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**MOLECULAR IDENTIFICATION  
OF  
CYTOTOXIC COMPOUNDS FROM A MARINE ORGANISM**

**Thesis submitted by**

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**for the degree of Master of Science**

**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 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 polycyclic 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<sub>50</sub> value of 5 µg/mL while armatol I and aplysiol C had moderate cytotoxic effects with IC<sub>50</sub> value of 18 and 25 µg/mL respectively.

Seven known compounds were also isolated from the alga included 3 halogenated C<sub>15</sub> 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<sub>15</sub> 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<sub>15</sub> acetogenins showed weak activity. Dihydrodisamide C was among the most potent of all the purified metabolites tested with an IC<sub>50</sub> 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 dihydrodisamide C was exuded by *D. herbacea* and absorbed into the alga.

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|                                     |   |
|-------------------------------------|---|
| <b>1D</b>                           | one dimensional                           |
| <b>2D</b>                           | two dimensional                           |
| <b>br d</b>                         | broad doublet                             |
| <b>C<sub>6</sub>D<sub>6</sub></b>   | deuterated benzene                        |
| <b>CDCl<sub>3</sub></b>             | deuterated chloroform                     |
| <b>CH<sub>2</sub>Cl<sub>2</sub></b> | dichloromethane                           |
| <b>CHCl<sub>3</sub></b>             | chloroform                                |
| <b>COSY</b>                         | Correlated Spectroscopy                   |
| <b>d</b>                            | doublet                                   |
| <b>dd</b>                           | doublet of doublets                       |
| <b>ddd</b>                          | doublet of doublet of doublets            |
| <b>dddd</b>                         | doublet of doublet of doublet of doublets |
| <b>ddq</b>                          | doublet of doublet of quartets            |
| <b>ESI-MS</b>                       | Electrospray Ionisation Mass Spectrometry |
| <b>EtOAc</b>                        | ethyl acetate                             |
| <b>FCS</b>                          | foetal calf serum                         |
| <b>HMBC</b>                         | Heteronuclear Multiple-Bond Coherence     |
| <b>HSQC</b>                         | Heteronuclear Single-Quantum Coherence    |
| <b>HPLC</b>                         | High-performance Liquid Chromatography    |
| <b>i.d.</b>                         | internal diameter                         |
| <b>IR</b>                           | Infrared                                  |
| <b>m</b>                            | multiplet                                 |
| <b>MeOH</b>                         | methanol                                  |
| <b>m.p.</b>                         | melting point                             |
| <b>NCI</b>                          | National Cancer Institute, Washington DC  |
| <b>NMR</b>                          | Nuclear Magnetic Resonance                |
| <b>nOe</b>                          | Nuclear Overhauser Effect                 |
| <b>NOESY</b>                        | Nuclear Overhauser Effect Spectroscopy    |
| <b>PDA</b>                          | photodiode-array                          |
| <b>ROESY</b>                        | Rotational Overhauser Effect Spectroscopy |
| <b>S</b>                            | singlet                                   |



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