

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E47338

**SYNTHESIS OF EUGENOL DERIVATIVES FOR ANESTHETIC TEST IN
SOME AQUATIC ANIMALS**

THITIPHONG KHAMKHEN

**A Thesis Submitted to the Graduate School of Naresuan University
In Partial Fulfillment of the Requirements
For the Master of Degree in Chemistry
May 2012
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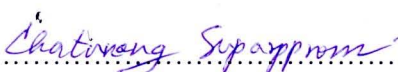
May 2012

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This thesis entitled "Synthesis of eugenol derivatives for anesthetic test in some aquatic animals" submitted by Thitiphong Khamkhen in partial fulfillment of the requirements for the Master of Science Degree in Chemistry is hereby approved.

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ACKNOWLEDGEMENT

I would like to express my sincere gratitude and appreciation to my advisor, Dr. Chaturong Suparpprom for his invaluable advice, guidance, kindness, and encouragement throughout the experimental work and make of the thesis. I also would like to all thesis committee members, and deeply grateful for their helpful comments. Furthermore, I would like to especially thank Dr. Anchalee Sirikulkajorn from the Department of Chemistry, Faculty of Science, Naresuan University for their co-advisor, Associate Professor Dr. Sakchai Wittaya-areekul from the Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Assistant Professor Dr. Boonyarath Pratoomchat and Dr. Thanomsak Boonphakdee from the Department of Aquatic Science, Faculty of Science, Burapha University for their important support, invaluable guidance, supervision and constant inspiration throughout this study. I would like to thank Miss. Haruthai Pansuwan, Miss. Sudarat Saenset, Miss. Salinee Khamseang, Miss. Pattawikorn Namrach and Mr. Suwichan Majakorn and Chaturong Research Group for assist in laboratory research. I would like to thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC) for financial support. I also express my gratitude to NU staff and all officers of the Faculty of Science, Naresuan University for facility support and sacrificing their time on this program. I am grateful to all my teachers for their instructions, my friends and colleagues of Department of Chemistry, Faculty of Science, Naresuan University for their precious helps and co-operation. Lastly, I would like to thank my beloved family for their love, care, financial support and encouragement throughout my life.

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Title	SYNTHESIS OF EUGENOL DERIVATIVES FOR ANESTHETIC TEST IN SOME AQUATIC ANIMALS
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Academic Paper	Thesis M.Sc. in Chemistry, Naresuan University, 2011
Keywords	<i>Litopenaeus vannamei</i> , <i>Lates calcarifer</i> , <i>Clarias macrocephalus</i> , <i>Eugenia caryophyllus</i> Bullock, <i>O</i> -alkylation

ABSTRACT

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Eugenol is an essential oil extracted from clove (*Eugenia caryophyllus* Bullock and Harriott MYRTACEAE) which exhibits local anesthetic properties in aquatic animals using in folk wisdom. Synthetic route of eugenol derivatives were studied to evaluate the effective candidates for anesthetic agent applied to valuable aquatic animal such as white shrimp (*Litopenaeus vannamei*), seabass (*Lates calcarifer*) and hybrid catfish female (*Clarias macrocephalus* x male *Clarias gariepinus*). In this research, the overall works were divided into three parts. First, the syntheses of eugenol derivatives have been reported in several methods such as *O*-alkylation at hydroxy group of eugenol, demethylation at methoxy group of eugenol, electrophilic aromatic substitution at aromatic ring of eugenol, smiles rearrangement and allylation *via* Grignard reaction. The hydroxyl group of eugenol was alkylated in one step with various hydrocarbon chains such as ethyl, propyl, isopropyl, butyl, *sec*-butyl, pentyl, hexyl and heptyl group to increase hydrophobicity in the molecule which afforded 9 alkylated eugenol derivatives (**5**, **7-14**) in high yield (55-98%). The hydroxyl group was changed to amino group *via* Smiles rearrangement (**4**) provided product in good yield (89%). Demethylation at hydroxyl group (**1-2**) by using MeMgI and the modification of allyl group of eugenol (**17**) *via* hydrogenation by using trifluoroacetic acid and hydrogen gas over Pd/C as reducing agent provided in good yield. In addition, electrophilic aromatic substitutions reaction at the *ortho*-position of eugenol such as nitration and bromination provide the desired product in 48% yield for bromine and nitro groups (**18**, **19**) and good yield (55% and 48%) for bromination by

using pyridinium hydrobromide perbromide (6, 15). Second, the emulsions of synthesized compounds (1-20) were prepared by the phase inversion technique. The water phase containing the emulsifier (Tween 80 and span 80) was heated to 75 °C and then added to the oily phase (eugenol derivatives) at 72 °C while continuously stirring. And, the prepared emulsion was homogenized for 5 min at 8500 rev./min. In final part, the aquatic animals were tested with eugenol derivatives and the results showed that low concentration can cause the unconscious in stage 2. The unconscious period of time was approximately 20-24 hours compared with eugenol standard. When considering the concentration at 3.5, 2.5, 2.5 and 10 ppm, it was found that derivative 8 is the most appropriate for transportation *L. vannamei* post larvae and adult, *L. calcarifer* post larvae and *C. macrocephalus* post larvae and adult with high percent survival rate. In summary, best anesthesia agent for aquatic animals was derivative 8 because of the short time of anesthetic, longer recovery period of time unconscious and high percent survival.

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ABBREVIATIONS

δ	=	chemical shift
AcOH	=	acetic acid
aq	=	aqueous
CT	=	control
CDCl_3	=	deuterated chloroform
18-crown-6	=	1,4,7,10,13,16-hexaoxacyclooctadecane
Cv%	=	coefficient of variation
d	=	doublet
dd	=	doublet of doublet
DABCO	=	1,4-diazabicyclo [2,2,2] octane
DBDMH	=	1,3-dibromo-5,5-dimethylhydantoin
DBU	=	1,8-diazabicyclo [5.4.0] undec-7-ene
DBN	=	1,5-diazabicyclo [4.3.0] non-5-ene
DMA	=	<i>N,N</i> -dimethyl acetamide
DMAP	=	4-dimethyl aminopyridine
DMF	=	<i>N,N'</i> -dimethylformamide
DMPU	=	1,3-dimethyl-2-oxo-hexahydropyrimidine
DMSO	=	dimethyl sulfoxide
ds	=	double strand
equiv	=	equivalent (s)
Et_2O	=	diethyl ether
EtOAc	=	ethyl acetate
EtI	=	ethyl iodide
FDA	=	food drug administration
g	=	gram
h	=	hour
Hz	=	hertz
<i>J</i>	=	coupling constant

ABBREVIATIONS (CONT.)

L	=	litre
LC ₅₀	=	lethal concentration 50
Max	=	maximum
m	=	multiplet
MeCN	=	acetonitrile
M	=	molar
mg	=	milligram
MHz	=	megahertz
Min	=	minute
mL	=	milliliter
mmol	=	millimol
MS222	=	tricanemethan sulfonate
NBS	=	<i>N</i> -bromosuccinimide
NMP	=	<i>N</i> -methylpyrrolidinone
NMR	=	nuclear magnetic resonance
°C	=	degree celsius
Pd	=	palladium
pH	=	potential of hydrogen ion
PHP	=	pyridinium hydrobromide perbromide
ppm	=	part per million
ppt	=	part per thousand
rev	=	revolution
rt	=	room temperature
s	=	singlet
sec	=	second
SD	=	standard deviation
Span 80	=	sorbitan (<i>Z</i>)-mono-9-octadecenoate
TBAB	=	<i>tetra-n</i> -buthylammonium bromide
t	=	triplet
TFA	=	trifluoroacetic acid

ABBREVIATIONS (CONT.)

THF	=	tetrahydrofuran
TLC	=	thin layer chromatography
Tween 80	=	polyoxyethylene (20) sorbitan monooleate
UV	=	ultraviolet