Ascidians and Sea Hares: Rich Sources of Bioactive Natural Products

by

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A thesis submitted

in fulfilment of the requirements for the degree of

Doctor of Philosophy

in Chemistry

November 2002

Department of Chemistry

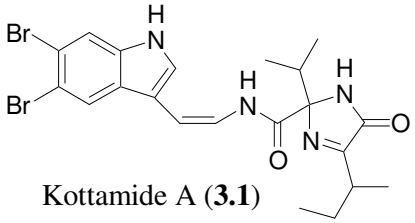
The University of Auckland

Abstract

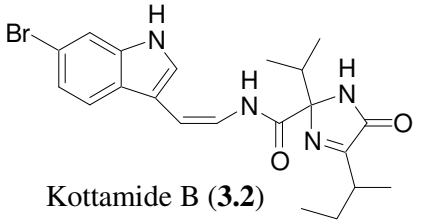
A survey of the metabolite content of fifty-two ascidians collected from New Zealand and Antarctica is presented. Using biological assays and reversed phase analytical C_{18} HPLC techniques, twenty-three specimens were selected for further work. Work on ten of the selected ascidians is presented in this study. Using standard chromatographic techniques combined with analytical C_{18} HPLC, eighteen metabolites were isolated of which sixteen exhibited biological activity. Ten of the eighteen metabolites were novel, with nine exhibiting biological activity. The New Zealand endemic ascidian *Pycnoclavella kottae*, collected from the Three Kings Islands yielded the novel 2,2,5-trisubstituted imidazol-4-ones, kottamides A - D (**3.1** - **3.4**). Three new pyridoacridines (**4.80**, **4.82**, **4.84**), along with two known pyridoacridines and the known benzopentathiepin, varacin were isolated from the New Zealand endemic ascidian *Lissoclinum notti*. Varacin was investigated for its use in ADEPT and a possible prodrug derivative was prepared. In addition, a novel biologically inactive purine, 8-oxo-1,3-dimethylisoguanine (**6.29**) was isolated from the New Zealand endemic ascidian *Pseudodistoma cereum*. The study of an ascidian of the genus *Aplidium*, collected from the Ross Sea, Antarctica resulted in the isolation of two novel quinone derivatives, rossinones A (**5.71**) and B (**5.72**), which exhibited potent cytotoxicity and antiviral activity. Rossinones A (**5.71**) and B (**5.72**) provide insights into the biosyntheses of several terrestrial plant natural products.

In addition, six sea hares of three species collected from the Auckland, New Zealand region were surveyed using the same methodology, with three novel and seven known compounds being isolated. The study of the sea hare *Aplysia dactylomela* resulted in the isolation of two inactive novel tryptophan dipeptides, dactylamides A (**7.9**) and B (**7.10**), the known ink pigment aplysioviolin and four known sesquiterpenes. A new malyngamide, S (**8.8**) along with an algal toxin, lyngbyatoxin A and its acetate were isolated from the sea hare *Bursatella leachii*. A survey of nine algae collected from the same locations as the sea hares resulted in the identification of several of the dietary sources of these sea hares.

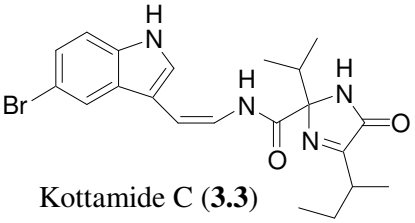
Standard spectroscopic techniques were used for structural elucidation, including the use of natural abundance ^{1}H-^{15}N 2-D NMR where required. All compounds were assayed for a range of biological activities.



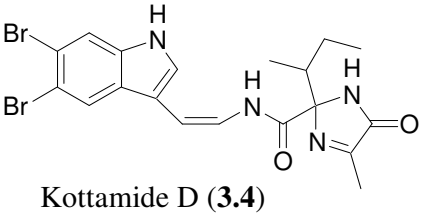
Kottamide A (**3.1**)



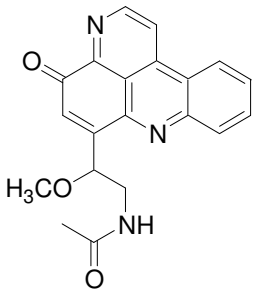
Kottamide B (**3.2**)



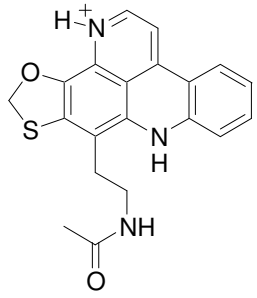
Kottamide C (**3.3**)



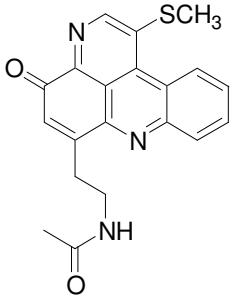
Kottamide D (**3.4**)



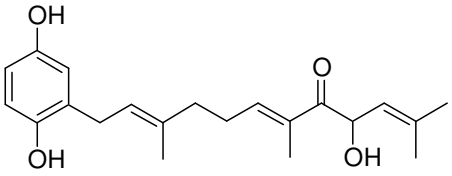
Cystodytin K (**4.80**)



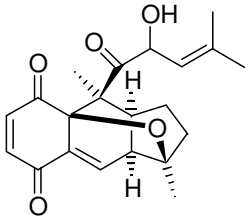
Lissoclinidine (**4.82**)



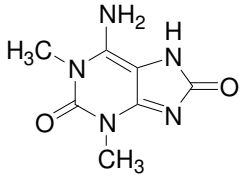
Isodiplamine (**4.84**)



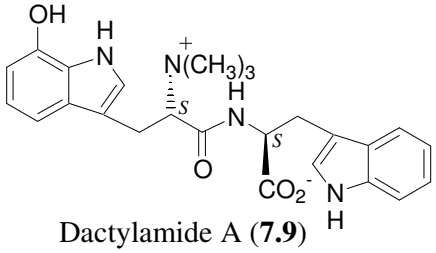
Rossinone A (**5.71**)



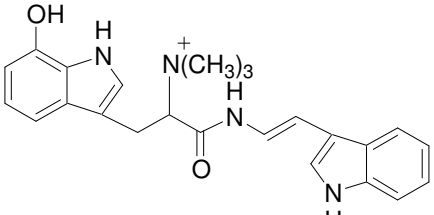
Rossinone B (**5.72**)



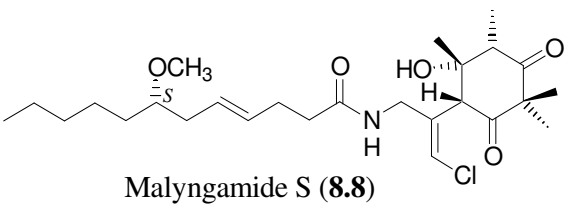
8-Oxo-1,3-DMiG (**6.29**)



Dactylamide A (**7.9**)



Dactylamide B (**7.10**)



Malyngamide S (**8.8**)

Acknowledgements

To my supervisor Dr Brent Copp: Your constant enthusiasm, interest and dedication amaze me. You have made this experience more than I could have ever hoped for. I have learnt so much from you. Thank you so much for everything - may all of your dreams and goals succeed in the future.

Well, what a surprise... I came back to university to further my education - who would have known that I would meet the girl of my dreams and be happily married by now? To the bestest wife Yu Huay (Michelle), you swept me off my feet. Thanks for making allowances for my idiosyncrasies during this work and then for reading and checking it through so well for me. I will always love you my SP. More proof that Chemistry CAN be fun.

I owe everything to my wonderful family. My endless thanks and love to Dad and Mum for all you have done for me and taught me over the years. You have always encouraged me to do whatever makes me happy, and to do it to the best of my ability, and then you've been there for me the whole way. Thank you so much! Thanks to my most excellent sister Debs, who has got closer to me as the years pass. I really appreciate your love for me, and for always being there.

To Dr Norrie Pearce, thank you so much for all the helpful advice and training, never complaining and being ever so patient with my many questions. Thanks also for your friendship, which I hope continues through the years to come, and for your significant contribution to making my last three years the best I have had so far. Thanks also to the others who have had the joy (???) of sharing a laboratory with me; Pierre Tulasne, Heather Wansborough, Katherine Shirley, Jamie Yim, Ming-Chun Wu and Tanya Grkovic.

My sincere thanks and gratitude go to Associate Professor Paul Woodgate, who is a truly gifted and inspiring teacher, for his useful advice along the way, and to Michael Walker for the many, many hours he has spent running mass spectra and NMR spectra for me and all the advice - it really is appreciated. Thanks also to the many other staff and students of the Department of Chemistry who have made this experience so enjoyable.

I would like to thank the Royal Society of New Zealand and especially The University of Auckland for the much appreciated funding during the last three years to make this financially possible.

Many thanks to Dr Michael Page of the National Institute of Water and Atmospheric research (NIWA), Associate Professor Russell Babcock, Dr Mary Sewell, David Todd and Nick Shears (UofA), and Dr Peter Northcote (Victoria University, Wellington) for assistance with specimen collections. Thanks also to Dr Gretchen Lambert (State University of Fullerton, USA), Dr Pat Kott (Queensland Museum, Australia) in addition to some of those above, for specimen identifications.

I would also like to thank Ms Gill Ellis (University of Canterbury) for the P388, cytotoxicity and antimicrobial assays, Dr V. Narayanan (NCI, Bethesda) for in vitro human antitumour assays, Tuberculosis Antimicrobial Acquisition and Co-ordinating Facility (TAACF) in contract to National Institute of Allergy and Infectious Diseases (USA) for antimycobacterial assays and Dr Michael Berridge and Mr An S. Tan (Malaghan Institute of Medical Research, Wellington) for anti-inflammatory and anti-metabolic assays. I am also extremely grateful to Professor Roberto Berlinck of the Universidade de Sao Paulo for obtaining circular dichroism spectra.

Thanks to God, for being so trustworthy, always putting me in the right place at the right time and for his limitless blessings that are too many to list.

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Abbreviations

Ac	Acetyl
ADEPT	Antibody-directed enzyme prodrug therapy
aq	Aqueous
atm	Atmospheres
b	Broad
BOC	t-Butyloxycarbonyl
BOP	Benzotriazol-1-yloxy tri(dimethylamine)phosphonium hexafluorophosphate
B.s.	*Bacillus subtilis*
C_{18}	Octadecyl-derivatised silica
C.a.	*Candida albicans*
Calcd	Calculated
CD	Circular dichroism
CI	Chemical ionisation
COSY	Gradient correlation spectroscopy (^{1}H-^{1}H)
d	Doublet
DBU	1,8-Diaza-bicyclo[5.4.0]undec-7-ene
dec	Decomposed
DEPT	Distortionless enhancement by polarisation transfer
DHA	Docosahexaenoic acid
DMG	Dimethylguanine
DMiG	Dimethylisoguanine
DNA	Deoxyribonucleic acid
DPPA	Diphenylphosphorylazide
E.c.	*Escherichia coli*
ED_{50}	Median effective dose
EI	Electron impact
EPA	Eicosapentaenoic acid
ETA	Eicosatetraenoic acid
Et	Ethyl
et al.	et alii
FAB	Fast atom bombardment
fMLP	*N*-Formyl-methionyl-leucyl-phenylalanine
GC-MS	Gas chromatography-mass spectrometry
GI_{50}	50% Growth inhibition
HMBC	Gradient heteronuclear multiple-bond correlation
HPLC	High performance liquid chromatography
HR	High resolution

HSQC	Gradient heteronuclear single-quantum correlation
HSV	Herpes simplex virus
IC_{50}	50% Inhibitory concentration
IR	Infrared
LC_{50}	50% Lethal dose concentration
m	Multiplet
M	mol/L
Me	Methyl
MIC	Minimum inhibitory concentration
mp	Melting point
MS	Mass spectrometry
M.t.	*Mycobacterium tuberculosis* $H_{37}Rv$
mult	Multiplicity
m/z	Mass to charge ratio
N	Normal
NCI	National Cancer Institute of America
NMR	Nuclear magnetic resonance
No.	Number
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser enhancement spectroscopy
Obsc	Obscurred
p	Pentet
PMA	Phorbol myristate acetate
PMS	1-Methoxy phenazinemethosulfate
PNBnzOC	*para*-Nitrobenzyloxycarbonyl
ppm	Parts per million
PV1	Polio virus, Type 1
q	Quartet
Rel int%	Relative intensity (%)
resp	Respectively
RNA	Ribonucleic acid
ROESY	Rotating frame Overhauser enhancement spectroscopy
s	Singlet
sat	Saturated
SCUBA	Self contained underwater breathing apparatus
sep	Septet
sp.	Species
spp.	Species (plural)
t	Triplet
TFA	Trifluoroacetic Acid
TGI	Total growth inhibition

TLC	Thin layer chromatography
T.m.	*Trichophyton mentagrophytes*
TMG	Trimethylguanine
TMiG	Trimethylisoguanine
TOCSY	Gradient total correlation spectroscopy
UV	Ultraviolet
Vis	Visible
v/v	Volume/volume
WST-1	2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt
^{1}H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon-13 nuclear magnetic resonance
^{15}N NMR	Nitrogen-15 nuclear magnetic resonance
2-D	Two-dimensional
δ_A	Chemical shift (ppm) for nucleus A
$[\alpha]^{20}_{x}$	Optical rotation at 20 °C at 'x' nm; D = sodium D-line (489 nm)
$^{n}J_{AB}$	Coupling constant between atoms A and B, 'n' bonds apart