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Oriental Pharmacy and Experimental Medicine 2004 **4(3)**, 211-214

Short Communications

Inhibitory effects of *dl*-Puerol A in the root of *Sophora japonica* on copper ion-induced protein oxidative modification of mouse brain homogenate *in vitro*

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SUMMARY

The inhibitory effect of *dl*-puerol A as but-2-enolide isolated from *Sophora japonica* was investigated on copper ion-induced protein oxidative modification *in vitro*. It inhibited copper-induced protein oxidative modification. However, its inhibitory effect was a little weaker than that of *dl*-α-tocopherol as an antioxidant.

The results demonstrated that *dl*-puerol A, one of but-2-enolides, might be of use in the oxidative stress.

Key words: Copper; Protein oxidative modification; *dl*-puerol A; *Sophora japonica*

Copper ion induces to generation of oxygen free-radical species. They have been a well-known cause of protein oxidative modification, leading to pathological conditions such as Wilson's disease (Halvorsen *et al.*, 1996). Recently, the natural antioxidants have been found in many plants, such as spices, vegetables and herbs (Nakatani, 2000). We found that isoflavones, licoisoflavones A and B have the antioxidative effects (Toda and Shirataki, 2002). These isoflavones were isolated from *Sophora plant* (Shirataki *et al.*, 1988). *dl*-Puerol A as but-2-enolide was isolated from *Sophora japonica*, which has been used in Traditional Medicine as a hemostatic (Shirataki *et al.*, 1987; Nohara *et al.*, 1993). In this paper, we investigated the inhibitory effect of *dl*-puerol A isolated from *Sophora japonica*, on copper ion-induced protein oxidative modification *in vitro*.

Sophora japonica L. (Leguminosae), were collected in Koishikawa Botanical Garden, Tokyo University in November 1972. The plants were verified by professor Yoshiaki Shirataki, Josai University in Japan. A voucher specimen was deposited at the Herbarium in Josai University in Japan.

Male ddY mice weighing between 25 and 30 g, 6-7 weeks old were obtained from SLC, Shizuoka, Japan. They were housed in a room maintained at 25 ℃ with a relative humidity of 60 %.

Copper chloride, 2,4-dithionitrophenylhydrazine, 5,5'-dithiobis(2-nitrobenzoic) acid (DTNB), disodium salt of ethylendiaminetetraacetic acid (EDTA), *dl*-α-tocopherol and urea were obtained from Wako Pure Chemicals Co., Osaka, Japan. Other

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common laboratory reagents were of analytical grade.

The dried roots of *Sophora japonica* L. were extracted 3 times with boiling methanol. The ether soluble part of the methanol extract was column chromatographed on silica gel by using benzene-ethyl acetate as eluents and checked by thin layer chromatography (solvent: benzen-ethyl acetate=1:1). *dl*-Puerol A was eluted in this chromatography. Crude *dl*-puerol *A* was subjected to rechromatography on silica gel to yield *dl*-puerol (Shirataki *et al.*, 1987; Nohara *et al.*, 1993).

The brains were removed from the mice immediately after killing and were subjected to homogenization in ice-cold 50 mM Tris-HCl buffer at pH 7.4, using a Potter-Elvehjem homogenizer. The brain homogenate containing 250 g protein/mL was prepared in the same buffer. The protein content of the brain homogenate was established by the procedure described by Lowry (Lowry *et al.*, 1951).

The brain homogenate described in the previous section was incubated to a total volume of 0.3 mL with 250 M copper chloride in 50 mM Tris-HCl pH 7.4 buffer. The reaction mixture was incubated at 37 ℃ for 2 h and then 1.6 mL of 0.125 M, pH 8.0 phosphate buffer containing 12.5 mM EDTA, 10.0 M urea and 0.1 mL of pH 7.0 phosphate buffer containing 10 mM DTNB were added. The resulting solution was allowed to stand at room temperature for 5 min, and then the absorbance of the cystein SH residue was read at 412 nm (Ellman, 1959). The inhibitory ratio of the test sample was evaluated by the following equation: I.R.%=(△Cys-SH/△ Cys-SH°). Where I.R.% is the inhibitory ratio, (%). △Cys-SH is the Cys-SH residue in treated sample Cys-SH residue in reaction blank and △ Cys-SH° is Cys-SH residue before incubation Cys-SH residue, untreated blank.

The inhibitory ratio was plotted against the log [F1] ([F1] = concentration of sample). The dose-response data were used to corresponding IC_{50}.

The values were expressed as the mean standard error of five experiments. The results were analysed by the non-parametric ANOVA-Scheffe *f*-test.

The results presented in Table 1 show that the percent inhibitory ratios of the inhibitory effects of *dl*-puerol A and *dl*-α-tocopherol as an antioxidant reached 89.8 ± 2.8% and 100 % at a concentration of 100 M, and that they increased in a concentration-dependent manner. Inhibitory ratios are lower than those of *dl*-α-tocopherol at concentrations of 100, 10 and 1.0 M (Table 1). The IC_{50} values of *dl*-puerol A and *dl*-α-tocopherol are 95.4 and 11.7 M, respectively.

The results showed that *dl*-puerol inhibited copper-induced protein oxidative modification. However, its inhibitory effect was a little weaker than that of *dl*-α-tocopherol as an antioxidant. *dl*-Puerol A was isolated from *Sophora japonica* (Shirataki *et al.*, 1987; Nohara *et al.*, 1993). *dl*-Puerol A is one of butenolides (Fig. 1). Three flavonol triglucosides, kaempferol 3-O-α-l-rhamnopyranosyl(1→6)-β-D-gluopyranosyl(1→2)-β-D-glucopyranoside, kaempferol 3-O-[α-l-rhamnopyranosyl (1→6)]-[β-D-gluopyranosyl(1→2)]-β-D-glucopyranoside and kaempferol 3-O-β-D-glucopyranosyl-(1→2)-β-D glucopyranoside-7-O-α-l-rhamnopyranoside were isolated from *Sophora japonica* and identified as antioxidants (Tang *et al.*, 2002). Rutin from *Sophora japonica* has been found to have antioxidant activity (Paniwnyk *et al.*, 2001). A butenolide compound, (1E, 3E, 5E,7E)-5-hydroxy-4-(8-phenyl-1,3,5,7-octatetraenyl)-2(5H)-furanone has been shown to have strong anti-tumor activity (Satomi *et al.*, 1992). Another butanolide, 2-buten-4-olide, has been also shown to inhibit type II collagen -induced arthritis in Lewis rats (Takeoka *et al.*, 1993). There have been no reports about the

antioxdant effects of butenolides isolated from *Sophora japonica* like *dl*-puerol A.

The present results demonstrated that butenolide as *dl*-puerol A isolated from *Sophora japonica* has inhibitory effect on copperinduced protein oxidative modification. There have been never reports about antioxidant activities of butenolides.

Table 1. Inhibitory effects of *dl*-puerol A on copper ion-induced protein oxidative modification of mouse brain homogenate in vitro

Test sample	Concentration (M)	Inhibitory ratio (%)
dl-puerol A	0.1	9.3 ± 2.5
1.0	14.4 ± 3.5[c]	
10	37.3 ± 4.4[b]	
100	89.8 ± 2.8[a]	
dl - α - tocopherol	0.1	13.4 ± 6.7
1.0	44.7 ± 2.6[c]	
10	95.6 ± 2.8[b]	
100	100[a]	

The inhibitory ratio, in %, is reported as the mean ± SE of five experiments. [a,b,c]$P<0.01$

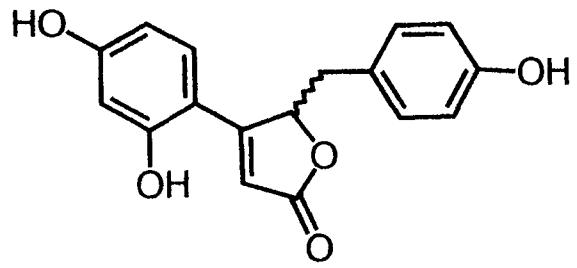

Fig. 1. Chemical structure of *dl*-Puerol A

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