CHAPTER I

INTRODUCTION

Rationale for the study

Aquaculture industry, such as an economic seafood export and ornamental fish, is the one of essential valuable industry in national and international level. The fresh aliveness aquaculture seafood product and lively ornamental fish can make a higher value than the dead product. However, the long-time transportation process by air, road or even ship unavoidably affect to the freshness and physical damage of these aquaculture products on the physiological stress in various fishes and crustacean. This is generally known that the aquatic animals especially fishes will sensitively respond to environmental stressors, called as general adaptation syndrome (GAS)[1]. The fishes can secrete the hematological response when was oppressed by the external factor like temperature, shaking, loud voice, congestion and stressful conditions. This respond will be affect to erythrocyte, platelet number, hemoglobin concentration or hematocrit, plasma electrolytes (Na^+, K^+ and Cl^-), glucose, cholesterol levels and cortisol hormone[2]. Especially, the cortisol hormones (Figure 1) will be leading to the increasing of glucose level in bloodstream and the rate of heartbeat. These are the cause of the hypertension and more volume of blood transport into the gill area in order to get enough oxygen and release electron through its gill. Furthermore, the cortisol hormone also has an effect on decreasing level of immunity, therefore the aquaculture is easy to get infection and the level of reproduction also decreases. Base on this phenomenon, it leads to the death rate of fish especially in small fishes.

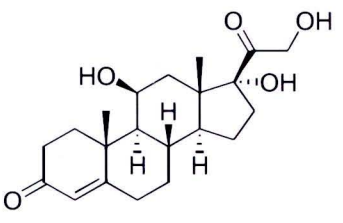


Figure 1 Chemical structure of cortisol hormone

For decreasing the death rate due to transportation procedure and preserving the physical properties of aquaculture product, there are many means to solve this problem such as the use of vaccine and other chemicals to relax the panic animals. Some anesthetics agent can reduce or block the activation of the hypothalamic-pituitary-interrenal (HPI) axis associated with stressors and thus decrease or prevent the release of the stress hormone cortisol to the bloodstream of fish[3].

The examples of these anesthetic substances are propofol, paridocain, benzocaine, tricanemethane-sulfonate (MS222), fentanyl, procaine derivatives and eugenol which have the potential anesthetic property especially in aquatic animals (Figure 2 and Figure 3).

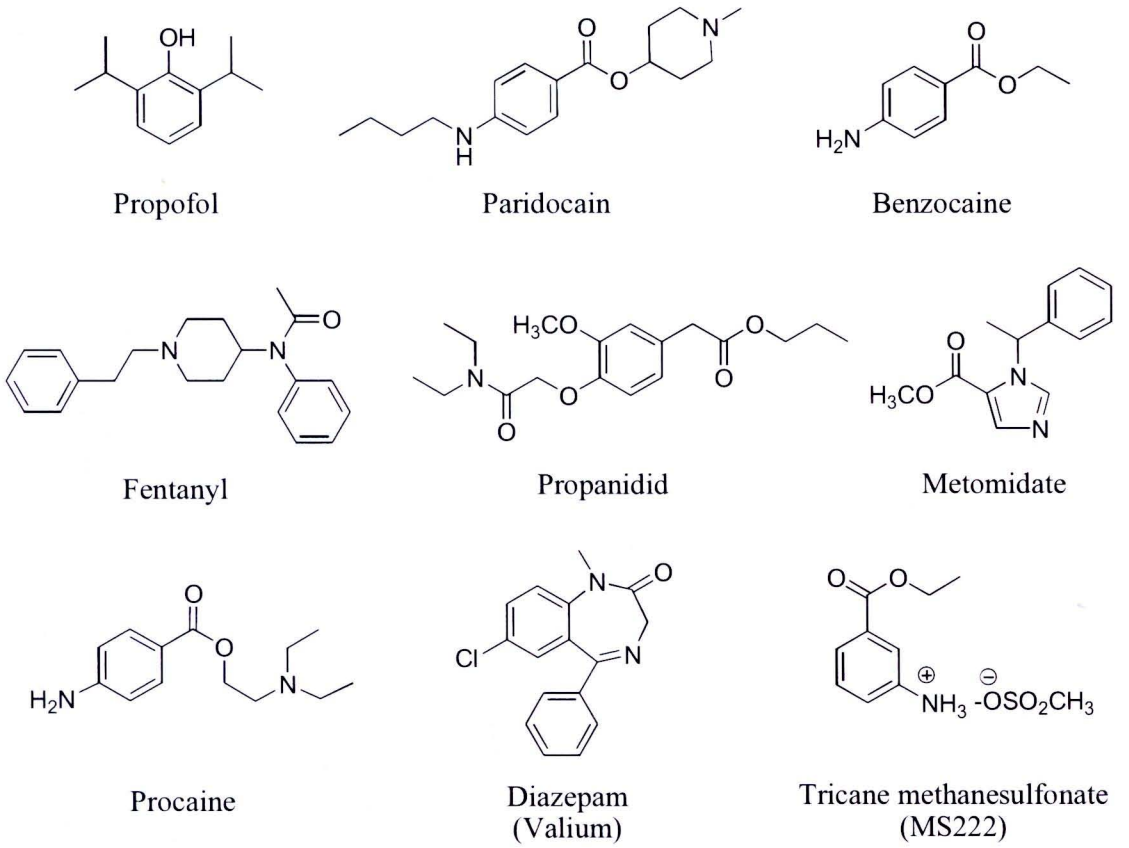


Figure 2 Example of anesthetic compounds

At the present, tricane methanesulfonate or MS222 is the synthetic compound that widely used to anesthetize aquaculture in transportation, although it is mildly

toxic and needed to obligatory keep the fish for withdrawal period (21 days) before sending to customer as a food. However, this compound is only one that has permission in food product according to the US Food and Drug Administration (FDA) [4, 5].

Unlike MS-222, Eugenol (4-allyl-2-methoxyphenol) is a natural product derivative of clove tree. It is a viscous liquid extracted from the leaves, buds and stem of the *Eugenia caryophyllata*[6]. Eugenol is approved as generally recognized as safe in direct food substance and in fish anesthetic[7]. Main advantages of eugenol are a low cost, efficiency, large margin of safety for fish and lack of toxicity to humans at the concentrations in use[8]. It is also rapidly metabolized and excreted from tissue, and thus it does not require a very long withdrawal time[9]. In addition, eugenol also possesses an antimycotic and antibacterial properties[10, 11]. Therefore, eugenol and its derivatives is an interesting candidate to use as anesthetic substance in aquaculture transportation. It is expected that the modification the eugenol structure will bring to high potential substances in term of very long anesthesia and safety.

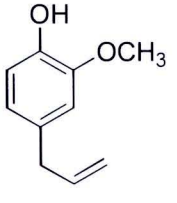


Figure 3 Chemical structure of Eugenol

In this research, the modification of eugenol was designed and synthesized to get the eugenol derivatives which was preliminarily tested an anesthetic activities in the aquatic animals.

Purpose of the study

1. To design and synthesize eugenol derivatives.
2. To investigate the property of synthesized compound for being the anesthetic in the aquatic animals for transportation.

Scope of the study

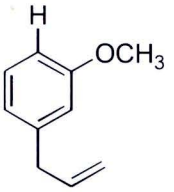
In this research, the overall works will be divided into three parts. The first part is the synthesis of eugenol derivatives in 7 groups. The second part is the preparation of eugenol derivatives recipe in solution form for use in next step. The third part is the preliminary anesthetic test of eugenol derivatives solution in aquatic animals.

Path 1 Synthesis of eugenol derivatives

Eugenol derivatives were designed and categorized to 7 groups in order to individually analyze as following;

1. Displacement of functional substituent on eugenol structure with hydrogen.

To study the important active site for anesthetic property in aquaculture.



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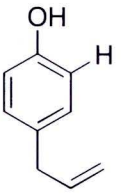

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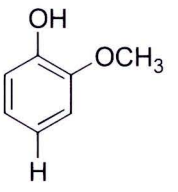
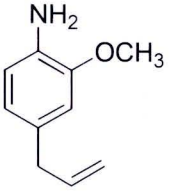


(3)

2. Displacement of hydroxyl group with other H-bond donor groups.

Amino group as H-bond donor will replace the hydroxyl group to study the effect of strength and type of H-bond.



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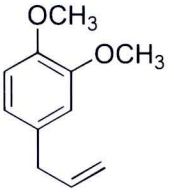
3. Displacement of hydroxyl group with other H-bond acceptor.

The hydroxyl group will replaced by the H-bond acceptor such as methoxy and bromine atom in order to study the effect of strength and type of H-bond.



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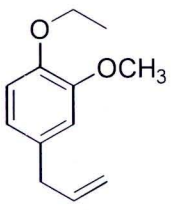
4. Alkylation of hydroxyl group of eugenol.

Hydroxyl group of eugenol will be alkylated with various hydrocarbon chains such as ethyl, propyl, isopropyl, butyl, sec-butyl, pentyl, hexyl and heptyl group to increase hydrophobicity in molecule.



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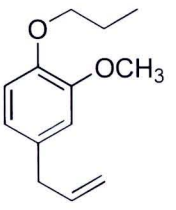

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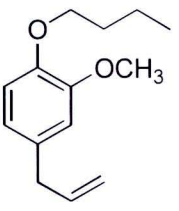

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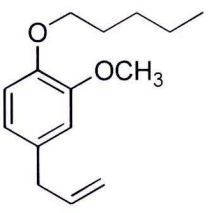

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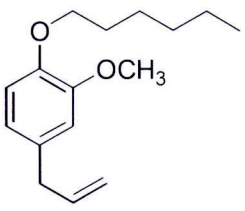

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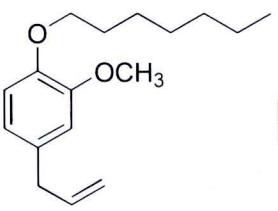

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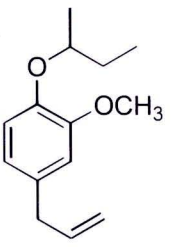

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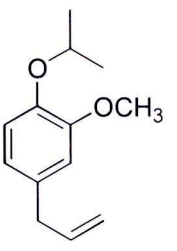
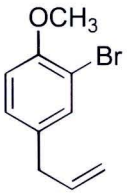


(14)

5. Displacement of methoxy group of eugenol.

Methoxy group of eugenol will be replaced by other heteroatom as H-bond acceptor such as bromine atom in order to study the effect of strength and type of H-bond.



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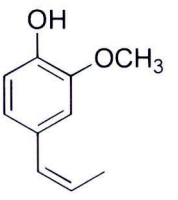
6. Modification of allyl group of eugenol.

Allyl group of eugenol will be modified with various functional groups to investigate the influence of allyl moiety.



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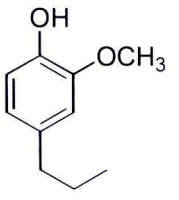

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7. Modification of eugenol.

Starting material as eugenol will be derivatized by various reaction such as halogenations and nitration reactions.

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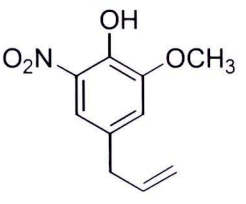

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Path 2 Preparation of eugenol derivatives solution

The preparation process of synthesized eugenol derivatives will be studied to avoid the solubility problem of hydrophobicity of the derivatives. This part of work is cooperating with associate professor Dr. Sakchai Wittaya-areekul at department of pharmaceutical technology, faculty of pharmaceutical science, Naresuan University.

Path 3 Preliminary anesthetic activity test in aquatic animals

The eugenol derivatives in the solution form will be preliminarily tested the anesthetic activity in 3 kinds of aquatic animals such as white shrimp (*Litopenaeus vannamei*), seabass (*Lates calcarifer*) and hybrid catfish female *Clarias macrocephalus* x male *Clarias gariepinus*). This part of work is cooperating with assistance professor Dr. Boonyarath Pratomchad and Dr. Thanomsak Boonphakdee at department of aquatic science, faculty of science, Burapha University.