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Antimutagens in *Citrus sudachi* Hort. et Shirai

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Antimutagens from *Citrus sudachi Hort. et* Shirai were examined. A methanol extract prepared from immature green fruit of this plant reduced the mutagenicity of 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) when *Salmonella typhimurium* TA98 was used in the presence of a rat liver microsomal fraction. From the methanol extract, two antimutagens were separated chromatographically while monitoring the antimutagenic activity on Trp-P-2 by a plate method of a modified Ames test, and were identified as 5, 7, 4'-trihydroxy-6, 8, 3'-trimethoxyflavone (sudachitin) and 5, 7, 3', 4'-tetrahydroxy-6, 8-dimethoxyflavone (3'-demethoxysudachitin).

Key Words: *Citrus sudachi*, sudachitin, 3'-demethoxysudachitin, Trp-P-2

C. sudachi is an evergreen tree cultured mainly in Tokushima prefecture in Japan. The immature fruits are often used for cooking. Regarding its constituents, several flavone glucosides[1-5] and their aglycones[6-7], as well as essential oils, are found in fresh green peelings. We recently found that a methanol extract prepared from immature fruits of this plant reduced the mutagenicity of the dietary carcinogen Trp-P-2, which causes frameshift type mutations in DNA[8]. The antimutagens of this fruit have not been reported.

In this report, the isolation and antimutagenicity of the antimutagens from *C. sudachi* Hort. et Shirai are described.

Materials and Methods

Materials: Green immature fruits of *C. sudachi* Hort. et Shirai were purchased in a supermarket in Nara City in 1997. Tangeretin (5, 6, 7, 8, 4'-pentamethoxyflavone) (**3**) was purchased from Funakoshi Co. (Tokyo, Japan). **Isolation of Active Substances:** Three point six hundred fourty five kg of fresh fruit (190 fruits) was extracted twice, for seven days each time, with 4.556 L of methanol at 5°C. The methanol extracts were filtered through a filter paper No. 2 (Advantec Toyo, Tokyo, Japan) and concentrated under reduced pressure to give a concentrate (200.4 g). Then, 190 g of this concentrate was partitioned between water and ethyl acetate. The ethyl acetate phase, showing an antimutagenic activity, was further purified by repeated Sephadex LH-20 column chromatography and preparative TLC. **Antimutagenicity test**: The antimutagenicity test was performed according to Ames et al.[9] with a minor modification. The details were

described in our previous paper[10].

Results and Discussion

The ethyl acetate phase reduced the mutagenic activity of Trp-P-2 by 88.4% in *S. typhimurium* TA98 without cytotoxicity. From the ethyl acetate phase, two active compounds, **1** and **2**, were isolated, and identified as sudachitin[6,11] and 3'-demethoxysudachitin[7,11], respectively, from spectral data and their physical properties. The yields were 22.8 mg and 12.1 mg, respectively. In the antimutagenicity test, **1**, **2** and **3** exhibited antimutagenic activities or inhibition % of over 67.6% at a dose of 50 μg/plate, but, the antimutagenic activities of these flavones were weaker than those of luteolin (5, 7, 3', 4'-tetrahydroxyflavone) and apigenin (5, 7, 4'-trihydroxyflavone) showing inhibition % of 94.5% and 93.3% at the same dose, respectively. We previously reported that the antimutagenic acticity of flavones such as luteolin and apigenin decreases when the hydroxyl groups were replaced by methoxyl groups, and that the hydroxyl groups is important for the antimutagenic activity[10]. **1** and **2** are 6, 8, 3'- and 6, 8- methoxylated derivatives

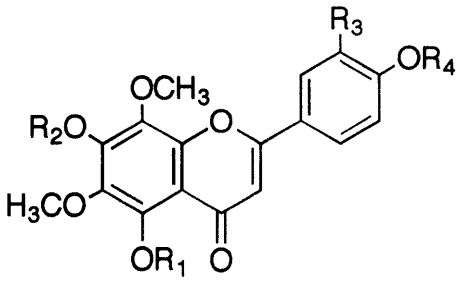

Figure 1. Structures of **1**, **2** and **3**

1 R_1=R_2=R_4=H, R_3=OCH_3 Sudachitin

2 R_1=R_2=R_3=R_4=H 3'-Demethoxysudachitin

3 R_1=R_2=R_4=CH_3, R_3=H Tangeretin

Table 1. Effects of *Citrus* Flavones on Frameshift Mutation Caused by Trp-P-2 (3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole in *Salmonella typhimurium* TA98[a]

sample	dose (μg/plate)	number of revertant colonies/plate	antimutagenic activity (inhibition %)	number of surviving cells/plate
control	0	3600	0	270
sudachitin	50	1165	67.6	267
	25	1272	64.7	272
	10	1856	48.4	288
	5	2811	21.9	266
	1	3281	8.9	277
demethoxy-	50	1072	71.5	260
sudachitin	25	1208	66.4	268
	10	1719	52.2	261
	5	2661	26.1	289
	1	3064	14.9	267
tangeretin	50	704	80.4	263
	25	864	76.0	278
	10	1786	50.4	280
	5	2733	24.1	264
	1	3251	9.7	266

[a]Dimethyl sulfoxide as a control solution was used in the presence of 0.15 μg of Trp-P-2 and 0.5 ml of S-9 mix. The number of spontaneous mutation colonies were 37/plate. The number of revertants, including spontaneously mutated colonies and surviving cells were the means of the three plates.

of apigenin. In addition, **3** has a structure that trihydroxyl groups of **2** were replaced by trimethoxyl groups. From the results of this experiment, we assumed that **1**, **2** and **3** exhibited lower antimutagenic activity than apigenin and luteolin by their polymethoxylated structures: methoxylation of proton and hydroxyl group.

We could not examine the antimutagenic mechanisms in this experiment because the amounts of **1** and **2** obtained were small. It is necessary to clarify with a pre-incubation method whether these antimutagenic flavones are desmutagen or bio-antimutagen.

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