CHAPTER I

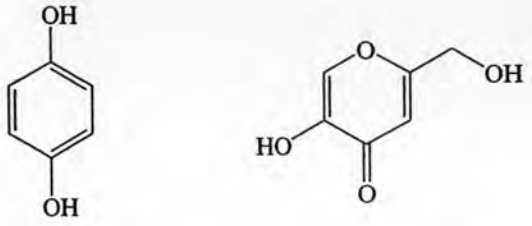
INTRODUCTION

Melanins are the insoluble heterogeneous polyphenol-like biopolymer with complex structure. It is one of the most widely distributed pigments found in animals, plants, and microorganisms. Malanin is secreted by melanocyte cells distributed in the basal layer of the dermis. The important role of melanin is to protect the skin from ultraviolet (UV) damage by absorbing UV sunlight and removing reactive oxygen species (ROS). The accumulation of excessive level of epidermal pigmentation cause various dermatological disorders such as melasma, age spots, lentigo, ephelis and sites of acitinic damage. Melanin is formed through a series of oxidative reactions involving the amino acid tyrosine in the presence of enzyme tyrosinase (Briton, 1983; Sanchez-Ferrer *et al.*, 1995; Lerner and Fitzpatrick, 1950).

Tyrosinase is a copper-containing enzyme. It is known as the key enzyme in melanin biosynthesis. Tyrosinase catalyzes two distinct reactions including the hydroxylation of tyrosine and oxidation of *o*-diphenol product, L-Dopa. Dopa oxidation produces a highly reactive intermediate that is further oxidized to form melanin by a series of non-enzymatic steps (Briton, 1983; Sanchez-Ferrer *et al.*, 1995; van Gelder, Flurky and Wichers, 1997; Seo, Sharma and Sharma, 2003). Melanin biosynthesis can be inhibited by avoiding UV exposure, inhibiting melanocyte metabolism and proliferation or inhibiting tyrosinase activity (Nerya *et al.*, 2003). Apart from avoiding UV exposure, application of tyrosinase inhibitors may be the least invasive procedure for maintaining skin whiteness. Tyrosinase may play a role in cancer and neurodegenerative diseases such as Parkinson's disease (Chen and Kubo, 2002). Furthermore, tyrosinase-induced unflavorable browning of wounded raw fruits, vegetables and beverages is a main problem in food industry.

Among the whithening agent commonly used for topical treatments of hyperpigmentation disorders, 1,4-dihydroquinones (1) is one of the most widely prescribed. It causes reversible inhibition of cellular metabolism by affecting both DNA and RNA synthesis. It is also a potent melanocyte cytotoxic agent and has been

reported as a mutagen (Kim and Uyama, 2005; Nerya *et al.*, 2003). As a result of these, the use of 1,4-dihydroquinones has been prohibited in cosmetics by most countries. Moreover, kojic acid (**2**), a well-known whitening agent in skin-care products, can cause contact dermatitis and is low stability in formulation (Nerya *et al.*, 2003). Thus, in the diverse areas of medical field, cosmetic industries and food processing, there is the increasing need to search for the alternative herbal and pharmaceutical depigmentating and antibrowning agents.



1,4-Hydroquinone (**1**) Kojic acid (**2**)

Figure 1. Chemical structure of whitening agents.

Licorice is derived from the roots and stolons of some *Glycyrrhiza* species of the family Leguminosae. It has been used for centuries as flavoring and sweetening agents, as well as demulcent and expectorant in the Western countries. It is also used as anti-allergic and anti-inflammatory agents in Japan and China (Fukai *et al.*, 2003). The two main bioactive components of licorice are glyzyrhhizin (**3**) and flavonoids (**4-15**) (Fu *et al.*, 2005; Fukai *et al.*, 1996; Fukai *et al.*, 2003; Kinoshita *et al.*, 1996a, 1996b; Kinoshita, Saitoh and Shibata, 1976; Mitscher, Park, and Clark, 1980; Saitoh, Kinoshita and Shibata, 1976). Licorice flavonoids have shown estrogenic-like activity (Somjen *et al.*, 2004), antitumorigenic (Kanzawa *et al.*, 2003), antimicrobial, antiviral, anti-inflammatory, antioxidant (Vaya, Berlinky and Aviram, 1997), antiulcer (Fukai *et al.*, 2002b) and anti-tyrosinase activities (Fu *et al.*, 2005; Nerya *et al.*, 2003; Yogota *et al.*, 1998). In Japan, the flavonoid rich fraction of licorice extract is used as a depigmentary agent in cosmetics (Fu *et al.*, 2005).

Glabridin (**4**) is a major pyranoisoflavan isolated from hydrophobic fraction of European licorice, *Glycyrrhiza glabra* L.var *typica* (Spanish and Italian licorice) and *G. glabra* L. var *glandulifera* (Russian licorice), but it is not found in Chinese and

Kirghiz *G. glabra* L. (Fukai *et al.*, 2003). It has been reported to exhibit a wide range of pharmacological activities such as antimicrobial (Fukai *et al.*, 2002a, 2002b; Mitscher, Park and Clark, 1980), protection of mitocondrial functions against oxidative stress (Haragugi *et al.*, 2000), prevention of low-density lipoprotein oxidation (Aviram, 2004; Belinky, Aviram, Fuhraman *et al.*, 1998; Belinky, Aviram, Mahmood *et al.*, 1998; Rosenblat *et al.*, 1999; Vaya, Belinky and Aviram, 1997), estrogenic and antiproliferative activities in human breast cancer cells (Somjen *et al.*, 2004; Tamir, 2000), inhibitor of adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase (Kusano *et al.*, 1991), inhibition of human cytochrome P450s 3A4, 2B6 and 2C9 (Kent *et al.*, 2002), inhibit melanogenesis and skin inflammation (Yokota *et al.*, 1998).

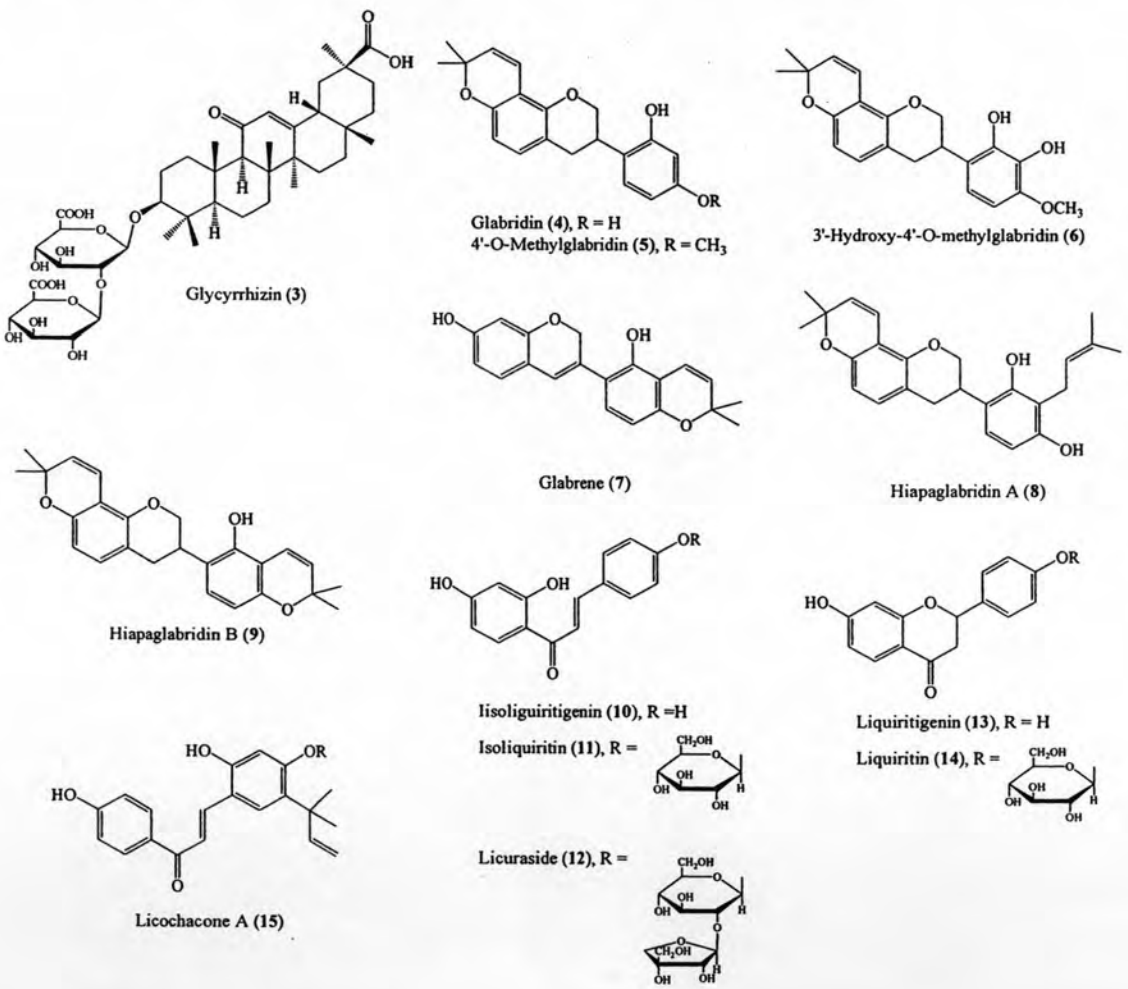
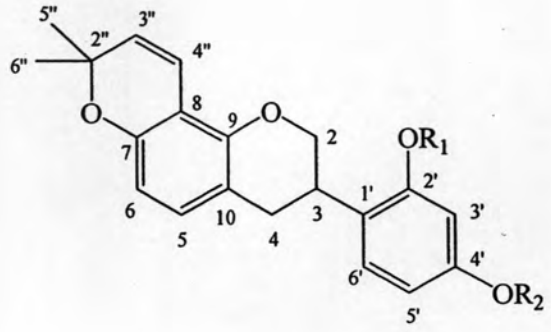


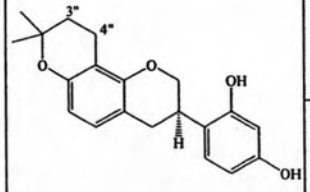
Figure 2. Chemical constituents in licorice extracts.

One of the most interested biological activity of flavonoids is antioxidant, which is due to their ability to reduce free radical formation and to scavenge free radical (Pietta, 2000). Oxygen free radicals, known as reactive oxygen species (ROS), particularly superoxide, hydroxyl and peroxyl radicals, are the common radicals generated during cell metabolism. They can also be generated by environmental exposure to prooxidants like cigarette smoke, ionizing radiation, organic solvents and pesticides. ROS can attack lipids in cell membranes, protein in tissues or enzymes, carbohydrates and DNA, to induce oxidation which cause membrane damage, protein modification and DNA damage. This oxidative damage is considered to play an important role in the promotion of aging and several diseases such as Alzeimers, atherosclerosis, cancer, Parkinsonism, cataract and other chronic diseases. Increasing antioxidant defenses may prove to be a useful intervention, not only to slowing the rate of aging process, but also to reduce the associated risk of chronic disease (Blumberg and Halpner, 1999).

Since glabridin is an antioxidant and has stronger tyrosinase inhibitory activity than kojic acid, suggesting the potential applications of this compound as a skin-whitening agent in cosmetic products or as an anti-browning agent in food of plant origin. In addition, the high quantity of glabridin in licorice extract provides a source for developing natural-product based useful bioactive compound. The main drawback of grabridin are poor skin penetration and instability in formulation. This prompted us to synthesize glabridin derivatives and study the structure-activity relationships of these compounds on tyrosinase activity, free radical scavenging activity and their lipophilicity and hydrolysis property. In this research, the effect of acyl and aroyl moieties at phenolic hydroxyl of the ring B and the effect of double bond between the carbon atom 3″ and 4″ on tyrosinase inhibition and free radical scavenging activity as well as the lipophilicity and hydrolysis property of the acyl derivatives would be investigated. The chemical structures of the desired compounds are shown in Table 1.

Table 1. Chemical structures of glabridin derivatives prepared in this study.



Compounds	R1	R2	Compounds	R1	R2
16	acetyl	acetyl	**22**	benzyl	benzyl
17	hexanoyl	hexanoyl	**23**	*m*-bromo benzyl	*m*-bromo benzyl
18	decanoyl	decanoyl	**24**	*p*-bromobenzyl	*p*-bromobenzyl
19	palmityl	palmityl	**25**	*m*-nitrobenzyl	*m*-nitrobenzyl
20	stearyl	stearyl	**26**	*p*-nitrobenzyl	*p*-nitrobenzyl
21			**27**	*o*-methoxy benzyl	*o*-methoxy benzyl
			28	*m*-methoxy benzyl	*m*-methoxy benzyl