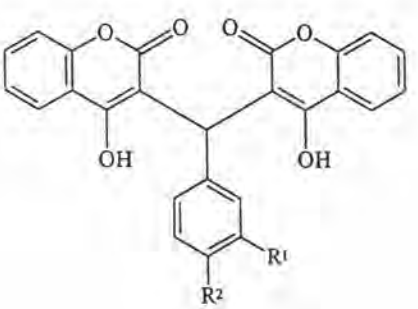
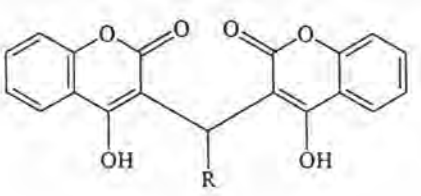
# CHAPTER IV
# CONCLUSION

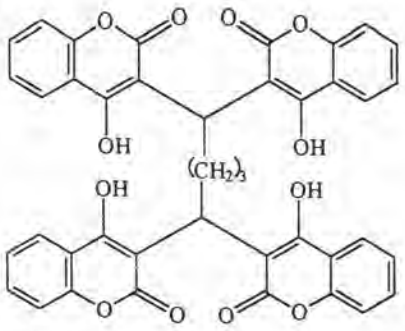
During the course of this research, dicoumarols and 3-alkyl-4-hydroxy coumarins categorized in the class of 3-substituted-4-hydroxycoumarins were synthesized and screened for the possibility of finding bioligically active compounds. Syntheses of these compounds were simple: 4-hydroxycoumarin was condensed with various aldehydes to provide forty-seven dicoumarols, four fused ring compounds, two tetramers and two unexpected compounds. Reductive fragmentation of some dicoumarols using sodium cyanoborohydride yielded fifteen 3-alkyl-4-hydroxy coumarins in high yield. All structures were well characterized using their physical properties and spectroscopic techniques such as IR, $^{1}$H-NMR and $^{13}$C-NMR and in some cases MS and elemental analysis were also performed. There are twelve compounds including **17**, **23**, **29**, **30**, **31**, **32**, **33**, **38**, **44**, **50**, **54** and **R12** that have not been reported in chemical literatures. The structures of new compounds are shown below:



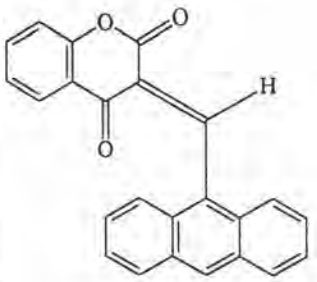

| Cpd | $R^1$ | $R^2$ |
|---|---|---|
| **17** | H | *t*-Bu |
| **23** | OPh | H |
| **29** | $OCH_3$ | $OC_2H_5$ |
| **30** | $OCH_3$ | $OC_4H_9$ |
| **31** | $OCH_3$ | $OC_6H_{13}$ |
| **32** | $OCH_3$ | $OC_8H_{17}$ |
| **33** | $OCH_3$ | $OC_{12}H_{25}$ |



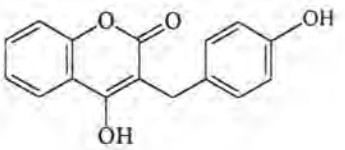

| Cpd | R |
|---|---|
| **38** | $CH(C_2H_5)_2$ |
| **44** | $(CH_2)_3Ph$ |



**50**



**54**



**R12**

In this research, 3-substituted-4-hydroxycoumarins were tested with three interesting bioassays. Brine shrimp lethality test was selected as the first choice for screening test among biological activity studies. For dicoumarols, structures which are of good tendency to be attractive for further study with anticancer, anticelline etc. were based on four high activity compounds, i.e., Compounds **31**, **43**, **50** and **55**. Six carbon atoms (Compound **31**) displayed high activity in series of 3-methoxy-4-alkoxy group substituent on benzylidene ring. The highest activity was shown when dicoumarol contains a cinnamoyl moiety, while alkyl tetramer also exhibited high activity. An unexpected compound that obtained from 1:1 condensation of 4-hydroxycoumarin and choral contained three chlorine atoms and one hydroxy atom also displayed high activity. 3-Alkyl-4-hydroxycoumarins having an ethyl or a cyclohexyl group at C-3 exhibited strongest activity. Most compounds showed high activity and all of these compounds exhibited higher activity than dicoumarols. This implies that 3-alkyl-4-hydroxycoumarins are more attractive for further study than dicoumarols because they exhibited higher activity.

Even though many reports mentioned the antibacterial activity of 4-hydroxy coumarin derivatives against many bacteria, the application of dicoumarols and 3-alkyl-4-hydroxycoumarins as antibacterial agent against seven food-poisoning bacteria including: *Bacillus cereus*, *Escherichia coli*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella derby*, *Staphylococus aureus* and Flat sour spoilage have never been addressed. These compounds were found to be selectively active with four bacteria, namely, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococus aureus* and Flat sour spoilage. In case of dicoumarols, the compound with a phenyl ring displayed high activity. The activity might be depended on

substituents on a benzylidene ring. The halo substituent on a benzylidene ring exhibited higher inhibition than other functional groups. Fused ring structures also showed attractive high activity. Comparison between dicoumarols with antibiotic drugs, it was found that those antibiotic drugs displayed higher activity than dicoumarols. 3-Alkyl-4-hydroxycoumarins were found to be active with three bacteria, namely, *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococus aureus*. These results showed that a phenyl linking with an alkyl chain substituent exhibited the activity as high as antibiotic drugs. A large group as cyclohexyl ring substituted at C-3 position of 4-hydroxycoumarin also exhibited high activity. The activity of 3-alkyl-4-hydroxycoumarins and dicoumarols, contained the same substituent was compared and showed that activities of 3-alkyl-4-hydroxycoumarins were higher than those of dicoumarols.

Considering in the case of antiviral activity against HSV-1 and HSV-2, some compounds were not tested but the results showed the relationship between structures and activity. Percent inhibition of dicoumarols with HSV-1 showed more potent and more interesting than HSV-2. For anti HSV-1, Compounds **3**, **18** and **46** displayed 100% inhibition. *Ortho* substituents on a benzylidene ring showed higher activity than those of *meta* and *para*. Nitro and fluoro groups seemed to show the potency on a benzylidene ring for inhibition of HSV-1. When a number of methoxy and hydroxy groups substituted on a benzylidene ring increased, the activity was dropped and in some cases it showed no activity. When a methoxy group was substituted on 3-position of fused ring compound, the activity showed 100% inhibition. In the case of 3-alkyl-4-hydroxy coumarins, the results were differed from those obtained from dicoumarols. To illustrate this, they inhibited HSV-2 more than HSV-1. However, none of the compounds showed 100% inhibition against both virus. The structures that contained cinnamoyl moiety showed high inhibition. 3,4-Methylenedioxybenzyl group also exhibited high activity. When a number of methoxy substituted on a benzylidene ring increased, the activity was also increased.

Finally, it was clearly disclosed that 3-substituted-4-hydroxycoumarins not only known for using as anticoagulant activity, but also showed broad spectrum activity. Brine shrimp lethality test, antibacterial activity against food poisoning bacteria and anti HSV-1 and HSV-2 of dicoumarols and 3-alkyl-4-hydroxycoumarins are three examples of bioassay results derived from this preliminary report. These

results could surely be utilized for further development as the applications for medicinal drugs or for agricultural purposes.

**Propose for the Future Work**

The discovery of 3-substituted-4-hydroxycoumarins displaying promising biological activities was highlighted. Synthetic methodologies of these compounds have been developed in the recent years[56] and still need for more simple and effective routes for preparing these structures. The investigation of brine shrimp lethality test was well-known as a preliminary indicator which could be used for further study on other sophisticated bioassays such as anticancer, antitumor, insecticide, etc. The antibacterial and antiviral activities should be carried on to obtain complete results. Other functional groups substituted on these structures were still attractive to be searched for more active compounds and attain the relationship between structures and activities. In addition, the molecular modeling studies of these compounds should be performed in order to find out the real active-site in the compound.