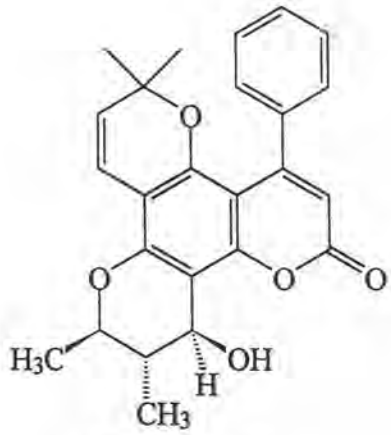
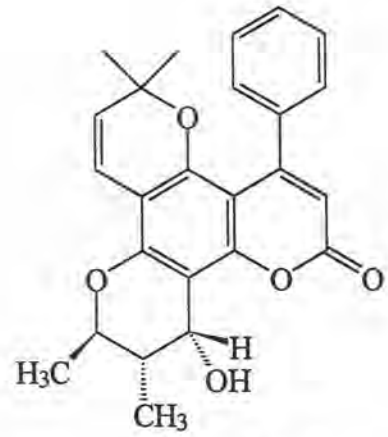
CHAPTER 1

INTRODUCTION

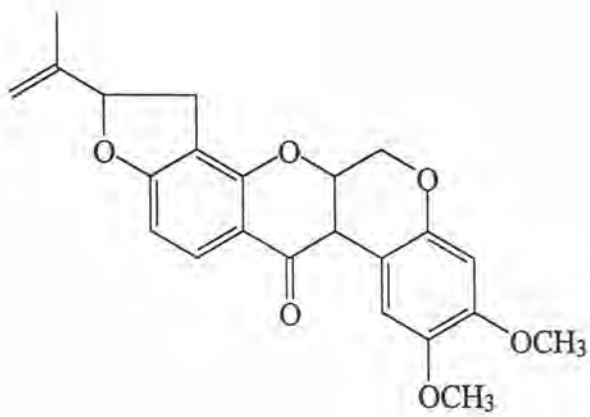
Naturally occurring organic chemical compounds (natural products) have always fascinated chemists. Interesting and intriguing chemistry is involved in their *in vivo* production and in their laboratory utilization, and their importance as structural materials and biologically active molecules (substrates for life processes, toxins, hormones, drugs, *etc.*) is of unparalleled importance. A knowledge of their biological properties, not only for the discovery of new therapeutic agents, but also because such information may be of value in disclosing new sources of economically important materials. In addition chemical substances from natural sources can be easily decomposed by biological pathway and no toxic waste remained to make pollution. There are many biological active compounds separated from plants and other natural sources, for example inophyllum B and inophyllum P from leaves of *Calophyllum inophyllum*. They act as HIV reverse transcriptase (HIV RT) inhibitors,[1] cause of AIDS symptom. Nicotine, a highly toxic alkaloid, from leaves of *Nicotiana tabacum* is used to get rid of aphids.[2] Rotenone, found in roots of some plants in Leguminosae particularly in *Derris* genus: *D. elliptica* and *D. malaccensis*, was used to kill insect, worm and fish, and was reported to have toxicity effect on cold-blooded animals, but not on warm-blooded animals.[3] Azadirachtin, a constituent of neem oil, revealed high antifeedant activity against many kinds of insects.[4]



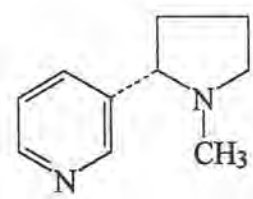
Inophyllum B



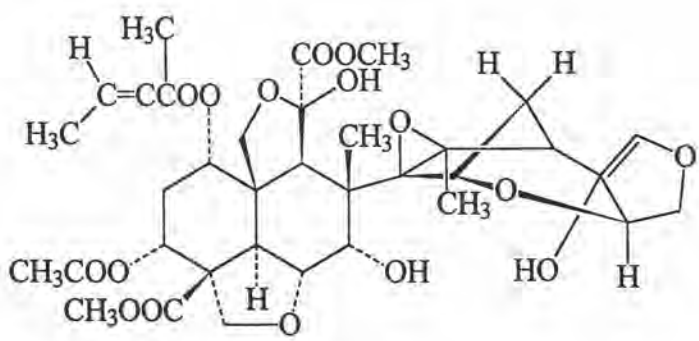
Inophyllum P



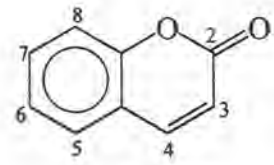
Rotenone



Nicotine



Azadirachtin



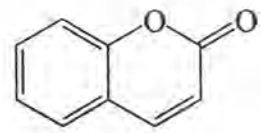
Coumarin

(2*H*-1-Benzopyran-2-one)

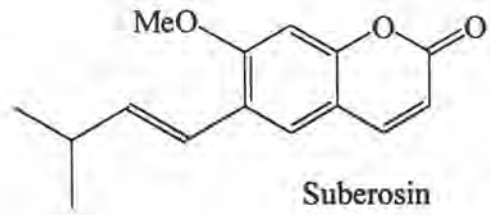
2*H*-1-benzopyran-2-one, commonly called coumarin, is benzo-fused analogue of pyrone ring. A coumarin, a kind of biologically active natural molecules, is the internal lactone of 2-hydroxy-*cis*-cinnamic acid, and the ring is opened with alkalis, yielding salts of coumaric acid.[5] The name coumarin originates from a Caribbean word '*coumarou*' for the tonka tree, which was known botanically at one time as *Coumarouna odorata* Aubl and the isolation of coumarin was first reported in 1820.[6] A considerable number of hydroxy- and methoxy-coumarins and their glycosides, have been isolated from plants, was known to have interesting pharmacology and toxicology. The notable physiological effects of coumarins are the acute hepatotoxicity and carcinogenicity of certain aflatoxins,[7] the anticoagulant action of dicoumarol[8] and the antibiotic activity of novobiocin and coumermycin A_1.[6]

The natural coumarins can be classified according to whether the structures were of one of the following types:

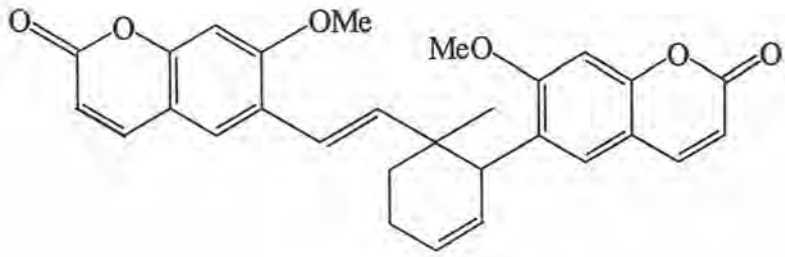
1. *simple*, which has implied coumarin (I) and hydroxylated, alkoxylated and alkylated derivatives and their glycosides: suberosin and thamnosin
2. *furanocoumarins*, of the linear (psoralen) or angular (angelicin) types with substituents at one or both of the remaining benzenoid positions and including dihydrofuranocoumarins: xanthotoxin, columbianetin and lomatin
3. *pyranocoumarins*, the six-membered ring analogues of coumarin (II): xanthyletin and suberenon
4. *coumarins substituted in the pyrone ring*, such as 4-hydroxycoumarins, 3-phenylcoumarins and 3,4-benzocoumarins: lonchocarpenin and isohalfordin



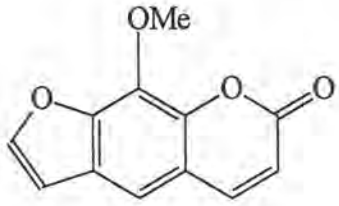
Coumarin (I)



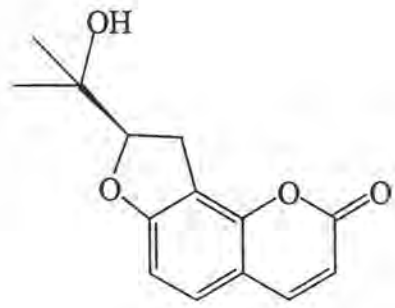
Suberosin



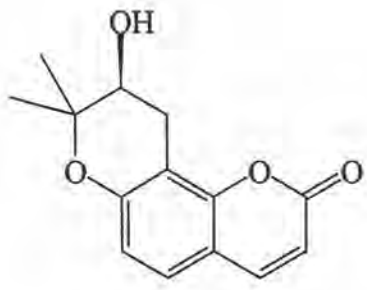
Thamnosin



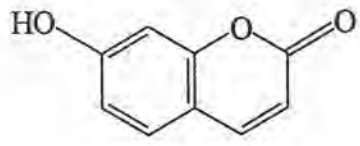
Xanthotoxin



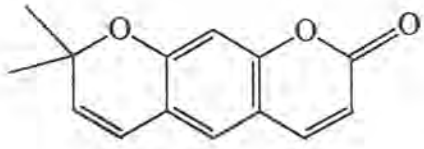
Columbianetin



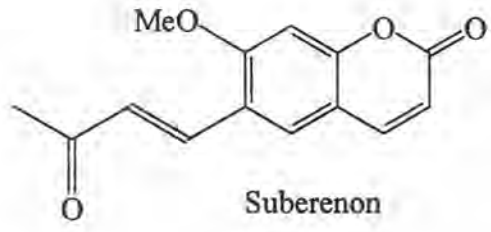
Lomatin



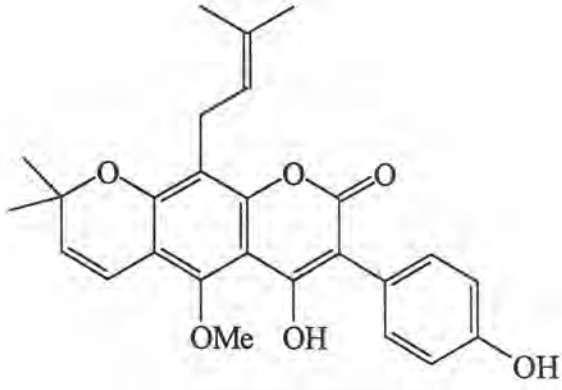
Coumarin (II)



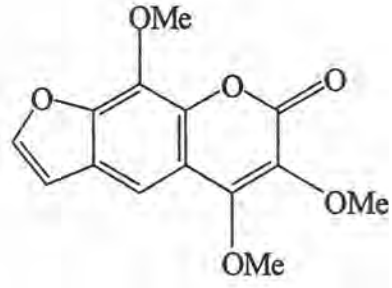
Xanthyletin



Suberenon

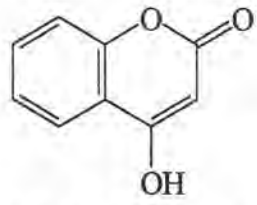


Lonchocarpenin



Isohalffordin

1.1 Literature Review



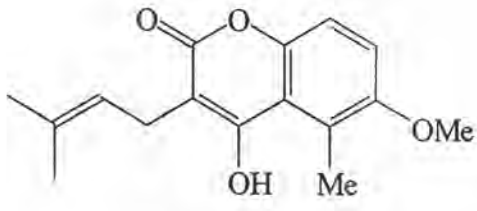
4-Hydroxycoumarin

The structure of 4-hydroxycoumarin is shown above. Although 4-hydroxycoumarin (2*H*-4-hydroxybenzopyran-2-one) is a small group in part of coumarins substituted in the pyrone ring, widespread interest in this group has been continuously recognized because of its anticoagulant effects. This property has been exploited in pharmaceuticals which are used in the treatment of cardiovascular disease.[6,8] For example, warfarin, originally developed as a powerful rodenticide that kills through its haemorrhagic properties, is useful as a anticoagulant drug and as the most valuable drug in the treatment of bronchial asthma and *etc.*[8b,8c,9]

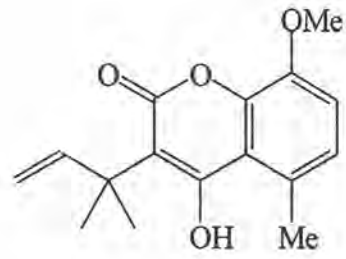
1.1.1 Sources of 4-Hydroxycoumarins

- Natural Source

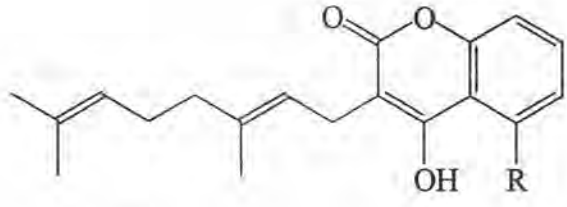
There are not less than 20 compounds found as natural 4-hydroxycoumarins. They were isolated from many parts of plants in the family "Compositae"; for instance, coumarins (III) and (IV) from *Bothriocline laxa*, piloselloidal, piloselloidan, and piloselloidol isovalerate from *Jungia herzogiana* Beauvard ex Koster and "Umbelliferae"; ammoresinol from *Dorema ammoniacum* D. Don[6] *etc.* Most natural 4-hydroxycoumarins have been assigned by trivial names which were generally derived from the family or species names, or a combination of both, of the plant which they were found. For example some natural 4-hydroxycoumarins were found from plants in the family "Leguminosae", genus "*Derris*"; scandenin from roots of *D. scandens*,[10] robustin from seeds of *D. robusta*,[11] derusnin and robustic acid from roots of *D. robusta*.[10d,11,12]



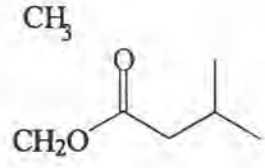
Coumarin (III)



Coumarin (IV)

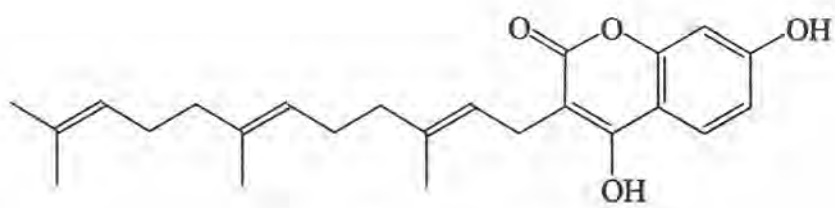


R = CHO — Piloselloidal

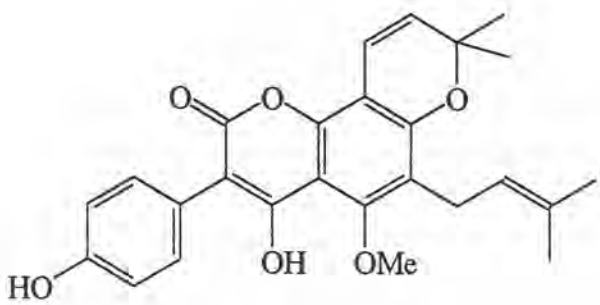


Piloselloidant

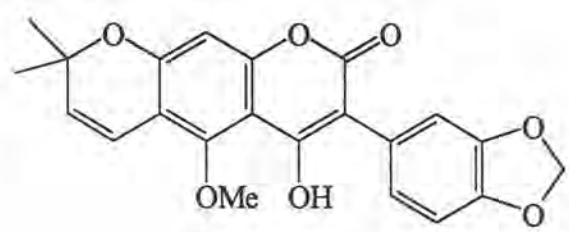
Piloselloidol isovalerate



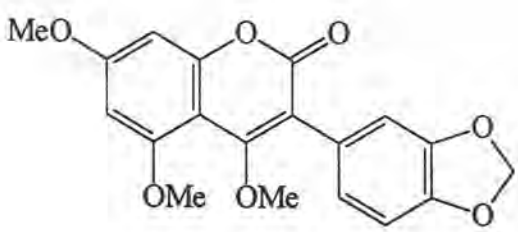
Ammoresinol



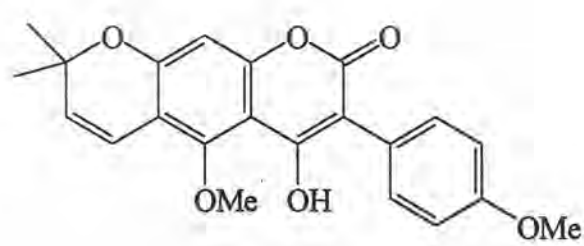
Scandenin



Robustin



Derrusnin



Robustic acid

- From Synthesis

Because of attractive bioactivities of 4-hydroxycoumarins, the synthesis of this class of compounds are continuously developed. Generally, the methodology for the synthesis of 4-hydroxycoumarins can be divided into 4 main categories. The first one is the principal known method, which is used in the past to provide considerable yield of the desired product with general processes and available reagents. These methods are: condensation of phenols with malonic acids in the presence of zinc chloride and

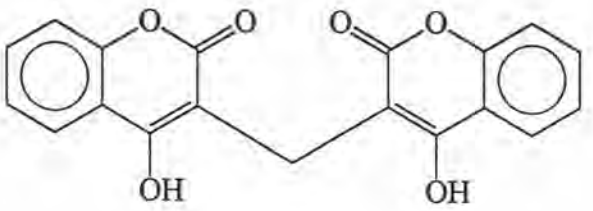
phosphorus oxychloride (method I),[8a,8b,13] condensation of phenols with malonyl chlorides in the presence of aluminum chloride (method II)[14] and condensation of 2-hydroxyacetophenones with diethyl carbonate in the presence of an alkali metal (method III).[8a,15] Other methodologies reported involved complicated experiments or using noncommercial reagents or giving low yield of products. The noticeable example is the condensation of phenols with carbon suboxide and aluminum chloride (method IV).[16] Another methodology was reported employing a one-pot reaction providing high to highest yield of product is method V.[17] This method included C-carbonylation of 2-hydroxyacetophenone with carbon monoxide in the presence of sulfur and base or selenium without base. The last group utilized specific methods for synthesizing 4-hydroxycoumarin derivatives that cannot be easily prepared (or gave little yields). For instance, fluorinated 4-hydroxycoumarins were prepared from condensation of fluorinated phenols with aluminum chloride, followed by decarboxylation and deacetylation with 90% sulfuric acid (method VI).[9]

For dicoumarols, the successfully simple process involved condensation of 4-hydroxycoumarin with interested aldehyde (method VII).[8a,8c,8f,18]

1.1.2 Biological Action of 4-Hydroxycoumarins

4-Hydroxycoumarins possess a wide range of biological activities, predominantly anticoagulant and HIV protease inhibition.[8,19] For example, coumadin (sodium warfarin) is the most widely prescribed anti-thrombotic in North America.[9] There are different actions of 4-hydroxycoumarin derivatives. The biological action of 4-hydroxycoumarins could be divided into 3 parts from different main structure-bioactivities as follows:

- Dicoumarol



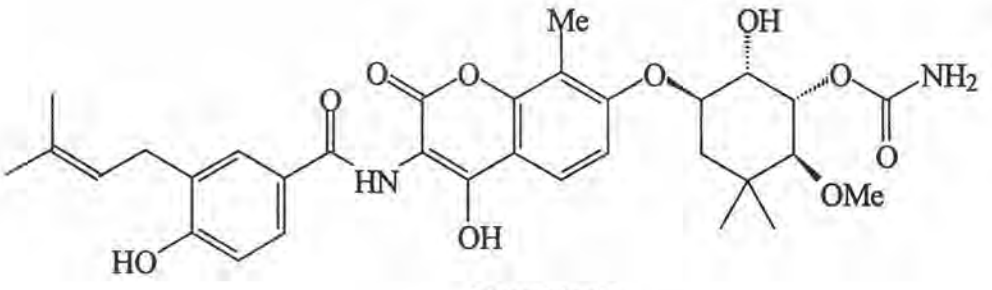
In 1924, a haemorrhagic disease was first studied in Canadian cattle due to the ingestion of moldy sweet clovers (*Melilotus alba* or *M. officinalis*) hay and dicoumarol was stemmed from them.[6,8a] A dicoumarol is provided as the leading structure for the family of oral anticoagulant. This compound is usefully introduced into medicine to lower blood coagulability in, for instance the treatment and prophylaxis of thromboembolic disorders in veins and, to a lesser extent, arteries by production abnormal prothrombins and as an antagonist of vitamin K.[6]

Moreover dicoumarol has been found in many investigations to exert a number of biological effects unrelated to blood coagulation, enzyme inhibition, antimicrobial action and *etc.*[6,8b] Important among these is an uncoupling agent as a potent uncoupler of NAD-linked oxidative phosphorylation.[6,20]

- Coumarin Antibiotics

Among 4-hydroxycoumarins categorized in this group: novobiocin and cumermycin A_1 have been most widely studied.

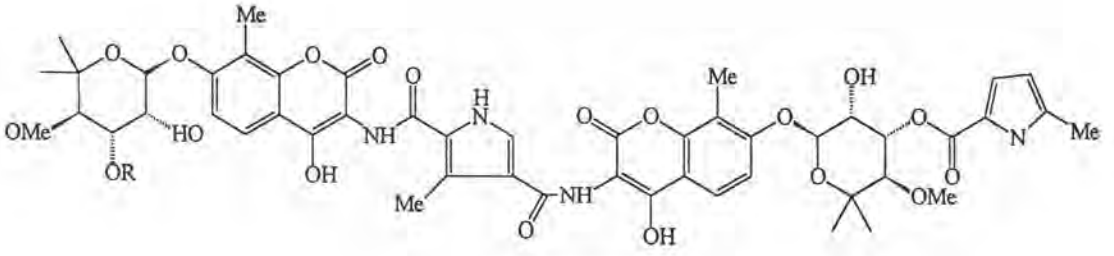
Novobiocin



Novobiocin

Novobiocin has been elucidated for the mechanism of action as antibiotic substance as follows: novobiocin binds to plasma proteins. It binds to human and bovine serum albumins; after incubation for 30 minutes at 37°C, novobiocin was almost completely bound, albumin has an enormous binding capacity for many drugs. It has relationship to magnesium ion. Magnesium ion reverses the activity of novobiocin against gram-negative bacteria, that it induced some effects also engendered by magnesium deficiency, and that it inhibited magnesium-requiring processes.[21] Furthermore, it has effects on enzymes, on metabolism and on nucleic acid information.[21]

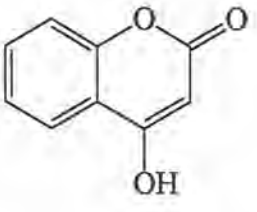
Coumermycin A_1



Coumermycin A_1

The newer antibiotic coumermycin A_1 has also been studied on its mode of action, which is similar to that of novobiocin.[22]

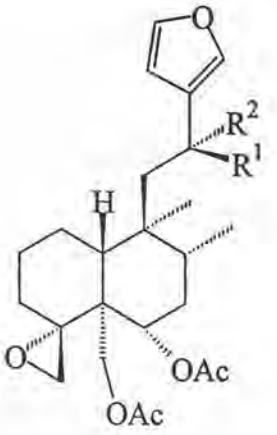
- 4-Hydroxycoumarin



4-Hydroxycoumarin inhibits oxygen uptake in young rice plants and has a little anticoagulant activity.[6]

1.2 Critical Review and Reason for Undertaking this Study

Many papers have cited that sometimes the natural product derivatives which have related structures, possessed better biological action and more interesting activities than parent molecules. For example in 1994, C. E. Tonn and coworkers reported the separation of 14 diterpenoids from plants in genus *Baccharis*, *Teucrium* and *Salvia*. Some of them have different antifeedant activity against *Tenebrio molitor*.



I : R^1 = OAc, R^2 = H

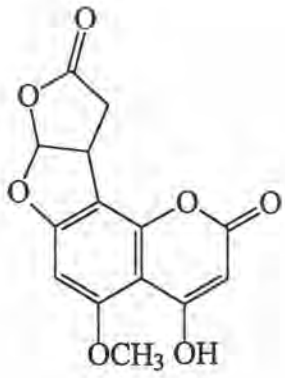
II : R^1 = OH, R^2 = H

III : R^1, R^2 = O

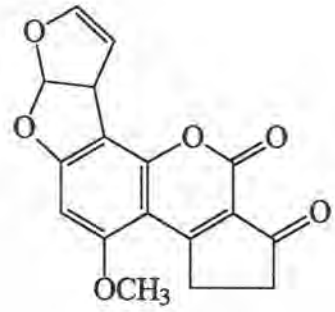
For example compound (I) from *Teucrium grisebachii* has PFI (percent feeding inhibition) 31.4% at 100 ppm, while compound (II) derived from specific deacetylation of compound (I) and compound (III) from oxidation with Jones' reagent of compound (II) exhibited better biological action with PFI 62.5% and 57.9% (at the same concentration), respectively.[23]

Therefore, "structure-activity relationship study" (SAR) of natural compounds is the manifestly useful and important methodology to improve the biological activity of these compounds.

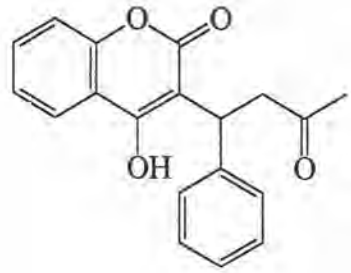
Following the above mentioned outcome, 4-hydroxycoumarins have been selected for SAR study in several research groups. For instance a research of the platelet aggregation inhibitors and anticoagulant of coumarin (V) analogues, containing the basic skeleton of alflatoxin B_1[7,19c] and warfarin analogues. The HIV-1 protease inhibitors and binding site in rat liver NAD(P)H of dicoumarols and 3-substituted-4-hydroxycoumarins.[8b,19d,24]



Coumarin V



Aflatoxin B_1



Warfarin

However, the studies of the relationship of 4-hydroxycoumarins and insect antifeedant of *Galleria mellonella* Linn. and antigrowth activity of *Mimosa pigra* Linn. have never been reported in chemical literature. Thereby, the study on SAR of 4-hydroxycoumarins is worth considering for increasing potential knowledge, understanding and utilizing of these compounds in agricultural purposes.

1.3 Goal of Research

This research is designed to develop 4-hydroxycoumarins in reverting behaviors of animals and plants. The approach is based upon the assumption that structure-activity relationship (SAR) study is not merely a knowledge of how much activities of each of them has, but also match structures and activities of studied compounds. In addition, the SAR study will permit a logical opportunity to predict

relationship of other molecules (analogues of 4-hydroxycoumarin) and these activities. Therefore, the goal of this research can be summarized as follows:

1. To synthesize 4-hydroxycoumarins and related compounds
2. To study the relationship between 4-hydroxycoumarin and dicoumarol structures and insect antifeedant against *Galleria mellonella* Linn. and weed growth inhibition against *Mimosa pigra* Linn.