Tyrosinase Inhibitory Prenylated Flavonoids from *Sophora flavescens*

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For the purpose of the development of a skin-whitening agent, *Sophora flavescens* was evaluated for tyrosinase inhibitory activity and its active principles were identified following activity-guided isolation. The ethanol extract and dichloromethane fraction from *S. flavescens* showed significant inhibition of mushroom tyrosinase. From the dichloromethane fraction, three known prenylated flavonoids, sophoraflavanone G, kuraridin, and kurarinone, were isolated. Compared with kojic acid (IC_{50}=20.5 μM), these compounds possessed more potent tyrosinase inhibitory activity. The IC_{50} values were 6.6, 0.6, and 6.2 μM for sophoraflavanone G, kuraridin, and kurarinone, respectively.

Key words　tyrosinase; skin whitening; *Sophora flavescens*; sophoraflavanone; kuraridin; kurarinone

Since melanin formation is the most important determinant of the color of mammalian skin, the inhibition of melanin formation may result in a reduction in skin darkness. Melanin is biosynthesized from tyrosine by enzymatic oxidation as well as an autooxidation process. Tyrosinase (EC 1.14.18.1), the rate-limiting enzyme, catalyzes tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and further oxidizes it to dopaquinone, which is used for the ultimate formation of melanin.[1] Therefore tyrosinase inhibitors may have potential for treating abnormal pigmentation disorders and for use as skin-whitening agents in the cosmetic industry.

Several tyrosinase inhibitors including arbutin and kojic acid have been widely used for the purpose of skin whitening, especially in Northeast Asia. Some plant extracts such as *Glycyrrhizae radix* and *Morus radix* have also been used. But there is always a need for new skin-whitening agents. During our continual search for new tyrosinase inhibitors,[2,3] the dichloromethane fraction from the ethanol extract of *Sophora flavescens* has been found to have potent inhibitory activity against mushroom tyrosinase. Although two flavanone derivatives, kurarinone and kushenol F, were recently reported to be tyrosinase inhibitors from *S. flavescens*,[4] further investigation may lead to the identification of other constituents responsible for the potent inhibitory activity of the dichloromethane fraction. Thus the present study was undertaken to establish the tyrosinase inhibitory activity of *S. flavescens* and to identify further its active principles.

MATERIALS AND METHODS

Plant Material and Isolation of Active Principles　*S. flavescens* AIT (Fabaceae) was collected in August 1998, in Yangku, Kangwon Province, Korea, and identified by Dr. T. J. Kim (Korea Research Institute of Bioscience and Biotechnology). The dried roots (3.65 kg) were extracted with ethanol at room temperature. After filtration, the filtrates were evaporated to dryness under a vacuum. The dried ethanol extract was dissolved in water and further partitioned in succession with dichloromethane, ethyl acetate, and then *n*-butanol affording 128.4, 25.5 and 280.3 g of the respective fractions. From the dichloromethane fraction, activity-guided isolation with silica gel chromatography eluted with dichloromethane–

ethyl acetate (gradient elution) gave 43 fractions. Fraction 2 was further purified over silica gel using benzene–ethyl acetate (10 : 1) to yield compound **I** (120 mg). Repeated silica gel chromatography of fraction 33 using benzene–ethyl acetate (3 : 2) gave compounds **II** (80 mg) and **III** (220 mg). The chemical structures of compounds **I**—**III** were identified as sophoraflavanone G, kuraridin, and kurarinone (Fig. 1), respectively, based on the spectral results of previously described.[5]
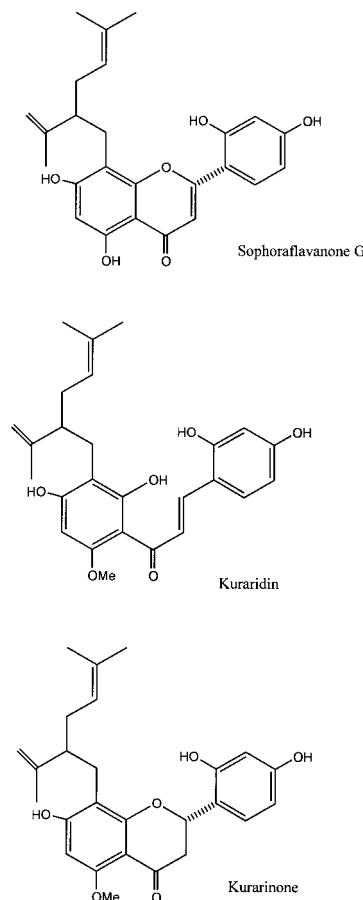

Fig. 1. Chemical Structures of the Prenylated Flavonoids Isolated in This Study

Tyrosinase Assay Tyrosinase activity was determined essentially based on the previously described procedure[6] with slight modification. In brief, the test reaction mixture comprised each plant extract, mushroom tyrosinase (105 units, Sigma-Aldrich) and L-tyrosine (0.55 mM) in 0.05 mM sodium phosphate buffer (pH 6.8). The reaction mixture (1.5 ml) was incubated at 37 °C for 10 min, and the absorption at 475 nm was measured. The absorbance of the same mixture without tyrosinase was used as the control. Each reaction was duplicated, and the data are expressed as arithmetic mean±S.D. The same experiment was repeated at least twice and they gave similar results.

RESULTS AND DISCUSSION

As shown in Fig. 2, the 75% ethanol extract of the roots of *S. flavescens* clearly showed tyrosinase inhibitory activity in a concentration-dependent manner (IC_{50}=75.1 μg/ml). Among the four fractions tested, the dichloromethane fraction exhibited potent inhibition of tyrosinase (IC_{50}=9.6 μg/ml). Following this fraction, activity-guided isolation was carried out and silica gel column chromatography finally led to the isolation of three known prenylated flavonoids, sophoraflavanone G, kuraridin, and kurarinone as active principles. IC_{50} values for sophoraflavanone G, kuraridin, and kurarinone were 6.6, 0.6, and 6.2 μM, respectively (Fig. 3). The IC_{50} value of the reference compound, kojic acid, was 20.5 μM. It is meaningful to note that the present investigation for the first time revealed sophoraflavanone G and kuraridin to be potent tyrosinase inhibitors.

Some flavonoid derivatives from plants such as quercetin and kaempferol were previously demonstrated to have tyrosinase inhibitory activity.[7] In another study, quercetin, myricetin, kaempferol, and theaflavin showed tyrosinase inhibition.[8] However, the inhibitory activities of these common flavonoids were not sufficiently potent for clinical use. On the other hand, the inhibitory activities of the prenylated flavonoids isolated from the present study were potent. Compared with kojic acid, these compounds exhibit very potent activity and are at least three times more potent. In particular, kuraridin has inhibitory activity comparable with that of oxyresveratrol (IC_{50}=1.0 μM), one of the most potent tyrosinase inhibitors in nature.[9] The common structural moieties of these prenylated flavonoids are A-ring C-7 hydroxyl and C-8 lavandulyl and B-ring 2′,4′-dihydroxyl groups. A similar observation was reported that 2′,4′,5,7-tetrahydroxy-6-prenylated flavones with the same B-ring 2′,4′-dihydroxyl moiety (4-substituted resorcinol) exhibited considerable tyrosinase inhibitory activity (IC_{50}=13.5—21.1 μM).[10] In addition, kurarinone and kushenol F, which have the same A-ring C-7 hydroxyl and B-ring 2′,4′-dihydroxyl groups, were recently demonstrated to be potent tyrosinase inhibitory flavonoids.[4] Although it is not fully understood at present how these prenylated flavonoids exert such potent activity, these flavonoids from *S. flavescens* may have potential for use as skin-whitening agents, and their *in vivo* activity should be evaluated for this purpose.

It is important to mention that some prenylated flavonoids exhibit cytotoxicity against normal cells and cell lines including tumor cells. For example, sanggenol and sanggenons show cytotoxicity against tumor cell lines and human gingi-
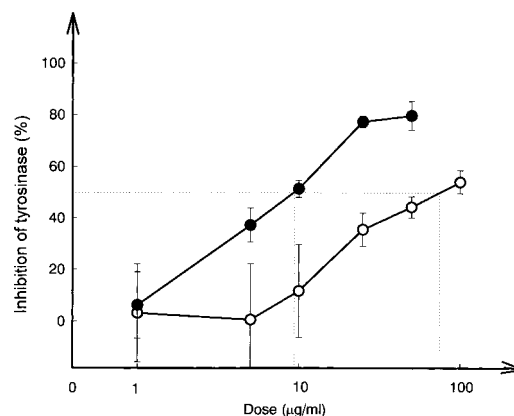

Fig. 2. Tyrosinase Inhibitory Activity of *S. flavescens*

○, ethanol extract; ●, dichloromethane fraction. The data are expressed as arithmetic mean±S.D. (n=2).
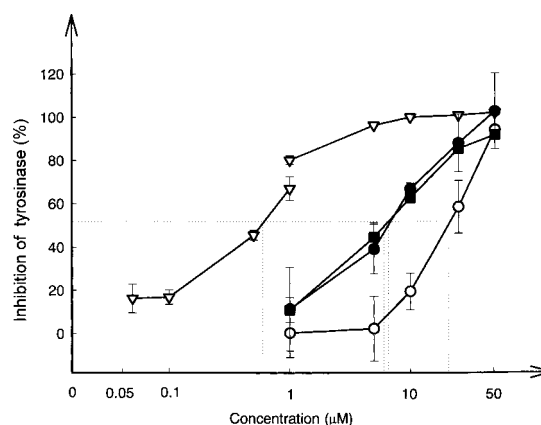

Fig. 3. Tyrosinase Inhibitory Activity of the Prenylated Flavonoids Isolated

○, kojic acid; ●, sophoraflavanone G; ▽, kuraridin; ■, kurarinone. The data are expressed as arithmetic mean±S.D. (n=2).

val fibroblasts.[11] C-8 lavandulyl flavonoids from *S. flavescens* such as sophoraflavanone G induce apoptotic death of HL-60 cells.[12] Regardless of apoptotic death or necrotic death, it is evident that certain prenylated flavonoids have *in vitro* cytotoxic effects against normal as well as tumor cells. However, it is not certain that these prenylated flavonoids are cytotoxic to animals and humans since there has been no report describing *in vivo* cytotoxicity using high concentrations of any prenylated flavonoid. To clarify their beneficial/harmful effects, this point should be investigated further.

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