CHAPTER I

INTRODUCTION

It has been long thought that human can use plants in many ways. Human can obtain numerously valuable benefits from their natural resources. Fortunately, Thailand is located in the tropical region of the world and has abundant kinds of plants, especially herbs which are used as medicinal plants.

Medicinal plants contain various bioactive secondary metabolites. They have especially pharmacological active principle which can be used as therapeutic drugs or herbal medicine. Therefore, medicinal plants still serve as sources for scientists to be developed into new lead and more active compounds.

There is growing interest in the contribution of free radical reaction participating in reactive oxygen species (ROS) to the overall metabolic perturbation that result in tissue injury and diseases. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are various forms of activated oxygen and nitrogen, which include free radicals such as superoxide anion ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), and nitric oxide ($NO^{\cdot}$) radicals as well as non-free radical species such as hydrogen peroxide (H_2O_2) and nitrous acid (HNO_2).[1] In living organisms various ROS and RNS can be formed by different ways. Normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages, and peroxisomes appear to the main endogeneous sources of most of the oxidants produced by cell. Exogeneous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides.

Normally phagocytic cells ingest and kill invading pathogens with free radicals including superoxide anion, hydrogen peroxides, nitric oxide and hypochlorite. A critical balance exists between the generation and detoxification of reactive oxygen species in cells.[2]

Furthermore, disease, aging and chemical environments such as drugs, pesticides, herbicides and various pollutants can disrupt this balance by inhibition of the cellular antioxidant defenses and/or by stimulation of the formation of reactive oxygen. However, ROS may be very damaging since they can attack lipid in cell membranes, proteins 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 a causative role in aging and several degenerative diseases associated with it such as heart disease, stroke, brain dysfunction, arthritis, inflammation, and cancer.[3] Free radicals can cause lipid peroxidation in food that leads to the deterioration of them. Oxidation does not affect only lipids. ROS and RNS may cause DNA damage that could lead to mutation. When produced in excess, ROS can cause tissue injury. Nevertheless, all aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages and numerous damage removal and repair enzymes that remove or repair damaged molecules. However, this natural antioxidant mechanism can be inefficient; hence, dietary intake of antioxidant compounds will become important. Although there are some synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), which are commonly used in processed foods. It has been reported that these compounds have some toxicity. Therefore, the search for natural antioxidants as alternatives to synthetic antioxidants is great interest among researchers. At the same time, they can serve as lead for the development of new drugs with prospect of improving the treatment of several diseases.

Antioxidants are compounds that can delay or inhibit the oxidation of lipid or other molecules by inhibiting the initiation or propagation of oxidizing chain reaction. According to Halliwell and Gutteridge,[4] mechanisms of antioxidant action can include (1) suppressing reactive oxygen species the formation of either by initiation of enzyme or chelating trace elements involved in free radical production (**Figure 1.1**); (2) scavenging reactive oxygen species (**Figure 1.2**): Various kinds of natural radical scavengers have received much attention as chain-breaking antioxidants which protect aerobic organisms from oxidation stress. The antioxidant activity has always been attributed to their phenolic constituents. These phenolic compounds can retard lipid peroxidation or protect against oxidative damages by donating a hydrogen atom or an electron chain initiating free radicals; (3) upregulating or protecting antioxidant defenses. Studies on the free radical scavenging properties of flavonoids have permitted characterization of the major phenolic components of naturally occurring phytochemicals as antioxidants. Flavonoids also have been identified as fulfilling most of the criteria described above.

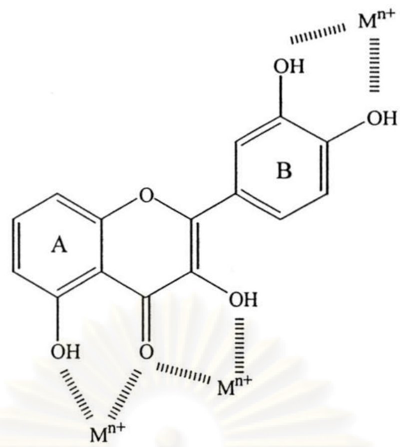


Figure 1.1 Binding sites for trace metals

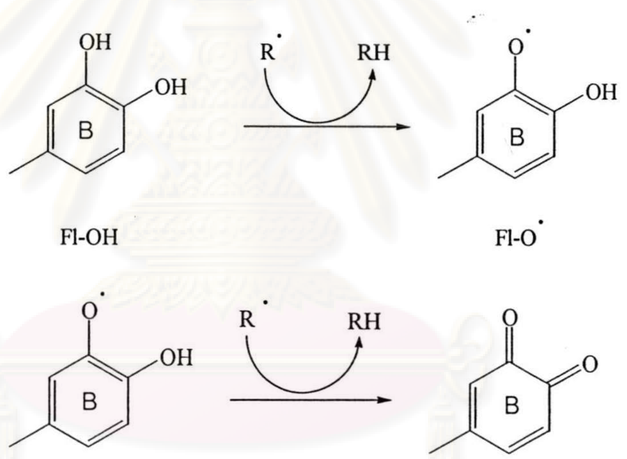
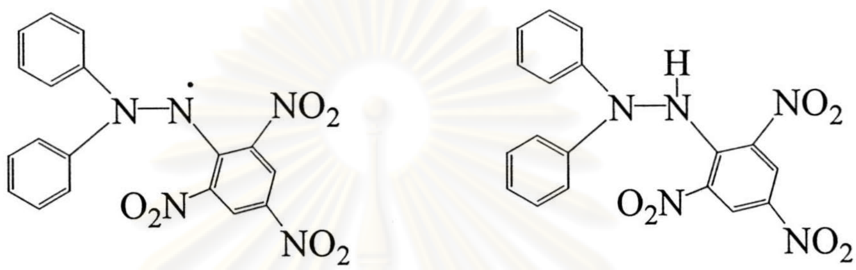


Figure 1.2 Scavenging of ROS (R·) by flavonoids.

There are many methods or models to determine the antioxidative properties of the compounds such as linoleic acid, β-carotene, xanthine oxidase, DPPH, and so on. In this study, DPPH is selected in activity directed fractionation of free radical scavenging activity because this model is rapid, convenient, reliable, inexpensive, sensitive, and require a little material. DPPH is a stable solid dye free radical, so it is easy to control the quantity of radicals. This is a kind of nitrogen-centered radical, but the reactivity is not so large as an oxygen-centered radical such as RO• and ROO• because of the widely spreading resonant system. When this radical reacts with polyphenols, dehydrogenation occurs on polyphenol molecules and DPPH changes into DPPHn, the structure of which is shown in **Figure 1.3**. DPPH is a deep purple substance, but DPPHn is colorless.[5]

In a primary screening for free radical scavenging activity among many plant materials, the stems of *Dalbergia cochinchinensis* were found to possess a strong activity on DPPH radical (the bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical) .



DPPH (2,2-diphenyl-1-picrylhydrazyl) DPPHn (2,2-diphenyl-1-picrylhydrazine)
deep purple colorless

Figure 1.3 Structure of DPPH and DPPHn

Xanthine oxidase (XO) is a complex enzyme, containing molybdenum and iron/sulfur redox center that has been known for at least 95 years. The intestinal mucosa and liver are the richest sources of XO.[6]

The enzyme xanthine oxidase catalyses the oxidation of hypoxanthine and xanthine to uric acid, and the accumulated uric acid caused hyperuricacidamia associated with gout. During the reoxidation of xanthine oxidase, molecular oxygen acts as electron acceptor, producing superoxide anion radicals or hydrogen peroxide.[7] These reactions can be written as follow (**Figure 1.4**): The isolated compounds were also tested with this assay.

$$\text{Xanthine} + 2O_2 + H_2O \longrightarrow \text{Uric acid} + 2O_2^{-\cdot} + 2H^+$$

$$\text{Xanthine} + O_2 + H_2O \longrightarrow \text{Uric acid} + H_2O_2$$

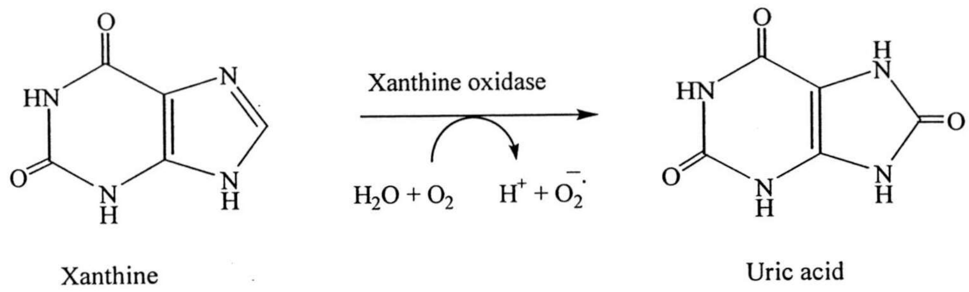


Figure 1.4 Xanthine oxidized to uric acid

Consequently, xanthine oxidase is considered to be an important biological source of superoxide anion radicals. Therefore, inhibition of xanthine oxidase is an effective therapeutic approach for treating hyperuricemia that causes gout, kidney stones, and myocardial ischemia.

In the present study, this research is focused on searching for bioactive compounds from the stem of *D. cochinchinensis* in the polar extracts. Due to the interesting screening results and a few information on the chemical constituents and their biological activities, the research on *D. cochinchinensis* should be further investigated.

The Objective of this Research :

1. To carry out a comprehensive chemical separation and structure determination of the polar extracts from the stems of *Dalbergia cochinchinensis* by chromatography and spectroscopic techniques.
2. To investigate biological activities of the isolated compounds

Botanical Aspect and Distribution

Dalbergia cochinchinensis Pierre is the plant in Leguminosae family. In Thailand it has been known as Payung or Payung Mai (Central region) Khayoong (East-North region) Pradoolai (Chonburi) and Pradoosein (Thrad). *D. cochinchinensis* is a medium to large evergreen tree, 25-30 m tall and 60-120 cm in diameter.[8]

Leaves : compound leaves, with 7-9 leaves.

Flowers : white, in axillary panicles.

Fruits : indehiscent, flat pod, 5-6 cm long, 1 cm wide , 1-3 seeds per pod.

Seeds : flat, brown , 35,000 seeds / kg.

Figure 1.5 Flowers, leaves, wood and tree of *Dalbergia cochinchinensis*

Chemical constituents of *Dalbergia cochinchinensis* Pierre.

There are a few parts of *D. cochinchinensis* Pierre which have been reported. Mostly of flavonoids were found. The chemical constituents of them are summarized in **Table 1.1** and chemical constituents of many other species of *Dalbergia* genus will be summarized in **Table 1.2**. Most of the chemical structures are shown in **Figure 1.6** and **Figure 1.7**.

Table 1.1 Chemical constituents of *D. cochinchinensis*

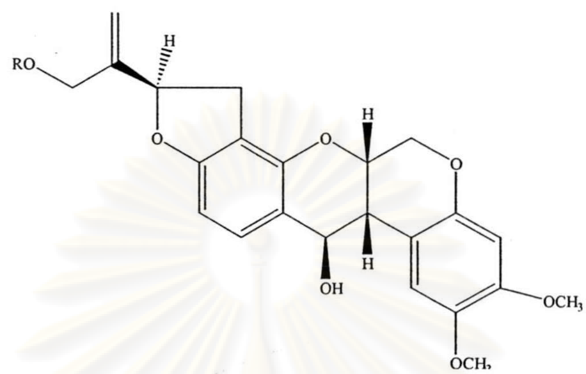
Parts of plant	**compounds**	**ref**
Seed	Dalcochinin-8'-O-β-D-glucoside	9
Stem	9-hydroxy-6,7-dimethoxydalbergiquinol 2,2',5-trihydroxy-4-methoxybenzophenone 7-hydroxy-6-methoxyflavone	10
Heartwood	(*R*)-latifolin (*R*)-4-methoxydalbergione	11

Table 1.2 Chemical constituents of *Dalbergia genus.*

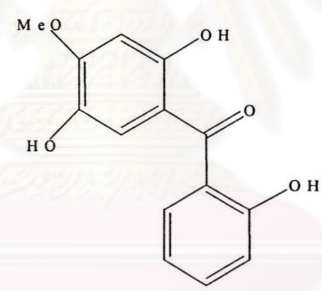
Species	**Part of plants**	**Name of compounds**	**ref**
D. sissoides	Stem	6-ketodehydroamorphigenin	12
D. nigra	Leaves	5-hydroxy-6,7-dimethoxy-4'-O-(6-O-D-apio-β-D-furanosyl-β-D-glucopyranosyl)isoflavone	13
D. odorifera	Heartwood	Ceroin Medicapin Meliotocarpan (*S*)-4-methoxydalbergione Koparin 3'-hydroxydaidzein 2',7-dihydroxy-4',5'dimethoxyisoflavone	14

		Xenognosin B Sativanone 3-hydroxy-9-methoxycoumestan Isoliquiritigenin Butein Stevein 3'-hydroxymelanettin Melanettin Dalbergin Liquiritigenin 3',4',7-trihydroxyflavanone (3R)-4'-methoxy-2',3,7-trihydroxyisoflavanone Bowdichione Fisetin 7-methoxy-3,3',4',6'tetrahydroxyflavone Sulfuretin 3'-O-methylviolanone (3R)-vestitol (3R)-claussequinone Formononetin Medicarpin (3R)-5'-methoxyvestitol (3R)-3',8-dihydroxyvestitol (3R)-2',3',7-trihydroxy-4'-methoxyisoflavone 2'-O-methylisoliquiritigenin Biisoflavonoid	
D. frutescens	Stem Bark	Fowmononetin Castanin Odoratin Glycitein Pseudobaptogenin	15

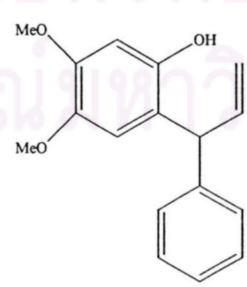
		Fujikinetin Cuneatin	
D. coromandeliana	Leaves	Prunetin-4'-O-apiosyl-(1-6)-glucoside	16



Dalcochinin-8'-O-β-D-glucoside

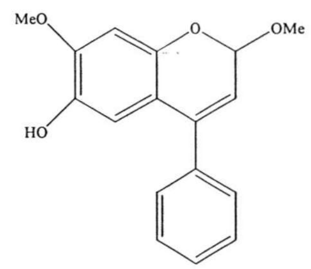


2,2',5-trihydroxy-4-
methoxybenzophenone

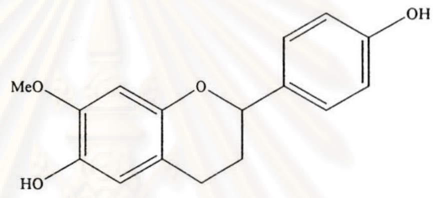


9-hydroxy-6,7-dimethoxydalbergiquinol

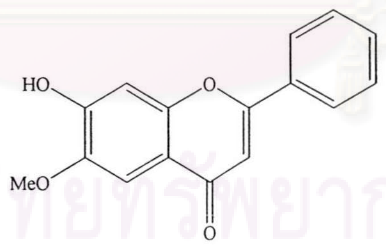
Figure 1.6 Structures of some chemical constituents of *Dalbergia cochinchinensis.*



6-hydroxy-2,7-dimethoxyneoflavene

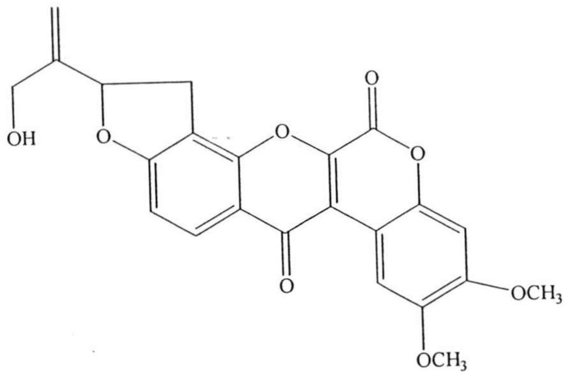


6,4'-dihydroxy-7-methoxyflavan

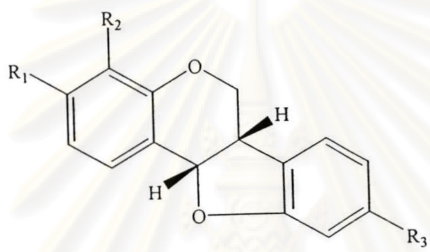


7-hydroxy-6-methoxyflavone

Figure 1.6 Structures of some chemical constituents of *Dalbergia cochinchinensis.* (continues)

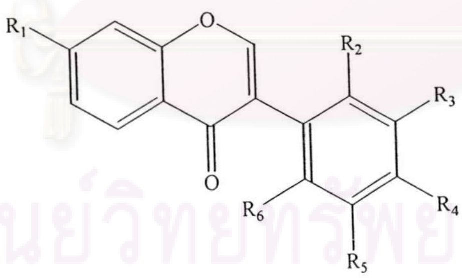


6-ketodehydroamorphigenin



Medicapin : $R_1 = OH$, $R_2 = H$, $R_3 = OCH_3$

Meliotocarpan : $R_1 = OCH_3$, $R_2 = OH$, $R_3 = OCH_3$



(*S*)-4-methoxydalbergione : $R_1 = OH$, $R_2 = R_3 = R_5 = R_6 = H$, $R_4 = OCH_3$

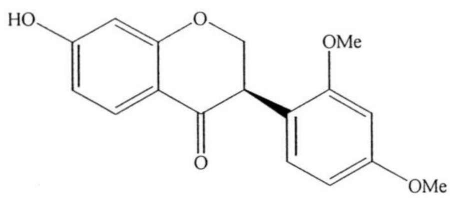
Koparin : $R_1 = R_2 = R_3 = OH$, $R_4 = OCH_3$, $R_5 = R_6 = H$

3'-hydroxydaidzein : $R_1 = R_3 = R_4 = OH$, $R_2 = R_5 = R_6 = H$

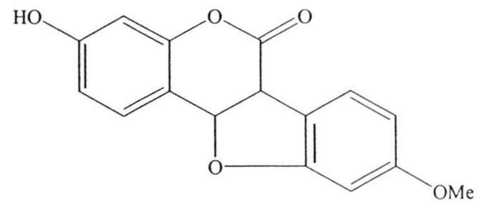
2',7-dihydroxy-4',5' dimethoxyisoflavone : $R_1 = R_2 = OH$, $R_3 = R_6 = H$, $R_4 = R_5 = OCH_3$

Xenognosin B : $R_1 = R_2 = OH$, $R_3 = R_5 = R_6 = H$, $R_4 = OCH_3$

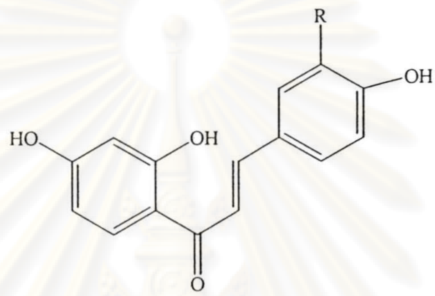
Figure 1.7 Structures of some chemical constituents of *Dalbergia* genus.



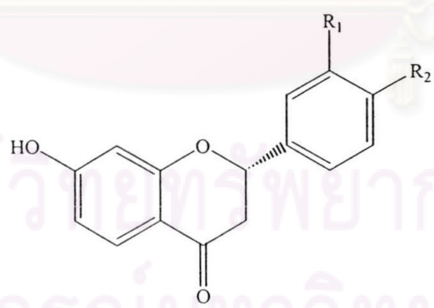
Sativanone



3-hydroxy-9-methoxycoumestan



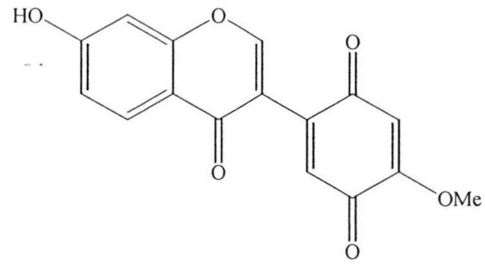
Isoliquiritigenin : R = H
Butein : R = OH



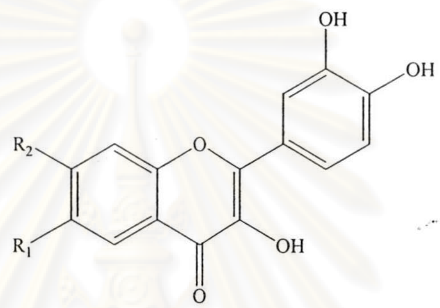
Liquiritigenin : $R_1 = H$, $R_2 = OH$

3’,4’,7-trihydroxyflavanone : $R_1 = R_2 = OH$

Figure 1.7 Structures of some chemical constituents of *Dalbergia genus.* (continues)

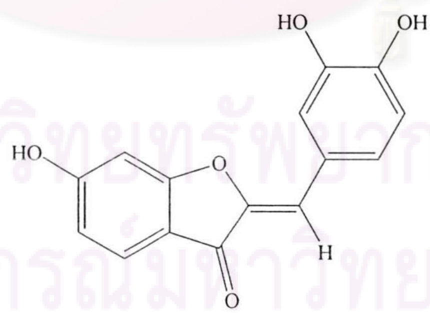


Bowdichione



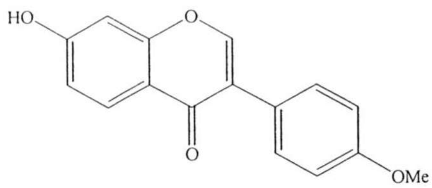
Fisetin : $R_1 = H$, $R_2 = OH$

7-methoxy-3,3’,4’,6’-tetrahydroxyflavone : $R_1 = OH$, $R_2 = OMe$

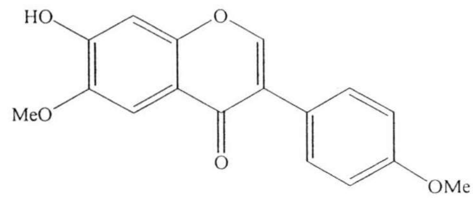


Sulfuretin

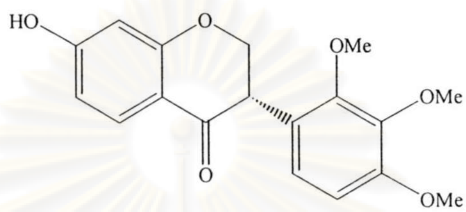
Figure 1.7 Structures of some chemical constituents of *Dalbergia genus.* (continues)



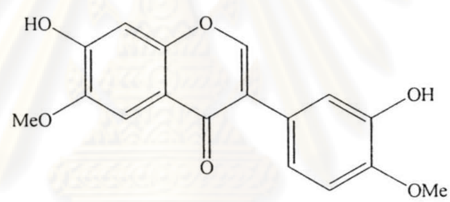
Formononetin



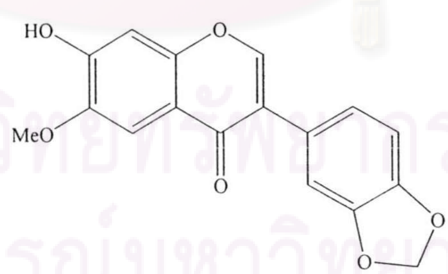
Castanin



3'-O-methylviolanone

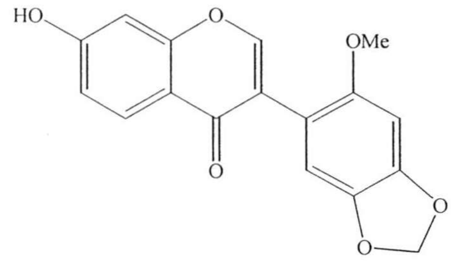


Odoratin

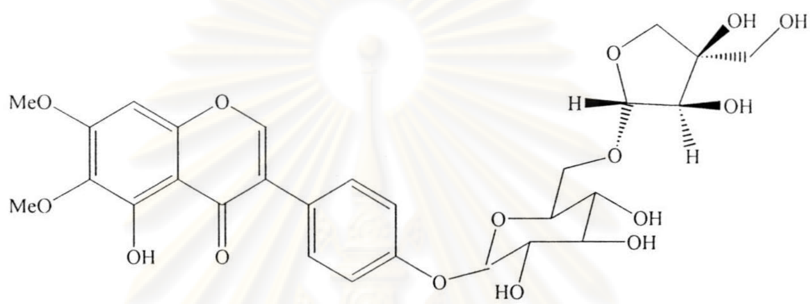


Fujikinetin

Figure 1.7 Structures of some chemical constituents of *Dalbergia genus.* (continues)



Cuneatin



5-hydroxy-6,7-dimethoxy-4'-O-(6-O-D-apio-β-D-furanosyl-β-D-glucopyranosyl)isoflavone

(3*R*)-4'-methoxy-2',3,7-trihydroxyisoflavanone

Figure 1.7 Structures of some chemical constituents of *Dalbergia genus.* (continues)