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

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Thitiphong Khamkhen

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ABSTRACT

Eugenol is an essential oil extracted from clove (*Eugenia caryophyllus Bullock* and Harrion 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. Frist, 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%). Demthylation 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	=	*N,N*-dimethyl acetamide
DMAP	=	4-dimethyl aminopyridine
DMF	=	*N,N'*-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
J	=	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	=	*N*-bromosuccinimide
NMP	=	*N*-methylpyrrolidinone
NMR	=	nuclear magnetic resonance
^{o}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 temparature
s	=	singlet
sec	=	second
SD	=	standard deviation
Span 80	=	sorbitan (Z)-mono-9-octadecenoate
TBAB	=	*tetra-n*-buthylammonium bromide
t	=	triplet
TFA	=	trifluoroacetic acid

ABBREVIATIONS (CONT.)

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