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SHORT COMMUNICATION

Antimutagenic activity of flavonoids from Sozuku

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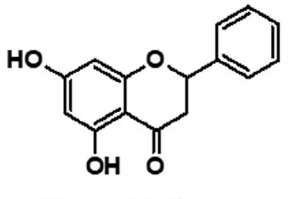
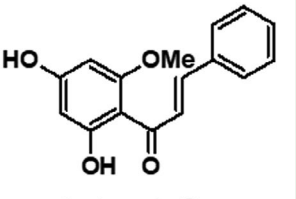
ABSTRACT

Pinocembrin (**1**) and cardamonin (**2**) from Sozuku showed a suppressive effect on *umu* gene expression of SOS response in *Salmonella typhimurium* TA1535/pSK1002 against the mutagen furylfuramide. Compounds **1** and **2** suppressed 52% and 36% of SOS-inducing activity at a concentration of 0.20 µmol/mL. The ID_{50} value of **1** was 0.18 µmol/mL. These compounds showed the suppression of 2-amino-3,4-dimethylimidazo-[4,5-f]quinolone (MeIQ) and UV irradiation-induced SOS response. Pinostrobin (**3**) and 5,7-dimethoxyflavanone (**4**), methyl ethers of **1**, showed similar activity to **1** against MeIQ-induced SOS response, but that of furylfuramide and UV irradiation were decreased. On the other hand, compounds **1**–**4** did not show the suppression of activated MeIQ-induced SOS response. Furthermore, compounds **1**–**4** showed potent antimutagenic activity against MeIQ mutagenesis in Ames test using the *S. typhimurium* TA100 and TA98 strains.



Pinocembrin (**1**)



Cardamonin (**2**)

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Sozuku; *Alpinia katsumadae* Hayata; pinocembrin; cardamonin; antimutagenic activity; *umu* test; Ames test

1. Introduction

Human epidemiology and animal studies have indicated that cancer risk may be modified by changes in dietary habits or dietary components. Human ingest large numbers of naturally occurring antimutagens and anticarcinogens with food. These antimutagens and

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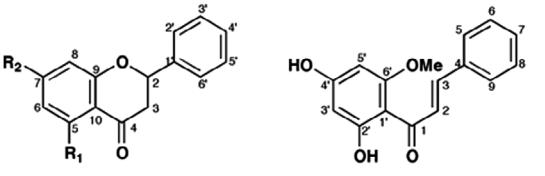
anticarcinogens may inhibit one or more stages of the carcinogenic process and prevent or delay the formation of cancer. Antimutagenic compounds have been found in several crude drugs, and some of these structures have been elucidated (Kim et al. 1991; Zheng et al. 1992). The SOS response is one of the DNA repair response to DNA damage induced by environmental factors and so on. The *umu* test system was developed to evaluate the genotoxic activities of a wide variety of environmental carcinogens and mutagens, using the SOS response to detect DNA damage (Oda et al. 1985). The results of this test are in agreement with the results of the Ames test and may be more useful with respect to simplicity, sensitivity and rapidity (Reifferscheid and Heil 1996).

Sozuku is Chinese crude drugs base on dried seed of *Alpinia katsumadae* HAYATA (synonym: *Alpinia hainanensis* K. Schum) (*Zingiberaceae*), and use as an aromatic stomachache and antiemetic medicine in China. This plant is native to Hainan Island in Southern China, but is widely ranged in shaded woodland in Hong Kong. Previous investigations of this plant have reported a variety of diarylheptanoids, chalcones and flavonoids, monoterpenes and sesquiterpenes (Yang et al. 1999; Hahm et al. 2003). Hahm et al. (2003) reported that 7,8-dihydroxyflavanone showed inhibitory effect on the Jun-Fos DNA complex formation. However, the compounds responsible for antimutagenicity from Sozuku has not been reported. In our search for new naturally occurring antimutagenic compounds in plants which have a history of safe use as Chinese crude drugs (Miyazawa et al. 2000), we found that the methanol extract of Sozuku exhibited a suppression of the SOS-inducing activity of furylfuramide. In this paper, we report on the isolation and identification of the antimutagenic compounds in Sozuku.

2. Results and discussions

The methanol extract from Sozuku was fractionated to search for the suppressive compound using the *umu* test as a guide. The chloroform fraction, partitioned from MeOH extract, showed a suppressive effect on *umu* gene expression of the SOS responses in *S. typhimurium* TA1535/pSK1002 against furylfuramide. The chloroform fraction was repeatedly fractionated by SiO2 column chromatography. Active compounds **1** and **2** were isolated and identified as pinocembrin and cardamonin by comparison of spectra (^{1}H-NMR, ^{13}C-NMR, EI-MS and IR) with literature data (Jaipetch et al. 1982; Ichino et al. 1988; Fukui et al. 1988; Liu et al. 1992; Ngo et al. 1998), respectively (Figure 1). The suppressive effects of compounds **1**, **2** and methylated compounds pinostrobin (**3**) and 5,7-dimethoxyflavanones (**4**), obtained by



	R1	R2
1	OH	OH
3	OH	OMe
4	OMe	OMe

2

Figure 1. Structures of compounds **1**–**4**.

methylation of **1**, were determined in the *umu* test. Compounds **1** and **2** suppressed 53% and 36% of the furylfuramide-induced SOS response at concentrations of 0.2 µmol/mL, but **3** and **4** did not (Figure S1). The ID50 value of **1** was 0.18 µmol/mL. These compounds were also assayed with MeIQ, which require the activation by liver-metabolising enzyme mixture (S9). Compounds **1**–**4** suppressed more than 75% of the MeIQ-induced SOS response at concentrations of 0.02 µmol/mL, and the ID50 values were 0.006, 0.003, 0.004 and 0.003 µmol/mL, respectively (Figure S1). However, these compounds did not show suppressive effects on activated MeIQ-induced SOS response (Figure S1). Whereas, **1** and **2** suppressed 70% and 50% of the UV irradiation-induced SOS response at concentrations of 0.2 µmol/mL, the ID_{50} values were 0.11 and 0.22 µmol/mol, respectively (Figure S2). The difference in structure between active compound **1** and negative compounds **3** and **4** is hydroxyl group at C-7 position. Therefore, it is indicated that a hydroxyl group at the C-7 position is important factor for suppressing furylfuramide and UV-irradiation-induced SOS response. The modes of action of antimutagens are mainly divided into desmutagen and bioantimutagen. The former inactivates or destroys mutagens directly or indirectly out of cell, and the latter suppresses the process of mutagenesis itself inside cells or act on the DNA repair system. From these results, **1** and **2** may have potency as bioantimutagens. However, the mechanism of bioantimutagen of these compounds requires more study.

Antimutagenic activity of **1**–**4** against furylfuramide, MeIQ and activated MeIQ were demonstrated by the Ames test using *S. typhimurium* TA100 and TA98 (Figure S3 and S4). Compound **1** suppressed only 53% of the mutagenicity of furylfuramide at 2.0 µmol/plate in TA100 strain, and ID_{50} value was 0.19 µmol/plate. While, **1**–**4** suppressed more than 90% of the mutagenicity of MeIQ at 0.2 µmol/plate in both strains. The ID_{50} values of **1**–**4** were 0.051, 0.049, 0.038 and 0.024 µmol/plate in TA100 strain and 0.048, 0.045, 0.034 and 0.026 µmol/plate in TA98 strain. The suppressive effects of flavanones increased with the methylation of hydroxyl group. However, these compounds did not inhibit the mutagenicity of activated MeIQ. These results were similar to the *umu* test. MeIQ is one of the heterocyclic amines, and requires the metabolic activation by P450 monooxygenase system in rat liver microsomes (S9) for mutagenicity. Trakoontivakorn et al. (2001) reported the antimutagenic activity of **1**–**3**, isolated from *Boesenbergia pandurate*, against heterocyclic amines, Trp-P-1, Trp-P-2 and PhIP, in *S. typhimuriumn* TA98, and their activity is due to the inhibition of *N*-hydroxylation of heterocyclic amines by S9. In this presence study, **1**–**4** exhibited a potent inhibition against MeIQ-induced mutagenesis, whereas they did not inhibit that of activated MeIQ in the both test. Therefore, it is suggested that antimutagenic activity of **1**–**4** against MeIQ-induced mutagenesis due to the inhibition of *N*-hydroxylation by P450 monooxygenase systems in S9.

3. Conclusion

The antimutagenic compounds in Sozuku were clearly pinocembrin (**1**) and cardamonin (**2**). These compounds showed suppressive effect and antimutagenic activity against chemical mutagens-induced SOS response and mutagenicity. Especially, these compounds had potent activity against MeIQ-induced SOS response and mutagenicity, and their activity was thought to due to inhibit the *N*-hydroxylation of MeIQ by S9. Further, in the comparison of structure in compounds **1**, **3** and **4**, it was suggested that the free hydroxyl group on C-7 is very important factor for suppressing the furylfuramide and UV irradiation-induced SOS response.

Disclosure statement

No potential conflict of interest was reported by the authors.

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