Inhibitory Activities of Flavanone Derivatives Isolated from *Sophora flavescens* for Melanogenesis

Tae Joung Ha, Min Suk Yang, Dae Sik Jang, Sang Uk Choi,† and Ki Hun Park*

Department of Agricultural Chemistry
†Central Laboratory, Gyeongsang National University, Chinju 660-701, Korea
Received August 1, 2000

Keywords: *Sophora flavescens*, Kurarinone, Kushnol F, Melanogenesis, Tyrosinase inhibitor, *Streptomyces bikiniensis.*

The melanin synthesis inhibitor has been interested as target molecular of natural product chemistry because they related with a localized hyper-pigmentation in humans such as lentigo, nevus, ephelis, post-inflammatary state and melanoma of pregnancy.[1,2] In the course of our investigation for melanogenesis inhibitor from herbal medicine, flavanone derivatives (**1**, **2**) from the roots of *Sophora flavescens*, were found to show significant inhibitory activity against *Streptomyces bikiniensis* and on mushroom tyrosinase. *Sophora flavescens* (Leguminosae) is Chinese medicinal herb whose leaves and roots have been applied in folk medicine as antipyretic, analgesic, anthelmintic, and a stomachic. Although the chemical constituents and their biological activity of this plant have been reported well,[3-7] there are no paper related with inhibitory activity of melanogenesis. In present study, we wish to report the isolation and identification of flavanone derivatives (**1**, **2**) from this species and their inhibitory activity of melanogenesis. Melanin synthesis inhibitory activity was determined by the paper-disc diffusion method using to inhibition of melanin production in *S. bikiniensis* and mushroom tyrosinase.[8,9]

In two compounds (**1**, **2**), an absorption maximum *ca* 290 nm in ultraviolet absorption spectra and strong bonds at 1625 cm^{-1} in the infrared absorption spectra revealed the presence of conjugated carbonyl group. From the analysis of the COSY spectra of both compounds (**1**, **2**), both of them have lavandulyl (5-methyl-2-isopropenyl-hex-4-enyl) side chain. HMQC spectra and DEPT experiments of both compounds (**1**, **2**) show that they have flavanone skeleton having two substituents. Putting these various spectroscopic data together, compound **1** {$[\alpha]_D^{25}$ = +9.2° (*c* 0.16, MeOH)} is in agreement with kurarinone,[4] while compound **2** {$[\alpha]_D^{25}$ = –59.8° (*c* 0.82, MeOH)} is in agreement with kushenol F.[3]

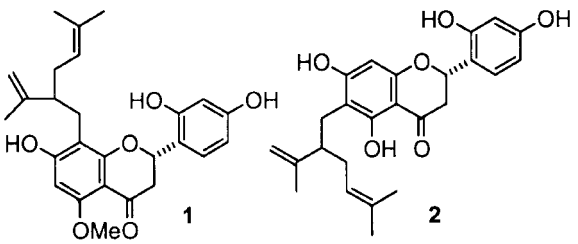

Figure 1. Chemical structures of **1** and **2**.

†Corresponding Author. Tel: +82-55-751-5472; Fax: +82-55-757-0178; e-mail: khpark@gshp.gsnu.ac.kr

Table 1. Inhibitory effects on mushroom tyrosinase and melanin formation of *Streptomyces bikiniensis*

Compound	*S. bikiniensis*	Mushroom Tyrosinase
	Inhobition zone (mm)[a]	IC_{50} (μg/mL)
1	18	4.6
2	17	9.0
Kojic acid	–[b]	14
4-hydroxyanisol	–	15
Arbutin	–	15

[a]compound 30 μg/paper disc. [b]not determined.

A higher inhibition of the melanine production of *S. bikiniensis* in the compounds (**1**, **2**) was evaluated than known melanogenesis inhibitors by the presence of clear zone in 30 μg/disk (Table 1). We also examined the inhibitor effect of mushroom tyrosinase activity using L-tyrosine as substrate. The compound **1** inhibited mushroom tyrosinase strongly with IC_{50} value of 4.6 μg/mL in comparison with kojic acid which is one of powerful depigmenting agents (Table 1). On the basis of the kinetic data, compound **1** was noncompetitive inhibitor ($Ki = 1.2 \times 10^{-5}$ M, $Km = 2 \times 10^{-3}$ M) on mushroom tyrosinase with L-tyrosine (Figure 2). The target compounds (**1**, **2**) was shown no antimicrobial activity at 5 mg/mL concentration against *Salmonella typhinarin* (ATCC 14038), *Bacillus cereus* (ATCC 27348), *Pseudomonas aeruginosa* (ATCC 33844) and *Staphylococcus epidermidis* (ATCC 12228) in paper disc agar diffusion method. The both compounds (**1**, **2**) inhibited the melanine production of *Streptomyces bikiniensis* strongly in comparison with known melanogenesis inhibitors. The kurarinone (**1**) exhibited significant inhibitory activity with IC_{50} value of 4.6 μg/ml on mushroom tyrosinase.

Experimental Section

Mushroom tyrosinase (EC 1.14.18.1) and L-tyrosine were purchased from Sigma Chemical Co. Specific rotation values were measured on JASCO DIP-370 polarimeter. Proton and carbon NMR spectra were measured down field relative

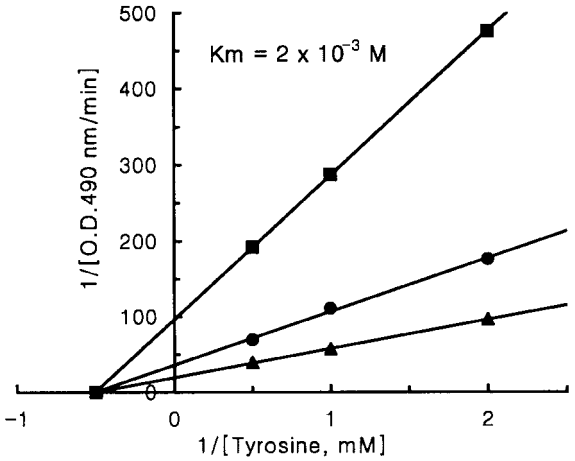

Figure 2. Lineweaver-Burk plots showing the reciprocal of the velocity (1/V) of the mushroom tyrosinase reaction *vs*. the reciprocal of the substrate concentration (1/S) with L-tyrosine as the substrate. This result shows the rate of the tyrosinase reaction in the presence of inhibitor kurarinone (■ , 0.05 mM; ● , 0.01 mM) and in absence of inhibitor (▲).

to tetramethyl silane in CD_3OD, ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC and COSY experiments were conducted on a Bruker AM-500 (500 MHz) spectrometer. Optical density was determined by the Dynatech MR-700 96-well microtiter plate reader.

Isolation. The sample of *Sophora flavescens* was collected in Hamyang (Korea) and identified by Prof. Myon Gi Chung. A voucher specimen of this raw material has been deposited at Herbarium of Gyeongsang National University (GNUC). The roots (2 kg) of *S. flavescens* were chopped and repeatedly extracted with MeOH (3 × 5 L) at room temperature. The extracts were combined and their solvent removed under reduced pressure to gain thickish mass (42 g). It was partitioned between water and ethylacetate (200 mL : 600 mL) and inhibitory activity of mushroom tyrosinase was found to accumulate in ethylacetate phase. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and then concentrated to gain a brown residue (17 g). The brown residue (17 g) was chromatographed over silica gel CC (5 × 50 cm, 230-400 mesh, 500 g) eluting with chloroform (2 L), then gradient $CHCl_3$-MeOH [100 : 1 (500 mL), 30 : 1 (900 mL)]. The fraction (900 mL) of $CHCl_3$-MeOH (30 : 1) was concentrated to gain a light yellow residue (2.7 g), which was chromatographed over silica gel CC (3.2 × 38 cm, 230-400 mesh, 160 g) eluting with gradient with $CHCl_3$-MeOH [50 : 1 (200 mL), 30 : 1 (150 mL) and then 20 : 1 (300 mL); 20 mL each]. Fraction 16-18 from this column were combined and evaporated to gain kurarinone (**1**, 128 mg) as a pale yellow amorphous powder. Fraction 27-28 from this column were combined and evaporated to gain kushenol F (**2**, 30 mg) as a pale yellow amorphous powder.

Kurarinone (1) $[\alpha]_D^{25}$ = +9.2° (*c* 0.16, MeOH); EIMS (70 eV) m/z 438 (9), 422 (22), 300 (30), 229 (100), 153 (47); UV (MeOH) λ_{max} 286 nm; IR (KBr) ν_{max} 3400, 2900, 1625, and 1520 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) δ 1.27 (3H, s), 1.54 (3H, s), 1.59 (3H, s), 2.00 (2H, m), 2.48 (1H, m), 2.61 (2H, m), 2.67 (1H, dd, *J* = 3.5, 17.1 Hz), 2.85 (1H, dd, *J* = 13.5, 16.5 Hz), 3.80 (3H, s), 4.51 (1H, m), 4.57 (1H, m), 4.95 (1H, m), 5.53 (1H, m), 6.09 (1H, s), 6.33 (1H, dd, *J* = 2.5, 4.1 Hz), 6.34 (1H, d, *J* = 2.5 Hz), 7.30 (1H, d, *J* = 2.5 Hz); ^{13}C-NMR (CD_3OD, 125 MHz) δ 17.8, 19.2, 25.9, 28.2, 32.3, 45.5, 48.2, 55.9, 75.4, 93.5, 103.3, 105.5, 107.6, 109.6, 111.2, 118.5, 124.8, 128.4, 131.9, 149.7, 156.5, 159.4, 161.9, 164.8, 165.4, 193.8.

Kushnol F (2) $[\alpha]_D^{25}$ = –59.8° (*c* 0.82, MeOH); EIMS (70 eV) m/z 424 (12), 406 (9), 301 (79), 283 (100), 153 (65), 109 (42); UV (MeOH) λ_{max} 292 nm; IR (KBr) ν_{max} 3400, 2900, 1625, and 1520 cm^{-1}; ^{1}H-NMR (500 MHz, CD_3OD) δ 1.56 (3H, s), 1.63 (3H, s), 1.69 (3H, s), 2.07 (2H, m), 2.54 (1H, m), 2.59 (2H, m), 2.71 (1H, dd, *J* = 2.8, 17.1 Hz,), 3.05 (1H, dd, *J* = 13.0, 17.2 Hz,), 4.53 (1H, m), 4.59 (1H, m), 5.04 (1H, m), 5.58 (1H, dd, *J* = 2.8, 13.1 Hz), 5.94 (1H, s), 6.32 (1H, s), 6.34 (1H, d, *J* = 2.4 Hz), 7.23 (1H, d, *J* = 8.1 Hz). ^{13}C-NMR (CD_3OD, 125 MHz) δ 18.3, 19.4, 26.3, 28.1, 32.9, 43.7, 48.4, 76.3, 95.8, 103.5, 103.9, 108.2, 109.5, 111.7, 118.5, 125.3, 129.3, 132.3, 150.1, 157.2, 160.1, 163.5, 166.8, 166.9, 198.9.

Determination of Inhibition of Melanin Production of *S. bikiniensis*. A preserved culture of *S. bikiniensis* NRRL B-1049 was inoculated a Papavizas' VDYA agar slant. After incubation at 28 °C for 2 weeks, the spore mass formed on the aerial mycelium was scraped with an inoculating loop. The spore suspension of *S. bikiniensis* was inoculated a agar medium ISP No.7. After drying of the agar surface, a paper disc (8 mm) soaked with sample solution was placed on the agar plate. The plate was incubated at 28 °C for 48 hours; the resulting zone of inhibition of melanin formation was measured from the reverse side of the plate.

Inhibition of Tyrosinase. To a 96-well microplate was added 0.1 M phosphate buffer (pH 6.5) 150 μL and 1.5 mM L-tyrosine solution 25 μL, and then 7 μL of 2100 unit/mL mushroom tyrosinase (Sigma, 0.05 mM phosphate buffer, pH 6.5). After incubation at 30 °C for 10 min, the amount of dopa produced in the reaction mixture was determined as the optical density at 490 nm. The inhibitory activity of the sample was expressed as the concentration which inhibits 50% of the enzyme activity (IC_{50}). Michaelis constant (Km) and maximal velocity (V_{max}) of the tyrosinase was determined by Lineweaver Burk's plot using various concentration of L-tyrosine.

Acknowledgment. This work was supported by a grant from High-technology Development Project for Agriculture, Forestry and Fisheries. We also thank the financial support of Brain Korea 21 program.

References

1. Griffiths, C. E. M.; Finkel, L. J.; Ditre, C. M.; Hamilton, T. A.; Ellis, C. N.; Voorhees, J. J. *Br. J. Dermatol*. **1993**, *129*, 415.
2. Kanwar, A. J.; Dhar, S.; Kaur, S. *Dermatology* **1994**, *188*, 170 .
3. Wu, L.; Miyase, T.; Ueno, A.; Kuroyanagi, M. *Yakugaku*

Zasshi **1985**, *105*, 736.
4. Ryu, S. Y.; Lee, H. S.; Kim, Y. K.; Kim, S. H. *Arch. Pharm. Res*. **1997**, *20*(5), 491.
5. Bae, K. H.; Kim, Y. K.; Min, B. S. *Arch. Pharm. Res*. **1997**, *20*(4), 342.
6. Yamaki, M.; Kashihara, M.; Takagi, S. *Phytother. Res*. **1990**, *4*, 235.
7. Yagi, A.; Fukunaga, M.; Okuzako, N.; Mifuchi, I.; Kawamoto, F. *Shoyakugaku Zasshi* **1989**, *43*, 343.
8. Tomita, K.; Oda, N.; Ohbayashi, M.; Kamei, H.; Miyaki, T.; Oki, T. *J. Antibiotics* **1990**, *12*, 1601.
9. Shin, N. H.; Ryu, S. Y.; Choi, E. J.; Kang, S. H.; Chang, I. M.; Min, K. R.; Kim, Y. S. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 801.