MOLECULAR IDENTIFICATION

OF

CYTOTOXIC COMPOUNDS FROM A MARINE ORGANISM

Thesis submitted by

Antonius R B Ola, S.Si (Gadjah Mada University)

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in the Department of Chemistry

James Cook University of North Queensland

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I, the undersigned of this work, acknowledge the contribution of others to this work. Substantial supervision was provided Assoc. Prof. Bruce Bowden (School of Pharmacy and Molecular Sciences, JCU). Editorial assistance in the preparation of this thesis was provided by Assoc. Prof. Bruce Bowden. Ms Cherrie Moti at the Australian Institute of Marine Sciences, Townsville, performed mass spectrometry (ESI-MS) on samples. Dr Anna-Marie Babey (School of Biomedical Sciences, JCU) performed cytotoxic assay.

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I, the undersigned and author of this work, declare that the research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *James Cook University Policy on Experimentation Ethics*, *Standard Practices and Guidelines on Research Practice (2001)*, and the *James Cook University Statement and Guidelines on Research Practice (2001)*. Research involving the use of animals followed the Australian code of Practice for the Care and Use of Animals for Scientific Purposes and the Queensland Animal Care and Protection Act 2001.

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I DEDICATE THIS WORK TO MY WIFE,

Ms. MARIA RENNY PRAPTIWI AND MY DAUGHTER GRACE

MICHELLE AUSSIOLA

ABSTRACT

The marine world has become an important source of anticancer lead drugs with novel mechanisms of action. Many of these are in active phase I or phase II clinical trials while Yondelis in 2007 was approved for clinical use for the treatment of advanced sarcoma. This study aims to identify the molecular structure of pharmacologically active metabolites from tropical marine organisms.

The study of pharmacological activity was focused on discovering novel cytotoxic compounds with potential as anticancer agents. Cytotoxicity was assessed *in vitro* using the P388D1 mouse lymphoma cell line. The structural elucidation was done via 1D and 2D NMR. From the marine red alga *Chondria armata*, seven new halogenated triterpene polycylic ethers, armatols G-J and aplysiols C-E were isolated. The absolute stereochemistry of armatol G was determined through an X-ray structure while the stereochemistry of armatol H was proposed from acetylation of armatol G which indicated the same absolute configuration as was present in armatol G. The relative stereochemistry of armatol I and J were established via nOe experiments and by using Chem 3D and its MM2 energy minimization program to predict the lowest energy conformations. The relative stereochemistry of aplysiols C-E was determined by comparison with the very closely related structure of aplysiol B and from gradient selective NOESY experiments. Armatol J showed potent cytotoxic activity with an IC_{50} value of 5 μg/mL while armatol I and aplysiol C had moderate cytotoxic effects with IC_{50} value of 18 and 25 μg/mL respectively.

Seven known compounds were also isolated from the alga included 3 halogenated C_{15} acetogenins, two diterpenes [(-) angasiol and (-) angasiol acetate], a triterpene, intricatetraol and a diketopiperazine (dihydrodisamide C). (-) Angasiol acetate is the enantiomer of the diterpene reported from the sea hare *Aplysia juliana* and (-) pinnatifidenyne is the enantiomer of the C_{15} acetogenin produced by the marine red alga *Laurencia pinnatifida* from Canary Island. (-) Angasiol and (-) angasiol acetate were found to be inactive while intricatetraol and C_{15} acetogenins showed weak activity. Dihydrodisamide C was among the most potent of all the purified metabolites tested with an IC_{50} value of 5 μg/mL. It is probable that the collection of *Chondria armata* may have been contaminated by the sponge *Dysidea herbacea* from the substrate on which the alga was growing, or that the dihydrodysamide C was exuded by *D.herbacea* and absorbed into the alga.

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LIST OF ABBREVIATIONS

1D	one dimensional
2D	two dimensional
br d	broad doublet
C_6D_6	deuterated benzene
$CDCl_3$	deuterated chloroform
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
dddd	doublet of doublet of doublet of doublets
ddq	doublet of doublet of quartets
ESI-MS	Electrospray Ionisation Mass Spectrometry
EtOAc	ethyl acetate
FCS	foetal calf serum
HMBC	Heteronuclear Multiple-Bond Coherence
HSQC	Heteronuclear Single-Quantum Coherence
HPLC	High-performance Liquid Chromatography
i.d.	internal diameter
IR	Infrared
m	multiplet
MeOH	methanol
m.p.	melting point
NCI	National Cancer Institute, Washington DC
NMR	Nuclear Magnetic Resonance
nOe	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
PDA	photodiode-array
ROESY	Rotational Overhauser Effect Spectroscopy
S	singlet

sp.	species (singular)
spp.	species (plural)
SRB	sulforhodamine B
TCA	trichloroacetic acid
t	triplet
Tris	tris(hydroxymethyl)aminomethane
UV	Ultraviolet
Vis	Visible Light