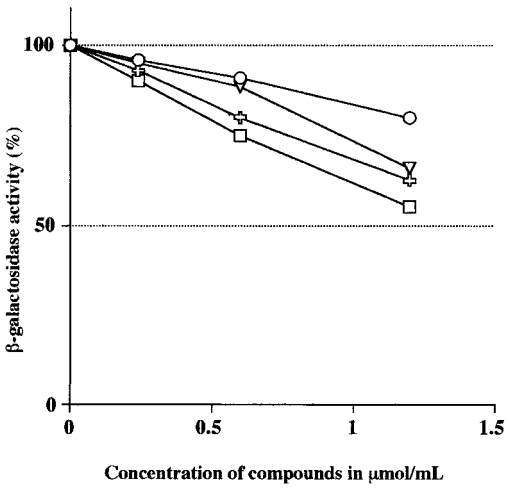

Figure 2. Suppression of furylfuramide-induced SOS responses by acetophenones which have methoxy substitution (**5**−**8**). Key: (○) effect of **5**; (✣) effect of **6**; (□) effect of **7**; (▽) effect of **8**. Furylfuramide (1 μg/mL in DMSO) was added at 60 μL.

tion occurred primarily (>93%) at the G:C base pair (Lambert et al., 1991).

MATERIALS AND METHODS

Materials. 2-Hydroxy-4-methoxy and 4-hydroxy-3-methoxyacetophenone were purchased from Tokyokasei Kougyou Co. Ltd. (Tokyo, Japan). 2-Hydroxy-5-methoxy and 2-hydroxy-6-methoxyacetophenone were purchased from Aldrich Chemical Co. (Milwaukee, WI). The *o*-, *m*-, and *p*-2,5-dimethoxyacetophenons were gifts of Dr. Takeo Nishiyama (Kinki University). Furylfuramide was purchased from Wako Pure Chemical Co. (Osaka, Japan).

***umu* Test.** An overnight culture of the tester bacteria strain (*S. typhimurium* TA1535/pSK1002) in Luria broth (1% Bactotryptone, 0.5% NaCl, and 0.5% yeast extract; supplemented with 20 mg/mL ampicillin) was diluted 50-fold with fresh TGA medium (1% Bactotryptone, 0.5% NaCl, and 2% glucose; supplemented with 20 mg/mL ampicillin) and incubated at 37 °C until the optical density at 600 nm of the culture reached 0.25−0.30. The culture was then aliquoted into 2.3 mL portions in test tubes, and the test compound (50 μL, diluted in DMSO), 0.1 m phosphate buffer (300 μL, pH 7.4), and furylfuramide (50 μL, 1 μL/mL in DMSO) were added to each 1.20, 0.60, and 0.24 μmol/mL. As positive controls, an equivalent volume of DMSO was added in stead of test compound, whereas with negative controls, an equivalent volume of DMSO were added instead of both test compound and mutagen. After 2 h of incubation at 37 °C with shaking, the culture was centrifuged to sediment the cells, and then the cells were resuspended in 2.5 mL of PBS. The optical density of the suspensions at 600 nm were recorded with one portion (0.25 mL), while the rest of the cell suspensions were used to measure the level of intracellular β-galactosidase activities using the method of Miller (1972).

RESULTS AND DISCUSSION

As shown in Figure 1, **1** showed a suppressive effect on *umu* gene expression of the SOS responses in *S. typhimurium* TA1535/pSK1002 against furylfuramide. **1** suppressed 60% of the SOS-inducing activity at concentrations less than 1.2 μmol/mL, and the EC_{50} (50% of effective concentration) value was 1.08 μmol/mL. **3** suppressed 50% of the SOS-inducing activity at concentration less than 0.60 μmol/mL, although this compound showed toxicity at 1.20 μmol/mL. **4** suppressed 23% of the SOS-inducing activity at a concentration of 1.2 μmol/mL. As shown in Figure 2, **5**−**7** also showed a suppressive effect of SOS-inducing activity, and these compounds showed 20, 37, and 45% suppressive effects at concentration less than 1.20 μmol/mL, respectively. **8** suppressed 34% of the SOS-inducing activity at similar concentration. **9** showed a weaker suppressive effect (15%) than its derivatives in *umu* test (Figure 3).

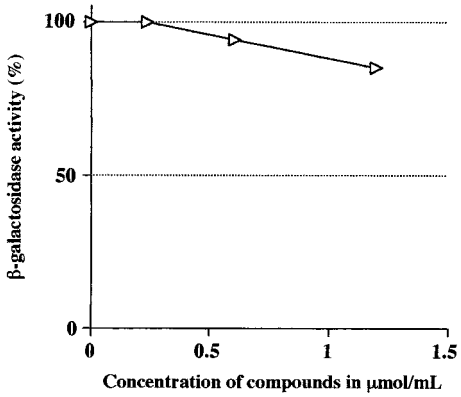

Figure 3. Suppression of furylfuramide-induced SOS response by acetophenone which has no substitution (**9**). Furylfuramide (1 μg/mL in DMSO) was added at 60 μL.

Figure 4 shows a suppressive effects on furylfuramide-induced SOS responses of acetophenones at a concentration of 0.6 μmol/mL in order to explain structure−activity relationship. Acetophenones which have 2-hydroxy substitution showed more than 25% suppressive effects at a concentration of 0.60 μmol/mL, and **3** (50%) is the most effective compound in this experiment. **4** showed a weak (12%) suppressive effect in comparison with acetophenones which have 2-hydroxy in the order *o*- < *m*- < *p*-acetophenone. **8** showed 11% suppressive effect of furylfuramide-induced SOS responses. Therefore, a 2-hydroxy substitution is important factor for this suppressive effect in comparison with **2** and **8**. **9** was used for the basic structure of acetophenones to compare with other compounds. This compound showed little (3%) suppressive effect on furylfuramide-induced SOS responses at a concentration of 0.60 μmol/mL.

For mutagenic activation of furylfuramide (cis form), cis−trans isomerization (Kalyanaraman et al., 1979, 1981; Koga et al., 1984) and reduction of the nitro group

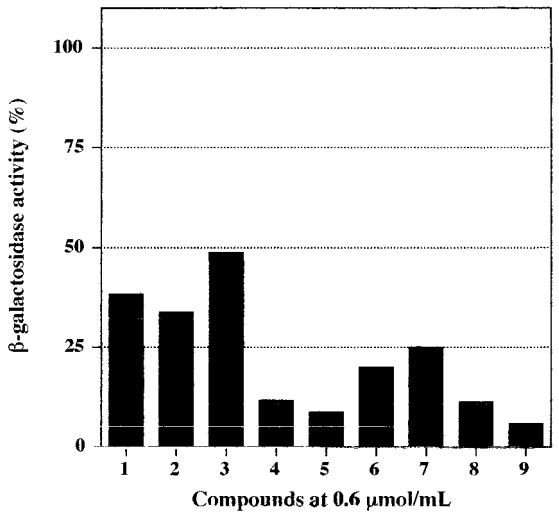

Figure 4. Suppression of furylfuramide-induced SOS responses by **1**−**8**. Concentration of compounds was 0.6 μmol/mL.

of 5-nitrofuran (Vroomen et al., 1988; Bertenyi et al., 1996) are important in the metabolic pathway. The cis−trans isomerization is based on the formation of nitro anion radicals. The *cis*-furylfuramide receives a single electron derived from an enzyme system to form the anion radical. Spin density on the olefinic double bond results in free rotation between the olefinic carbons followed by conversion to its thermodynamically more stable trans isomer. The nitro group of 5-nitrofuran is activated by the reductive metabolism associated with nitroreductases in bacteria. The main pathway for nitrofuran activation would be via reduction to a hydroxylamine intermediate which could react with DNA though a nitrenium ion. An alternative reactive intermediate, the ring-opened acrylonitrile derivative, could form through rearrangement of the hydroxylamine intermediate. It has been shown that the acrylonitrile derivative readily forms conjugates with glutathione, nercaptoethanol, and thiol groups of proteins. These conjugates increase the mutation frequency in *S. typhimurium* TA100, suggesting that the acrylonitryle derivative is also capable of interacting with DNA. Therefore, acetophenones may protect these reactive metabolic pathway. Apocynin showed the inhibition of NADPH oxidase activation (Suzuki et al., 1992; Stolk et al., 1994). Paeonol showed the inhibition of xanthine oxidase activity (Chang et al., 1994). These reports may support a part of this hypothesis. In addition, the low-molecular-weight phenolic compounds induced genetic evidence, the vir genes of one *Agrobacterium tumefaciens* are induced by several acetophenones, benzaldehydes, benzoic acids, cinnamic acids, and phenols (Lee et al., 1995). Therefore, it is a possibility that acetophenones may potently influence genetic repair system against mutagenesis. Indeed, the antimutagenic effect of vanillin was explained to cause enhancement of a recA-dependent, error-free pathway of post-replication repair (Ohta et al., 1998).

Antimutagenic properties of low-molecular-weight phenolic compounds have been explored through the use of several mutagens and assay systems. Anisaldehyde (*p*-methoxybynzaldehyde), ethyl vanillin (4-hydroxy-3-ethoxybenzaldehyde), and vanillin (4-hydroxyy-3-methoxybenzaldehyde) showed antimutagenic effects on mutagenesis induced by 4-nitroquinoline 1-oxide (4NQO), furylfuramide, acaptan, and methylglyoxal in *E. coli* WP2s, though they were not effective against mutations by 3-amino-1-methy-5*H* pyrido[4,3-*b*]indole (Trp-P-2) or 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ) in *S. typhimurium* TA98 (Ohta et al., 1986, 1988). In the case of benzaldehydes, 4-hydroxy-3-methoxy substitution showed more suppressive effects than *p*-methoxy substitution against furylfuramide-induced mutagenicity.

o-Vanillin suppressed furylfuramide- and 4NQO-induced mutagenicity in *E. coli* WP2s, but it increased MNNG (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine)- and MNU (*N*-methyl-*N*-nitrosourea)-induced mutagenesis in the same strain (WP2s) (Watanabe et al., 1989). Recently, cinnamic acid derivatives were isolated as the suppressive compounds for furylfuramide-induced SOS responses in *S. typhimurium* TA1535/pSK1002 in our laboratory, and *trans*-cinnamic acid, *p*-methoxy cinnamic acid were isolated (Miyazawa et al., 1998). In this case, all of them showed similar suppressive effects slightly more suppressive than those of others. From these observations, the relationship between substitutions of the benzene ring and acetophenone, benzaldehyde, and cinnamic acid for their suppressive effects against furylfuramide could not clearly be explained. The structure−activity relationship in acetophenones were confirmed from our experiment. The structural requirements for the suppressive effect on furylfuramide-induced SOS responses were explained in that hydroxy and methoxy substitutions were required for their suppressive effects and 2-hydroxyacetophenones which have methoxy substitution showed effective suppression for furylfuramide SOS responses.

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