CHAPTER III

RESEARCH METHODOLOGY

General Procedure

1. Measurement

All reactions were performed in oven-dried glassware. The weight of all chemical substances was determined on a Sartorius electronic analytical balance. Evaporation of solvents was carried out on Büchi Rotavapor R-124 with a water aspirator model B-490 or a Refco Vacuubrand pump or diaphragm pump. The magnetic stirrers and heater were of Heidolph and HARMONY. The progress of the reaction was followed by thin layer chromatography (TLC) performed on Merck D.C. silica gel F_{254} 0.2 mm. precoated aluminium plates cat. No. 1.05554.Visualization was accomplished using either a UV light at 254 nm, potassium permanganate stain (1.5 g of $KMnO_4$, 10 g of K_2CO_3, 1.25 mL of 10% (w/w) NaOH in 200 mL of water) and ninhydrin stain (1.5 g of ninhydrin in 100 mL of *n*-butanol and 3 mL of AcOH). Column chromatography was performed on silica gel 70-230 mesh for column chromatography. Solvent mixtures used for TLC and column chromatography are reported in v/v ratio.^{1}H and ^{13}C NMR spectra were reported in ppm and recorded on Bruker Merury-400 plus or Bruker/Avance 400 NMR spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. The preparation of anesthetic agent foundation was operated at Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University. Anesthetic test in white shrimp (*Litopenaeus vannamei*), seabass (*Lates calcarifer*) and hybrid catfish female *Clarias macrocephalus* x male *Clarias gariepinus*) was performed at Department of Aquatic Science, Faculty of Science, Burapha University.

2. Materials

All chemicals were purchased from Fluka, Merck, Acros Organics or Aldrich Chemical Co., Ltd., and were used as received without further purification. Commercial grade solvents were distilled before use for column chromatography. Solvents for reactions used without purification. Preparation of tetrahydrofuran (THF) anhydrous has been using sodium metal (Na) dispersion in paraffin wax, it cuts with a

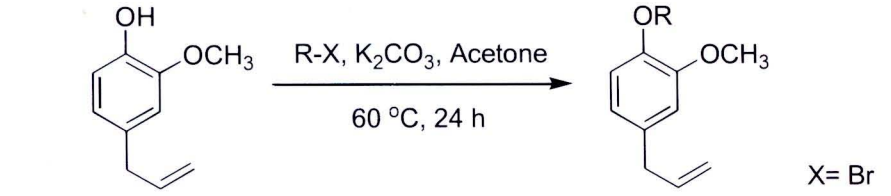
knife fire hazzard and due to huge surface area the benzophenone key color develops promptly, with one hour.

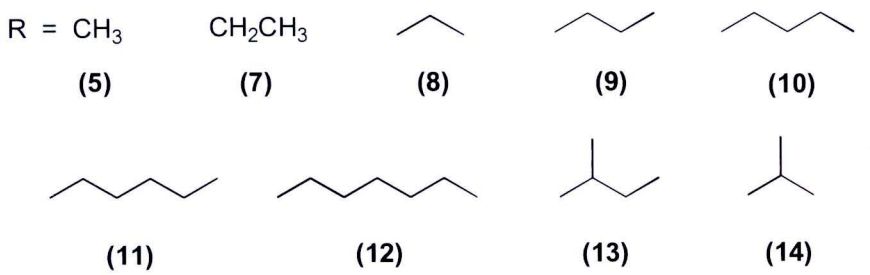
3. Research Methodology

In this research, the overall works are divided into three parts. The first part is the synthesis of eugenol derivatives in 7 groups according to the plan in previous topic. The second part is the preparation of eugenol derivatives to the solution form for use in next step. The third part is the preliminary anesthetic test of eugenol derivatives solution in white shrimp (*Litopenaeus vannamei*), seabass (*Lates calcarifer*) and hybrid catfish female *Clarias macrocephalus* x male *Clarias gariepinus*).

Part 1 Synthesis of eugenol derivatives

1. Synthesis of eugenol derivatives via *O*-alkylation reaction (5, 7-14)





4-allyl-1,2-dimethoxybenzene (Methyleugenol) (5) A mixture of anhydrous K_2CO_3 (4.9 g, 30 mmol), eugenol (3.2 g, 20 mmol), and acetone (30 mL) was stirred for 30 min then methyl bromide (8.2 g, 60 mmol) was added and refluxed for 6 h. The mixture was extracted with ethyl acetate (3x20 mL). The combined extracts were washed with water (3x20 mL), dried over anhydrous Na_2SO_4 and the solvent was evaporated. Purification on silica gel with EtOAc:hexane (1:6) as eluent and the

solvent removed in vacuo. The product 4-allyl-1,2-dimethoxybenzene receive as a pale yellow oil. (3.19 g, 89.7% yield)

^{1}H NMR (400 MHz, $CDCl_3$):δ_H 3.33 (d, *J*=6.6 Hz, 2H, C<u>H</u>$_2$ benzylic), 3.85 (s, 3H, OC<u>H</u>$_3$ methyl), 3.86 (s, 3H, OC<u>H</u>$_3$), 5.07 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.12 (d, *J*=12.9 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.96 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.80 (s, 1H, Ar<u>H</u>), 7.26 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>)

4-allyl-1-ethoxy-2-methoxybenzene (Ethyleugenol) (7) This compound was performed with the same procedure to synthesize compound **5** but use bromoethane (10.9 g, 100 mmol) as alkylating agent and eugenol (6.5 g, 40 mmol) as starting material and received as a pale yellow oil (4.20 g, 54.7% yield)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.44 (t, *J*=6.8 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 3.33 (d, *J*=6.6 Hz, 2H, C<u>H</u>$_2$ benzylic), 3.86 (s, 3H, OC<u>H</u>$_3$), 4.06 (quartet, *J*=7.0 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 5.07 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.12 (d, *J*=13.0 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.97 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.80 (s, 1H, Ar<u>H</u>), 7.26 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>)

4-allyl-2-methoxy-1-propoxybenzene (propyleugenol) (8) This compound was performed with the same procedure to synthesize compound **5** but use 1-bromopropane (9.8 g, 80 mmol) as alkylating agent and eugenol (6.5 g, 40 mmol) as starting material and received as a pale yellow oil (7.12 g, 86.2 %yield)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.03 (t, *J*=7.4 Hz, 3H, OCH$_2$CH$_2$C<u>H</u>$_3$), 1.85 (sextet, *J*=7.2 Hz, 2H, OCH$_2$C<u>H</u>$_2$ CH$_3$), 3.33 (d, *J*=6.6 Hz, 2H, C<u>H</u>$_2$ benzylic), 3.85 (s, 3H, OC<u>H</u>$_3$), 3.95 (t, *J*=6.8 Hz, 2H, OC<u>H</u>$_2$CH$_2$CH$_3$), 5.07 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.12 (d, *J*=13.0 Hz, 1H, *trans*-CH terminal alkene), 5.95 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.80 (s, 1H, Ar<u>H</u>), 7.25 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>)

4-allyl-1-butoxy-2-methoxybenzene (butyleugenol) (9) This compound was performed with the same procedure to synthesize compound **5** but use 1-bromobutane (8.2 g, 60 mmol) as alkylating agent and eugenol (4.9 g, 30 mmol) as starting material and received as a pale yellow oil (6.60 g, 90.5 %yield)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.97 (t, *J*=7.3 Hz, 3H, $O(CH_2)_3C\underline{H}_3$), 1.48 (sextet, *J*=7.5 Hz, 2H, $O(CH_2)_2C\underline{H}_2CH_3$), 1.81 (quintet, *J*=7.0 Hz, 2H, $OCH_2C\underline{H}_2CH_2CH_3$), 3.34 (d, *J*=6.5 Hz, 2H, $C\underline{H}_2$ benzylic), 3.85 (s, 3H, $OC\underline{H}_3$), 4.00 (t, *J*=6.8 Hz, 2H, $OC\underline{H}_2(CH_2)_2CH_3$), 5.07 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.12 (d, *J*=13.0 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.94 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.81 (s, 1H, Ar<u>H</u>), 7.25 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>)

4-allyl-2-methoxy-1-pentoxy benzene (pentyleugenol) (10) This compound was performed with the same procedure to synthesize compound **5** but use 1-bromopentane (7.5 g, 50 mmol) as alkylating agent and eugenol (4.9 g, 30 mmol) as starting material and received as a pale yellow oil (5.70 g, 81.0 %yield)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.93 (t, *J*=7.0 Hz, 3H, $O(CH_2)_4C\underline{H}_3$), 1.38 (m, 2H, $O(CH_2)_3C\underline{H}_2CH_3$), 1.43 (m, 2H, $O(CH_2)_2C\underline{H}_2CH_2CH_3$), 1.82 (quintet, *J*=7.4 Hz, 2H, $OCH_2C\underline{H}_2(CH_2)_2$ CH_3),3.33 (d, *J*=6.6 Hz, 2H, $C\underline{H}_2$ benzylic), 3.85 (s, 3H, $OC\underline{H}_3$), 3.98 (t, *J*=6.8 Hz, 2H, $OC\underline{H}_2(CH_2)_3CH_3$), 5.05 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.12 (d, *J*=13.0 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.96 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.81 (s, 1H, Ar<u>H</u>), 7.26 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>)

4-allyl-1-hexyloxy-2-methoxy-benzene (hexyleugenol) (11) This compound was performed with the same procedure to synthesize compound **5** but use 1-bromohexane (8.2 g, 50 mmol) as alkylating agent and eugenol (4.9 g, 30 mmol) as starting material and received as a pale yellow oil (7.30 g, 98.0 %yield)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.89 (t, *J*=6.9 Hz, 3H, $O(CH_2)_5C\underline{H}_3$),1.32 (m,2H, $O(CH_2)_4C\underline{H}_2CH_3$), 1.44 (m, 2H, $O(CH_2)_3C\underline{H}_2CH_2CH_3$), 1.61(m, 2H, $O(CH_2)_2C\underline{H}_2$ $(CH_2)_2CH_3$), 1.81 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_3CH_3$), 3.32 (d, *J*=6.6 Hz, 2H, $C\underline{H}_2$ benzylic), 3.84 (s, 3H, $OC\underline{H}_3$), 3.98 (t, *J*=6.9 Hz, 2H, $OC\underline{H}_2(CH_2)_4CH_3$), 5.05 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.12 (d, *J*=13.0 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.96 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.81 (s, 1H, Ar<u>H</u>), 7.26 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>)

4-allyl-1-(heptyloxy)-2-methoxybenzene (heptyleugenol) (12) This compound was performed with the same procedure to synthesize compound **5** but use

1-bromoheptane (8.9 g, 50 mmol) as alkylating agent and eugenol (4.9 g, 30 mmol) as starting material and received as a pale yellow oil (5.91g, 75.1 %yield)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.88 (t, *J*=6.7 Hz, 3H, $O(CH_2)_6C\underline{H}_3$), 1.33 (m, 2H, $O(CH_2)_5C\underline{H}_2CH_3$), 1.34 (m, 2H, $O(CH_2)_4C\underline{H}_2CH_2CH_3$), 1.46 (m, 2H, $O(CH_2)_3C\underline{H}_2(CH_2)_2CH_3$), 1.81 (m, 2H, $O(CH_2)_2C\underline{H}_2(CH_2)_3CH_3$), 1.83 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_4CH_3$), 3.33 (d, *J*=6.6 Hz, 2H, $C\underline{H}_2$ benzylic), 3.84 (s, 3H, $OC\underline{H}_3$), 3.97 (t, *J*=6.9 Hz, 2H, $OC\underline{H}_2(CH_2)_5CH_3$), 5.05 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.12 (d, *J*=13.0 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.95 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.81 (s, 1H, Ar<u>H</u>), 7.26 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>)

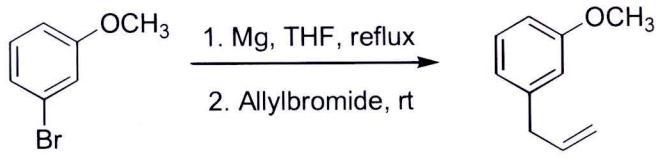
4-allyl-1-*sec*-butoxy-2-methoxybenzene (*sec*-butyleugenol) (13) This compound was performed with the same procedure to synthesize compound**5** but use *sec*-butylbromide (6.8 g, 50 mmol) as alkylating agent and eugenol (4.9 g, 30 mmol) as starting material and received as a pale yellow oil. (3.73 g, 57% yield)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.99 (t, *J*=7.4 Hz, 3H, $OCH(CH_3)CH_2C\underline{H}_3$), 1.30 (d, *J*=6.1 Hz, 3H, $OCH(C\underline{H}_3)CH_2CH_3$), 1.62 (septet, *J*=7.3 Hz, 2H, $OCH(CH_3)C\underline{H}_2CH_3$), 3.33 (d, *J*=6.6 Hz, 2H, $C\underline{H}_2$ benzylic), 3.84 (s, 3H, $OC\underline{H}_3$), 4.23 (sextet, *J*=6.1 Hz, 1H, $OC\underline{H}(CH_3)CH_2CH_3$), 5.06 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.11 (d, *J*=13.0 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.98 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.81 (s, 1H, Ar<u>H</u>), 7.26 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>)

4-allyl-1-isopropoxy-2-methoxybenzene (isopropyleugenol) (14) This compound was performed with the same procedure to synthesize compound**5** but use *iso*-propylbromide (12.3 g, 100 mmol) as alkylating agent and eugenol (6.5 g, 40 mmol) as starting material and received as a pale yellow oil (5.57 g, 68%)

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.35 (d, *J*=6.0 Hz, 6H, $OCH(C\underline{H}_3)_2$), 3.33 (d, *J*=6.6 Hz, 2H, $C\underline{H}_2$ benzylic), 3.84 (s, 3H, $OC\underline{H}_3$), 4.47 (septet, *J*=6.0 Hz, 1H, $OC\underline{H}(CH_3)_2$), 5.07 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.11 (d, *J*=12.9 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.98 (m, 1H, C<u>H</u> alkenyl), 6.70 (d, *J*=8.0 Hz, 2H, Ar<u>H</u>), 6.81 (s, 1H, Ar<u>H</u>), 7.26 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>).

2. Synthesis of 3-allylanisole (1)

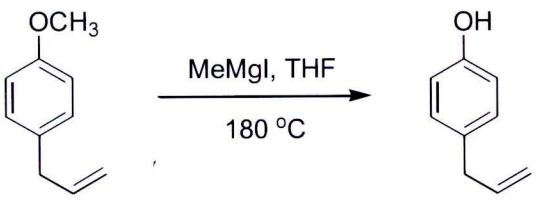


(1)

Magnesium powder (0.43 g, 18 mmol) was suspended in dry tetrahydrofuran (THF) (10 mL) and 3-bromoanisole (2.80 g, 15 mmol) was added. After the reaction has started, the remaining 3-bromoanisole dissolved in dry THF was added dropwise, maintaining the Grignard solution at reflux condition. Stirring was continued under reflux for 2 h, then the mixture was cooled to 25 °C and transferred into another flask under nitrogen atmosphere at room temperature, allylbromide (2.17 g, 18 mmol) was dissolved in dry THF. Stirring of the mixture was continued for additional 3 h at room temperature. After the reaction completed, the reaction mixture was isolated by extraction into ethyl acetate with water and then dried over Na_2SO_4 anhydrous and purified by column chromatography on silica gel with hexane 100% as eluent and the solvent removed in vacuo. (1.47 g, 96.1 % yield)

^{1}H NMR (400 MHz, $CDCl_3$):δ_H 3.40 (d, *J*=6.7 Hz, 2H, C<u>H</u>$_2$ benzylic), 3.81 (s, 3H, OC<u>H</u>$_3$), 5.07 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.11 (d, *J*=12.9 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.98 (m, 1H, C<u>H</u> alkenyl), 6.80 (m, 3H, Ar<u>H</u>), 7.23 (t, *J*=2.4 Hz, 1H, Ar<u>H</u>)

3. Synthesis of 4-allylphenol (2)



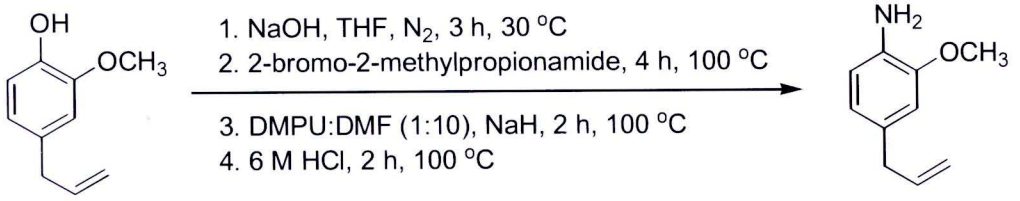
(2)

The mixture of 4-allylanisole (2.20 g, 15 mmol) and methyl magnesium iodide (4 mL, 25 mmol) in THF (10 mL) was stirred for 4 h. at 180 °C. After stirring

aqueous 5% $NaHCO_3$ was added, and the mixture was stirred vigorously for 30 min. the solution was extracted with water (3x20mL).The organic layer was separated after acidification 5% HCl solution, washed with aqueous NH_4Cl (2x30 mL) and H_2O (1x30 mL), and dried over Na_2SO_4 anhydrous. The crude product was purified by column chromatography on silica gel with EtOAc:hexane (1:3) as eluent and the solvent removed in vacuo (1.50 g, 70.3 % yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.33 (d, *J*=6.6 Hz, 2H, C<u>H</u>$_2$ benzylic), 4.88 (br, 1H, O<u>H</u>), 5.04 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.12 (d, *J*=12.9 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.99 (m, 1H, C<u>H</u> alkenyl), 6.77 (d, *J*=6.8 Hz, 2H, Ar<u>H</u>), 7.04 (d, *J*=8.4 Hz, 2H, Ar<u>H</u>)

4. Synthesis of 4-allyl-2-methoxyaniline (4)



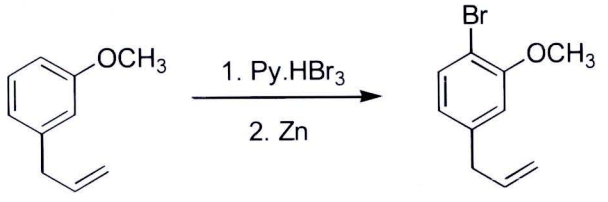
(4)

To a solution of eugenol (3.80 mL, 6mmol) was stirred in dry THF (15 ml) with sodium hydroxide (0.30 g, 7.4mmol) for 3 h at 30°C in a nitrogen atmosphere. Then, 2-bromo-2-methylpropionamide was added and the reaction mixture was kept at 100 °C and stirred for 4 h. After cooling at room temperature, the mixture of *N,N*-dimethylformamide (DMF) and 1,3-dimethyltetrahydropyrimidin-2-(1*H*)-one (DMPU) 1:10 was added sodium hydride (0.20 g, 12.3 mmol) and the reaction mixture was kept at 100 °C for 2 h. After the period of time, the temperature was cooled to 25 °C and the solution was refluxed in 6 M HCl solution for 2 h, then the reaction mixture was cooled to 25 °C and treated with NaOH solution until neutrality. The aqueous solution was extracted with EtOAc, dried over Na_2SO_4 anhydrous, after solvent removal, the crude was purified on silica gel using hexane/ethyl acetate 3:1 as eluent to give the compound **4** (0.4 g, 89.0% yield) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$):δ_H 3.33 (d, *J*=6.4 Hz, 2H, C<u>H</u>$_2$ benzylic), 3.79 (s, 3H, OC<u>H</u>$_3$), 5.04 (m,1H, *cis*-terminal alkene), 5.07 (dd, *J*=6.6, 1.5 Hz, 1H, *trans*-

terminal alkene), 5.93 (m, 1H, C<u>H</u> alkenyl), 6.33 (br, 1H, N<u>H</u>$_2$), 6.67 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>), 6.71 (s, 1H, Ar<u>H</u>), 6.90 (d, *J*=8.0 Hz, 1H, Ar<u>H</u>), 7.45 (br, 1H, N<u>H</u>$_2$)

5. Synthesis of 4-allyl-1-bromo-2-methoxybenzene (6)

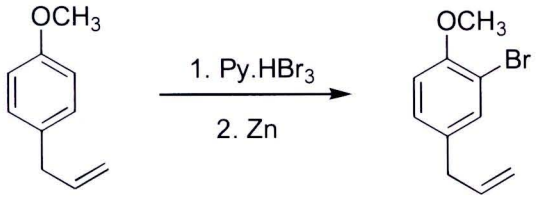


(1) **(6)**

To a solution of 3-allylanisole **(1)** (0.30 g, 2 mmol) was dissolved in 10 mL acetic acid. This solution was added (0.70 g, 3 mmol) of pyridinium hydrobromide perbromide (PHP) and the mixture stirred until the crystals of the PHP had dissolved for 4 h. More PHP (0.70 g, 3 mmol) was added to the acetic acid solution and the mixture stirred overnight. Most of the acetic acid was removed *in vacuo* leaving red oil that was taken up in toluene (8 mL), washed once with 20 mL of water three times, with saturated aqueous $NaHCO_3$ solution, and finally dried over anhydrous Na_2SO_4. Treatment of this oil in 8 mL diethylether with 0.20 g zinc dust and 0.20 mL of acetic acid under reflux for 4 h followed by stirring at room temperature for 18 h. After the reaction completed was isolated by extraction into ethyl acetate with water and then dried over Na_2SO_4 anhydrous and purified by column chromatography on silica gel with hexane 100% as eluent and the solvent removed in vacuo (0.078 g, 55.0% yield).

^{1}H NMR (400 MHz, $CDCl_3$); δ_H: 3.38 (d, *J*=6.8 Hz, 1H, C<u>H</u>$_2$ benzylic), 3.48 (d, *J*=6.4 Hz,1H, C<u>H</u>$_2$benzylic), 3.77 (s, 3H, OC<u>H</u>$_3$), 5.07 (d, *J*=5.8 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.11 (d, *J*=12.9 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.95 (m, 1H, C<u>H</u> alkenyl), 6.64 (d, *J*=3.2 Hz, 1H, Ar<u>H</u>), 6.79 (d, *J*=2.8 Hz, 1H, Ar<u>H</u>), 7.43 (d, *J*=8.8 Hz, 1H, Ar<u>H</u>)

6. Synthesis of 4-allyl-2-bromo-1-methoxybenzene (15)

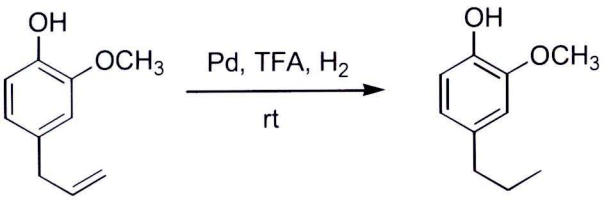


(15)

To a solution of 4-allylanisole (0.40 g, mmol) was dissolved in 10 mL acetic acid and to this solution, PHP (1.90 g, 5 mmol)was added and the mixture stirred until the crystals of the PHP had dissolved for 4 h. More PHP (1.90 g, 5 mmol) was added to the acetic acid solution and the mixture stirred overnight. Most of the acetic acid was removed in vacuo leaving red oil that was taken up in toluene (8 mL), washed once with 20 mL of water and three times, with saturated aqueous $NaHCO_3$ solution, and finally dried over anhydrous Na_2SO_4. Treatment of this oil in 8 mL diethylether with 0.20 g zinc dust and 0.20 mL of acetic acid under reflux for 4 h followed by stirring at room temperature for 18 h. After the reaction completed was isolated by extraction into ethyl acetate with water and then dried over Na_2SO_4 anhydrous and purified by column chromatography on silica gel with hexane 100% as eluent and the solvent removed in vacuo (0.82 g, 48% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ_H : 3.38 (d, *J*=6.8 Hz, 1H, C<u>H</u>$_2$ benzylic), 3.48 (d, *J*=6.4 Hz,1H, C<u>H</u>$_2$benzylic), 3.86 (s, 3H, OC<u>H</u>$_3$), 5.05 (d, *J*=11.2 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.08 (d, *J*=4.4 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.89 (m, 1H, C<u>H</u> alkenyl), 6.84 (d, *J*=8.4 Hz, 1H, Ar<u>H</u>),7.11 (dd, *J*=8.4, 2.4 Hz, 1H, Ar<u>H</u>), 7.36 (d, *J*=2.0 Hz, 1H, Ar<u>H</u>)

7. Synthesis of 2-methoxy-4-propylphenol (PMP) (17)

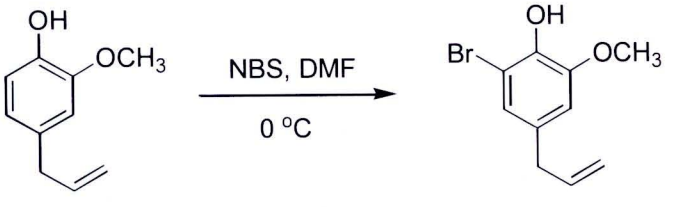


(17)

To a mixture of eugenol (20 mmol) 3.08 mL was dissolved in ethanol 30 mL and 0.23 g of Pd on activated carbon was added along with 0.30 mL of trifluoroacetic acid (TFA). The reaction flask was stirred under a H_2 atmosphere for 24 h. The solution was filtered through celite and the residue was isolated by extraction into ethyl acetate with water, and then dried over Na_2SO_4 anhydrous and purified by column chromatography on silica gel with hexane as eluent and the solvent removed in vacuo. 2-methoxy-4-propylphenol (PMP) obtained as pale yellow oil (2.7 g, 82%).

^{1}H NMR ($CDCl_3$): δ_H 0.95 (t, J=7.2 Hz, 3H, $CH_2CH_2C\underline{H}_3$), 1.63 (sextet, J=7.4 Hz, 2H, $CH_2C\underline{H}_2CH_3$), 2.54 (t, J=7.6 Hz, 2H, $C\underline{H}_2CH_2CH_3$), 3.88 (s, 3H, $OC\underline{H}_3$), 6.96 (d, J=3.2 Hz, 1H, Ar$\underline{H}$), 6.72 (d, J=2.8 Hz, 1H, Ar$\underline{H}$), 7.86 (d, J=7.7 Hz, 1H, Ar$\underline{H}$)

8. Synthesis of 4-allyl-2-bromo-6-methoxyphenol (bromoeugenol) (18)

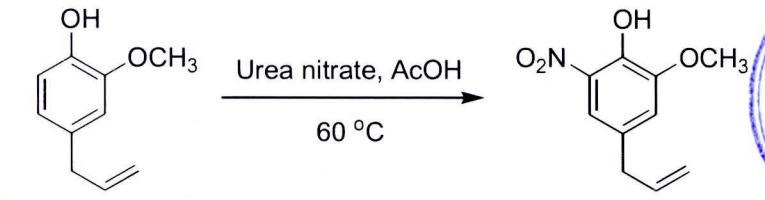


(18)

N-Bromosuccinimide (NBS) (1.00 g, 5.99 mmol) was dissolved in DMF (5 mL). After 15 minutes of stirring, a solution of eugenol (0.10 g, 1.0 mmol) in DMF was added and the mixture was stirred at 0 °C for 1 h. The residue was isolated by extraction with ethyl acetate and water then dried over Na_2SO_4 anhydrous and purified by column chromatography on silica gel with EtOAc:hexane (1:6) and the solvent was removed in vacuo (48% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.26 (d,*J*=6.8 Hz, 2H, C<u>H</u>$_2$ benzylic), 3.86 (s, 3H, OC<u>H</u>$_3$), 5.05 (d, *J*=11.2 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.08 (d, *J*=4.4 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.89 (m, 1H, C<u>H</u> alkenyl), 6.59 (d, *J*=1.6 Hz, 1H, Ar<u>H</u>), 6.88 (d, *J*=1.6 Hz, 1H, Ar<u>H</u>)

9. Synthesis of 4-allyl-2-methoxy-6-nitrophenol (nitroeugenol) (19)



(19)

Urea nitrate (0.20 g, 1.63 mmol), acetic acid (50 mL) and eugenol (0.10 g, 0.65 mmol) were stirred at RT for 30 min. Then the reaction mixture was stirred at 60 °C for 24 h. The residue was neutralized with $NaHCO_3$ until pH=7 and isolated by extraction with diethylether and water. After that, the aqueous phase was extracted with ethyl acetate, then dried over Na_2SO_4 anhydrous and purified using column chromatography on silica gel with EtOAc:hexane (1:6) and the solvent was removed in vacuo (48% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ_H 3.36 (d, *J*=6.0 Hz, 2H, C<u>H</u>$_2$ benzylic), 3.92 (s, 3H, OC<u>H</u>$_3$), 5.05 (d, *J*=11.2 Hz, 1H, *trans*-C<u>H</u> terminal alkene), 5.08 (d, *J*=4.4 Hz, 1H, *cis*-C<u>H</u> terminal alkene), 5.90 (m, 1H, C<u>H</u> alkenyl), 6.95 (d, *J*=1.6 Hz, 1H, Ar<u>H</u>), 7.49 (d, *J*= 1.6 Hz, 1H, Ar<u>H</u>)

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 eugenolderivatives. This part of work is cooperating with Associate Professor Dr.Sakchai Wittaya-areekul at Department of Pharmaceutical Technology, Faculty of Pharmaceutical Science, Naresuan University. The solutions of synthesized compounds were prepared in oil/water emulsion form by the phase inversion technique. The steps are as following.

1. The water phase was prepared by mixture emulsifier Tween 80 2.66 g and water 91 g. Then the solution was heated up to 75 °C with continually stirring.

2. The oil phase was prepared by mixture eugenol derivatives span 80 2.33 g and derivatives 3.99 g. Then the solution heated up to 72 °C with continually stirring.

3. Mix water phase into oil phase and then homogenized for 5 min at 8500 rev./min. by homogenized device.

Path 3 preliminary anesthetic activity test in aquatic animals

The anesthetic activity in aquatic animals such as white shrimp (*L. vannamei*), seabass (*L. calcarifer*) and hybrid catfish (female *C. macrocephalus* x male *C. gariepinus*) using the eugenol derivatives in emulsion form were tested. This part operated at department of aquatic science, faculty of science, Burapha University.

1. Post larvae *L. vannamei*

Post larvae (PL) of *L. vannamei* (1.12±0.11 cm in total length) were obtained from the private hatchery. They were acclimated in 30 ppt water, 27-29 °C; pH 7.8-8.2 and 4.6 ppm D.O. in 1000 L fiber glass tank.

1.1 Eugenol derivatives assay

1.1.1 Ten *L. vannamei* PL were randomly selected to the static 3.5 L aquaria containing 1 L of 30 ppt water.

1.1.2 Eugenol derivatives from **1** to **20** at concentrations of 25 and 50 ppm were introduced to each aquaria and control (**CT**) (without anesthetic agent).

1.1.3 Anesthetic (stage 2 of anesthesia) [18, 19] and recovery periods of individual PL were recorded. Percent survival rate of *L. vannamei* PL was examined after acclimation them to normal condition.

1.1.4 The three replications were operated.

1.1.5 Water quality at 27-28 °C pH 7.8 and 4.6 ppm D.O were controlled.

1.2 Concentration determination for eugenol derivative **8**.

1.2.1 *L. vannamei* PL samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

1.2.2 Five PL at stage 2 of anesthesia were randomly selected to each 3.5 L aquaria containing 0.5 L of 30 ppt water containing derivative **8** at 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6 and 6.5 ppm and positive control (PC, without derivative **8**). Group of without derivative **8** was negative control (NC).

1.2.3 The three replications were operated.

1.2.4 Water quality at 27-28 °C pH 7.8 and 4.6 ppm D.O were controlled.

1.2.5 Percent survival rate of *L. vannamei* PL was examined after acclimation them to normal condition.

1.3 Live transportation of *L. vannamei* PL in plastic bag.

1.3.1 PL samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

1.3.2 Twenty PL at stage 2 of anesthesia were randomly selected to 4.5"x7" plastic bag with 100mL of 30 ppt water containing 3.5 ppm derivative **8** and positive control (PC, without derivative **8**). Group of without anesthesia and derivative **8** was negative control (NC).

1.3.3 Shaking the transporting plastic bags every 1 hour for 24 hours was operated. The three replications were done.

1.3.4 Water quality at 27-28 °C pH 7.8 and 4.6 ppm D.O were controlled.

1.3.5 Percent survival rate of *L. vannamei* PL was examined after acclimation them to normal condition.

2. Adult of *L. vannamei*

Adult of *L. vannamei* (10.64±0.42 g in total weight and 11.13 ±0.56 cm in total length) were obtained from the private hatchery. They were acclimated in 30 ppt water , 27-29 °C; pH 7.8-8.2 and 5.2 ppm D.O. in 1000 L fiber glass tank.

2.1 Eugenol derivatives assay

2.1.1 Two *L. vannamei* adult were randomly selected to the static 3.5 L aquaria containing 1 L of 30 ppt water.

2.1.1 Eugenol derivatives from **1** to **20** at concentrations of 25 and 50 ppm were introduced to each aquaria and control (**CT**) (without anesthetic agent).

2.1.2 Anesthetic (stage 2 of anesthesia) [18, 19] and recovery periods of individual adult were recorded. Percent survival rate of *L. vannamei* adult was examined after acclimation them to normal condition.

2.1.3 The three replications were operated.

2.1.4 Water quality at 27-28 °C pH 7.8 and 4.6 ppm D.O were controlled.

2.2 Concentration determination for eugenol derivative **8**.

2.2.1 *L. vannamei* adult samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

2.2.2 Two adult at stage 2 of anesthesia were randomly selected to each 3.5 L aquaria containing 1 L of 30 ppt water containing derivative **8** at 2.5, 5 and 10 ppm and positive control (PC, without derivative **8**). Group of without derivative **8** was negative control (NC).

2.2.3 The three replications were operated.

2.2.4 Water quality at 27-28 °C pH 7.8 and 4.6 ppm D.O were controlled.

2.2.5 Percent survival rate of *L. vannamei* adult was examined after acclimation them to normal condition.

2.3 Live transportation of *L. vannamei* adult in plastic bag.

2.3.1 Adult samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

2.3.2 Ten adult at stage 2 of anesthesia were randomly selected to 7"x11" plastic bag with 200 mL of 30 ppt water containing 2.5 ppm derivative **8** and positive control (PC, without derivative **8**). Group of without anesthesia and derivative **8** was negative control (NC).

2.3.3 Shaking the transporting plastic bags every 1 hour for 24 hours was operated. The three replications were done.

2.3.4 Water quality at 27-30 °C pH 7.8 and 5.2 ppm D.O were controlled.

2.3.5 Percent survival rate of *L. vannamei* adult was examined after acclimation them to normal condition.

3. Post larvae *L. calcarifer*

Post larvae (PL) of *L. calcarifer* (1.12±0.15 cm in total length) were obtained from the private hatchery. They were acclimated in 30 ppt water, 27-29 °C; pH 7.8-8.2 and 5.2 ppm D.O. in 1000 L fiber glass tank.

3.1 Eugenol derivatives assay

3.1.1 Ten *L. calcarifer* PL were randomly selected to the static 3.5 L aquaria containing 1 L of 30 ppt water.

3.1.2 Eugenol derivatives from **1** to **20** at concentrations of 25 and 50 ppm were introduced to each aquaria and control (**CT**) (without anesthetic agent).

3.1.3 Anesthetic (stage 2 of anesthesia) [18, 19] and recovery periods of individual PL were recorded. Percent survival rate of *L. calcarifer* PL was examined after acclimation them to normal condition.

3.1.4 The three replications were operated.

3.1.5 Water quality at 27-28 °C pH 7.8 and 5.2 ppm D.O were controlled.

3.2 Concentration determination for eugenol derivative **8**.

3.2.2 *L. calcarifer* PL samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

3.2.3 Five PL at stage 2 of anesthesia were randomly selected to each 3.5 L aquaria containing 0.5 L of 30 ppt water containing derivative **8** at 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6 and 6.5 ppm and positive control (PC, without derivative **8**). Group of without derivative **8** was negative control (NC).

3.2.4 The three replications were operated.

3.2.5 Water quality at 27-28 °C pH 7.8 and 5.2 ppm D.O were controlled.

3.2.6 Percent survival rate of *L. calcarifer* PL was examined after acclimation them to normal condition.

3.3 Live transportation of *L. calcarifer* PL in plastic bag.

3.3.1 PL samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

3.3.2 Five PL at stage 2 of anesthesia were randomly selected to 4.5"x7" plastic bag with 100 mL of 30 ppt water containing 2.5 ppm derivative **8** and positive control (PC, without derivative **8**). Group of without anesthesia and derivative **8** was negative control (NC).

3.3.3 Shaking the transporting plastic bags every 1 hour for 24 hours was operated. The three replications were done.

3.3.4 Water quality at 27-30 °C pH 7.8 and 5.2 ppm D.O were controlled.

3.3.5 Percent survival rate of *L. calcarifer* PL was examined after acclimation them to normal condition.

4. Post larvae *C. macrocephalus*

Post larvae (PL) of *C. macrocephalus* (1.1±0.1 cm in total length) were obtained from the private hatchery. They were acclimated in 30 ppt water, 27-29 °C; pH 7.8-8.2 and 6.5 ppm D.O. in 1000 L fiber glass tank.

4.1 Eugenol derivatives assay

4.1.1 Ten *C. macrocephalus* PL were randomly selected to the static 10 L aquaria containing 20 L of 30 ppt water.

4.1.1 Eugenol derivatives **5**, **7** to **14**, **17** and **20** at concentrations of 25 and 50 ppm were introduced to each aquaria and control (**CT**) (without anesthetic agent).

4.1.2 Anesthetic (stage 2 of anesthesia) [18, 19] and recovery periods of individual PL were recorded. Percent survival rate of *C. macrocephalus* PL was examined after acclimation them to normal condition.

4.1.3 The three replications were operated.

4.1.4 Water quality at 27-29 °C pH 7.8 and 6.5 ppm D.O were controlled.

4.2 Concentration determination for eugenol derivative **8**.

4.2.1 *C. macrocephalus* PL samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

4.2.2 Ten PL at stage 2 of anesthesia were randomly selected to each 500 L aquaria containing 100 L of 30 ppt water containing derivative **8** at 5, 10 and 20 ppm and positive control (PC, without derivative **8**). Group of without derivative **8** was negative control (NC).

4.2.3 The three replications were operated.

4.2.4 Water quality at 27-29 °C pH 7.8 and 6.5 ppm D.O were controlled.

4.2.5 Percent survival rate of *C. macrocephalus* PL was examined after acclimation them to normal condition.

4.3 Live transportation of *C. macrocephalus* PL in plastic bag.

4.3.1 PL samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

4.3.2 Twenty PL at stage 2 of anesthesia were randomly selected to 12"x24" plastic bag with 100 mL of 30 ppt water containing 10 ppm derivative **8** and positive control (PC, without derivative **8**). Group of without anesthesia and derivative **8** was negative control (NC).

4.3.3 Shaking the transporting plastic bags every 1 hour for 24 hours was operated. The three replications were done.

4.3.4 Water quality at 27-29 °C pH 7.8 and 6.5 ppm D.O were controlled.

4.3.5 Percent survival rate of *C. macrocephalus* PL was examined after acclimation them to normal condition.

5. Adult of *C. Macrocephalus*

Adult of *C. macrocephalus* (7.2±0.2 cm in total length and 80.5±3.5 g in total weight) were obtained from the private hatchery. They were acclimated in 30 ppt water, 27-29 °C; pH 7.8-8.2 and 6.5 ppm D.O. in 1000 L fiber glass tank.

5.1 Eugenol derivatives assay

5.1.1 Ten *C. macrocephalus* adult were randomly selected to the static 10 L aquaria containing 20 L of 30 ppt water.

5.2.1 Eugenol derivatives **5**, **7** to **14**, **17** and **20** at concentrations of 25 and 50 ppm were introduced to each aquaria and control (**CT**) (without anesthetic agent).

5.2.2 Anesthetic (stage 2 of anesthesia) [18, 19] and recovery periods of individual adult were recorded. Percent survival rate of *C. macrocephalus* adult was examined after acclimation them to normal condition.

5.2.3 The three replications were operated.

5.2.4 Water quality at 27-28 °C pH 7.8 and 6.5 ppm D.O were controlled.

5.2 Concentration determination for eugenol derivative **8**.

5.2.1 *C. macrocephalus* adult samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

5.2.2 Ten adult at stage 2 of anesthesia were randomly selected to each 500 L aquaria containing 200 L of 30 ppt water containing derivative **8** at 5, 10 and 20 ppm and positive control (PC, without derivative **8**). Group of without derivative **8** was negative control (NC).

5.2.3 The three replications were operated.

5.2.4 Water quality at 27-29 °C pH 7.8 and 6.5 ppm D.O were controlled.

5.2.5 Percent survival rate of *C. macrocephalus* adult was examined after acclimation them to normal condition.

5.3 Live transportation of *C. macrocephalus* adult in plastic bag.

5.3.1 Adult samples were anesthetized by 25 ppm of derivative **8** for 24 hour.

5.3.2 Ten adult at stage 2 of anesthesia were randomly selected to 12"x24" plastic bag with 100 mL of 30 ppt water containing 10 ppm derivative **8** and positive control (PC, without derivative **8**). Group of without anesthesia and derivative **8** was negative control (NC).

5.3.3 Shaking the transporting plastic bags every 1 hour for 24 hours was operated. The three replications were done.

5.3.4 Water quality at 27-29 °C pH 7.8 and 6.5 ppm D.O were controlled.

5.3.5 Percent survival rate of *C. macrocephalus* adult was examined after acclimation them to normal condition.