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Prenylated Flavonoids as Tyrosinase Inhibitors

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In order to find new tyrosinase inhibitors and the effects of prenyl residue on flavonoid molecules, eight prenylated and three synthetic vinylated flavonoids were examined on their inhibitory effect against tyrosinase activity. From the results, kuwanon C, papyriflavonol A, sanggenon D and sophoflavescenol were found to possess the considerable inhibitory activity. Especially, sanggenon D is revealed as a potent inhibitor (IC_{50} = 7.3 μM), compared to the reference compound, kojic acid (IC_{50} = 24.8 μM). However, the prenylation with isoprenyl group or the vinylation to flavonoid molecules did not enhance tyrosinase inhibitory activity.

Key words: Flavonoid, Tyrosinase, Skin, Sanggenon, Kuwanon, Prenylation

INTRODUCTION

Because melanin formation is the most important factor to determine the mammalian skin color, the inhibition of melanin formation may result in a reduction of skin darkness. On the sequential pathway to melanin formation, tyrosinase (EC 1.14.18.1) acts as a rate-limiting enzyme, which catalyzes tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and further oxidizes it to dopaquinone (Sanchez-Ferrer *et al.*, 1995). Therefore, tyrosinase inhibitors may be of importance to treat abnormal pigmentation disorders and to use as skin whitening agents in cosmetics. Although the chemicals such as arbutin and plant extracts such as Glycyrrhizae Radix and Mori Cortex Radicis are currently used, it is always necessary to develop new potent and safe agents for the purpose of skin whitening.

As potential candidates, naturally occurring flavonoids have been examined. For instance, several flavonoid derivatives including quercetin, myricetin, myricetin-glycoside and several others have been found to have varying degrees of inhibitory activity toward tyrosinase (Matsuda *et al.*, 1996). Glabridin, an isoflavan from Licorice roots, inhibited melanogenesis (Yokota *et al.*, 1998). Another flavonoids from Licorice roots, glabrene (isoflavene) and isoliquiritigenin (chalcone), were also found to be strong tyrosinase inhibitors (Nerya *et al.*, 2003). (+)-Catechin-3-*O*-gallate was reported to inhibit tyrosinase activity (No *et al.*, 1999). In addition, flavonols and flavones such as quercetin, kaempferol, galangin, luteolin, chrysin, baicalein and luteolin-7-*O*-glucoside were established to weakly inhibit tyrosinase activity (Kubo *et al.*, 2000). Structure-activity relationships with various flavonoids and stilbenes were demonstrated that 4-substituted resorcinol moiety was essential for showing the strong inhibitory activity against tyrosinase activity (Shimizu *et al.*, 2000). Many flavonoids and theaflavins were evaluated for tyrosinase inhibition and some of them were repeatedly found to be inhibitory (Badria and ElGayyar, 2001). In particular, several prenylated flavonoids were demonstrated as potent tyrosinase inhibitors. The prenylated flavanones from *Sophora flavescens* such as kurarinone and kushenol F were reported to strongly inhibit tyrosinase activity noncompetitively (Ha *et al.*, 2001). Some other prenylated flavonoids including sophoraflavanone G from the same plant were also found to be potent inhibitors, being more potent than kojic acid (Son *et al.*, 2003; Kim *et al.*, 2003). These previous findings suggest that the prenyl residues on flavonoid molecules may lead to the potent inhibition of tyrosinase activity. Therefore, for a continual search, some prenylated flavonoids isolated from several plants, and some synthetic vinylated flavonoids were examined on tyrosinase inhibitory activity in this study.

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MATERIALS AND METHODS

Materials

Kojic acid, chrysin and quercetin were purchased from Sigma Chemical (St. Louis, MO). Isosophoranone and sophoflavescenol were isolated from the roots of *Echinosophora koreensis* Nakai (Fabaceae) and *Sophora flavescens* Ait. (Fabaceae), respectively (Kim *et al.*, 2002; Kang *et al.*, 2000). Kuwanon C and morusin were obtained from the root barks of *Morus alba* L. (Moraceae). Sanggenon D was separated from the root barks of a commercial crude drug of *Morus mongolica* Schneider (Moraceae) (Nomura *et al.*, 1988). Broussochalcone A, kazinol B and papyriflavonol A were isolated from the root barks of *Broussonetia papyriferra* (L.) Vent. (Moraceae) (Son *et al.*, 2001). 4'-, 6- and 8-Vinylflavone were synthesized according to the previously described procedure (Dao *et al.*, 2003). The chemical structures of flavonoids tested are shown in Fig. 1.

Tyrosinase assay

Tyrosinase activity was determined essentially based on the previously described procedure (Kim *et al.*, 2003). In brief, the test reaction mixture comprised each test compound, mushroom tyrosinase (105 units, Sigma Chemical) 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. The test compounds were initially dissolved in DMSO and subsequently diluted to the appropriate concentrations with the above sodium phosphate buffer. The final concentrations of DMSO in the final reaction mixture never exceeded 0.1% (v/v). Each reaction was duplicated. The same experiment was repeated at least twice and they gave similar results.

RESULTS AND DISCUSSION

Initially, the inhibitory activity of each prenylated flavonoid against tyrosinase was examined at 30 and 100 μM, using tyrosine as a substrate. In this study, kojic acid was used as a reference compound of tyrosinase inhibitor. As demonstrated in Table I, broussochalcone A, isosophoranone, kazinol B and morusin did not show the considerable inhibitory activity (less than 15% inhibition at 100 μM). Three synthetic flavonoids having a vinyl residue at different positions were not inhibitory. In contrast, kuwanon C, papyriflavonol A, sanggenon D and sophoflavescenol showed the significant inhibition at 30 and 100 μM. Fig. 2 illustrated the concentration-dependent inhibition of the selected compounds, and the IC_{50} values of kuwanon C and sanggenon D were 49.2 and 7.3 μM, respectively. Particularly, sanggenon D was found to possess more potent inhibitory activity, compared to kojic acid (IC_{50} = 24.8 μM).

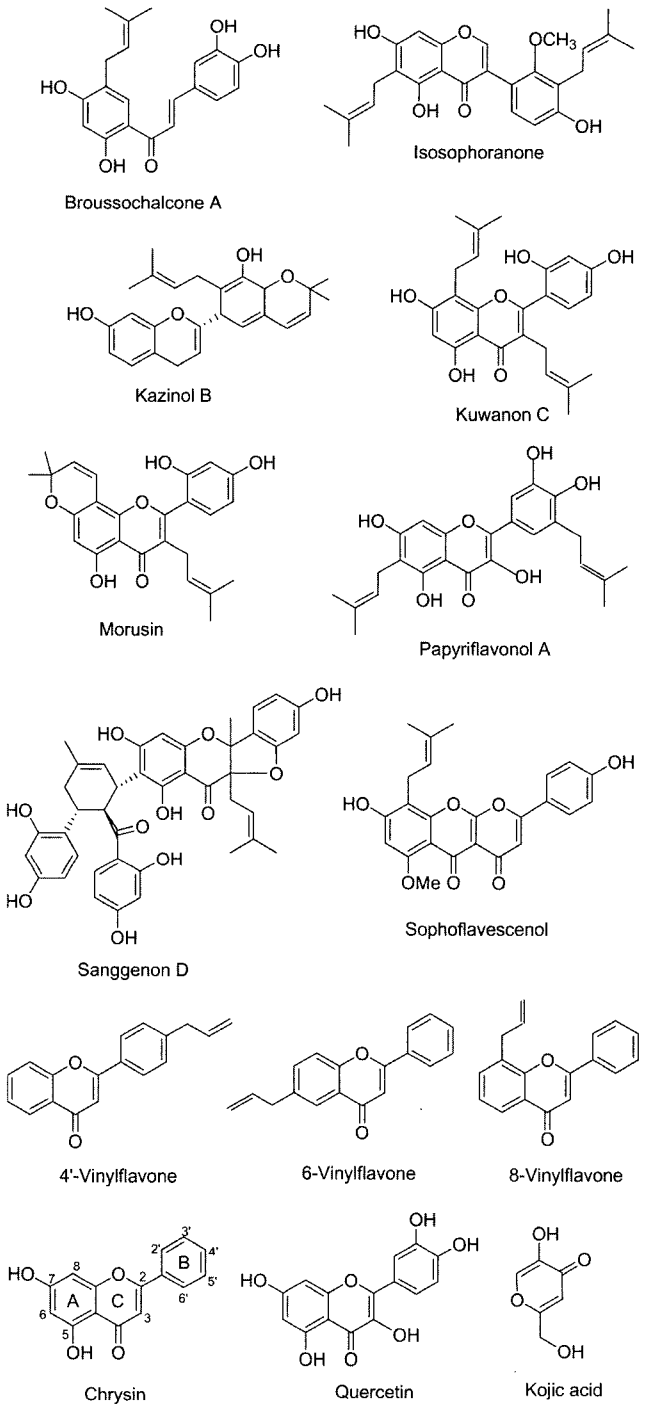

Fig. 1. The chemical structures of flavonoids tested in this study

Previously, several prenylated flavonoids from *Sophora flavescens* exhibited potent inhibitory effect on tyrosinase activity (Ha *et al.*, 2001; Son *et al.*, 2003; Kim *et al.*, 2003). These findings prompted us to examine other prenylated

Table I. Tyrosinase inhibitory activity of flavonoids

Compounds	% inhibition at	
	30 μM	100 μM
Broussochalcone A	3[a]	7
Isosophoranone	-[b]	-
Kazinol B	15	10
Kuwanon C	54	58
Morusin	-	10
Papyriflavonol A	16	39
Sanggenon D	91	98
Sophoflavescenol	30	49
4'-Vinylflavone	-	-
6-Vinylflavone	7	9
8-Vinylflavone	3	2
Chrysin	-	-
Quercetin	29	42
Kojic acid	92	93

[a]Arithmetic mean of two separate measurement (n = 2), [b]Not active

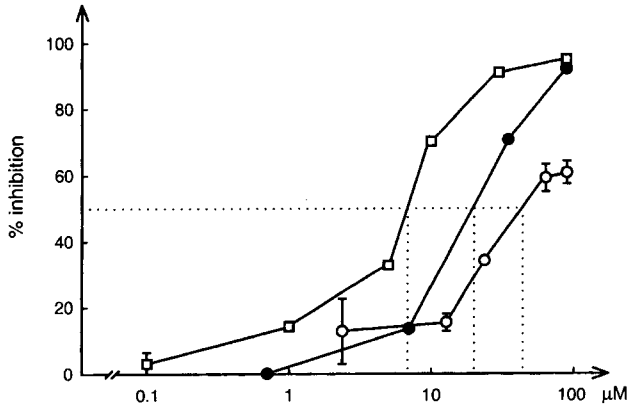

Fig. 2. The concentration-dependent tyrosinase inhibition of kuwanon C and sanggenon D. The data points and bars represent arithmetic mean± SD from two separate experiments (n = 2), Kuwanon C (○), sanggenon D (□) and kojic acid (●).

flavonoids in plant extracts. By standard tyrosinase inhibition assay, we have demonstrated in this study that several prenylated flavonoids such as kuwanon C, papyriflavonol A and sanggenon D were found to be tyrosinase inhibitors. But, structure-activity relationships among the flavonoids tested are poorly understood. Prenyl groups comprise isoprenyl, geranyl and lavandulyl moieties. The prenylated flavonoids having potent inhibitory activity previously found are the derivatives with a lavandulyl moiety on C-6 or C-8 position of flavonoid moiety (Ha *et al.*, 2001; Son *et al.*, 2003; Kim *et al.*, 2003). And one C-6 isoprenylated flavonoid, artocarpesin, was found to possess significant inhibitory activity (IC_{50} = 13.5 μM) (Shimizu *et al.*, 2000). However, in this study, the prenylation with a isoprenyl group at C-3 or C-6 did not increase the inhibitory activity. The fact that three synthetic vinylated flavones did not significantly inhibit tyrosinase activity regardless of the positions of substitution may also indicate that other structural requirement(s) instead of the prenyl/vinyl moiety is more important for tyrosinase inhibition by flavonoids. When the inhibitory activities of papyriflavonol A and quercetin (non-prenylated counterpart) were compared, isoprenylation did not increase the inhibitory activity. Rather, 4-substituted resorcinol moiety seems to be more contributable to the significant inhibitory activity as in sanggenon D and kuwanon C. The similar structure-activity relationship was reported previously (Shimizu *et al.*, 2000; Son *et al.*, 2003). It is also found that cyclization of C-8 isoprenyl substitution decreased the inhibitory activity (morusin vs kuwanon C).

From the results of the present investigation, it is concluded that several prenylated flavonoids including kuwanon C, papyriflavonol A, sanggenon D and sophoflavescenol are found as tyrosinase inhibitors. Especially, sanggenon D is revealed as more potent inhibitor than kojic acid. However, the prenylation (isoprenyl residue)/vinylation to the flavonoid molecule may be not favorable to exhibit tyrosinase inhibition.

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