CHAPTER I

Introduction

Coumarins, a large group of natural occurring compounds possessing a 2*H*-1-benzopyran-2-one, are commonly found from plants in the family Rutaceae and Umbelliferae and sporadically reported in Leguminosae, Moraceae and Miliaceae (Gray and Waterman, 1987). Coumarins have been investigated and evaluated for biological activity and some of them have been used in therapeutic purpose. For example, warfarin is used as antithrombotic agent for atherosclerosis, acute myocardial infarction, etc.

Reveiro and colleague (2004) evaluated the trioxygenated coumarins, 5-methoxy-6,7-methylenedioxycoumarin and 5-(3-methyl-2-butenyloxy)-6,7-methylenedioxy-coumarin, from *Ptercaulon polystachyum* for antiproliferation and induction of cell differentiation of U-937 cell, a human myelocytic leukemia cell lines. They found that two trioxygenated coumarins successively inhibited proliferation of U-937 cell without cytotoxic effect. In addition, two trioxygenated coumarins were able to induce cell differentiation which monitored by two important parameters: (*i*) mobilization of intracellular Ca^{2+} from endoplasmic recticulum to cytoplasm that necessary for cell movement of monocytic cell and (*ii*) oxidative burst capacity that is the marker of monocytic differentiation. The effects of trioxygenated coumarins on U-937 cell were performed and compared with two positive controls, dibutyryl cAMP and all-trans-retinoic acid, which were used as therapeutic agents for myelocytic leukemia (Jiménez-Lara *et al*, 2004). The 5-methoxy-6,7-methylenedioxycoumarin was more effective than -(3-methyl-2-butenyloxy)-6,7-methylenedioxycoumarin in inhibition of cell proliferation and differentiation.

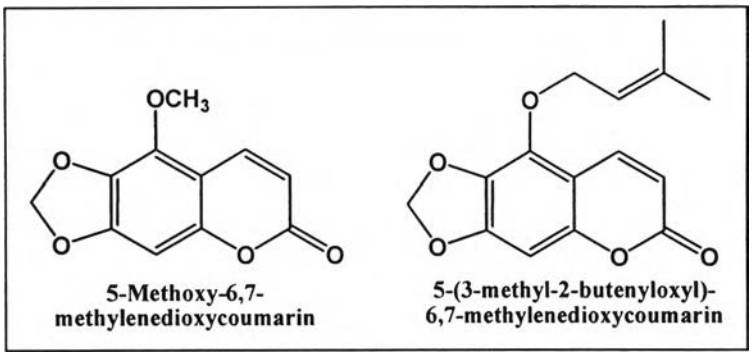

Figure 1.1 Structure of trioxygenated coumarins from *Pterocaulon polystachyum*.

Platelet aggregation is the process that platelet interacts with one another to form hemostatic plug or thrombus. Under normal condition, platelets freely circulate in the blood stream and do not adhere to each other. Following blood vessel wall has been injured, platelets adhere to the exposed subendothelium, become activated and aggregated (Gachet, 2005). The platelet aggregation can be induced or initiated by several agonists such as arachidonic acid, adenosine diphosphate (ADP), collagen, thrombin, epinephrine, etc. Inappropriate platelet activations and subsequent thrombus formation are important in the clinical complication of arterial atherosclerosis and thrombosis (McNicol and Israels, 2003).

At the site of a vulnerable coronary plague, platelets attach to the vessel wall and initiate thrombotic occlusion of coronary vessel leading to myocardial ischemia and infarction. Besides thrombus formation in the epicardial arteries, platelet microembolization and accumulation within the microcirculation of the ischemic myocardium play a major role in microcirculation arrest, thus promoting tissue damage and leading to myocardial infarction (Gawaz, 2004). The antiplatelet drugs interfere with certain steps in the activation process by selectively blocking key platelet enzyme or receptors reducing the risk of arterial thrombosis (Patrono *et al*, 2005). For this reason, coumarins from medicinal plants have been evaluated for antiplatelet aggregation.

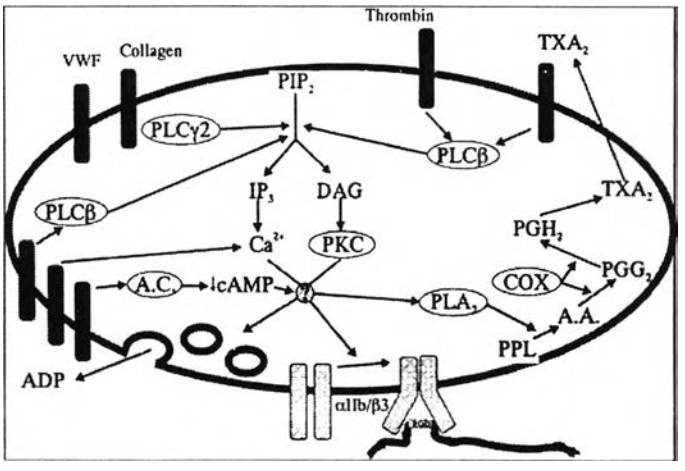

Figure 1.2 Mechanisms of agonist-induced platelet activation and site of action of inhibitors. Platelet is activated by collagen, thrombin, and ADP including intracellular signaling pathways leading to TXA2 production, ADP release from dense granules and conformational changes in the αIIb/ß3 integrin allowing fibrinogen binding.

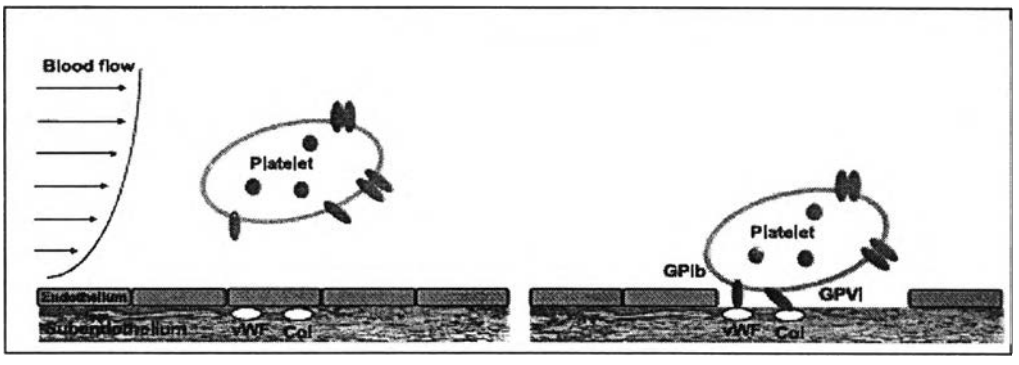

Figure 1.3 Platelet-dependent formation of thrombus at an atherosclerotic plaque. Platelets do not adhere to the intact endothelial monolayer under physiological conditions. At site of vascular lesions, extracellular matrix proteins like von Willebrand factor (vWF) and collagen (Col) are exposed to the blood. Via the membrane adhesion receptors GPIba and GPVI platelets loosely adhere to the subendothelium.

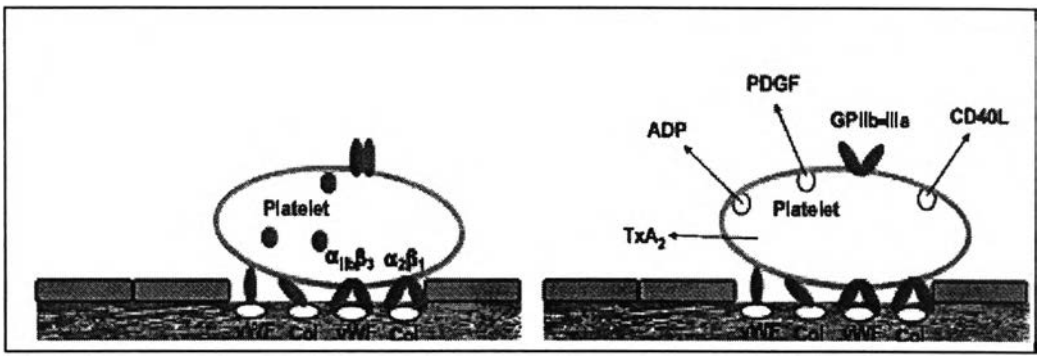

Figure 1.4 This initial adhesion results in platelet activation and ''opening'' of the integrin receptors $\alpha_{IIb}\beta_3$ (fibrinogen receptor) and $\alpha_2\beta_1$ (collagen receptor). Interaction of $\alpha_{IIb}\beta_3$ and $\alpha_2\beta_1$ with extracellular matrix proteins stabilizes platelet adhesion (firm adhesion). Subsequently, platelets spread and degranulate and thereby recruit additional platelets to the already adherent ones.

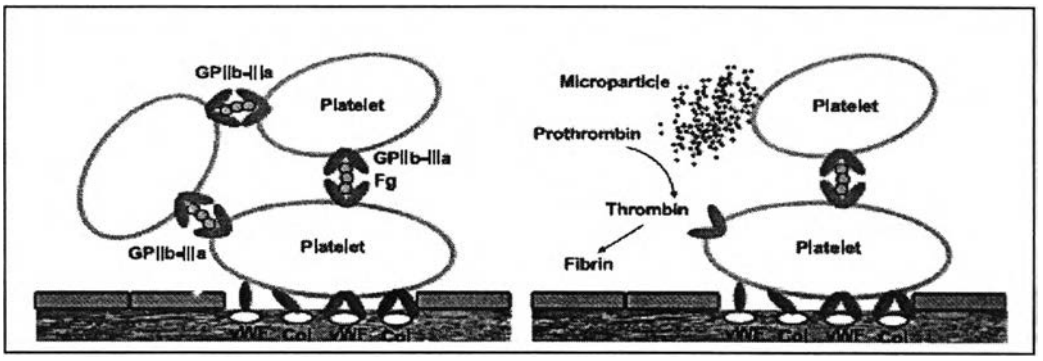

Figure 1.5 Platelets form microaggregates via fibrinogen ''bridges'' between two $\alpha_{IIb}\beta_3$ receptors. Formation of microparticles around the platelet aggregates catalyses thrombin generation and thus fibrin formation that stabilizes the platelet thrombus.

Chen and colleague (1995) isolated coumarins from *Zanthozylum schinifolium* and evaluated for anti-platelet aggregation. The active components were identified to be schinicoumarin, acetoxyaurapten, schininallylol, aurapten, collinin and (-)-acetoxycollinin. Tsai *et al* (1998) reported that isopimpinellin, a dimethoxyfuranocoumarin, from *Toddalia asiatica* completely inhibited arachidonic acid-induced platelet aggregation. Moreover, Wu *et al* (2001) investigated chemical constituents and evaluated for anti-platelet aggregation of *Artemisia capillaris*. The results show that scoperone and scopoletin inhibited both arachidonic acid- and collagen-

induced platelet aggregation whereas artermicapin B and aesculetin inhibited only arachidonic acid-induced platelet aggregation.

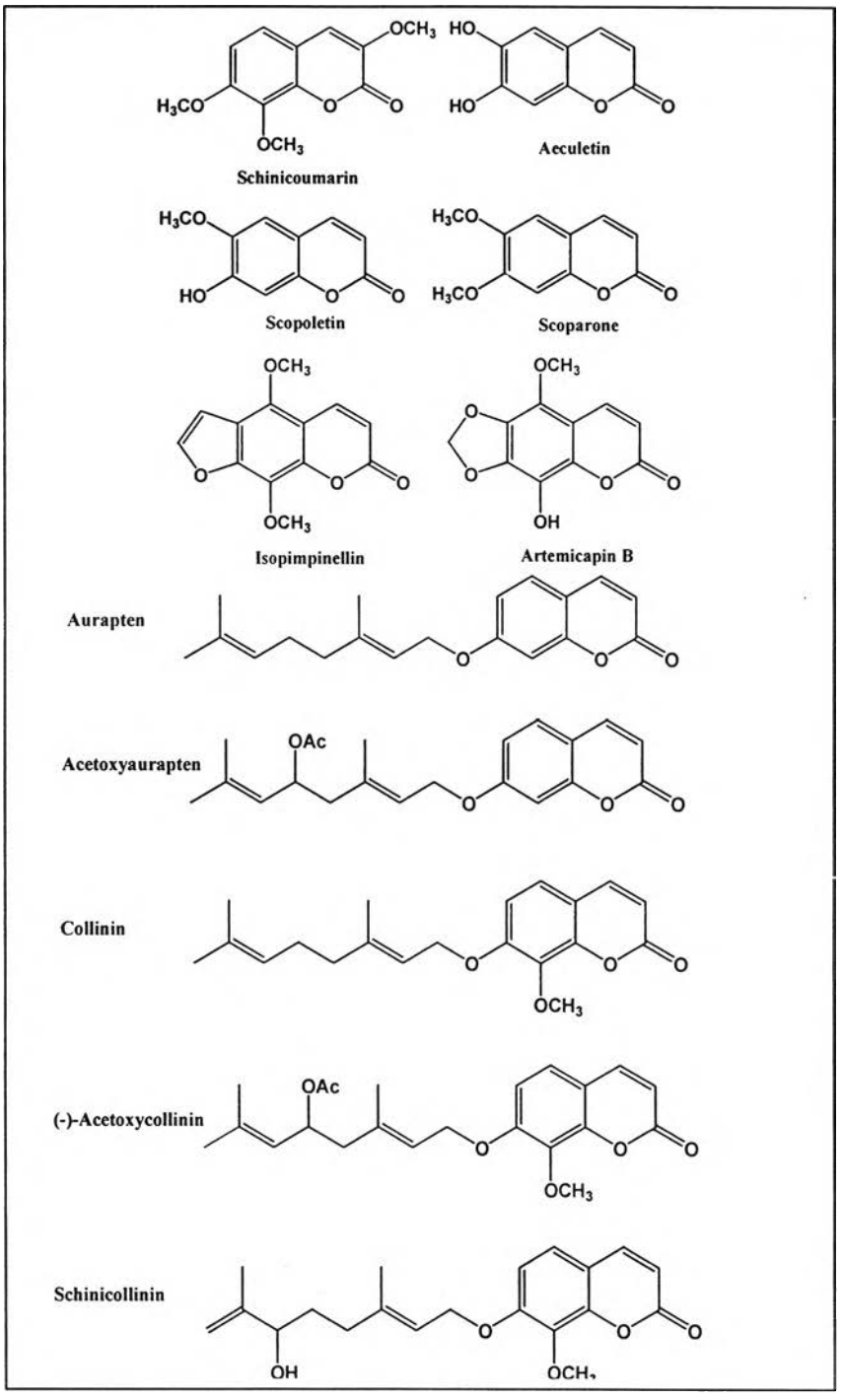

Figure 1.6 Antiplatelet aggregation coumarins from *Zanthozylum schinifolium*, *Taddalia asiatica* and *Artemisia capillaris*.

According to biological activities of coumarins, the author was interested in investigation of phytochemical constituents of plant from the family Rutaceae. *Feroniella lucida* (Ma-Sang) is classified in the subtribe Balsamocitrinae, tribe Citrinae, subfamily

Aurantioideaem (Dreyer *et al*, 1972). Although the family *Feroniella* comprises 3 species, *F. lucida* is the only species found in Thailand. Other related genera include *Feronia* and *Aegle*. To date, there is no phytochemical report for this species.

Phytochemical investigations of *Feronia* and *Aegle* have been reported since 1970s. Talapatra and colleague (1973) isolated coumarins from the roots bark of *Feronia limonia* (Syn. *F. elephantum*). They were consisted of aurapten, bergapten, isopimpinellin, 6-methoxy-7-geranyloxycoumarin and marmesin. Subsequent investigations resulted in the isolation of psoralen and bergapten from leaves (Gupta *et al*, 1979) and isopimpinellin, 7-demethylsuberosin, and xanthotoxin from stem bark (Rahman and Gray, 2002).

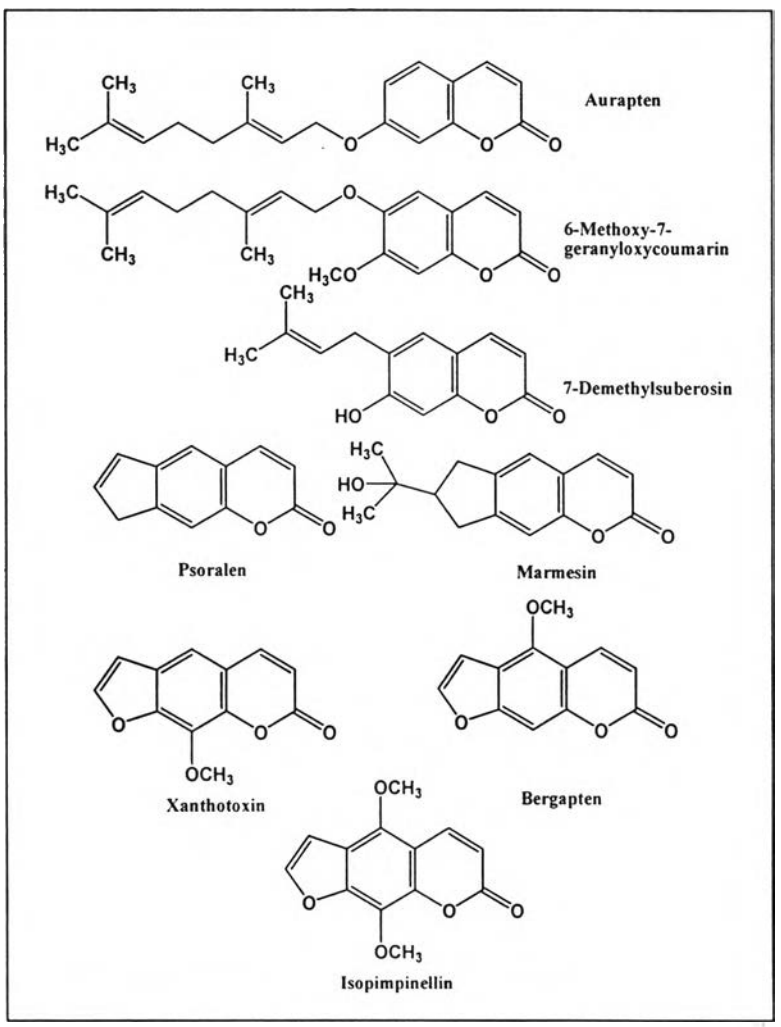

Figure 1.7 Coumarins from *Feronia limonia*.

Shoeb et al (1973) isolated eight coumarins, including xanthotoxin, psoralen, 6, 7-dimethoxycoumarin, scopoletin, umbelliferone, skimmin, marmesin and marmin from the roots of *Aegle marmelos*. Basu and Sen (1974) isolated decursinol from the root bark of

A. marmelos. Finally, Chatterjee and colleague (1978) isolated aegelinol, anhydromarmesin and marmin from the roots and stem bark of *A. marmelos*.

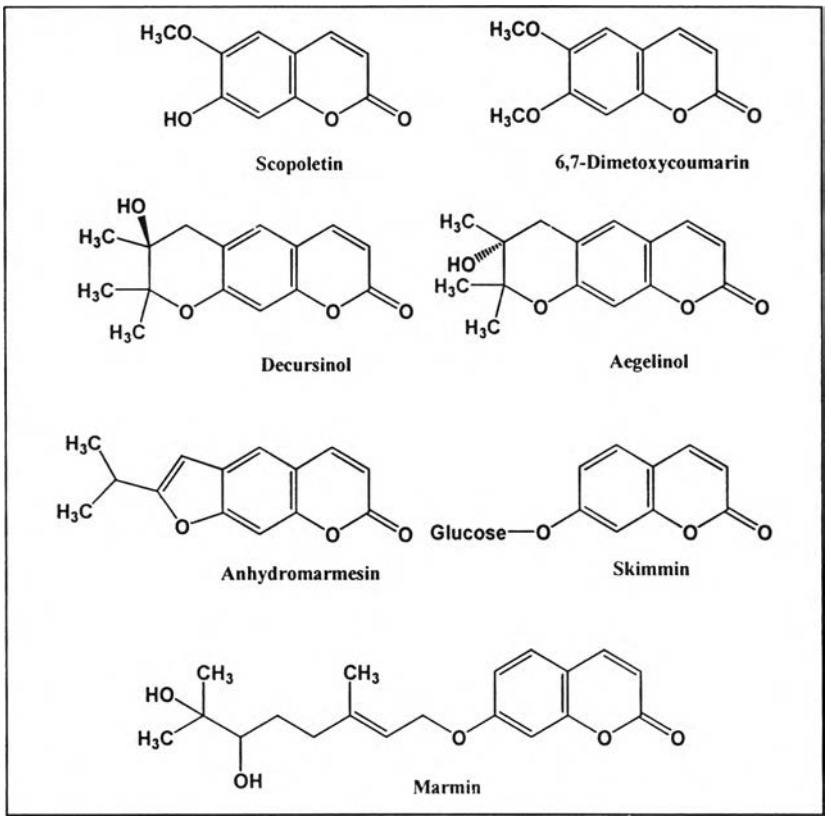

Figure 1.8 Coumarins from *Aegle marmelos*.

From the attractively biological activities of coumarins from the plants in the family Rutaceae, *F. lucida* (Ma Sang) was selected for further investigation of phytochemical constituents and their effects on platelet aggregation. The goals of this research can be summarized as following:

1. To extract and isolate the comarins from the roots of *F. lucida*
2. To elucidate the structures of the isolated coumarins
3. To determine the antiplatelet activities of the isolated coumarins