Larvicidal Principles From Some Insecticidal Plants of Sarawak

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A Dissertation
Submitted for the Degree of
Doctor of Philosophy

April, 1996
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ACKNOWLEDGEMENTS

I would like to express my deepest gratitude and sincere appreciation to Professor Dr Goh Swee Hock for his invaluable guidance, constant supervision, intellectual advice and constructive criticisms throughout the course of this study and during the preparation of this thesis. I also wish to thank Dr. Chuah Cheng Hock and Professor Yong Hoi Sen for their supervision during Professor Goh's sabbatical leave. I also benefited from Dr. Chuah's supervision and help towards the later part of this study.

My sincere thanks and deepest gratitude are also extended to Mr. Lee Han Lin of the Institute of Medical Research, Kuala Lumpur for guidance on bioassay work involving *Aedes aegypti*. I am also grateful to Mr. K. T. Wong from the Pharmacology Department, National University of Singapore for conducting *in vitro* bioassays. Many thanks also go to Gnanamalar Panandam for typing this thesis. To K. Yoganathan, Lee Kee Huat, Ooi Kay Eng, Hew Nam Fong and Dr. Richard Wong thank you for all the help you have given me throughout my course of study.

I also wish to thank my parents, my two sisters Evelyn and Susan and my two sons Joey and Lance for their patience, tolerance and moral support. To them I dedicate this work. Finally, I would like to thank my husband Jegak Uli for encouraging me to undertake this study. I also thank him for collecting plant samples and for preparation of this thesis for without him I would never have achieved this piece of work.

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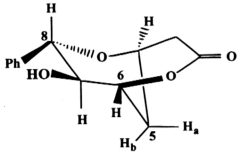
ABSTRACT

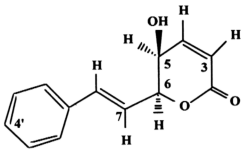
Chemical and cytotoxicity studies were made on twelve plant species of the genus *Goniothalamus* and one plant species each of the genera *Disepalum* and *Mezzetia* (Annonaceae). These plants were mostly used as insect repellents by the Iban community in Sarawak, East Malaysia.

The chemical investigations on the plant species covered larvicidal principles such as annonaceous acetogenins, styrylpyrones, flavonoids, alkaloids and essential oils (mainly sesquiterpenes). Detail studies were carried out on four *Goniothalamus* species (*G. andersonii*, *G. dolichocarpus*, *G. malayanus* and *G. velutinus*), *Disepalum anomalum* and *Mezzetia umbellata*. Natural products were isolated using chromatographic techniques and identified using spectroscopic methods (including 2-D NMR). Eight other *Goniothalamus* species (*G. ridleyi*, *G. macrophyllus*, *G. uvarioides*, *G. macranii*, *G. sinclairinius*, *G. gigantifolius*, *G. umbrosus* and *G. montanus*) were screened for selected larvicidal principles using thin layer chromatography, gas chromatography and gas chromatography-mass spectrometry.

The four *Goniothalamus* species which were studied in detail provided styrylpyrone derivatives (+)-goniothalamin, (+)-goniothalamin epoxide, (+)-goniodiol, (+)-goniothalenol, two new natural products (-)-iso-5-deoxygoniopypyrone and (+)-5β-hydroxygoniothalamin and essential oils which mainly contained a mixture of sesquiterpenes. The two new natural products were characterised spectroscopically.



(-)-Iso-5-deoxygoniopypyrone



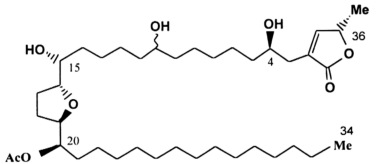
(+)-5β-Hydroxygoniothalamin

The structure assigned for (-)-iso-5-deoxygoniopypyrone is consistent with a stereoisomer of the known (+)-5-deoxygoniopypyrone previously isolated from *G. giganteus*. NOE difference spectra of the acetylated compound show NOE enhancement between the phenyl protons and H-7 and H-8, these effects suggesting that H-7 and the phenyl group are in close proximity. For an assumed preferential average conformation of the phenyl group in a quasi-equatorial position H-7 and H-8 will assume a transoid geometry. A coupling constant value of 10.4 Hz between H-7 and H-8 is in agreement of an almost antiparallel geometry of the two hydrogens. In contrast, a singlet is reported for H-8 in the compound (+)-5-deoxygoniopypyrone due to an almost orthogonal H-7 - H-8 orientation. This new compound was synthesised from the *erythro*-(6*R*, 7*S*, 8*S*)-goniodiol using a catalytic amount of DBU in THF.

The structure of (+)-5β-hydroxygoniothalamin was assigned using ^{1}H-^{1}H COSY, ^{1}H-^{13}C HETCOR and NOE difference spectra. A coupling constant value of 2.9 Hz between H-5 and H-6 indicates a dihedral angle of approximately 50°. Moreover, the acetate derivative also shows a similar $J_{5,6}$ coupling of 2.9 Hz. There is no NOE enhancement between H-7 and H-5. This new compound and its diastereomer (+)-5α-hydroxygoniothalamin were synthesised by reacting

goniothalamin with selenium dioxide and refluxing in dioxane for three and a half hours.

Goniothalamus dolichocarpus furnished two very cytotoxic flavonoids naringenin and pinocembrin. The four *Goniothalamus* species also provided very cytotoxic annonaceous acetogenins, annonacin and goniothalamicin along with the phenanthrene lactam, aristolactam BII and the dioxoaporphine ouregidione. Annonacin and aristolactam BII were also present in *Mezzetia umbellata*. *Disepalum anomalum* gave a common oxoaporphine liriodenine and a new annonaceous C-39 acetogenin was found for the first time in this genus. The new natural product disepalin has a mono-THF ring with an adjacent acetate group at the C-20 position. The structural assignments of this compound were determined using 2D-NMR (HMBC, COSY, H-C HETCOR, NOESY), NOE difference spectra and mass spectrometric techniques. Stereochemical assignments were by correlations with reported NMR data in addition to the new NMR data obtained.



Disepalin

The eight other *Goniothalamus species* were also found to have (+)-goniothalamin, (+)-goniothalamin epoxide, (+)-goniothalenol, (+)-goniodiol, (+)-

goniodiol diacetate and the complex mixture of essential oils containing sesquiterpenes. Other natural products such as (-)-iso-5-deoxygoniopypyrone, naringenin and pinocembrin, aristolactam BII and ouregidione were also found to be present in some of these plants. Annonacin was detected only in *Goniothalamus macranii*. The various styrylpyrones, alkaloids, flavonoids and acetogenins isolated were tested on the larvae of *Aedes aegypti* using the WHO (1981a) standard procedures with slight modifications. The pure compounds tested showed moderate to good larvicidal activity, the most cytotoxic compounds being naringenin and annonacin (LC_{50} values 3.7 and 9.5 µg/ml respectively).

Crude stem bark extracts of all twelve *Goniothalamus* species, *Disepalum anomalum* and *Mezzetia umbellata* were screened for their larvicidal activity against the larvae of *Aedes aegypti*. Most crude hexane extracts were moderately cytotoxic except for that from *G. gigantifolius*, while that of *Disepalum anomalum* and *Mezzetia umbellata* mildly cytotoxic. The crude ethanol extracts of *G. sinclairinius* and *G. montanus* were not cytotoxic to the larvae of *Aedes aegypti*, while the rest of the extracts showed larvicidal activity. The mosquito larvae of *Aedes aegypti* were susceptible to the methanol-soluble fractions partitioned from the ethanol extracts and good activity was found. Preliminary *in vitro* cytotoxicity screening against P388 cell lines was also carried out on the crude extracts and some bioactivity was detected which corroborates the presence of bioactive acetogenins and some of the styrylpyrone derivatives.

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ACRONYMS/ABBREVIATIONS USED IN TEXT

α	alpha
β	beta
δ	chemical shift in ppm
γ	gamma
μg	micro gram
^{0}C	degree in celsius
ATP	adenosine triphosphate
brd	broad doublet
brs	broad singlet
BSTFA	N,O-bis (trimethylsilyl)-trifluoroacetamide
CIMS	Chemical ionization mass spectroscopy
cm	centimetre
COSY	Correlated Spectroscopy
^{13}C	carbon-13
CL	confidence limit
d	doublet
DBU	1,8-diazabicyclo [5.4.0] undec-7-ene
dd	doublet of doublet
ddd	doublet of doublet of doublet
dddd	doublet of doublet of doublet of doublet
DEPT	Distortionless enhancement by polarization transfer
DMSO	dimethylsulfoxide
DNP	dinitrophenol
dt	doublet of triplet
ED	effective dose
EIMS	Electron impact-mass spectroscopy
FAB	Fast Atom Bombardment
Fe-S	ferrous sulphide
FI	fluorescence inhibition
FP	frond proliferation
g	gram(s)
GC	Gas Chromatography
GC-MS	Gas Chromatography-mass spectroscopy
GI	growth inhibition
^{1}H	proton
HETCOR	Heteronuclear chemical shift-correlation
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum NMR
HMQC	^{1}H-Detected Heteronuclear Multiple-Quantum Coherence via Direct Coupling
HOAc	acetic acid
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrum
Hz	hertz

id	internal diameter
IR	infrared
J	coupling constant in Hz
l	litre
LC	lethal concentration
LD	lethal dose
Lit.	literature
LPRL	log-probit regression lines
LR	long range coupling constant
m	multiplet
MCPBA	*m*-chloroperoxybenzoic acid
MHz	megahertz
ml	millilitre(s)
mm	millimetre(s)
mol	moles
Mol. wt.	molecular weight
m.p.	melting point
MS	mass spectrum/spectra/spectrometer/spectroscopy
MTBE	methyl tert-butyl ether
MTPA	methoxytrifluoromethylphenylacetate
NA	not available
NADH	nicotinamide adenine dinucleotide, reduced form
NCI	National Cancer Institute
nm	nanometre(s)
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Enhancement
NOESY	Nuclear Overhauser and Exchange Spectroscopy
OAc	acetate
PCN	propionitrile
Ph	phenyl
PLC	preparative layer chromatography
ppm	parts per million
q	quartet
dq	doublet of quartet
[R]	corresponding alkyl group
RI	refractive index
S	singlet
SE	standard error
t	triplet
td	triplet of doublets
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
TMPD	N,N,N,N'-tetramethyl-p-phenylenediamine
UV	ultra violet
WHO	World Health Organization