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# Honokiol : A Noncompetitive Tyrosinase Inhibitor from Magnoliae Cortex

**Yu-Hua Tian, Tai-hyun Kang, Hyun-Chul Kim, and Youn-Chul Kim***

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

**Abstract** – Effect of the neolignans, honokiol (**1**) and magnolol (**2**), isolated from Magnoliae Cortex on mushroom tyrosinase activity was investigated *in vitro* using L-tyrosine as a substrate. Honokiol (**1**) inhibited tyrosinase activity significantly in a concentration-dependent manner, on the other hand, magnolol (**2**) did not show tyrosinase inhibitory effect. Honokiol exhibited tyrosinase inhibitory effect with $IC_{50}$ value of 67.9 μM, and proved to act as a non-competitive inhibitor by the analysis of Lineweaver-Burk plot.
**Keywords** – Honokiol; Tyrosinase inhibitor; Magnoliae Cortex

## Introduction

The neolignans, honokiol and magnolol, are significant bioactive constituents of Magnoliae Cortex which has been used in traditional Oriental medicine for the treatment of distention and pain of the abdomen, dyspepsia, and asthmatic cough (Chang and But, 1987). Up to date the various bioactivities of honokiol and magnolol have been reported including antibacterial (Park *et al.*, 2004), smooth muscle relaxative (Ko *et al.*, 2003), hepatoprotective (Park *et al.*, 2003), anti-platelet (Pyo *et al.*, 2002), anti-histamine release (Ikarashi *et al.*, 2001), antifungal (Bang *et al.*, 2000), and antiarrhythmic (Tasi *et al.*, 1999). To search for the other biological activity on honokiol and magnolol, we isolated above two compounds from Magnoliae Cortex, and subjected to tyrosinase *in vitro* inhibitory assay.

Melanin is the molecule responsible for pigmentation and plays an important role in prevention to 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) and the oxidation of the L-DOPA to dopaquinone (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. This paper describes the isolation of honokiol and magnolol and their inhibitory effects on mushroom tyrosinase activity.

*Author for correspondence
Fax: +82-63-852-8837; E-mail: yckim@wonkwang.ac.kr

## Experimental

**Plant material and isolation** – Magnoliae Cortex was purchased from the University Oriental herbal drugstore, Iksan, Korea, in October 2003, and the voucher specimen (No. WP 03-017) was deposited at the Herbarium of the College of Pharmacy, Wonkwang University (Korea). Dried and pulverized Magnoliae Cortex (1.2 kg) was extracted with EtOH (2×5 L) for 3 h under reflux. EtOH extract (43.5 g) was dissolved in 60% aqueous MeOH (1 L), and partitioned with $CH_2Cl_2$ (2×800 mL) to give 31.0 g of $CH_2Cl_2$-soluble fraction. $CH_2Cl_2$-soluble fraction was chromatographed on silica gel column with elution of *n*-hexane:iPrOH (10 :1) followed by $CH_2Cl_2$:MeOH (4 : 1) to obtain five subfractions (Fr. A-E). Fr. C (8.83 g) was further chromatographed on Sephadex LH-20 column [eluent: $CHCl_3$ : MeOH (1 : 0) → $CHCl_3$ : MeOH (0 : 1)] to afford four subfractions (Fr. C1-C4). Fraction C3 (4.35 g) was purified by Sephadex LH-20 column chromatography (eluent: *n*-hexane : $CHCl_3$ : MeOH, 4 : 3 : 1) to yield compound **1** (2.04 g, 0.17 w/w%) and compound **2** (1.39 g, 0.12 w/w%). The structures of compounds **1** and **2** were identified as honokiol and magnolol, respectively, by comparison with reported spectral data (MS, $^1H$- and $^{13}C$-NMR) (Bang *et al.*, 2000). Copies of the original spectra for compounds **1** and **2** are obtainable from the author of correspondence.

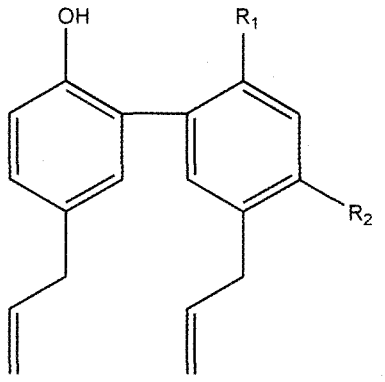
**Tyrosinase inhibitory assay** – Tyrosinase activity was

spectrophotometrically determined as described previously with minor modifications (Matsuda *et al.*, 1994). In brief, 40 μl of 5 mM L-tyrosine, 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 10 min, and the absorption at 492 nm was measured using a microplate reader (Molecular Devices Corp., USA). Michaelis constant ($K_m$) and maximal velocity ($V_{max}$) of the tyrosinase was determined by Lineweaver-Burk plot using various concentrations of L-tyrosine.

## Results and Discussion

Two neolignans, honokiol and magnolol, have known to contain in *Magnolia officinalis* and *M. obovata* as the characteristic major phenolic constituents. Various pharmacological activities have been reported on both honokiol and magnolol. These broad-spectrum of biological activity for honokiol and magnolol encouraged us to find new biological activity on them. First, we isolated honokiol (**1**) and magnolol (**2**) from the $CH_2Cl_2$-soluble fraction of EtOH extract of Magnoliae Cortex using silica gel and Sephadex LH-20 column chromatography (Fig. 1).

Compounds **1** and **2** were tested the inhibitory effects on mushroom tyrosinase activity using L-tyrosine as a substrate. As shown in Fig. 2, honokiol showed a concentration-dependent reduction in tyrosinase activity with $IC_{50}$ value of 67.9 μM. Arbutin and kojic acid, well-known tyrosinase inhibitors, were also tested as the positive controls, and exhibited the tyrosinase inhibitory




Honokiol (**1**) : $R_1$=H, $R_2$=OH
Magnolol (**2**) : $R_1$=OH, $R_2$=H

**Fig. 1.** Chemical structures of honokiol (**1**) and magnolol (**2**).

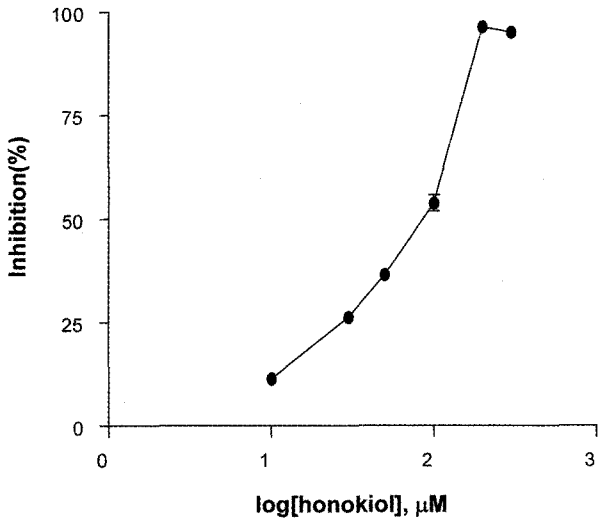



**Fig. 2.** Inhibitory effect on mushroom tyrosinase by honokiol (**1**). Each data point represents the mean ± S.D. from three independent experiments.

**Table 1.** Inhibitory effects of honokiol (**1**) and magnolol (**2**) on mushroom tyrosinase activity

| Compound | $IC_{50}$ value (μM) |
|---|---|
| Honokiol (**1**) | 67.9 |
| Magnolol (**2**) | > 300 |
| Arbutin | 121.6 |
| Kojic acid | 41.8 |

Values are presented as means of three independent experiments.

effects with $IC_{50}$ values of 121.6 and 41.8 μM, respectively (Table 1). On the other hand, magnolol did not show tyrosinase inhibitory effect ($IC_{50}$ value > 300 μM).

The chemical structure of both honokiol and magnolol consists of biphenyl skeleton with phenolic and allylic functionalities. Since the structures of these two compounds have the same functionalities, both of them known to have the similar biological activities in many cases. These two analogs, however, showed the significantly different effects on mushroom tyrosinase inhibitory assay (Table 1). The structure of honokiol (**1**) consists of *para*-allyl-phenol and an *ortho*-allyl-phenol which link together through *ortho, para*- C-C-couplng, whereas, the structure of magnolol (**2**) is an *ortho, ortho*- C-C dimmer of 4-allyl-phenol which consists a biphenyl skeleton with phenolic functionalities and two *p*-allyl groups as side chains. Although it has not sufficient information to evaluate the structure-activity relationship for compounds **1** and **2**, it seems the position of phenolic hydroxyl group in biphenyl skeleton might play an important role to tyrosinase inhibitory activity. It is also reported that some biological activities of honokiol was more potent than

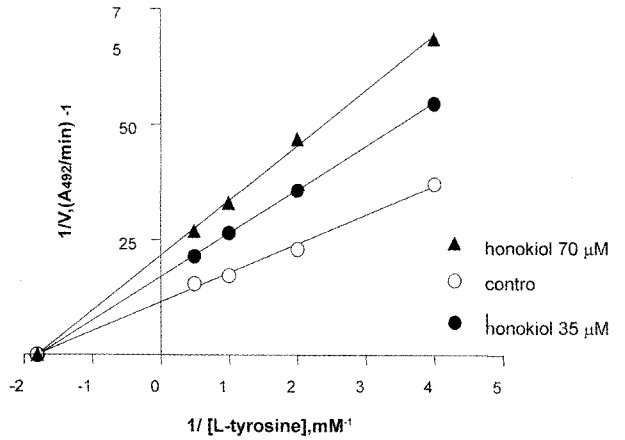



**Fig. 3.** Lineweaver-Burk plot of mushroom tyrosinase in the absence or presence of honokiol (1) ( ○ : 0 μM, ● : 35 μM, and ▲ : 70 μM).

those of magnolol such as in cases of anti-platelet effect (Pyo *et al.*, 2002) and anti-peroxidative activity (Haraguchi *et al.*, 1997).

In a kinetic study with L-tyrosine as a substrate, honokiol (**1**) decreased the $V_{max}$ value of tyrosinase in a concentration-dependent manner but did not change the $K_m$ value, indicating that honokiol (**1**) was acting as a noncompetitive inhibitor with $K_i$ value of $1.3\times10^{-7}$ M (Fig. 3). In conclusion, we have demonstrated that a neolignan honokiol (**1**) isolated from Magnoliae Cortex has the concentration-dependent and noncompetitive inhibitory activity on mushroom tyrosinase using L-tyrosine as a substrate.

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