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Isoliquiritigenin : A Competitive Tyrosinase Inhibitor from the Heartwood of *Dalbergia odorifera*

Tai Hyun KANG, Yu Hua TIAN and Youn Chul KIM*

College of Pharmacy and Phytofermentation Research Center, Wonkwang University, 344-2, Shinyong-Dong, Iksan, Jeonbuk 570-749, Korea

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Abstract – Effect of isoliquiritigenin isolated from the heartwood of *Dalbergia odorifera* T. Chen (Leguminosae) on mushroom tyrosinase activity was investigated *in vitro* using L-tyrosine and L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrates. When L-tyrosine was used as a substrate, both isoliquiritigenin and kojic acid, a positive control, inhibited tyrosinase activity in a concentration-dependent manner. IC_{50} values of isoliquiritigenin and kojic acid were 61.4 and 52.2 μM, respectively. However, isoliquiritigenin showed week inhibitory effect on the oxidation of L-DOPA by tyrosinase with inhibition ratio of 9.1 ± 7.1% at 100 μM. It is also suggested that 3-unsubstituted and 4-hydroxyl phenyl group in isoliquiritigenin plays an important role on the inhibition of tyrosinase activity when L-tyrosine was used as a substrate. Analysis of Lineweaver-Burk plot showed that isoliquiritigenin acts as a competitive inhibitor in case of L-tyrosine as a substrate.

Keywords □ *Dalbergia odorifera*, isoliquiritigenin, L-tyrosine, L-DOPA, tyrosinase inhibitor

Melanin is the molecule responsible for pigmentation and plays an important role in prevention of sun-induced skin injury. Tyrosinase (monophenol, dihydroxyphenylalanine: oxygen oxidoreductase EC 1.14.18.1) is known to be the key enzyme implicated in the biosynthesis of melanin in melanocytes (Hearing and Jimenez, 1989). This enzyme catalyzes two distinct reactions; the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) (tyrosine hydroxylase activity) and the oxidation of the L-DOPA to dopaquinone (DOPA oxidase activity) (Hearing and Tsukamoto, 1991; Iwata *et al.*, 1990). Dopaquinone rapidly and spontaneously polymerize to form brown or black pigments. Therefore, tyrosinase inhibitors have become increasingly important in cosmetic and medicinal products in relation to hyperpigmentation.

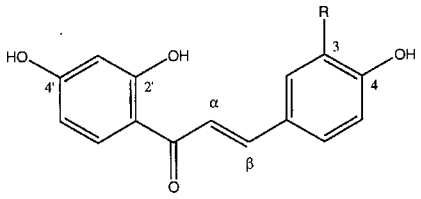
The heartwood of *Dalbergia odorifera* T. Chen (Leguminosae) has been used for the treatment of blood stagnation syndrome, ischemia, swelling, necrosis, and rheumatic pain in China and Korea (Chang, 1981; Su, 1977). Previous reports demonstrated that *D. odorifera* showed significant anti-inflammatory activity and inhibited prostaglandin biosynthesis as well as platelet aggregation induced by arachidonic acid (Chan *et al.*, 1998; Goda *et al.*, 1992). Moreover, isoliguiritigenin isolated from *D. odorifera* suppressed the superoxide anion formation from rat neutrophils (Chan *et al.*, 1998). In the present study, we investigated the inhibitory effect of isoliquiritigenin on mushroom tyrosinase activity using L-tyrosine or L-DOPA as the substrates.

*Corresponding author
Tel: +82-63-850-6823, Fax: +82-63-852-8837
E-mail: yckim@wonkwang.ac.kr

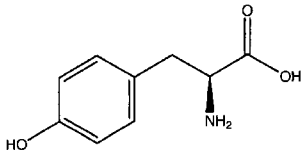
MATERIALS AND METHODS

Plant material and isolation

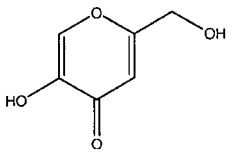
The heartwood of *D. odorifera* was purchased from the herbal medicine co-operative association of Jeonbuk Province, Korea, in October 2002, and the voucher specimen (No. WP 02-008) was deposited at the Herbarium of the College of Pharmacy, Wonkwang University (Korea). Dried and pulverized heartwoods of *D. odorifera* (60 g) were extracted with ethanol (1 L) for 3 h. EtOH extract (8 g) was concentrated and chromatographed on Sephadex LH-20 column with elution of 70% aqueous MeOH followed by MeOH to obtain 3 subfractons (Fr. A-C). Fr. B (98 mg) was chromatographed on silica gel (500 g) column with $CHCl_3$:MeOH (10:1) to yield compound **1** (32 mg, 0.053 w/w%). The structure of compound **1** was identified as isoliquiritigenin (Fig. 1) by comparison with reported spectral data (MS, ^{1}H- and ^{13}C-NMR) (Namikoshi *et al.*, 1987).



Isoliquiritigenin(1): R=H
Butein : R=OH



L-Tyrosine



Kojic acid

Fig. 1. Chemical structures of isoliquiritigenin, butein, L-tyrosine, and kojic acid.

Isoliquiritigenin (1)

Yellow needles (50% aqueous EtOH). mp 200-202°C. UV λmax : 368 nm (MeOH). EI-MS *m/z*: 256 (M^+), 255, 134. ^{1}H-NMR(500 MHz, CD_3OD) : δ 6.27(1H, d, *J*=2.2Hz, H-3'), 6.40(1H, dd, *J*=2.2, 9.1Hz, H-5'), 6.83(2H, d, *J*=8.7Hz, H-3 and 5), 7.60(1H, d, *J*=15.1Hz, H-α), 7.61(2H, d, *J*=8.7Hz, H-2 and 6), 7.77(1H, d, *J*=15.1Hz, H-β), 7.96 (1H, d, *J*=9.1Hz, H-6'). ^{13}C-NMR(125 MHz, CD_3OD) : δ 126.5(C-1), 130.4(C-2 and 6), 115.6(C-3 and 5), 160.2(C-4), 113.3(C-1'), 165.0(C-2'), 102.5(C-3'), 166.1(C-4'), 107.8(C-5'), 132.0(C-6'), 117.0(C-α), 144.3(C-β), 192.2(C=O).

Tyrosinase inhibitory assay

Tyrosinase activity was spectrophotometrically determined as described previously with minor modifications (Matsuda *et al.*, 1994). In brief, 40 μl of 5 mM L-tyrosine or L-DOPA, 80 μl of 1/15 mM phosphate buffer (pH 6.8) and 40 μl of the same buffer with or without test sample were added to a 96 well microplate (Falcone, USA), and then 40 μl of mushroom tyrosinase (150 U/ml) was mixed. The reaction mixture was incubated at 37°C for 30 or 10 min, and the absorption at 492 nm was measured using a microplate reader (Molecular Devices Corp., USA). Michaelis constant (Km) and maximal velocity (Vmax) of the tyrosinase was determined by Lineweaver-Burk plot using various concentrations of L-tyrosine.

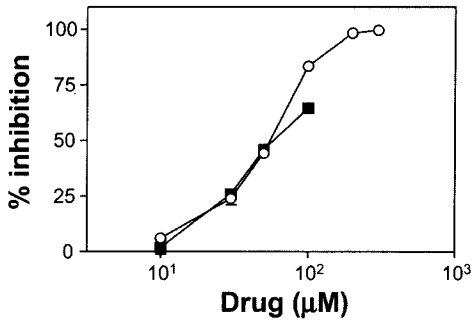


Fig. 2. Inhibitory effects on mushroom tyrosinase by isoliquiritigenin(■) and kojic acid (○) Each data point represents the mean ± S.D. from three independent experiments.

RESULTS AND DISCUSSION

First, we investigated the effect of isoliquiritigenin isolated from *D. odorifera* on mushroom tyrosinase activity using L-tyrosine as a substrate. As shown in Fig. 2, isoliquiritigenin showed a concentration-dependent reduction in tyrosinase activity when L-tyrosine was used as a substrate. Isoliquiritigenin at a concentration of 100 μM exhibited 64.6 ± 0.9 % inhibition of mushroom tyrosinase activity, where 50 % of inhibition (IC_{50}) was shown at the concentration of 61.4 μM. Kojic aicd, a positive control, also reduced the activity of tyrosinase by 83.4 ± 1.6 % at 100 mM, with an IC_{50} value of 52.2 μM (Fig. 2). Therefore, isoliquiritigenin exhibited similar potency compared with kojic acid (P=0.1779, *t*-test).

Isoliquiritigenin was also examined for the tyrosinase inhibitory activity using L-DOPA as a substrate. This compound, however, did not show tyrosinase inhibitory effect compared with that of kojic acid. As summarized in Table 1, isoliquiritigenin and kojic acid at 100 μM inhibited tyrosinase activity by 9.1 ± 7.1 and 80.9 ± 2.4 %, respectively.

In a kinetic study with various concentrations of L-tyrosine, isoliquiritigenin decreased the K_m value of tyrosinase in a concentration dependent manner but did not change the V_{max} value, indicating that isoliquiritigenin was acting as a competitive inhibitor with K_i value of 2.8×10^{-5} M (Fig. 3). The inhibition mechanism is different from that of kojic acid. Kojic acid was reported as a competitive inhibitor with L-tyrosine as a substrate and as a mixed-type inhibitor with L-DOPA, respectively (Chen *et al.*, 1991).

To study structure-activity relationships on tyrosinase inhib-

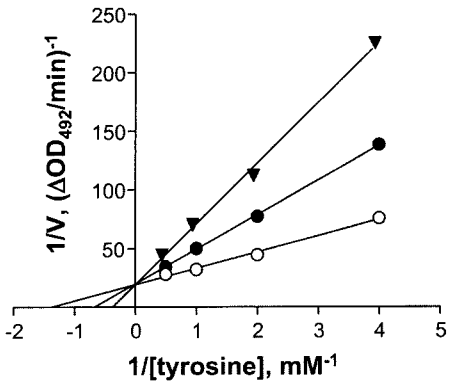


Fig. 3. Lineweaver-Burk plot of mushroom tyrosinase in the absence or presence of isoliquiritigenin (○: 0 μM, ●: 30 μM and ▼: 60 μM).

itory effects of liquiritigenin and its derivative, butein (Fig. 1) was tested for inhibitory activity on tyrosinase. We previously reported that butein was isolated and identified from *Rhus verniciflua* (Lee *et al.*, 2003). According to chemical structure, butein (2',3,4,4'-tetrahydroxychalcone) has one more hydroxyl group in comparison with isoliquiritigenin (2',4,4'-trihydroxychalcone). Butein did not show the inhibitory effect on the activity of tyrosinase using L-tyrosine as well as L-DOPA (Table I). Considering structural property of these chalcones, the present findings suggest that 3-unsubstituted and 4-hydroxyl phenyl skeleton plays an important role in inhibition of tyrosinase activity when L-tyrosine was used as a substrate. However, further studies are required to elucidate the exact mechanism underlying the inhibitory action of isoliquiritigenin and to evaluate the effect of this compound on melanogenesis in melanocytes. Although isoliquiritigenin had been isolated from licorice roots as a tyrosinase inhibitor (Nerya *et al.*, 2003), this is first report on its kinetic and structure-activity relationship study about tyrosinase.

In conclusion, we have demonstrated that isoliquiritigenin dose-dependently and competitively inhibited mushroom tyrosinase activity using L-tyrosine as a substrate. From these results it is expected that isoliquiritigenin would be useful in the development of cosmetic materials and depigmenting agents for hyperpigmentation.

Table I. Effects of isoliquiritigenin, butein and kojic acid on mushroom tyrosinase activity using L-tyrosine or L-DOPA as the substrates

Compound	L-tyrosine		L-DOPA	
	Inhibition %*	IC_{50}	Inhibition %*	IC_{50}
Isoliquiritigenin	64.6 ± 0.9	61.4	9.1 ± 7.1	> 100
Butein	-7.77 ± 7.1	> 100	9.6 ± 2.6	> 100
Kojic acid	83.4 ± 1.6	52.2	80.9 ± 2.4	29.7

*Data are represented as inhibition %, mean±S.D. of three independent tests at 100 μM.

ACKNOWLEDGMENTS

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