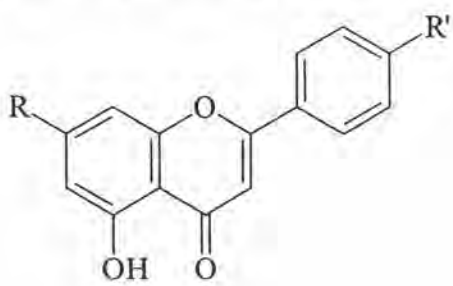
CHAPTER I
INTRODUCTION

Every race in the world has practised the ancient art of medicinal plants. The earliest record of the medicinal usage of plants, the Chinese pharmacopoeia, was published in approximately 3000 BC. Herbal medicine has also been an integral part of Thai culture for a long period of time, not only used as drugs but also applied as food and constituent in cosmetics. Although, the interest in medicinal plants has sometimes declined, studies of natural products in recent years have been gaining much attention because of their biological activities such as anticancer, anti-HIV, antifungus etc. The search for bioactive components from plants as a source of lead compounds in drug development, is therefore an important endeavor in phytochemistry. Several isolated compounds were identified as novel compounds and a number of known compounds were reisolated and proved to possess biological activities. However, the isolation of biologically active organic compounds in several plants has some limitations. In some case, pure compounds separated from plants were not enough for further investigation and/or commercial utilization. Thus, syntheses of the compounds which are of structures based on biologically active compounds isolated from plants are worth concern. Various reports have cited that the synthetic compounds whose structures were based on natural lead compounds showed higher activity than the original substances. For instance, a natural flavone, Chrysin **(I)**, exhibited IC_{50} = 5 μg/mL against T cell Protein-tyrosine kinases ($p56^{lck}$), whereas the synthetic flavone **(II)** showed IC_{50} = 4 μg/mL.[I] This indicated that the synthesis of natural lead compounds and their derivatives are essential for achieving more interesting biologically active structures that would be available to use efficiently as therapeutic drugs.

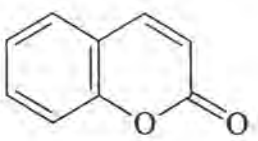


(I) (R = OH, R' = H)

(II) (R = H, R' = OH)

1.1 Literature Reviews

Among important natural compounds found in a number of plant sources including the sweet clover and the tonka bean, coumarin **(III)** and its derivatives, was first reported in 1820.[2] The carbocyclic ring of the nucleus is usually oxygenated at C-7, less frequently at C-5, C-6 and C-8. Examples are umbelliferone **(IV)**, which occurs in many different plants, and skimmin, its *β*–glycoside, which is found in *Skimmia japonica* Thumb. Derivatives of umbelliferone are of special interest as sun-burn preservatives on account of their wide spectrum of ultraviolet absorption. Other substituted coumarins peculiar to the coumarin family are found in both 4-aryl and 4-alkyl derivatives represented by dalbergin **(V)** (from the heartwood of *Dalbergia sissoo*) and mammien **(VI)** (from the seeds of *Mammea americana* L.) which has attracted interest on account of its insecticidal properties.[3] The recent report indicated that (+)-calanolide A **(VII)** and (-)-calanolide B **(VIII)**, isolated from the leaves of *Calophyllum lanigerum* provided complete protection against the *in vitro* replication and cythopathicity of HIV-1 in lymphoblastic cells.[4] Another important group of naturally occurring coumarins is the furano coumarins, which has interesting physiological properties, represented here by psoralen **(IX)**, xanthotoxin **(X)** and angelicin **(XI)**. For example, psoralen has a photosensitizing action on the human skin which has been exploited in medicine.



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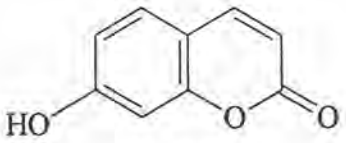

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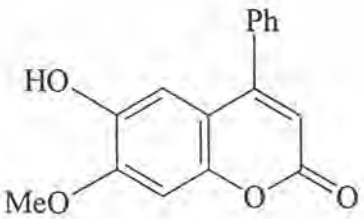

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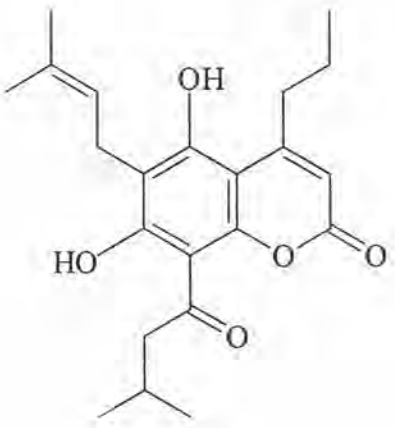

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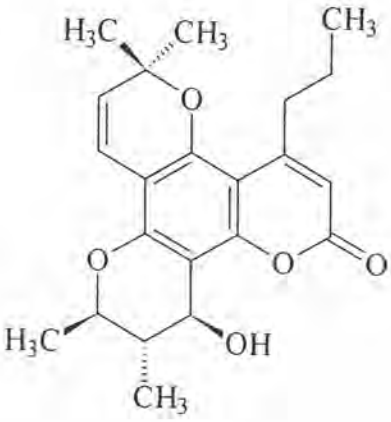

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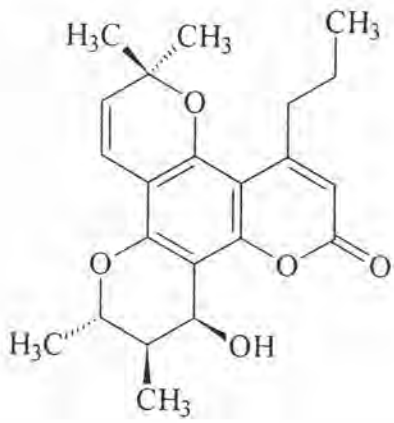

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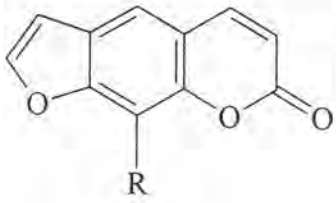
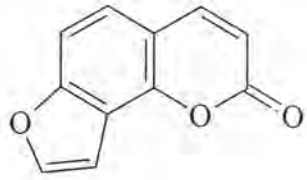


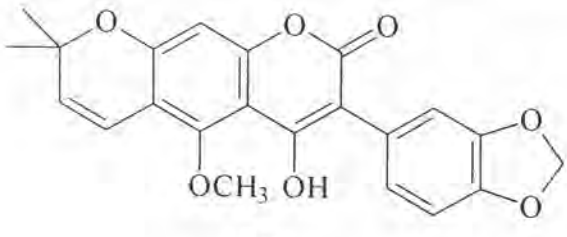
(IX) R = H

(X) R = OMe



(XI)

4-Hydroxycoumarins are a small group in part of natural coumarins. Some members bearing the substituent at 3-position exhibited strongly biological activity especially anticoagulant effects and antibiotic drugs. 3-Substituted-4-hydroxycoumarins were found from many parts of plants. From the family Leguminosae in the genus *Derris*,[2] and the genus *Millettia*,[5] the 3-aryl-4-hydroxy coumarins (a class of isoflavonoid natural products) have been isolated. For instance, robustin **(XII)** from roots of *D. robusta*,[6] scandenin **(XIII)** from roots of *D. scandens*[7] and robustic acid **(XIV)** from seeds of *M. thonningii*.[8] 3-Alkyl-4-hydroxycoumarins were extracted from the family Compositae; for example, piloselloidal **(XV)** and piloselloidan **(XVI)** from *Jungia herzogiana* Beauvard ex Koster, coumarin **(XVII)** and **(XVIII)** from *Bothriocline laxa*, *etc.*[2]



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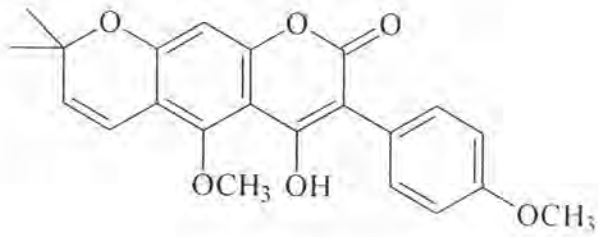
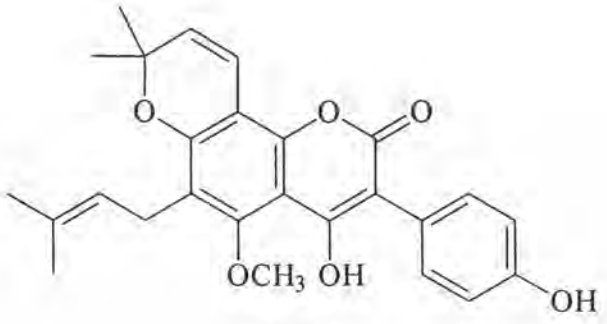
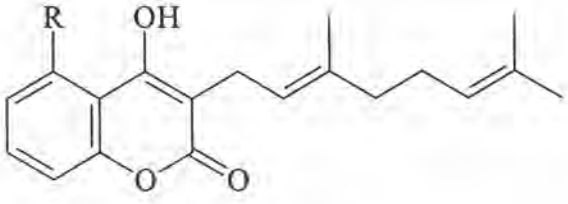


(XIII)

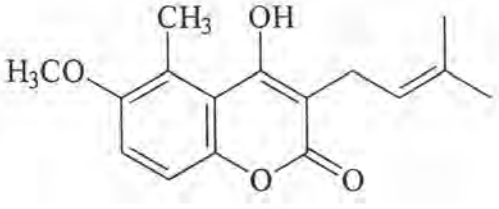


(XIV)



(XV) R = CHO

(XVI) R = CH_3



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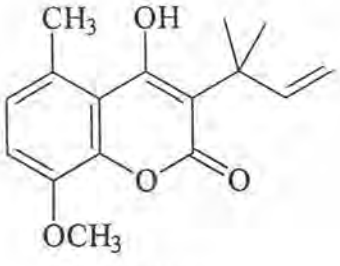
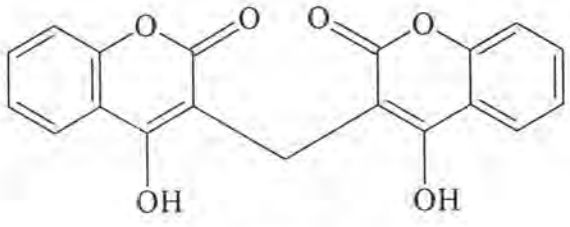


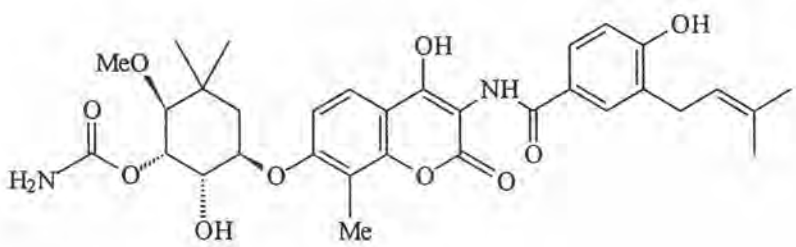
(XVIII)

For a 3,3′-methylene*bis*-4-hydroxy coumarin[9] (Dicoumarol) **(XIX)**, which is the best known for its anticoagulant effect on blood, was present in the ingestion of mouldy sweet clover (*Melilotus alba* or *M. officialis*) hay and caused the sweet clover disease in cattle. This condition, which is brought on by eating spoiled sweet clover, produces serious and often fatal haemorrhages in affected beasts.



(XIX)

There are a number of biologically interesting compounds containing 4-hydroxycoumarin residue. Novobiocin **(XX)** and coumermycin **(XXI)** are the ones which have been long recognized, and has been more intensively studied because of their pharmacological action as antibiotic drugs.



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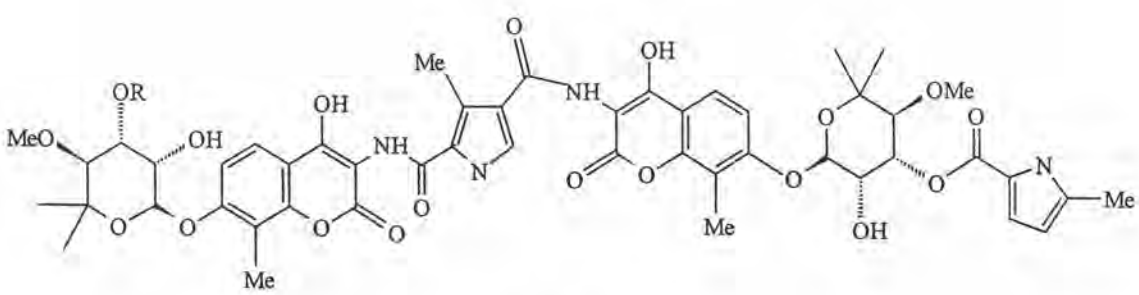


(XXI)

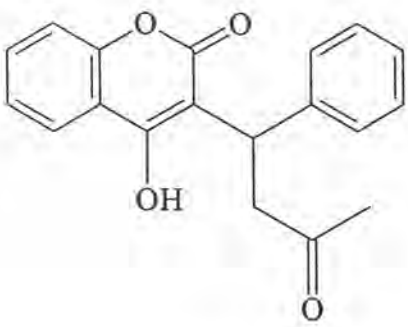
1.1.1 Synthesis of 3-Substituted-4-hydroxycoumarins

There are numerous methods applied for the synthesis of 3-substituted-4-hydroxycoumarins because of their biological importance and considerable therapeutic potential especially anticoagulant action. The early reported routes to synthesize these compounds are, for instance, treating methyl acetylsalicylate with sodium metal at high temperature to obtain the ring closure *via* an intramolecular Claisen condensation,[10] the condensation of substituted phenols with substituted malonate esters in the presence of zinc chloride and phosphorus oxychloride[11] and the condensation of a single carbon atom in the form of diethyl carbonate or ethyl chloroformate with deoxybenzoin in the presence of an alkali metal.[12] Dicoumarol, a class of 3-substituted-4-hydroxycoumarin which contained another group of 4-hydroxycoumarin substituted at 3-position, was synthesized by treatment of two

equimolar of 4-hydroxycoumarin with aldehyde derivatives.[13] The intensive investigation of these compounds still goes on, recent reports have shown that treatment of dicoumarol derivatives with sodium cyanoborohydride yielding 3-alkyl-4-hydroxycoumarins by cleaving a 4-hydroxycoumarin moeity.[14] 3-Aryl-4-hydroxy coumarins were synthesized by arylation of 4-hydroxycoumarin using aryllead triacetate. This methodology was used to exploit for the total synthesis of robustin and robustic acid.[5,15] In the last few years, the preparation of regiospecific 3-substituted-4-hydroxycoumarin was succeeded *via* arylketone intermediates in three steps.[16]

1.1.2 Biological Activity of 3-Substituted-4-hydroxycoumarins

Most 3-substituted-4-hydroxycoumarins were reported to have interesting biological activity in addition to their anticoagulant and antibiotic effects. For example, warfarin **(XXII)** as its sodium salt, can be used in the treatment of cardiovascular disease. The high activity as an anticoagulant caused it to be originally proposed for rodent control. It became the world's most useful rodenticide.[9] Diphenacoum **(XXIII)** and brodifacoum **(XXIV)**, showed outstanding activity against both warfarin-sensitive and warfarin-resistant rats, and are now also used commercially as rodenticides.[17] To aid the development of anti-HIV drugs that has attracted much attention recently, dicoumarols were used *in vitro* for the design of non-peptide HIV-1 enzyme inhibitors such as protease and integrase inhibitors.[18-20] Besides, some 4-hydroxycoumarins and dicoumarols were synthesized for SAR study on insect antifeedant against *Galleria mellonella* Linn. and weed growth inhibition against *Mimosa pigra* Linn. It was observed that when hydroxy and methoxy substituents were present on a benzylidene ring of dicoumarols, those compounds displayed high activity.[21] Some of them were claimed to have promise for the development of new agrochemicals.



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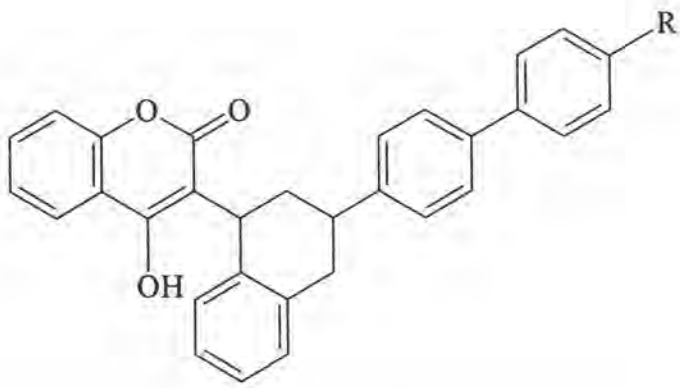

(XXIII) R = H

(XXIV) R = Br

1.2 Goal of Research

Although 3-substituted-4-hydroxycoumarins, which are part of natural lead compounds, were reported to have broad spectrum activities, the investigation of some biological activities of these compounds has still been of interest. Thus, it must be emphasized that the synthesis of 3-substituted-4-hydroxycoumarins would be important for understanding the chemistry of these compounds and gaining the relationship between structure and activities for further development as new drugs. In summary, the aim of this investigation is:

1. To synthesize 3-substituted-4-hydroxycoumarins and dicoumarols
2. To study the biological activity of 3-substituted-4-hydroxycoumarins and dicoumarols: cytotoxicity against *Artemia salina* Linn. (brine shrimp), antibacterial activity and antiviral against HSV-1 and HSV-2.