Chapter One
Introduction

1.1 Natural Products

Natural products, or secondary metabolites, are organic compounds that are formed by plants and animals and have no apparent function within the organism.[1] A limited number of species produce secondary metabolites. These compounds attract scientific interest because they usually exert an influence over other living organisms; often playing a part in directing interactions between plants, microorganisms, insects and animals. Defence chemicals, anti-feedants, attractants and pheromones are all natural products.[2] Mankind has been using plant extracts to soothe ailments since ancient times; in fact it is estimated that over 40 % of medicines currently in use originated from natural products and their derivatives.[2]

Marine natural products have become an increasingly popular field of research over the past 40 years. Over 12 000 novel compounds have already been described from marine plants, microorganisms and animals,[3] yet the marine environment remains relatively unexplored in comparison to its terrestrial counterpart.[4]

The ocean provides potential for a pool of structurally diverse natural products as 34 of the 36 phyla are represented in the biodiversity of the marine environment.[3] This biodiversity gives rise to ecological pressures due to factors such as competition for space, food and reproductive success as well as prevention of

overgrowth and predation. The result of this is the evolution of a large range of natural products with various degrees of biological activity and unique actions.[3] The occurrence of natural products is most common in soft-bodied sessile organisms, a lower occurrence is seen in mobile and/or armoured animals which have alternative means of defence.[5] Marine natural products need to be extremely potent, since they are rapidly diluted by the medium that they are in. This suggests drugs based on marine natural products have the potential to be more efficient and specific than many of the drugs used currently.[6] Programmes set up with the aim to discover marine based natural products, such as that run by the National Cancer Institute (NCI) of the National Institute of Health (NIH) in the United States of America (USA), have already resulted in the discovery of several clinically useful drugs as well as possible drug leads and pharmacological tools.[3] The majority of recent natural product investigations that give rise to new leads into anticancer drugs come from research into marine sources.[7]

1.2 Biology of Bryozoans

The phylum Bryozoa contains exclusively aquatic sedentary animals. Species are found in fresh and brackish water although they are predominantly marine.[8,9] The name for the phylum, Bryozoa, was coined because of the moss-like appearance of the colonies of these animals, the term Ectoprocta (from the Greek *ecto*-outside, *procta*-anus) has been used in the literature instead of Bryozoa by some authors.[8] There are over 4 000 living species in this phylum and many more that have been observed in fossil records dating back to the early Palaeozoic period.[10]

Although a few are solitary, most bryozoan species form colonies (also called zoarium)[11] that are made up of large numbers of intercommunicating individuals called zooids. The zooid structure, illustrated in Figure 1.1, has little variation between species. Bryozoans are widely distributed throughout the oceans of the world, typically in the littoral zone of the continental shelf but they are also found down to the depths of the ocean and in the intertidal region.[11] Being filter feeders, bryozoans require food-bearing water but are not restricted to depths penetrable by sunlight. Their global distribution is also as diverse; bryozoans are found from polar regions to tropical waters.[11] The range of habitats is so great due to the ability of the larvae to travel on ocean currents, attached to floating debris or migrating animals.[11]

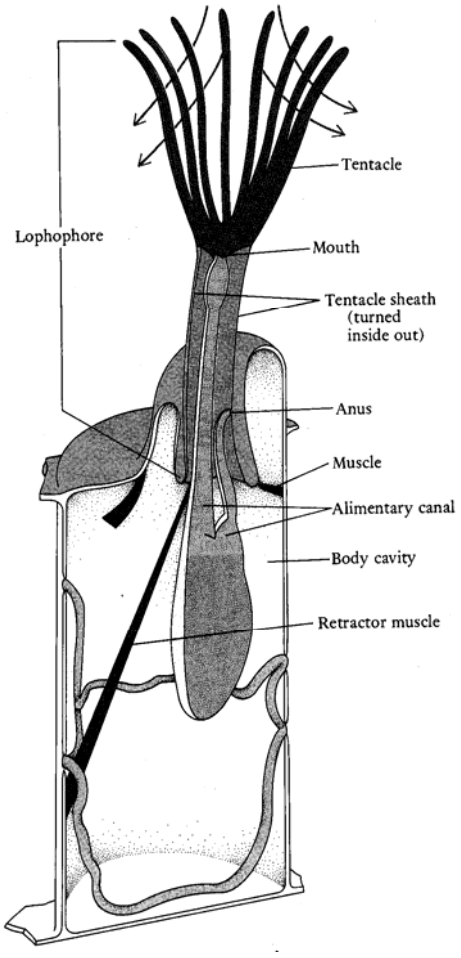


Figure 1.1: A Typical Structure of a Bryozoan Zooid.[12]

The minute size of bryozoans means that they have no need for internal transport systems, for example a circulatory system. Transport of nutrients is accomplished by movement of the coelomic fluid or by diffusion.[12] The surface area to mass ratio is such that gas exchange and excretion of ammonia can be performed at the surface, therefore there is no need for excretory or gas exchange organs either.[12]

A colony grows by asexual reproduction or budding from a primary zooid that is formed by the metamorphosis of a sexually produced larva.[12] Bryozoans are typically hermaphrodites. The gonads shed gametes into the coelom, the sperm exits through pores found at the end of the tentacles whereas eggs exit through a specific pore at the base of the tentacle.[12]

All zooids have a similar body plan but the formation of colonies has no set arrangement. There can be huge variation in the appearance of colonies between different species. Colonies are normally anchored by the substratum; the zooids grow upward from this in varying arrangements.[9] The texture of a bryozoan colony can vary greatly between species. Some bryozoans are heavily calcified making them completely rigid whereas others can be soft or even gelatinous such as some *Alcyonidium* species.[9]

The zooids have belts of tentacles, that emerge for feeding, with beating cilia that direct flow toward the mouth.[9] These tentacles are part of the lophophore, which is typically funnel shaped and protrudes from the skeletal encasement by elevated pressure of the coelomic fluid.[12]

The phylum Bryozoa has three classes divided into different orders as seen in Figure 1.2.

Phylum	***Class***	***Order***
Bryozoa	I Phylactolaemata	
	II Stenolaemata	II.1 Cyclostomata
		II.2 Cystoporata
		II.3 Trepostomata
		II.4 Cryptostomata
	III Gymnolaemata	III.1 Ctenostomata
		III.2 Cheilostomata

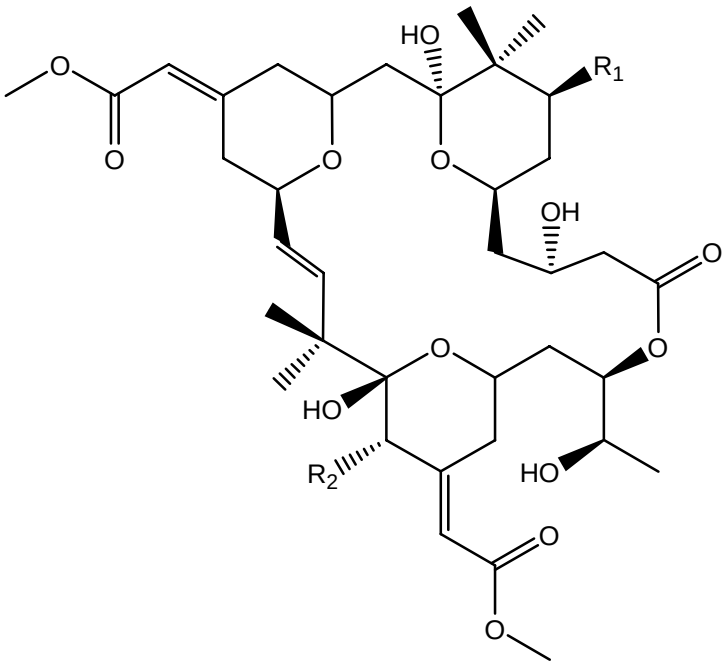
Figure 1.2: Classification of the Phylum Bryozoa. [9]

1.3 Natural Products Isolated from Bryozoans

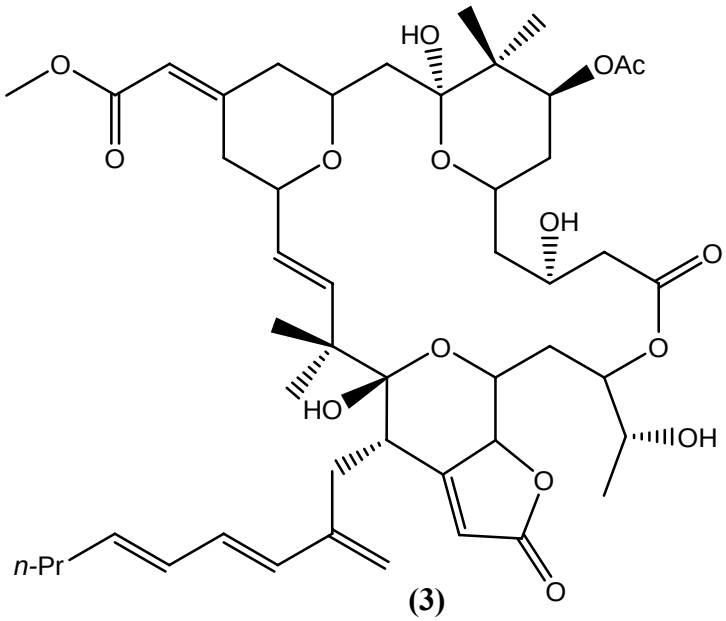
Bryozoans have been shown to produce many secondary metabolites that are structurally diverse.[13] A comprehensive review of natural products from bryozoans was published in 1985.[10] Another comprehensive review of alkaloids from marine bryozoans was published in 2001.[14] Reviews detailing the available research on marine natural products, classified by phylum, have been published yearly in *Natural Product Reports*.[15-35] A discussion of the literature concerning natural products obtained from marine bryozoans is presented here.

The bryostatins are based on a 26 membered ring system called a bryopyran system[10] and are probably the most well known natural products isolated from a bryozoan. Fourteen years after the Pettit group found that extracts from *Bugula neritina* exhibited excellent antineoplastic activity, they reported the isolation and X-ray crystal structure of bryostatin 1 (**1**).[36] Since the isolation of bryostatin 1(**1**), Pettit's group have isolated bryostatins 2-18 (**2-18**) from a range of collections from the Californian coast, the Gulf of California (Mexico), the Gulf of Mexico

and the Gulf of Sagami (Japan).[36-41] The structures of these compounds (**2-18**) were elucidated using one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) techniques, various forms of mass spectrometry (MS) including solution phase secondary mass spectrometry (SPSMS) and chemical methods such as acid catalysed hydrolysis and selective acetylation, dehydration and oxidation experiments. Bryostatins are typically present in ca 10^{-6} % -10^{-8} % dry weight. [42]



	R_1	R_2
(1)	OAc	$OCO(CH)_4$*n*-Pr
(2)	OH	$OCO(CH)_4$*n*-Pr
(4)	$OCOC(CH_3)_3$	OCOn-Pr
(5)	$OCOC(CH_3)_3$	OAc
(6)	OCO*n*-Pr	OAc
(7)	OAc	OAc
(8)	OCO*n*-Pr	OCO*n*-Pr
(9)	OAc	OCO*n*-Pr
(10)	$OCOC(CH_3)_3$	H
(11)	OAc	H
(12)	$OCO(CH)_4$*n*-Pr	OCO*n*-Pr
(13)	OCO*n*-Pr	H
(14)	$OCOC(CH_3)_3$	OH
(15)	OAc	$OCO(CH)_4CH(OH)Et$



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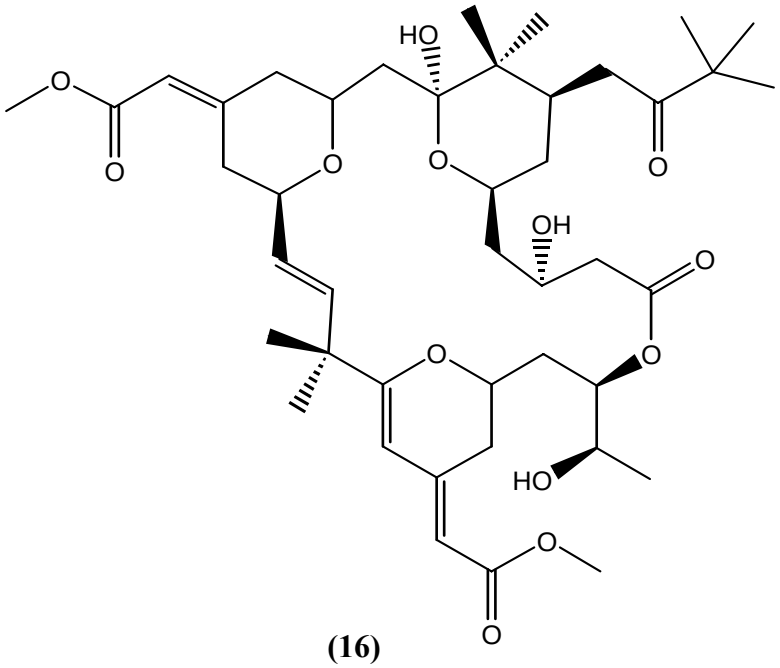

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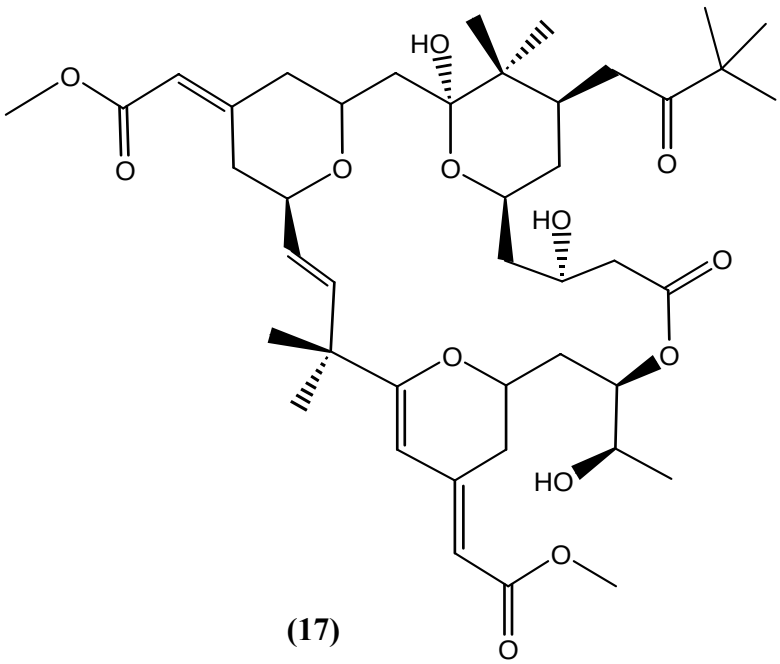

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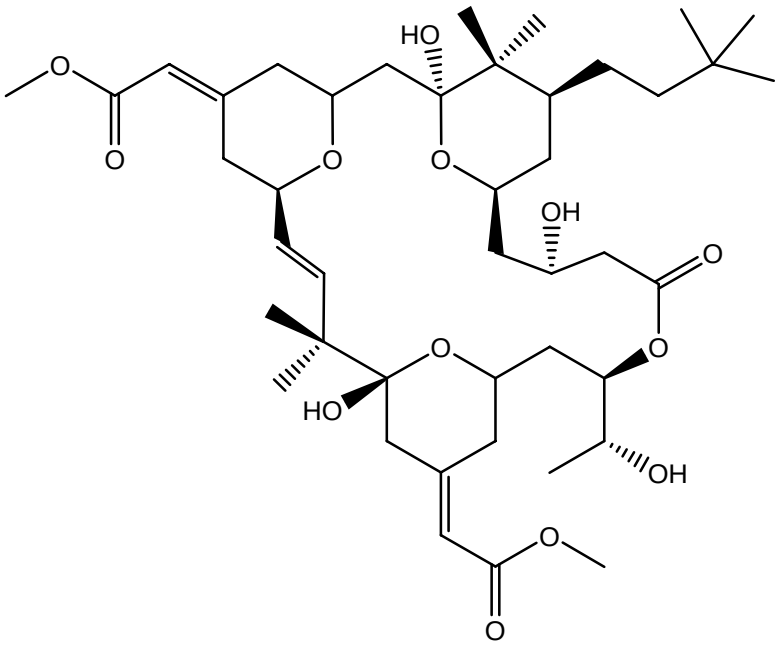
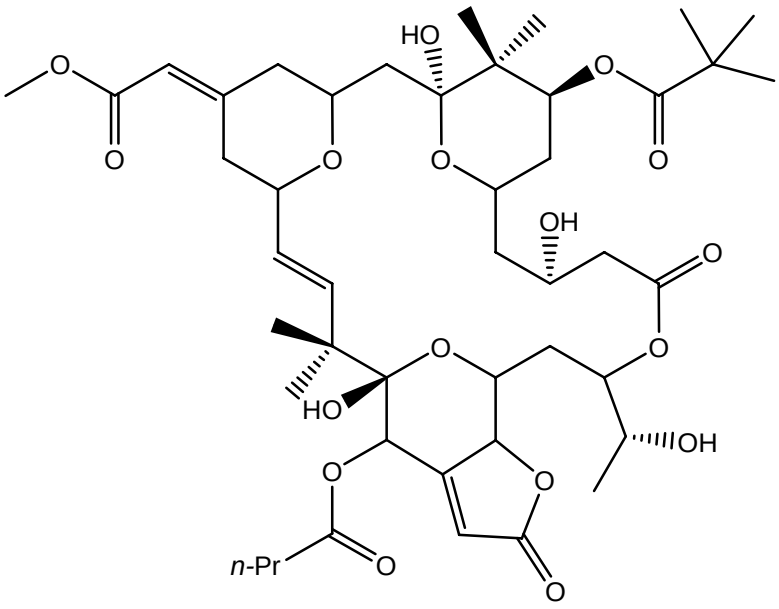


(18)

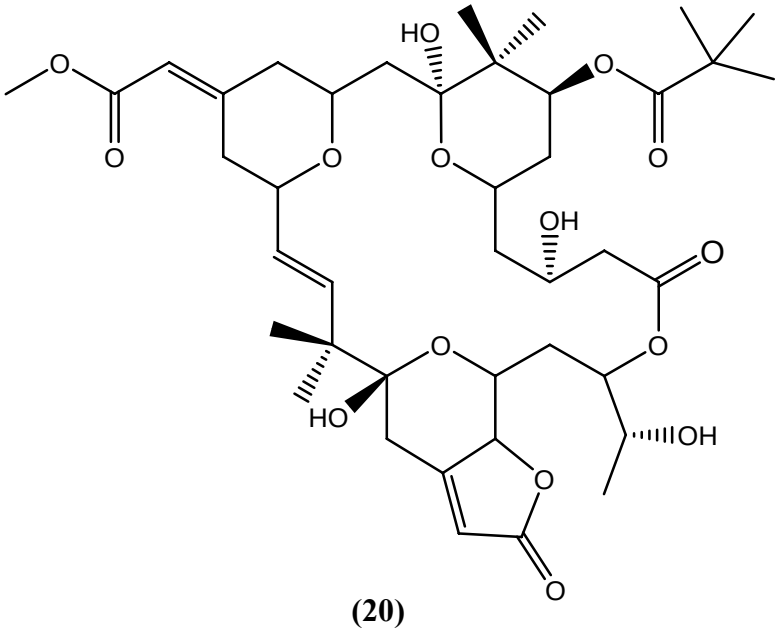
While bryostatins 1-3 (**1-3**) were found in animals collected from the Californian coast, bryostatins 4-7(**4-7**) were found in collections from the Gulf of California (Mexico), the Gulf of Mexico and the Gulf of Sagami (Japan) with little evidence of bryostatins 1-3 (**1-3**) being present.[36] Bryostatin 3 (**3**) was found to be identical to bryostatin 1 (**1**) except for a profound change on the bryopyran C ring. Bryostatins 10 (**10**), 11 (**11**) and 16-18 (**16-18**) are 20-desoxy bryostatins.[40]

Unlike the other bryostatins, bryostatin 8 (**8**) was first isolated from a collection of *Amathia convoluta* which also contained bryostatins 4-6 (**4-6**).[43] Investigation into a relationship between the two species revealed that *B. neritina* colonies were seen growing from *A. convoluta* samples in a parasitic or epiphytic manner in amounts up to 3 % dry weight.[43] Bryostatin 8 (**8**) was found in high enough levels in *A. convoluta* that it was concluded that this organism and not the invading *B. neritina* was responsible for producing it. It was unclear if the same could be said for bryostatins 4 (**4**) and 6 (**6**).



(19)

Bryostatin 19 (**19**) was isolated from a collection of *B. neritina* from the South China Sea and the structure was attained based on chemical evidence and spectroscopic data. It has significant antineoplastic activity against U937 cells (ED_{50} 2.8 x 10^{-3} μg/mL) as well as against HL-60 and K562 leukaemia cells *in vitro*.[44] Bryostatin 20 (**20**) was found in a collection of larvae of *B. neritina* and the structure was determined by spectral comparison with previously described bryostatins.[45]



(20)

These molecules offer a challenge to synthetic chemists. The first total synthesis of bryostatin 2 (**2**) was reported in 1998.[46] Bryostatin 3 (**3**) has been synthetically prepared by a Japanese group[47] and a full synthesis of bryostatin 7 (**7**) has also been accomplished.[39]

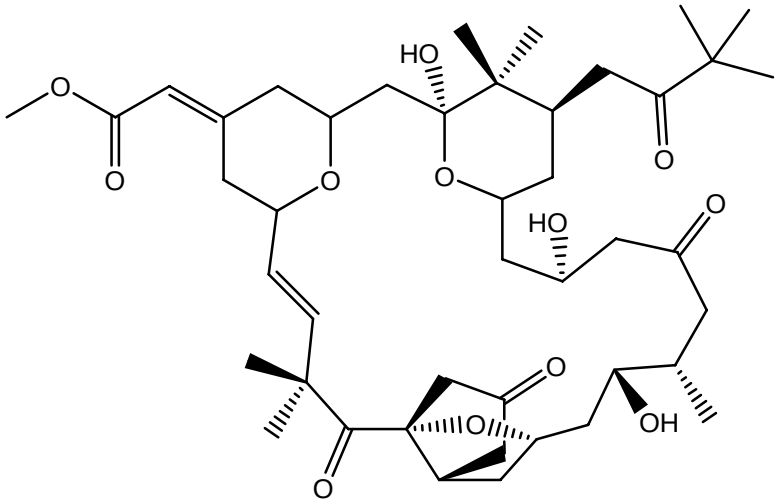
All of the bryostatins possess antineoplastic activity. Bryostatin 1 (**1**) has an ED_{50} value of 0.89 μg/mL against the P388 *in vitro* cell line,[48] while bryostatins 16-18 (**16-18**) showed significant growth inhibitory activity against the P388 cell line, (ED_{50} values 9.3 x 10^{-3} μg/mL, 1.9 x 10^{-2} μg/mL and 3.3 x 10^{-3} μg/mL respectively).[40] Bryostatin 1 (**1**) in the crystalline state exhibits a large oxygen rich cavity and it has been suggested that this could give it antibiotic potential.[10]

Bryostatins A and B have been found in a sample of the yellow sponge *Lissodendoryx isodictyalis.*[36] Full characterisation was not possible due to insufficient quantities (only 900 μg of bryostatin A and 600 μg of bryostatin B were isolated),[36] but there is evidence that these are desoxybryostatin 5 and 4 respectively.[42] Later examinations of *L. isodictyalis* samples were found to contain up to 5 % by weight *B. neritina* which indicated a possible symbiotic relationship.[36]

Due to the cytotoxicity and antitumour activity of bryostatin 1 (**1**), investigations have been carried out into its mode of action. Results indicate that it interacts with protein kinase C (PKC). Bryostatin 1 (**1**) bound PKC is translocated to the cell membrane, where it is then down regulated and degraded.[49]

Some evidence suggests that PKC modulation is not the only pathway by which bryostatins exhibit their cytotoxicity; Protein kinase D has also been implicated in mediating some of the activity of the bryostatins.[49] Bryostatin 1 (**1**) has been shown to cause reversal of multi-drug resistance (MDR) in some cancer cell lines[49] and research has demonstrated that it has influence over the immune system. [49] Results from preclinical trials illustrated that bryostatin 1 (**1**) possessed activity *in vitro* and *in vivo* against leukaemia, lymphoma, melanoma and lung cancers.[50] The first clinical trial started in 1990 and since then bryostatin 1 (**1**) has been in over eighty Phase I and Phase II trials[51] and is currently still in one Phase I trial and two Phase II trials.[52]

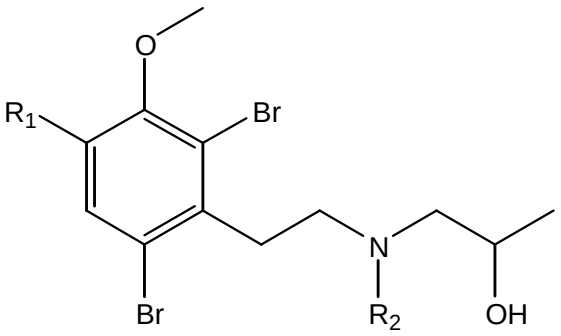
A study into possible biosynthetic precursors and degradation products of the bryostatins resulted in the isolation and characterisation of neristatin 1 (**21**). Neristatin 1 (**21**) does not possess a bryopyran ring and crystallisation attempts showed it to be a lot less stable than the bryostatins. Neristatin 1 (**21**) is only weakly active against the P388 cell line (ED_{50} of 10 μg/mL) and although it also has affinity for PKC, this affinity is two orders of magnitude less than that of bryostatin 4 (**4**).[53]



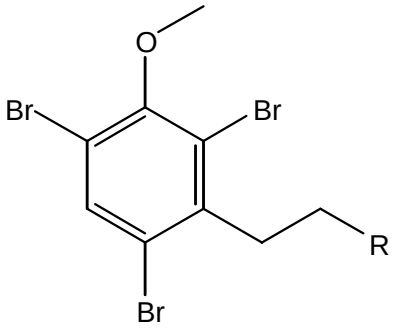
(21)

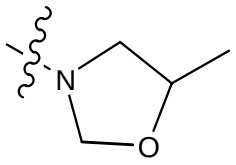
It has been shown that the bryostatins are produced by a symbiotic bacterium *Endobugula sertula* that is found in *B. neritina*.[54] The bryostatins are produced in the larval stages of *B. neritina* and protect them against predation by fish. Bryostatins deter feeding by a common planktivorous fish that co-occurs with *B. neritina* but adult colonies are not protected by the bryostatins. *B. neritina* larvae hold rod shaped bacteria within a surface invagination called a pallial sinus.[54] Evidence for the bacterial production of bryostatins was reported from an experiment where a group of larvae was treated with antibiotics to kill their population of *E. sertula*. The treated group were shown to be significantly more palatable to fish compared with the control group. High performance liquid chromatography (HPLC) analysis of the treated group showed no sign of any bryostatins present.[54]

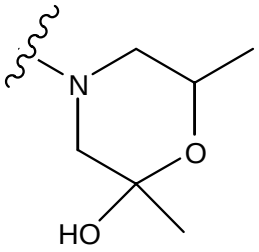
Pettit has also described a family of β-phenyl ethylamine alkaloids, convolutamines A-E (**22-26**) and the biosynthetically related convolutamides A-F (**27-32**) from the Floridian bryozoan *Amathia convoluta.*[43]

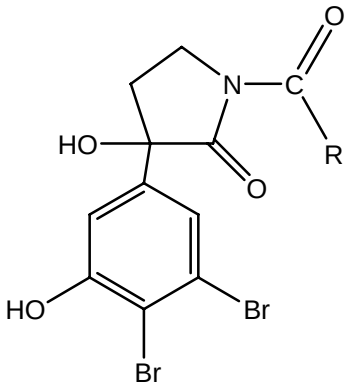


(22) R_1 = Br, R_2 = Me
(23) R_1 = H, R_2 = Me
(24) R_1 = Br, R_2 = H



(25) R= 

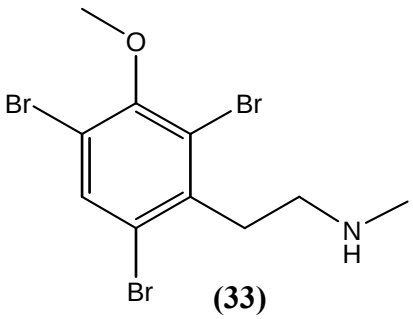
(26) R= 



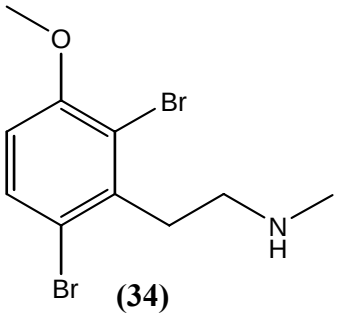
(27) R = $(CH_2)_{12}CH_3$
(28) R = $(CH_2)_7CH{=}CH(CH_2)_5CH_3$
(29) R = $(CH_2)_{14}CH_3$
(30) R = $(CH_2)_7CH{=}CH(CH_2)_7CH_3$
(31) R = $(CH_2)_{16}CH_3$
(32) R = $(CH_2)_6CH{=}CH(CH_2)_{10}CH_3$

This same bryozoan was later shown to yield convolutamines F (**33**) and G (**34**).[55] Convolutamine F (**33**) showed activity against KB, KB/VJ-300 and U937 cell lines with IC_{50} values of 27, 9.6 and 13 μg/mL respectively. It also possessed inhibitory activity against fertilised sea urchin eggs with an IC_{50} value of 82 μg/mL.[55] Convolutamides possess a rare γ-lactam and a dibromophenol ring system. These two classes of compounds are moderately cytotoxic against L1210,

KB, P388, KB/VJ-300 and U937 cell lines with IC_{50} values in the range of 2-10 μg/mL.



(33)



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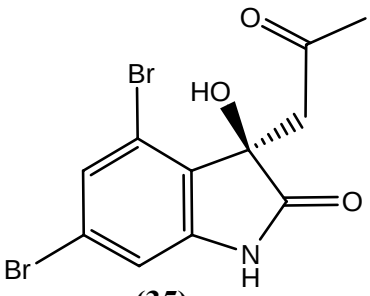

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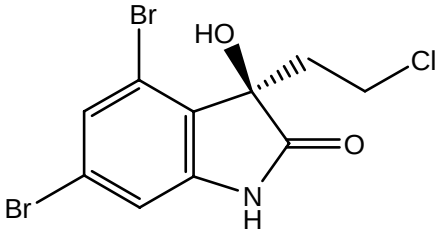

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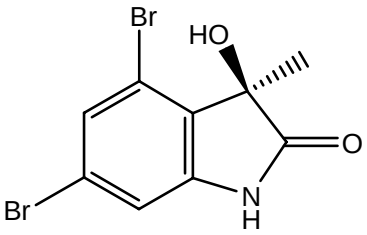
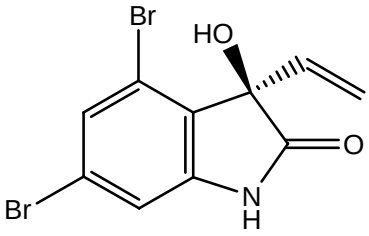


(37)



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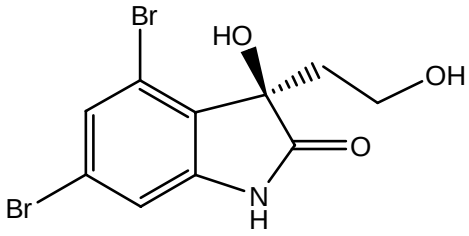
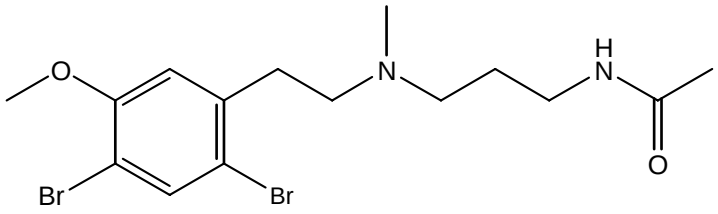


(39)

Convolutamydines A (**35**),[56] B-D (**36-38**)[57] and E (**39**) [55] were also isolated from this species. These compounds are a series of dibromohydroxyindole alkaloids.

Convolutamydines A-B (**35-36**) were shown to inhibit differentiation of HL-60 cells at concentrations of 0.1-2.5 μg/mL.[56,57]



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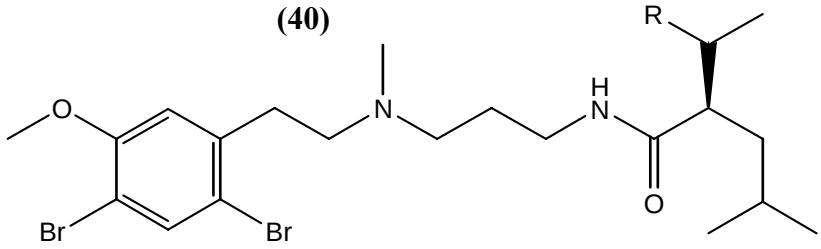
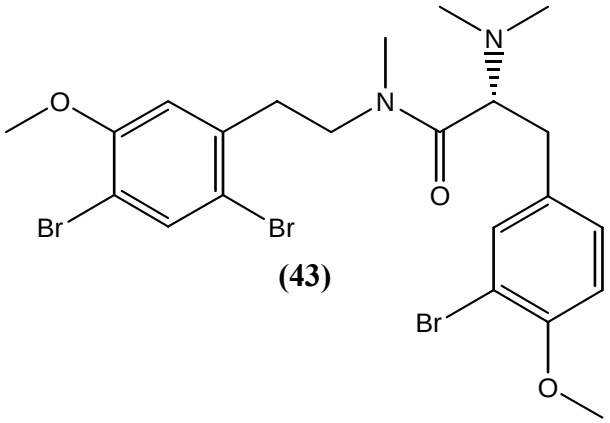


(41) R = H
(42) R = Me



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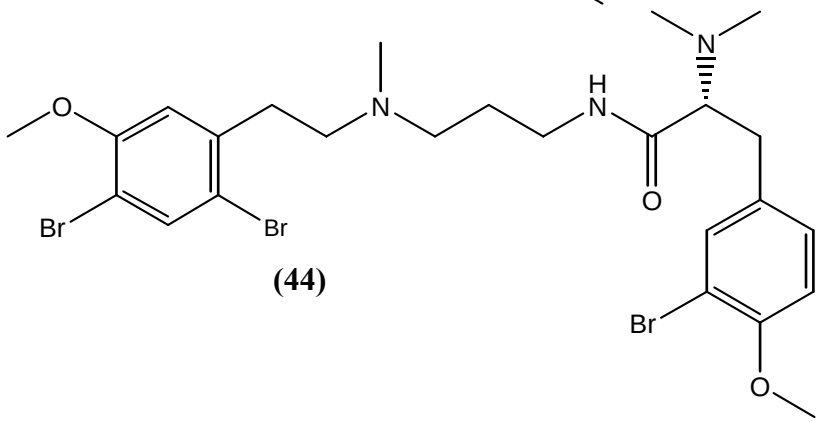
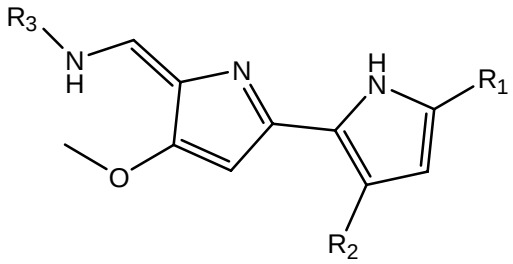


(44)

Volutamides A-E (**40-44**) were also isolated from a sample of *A. convoluta* collected from the temperate Atlantic coast of North Carolina, USA. These compounds have anti-feedant activity against generalist predators such as the

omnivorous pinfish *Lagodon rhomboides.* They are also anti-fouling agents, being toxic to the larva of a co-occurring hydroid. The structures of these compounds resulted from evidence gathered by NMR spectroscopic experiments, mass spectrometry and chemical methods.[58]

The tambjamines are a family of bipyrrole-based metabolites. Tambjamines A-D (**45-48**) were isolated from the bryozoan *Sessibugula translucens*. They have also been isolated from other organisms, including nudibranchs and ascidians that feed on this bryozoan.[59] The tambjamines have promising antitumour activity and have been shown to bind and facilitate the cleavage of DNA in the presence of copper (II) and molecular oxygen.[60]

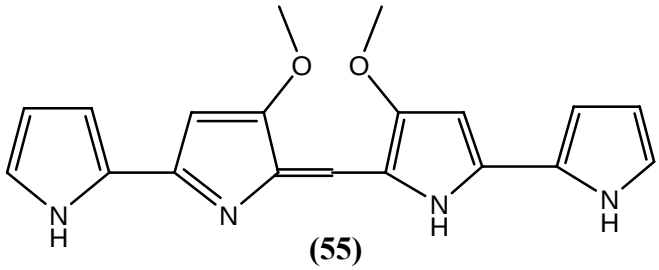


	R_1	R_2	R_3
(45)	H	H	H
(46)	Br	H	H
(47)	H	H	$CH_2CH(CH_3)_2$
(48)	H	Br	$CH_2CH(CH_3)_2$
(49)	H	H	CH_2CH_3
(50)	H	H	CH_2CH_2Ph
(51)	Br	H	CH_2CH_3
(52)	Br	H	$CH_2CH_2CH_3$
(53)	Br	H	$CH_2CH(CH_3)_2$
(54)	Br	H	$CH_2CH(CH_3)CH_2CH_3$

Four tambjamines, A (**45**), C (**47**), E (**49**) and F (**50**) were also found in the ascidian *Atapozoa sp.*[61] Blackman identified four novel tambjamines, tambjamines G-J (**51-54**), as well as known tambjamines C and E (**47** and **49**) from a Tasmanian collection of the bryozoan *Bugula dentata.*[62]

Because of the widespread occurrence of these alkaloids, it has been proposed that they are of microbial origin, however Lindquist and Fenical have indicated that the ascidian *Atapozoa sp.* may be capable of *de nove* tambjamine biosynthesis based on a microscopic examination of this animal. It has been reported that the nudibranch obtains tambjamines from the ascidian and they both use them as a form of chemical defence.[61]

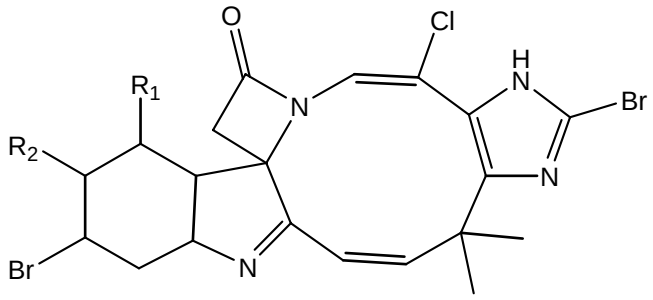
A tetrapyrrole blue pigment (**55**) has also been isolated from *B. dentata.* This compound displays antimicrobial activity.[63]



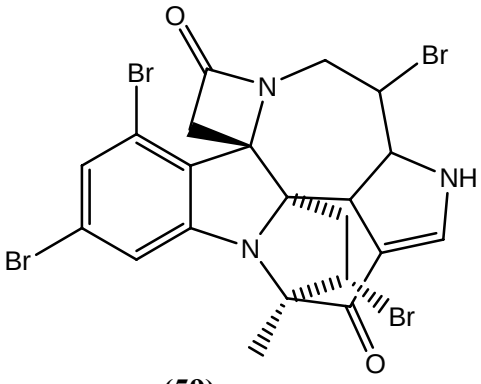
(55)

Chartelline A (**56**) was isolated from the marine bryozoan *Chartella papyracea* which is closely related to *Flustra foliacea.*[10] MS, NMR spectroscopy and X-ray crystallography were used to establish the structure of chartelline A (**56**) which appears to be composed of modified tryptamine and histamine residues linked via an isoprene unit, as is suggested by the literature.[64] Chartellines A-C (**56-58**) as well as chartellamides A and B (**59-60**)[65] have been isolated from this species. Chartellines are β-lactam indole alkaloids that only differ in the number and position of the bromo substituents. These compounds exhibited no significant biological activity in antimicrobial and antitumour assays.[66] A recent attempt at total synthesis of chartelline A (**56**) was unsuccessful due to the resulting product

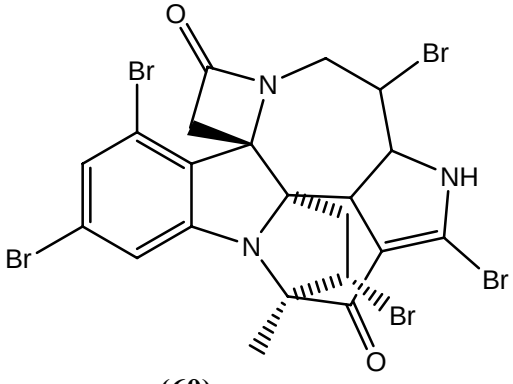
having a 7-membered ring instead of the 10-membered ring seen in the natural product.[67]



(56) $R_1 = R_2 = Br$
(57) $R_1 = Br$ $R_2 = H$
(58) $R_1 = R_2 = H$

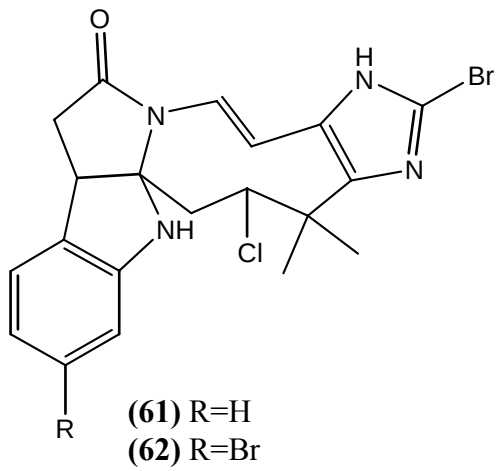


(59)

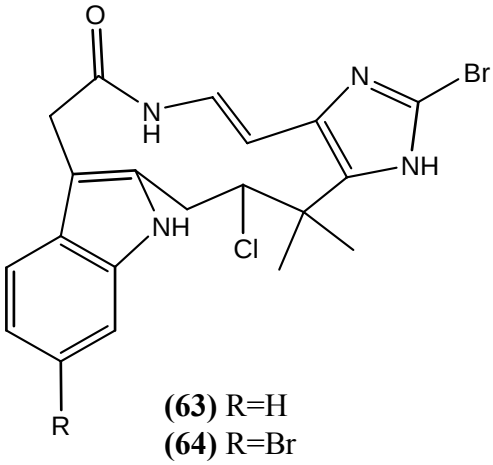


(60)

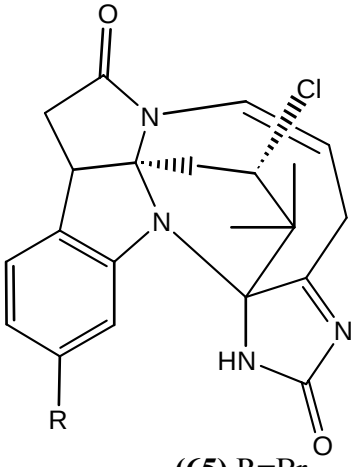
Securamines and securines are a class of halogenated indole-imidazole alkaloids that have been isolated from the marine bryozoan *Securiflustra securifrons* collected from the North Sea. Securamines A (**61**) and B (**62**) are in equilibrium with securines A (**63**) and B (**64**) respectively when dissolved in DMSO-d_6.[68] The biosynthesis of these alkaloids appears to originate from the modification of tryptamine, histamine and isoprene units. These compounds were structurally determined using NMR spectroscopy and MS and combine structural features of the flustramines with some of those from the chartellines. Securamines A-B (**61-62**) only differ in the presence (**62**) or absence (**61**) of a bromine substituent in the benzene ring, as do C (**65**) (present) and D (**66**) (absent). Securamines C-D (**65-66**) appear to be derived from securamines A-B (**61-62**) via the attack of the indole nitrogen at C8 of the imidazole ring. [68]



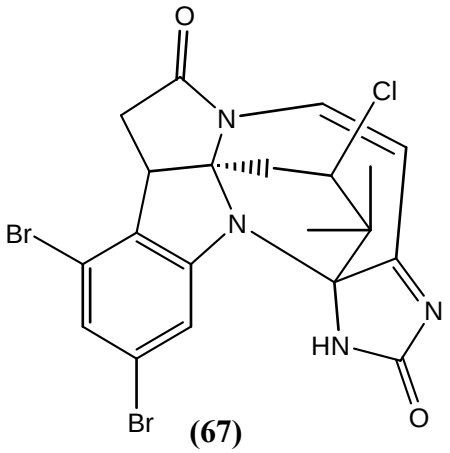
(61) R=H
(62) R=Br



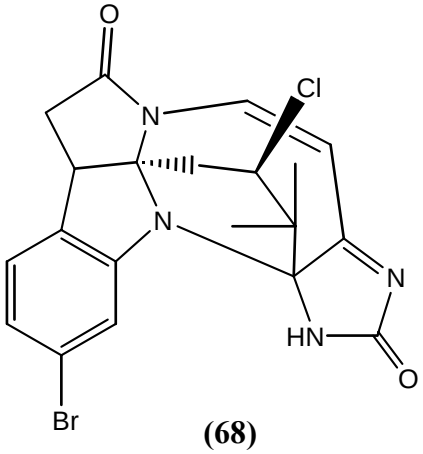
(63) R=H
(64) R=Br



(65) R=Br
(66) R=H



(67)



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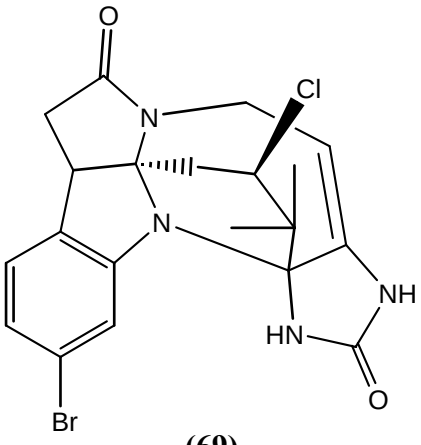
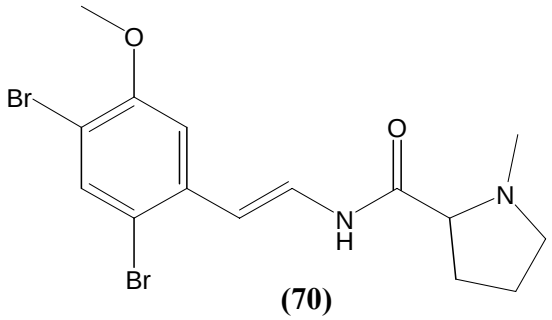


(69)

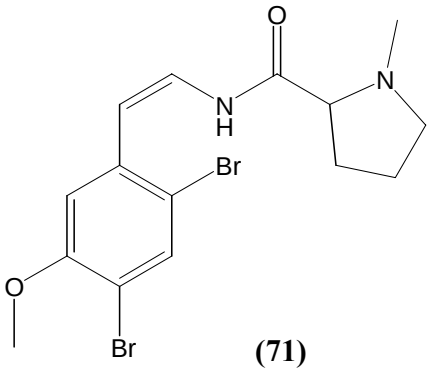
Three new securamines E-G (**67-69**) were isolated from the same bryozoan and structurally determined using NMR spectroscopic and MS techniques. These three compounds are structural variations of securamine C (**65**). Securamine E (**67**) has an extra bromine substituent on the indole ring system, while securamine F (**68**) is

a diastereoisomer differing in configuration at one of the four asymmetric carbon centres and securamine G (**69**) is a hydrogenated form of securamine C (**65**).[69] No biological activity was reported for these compounds.

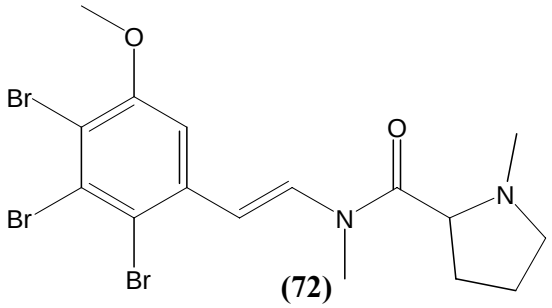
Amathia wilsoni is another bryozoan from which a number of secondary metabolites have been isolated. Amathamides A-B (**70-71**) were isolated from a Tasmanian collection of this bryozoan. The structures of these two compounds were determined using high resolution mass spectrometry (HRMS) and NMR spectroscopic data.[70] Amathamides are brominated proline-derived alkaloids. Further studies have resulted in the isolation of amathamides A-F from *A. wilsoni* (**70-75**).[71] Amathamide G (**76**) was isolated from the closely related species *A. convoluta*.[72]



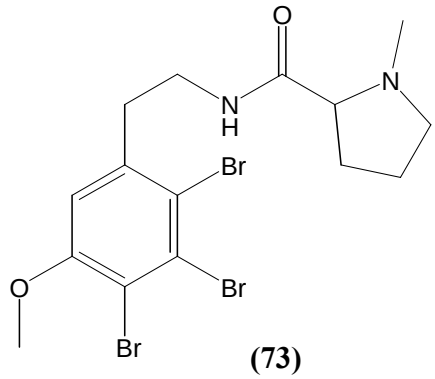
(70)



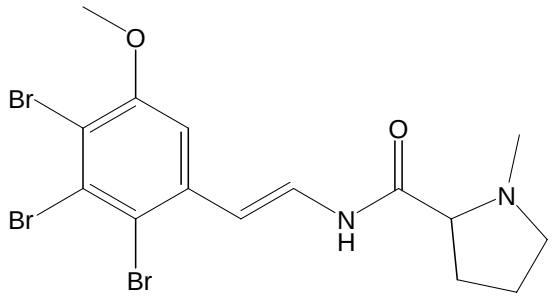
(71)



(72)



(73)



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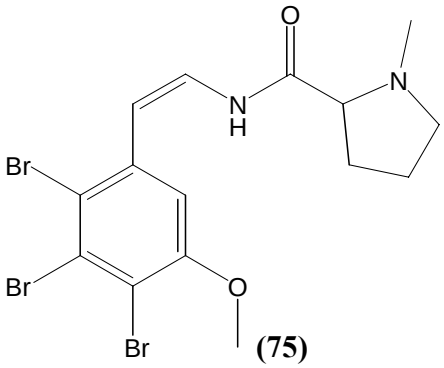

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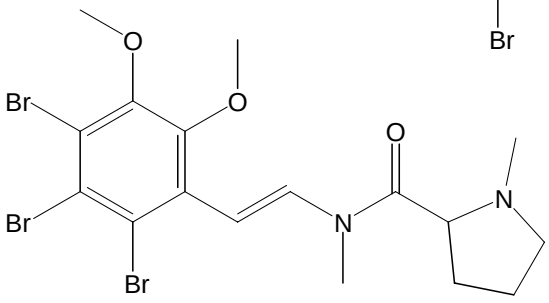
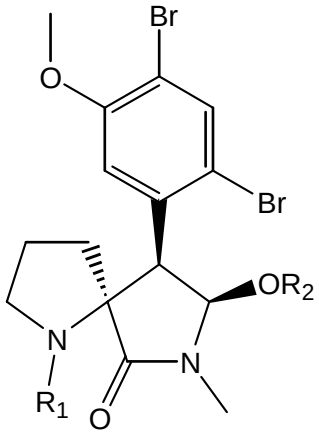


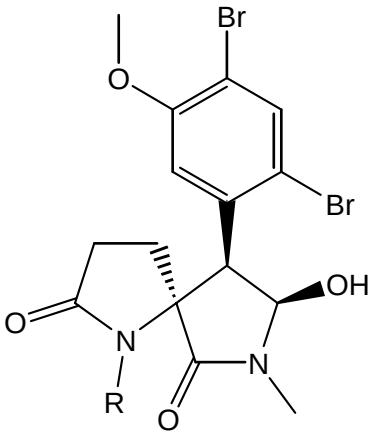
(76)

Amathaspiramides A-F (**77-82**) were isolated from a New Zealand collection of *Amathia wilsoni*.[73] A combination of spectroscopic data and X-ray crystallography was used to establish the structures of these compounds including

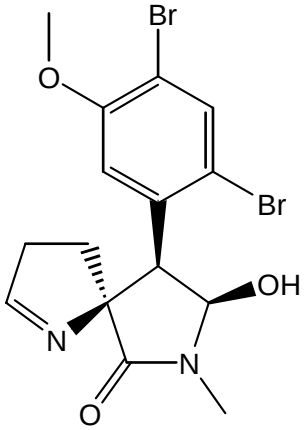
absolute configuration. Amathaspiramide E (**81**) shows strong activity against the *Polio* virus type I. No amathamides were present in this collection.[73]



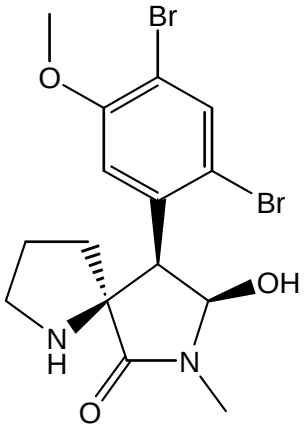
(77) R_1 = Me, R_2 = H
(79) R_1 = H, R_2 = H



(78) R = Me
(80) R = H

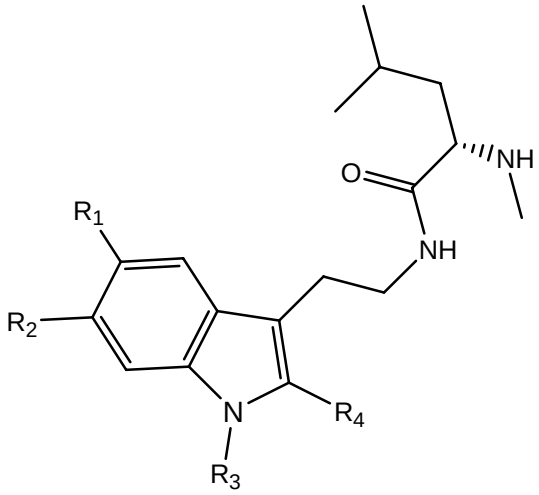


(81)



(82)

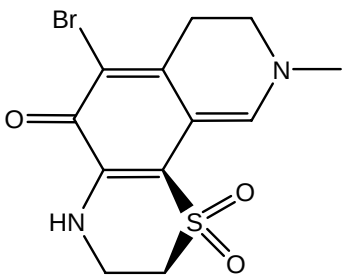
Alternatamides A-D (**83-86**), four bromotyptamine peptides, were isolated from the marine bryozoan *Amathia alternata.* The structures of these were established predominantly with 2D NMR spectroscopy but also with MS and 1D NMR spectral information.[74]



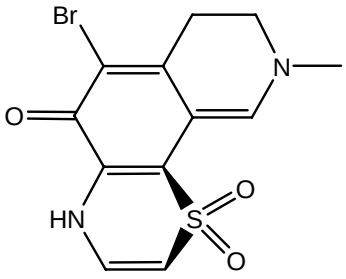
	R_1	R_2	R_3	R_4
(83)	Br	Br	CH_3	Br
(84)	Br	Br	H	Br
(85)	Br	H	H	Br
(86)	Br	Br	H	H

Alternatamides A-C (**83-85**) show modest antibacterial activity against *Staphylococcus aureus*, *S. epidermidis*, *S. haemolyticus*, *Bacillus subtilis*, *Enterococcus faecalis*, *E. faecium*, and *Streptococcus pyogenes* with minimum inhibitory concentration (MIC) values ranging between 4 and 32 μg/mL. These compounds do not show any activity against Gram-negative bacteria. Alternatamide D (**86**) was not tested.[74]

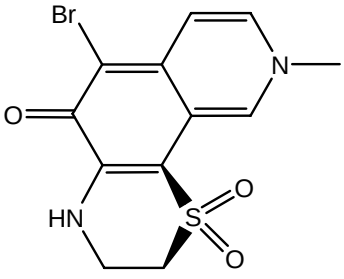
Three novel brominated quinone methides, euthyroideones A-C (**87-89**) were isolated from a New Zealand collection of the bryozoan *Euthyroides episcopalis*. The structures of these three compounds were determined using MS, NMR spectroscopic data and X-ray crystallography.[75]



(87)

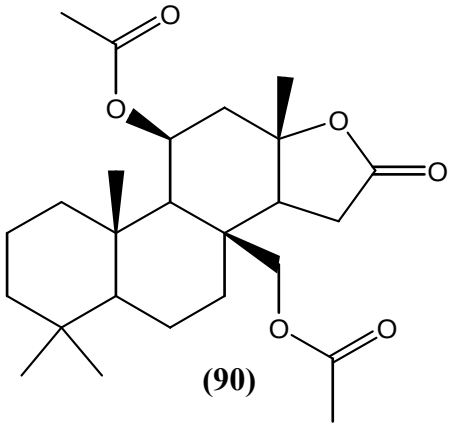


(88)



(89)

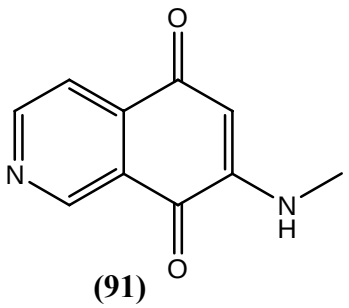
Secondary metabolites from bryozoans have a large variety of structures. A unique C_{21} tetracyclic terpenoid lactone, murrayanolide (**90**), was isolated from *Dendrobeania murrayana.* Terpenes are not commonly found in bryozoans but murrayanolide is thought to be biosynthesised by the degradation of a higher terpenoid chain.[76]



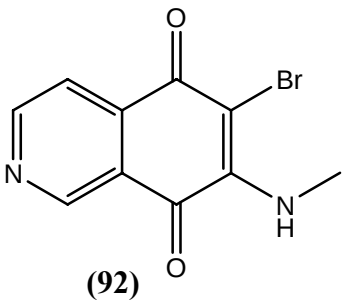
(90)

An Indo-Pacific collection of the marine bryozoan *Caulibugula intermis* led to the isolation of six novel cytotoxic isoquinoline quinones and iminoquinones. Bioactivity directed fractionation guided the isolation of caulibugulones A-F (**91-96**). The structures of these compounds were determined by spectrochemical analysis including liquid chromatography-mass spectrometry (LCMS), high resolution fast atom bombardment mass spectrometry (HRFABMS), 1D and 2D NMR spectroscopy as well as comparison with related compounds.[77]

Caulibugulones E-F (**95-96**) are thought to be the first compounds with an isoquinoline iminoquinone skeleton to be isolated from a natural source.



(91)



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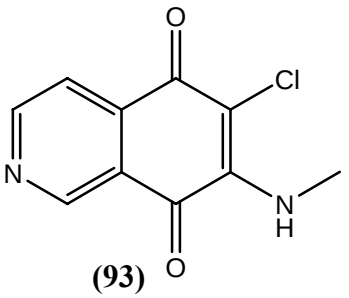
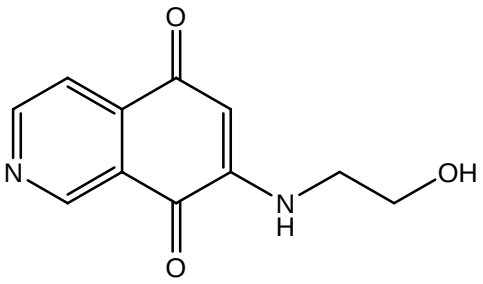
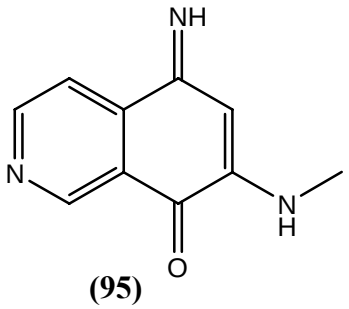


(93)



(94)



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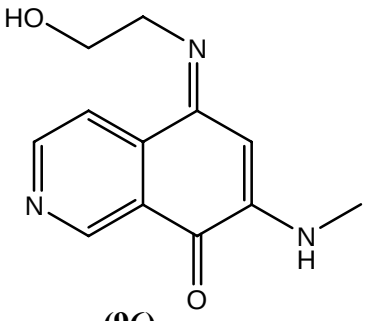
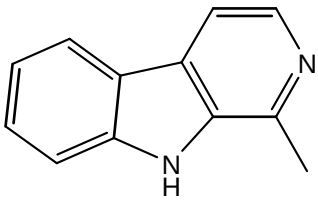


(96)

Evaluation of the biological activity of these compounds revealed IC_{50} values ranging between 0.03 and 1.67 μg/mL against the murine IC2 cell line. Caulibugulones A-C (**91-93**) showed similar activity indicating that halogen substitution at C6 does not affect cytotoxicity. Caulibugulone E (**95**) is roughly an order of magnitude more cytotoxic than its corresponding isoquinoline quinone caulibugulone A (**91**). [78]

β-Carbolines are known to occur in terrestrial plants,[79] but examples have also been found in marine bryozoans. Two collections of the orange foliose bryozoan *Costaticella hastata* from southern Tasmania contained a range of 1-substituted β-carboline alkaloids. The major β-carboline alkaloid in both collections was 1-methyl-β-carboline (harman) (**97**). Three minor β-carbolines were also found. The first collection yielded 1-ethyl-β-carboline (**98**) and (*S*)-1-(1'hydroxyethyl)-β-carboline (**99**) while the second collection yielded both these compounds (**98-99**) as well as a third minor constituent, 1-vinyl-β-carboline (pavettine) (**100**).[80]



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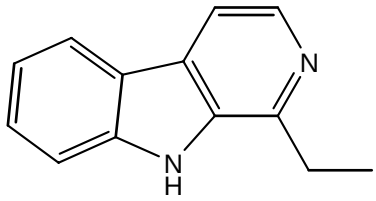

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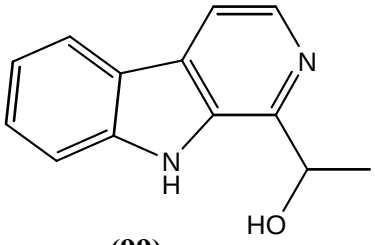

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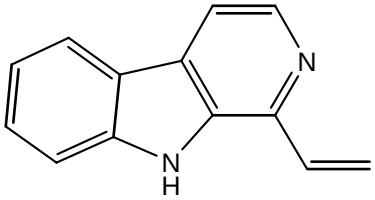

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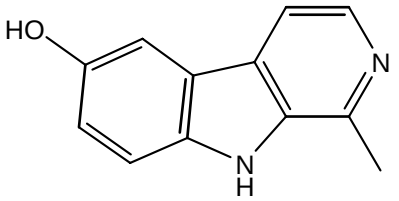

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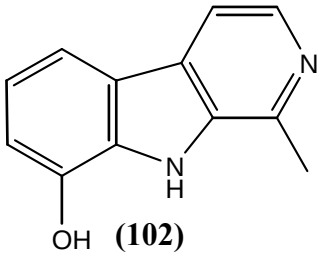

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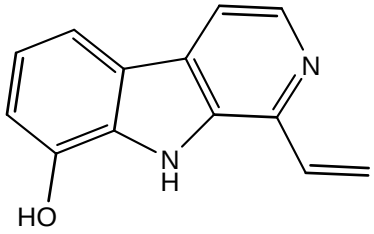

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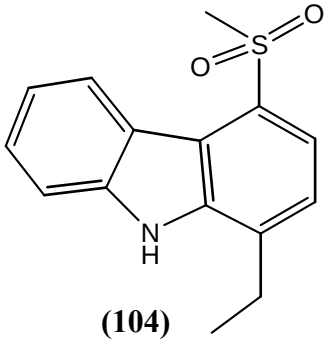
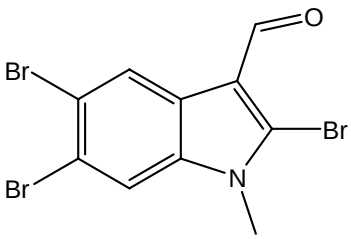


(104)

Another marine bryozoan *Cribricellina cribraria* has been shown to produce both known and new β-carboline alkaloids. Investigation into the cytotoxic extract of this bryozoan revealed 1-vinyl-8-hydroxy-β-carboline (**103**) was the major component. Harman (**97**) and pavettine (**100**) were also present as well as 1-ethyl-4-methyl-sulfone-β-carboline (**104**).[81] These alkaloids differ significantly in their degree of biological activity in the P388 assay. β-Carbolines with a vinyl group at

C1 exhibit IC_{50} values of 100 ng/mL whereas those lacking this substituent are virtually inactive.[81] Further investigation of other collections of this bryozoan yielded more novel β-carboline alkaloids including 6-hydroxyharman (**101**), a β-carboline previously reported from terrestrial origins, and 8-hydroxyharman (**102**), the latter being a new β-carboline alkaloid. The structures of these two compounds were determined using NMR spectroscopy and HRMS. These compounds showed no significant activity against the P388 murine leukaemia cell line (both had an IC_{50} > 12500 ng/mL). 6-Hydroxyharman (**101**) did show activity against the *Herpes simplex* type 1 virus with no significant cytotoxicity.[82]

The brominated indole alkaloid 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde, also known as 2,5,6-tribromo-1-methylgramine (**105**) was isolated from a Spanish collection of the marine bryozoan *Zoobotryon verticullatum*. This extract also contained a number of known gramine alkaloids.[83] Structural elucidation was based on spectroscopic information and confirmed by comparisons with synthetic versions.[10] This compound was shown to delay metamorphosis in fertilised sea urchin eggs with an ED_{50} value roughly equal to 16 μg/mL.[10] There is a taxonomic interest in this compound because *Z. verticullatum* is of the order Ctenostomata and an unrelated bryozoan *Flustra folicea* from the order Cheilostomata also produces similar bromoindole alkaloids.[10]



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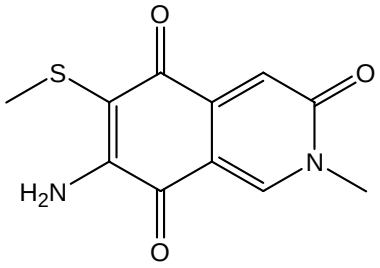

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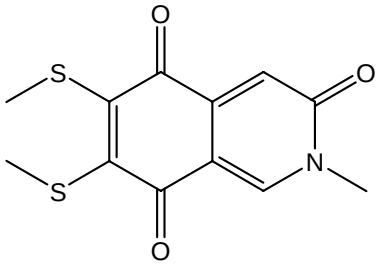
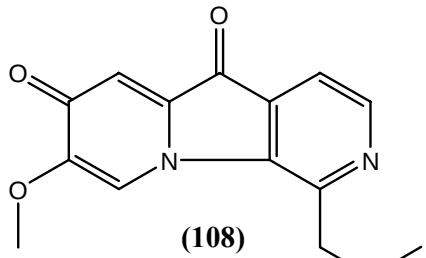


(107)

Two novel compounds have been isolated from the marine bryozoan *Membranipora perfragilis.* These compounds, perfragilins A-B (**106-107**), are isoquinoline quinones and their structures were determined by X-ray crystallography. They are cytotoxic to P388 murine leukaemia cells with IC_{50} values of 0.8 and 0.07 μg/mL respectively but have no antiviral activity. It has been suggested that these compounds are of bacterial origin because of their structural relationship to mimosamycin, a secondary metabolite produced by the terrestrial actinomycete *Streptomyces lavendulae.*[84]



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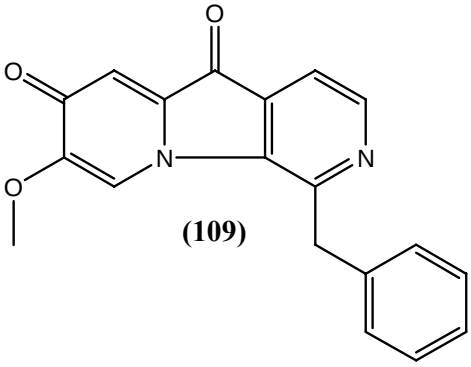

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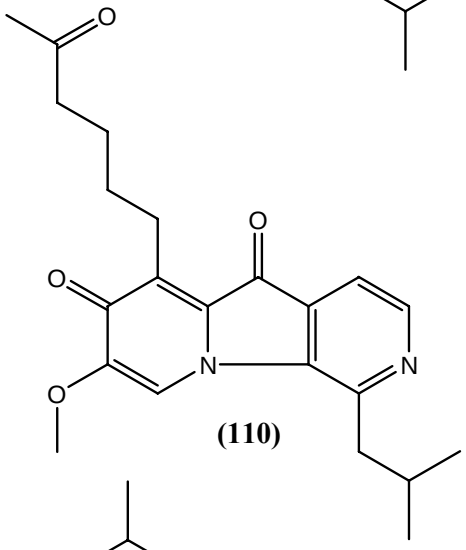

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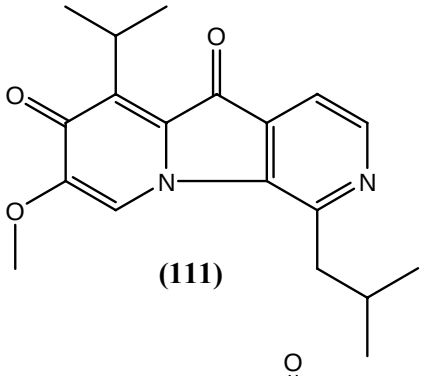

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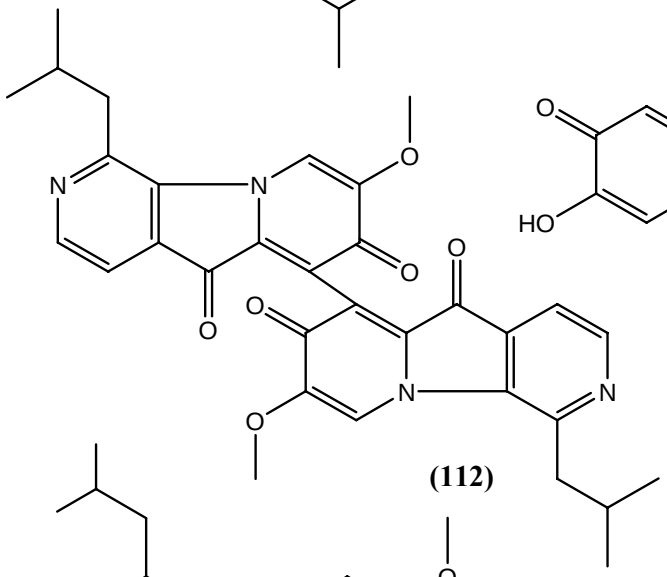

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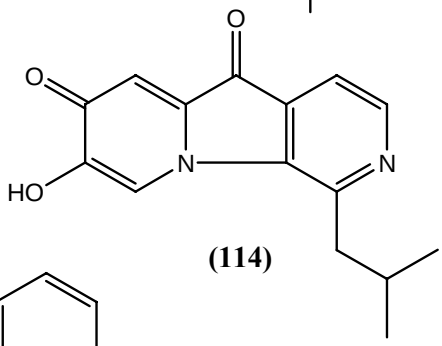

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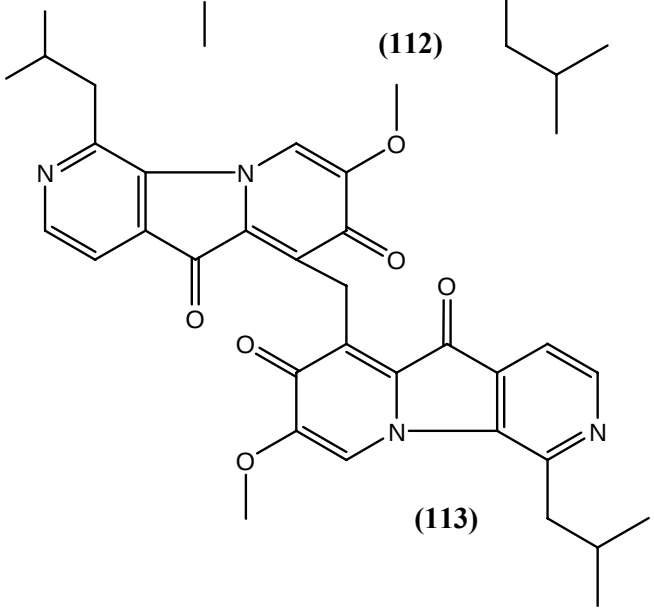
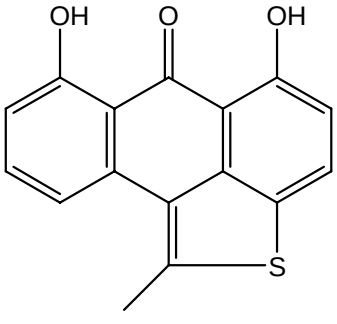


(113)

A New Zealand collection of *Pterocella vesiculosa* yielded two novel cytotoxic compounds. Pterocellins A-B (**108-109**) belong to a new class of alkaloids with a unique tricyclic pyrido[4,3-*b*]indolizine ring system.[85] Structural elucidation was achieved by NMR spectroscopy and MS data analysis as well as X-ray diffraction studies of pterocellin A (**108**).[86] Both compounds exhibit potent antitumour activity *in vitro* against P388 murine leukaemia cells, but only modest activity in the *in vivo* hollow fibre assay at the NCI.[86] Pterocellins C-G (**110-114**) have been isolated and characterised from the same bryozoan.[87] Pterocellin A (**108**) has been synthesised.[85]

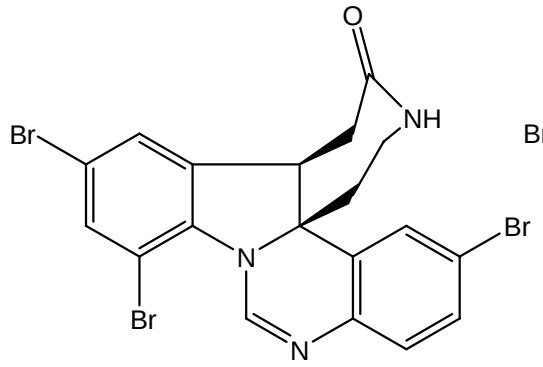
Bryoanthrathiophene (**115**) was isolated from a Japanese sample of the bryozoan *Watersipora subtorquata* by bioassay guided fractionation. The structure was characterised using MS and NMR spectral data. This compound has antiangiogenic activity, so stops the formation of new blood vessels thus preventing the growth of tumours.[88]



(115)

Hinckdentine A (**116**) was isolated from a Tasmanian collection of the marine bryozoan *Hincksinoflustra denticulata.* Spectral analysis along with X-ray crystallography gave the structure with absolute stereochemistry.[89] Although hinckdentine A (**116**) has not been successfully synthesised to date, both the

hinckdentine framework and 8-desbromohinckdentine A (**117**) have been synthesised.[90]



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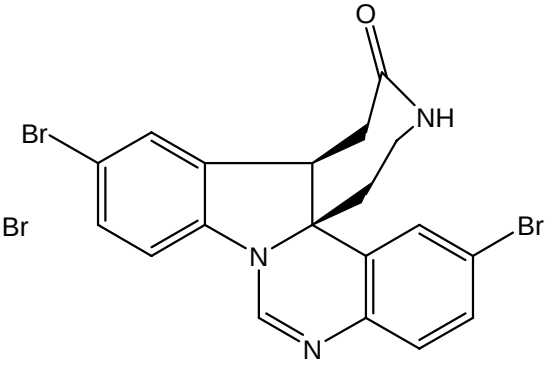
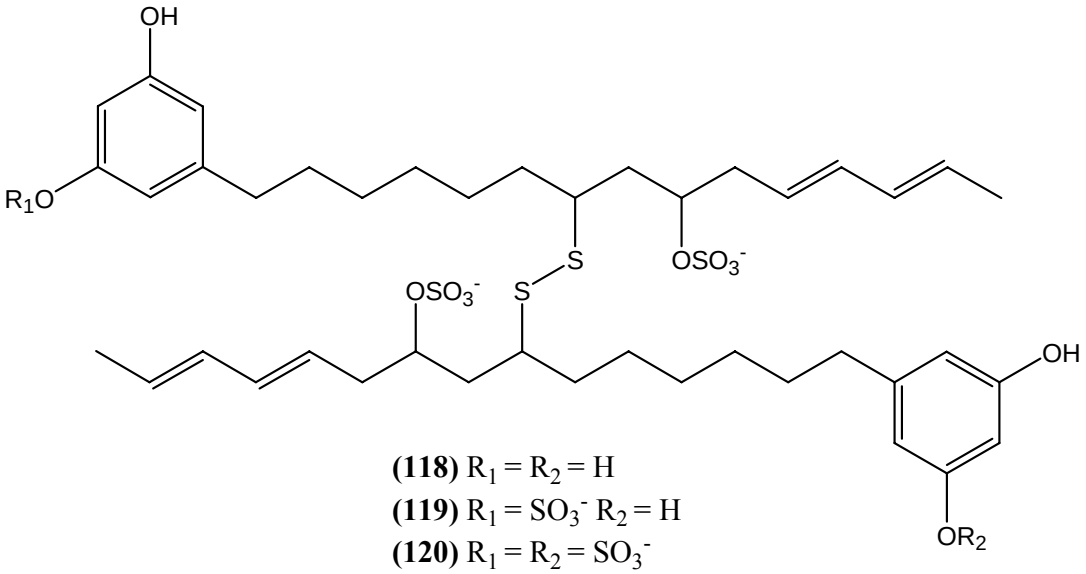


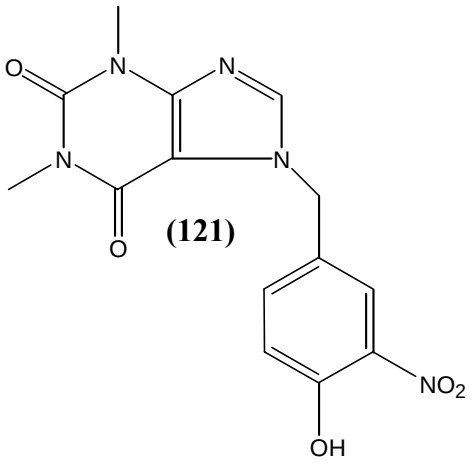
(117)

The crude extract of the marine bryozoan *Pentapora fascialis* showed antihelmintic activity against *Trichenella spiralis*. Bioassay guided fractionation led to the isolation of three new sulfur containing compounds, pentaporins A-C (**118-120**). Pentaporins A-C (**118-120**) were structurally determined through 1D and 2D NMR spectroscopy as well as mass spectral information and energy dispersive X-ray (EDX) analysis.[91] Structure activity investigation led to the assumption that the sulfate ester groups are responsible for the antihelmintic activity of the pentaporins.[91]



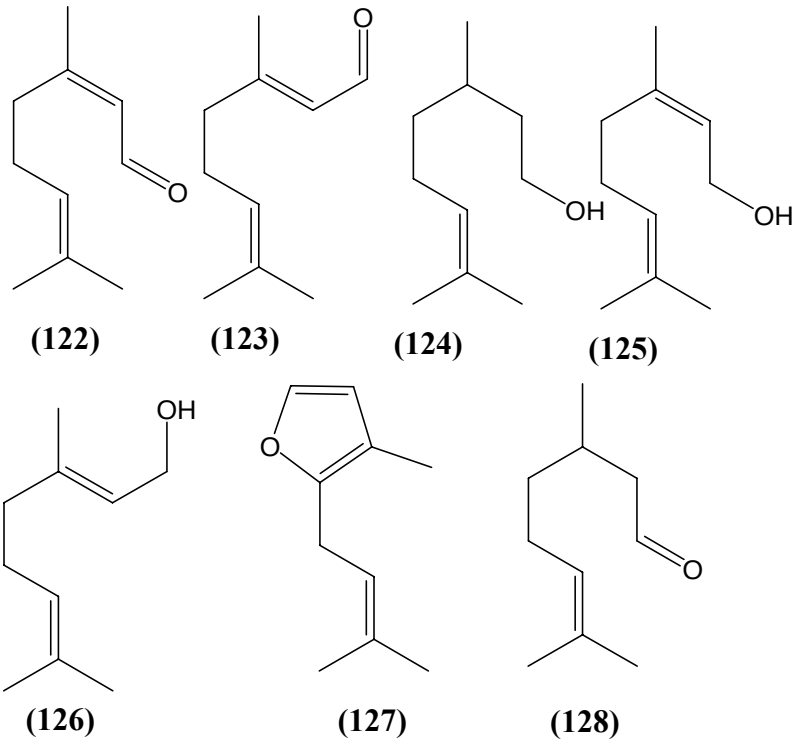
(118) $R_1 = R_2 = H$
(119) $R_1 = SO_3^-$ $R_2 = H$
(120) $R_1 = R_2 = SO_3^-$

Phidolopin (**121**) is a purine derivative isolated from the marine bryozoan *Phidolopora pacifica* collected in British Columbia.[92] The crude extract of *P. pacifica* showed strong *in vitro* antifungal and antialgal activity for which phidolopin (**121**) was largely responsible. The structure of phidolopin (**121**) was solved through X-ray crystallography with supporting evidence provided by high resolution electron ionisation mass spectrometry (HREIMS), NMR and infrared (IR) spectroscopic information.[92]



(121)

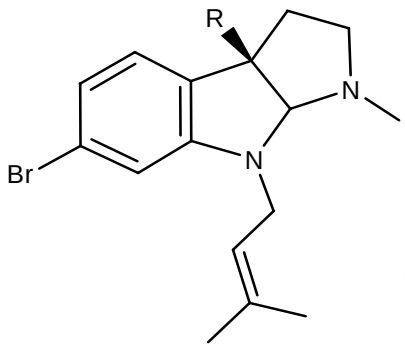
Flustra foliacea is a marine bryozoan that has yielded a vast number of secondary metabolites. Several monoterpenes have been isolated from *F. foliacea* including *cis*- and *trans*-citral (**122-123**), citronellol (**124**), nerol (**125**), geraniol (**126**), rosefuran (**127**) and citronellal (**128**).[93]



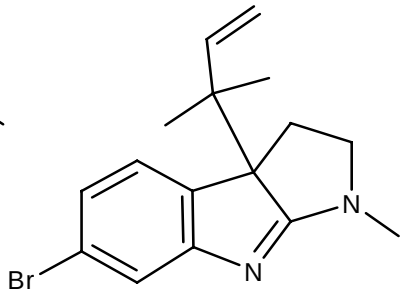
(122) **(123)** **(124)** **(125)**

(126) **(127)** **(128)**

Flustramines A and B (**129-130**) were the first secondary metabolites isolated from *F. foliacea*. The structures were elucidated using HRMS and NMR spectroscopic data.[94,95] They have a physostigmine skeleton and flustramine A possesses a rare inverted isoprene unit.[94] Flustramines A-B (**129-130**) show strong muscle relaxant effects *in vivo*, causing 50 % inhibition of contractions of rat diaphragm muscle at concentrations of 59 and 63 μg/mL respectively.[96] Flustramine C (**131**) and flustraminols A and B (**132-133**) were isolated from another extract of *F. foliacea*. Their structures were determined using HRMS data, NMR spectroscopic information and ultra violet (UV) absorption results.[97] Another sample of *F. foliacea* yielded 7-bromo-4-(2-ethoxyethyl)quinoline (**134**), the first naturally occurring bromo-quinoline to be isolated. The structure of this compound was elucidated using HRMS, NMR spectroscopy and UV spectroscopy.[98]



(129) R = $CH(CH_3)_2CH=CH_2$
(130) R = $CH_2CH=C(CH_3)2$



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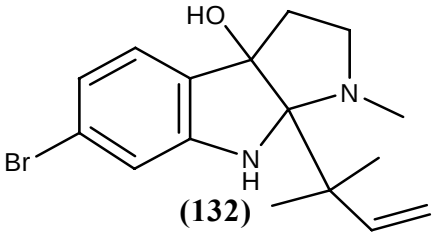

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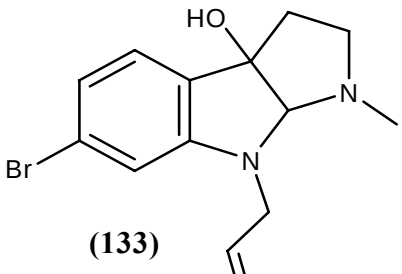

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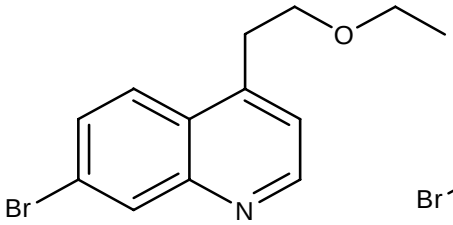

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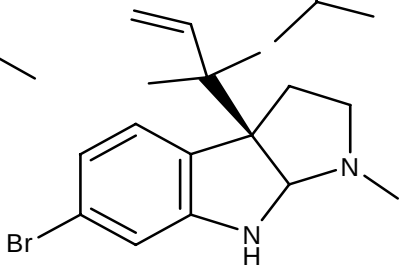

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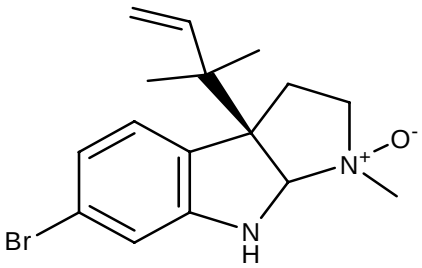

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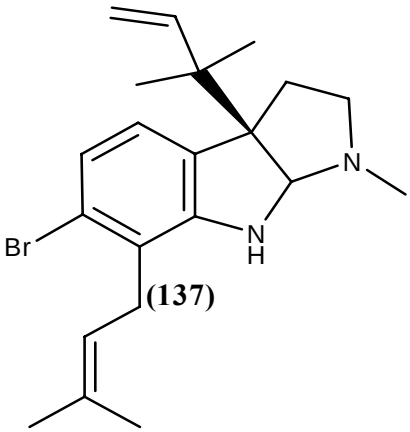

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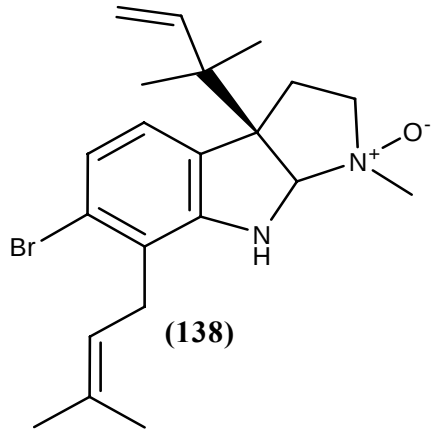
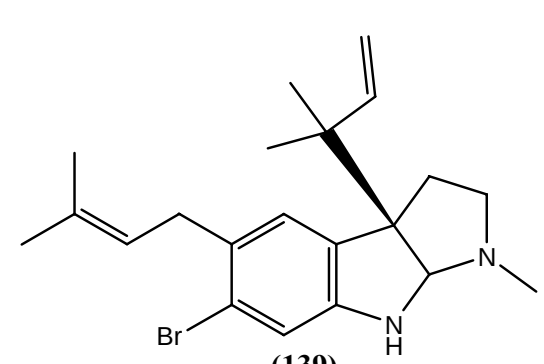


(138)

Dihydroflustramine C (**135**) was identified as the principle antibacterial agent in an extract of *F. foliacea*. The structural assignment was based on mass spectral evidence along with NMR spectroscopic information.[99] Dihydroflustramine C (**135**), its *N*-oxide (**136**), flustramine D (**137**), its *N*-oxide (**138**) and isoflustramine D (**139**), have been isolated from *F. foliacea.* It is rare to isolate *N*-oxides from marine organisms.[100] An extract mixture containing mainly dihydroflustramine C (**135**) and flustramine D (**137**) showed activity against a wide range of bacteria in disk diffusion assays.[100] Flustramines A and B (**129-130**) and dihydroflustramine C (**135**) are weakly cytotoxic against the HCT-116 cell line with IC_{50} values of roughly 10 μg/mL.[101]



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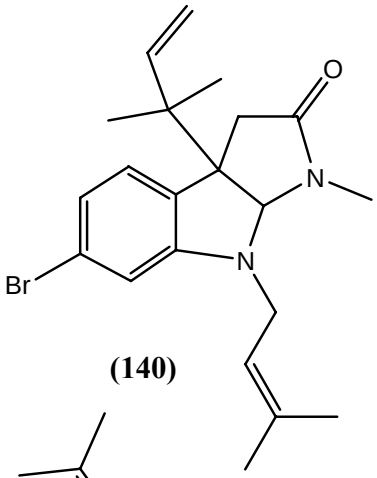

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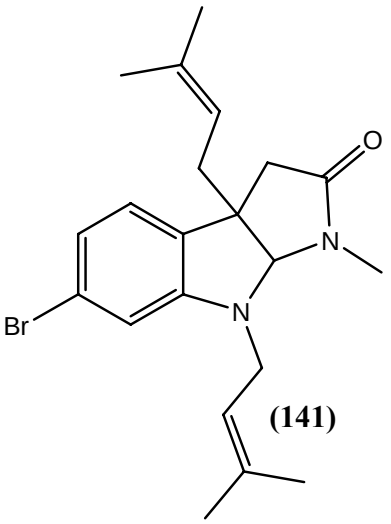

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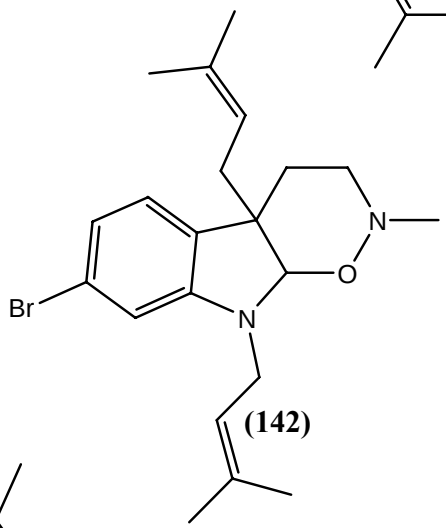

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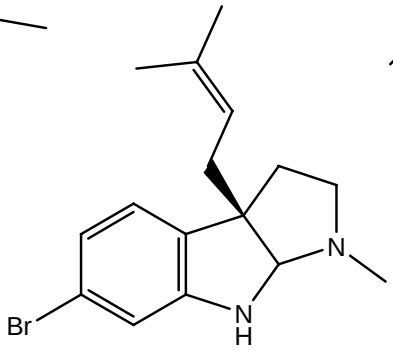


(143)

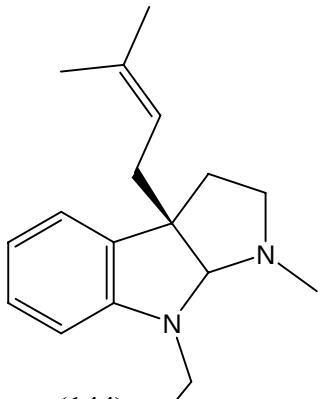
Flustramide A (**140**) flustramide B (**141**) and flustrarine B (**142**) have also been isolated from *F. foliacea*.[98,102] Structural elucidation was based on IR, MS and NMR spectral information. Flustrarine B (**142**) was synthesised by oxidation of flustramine B (**130**).[102]

Flustramine E (**143**) was isolated from a North Sea sample of *F. foliacea* and characterised based on spectroscopic investigation. Debromoflustramine B (**144**) was also present in trace amounts in the same extract and was identified based on

comparison with a synthetic version.[103] Flustramine E (**143**) showed marginal activity against *Bacillus subtilis* and good activity against *Botrytis cinerea* and *Rhizotonia solani.*[103]

Deformylflustrabromine (**145**) was isolated from a North Sea collection of *F. folicea*. It appears to be a link in the biosynthetic pathway from flustrabromine (**146**) to flustraminol A (**132**). HRMS information along with 1D and 2D NMR spectroscopic data was used to determine the structure and compound (**145**) is cytotoxic to the HCT-116 cell line with an IC_{50} value of 1.87 μg/mL.[101]

New natural products were reported from a North Sea collection of *F. foliacea* along with known metabolites. The three new indole alkaloids (**147-149**) were isolated and their structure determined with MS information accompanied by 1D and 2D NMR data and UV absorption information.[104]



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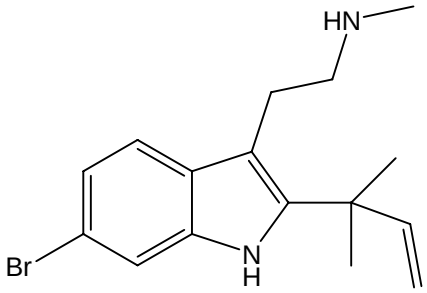

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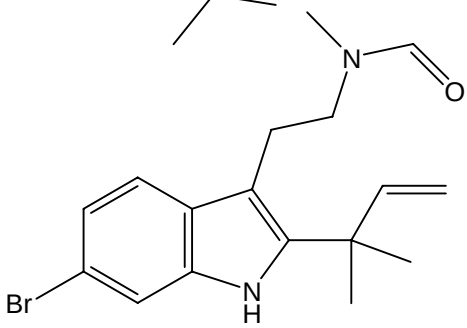

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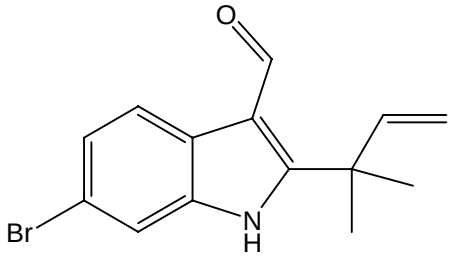

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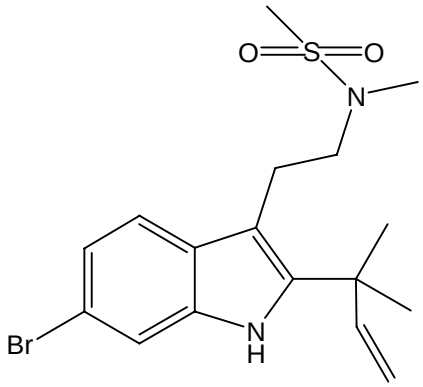

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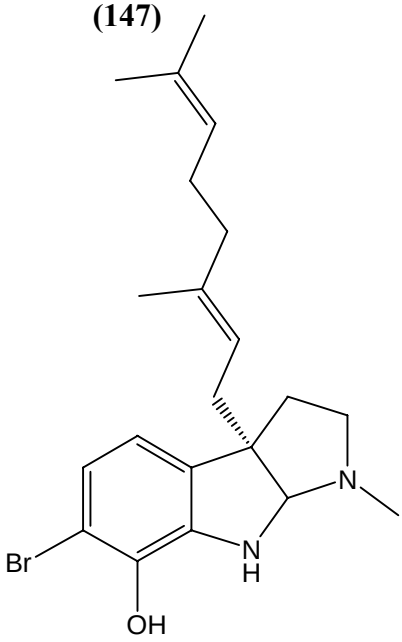
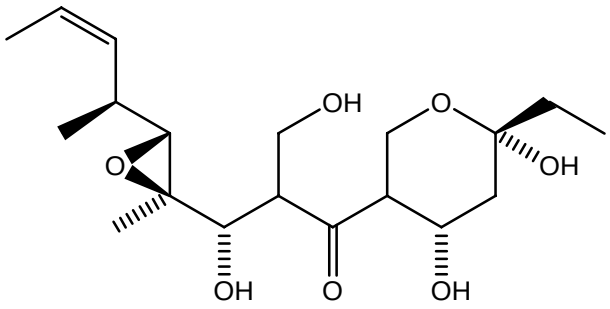


(149)

Flustramines A-B (**129-130**), flustramides A (**140**) and B (**141**) and debromoflustramine B (**144**) have been synthesised.[105]

The number of brominated alkaloids in a single colony of *F. foliacea* can vary significantly and may be as high as eleven. Colonies at a certain location possess a distinct and fairly constant alkaloid profile.[93]

A strongly cytotoxic compound (**150**) and its isomer (**151**) were isolated from an extract of the marine bryozoan *Myriapora truncata.* They are polyketide derived metabolites which both showed 88 % inhibition of L1210 murine leukaemia cells at a concentration of 0.2 μg/mL.[106]



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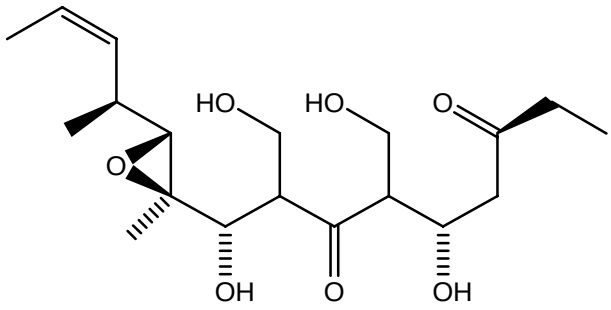


(151)

1.4 Biological Assay Systems Used in this Thesis

Biological assays were performed at the University of Canterbury. All crude extracts were tested for activity over the whole range of assays. Bulk extracts and subsequent fractions from those were tested in the P388 antitumour assay system only.

P388 Antitumour Assay

This assay is used to determine the concentration of the test sample required to reduce the P388 murine leukaemia cell line growth by 50 %, the IC_{50} value. The test solution is added to wells containing the leukaemia cells in a series of eight two fold dilutions, and then the cells are incubated (35 °C, 72 hours). The number of viable leukaemia cells is determined by adding 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the wells and then this is incubated for a further four hours. Normal leukaemia cells reduce MTT, which is a yellow colour, to the purple formazan product. The quantity of the formazan product is determined with a spectrophotometer at 540 nm. The percentage inhibition of P388 cell growth by a sample can be determined by comparing the absorbance of a test well to that of the control. A plot of absorbance against the logarithm of the sample concentration allows the IC_{50} value to be obtained (in ng/mL) from the antilog of the 50 % value.

The Antimicrobial Assay

The antimicrobial assay carried out at the University of Canterbury determines if a test solution has the ability to inhibit the growth of bacteria and/or fungi. Six microorganisms are used in these tests: two Gram-negative bacteria

(*Pseudomonas aeruginosa* and *Escherichia coli*) a Gram-positive bacterium (*Bacillus subtilis*) and three fungi (*Candida albicans, Trichophyton mentagrophytes,* and *Cladosporium resinae*). A known concentration of bacteria or fungi is mixed with Mueller Hinton or potato dextrose agar and poured into a Petri dish and a "lawn" of the microorganism growth over the dish is observed.

Filter paper disks (6 mm diameter) are impregnated with the sample of interest then air dried at room temperature for twenty minutes to allow the solvent to evaporate. These disks, along with antibiotic and control disks are placed onto the seeded agar dishes. The disks are then incubated (35 °C, 24 hours for most but for *T. mentagrophytes* and *C. resinae* it is 48 hours). If a sample is active against the microorganism in that dish, there will be a zone of inhibition around the disk. The radius of this inhibition zone is measured to determine the extent of inhibition from that sample. "ND" indicates no activity was observed and an asterisk indicates slight activity.

The Antiviral/Cytotoxicity Assay

These assays use a continuous BSC-1 cell line derived from African Green Monkey kidney cells infected with either *Herpes simplex* type 1 virus (ATCC VR 733) or *Polio* virus type 1 (Pfizer vaccine strain) to coat assay wells. A layer of methylcellulose is then put down to cover the well. The sample of interest is applied onto a paper disk 6 mm in diameter, and then the disk is air dried at room temperature (20 minutes) and advanced through the methylcellulose layer to be directly contacting the monolayer of cells. Virus and cell controls are also included with each run. All of the wells are incubated (35 °C, 24 hours) with a 5

% CO_2 enriched atmosphere. The assays are viewed under an inverted microscope and examined for the zone of inhibition around the paper disk. Table 1.1 lists the abbreviations used to describe the extent of the antiviral or cytotoxic zone, while Table 1.2 lists the terms used to describe the cytotoxicity types.

Table 1.1: Scale Used for Antiviral and Cytotoxicity (zone) Results.

ND	No discernable antiviral or cytotoxic zones.
?	Indeterminate activity due to strong cytotoxicity.
?*	Virus growing right up to cytotoxic zone.
±	Minor effects located under the disk.
+	Antiviral or cytotoxic zone extending 1-2 mm radius from disk edge. (25 % zone)
2+	Antiviral or cytotoxic zone extending 2-4 mm radius from disk edge. (50 % zone)
3+	Antiviral or cytotoxic zone extending 4-6 mm radius from disk edge. (75 % zone)
4+	Antiviral or cytotoxic zone extending over whole well. (100 % zone)

Table 1.2: Interpretation of Cytotoxicity Types.

1	Tightly packed small and round cells with well defined nuclei.
2	Cells appear elongated and stringy.
3	A scruffy mixture of rounded and elongated cells.
4	Well separated cells that are small and round.
5	Clumps of rounded cells.
6	Cells are distended and scattered throughout the whole well.
7	Cells are slightly elongated and appear granular.
7*	Indistinct individual cells resulting in a misty appearance.
8	Elongated cells with pronounced nuclei.
9	Small and rounded cells with ragged edges and two prominent nuclei.
10	Cells are extremely small and appear as dots.
11	Enlarged cells with square ends packed together in a mosaic pattern.
12	An enlarged version of type 3 cytotoxicity.

1.5 Work in this Thesis

A study of the bryozoan *Pterocella vesiculosa* resulted in the isolation and structural elucidation of three new novel compounds pterocellin H (**188**), pterocellin I (**189**) and 1-methyl-5-bromo-8-methoxy-β-carboline (**190**). Isolation, characterisation and the evaluation of the biological activity of these three new compounds are discussed.

A survey of the biological activity and chemical components of 85 marine organisms was undertaken using various analytical techniques. Results indicated that some samples of the same species have varying chemical compositions based on factors such as location.

Results from the chemical survey led to the further investigation of two marine organisms including one sponge sample from the family Darwinellidae. The study was aimed at isolating bioactive and/or novel compounds and analysing the sterol composition of these organisms. Due to lack of material no novel compounds were isolated.

Chapter Two

Studies of the Marine Bryozoan *Pterocella vesiculosa*

2.1 Introduction

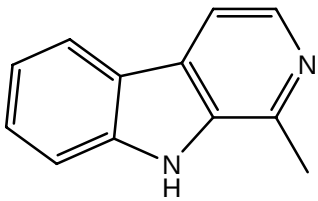
Pterocella vesiculosa (order Cheilostomatida, suborder Ascophorina, family Catenicellidae) is a marine bryozoan found in the waters of the upper North Island of New Zealand and South Australia.[86] Bryozoans are a relatively unexplored phylum in natural product chemistry, although many secondary metabolites with significant biological activity have been found from these animals. The most well known examples are the bryostatins. Bryostatin 1 (**1**) has successfully been through over eighty Phase I and Phase two clinical trials and is currently in one Phase I and two Phase II clinical trials.[42,50-52]

The natural product research group at the University of Waikato, led by Dr. Michèle Prinsep, have been successful in the isolation of new metabolites from bryozoans. The amathaspiramides A-F (**72-77**),[73] the euthyroideones A-C (**82-84**)[75] and the pterocellins A-G (**108-114**)[86,87] have all been isolated from endemic bryozoan species.

Pterocellins A and B (**108-109**) were isolated and characterised from a sample of *P. vesiculosa*.[86] They have excellent *in vitro* activity against the P388 murine leukaemia cell line, but were inactive in the *in vivo* hollow fibre assay performed

by the National Cancer Institute (NCI).[86] Pterocellins C-G (**110-114**) have also been isolated and characterised, although remain unpublished to date.[87]

Other members of the family Catenicellidae such as *C. hastata* and *C. cribraria* have between them yielded six novel β-carbolines. 1-Methyl-β-carboline (harman) (**97**) and 1-vinyl-β-carboline (pavettine) (**100**) were common to both species. *C. hastata* also yielded (*S*)-1-(1'hydroxyethyl)-β-carboline (**99**) and 1-ethyl-β-carboline (**98**)[80] while *C. cribraria* yielded 6-hydroxyharman (**101**),[82] 8-hydroxyharman (**102**),[82] 1-vinyl-8-hydroxy-β-carboline (**103**)[81,107] and 1-ethyl-4-methylsulfone-β-carboline (**104**).[81] Many of these alkaloids had been isolated from terrestrial sources but the latter two were previously unknown.



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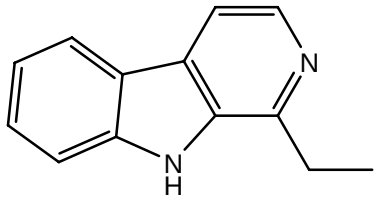

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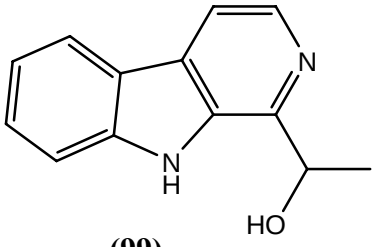

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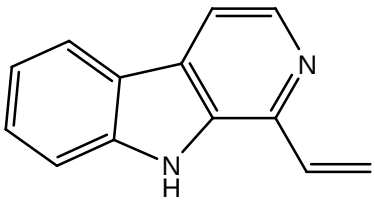

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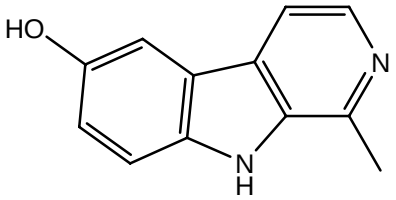

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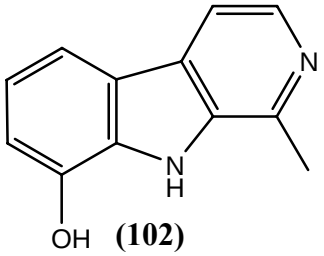

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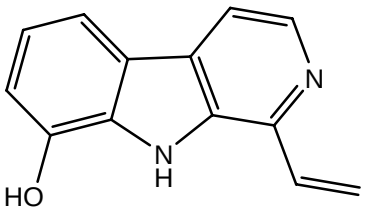

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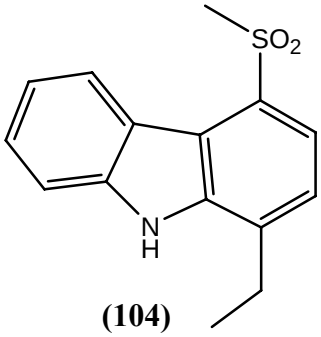
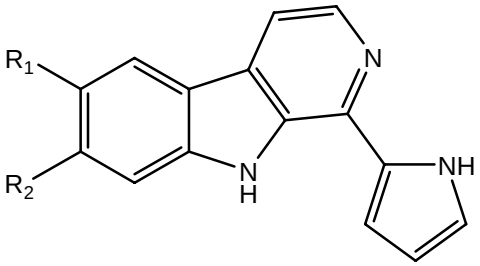
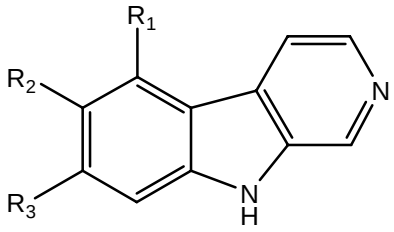


(104)

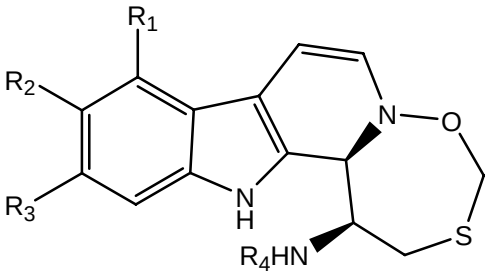
Brominated β-carboline alkaloids have been reported from marine organisms, some with interesting biological activity. The series of compounds called the eudistomins have been isolated from organisms belonging to several families and genera.[108-115]



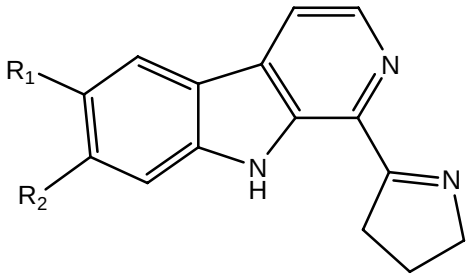
	R_1	R_2
(152)	OH	Br
(153)	unassigned	
(164)	OH	H



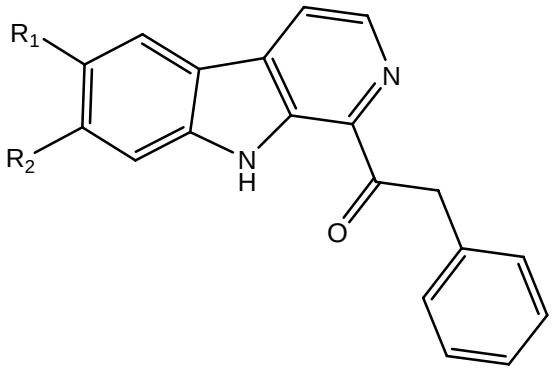
	R_1	R_2	R_3
(155)	Br	OH	H
(161)	H	OH	Br
(165)	H	Br	H
(166)	H	H	Br



	R_1	R_2	R_3	R_4
(154)	H	OH	Br	H
(156)	Br	OH	H	H
(157)	H	OH	OH	$C_2H_3O_2$
(162)	H	H	Br	H
(163)	H	Br	H	H

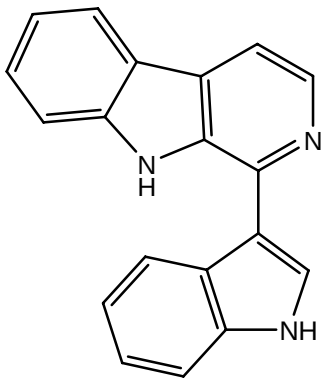


	R_1	R_2
(158)	H	Br
(159)	Br	H
(160)	H	H
(167)	OH	Br
(168)	OH	H



	R_1	R_2
(169)	H	Br
(170)	Br	H
(171)	H	H

To date, eudistomins A-X (**152-175**)[108-111] have been reported. Eudistomins A-T (**152-171**) have been isolated from the tunicate *Eudistoma olivaceum*.[108,110] Of these, the structure of eudistomin B (**153**) has not been fully assigned, the ^{1}H NMR spectroscopic data suggests that it is a 1,6,7-trisubstituted β-carboline with a methoxyl group.[110] Eudistomins C (**154**), K (**162**) and O (**166**) along with eudistomin K-sulfoxide (**176**) and debromoeudistomin K (**177**) have been isolated from the tunicate *Ritterella sigillinoides*.[113-115] Eudistomin U (**172**) was isolated from the ascidian *Lissoclinum fragile* along with isoeudistomin U (**178**).[109] Eudistomin V (**173**) was isolated from the ascidian *Pseudodistoma aureum*,[112] while eudistomins W (**174**) and X (**175**) were also isolated from a *Eudistoma sp.*[111]



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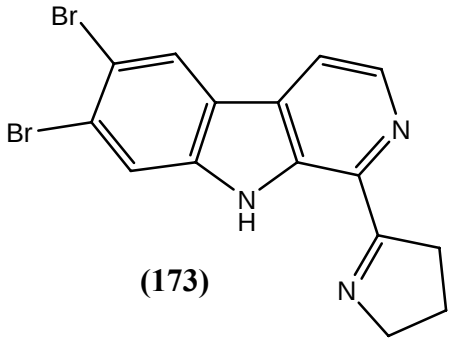

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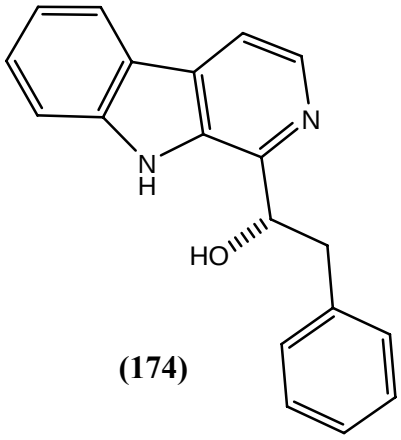

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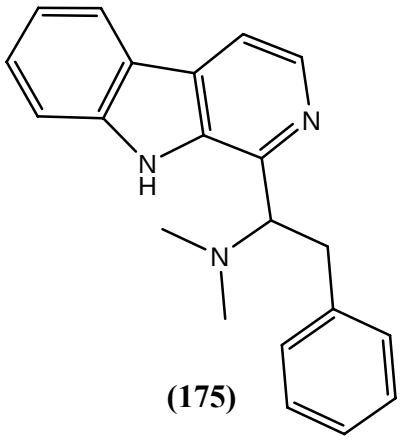

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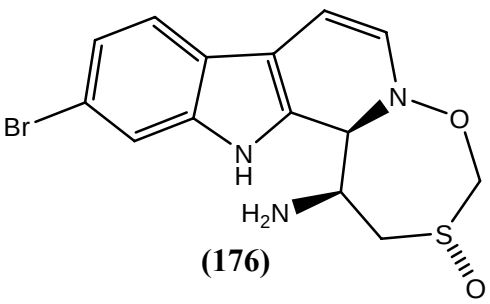

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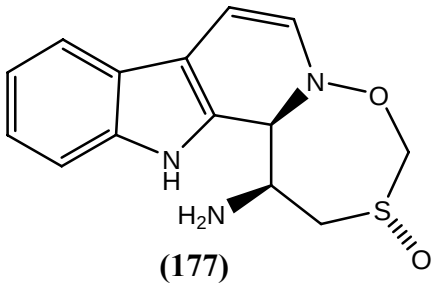

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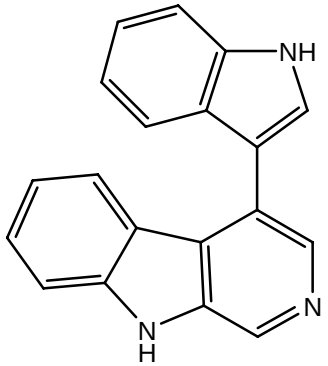
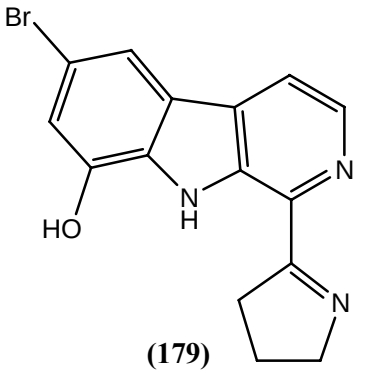


(178)

A series of brominated β-carboline alkaloids, eudistomidins A-F (**179-184**) were isolated from an ascidian *Eudistoma glaucus*.[116] Eudistomidins B-D (**180-182**) showed promising antitumour activity against the cell lines L1210 (IC_{50} values of 3.4, 0.36 and 2.4 μg/mL respectively) and L5178Y (IC_{50} values of 3.1, 0.42 and 1.8 μg/mL respectively).[116]



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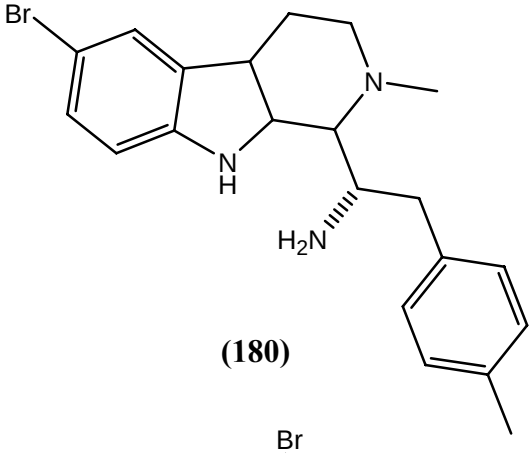

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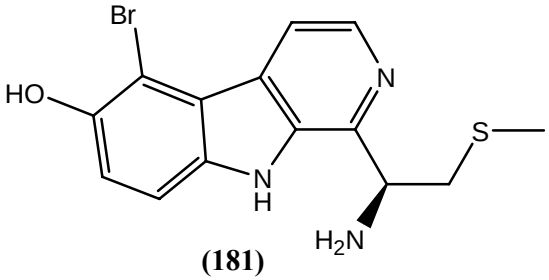

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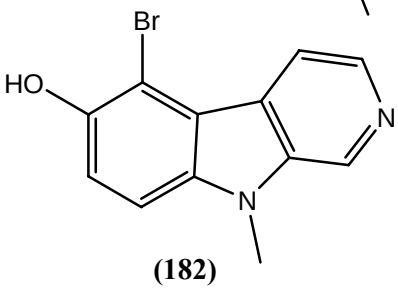
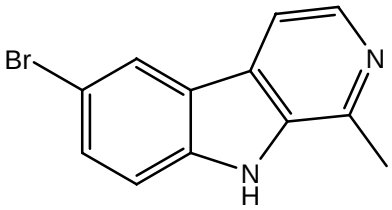


(182)

The hydroid *Aglaophenia pluma* has yielded three novel brominated β-carboline alkaloids, 6-bromo-1-methyl-β-carboline (**185**), 6-bromo-1-ethyl-β-carboline (**186**) and 6,8-dibromo-1-ethyl-β-carboline (**187**). No biological activity was reported for these compounds.[117]



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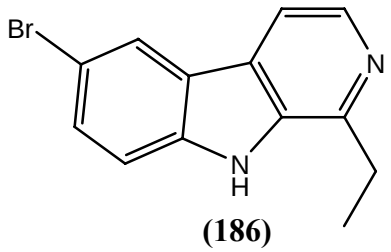
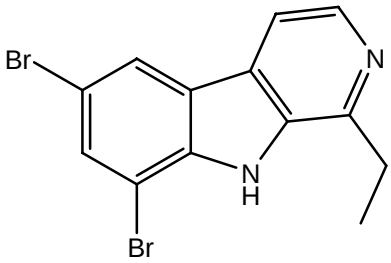


(186)



(187)

No brominated β-carbolines have previously been reported from a bryozoan species.

A sample of *P. vesiculosa* was chosen for further investigation with the aim of isolating a larger quantity of pterocellin G so further characterisation could be completed. Pterocellin G (**114**) was not found in this sample but three new compounds, pterocellin H (**188**), pterocellin I (**189**) and 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) were isolated and characterised. A discussion of the structure-activity relationship of selected members of these classes of compounds is also presented.

2.2 Isolation and Structural Elucidation of Pterocellin H (188)

2.2.1 Isolation of Pterocellin H (188)

The crude dichloromethane extract of *P. vesiculosa* was subject to fractionation by reversed phase and size exclusion column chromatography monitored by TLC analysis. This resulted in the isolation of a fraction containing the new compound pterocellin H (**188**). The structure of pterocellin H (**188**) was elucidated using one-dimensional (1D) and two-dimensional (2D) NMR spectroscopic techniques and high resolution electrospray mass spectrometry (HRESMS). The separation tree for this extract is presented in Appendix Five.

2.2.2 Characterisation of Pterocellin H (188)

The ^{1}H NMR spectrum of pterocellin H (**188**) in $CDCl_3$ (Figure 2.1) contained ten signals. These signals included four aromatic proton signals (two singlets at 6.57 and 7.81 ppm and two mutually coupled doublets at 7.30 and 8.39 ppm). A methyl signal at 2.00 ppm, a methoxyl resonance (3.74 ppm), two methylene signals at 3.31 ppm and 3.02 ppm, a methine proton multiplet at 2.17 ppm and a doublet at 1.02 ppm were also present.

The ^{13}C NMR spectrum of pterocellin H (**188**) in $CDCl_3$ contained eighteen carbon signals. Four signals represented protonated aromatic carbons, while there were also six quaternary aromatic carbon resonances, and two carbonyl signals at 172.5 ppm and 205.0 ppm. Of the six aliphatic protonated carbon resonances, the signal at 22.4 ppm was significantly more intense than the others, indicating that it represented two carbon atoms.

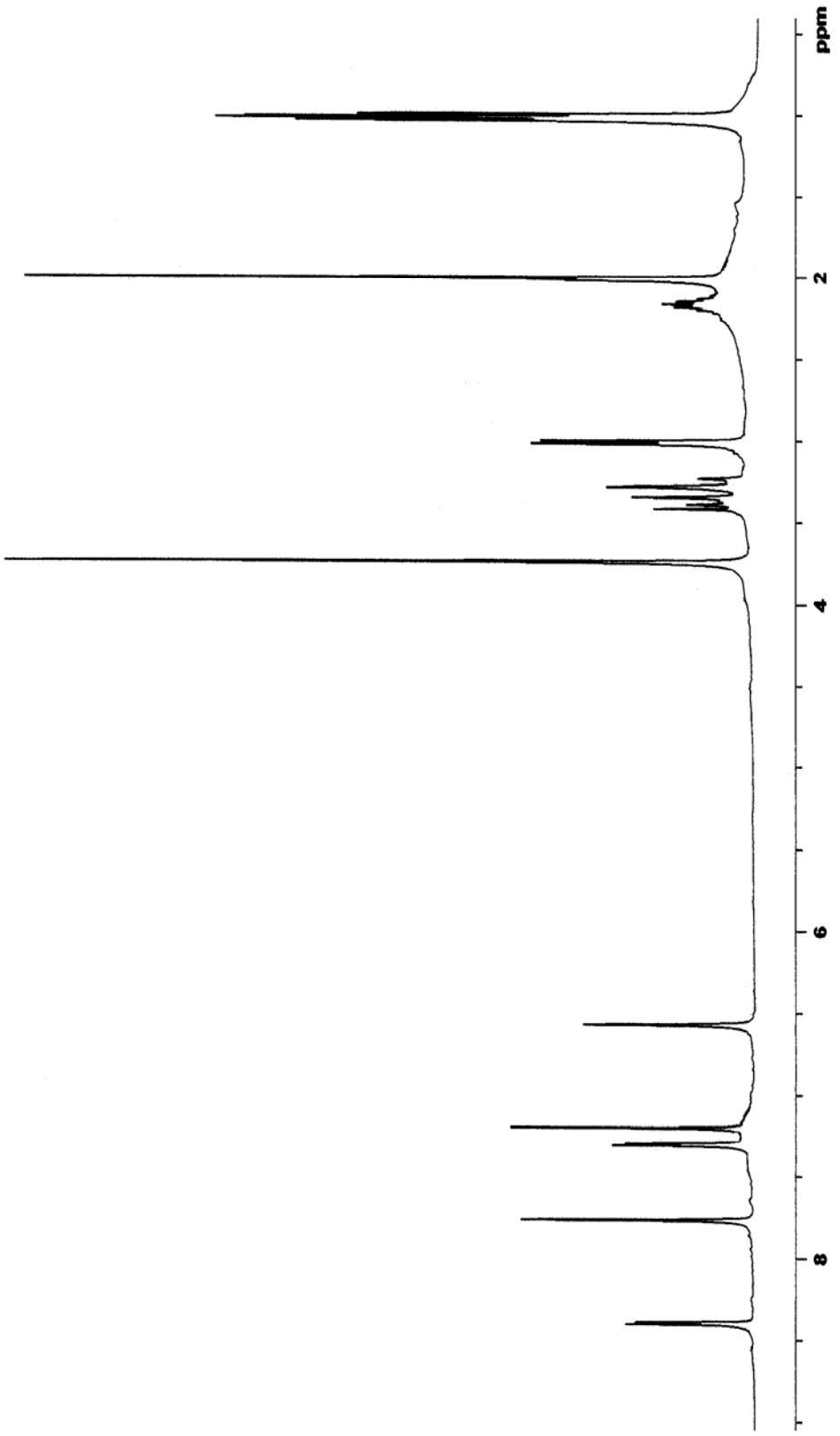


Figure 2.1: ^{1}H NMR Spectrum of Pterocellin H (**188**) in $CDCl_3$

The HRESMS of compound (**188**) contained a $[M+Na]^+$ peak at *m/z* 365.1512, with no obvious isotope pattern and the $[M+H]^+$ ion was also visible at *m/z* 343.1692. These two peaks are consistent with the molecular formula $C_{19}H_{22}N_2O_4$ for pterocellin H (**188**).

Atom connectivity was established by COSY, ^1H-^{13}C HSQC and ^1H-^{13}C HMBC NMR spectroscopic experiments, as well as by comparisons with molecules of closely related structure. For example, the proton signals at 3.02, 2.17 and 1.02 ppm show correlations that indicate they form an isobutyl group as depicted in Figure 2.2. The correlations seen in the HMBC NMR experiment from H1' to C1 and C13 and from H2' to C1 indicate that the isobutyl group is attached to the C1 position.

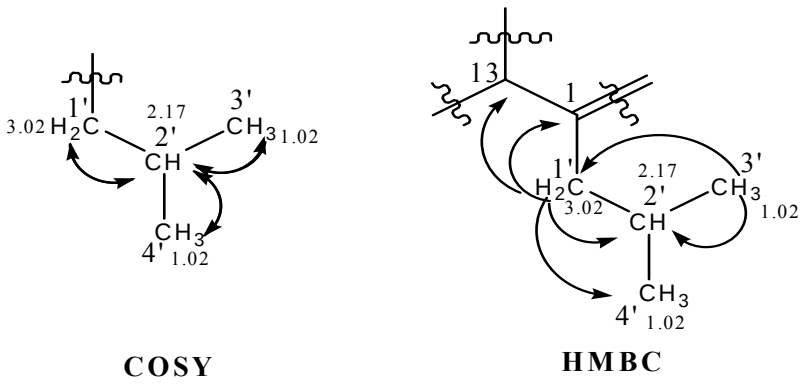


Figure 2.2 COSY and HMBC Interactions of a Substructure of Pterocellin H (**188**).

The HMBC NMR spectroscopic data indicates that the two aromatic proton signals at 6.57 and 7.81 ppm represent protons that are positioned *para* to each other. HMBC NMR correlations are seen from H8 to C9 and C10 and also from H11 to C9 and C10. The methoxyl group is attached at C10 as indicated by the

HMBC correlation from H14 to C10, which was the only HMBC correlation observed for H14. This leads to the fragment structure depicted in Figure 2.3.

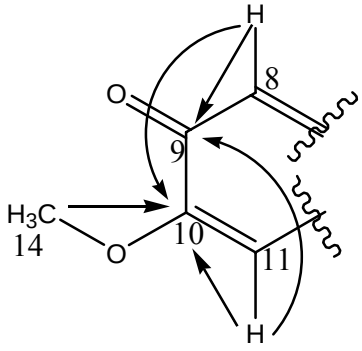


Figure 2.3: HMBC Interactions of a Pterocellin H (**188**) Fragment.

The proton resonances of H1" and H3" (3.31 and 2.01 ppm respectively) show correlations in the 2D NMR experiments that indicate they are part of a propanoyl sidechain attached at C6. The HMBC NMR spectrum contains a correlation from H3" to C2", the carbonyl carbon, and C1", it also shows correlations from H1" to C2" indicating that the carbonyl is positioned between these two. Additional correlations are observed in the HMBC experiment from H1" to both C6 and to the quaternary aromatic signal of C7, indicating that this propanoyl group is attached at the C6 position.

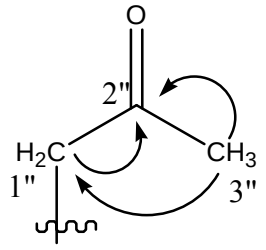
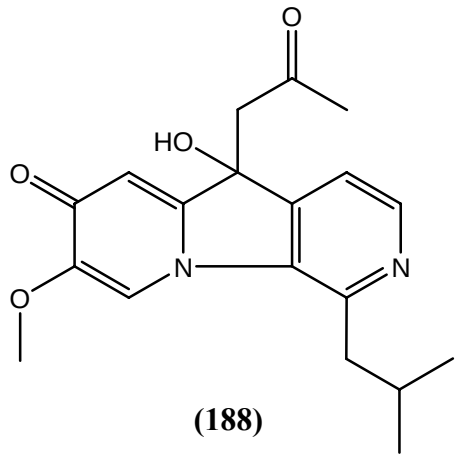


Figure 2.4: HMBC Correlations of the C6 Sidechain of Pterocellin H (**188**).

The NMR spectroscopic data, along with comparisons with reported data on closely related compounds, indicates that pterocellin H (**188**) possesses the same skeleton common to the pterocellin series of compounds and has an isobutyl group at position 1 and an hydroxyl group and a propanoyl side chain at position 6.

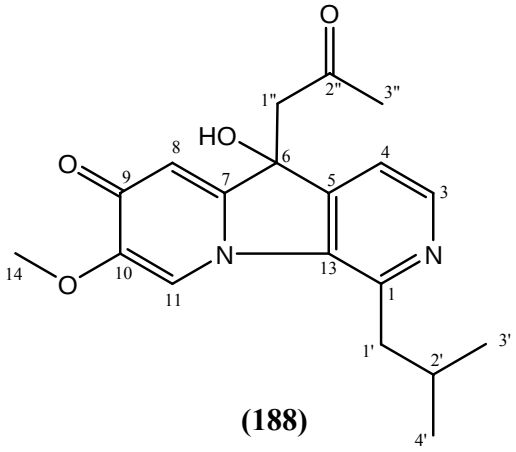


(188)

The NMR spectroscopic data for pterocellin H (**188**) is summarised in Table 2.1

The resonance of the C6 carbonyl group in pterocellin A is 184.3 ppm,[86] the replacement of this carbonyl group with a hydroxyl group and a propanoyl group shifts the resonance significantly upfield to 75.9 ppm as is seen in pterocellin H. The proton resonance of H8 seen at 7.07 ppm in pterocellin A[86] is also moved significantly upfield to its position at 6.57 ppm in the ^{1}H NMR spectrum of pterocellin H.

Table 2.1: ^{1}H and ^{13}C NMR Spectroscopic Data for Pterocellin H (**188**) Recorded in $CDCl_3$.



(188)

Position	^{1}H δ (ppm) (*J*, Hz)	^{13}C δ (ppm)	HMBC (H to C)
1		145.2	
3	8.39 d (4.6)	146.5	1, 4,
4	7.31 d (4.6)	117.1	3, 6, 13
5		145.5	
6		75.9	
7		149.6	
8	6.57 s	111.8	6, 7, 9, 10
9		172.5	
10		153.3	
11	7.76 s	115.8	7, 9, 10, 13
13		136.1	
14	3.74 s	56.5	10
1’	3.00 d (7.2)	44.6	1, 13, 2’, 3’/4’
2’	2.17 m	28.4	1’, 3’/4’
3’	1.02 d (6.5)	22.4	1’, 2’, 4’
4’	1.02 d (6.5)	22.4	1’, 2’, 3’
1a”	3.37 d (18.0)	52.1	6, 7, 2”
1b”	3.26 d (18.0)	52.1	6, 7, 2”
2”		205.0	
3”	2.01 s	30.7	1”, 2”

2.3 Isolation and Structural Elucidation of Pterocellin I (189)

2.3.1 Isolation of Pterocellin I (189)

TLC directed fractionation of the crude extract of *P. vesiculosa* led to the isolation of a fraction containing pterocellin I (**189**). The structure of pterocellin I (**189**) was determined by HRESMS and 1D and 2D NMR spectroscopic data analysis.

2.3.2 Characterisation of Pterocellin I (189)

The ^{1}H NMR spectrum of pterocellin I (**189**) in $CDCl_3$ (Figure 2.5) strongly resembled that of pterocellin H (**188**) but the resonances for the isobutyl group were absent, replaced by a methylene singlet at 4.64 ppm and extra signals in the aromatic region consisting of a three-proton multiplet at 7.34 ppm and a two-proton doublet at 7.16 ppm.

The ^{13}C NMR spectrum of pterocellin I (**189**) contained twenty carbon resonances. Seven of these signals were aromatic protonated carbons, two of which were considerably more intense than the others, indicating that they each represented two carbon atoms. There were two carbonyl carbon signals at 172.4 ppm and 204.9 ppm. The spectrum also contained four aliphatic carbon signals and seven quaternary aromatic carbon signals. Of these resonances, most were analogous to those seen in the ^{13}C NMR spectrum of pterocellin H. The resonances of the carbon atoms from the isobutyl group in pterocellin H (**188**) were absent and there were five additional resonances representing an aromatic quaternary carbon, three protonated aromatic carbons and a protonated aliphatic carbon.

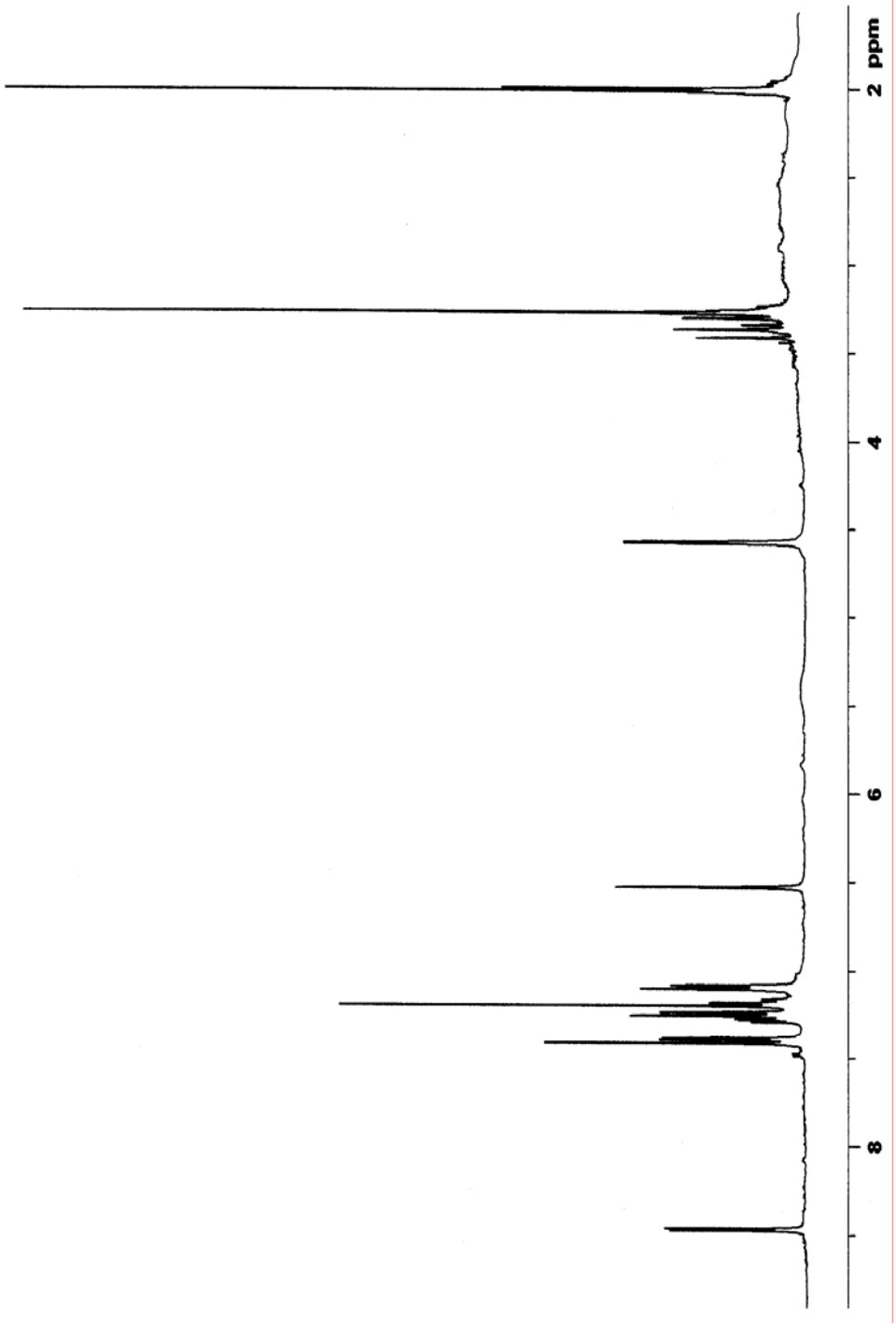


Figure 2.5: ^{1}H NMR Spectrum of Pterocellin I (**189**) in $CDCl_3$.

The HRESMS of compound (**189**) contained a $[M+Na]^+$ peak at *m/z* 399.1240, with no obvious isotope pattern, the $[M+H]^+$ ion was also visible at *m/z* 377.1436. These two peaks are consistent with the molecular formula $C_{22}H_{20}N_2O_4$ for pterocellin I (**189**).

The differences in the ^1H and ^{13}C NMR spectra between pterocellin H and pterocellin I indicate that the isobutyl substituent at C1 in pterocellin H is replaced by a benzyl group in pterocellin I. COSY, ^1H-^{13}C HMBC and ^1H-^{13}C HSQC NMR data were used to establish the connectivity of the new atoms. The COSY NMR spectrum contained correlations between the multiplet of H4'/H5' and the resonance of H3'. The HMBC NMR spectrum contained correlations from H1' to both C1 and C13, indicating the sidechain attachment at position 1, in addition to a correlation to the quaternary aromatic signal of C2' (137.0 ppm). The HMBC spectrum also contained correlations from H3' and H4'/H5' to C2' indicating the benzyl sidechain as depicted in Figure 2.6.

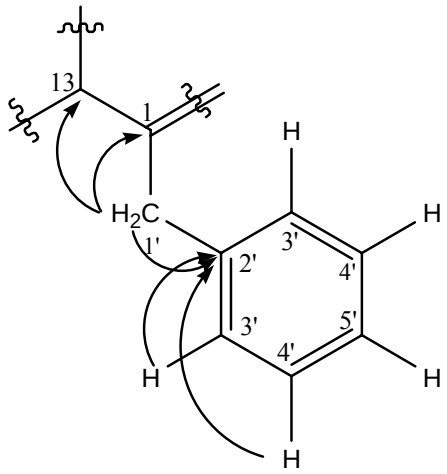
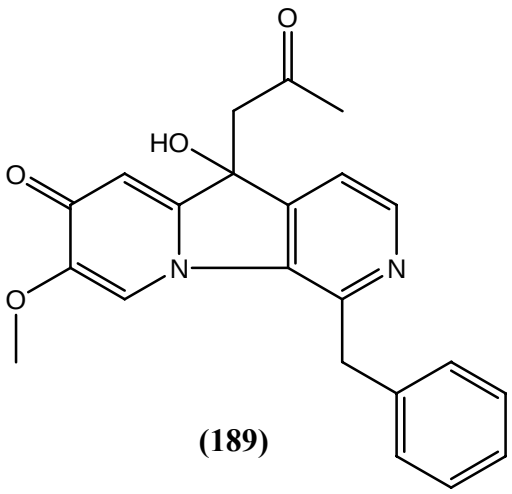


Figure 2.6: C1 Sidechain of Pterocellin I (**189**) with HMBC Interactions.

Other correlations observed in the HMBC NMR spectrum indicated that this compound possessed the same skeleton as pterocellin H with the same

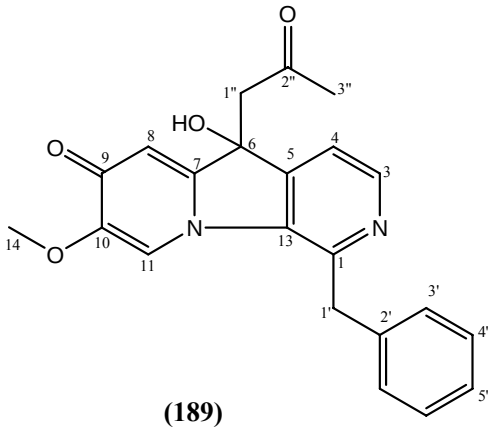
substitution of groups except that the benzyl group replaced the isobutyl group at C1. This allowed complete structural elucidation of pterocellin I (**189**).



(189)

A summary of the NMR data for pterocellin I (**189**) is presented in Table 2.2.

Table 2.2: ^{1}H and ^{13}C NMR Spectroscopic Data for Pterocellin I (**189**) in $CDCl_3$



(189)

Position	^{1}H δ (ppm) (*J*, Hz)	^{13}C δ (ppm)	HMBC (H to C)
1		145.9	
3	8.53 d (4.6)	147.0	1, 4,
4	7.45 d (4.6)	117.8	3, 6, 13
5		142.6	
6		76.0	
7		149.6	
8	6.56 s	111.3	6, 7, 9, 10
9		172.4	
10		152.8	
11	7.76 s	115.7	7, 9, 10, 13
13		136.7	
14	3.34 s	56.1	10
1’	4.64 s	41.9	1, 13, 2’, 3’
2’		137.0	
3’	7.16 d (7.2)	127.8	1’, 2’, 4’, 5’
4’	7.34 m	129.3	2’, 3’, 5’
5’	7.34 m	127.3	
1a”	3.39 d (18.3)	52.2	6, 7, 2”
1b”	3.28 d (17.8)	52.2	6, 7, 2”
2”		204.9	
3”	2.08 s	30.6	1”, 2”

2.4 Biological Activity of the Pterocellins.

The pterocellin series of compounds show activity in the P388 antitumour assay and also in two of the antimicrobial assays performed at the University of Canterbury (for assay details see Section 1.4). Table 2.3 outlines the biological activity of the pterocellins in these assay systems. Biological assay information for pterocellins A-F (**108-113**) has been previously obtained.[86,87]

Table 2.3: Biological Activity of Pterocellins A-I.

Pterocellin	**P388** IC_{50} (ng/mL)	**Bs**[a] MID (μg/disk)	**Tm**[b] MID (μg/disk)
A (**108**)	477	0-0.3	3.9-7.5
B (**109**)	323	0-0.3	3.9-7.5
C (**110**)[c]	>6250	0-0.5	ND[d]
D (**111**)	4773	0.5-1	10-15
E (**112**)[c]	>6250	0-0.5	ND
F (**113**)[c]	>6250	0-0.5	ND
G (**114**)	not yet tested		
H (**188**)	>6250	ND	ND
I (**189**)	>6250	ND	ND

[a] *Bacillus subtilis,* [b] *Trichophyton mentagrophytes,* [c] MID values yet to be confirmed, [d] no activity was observed.

It is evident that pterocellins A (**108**) and B (**109**) are the most active of this series of compounds. Based on the information in Table 2.3 there appear to be relationships between the structures of the pterocellins and the activity they exhibit. All pterocellins tested to date display antibacterial activity other than pterocellins H (**188**) and I (**189**). This indicates that the carbonyl group at position 6 is important in the mechanism by which these compounds exert their

antibacterial activity. The level of antitumour activity against the P388 cell line exhibited by the pterocellins would appear to be influenced by the size of the group at position 8 excluding pterocellins H (**188**) and I (**189**). Pterocellins A (**108**) and B (**109**) exhibit the strongest activity and they both have a hydrogen at position 8, pterocellin D (**111**), with an isopropyl group at this position, possesses intermediate activity and the other pterocellins C (**110**), E (**112**) and F (**113**) are essentially inactive in this assay and have larger groups at position 8. Pterocellins H (**189**) and I (**189**) also have a hydrogen at position 8 yet show no activity. This could be due to the propanoyl group hindering access to position 8 or alternatively, the carbonyl at position 6 could also be important for the antitumour activity. It is likely that the mechanism of action by which the pterocellins exhibit their antitumour activity is intercalation, so the bulkier the side chains, as in pterocellin C (**110**), or the more they deviate from the plane of the molecule, as the C6 sidechain does in pterocellins H (**188**) and I (**189**), the less likely the molecule is able to effectively intercalate into DNA. Antifungal activity appears to follow the same trend as antitumour activity, pterocellins A (**108**) and B (**109**) display good activity, pterocellin D (**111**) has intermediate activity and the remaining pterocellins that have been tested exhibit no activity in this assay.

2.5 Isolation and Structural Elucidation of 1-Methyl-5-bromo-8-methoxy-β-carboline (190)

2.5.1 Isolation of 1-Methyl-5-bromo-8-methoxy-β-carboline (190)

TLC monitored separation of the extract of *P. vesiculosa* yielded a fraction containing the novel compound, 1-methyl-5-bromo-8-methoxy-β-carboline (**190**). The structure of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) was elucidated using HRESMS, 1D and 2D NMR experiments and X-ray crystallography.

2.5.2 Characterisation of 1-Methyl-5-bromo-8-methoxy-β-carboline (190)

The ^{1}H NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$ (Figure 2.7) contained six resonances. Three signals represent aromatic protons, (a two proton singlet at 8.33 ppm and two mutually coupled doublets at 7.25 ppm and 6.80 ppm). The spectrum also contained a methoxyl signal at 3.92 ppm and a methyl signal at 2.77 ppm.

The ^{13}C NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) contained 13 signals, including six protonated carbon resonances (two aliphatic and four aromatic) and seven quaternary aromatic carbons.

The HRESMS of compound (**190**) contained a $[M+ H]^+$ pair of peaks at *m/z* 291.0147 and 293.0049, an isotope pattern that indicated the presence of a halogen atom. The $[M +Na]^+$ pair of peaks was also visible at *m/z* 312.9886 and 314.9866. These peaks are consistent with a molecular formula $C_{13}H_{11}N_2OBr$ for compound (**190**).

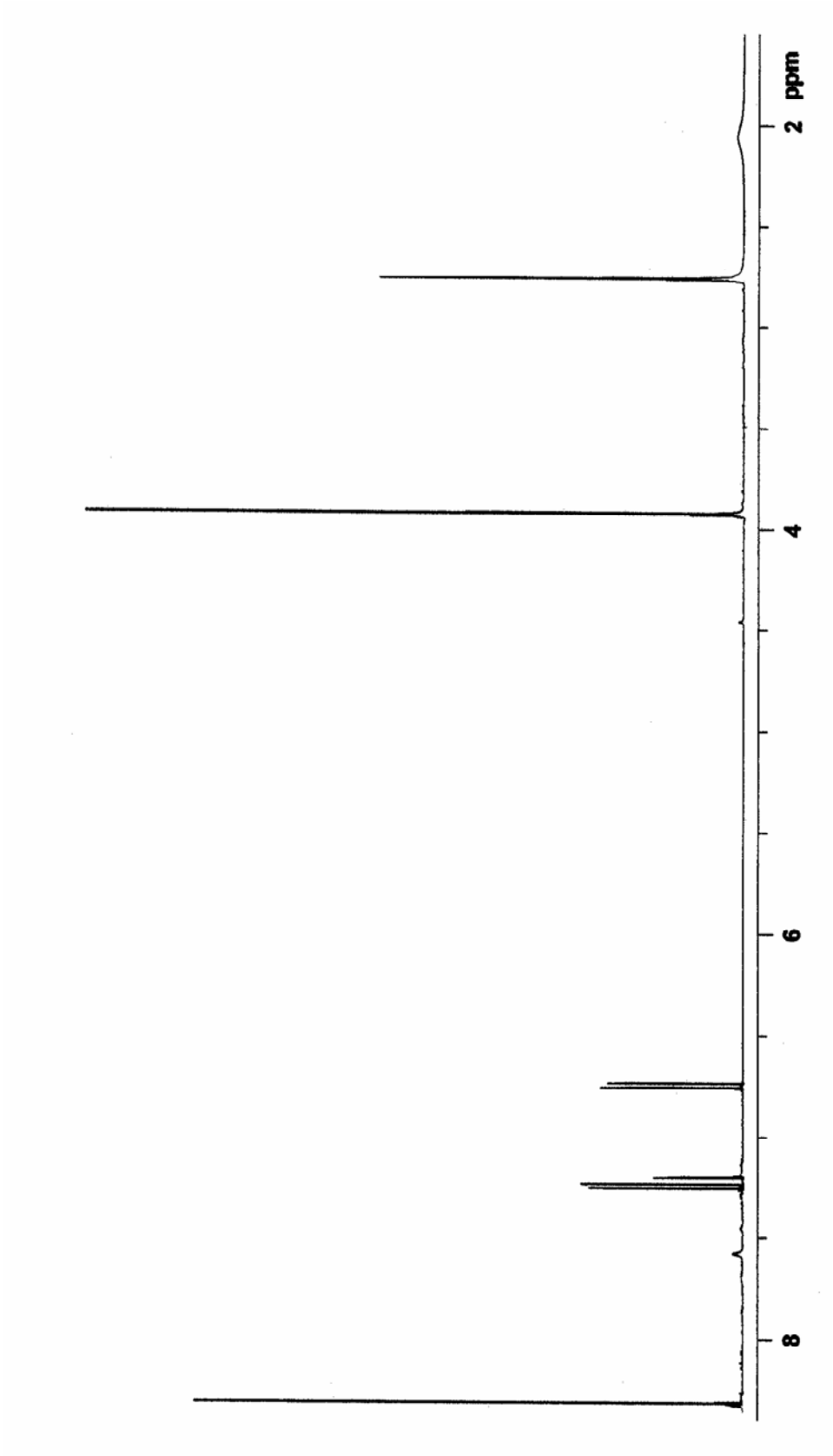


Figure 2.7: ^{1}H NMR Spectrum of 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$.

Atom connectivity was established through NOESY, ^{1}H-^{13}C HMBC and ^{1}H-^{13}C HSQC NMR spectroscopic data. For example, in the HSQC NMR spectrum, the two coincidental aromatic protons at 8.33 ppm show correlations to two different carbon atoms, C3 and C4 (114.7 ppm and 139.0 ppm respectively).

The NOESY NMR experiment showed correlations between the methoxyl group at 3.92 ppm and H7 at 6.80 ppm, and between H6 at 7.25 ppm and H7 at 6.80 ppm. The methoxyl signal in the HMBC NMR experiment correlated to the quaternary carbon resonance of C8 (145.8 ppm). This confirmed that it is attached at position 8 as indicated in Figure 2.8.

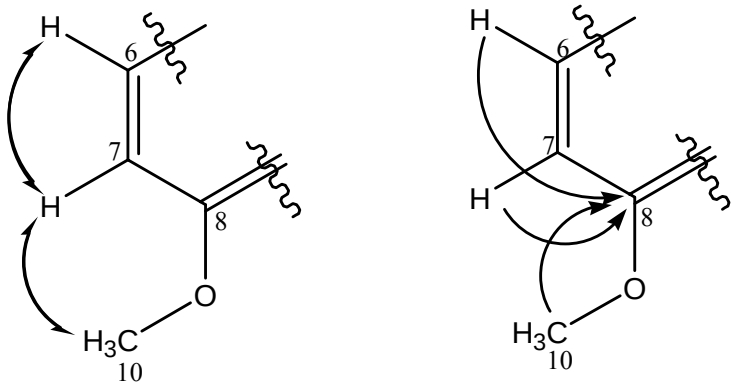


Figure 2.8: NOESY and HMBC Correlations for a Structural Fragment of 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**)

In the HMBC NMR experiment, the methyl proton resonance was correlated to C1, C4b and also showed a weak correlation to C3, indicating that it was attached at C1. The HRESMS information indicated the presence of a bromine atom and a combination of ^{1}H NMR and NOESY spectroscopic experiments indicated that there was no proton at C5; therefore the bromine is attached at this position. Full HMBC NMR correlations can be seen in Figure 2.9.

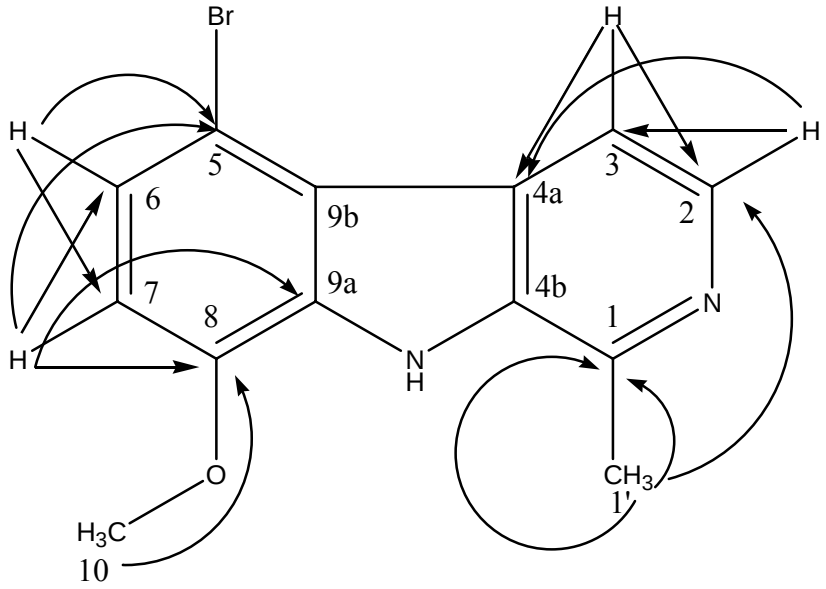
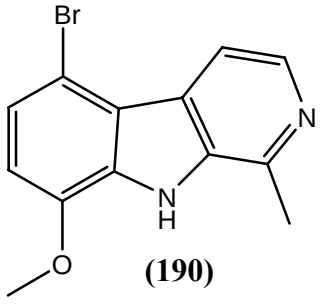


Figure 2.9: HMBC Correlations of 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**).

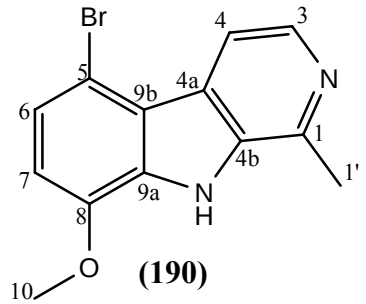
The NMR spectroscopic data was slightly ambiguous, so the structure of 1-methyl-5-bromo-8-methoxy-β-carboline (**190)** was confirmed with the aid of an X-ray crystal structure solved by direct methods (Section 2.5.3).



(190)

The NMR spectroscopic data for 1-methyl-5-bromo-8-methoxy-β-carboline (**190)** is summarised in Table 2.4.

Table 2.4: ^{1}H and ^{13}C NMR Spectroscopic Data for 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**) Recorded in $CDCl_3$.



(190)

Position	^{1}H δ (ppm) (*J*, Hz)	^{13}C δ (ppm)	HMBC (H to C)
1		142.4	
3	8.33 s	114.7	1, 4, 4a,
4	8.33 s	139.0	3, 4a, 4b, 9b
4a		128.5	
4b		134.1	
5		108.1	
6	7.25 d (8.3)	123.6	5, 7, 8, 9b
7	6.80 d (8.3)	109.0	5, 6, 8, 9a
8		145.8	
9a		131.5	
9b		121.3	
10	3.92 s	55.6	8
1’	2.77 s	20.4	1, 3, 4b

The signals of H3 and H4 were coincidental although their chemical environments appear to be quite different. It has been seen previously in 5-bromo-β-carbolines that the H3 and H4 protons resonate closer together than is typical of any other β-carboline alkaloid. In the reported NMR spectroscopic data for eudistomin D (**155**) (5-bromo-6-hydroxy-β-carboline), the signals for H3 and H4 are at 8.55

ppm and 8.34 ppm respectively,[118] for the acetylated derivative 5-bromo-6-acetoxy-9-acetyl-β-carboline, the resonances for H3 and H4 were even closer together at 8.71 and 8.67 ppm respectively.[118] This is in contrast to a β-carboline without a 5-bromo substituent such as harman (1-methyl-β-carboline) with H3 and H4 resonances appearing at 8.43 and 7.83 ppm respectively.[119] This data, along with the NMR data for (**190**) indicates that the presence of a bromine substituent at C5 on a β-carboline ring system has the effect of moving the resonances of H3 and H4 closer together. In the case of the new compound (**190**) they are coincidental.

The addition of the 5-bromo and the 8-methoxyl groups to the base structure of 1-methyl-β-carboline (harman) (**97**) results in the resonances of C5, C7, C8 and C9a shifting significantly as would be expected. In harman (**97**), the C5 resonance appears at 122.1 ppm,[120] with the addition of a bromine at that point as in (**190**), the resonance shifts significantly upfield to 108.1 ppm. The 5-bromo substituent affects the chemical shift of C5 but has little effect on the neighbouring carbon atoms C6 and C9b. The addition of the methoxyl group at position 8 shifts the resonance of C8 from 111.9 as seen in harman (**97**)[120] to 145.8 in 1-methyl-5-bromo-8-methoxy-β-carboline (**190**). The addition of the methoxyl group also significantly affects the resonance of the neighbouring carbon atoms at C7 and C9b. C7 resonates at 128.5 ppm in harman (**97**),[120] while in 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) it is shifted upfield to 109.0 ppm. The C9b signal appears in the ^{13}C NMR spectrum of harman (**97**) at 140.5 ppm,[120] but is shifted upfield to 131.5 ppm in 1-methyl-5-bromo-8-methoxy-β-carboline (**190**).

2.5.3 X-ray Crystal Structure of 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**)

Small block crystals were grown from toluene/heptane at room temperature over seven days. The unit cell dimensions and intensity data were obtained on a Bruker-Nonius Apex II CCD diffractometer. The structure was solved by direct methods using the SHELXS-97[121] programme and Full matrix least-squares refinement was completed using the programme SHELXL-97.[122]

The crystal was shown to have the formula $C_{13}H_{11}N_2OBr.CH_3OH$ and was monoclinic with the space group P2(1)/c. There are four molecules in a unit cell. Crystal data and structural refinement detail are outlined in Table 2.5. Atomic coordinates and equivalent isotropic displacement parameters are presented in Table 2.6. Bond lengths and bond angles are displayed in Tables 2.7 and 2.8 respectively.

Table 2.5: Crystal Data and Structure Refinement for 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**).

Empirical formula	$C_{14}H_{15}N_2O_2Br$	
Formula weight	323.19	
Temperature	93(2) K	
Wavelength	0.71073 Å	
Crystal system, space group	Monoclinic, P2(1)/c	
Unit cell dimensions	a = 9.6025(5) Å	alpha = 90°
	b = 10.9301(5) Å	beta = 107.645(2) °
	c = 13.3928(6) Å	gamma = 90°
Volume	1339.53(11) $Å^3$	
Z, Calculated density	4, 1.603 mg/m^3	
Absorption coefficient	3.068 mm^{-1}	
F(000)	656	
Crystal size	0.60 x 0.56 x 0.30 mm	
Theta range for data collection	2.90 < θ < 28.00°	
Limiting indices	-12 <= h <= 8, -13 <= k <= 14,	
	-15 <= l <= 17	
Reflections collected / unique	11505 / 3236 [R(int) = 0.0382]	
Completeness to θ = 28.00	99.9 %	
Absorption correction	Semi-empirical	
Max. and min. transmission	0.4596 and 0.2604	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3236 / 0 / 199	
Goodness-of-fit on F^2	1.074	
Final R indices [I > 2sigma(I)]	R_1 = 0.0316, wR_2 = 0.0827	
R indices (all data)	R_1 = 0.0364, wR_2 = 0.0848	
Largest diff. peak and hole	1.212 and -0.448 e. $Å^{-3}$	

Table 2.6: Atomic Coordinates (x 10^4) and Equivalent Isotropic Displacement Parameters ($Å^2$ x 10^3) for 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**). (U(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor).

Atom	x	y	z	U(eq)
Br (1)	7292 (1)	983 (1)	1311 (1)	32 (1)
O (1)	9883 (2)	-3364 (1)	-332 (1)	23 (1)
N (1)	13724 (2)	710 (2)	2872 (1)	22 (1)
N (2)	11616 (2)	-1691 (2)	1139 (1)	17 (1)
C (1)	13505 (2)	-331 (2)	2319 (1)	20 (1)
C (3)	12559 (2)	1386 (2)	2910 (2)	24 (1)
C (4)	11119 (2)	1088 (2)	2406 (2)	21 (1)
C (4a)	10853 (2)	13 (2)	1803 (1)	17 (1)
C (4b)	12076 (2)	-690 (2)	1776 (1)	17 (1)
C (5)	8070 (2)	-396 (2)	799 (2)	21 (1)
C (6)	7150 (2)	-1175 (2)	95 (2)	23 (1)
C (7)	7709 (2)	-2198 (2)	-302 (2)	21 (1)
C (8)	9195 (2)	-2439 (2)	11 (1)	18 (1)
C (9a)	10118 (2)	-1642 (2)	739 (1)	16 (1)
C (9b)	9581 (2)	-604 (2)	1137 (1)	18 (1)
C (10)	9001 (2)	-4161 (2)	-1121 (2)	25 (1)
C (1')	14797 (2)	-1084 (2)	2291 (2)	27 (1)
O (Methanol)	3521 (2)	6457 (1)	859 (1)	27 (1)
C (Methanol)	3419 (3)	5987 (2)	-148 (2)	28 (1)

Table 2.7: Bond Lengths (Å) from X-ray Crystal Data for 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**).

Bond	**(Å)**	**Bond**	**(Å)**
Br(1)-C(5)	1.8998 (19)	C(4)-C(4a)	1.405 (3)
O(1)-C(8)	1.361 (2)	C(4)-H(4)	0.93 (2)
O(1)-C(10)	1.432 (2)	C(4a)-C(4b)	1.414 (3)
N(1)-C(1)	1.339 (2)	C(4a)-C(9b)	1.443 (3)
N(1)-C(3)	1.354 (3)	C(9b)-C(5)	1.402 (3)
N(2)-C(4b)	1.375 (2)	C(9b)-C(9a)	1.415 (3)
N(2)-C(9a)	1.375 (2)	C(5)-C(6)	1.375 (3)
N(2)-H(1)	0.75 (3)	C(6)-C(7)	1.412 (3)
C(1)-C(4b)	1.400 (3)	C(6)-H(6)	0.92 (3)
C(1)-C(1')	1.499 (3)	C(7)-C(8)	1.385 (3)
C(3)-C(4)	1.381 (3)	C(7)-H(7)	1.00 (2)
C(3)-H(3)	0.98 (2)	C(8)-C(9a)	1.404 (2)

Table 2.8: Bond Angles (°) from X-ray Crystal Data for 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**).

Angle	(°)	Angle	(°)
C(8)-O(1)-C(10)	117.52 (15)	C(9a)-C(9b)-C(4a)	105.74 (16)
C(1)-N(1)-C(2)	119.45 (17)	C(6)-C(5)-C(9b)	120.58 (18)
C(4b)-N(2)-C(9a)	108.22 (16)	C(6)-C(5)-Br(1)	119.85 (15)
C(4b)-N(2)-H(1)	129.00 (2)	C(9b)-C(5)-Br(1)	119.57 (14)
C(9a)-N(2)-H(1)	123.00 (2)	C(5)-C(6)-C(7)	120.64 (18)
N(1)-C(1)-C(4b)	119.37 (18)	C(5)-C(6)-H(6)	120.30 (16)
N(1)-C(1)-C(1')	119.23 (17)	C(7)-C(6)-H(6)	119.10 (16)
C(4b)-C(1)-C(1')	121.40 (17)	C(8)-C(7)-C(6)	120.74 (18)
N(1)-C(3)-C(4)	124.67 (18)	C(8)-C(7)-H(7)	120.60 (13)
N(1)-C(2)-H(2)	116.30 (13)	C(6)-C(7)-H(7)	118.70 (13)
C(3)-C(2)-H(2)	118.90 (13)	O(1)-C(8)-C(7)	126.85 (17)
C(3)-C(4)-C(4a)	117.32 (19)	O(1)-C(8)-C(9a)	115.25 (16)
C(3)-C(4)-H(4)	120.00 (15)	C(7)-C(8)-C(9a)	117.87 (17)
C(4a)-C(4)-H(4)	122.70 (15)	N(2)-C(9a)-C(8)	127.71 (17)
C(4)-C(4a)-C(4b)	117.54 (17)	N(2)-C(9a)-C(9b)	109.96 (16)
C(4)-C(4a)-C(9b)	135.97 (18)	C(8)-C(9a)-C(9b)	122.28 (17)
C(4b)-C(4a)-C(9b)	106.47 (16)	N(2)-C(4b)-C(1)	128.75 (17)
C(5)-C(9b)-C(9a)	117.87 (17)	N(2)-C(4b)-C(4a)	109.59 (16)
C(5)-C(9b)-C(4a)	136.35 (17)	C(1)-C(4b)-C(4a)	121.64 (17)

Figure 2.10 shows an Oak Ridge thermal ellipsoid plot (ORTEP) representation of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**).

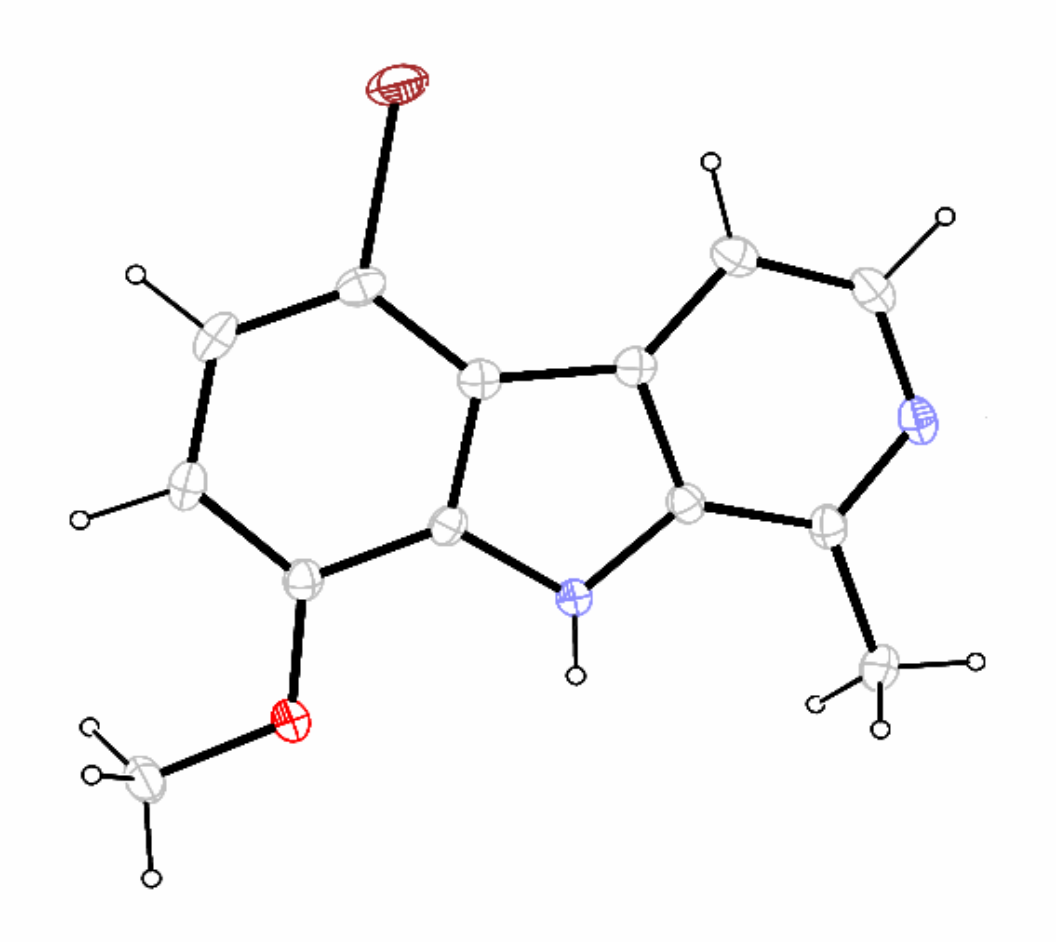


Figure 2.10: ORTEP Projection of 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**).

The packing in 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) is such that a molecule is reflected then moved up half a molecule. Hydrogen bonding occurs between the oxygen on a methanol molecule and the pyridol hydrogen of this compound and also between the hydroxyl hydrogen of that same methanol molecule with the tertiary nitrogen of another 1-methyl-5-bromo-8-methoxy-β-carboline molecule. This helps to stack and align the molecules in the crystal. A diagram of the stacking and another of the hydrogen bonding of (**190**) are presented in Figures 2.11 and 2.12 respectively.

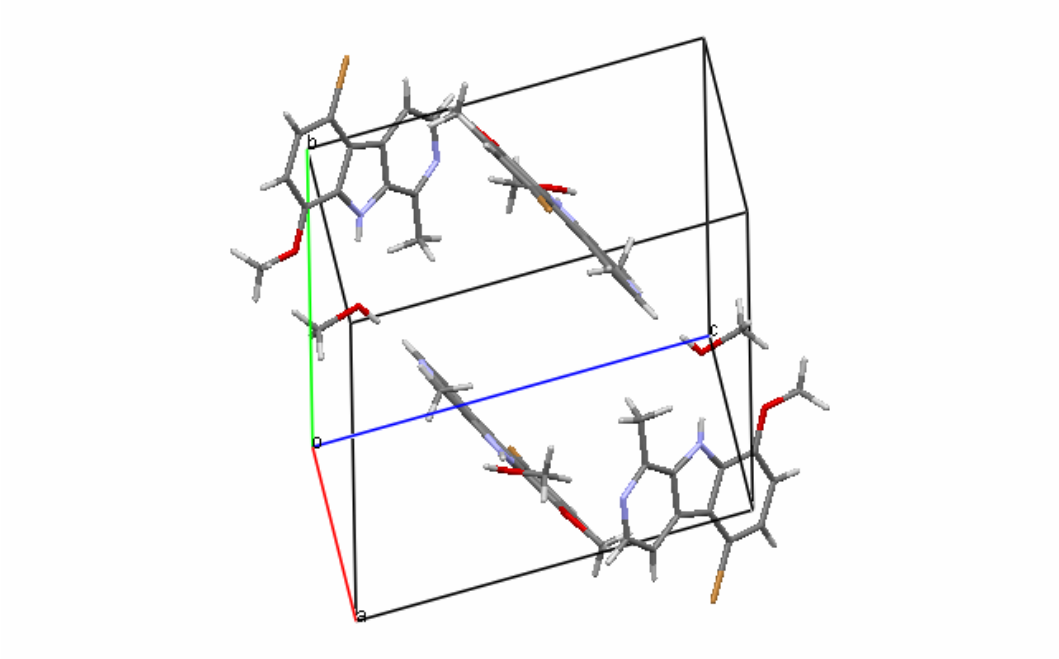


Figure 2.11: Stacking of 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**) within the Unit Cell.

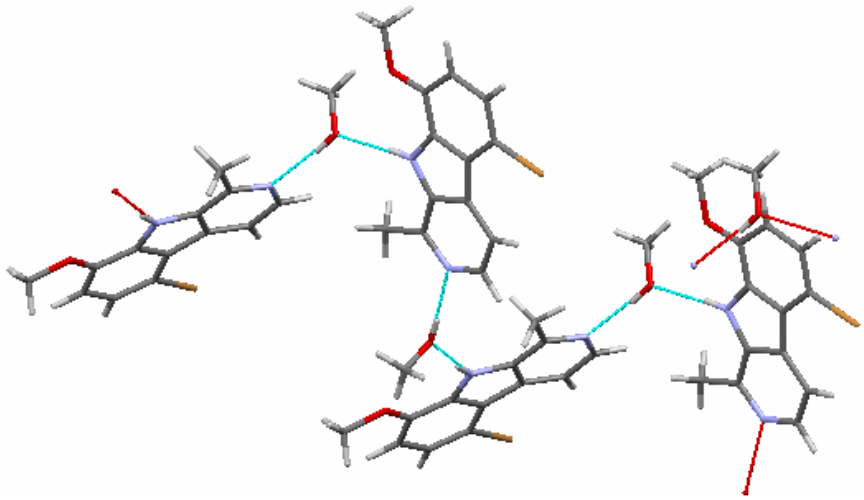


Figure 2.12: Hydrogen Bonding Interactions of 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**) with Methanol within the Crystal Structure.

Crystal structure data indicated that 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) is planar throughout other than the methoxyl sidechain, which deviates from the molecular plane. In general, the bond lengths and angles are as would be

expected for a molecule with this structure. The bond length C(6)-C(7) is longer than the median length for this type of system and the bond angle O(1)-C(8)-C(7) is slightly smaller than the median for that angle, although both fit within previously observed limits.

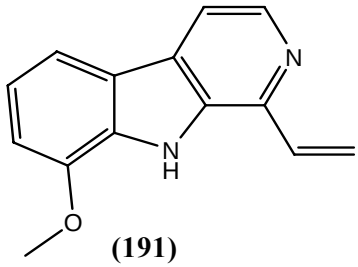
2.6 Biological Evaluation of Selected β-Carboline Alkaloids.

β-Carboline alkaloids have been found from the family Catenicellidae to which *P. vesiculosa* belongs, although this is the first example of a β-carboline alkaloid in the genus *Pterocella*. Previous work on *Costaticella hastata* and *Cribricellina cribraria,* has led to the isolation of β-carboline alkaloids. These alkaloids only show significant activity in the P388 biological assay when there is a vinyl group present at C1. Pavettine (1-vinyl-β-carboline) (**100**) has an IC_{50} value of 100 ng/mL whereas harman (1-methyl-β-carboline) (**97**) is virtually inactive with an IC_{50} value of >12500.[81] Table 2.9 presents the activity of some relevant β-carboline alkaloids including the new compound (**151**) in the P388 antitumour assay. Other β-carboline alkaloids show different types of biological activity. The results are not directly comparable due to the use of different assay systems but they are discussed below.

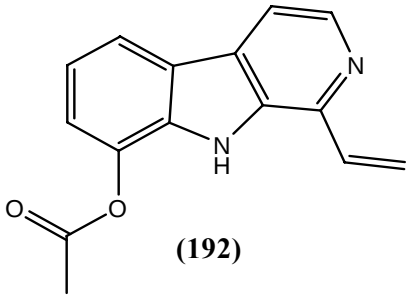
Table 2.9: Activity o f Selected β-Carboline Alkaloids in the P388 Antitumour Assay.[81,119]

Alkaloid	**P388** IC_{50} (ng/mL)
1-Methyl-β-carboline (**95**)	>12500
1-Methyl-5-bromo-8-methoxy-β-carboline (**190**)	5089
1-Methyl-6-hydroxy-β-carboline (**99**)	>12500
1-Methyl-8-hydroxy-β-carboline (**100**)	>12500
1-Vinyl-β-carboline (**98**)	100
1-Vinyl-8-hydroxy-β-carboline (**101**)	100
1-Vinyl-8-methoxy-β-carboline (**191**)	100
1-Vinyl-8-acetoxy-β-carboline (**192**)	650
1-Ethyl-β-carboline (**96**)	>12500
1-Ethyl-4-methylsulfone-β-carboline (**102**)	>12500
1-Ethyl-8-hydroxy-β-carboline (**193**)	>12500
1-Ethyl-8-methoxy-β-carboline (**194**)	>12500
1-Propyl-β-carboline (**195**)	>12500

β-Carbolines with a vinyl substituent at C1 have strong activity in this assay, while the other compounds are essentially inactive except for 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) which has intermediate activity. This suggests the bromine substituent is influencing the mechanism by which β-carboline alkaloids exert their inhibitory activity on the P388 cells.



(191)



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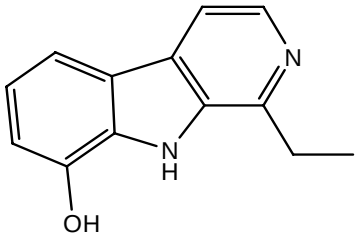

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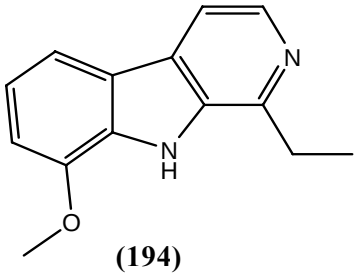

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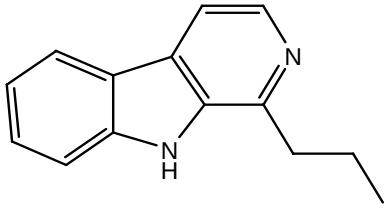
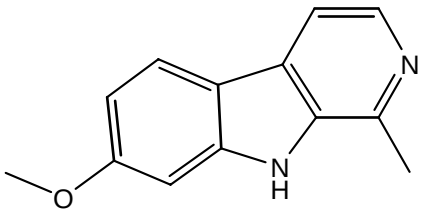


(195)

Investigation into the cytotoxicity of the β-carboline alkaloid harmine (1-methyl-7-methoxy-β-carboline) (**196**) and its analogues (**197-200**) across a range of assays representative of different cancer types including an epidermoid carcinoma (KB), a lung carcinoma (A549), renal cancer (CAKI-1), breast cancer (MCF-7), ovarian cancer (1A9), a glioblastoma (U-87-MG), and a melanoma cancer (SK-MEL-2) allowed structure-activity relationships to be established.[123]



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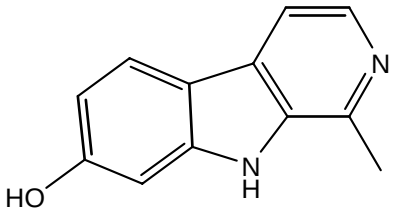

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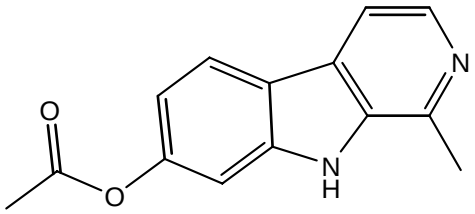

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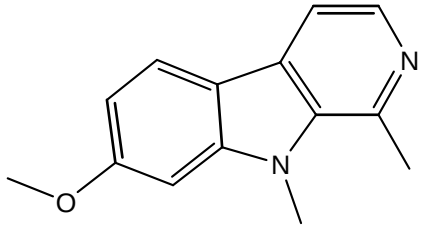

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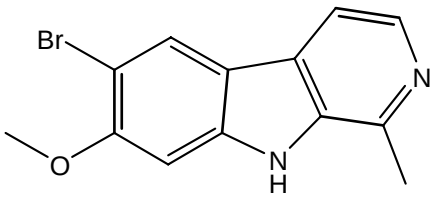


(200)

The 6-bromo analogue (**200**) of harmine (**196**) shows decreased activity across this range of antitumour assays but it shows increased activity against the glioblastoma cell line U-87-MG with an ED_{50} of 7.8 μg/mL compared with the ED_{50} of harmine (**196**) at 14.5 μg/mL.[123] This indicated the bromination of harmine causes the compound to become more selective and increasingly toxic to this cell line whilst decreasing its activity against all other cell lines tested. Analogues of harmine with hydroxyl (**197**) or acetoxyl (**198**) substituents instead of a methoxyl group at C7 generally display decreased activity but are selectively more active against the MCF-7 breast cancer cell line. The ED_{50} of harmine (**196**) against MCF-7 is 10.5 μg/mL whereas the C7 hydroxyl analogue (**197**) has an ED_{50} of 9.2 μg/mL and the C7 acetoxyl analogue (**198**) has an ED_{50} of 5.0

μg/mL.[123] Harmine (**196**) displays better activity across the whole range of assay cell lines tested than its C7 demethoxyl equivalent harman (**97**).[123]

The strength of the antimicrobial activity of β-carboline alkaloids varies significantly depending on the substituents. 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**) displayed inhibitory action towards *B. subtilis, C. albicans* and *T. mentagrophytes* with MID ranges of 2-4, 4-5 and 4-5 μg/mL respectively. Against the same three microorganisms 1-methyl-β-carboline (**97**) has MID values of 7.5-15, 1.9-3.8 and 3.7-7.5 μg/mL respectively, 1-ethyl-8-methoxy-β-carboline (**194**) has MID values of >60, 7.5-15 and 7.5-15 μg/mL respectively and 1-ethyl-8-hydroxy-β-carboline (**193**) has an MID of 30-60 μg/mL for all three. This data indicates that the ethyl group at the C1 position decreases the potency of the β-carboline across the range of microorganisms, it also indicates that the 5-bromo and/or 8-methoxyl substituents increases the potency of the β-carboline toward *B. subtilis* while decreasing the potency against *C. albicans* and has little effect on the potency against *T. mentagrophytes*. 1-Vinyl-β-carboline (**98**) has MID ranges of 1.9-3.8, 1.9-3.8 and 0.1-0.2 μg/mL against the three previously mentioned microorganisms respectively while the 8-methoxyl analogue (**191**) had MID ranges of 7.5-15, 15-30 and 0.45-0.9 μg/mL respectively. This indicates that the 8-methoxy substituent decreases the potency of the β-carboline across this range of microorganisms, which could indicate the bromo substituent in (**190**) is responsible for the increased activity compared to that of (**97**) against *B. subtilis*.

2.7 Further work

Thin layer chromatography (TLC) analysis of the crude extract of this sample of *P. vesiculosa* indicated the presence of a number of coloured compounds not previously observed in studies of this organism implying that there are potentially more novel compounds that could be isolated from this extract. Mass spectral evidence indicated the presence of additional β-carboline alkaloids in this extract. The data indicated the demethoxyl and debromo analogues of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) were present. Further investigation needs to be carried out to isolate and fully characterise these compounds. If these prove to be 1-methyl-5-bromo-β-carboline and 1-methyl-8-methoxy-β-carboline respectively, the former would be a new compound.

UV data and optical rotation experiments are still to be carried out on the new compounds pterocellins H (**188**) and I (**189**). Pterocellins H (**188**) and I (**189**) also need to be isolated in larger quantities so that derivatisation of the 6-hydroxyl group can be performed and structure-activity relationships can be investigated.

Larger quantities of pterocellin G (**114**) still need to be isolated in order for biological evaluation and other analytical experiments to be completed.

Chapter Three

Chemical Survey of New Zealand Marine Organisms

3.1 Introduction

A survey of eighty-five marine samples collected from three different sites around the upper North Island of New Zealand was carried out. This involved extracting a sub-sample of each specimen and testing the crude extract in a variety of assays. A portion of each crude extract was subject to analysis by liquid chromatography mass spectrometry (LCMS) equipped with a photodiode array (PDA) detector and mass spectrometer, as well as thin layer chromatography (TLC).

3.2 Biological Survey

The crude extracts of all samples in the survey were tested in the biological assays run at the University of Canterbury (see Section 1.4).

The bioassay results showed that twelve samples had significant activity against the P388 tumour cell line. Eight of those samples also showed slight antimicrobial activity. Of those eight, three were active in the antiviral assay as well. There was no activity seen in either assay of Gram-negative bacteria (*P. aeruginosa* and *E. coli*) and also no activity against the fungus *C. resinae*. Most samples showed either no activity or limited activity in the antimicrobial assays. Full biological assay results are presented in Appendix Four.

3.3 Chemical Survey

As part of the chemical survey, samples were analysed by LCMS with a PDA detector scanning wavelengths from 190 to 800 nm, as well as a MS detector scanning *m/z* range 150 to 1400. This allowed inspection of the UV spectra for individual chromatographic peaks, as well as the equivalent mass spectrum. LCMS was the technique chosen instead of HPLC for this reason.

The LCMS analysis produced a lot of data about the contents of the crude extracts. The MS chromatograms were difficult to analyse in the area between roughly 10 and 24 minutes as the spectra produced during these periods are swamped with large clusters of peaks between *m/z* 150-200, probably as a result of solvent mixing. Anything that elutes during this time does not have a suitable mass spectrum to accompany the PDA spectrum unless it has a mass spectral peak above the area where the peak clusters occur with significant intensity.

Seventeen samples in the collection were preliminarily identified as *Pterocella vesiculosa.* The samples were collected from the Alderman Islands and White Island. On chemical investigation it was clear that one of these samples was not *P. vesiculosa*. This sample (05-TS1-01) displayed no similarities in its TLC spotting patterns or LCMS chromatogram to the other samples. The spotting pattern on TLC plates was very similar throughout the other sixteen samples. Analyses of the LCMS chromatograms (both PDA and MS) showed that the chemical compositions of these samples were reasonably similar. A combination of the PDA UV spectrum and the mass spectral information indicated that the chromatographic peak at a retention time of 25.7 minutes in sample 05-HC1-01 is

pterocellin A (**108**), while the closely related peak at retention time 26.5 minutes appears to be pterocellin B (**109**). A representative chromatogram for this group of samples is presented in Figure 3.1. The analogous peaks appear in all of the chromatograms of the above samples. Both compounds have been previously isolated from *P. vesiculosa* but not reported from any other source, therefore this is good evidence that all of these sixteen samples are indeed *P. vesiculosa*.

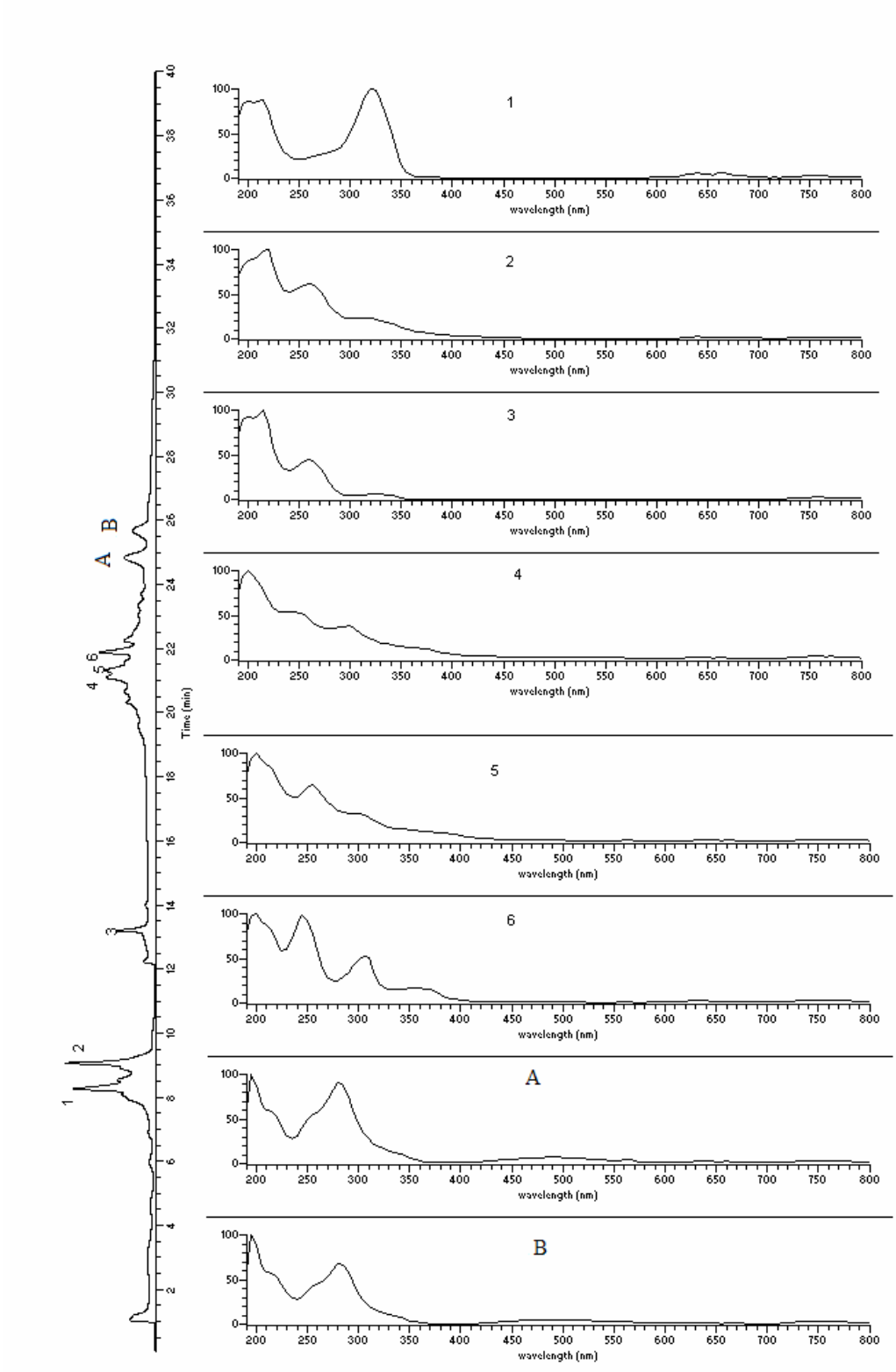


Figure 3.1: A Chromatogram of Sample 05-HC1-01 with UV traces, Representative of the *P. vesiculosa* Samples.

The biological activity of these samples varies significantly. Only three samples showed activity against the P388 tumour cell line, 05-HR1-02, 05-SA1-06 and 05-TT1-03 (IC_{50} values of 82403, 75361 and 92827 ng/mL respectively). Antimicrobial assay results showed that the majority of samples have activity against *B. subtilis*. There is correlation between the collection location of the sample and the strength of its crude extract activity, with the samples that had the strongest effect being those from the White Island collection. Tables 3.1 and 3.2 show complete biological assay results for the *P. vesiculosa* samples.

Table 3.1: Antitumour and Antimicrobial Assay Results for *P. vesiculosa* Samples.[a]

	Antitumour	Anti-microbial
Sample	P388 (IC_{50} μg/mL)	*B. subtilis inhibition zone (mm)*
05-TS1-02	> 125000	1
05-TT1-01	> 125000	0
05-TT1-02	> 125000	2
05-TT1-03	92827	1
05-TT1-04	> 125000	1
05-MC1-02	> 125000	1
05-MC1-03	> 125000	1
05-MC1-04	> 125000	1
05-MC1-05	> 125000	1
05-OB1-01	> 125000	1
05-OB1-02	> 125000	2
05-HC1-01	> 125000	2
05-HC1-02	> 125000	2
05-HC1-03	> 125000	2
05-HR1-02	82403	3
05-SA1-06	75361	3

[a] *Refer to Section 1.4 for details of assay.*

Table 3.2: Antiviral/Cytotoxicity Assay Results for *P. vesiculosa* Samples.[a]

	Antiviral/Cytotoxicity Assay			
Sample	HSV	HCyt	PV1	PCyt
05-TS1-02	ND	ND	ND	ND
05-TT1-01	ND	ND	ND	ND
05-TT1-02	ND	ND	ND	ND
05-TT1-03	?*	2+	ND	ND
05-TT1-04	ND	ND	ND	ND
05-MC1-02	ND	ND	ND	ND
05-MC1-03	ND	ND	ND	ND
05-MC1-04	ND	ND	ND	ND
05-MC1-05	ND	ND	ND	ND
05-OB1-01	ND	ND	ND	ND
05-OB1-02	ND	ND	3+	+*,7
05-HC1-01	ND	ND	ND	ND
05-HC1-02	ND	ND	ND	ND
05-HC1-03	ND	ND	ND	ND
05-HR1-02	ND	ND	ND	ND
05-SA1-06	ND	ND	ND	ND

[a] ***Refer to section 1.4 for details of assay. Tables 1.1 and 1.2 have an explanation of the results.***

Samples 05-DP1-03 and 05-DP1-04 were both identified as being the sponge, *Raspailia sp*. The samples were both collected from Devil's Point in the Mercury Islands and showed similar spots on TLC plates, although the intensity of the spots differed in some cases. Furthermore, comparison of their LCMS chromatograms and UV and MS profiles indicated that the chemical composition of the two samples is reasonably similar. For example, the UV spectrum of the chromatographic peak at retention time 30.39 minutes of sample 05-DP1-03 is very similar to the analogous peak of sample 05-DP1-04, although the

chromatogram of sample 05-DP1-04 had more peaks compared with that of 05-DP1-03. Sample 05-DP1-04 showed activity against the P388 cell line (IC_{50} value of 77639 ng/mL) whereas 05-DP1-03 was inactive. Both samples had inhibitory activity in the antimicrobial assay against the fungus *T. mentagrophytes* (3 mm inhibition zone for both). Neither showed any activity in the antiviral cytotoxicity assay.

Four samples were thought to be the sponge *Polymastia sp.,* 05-OI1-08, 05-DP1-02, 05-DP1-08 and 05-SA1-12. All samples were inactive in the antitumour and antiviral/cytotoxicity assays but had very different activities in the antimicrobial assay. Sample 05-OI1-08 was inactive, 05-DP1-02 and 05-DP1-08 showed antimicrobial activity against *T. mentagrophytes* (3 mm and 1 mm inhibition zones respectively), while 05-SA1-12 showed antimicrobial activity against *C. albicans* (3 mm inhibition zone). The TLC chromatograms for these four samples revealed similar contents with 05-OI1-08 and 05-SA1-12 having additional compounds in their respective traces unique to that sample. The LCMS chromatograms of samples 05-OI1-08, 05-DP1-02, 05-DP1-08 showed strong similarities, especially in the cluster of peaks between retention times of 23.50 and 26.00 minutes. The LCMS data indicates that these three samples contain at least some of the same metabolites. The chromatogram of sample 05-SA1-12 was different to that of the other three.

The survey collection included five algal species (05-DP1-05, 05-HR1-03, 05-SA1-08, 05-MC1-06 and 05-HC1-04). Three of these, 05-HR1-03, 05-SA1-08 and 05-MC1-06 were red, feathery algal species, 05-DP1-05 was a pink coralline

algal species and 05-HC1-04 was a dark red alga. The TLC spotting patterns of these five samples were quite different, although there were two spots that were consistent across all five samples, which could represent common primary metabolites. The LCMS trace of all five samples was very simple indicating that these samples contain fewer compounds than most other samples or that the chromatographic separation is not optimised to separate the compounds contained in these samples. There was no biological activity exhibited by any of these samples.

Eight samples were hydroids (05-HR1-04, 05-HR1-05, 05-HR1-06, 05-HR1-08, 05-HR1-09, 05-HR1-10, 05-SA1-04 and 05-TT1-05). These samples differed in colour, 05-HR1-04 was white, 05-HR1-05 was yellow, 05-HR1-06 and 05-HR1-09 were brown, 05-HR1-08 and 05-TT1-05 were orange, 05-HR1-10 was green and 05-SA1-04 appeared to be a mixture of hydroids. Five of the hydroid samples showed no biological activity, sample 05-HR1-06 showed significant inhibitory activity against the P388 tumour cell line (IC_{50} value of 65196 ng/mL), sample 05-SA1-04 showed slight activity in the same assay (IC_{50} value of 121357 ng/mL) and sample 05-TT1-05 showed marginal activity in the antiviral/cytotoxicity assay against the *Polio* virus strain with the virus growing right up to the cytotoxic zone which extended 1-2 mm radius from the edge of the disk. TLC analysis supported these samples being different species as all samples gave very different spotting patterns. The LCMS chromatograms were also very different; there seemed to be no consistency across this group in either the number of peaks in the chromatogram or the retention times of any of the peaks.

Samples 05-OI1-10, 05-OI1-11 and 05-OI1-12 were all identified as being the bryozoan *Bugula dentata*. The samples were collected from Ohinau Island in the Mercury Islands and gave similar spots on the TLC plates, although sample 05-OI1-11 had fewer spots than the other two samples. Comparison of the LCMS chromatograms and UV and MS profiles of the three samples indicated that the chemical composition is reasonably similar again with sample 05-OI1-11 having fewer peaks than the others. There appeared to be a series of compounds common to all three samples. The compounds had no obvious chromophore but showed strong peaks in the mass spectral chromatogram, these compounds had *m/z* values of 312.7, 340.9, 519.3 and 547.7, with retention times ranging from 36 to 42 minutes. Sample 05-OI1-12 contained an extra compound with an *m/z* of 326.8 not apparent in the other two samples. All three samples showed inhibitory activity against the P388 cell line, with IC_{50} values of 114341 ng/mL, 110986 ng/mL and 61181 ng/mL respectively. Clearly sample 05-OI1-12 was significantly more active than the other two. Sample 05-OI1-12 was the only sample to show activity in the antimicrobial assay (1 mm inhibition zone against *T. mentagrophytes*). No activity was seen in the antiviral/cytotoxicity assay.

There were five samples that appeared to be *Steginoporella neozelanica* (05-HR1-11, 05-TS1-04, 05-MC1-01, 05-OB1-03 and 05-HC1-05). Samples 05-TS1-04 and 05-OB1-03 showed slight activity against the P388 cell line in the antitumour assay (121357 and 117797 ng/mL respectively). Sample 05-MC1-01 was the only one of these samples to show activity in the antimicrobial (1 mm inhibition zone against *B. subtilis*) and antiviral/cytotoxic bioassays with activity against both *Herpes simplex* type 1 virus and *Polio* virus type 1. The TLC traces of these

samples are essentially identical, other than additional spots were seen under UV light in the trace of sample 05-MC1-01. The LCMS chromatogram varied across these samples. They all had a similar group of peaks between retention times of 8.30-9.50 minutes. The chromatogram of sample 05-TS1-04 contained a lot more peaks than the other chromatograms. These peaks were typically small and had retention times between 22 and 30 minutes.

Six samples were identified as the soft coral *Alcyonium* species (05-OI1-09, 05-DP1-12, 05-DP1-13, 05-DP1-15, 05-DP1-18 and 05-SA1-05). Samples 05-OI1-09, 05-DP1-12 and 05-DP1-13 appear to have very similar spotting patterns. The other three samples had unique spotting patterns, with no correlation between them or with the patterns of the first three samples. Unexpectedly there were no obvious similarities between any of the chromatograms for these six species.

The biological activity of these samples varied, with samples 05-OI1-09, 05-DP1-18 and 05-SA1-05 showing activity in the P388 assay (IC_{50} values of 114341, 68920 and 121357 ng/mL respectively), while samples 05-OI1-09, 05-DP1-12 and 05-DP1-13 all showed activity in the antiviral/cytotoxicity assay although the antiviral activity was indeterminable due to the strong cytotoxicity in all three samples against both virus types.

Samples 05-OI1-01, 05-OI1-06, 05-OI1-07 and 05-OI1-13 appeared to be the same species of red encrusting sponge. All samples showed no biological activity except 05-OI1-13, which showed limited activity in the antimicrobial assay

against the fungus *T. mentagrophytes* (1 mm inhibition zone). Samples 05-OI1-01, 05-OI1-06 and 05-OI1-07 had very similar TLC spotting patterns. TLC analysis indicated sample 05-OI1-13 had less compounds present in its crude extract than the other three samples.

LCMS analysis showed samples 05-OI1-01 and 05-OI-06 contained many of the same compounds. Comparisons of the peaks in the chromatogram of 05-OI1-01 at 27.7, 31.86, 33.40, 35.94, 40.85 and 42.56 minutes with the analogous peaks in the chromatogram of 05-OI1-06 were similar. Sample 05-OI1-07 did not appear to contain these peaks, while sample 05-OI1-13 contained peaks analogous to those from sample 05-OI1-01 at 33.40, 35.94, 40.85 and 42.56 minutes.

Five brown samples identified as being bryozoans appeared similar (05-TS1-03, 05-HC1-06, 05-HC1-07, 05-OB1-06 and 05-OB1-07). TLC analysis showed samples 05-TS1-03, 05-HC1-06 and 05-HC1-07 had the same TLC spotting patterns. Samples 05-OB1-06 and 05-OB1-07 had fewer spots than the other three samples, while 05-OB1-06 had an additional spot visible under UV light at 254 nm that was unique to that sample. The LCMS chromatogram of sample 05-OB1-06 was very simple, with the only distinguishing feature, a strong peak at a retention time of 25.01 minutes. Samples 05-HC1-06, 05-HC1-07 and 05-OB1-07 all had similar chromatograms with minimal distinguishable peaks other than the strong peak analogous to the one seen in the chromatogram of 05-OB1-06. Sample 05-TS1-03 produced a chromatogram that along with this analogous peak contained a lot more peaks. None of these samples showed activity in any of the biological assays.

Chapter Four

Studies of Two Marine Organisms

4.1 Introduction

Results from the survey (work described in Chapter Three) indicated which samples exhibited cytotoxicity toward the P388 cell line. Of the active samples, two were identified as promising leads for further investigation. The crude extracts of samples 05-OI1-02 and 05-SA1-10 showed strong inhibition in the P388 antitumour assay with IC_{50} values of 39141 and 52717 ng/mL respectively. The other samples that showed activity in the P388 assay were either samples of *P. vesiculosa* or showed weaker inhibition.

4.2 Studies of a Sponge from the Family Darwinellidae

4.2.1 Bioactivity Directed Separation

The crude methanol/dichloromethane extract taken from a sponge belonging to the family Darwinellidae, from the 2005 collection at the Spanish Arch, White Island showed activity against the P388 murine leukaemia cell line and also slight antibacterial activity against *B. subtilis*. Separation of the crude extract by reversed phase column chromatography resulted in one active fraction (IC_{50} of 1522 ng/mL). Size exclusion chromatography was performed on the active fraction, resulting in the activity concentrating into two consecutive fractions with IC_{50} values of 751 and 508 ng/mL respectively. No spots were observed on the TLC plate in visible light or under UV light. Analysis by high resolution electrospray mass spectrometry (HRESMS) indicated that there was a complex

mixture present; ^{1}H NMR spectroscopic experiments also supported the presence of a complex mixture. The NMR spectroscopic data on these two fractions was very similar indicating that they contained the same mixture.

The ^{1}H NMR spectrum of these fractions in $CDCl_3$ (Figure 4.1) showed a number of clearly defined signals downfield of 3 ppm. The signals varied in intensity indicating that there was a mixture of compounds in the fraction. The region upfield from 3 ppm was ambiguous, it contained multiple signals overlapping which were unable to be differentiated from each other and separate multiplicity was impossible to determine. The COSY NMR spectrum contained a lot of correlations, most of which would correlate to at least one undeterminable signal upfield of 3 ppm. The HMBC NMR spectrum again was not helpful in elucidation of the structure of these compounds. It did contain a lot of variation in signal intensity adding to the evidence for a mixture of compounds, possibly a series of compounds. The HMBC NMR spectrum contained strong signals with weaker signals in close vicinity to them, indicating another molecule with the same sub-structure but with NMR signals that were slightly altered in their shift due to changes elsewhere in the molecule.

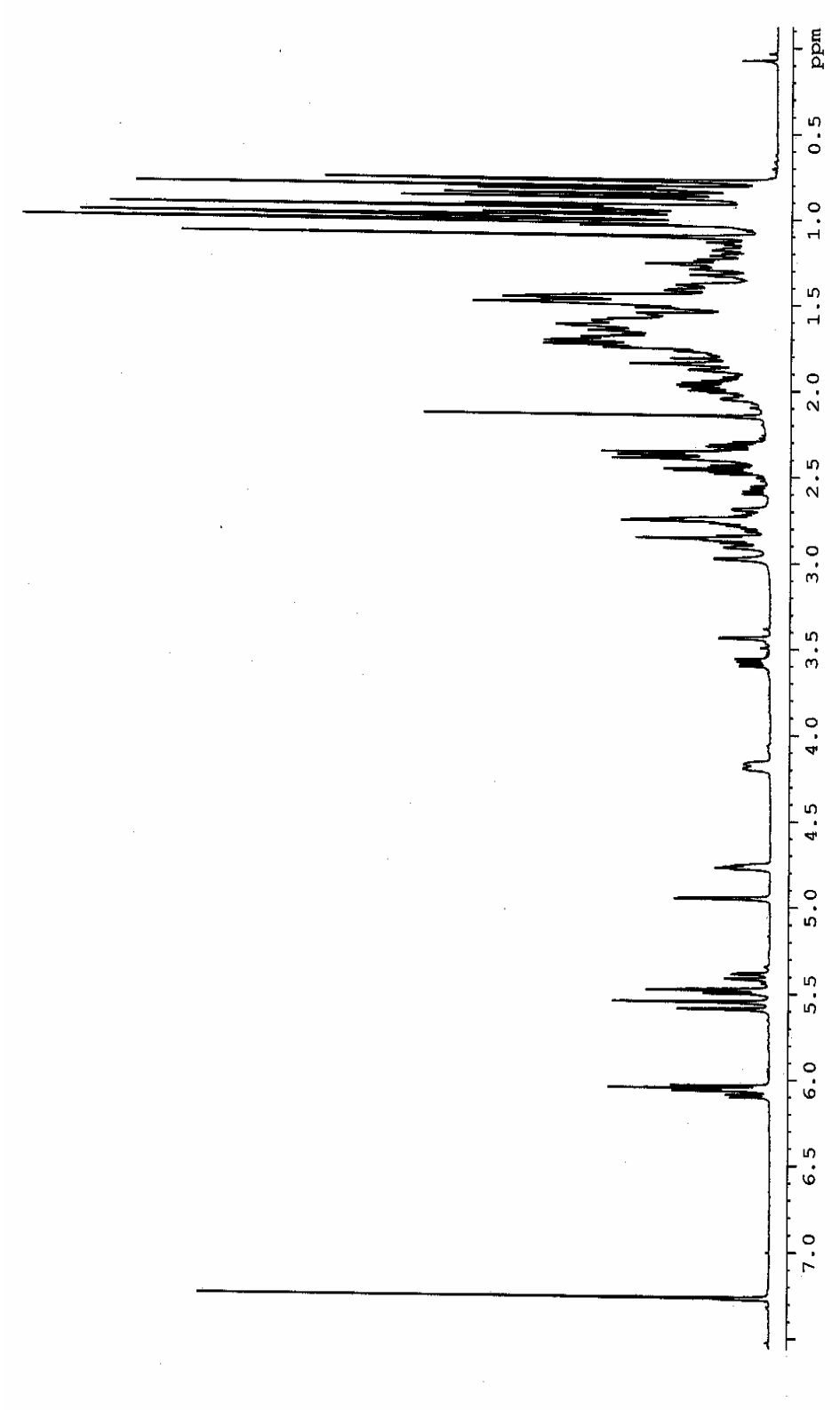


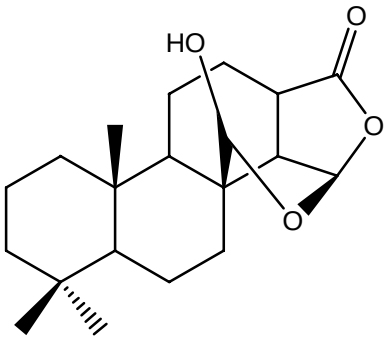
Figure 4.1: ^{1}H NMR Spectrum of the Active Fraction of a Sponge from the Darwinellidae family.

The two active fractions were combined and subjected to further size exclusion chromatography. The resulting fractions were analysed by HRESMS and also NMR spectroscopy, which indicated that the mixture of active compounds co-eluted from the column over several fractions. The separation tree for this fraction is presented in Appendix Five.

Due to the complex spectra obtained from this mixture, no definite structural elucidation could be carried out. The mixture was unable to be separated further due to insufficient quantities and a further collection of the sponge was deemed impractical. Taxonomic information and spectral data were used to identify possible compounds in the mixture from the literature.

Within the order Dendroceratida, family Darwinellidae, the genera of *Dendrilla* or *Chelonaplysilla* have been shown to produce many novel diterpenes (some occurring in species of both genera), with varying biological activity.

The diterpenoids dendrillols 1-4 (**201-204**) as well as the aplyroseols 1-6 (**205-210**) were extracted from a sample of *Dendrilla rosea* collected from New Zealand. No biological activity was reported for these compounds. Structural elucidation resulted from NMR spectroscopic and MS data as well as X-ray crystallographic data.[124]



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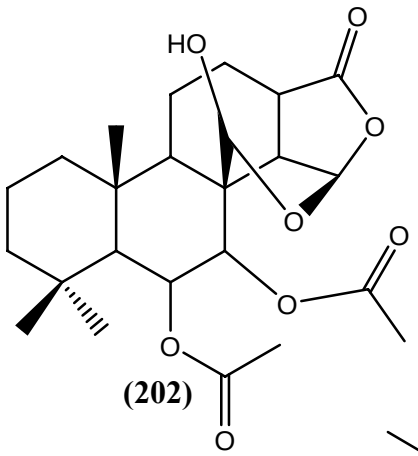

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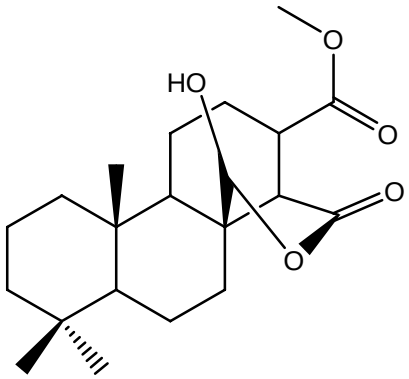

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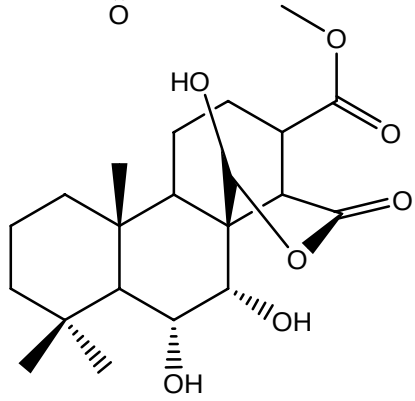

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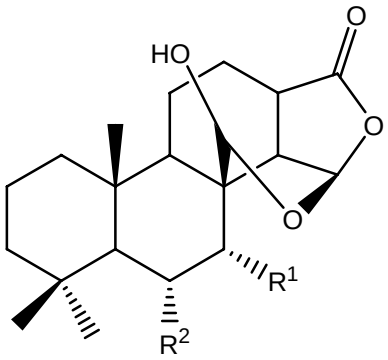
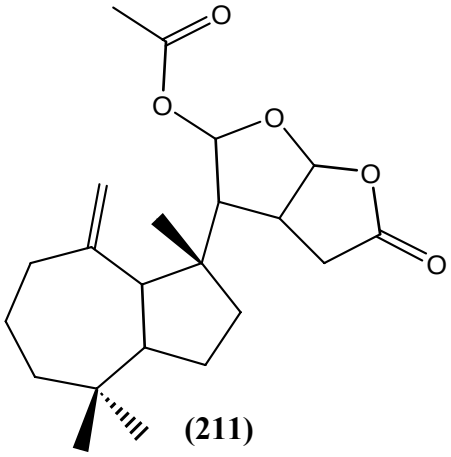


	R^1	R^2
(205)	OCOPr	H
(206)	OAc	H
(207)	OCOPr	OH
(208)	OCOPr	OAc
(209)	OH	OCOPr
(210)	OAc	OCOPr

A series of compounds dendrillolides A (**211**), B and C – E (**212-214**) have been isolated from a *Dendrilla sp*.[125] Dendrillolides A (**211**) and D (**213**) were isolated from a sample preliminarily identified as a *Dendrilla* species then later reidentified as a *Chelonaplysilla* species.[126] The structure of dendrillolide B remains undetermined.



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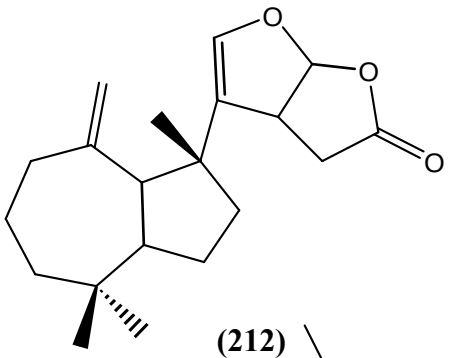

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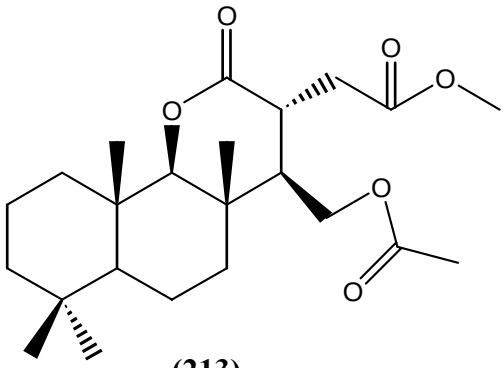

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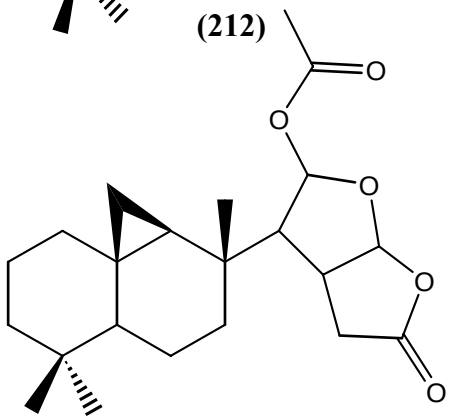

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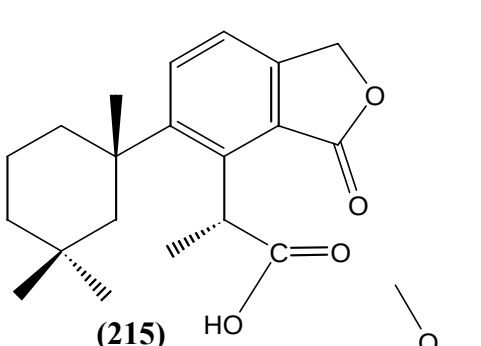
The Antarctic sponge *Dendrilla membranosa* has yielded novel diterpenes membranolides A-D (**215-218**).[127,128] Membranolide A (**215**) inhibited the growth of *B. subtilis* at 100 μg/disk and was also mildly active against S. *aureus.*[127] Membranolides C (**217**) and D (**218**) also display antibacterial and antifungal activity.[128]



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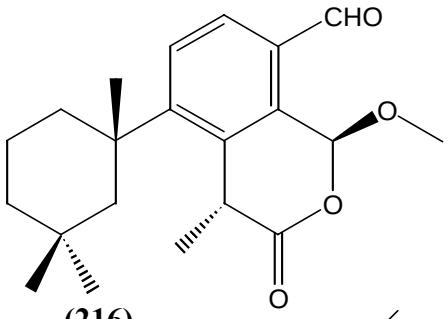

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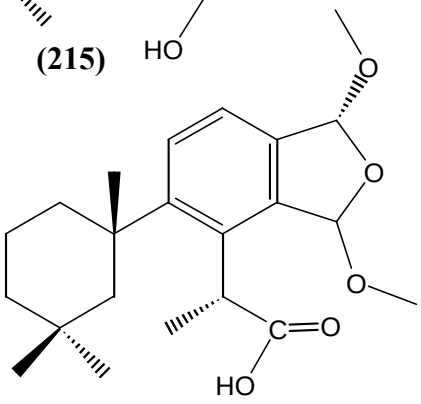

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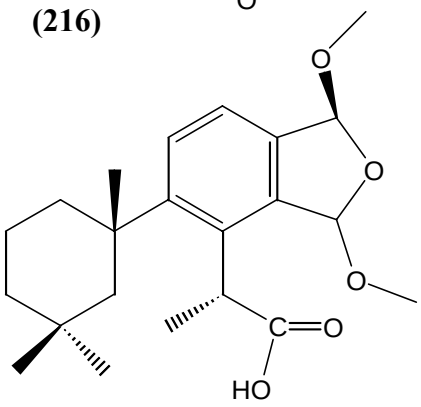

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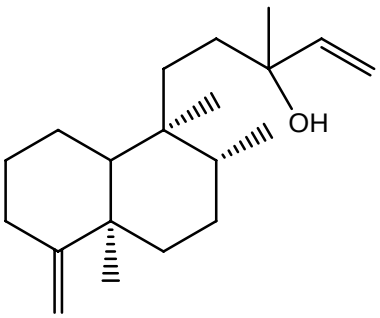
Three new diterpenoid metabolites, chelodane (**219**), barekoxide (**220**) and zaatirin (**221**), were isolated from the cytotoxic extract of *Chelonaplysilla erecta*. Structures were determined predominantly by 1D and 2D NMR spectroscopy. These compounds were not responsible for the cytotoxicity of the crude extract.[129]



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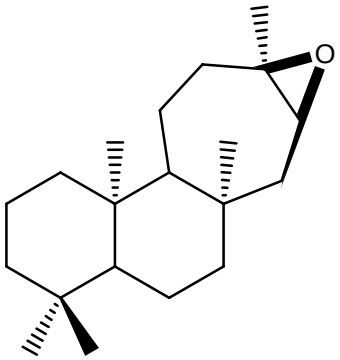

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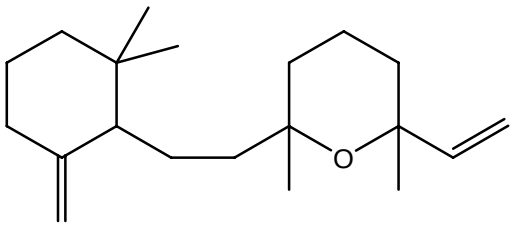

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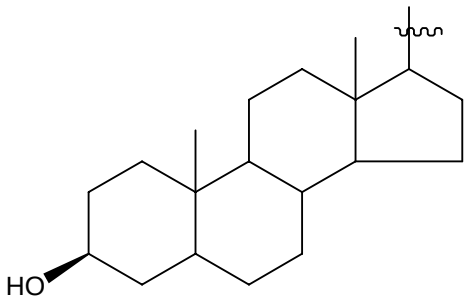
4.2.2 Sterols from a Sponge of the Family Darwinellidae

Three non-polar fractions from the initial reversed phase column on the crude extract of the Darwinellidae species contained sterols as indicated by TLC analysis. These fractions were purified by normal phase column chromatography to yield fractions containing relatively pure sterols, which were derivatised to form the trimethylsilyl (TMS) ethers. The mixture of TMS ethers was subject to separation and analysis by gas chromatography mass spectrometry (GCMS). They were identified by comparison of fragmentation patterns with literature values.[130-134] The composition percentage was determined based on peak area with the assumption that all sterols had the same response factor. The sterol composition of this sponge is shown in Table 4.1.

Table 4.1: Sterol Composition of a Sponge from the Darwinellidae Family.

Sterol	Structure	Composition (%)
(22*Z*)-Cholest-22-en-3β-ol	**222a**	56.2
Cholesterol	**223b**	10.2
24-Methylcholest-5,22-dien-3β-ol	**223c**	7.5
Cholest-5,22-dien-3β-ol	**223d**	6.4
24-Ethylcholest-5-en-3β-ol	**223e**	6.4
24-Methylcholest-5,24(28)-dien-3β-ol	**223f**	2.0
24-Ethylcholest-5,24(28)-dien-3β-ol	**223g**	1.7
24-Methylcholest-7-en-3β-ol	**224h**	1.3
Cholestanol	**222b**	1.2
24-Methylcholest-22-en-3β-ol	**222i**	1.0
Unidentified		6.1



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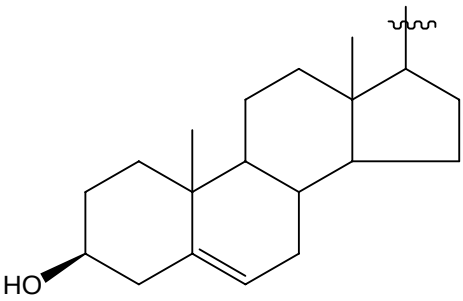

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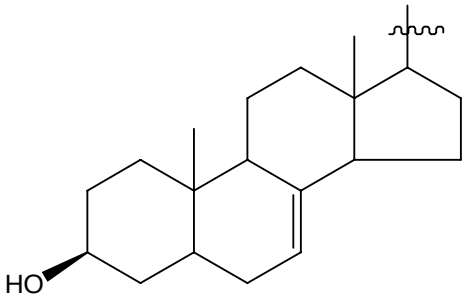

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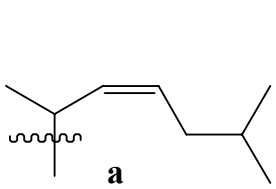

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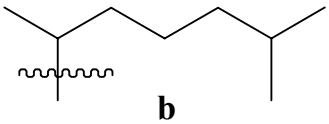

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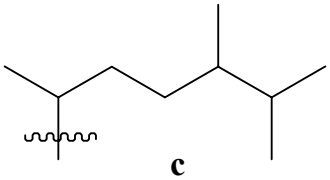

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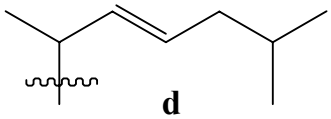

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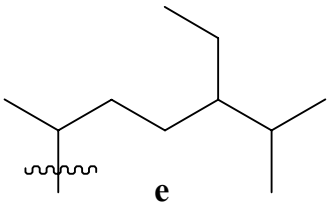

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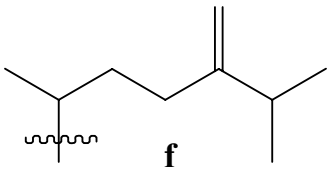

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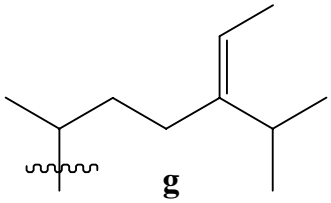

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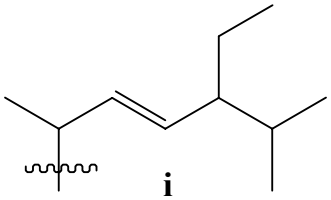

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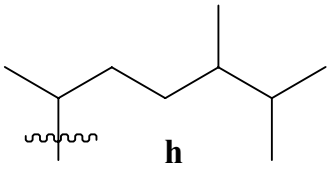

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4.3 Studies of an Unidentified Marine Organism

4.3.1 Bioactivity Directed Separation

The methanol/dichloromethane crude extract of an unidentified, dark blue, amorphous sample collected from Ohinau Island in the Mercury Islands in 2005, displayed strong activity against the P388 murine leukaemia cell line with an IC_{50} of 39141 ng/mL as well as moderate antibacterial activity against *B. subtilis, C. albicans* and *T. mentagrophytes*, so was chosen for further investigation. Attempts at identifying this marine organism were unsuccessful.

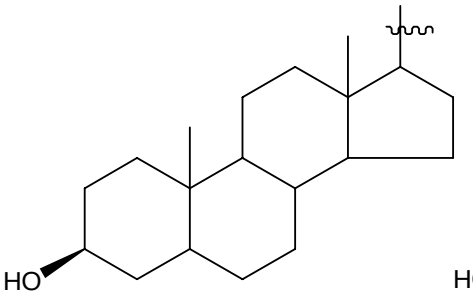
The crude extract was subjected to reversed phase column chromatography. The activity spread across six fractions but was clearly concentrated into two of these. The two most active fractions, which had IC_{50} values of 3032 and 4215 ng/mL, were combined and subject to size exclusion chromatography which concentrated the activity in two fractions. These fractions were subjected to TLC, HRESMS and NMR spectroscopic analysis, the results of which indicated the presence of a mixture of compounds. ^{1}H NMR experiments showed little, due to the spectra being swamped with a peak indicative of 'grease'. The fractions were combined and attempts made to remove the grease, however they were unsuccessful. The combined fractions were subjected to a further size exclusion column. Biological assay indicated the activity had spread across six fractions. HRESMS analysis of these fractions revealed a mixture of compounds. ^{1}H NMR data was unhelpful as it showed the large grease signal and little else. Due to the activity spreading, insufficient quantities and lack of taxonomic information, no further investigation of this species was pursued. The separation tree for this organism is displayed in Appendix Five.

4.3.2 Sterols Identified from the Unidentified Marine Organism

TLC analysis revealed the presence of sterols in two non-polar fractions from the initial reversed phase column on the crude extract. These two fractions were subjected to normal phase column chromatography to purify the sterols. The resulting sterol containing fractions were derivatised to the corresponding TMS ethers. These were then analysed by GCMS and identified by literature comparisons.[130-134] The sterol composition of this organism can be seen in Table 4.2. Cholesterol was the main sterol identified in this organism. As cholesterol is a very common sterol, no taxonomic clues could be gathered from the sterol composition of this organism.

Table 4.2: Sterol Composition of Unidentified Marine Organism.

Sterol	Structure	Composition (%)
Cholesterol	**223b**	54.6
23,24-Dimethylcholest-5,24-dien-3B-ol	**223j**	12.6
24-Methylcholest-5,24(28)-dien-3B-ol	**223f**	5.4
Cholestanol	**222b**	4.4
24-Ethylcholest-5-en-3B-ol	**223e**	4.4
24-Methylcholest-5-en-3B-ol	**223h**	4.3
24-Methylcholest-3B-ol	**222h**	4.0
24-Methylcholest-5,22-dien-3B-ol	**223c**	2.1
24-Ethylcholest-5,24(28)-dien-3B-ol	**223g**	1.7
24-Ethylcholest-22-en-3B-ol	**222i**	1.2
24-Ethylcholest-5,22-dien-3B-ol	**223i**	1.1
Cholesta-5,24-dien-3B-ol	**223k**	1.0
Unidentified		3.2



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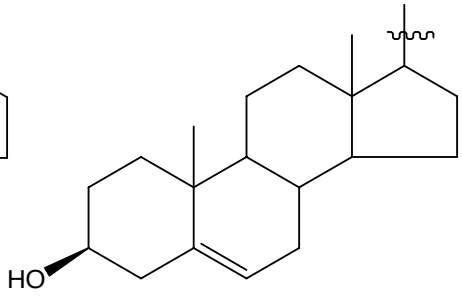

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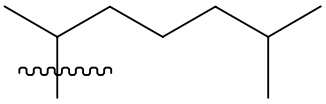

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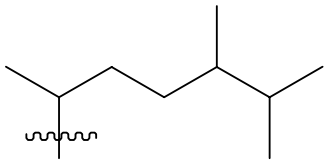

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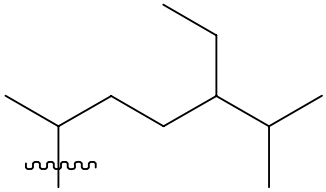

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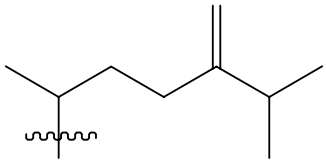

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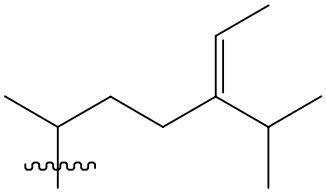

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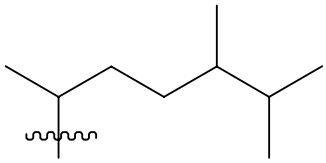

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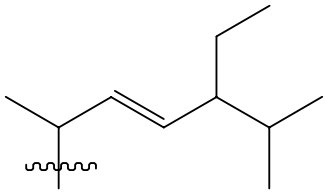

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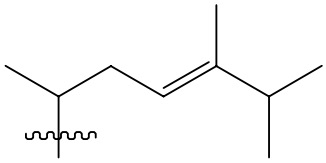

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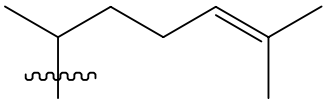

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Chapter Five

Experimental

5.1 General Experimental Methods

Nuclear Magnetic Resonance (NMR) spectra were acquired on a Bruker DRX-400 spectrometer (^{1}H NMR data at 400.13 MHz and ^{13}C NMR data at 100.62 MHz). Chemical shifts recorded in ppm were referenced to the appropriate solvent signal: $CDCl_3$ ^{1}H 7.26 ppm, ^{13}C 77.06 ppm. Standard pulse sequences were used for HMBC, HSQC, COSY and NOESY experiments.

High Resolution Electro Spray Mass Spectrometry (HRESMS) data was acquired on a Bruker Daltonics MicrOTOF™ spectrometer which uses electrospray ionisation and time-of-flight for ion separation. Samples were dissolved in distilled methanol at a concentration of <0.1 mg/mL. The solution was filtered before being injected directly into the machine. The machine was calibrated before each session with a sodium formate standard solution. Between samples the system was flushed with methanol until no residual peaks could be seen**.**

Unit cell dimensions and intensity data for X-ray Crystallography were obtained on a Bruker-Nonius Apex II CCD diffractometer, at the University of Canterbury, at 93 K.

Thin Layer Chromatography (TLC) was performed by applying a solution of a sample dissolved in methanol to Macherey-Nagel aluminium backed silica TLC plates with drawn glass capillaries.

All Liquid Chromatography Mass Spectrometry (LCMS) samples were run using a ThermoFinnigan LCQ Advantage ion trap mass spectrometer with electrospray ionisation, a Surveyor HPLC with auto sampler and a photodiode array UV detector using a Phenomex® prodigy 5µL ODS 3100A column with a matching guard. The tuning file for the mass spectrometer was optimised using cholesterol. The liquid chromatography gradient was optimised using a sample of *Pterocella vesiculosa* (05-TT1-01 crude extract) in order to distinguish pterocellin A and pterocellin B separately.

Gas Chromatography Mass Spectrometry (GCMS) analysis was performed on a Hewlett Packard 6890 gas chromatograph instrument coupled to a HP 5973 mass selective detector. The column used was a Phenomenex® ZB-5 Zebron capillary GC column (30 m x 0.25 mm diameter x 0.25 µL film thickness.)
For derivatised sterol analysis, the GC oven temperature programme is outlined in Table 1.

Table 5.1. GC Oven Temperature Programme for Derivatised Sterols.

Level	Rate (°C/min)	Final temperature (°C)	Hold (min)
1	0	100	0.5
2	20	220	2
3	2	295	10

Ultraviolet/visible absorbance data was obtained using a Varian CARY 100 Ultra-violet/visible spectrophotometer. The wavelengths between 800-200 nm were scanned at a rate of 60 nm/second.

For reversed phase column chromatography C-18 material (30 g) was packed into the glass column in a methanol slurry. The column was equilibrated from methanol to water prior to loading. The material was applied dry to the top of the column which was run under N_2 (g) pressure with a steep steeped gradient from water to methanol to dichloromethane.

For size exclusion column chromatography Sephadex LH-20 material in a methanol slurry was packed into size exclusion columns. Prior to use the column was flushed with methanol (250 mL). The material was applied to the top of the column as a concentrated solution and then eluted with methanol. The collected fractions were rotary evaporated to remove solvent and then blown down with N_2 (g) to ensure dryness.

For Normal Phase column chromatography the columns were packed with Davisil 35-70 µm silica, (20-25 g). The material was added to the column dissolved in the minimum amount of petroleum ether. The elution gradient started at 100 % petroleum ether then graduated to 100 % ethyl acetate. The fractions were dried down under N_2 (g**).**

All solvents used were bulk drum grade solvents that had been distilled prior to use. All lyophilisation was carried out using a LABCONCO® freeze dry system.

Rotary evaporation was carried out using a BÜCHI Rotovapor R110 rotary evaporator with a BÜCHI 461 water bath at 40 °C. Samples were macerated with a Kunkel Ultra Turrax T25 handheld blender.

5.2 Work Described in Chapter Two

5.2.1 Collection and Extraction of *Pterocella vesiculosa*

The red feathery bryozoan identified as *P. vesiculosa* was collected from the Alderman Islands by self contained underwater breathing apparatus (SCUBA) in 2001. A voucher (AI201-03) is kept in the Chemistry department of the University of Waikato. The bryozoan (1.44 kg wet weight) was exhaustively extracted by soaking in dichloromethane (800 mL per extraction). The solvent was removed and replaced daily for 7 days. The majority of the solvent was removed by rotary evaporation and then the sample was lyophilised to yield a dark reddish purple extract (1.42 g).

5.2.2 Isolation of Pterocellin H (188) and Pterocellin I (189)

The crude extract of *P. vesiculosa* (1.42 g) was fractionated using a C-18 reversed phase column (75 g) with a water to methanol to dichloromethane solvent gradient.

The third fraction from this column showed coloured spots on the TLC indicative of pterocellins. This fraction was then subjected to size exclusion chromatography on a Sephadex LH-20 gel column (35 g) eluted with methanol. Fractions 4-6 from this column showed a purple dot on TLC (ethyl acetate: methanol, 5:1). Analysis of these fractions by NMR spectroscopy indicated that fraction 4 contained

pterocellin H (**188**) with minor impurities, fraction 5 contained a mixture of pterocellins H (**188**) and I (**189**) and fraction 6 contained pterocellin I (**189**) with minor impurities.

Pterocellin H (**188**) was isolated as a dark red amorphous solid (6.0 mg) 4.3 x 10^{-4} % wet weight. For ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 2.1. COSY H3↔H4, H1'↔H2', H2'↔H3', H2'↔H4'. HRESMS *m/z* $[M+Na]^+$ 365.1512 (100 %) (Calculated for $C_{19}H_{22}N_2O_4Na$ = 365.1471) *m/z* $[M+H]^+$ 343.1692 (71.9 %) (Calculated for $C_{19}H_{23}N_2O_4$ = 343.1652). UV data and optical rotation measurements are yet to be obtained for this compound.

Pterocellin I (**189**) was isolated as a dark red amorphous solid (7.2 mg) 5.1 x 10^{-4} % wet weight. For ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 2.2. COSY H3↔H4, H3'↔H4'. HRESMS *m/z* $[M+Na]^+$ 399.1301 (100 %) (Calculated for $C_{22}H_{20}N_2O_4Na$ = 399.1315) *m/z* $[M+H]^+$ 377.1436 (36.8 %) (Calculated for $C_{22}H_{21}N_2O_4$ = 377.1495). UV data and optical rotation measurements are yet to be obtained for this compound.

5.2.3 Isolation of 1-methyl-5-bromo-8-methoxy-β-carboline (190)

The crude extract of *P. vesiculosa* (1.41 g) was fractionated using a C-18 reversed phase column (75 g) with a water to methanol to dichloromethane solvent gradient.

Fraction 10 from this separation (240.8 mg) was subjected to size exclusion chromatography using a column packed with Sephadex LH-20 (150 g). The

fractions were eluted with methanol. Fraction 10 from this column (42.6 mg) showed an intense orange spot on the TLC plate (ethyl acetate:methanol, 5:1). ^{1}H NMR spectroscopy of this fraction indicated a sample containing 1-methyl-5-bromo-8-methoxy-β-carboline (**190**).

1-Methyl-5-bromo-8-methoxy-β-carboline (**190**) was isolated as small square yellow crystals (42.6 mg) 3.0 x 10^{-3} % wet weight. For ^{1}H and ^{13}C NMR data ($CDCl_3$) see Table 2.4. COSY H6↔H7, NOESY H6↔H7, H7↔H10. HRESMS *m/z* $[M+H]^+$ 291.0142 (100 %) (Calculated for $C_{13}H_{12}N_2OBr^{79}$ = 291.0128) and 293.0124 (94.2 %) (Calculated for $C_{13}H_{12}N_2OBr^{81}$ = 293.0108). $[M+Na]^+$ 312.9971 (26.2 %) (Calculated for $C_{13}H_{11}N_2OBr^{79}Na$ = 312.9947) and 314.9989 (24.1 %) (Calculated for $C_{13}H_{11}N_2OBr^{81}Na$ = 314.9927). λ max (MeOH) 487, 353, 340, 293, 288 nm (log ε 3.27, 4.61, 4.66, 4.60, 4.72), λ max (KOH/MeOH) 388, 373, 357, 298, 290 nm (log ε 4.39, 4.48, 4.43, 4.74, 4.72).

5.2.4 Recrystallisation of 1-Methyl-5-bromo-8-methoxy-β-carboline (190)

Yellow square crystals suitable for X-ray diffraction were obtained by vapour diffusion of heptane into a toluene solution containing the sample at room temperature. These were sent to the University of Canterbury for X-ray crystallography.

5.2.5 Solving of X-ray crystal structure of 1-methyl-5-bromo-8-methoxy-β-carboline (190)

The structure was solved by the direct methods option of SHELXS-97,[121] with the position of the bromine atom initially found. All other atoms (excluding the

methyl hydrogens) of the main molecule and solvent molecule were found by a series of difference maps. Full matrix least-squares refinement (SHELXL-97)[122] was based upon F_0^2 with all non-hydrogen atoms anisotropic and methyl hydrogens in calculated positions. Other hydrogen atoms were located from difference maps and were refined normally. Convergence gave $R_1 = 0.0316$ [$I < 2\sigma(I)$] and $wR_2 = 0.0848$ (all data). Goodness of fit = 1.074.

5.3 Work Described in Chapter Three

5.3.1 Collection and Extraction for Chemical Survey

Samples were collected by SCUBA from Ohinau Island and Devil's Point in the Mercury Islands, from Homestead Reef and Spanish Arch at White Island, and also from the Sieve, Tom's Tool, Maori Chief, Orca Bay and Honeycomb in the Alderman Islands in 2005. A sub-sample (about 2 g) was taken from each sample in the collection. The material was extracted exhaustively, each time being macerated in methanol:dichloromethane (3:1, 25 mL). The extracts were combined then freeze-dried. About 10 mg of the crude extract was submitted to the University of Canterbury for bioassay as described in Section 1.4.

5.3.2 TLC Analysis of Crude Extracts

Each sample was put onto three plates, each of which was run with a different solvent system. Solvent systems that were used were ethyl acetate:methanol (5:1), petroleum ether:ethyl acetate (2:1) and petroleum ether:ethyl acetate (4:1). The plates were air dried and analysed by eye as well as under UV light at 254 nm and 312 nm.

5.3.3 Chemical Survey of New Zealand Marine Organisms

Crude extract (20 mg) was dissolved in methanol (1 mL) and this solution was then filtered. The filter was rinsed with methanol (2 x 0.5 mL).

Two gradient programs were used for LCMS analysis of the crude extracts. All *Pterocella vesiculosa* samples were run on the gradient program set out in Table 5.2. All other samples were run on a slightly longer program set out in Table 5.3. Solvent A was acetonitrile and Solvent B was deionised water with 0.05 % trifluoroacetic acid (TFA). The photodiode array detector acquired data from 190 to 800 nm, the mass spectrometer obtained data in the *m/z* range 150 to 1400. A three minute isocratic methanol blank (10 % acetonitrile, 90 % deionised water containing 0.05 % TFA) interceded each run to assist with reproducibility. All samples were run in duplicate.

Table 5.2: LCMS Gradient Programme for *Pterocella vesiculosa*.

Time (min)	Solvent A (%) (acetonitrile)	Solvent B (%) (deionised water with 0.05 %TFA)
0	10	90
2	10	90
22	100	0
35	100	0
40	10	90

Table 5.3: LCMS Gradient Programme for all Other Survey Samples.

Time (min)	Solvent A (%) (acetonitrile)	Solvent B (%) (deionised water with 0.05 %TFA)
0	10	90
2	10	90
27	100	0
39	100	0
45	10	90

5.4 Work Described in Chapter Four

5.4.1 Collection and Extraction of a Sponge from the Darwinellidae Family (05-SA1-10)

The purple finger sponge was collected from the Spanish Arch, White Island in 2005 by SCUBA and was stored frozen. The sample (48.1 g wet weight) was exhaustively extracted by blending in methanol: dichloromethane (3:1) (200 mL per extraction). The majority of the solvent was removed by rotary evaporation before the sample was freeze-dried to yield a dark brown-red extract (1.94 g). A voucher sample (2 g) of this animal (05-SA1-10) is kept in the Chemistry Department at the University of Waikato.

5.4.2 Studies of a Sponge from the Family Darwinellidae

The crude extract (1.94 g) of the sponge was separated by flash column chromatography on C-18 reversed phase silica (30 g) eluted with a solvent gradient from water to methanol to dichloromethane. Fraction 5 showed considerable activity against the P388 cell line (IC_{50} = 1522 ng/mL). TLC analysis revealed no distinctive spots. Fraction 5 (37.4 mg) was then subjected to further separation on a Sephadex LH-20 size exclusion column (35 g) using methanol as the solvent. Fractions 6 and 7 from this column showed good activity against the P388 cell line (IC_{50} values = 751 and 508 ng/mL respectively), TLC analysis did not reveal any significant spots. ^{1}H NMR and HRESMS analysis indicated that a mixture of compounds was present in these two fractions. In an attempt to separate this mixture, fractions 6 and 7 (11.9 mg) were combined and subjected to further size exclusion chromatography (Sephadex LH-20, 35 g) resulting in the compounds of interest co-eluting in fractions 17-20. These fractions were again combined (8.7 mg) and put through a size exclusion column (Sephadex LH-20, 35 g) with smaller aliquots being collected. HRESMS and ^{1}H NMR revealed that these compounds co-elute and could not be separated solely by size exclusion chromatography. The separation tree for this sample can be seen in Appendix Five.

5.4.3 Sterols from a Sponge of the Family Darwinellidae

Analysis by TLC of fractions 134.7, 134.8 and 134.9 indicated the presence of sterols. These three fractions were combined (total weight 107.9 mg) and run through a silica column (Davisil 35-70 μm silica, 23.14 g). TLC analysis of fractions 165.7-165.10 indicated the presence of sterols. These fractions were

combined for derivatisation (total weight 26.5 mg). The combined fraction was dissolved in trimethylsilyl (TMS) imidazole (300 μL). The mixture was sonicated (2 minutes) then heated (60 °C for 1 hour). Heptane was added to the solution (1 mL), shaken and then the heptane removed into a new vial. Distilled water (1 mL) was added to the heptane mixture. This was then put on the vortex shaker (5 seconds) and then centrifuged (5 minutes). The layer of water was removed, $CaCO_3$ was added to further dry the solution then the heptane layer was transferred into a vial for GCMS analysis.

5.4.4 Collection and Extraction of an Unidentified Marine Organism (05-OI1-02)

Sample 05-OI1-02, an amorphous blue lump, was collected by SCUBA from Ohinau Island in the Mercury Islands in 2005 and stored frozen until required. The sample (19.01g wet weight) was exhaustively extracted by repeatedly blending in methanol/dichloromethane (3:1), (200 mL per extraction) until the solvent was colourless. The majority of the solvent was removed by rotary evaporation before the sample was lyophilised to yield a dark brown green extract (1.65 g). A voucher sample (2 g) of this animal (05-OI1-02) is kept in the Chemistry Department at the University of Waikato.

5.4.5 Studies of Unidentified Marine Organism

The crude extract (1.65 g) of this sample was separated on C-18 reversed phase silica (30 g) eluted with a solvent gradient of water to methanol to dichloromethane. Fractions 4-7 showed activity in the P388 antitumour assay, (IC_{50} values were 12030, 3032, 4215, 17760 ng/mL respectively). These four

fractions were combined (195.9 mg) and subjected to size exclusion chromatography on Sephadex LH-20 material (150 g) with methanol as the solvent. Fractions 4-5 displayed activity in the P388 assay. TLC analysis of these fractions under UV light indicated that multiple spots were present and ^{1}H NMR spectroscopic experiments showed a dominance of a 'grease' signal in the spectrum of both fractions. These fractions were combined (118.6 mg) and dissolved in 95 % ethanol, then partitioned against petroleum ether, ^{1}H NMR analysis of the resulting fractions indicated removal of grease had been unsuccessful so the fractions were recombined and subject to further size exclusion chromatography (Sephadex LH-20, 150 g) with methanol as the solvent. Biological assay results indicated that the P388 inhibitory activity was present in fractions 9-18. TLC analysis of these compounds revealed multiple spots in all fractions. The ^{1}H NMR spectrum contained a dominant peak at 1.25 ppm, typical of grease, as well as small, indeterminable signals. The separation tree for this unidentified marine organism can be seen in Appendix Five.

5.4.6 Sterols Identification of Unidentified Marine Organism (05-OI1-02)

TLC analysis of fractions 129.8 and 129.9 indicated the presence of sterols. These two fractions were combined (total weight 127.2 mg) and then subjected to normal phase chromatography on a silica column (Davisil 35-70 μm silica, 21.46 g). The combined fractions were added to the column dissolved in the minimum amount of petroleum ether. TLC analysis indicated the presence of sterols in fractions 162.8-162.11. These fractions were combined (38.7 mg) and sterol derivatisation was performed as detailed in Section 5.4.3.

Appendices

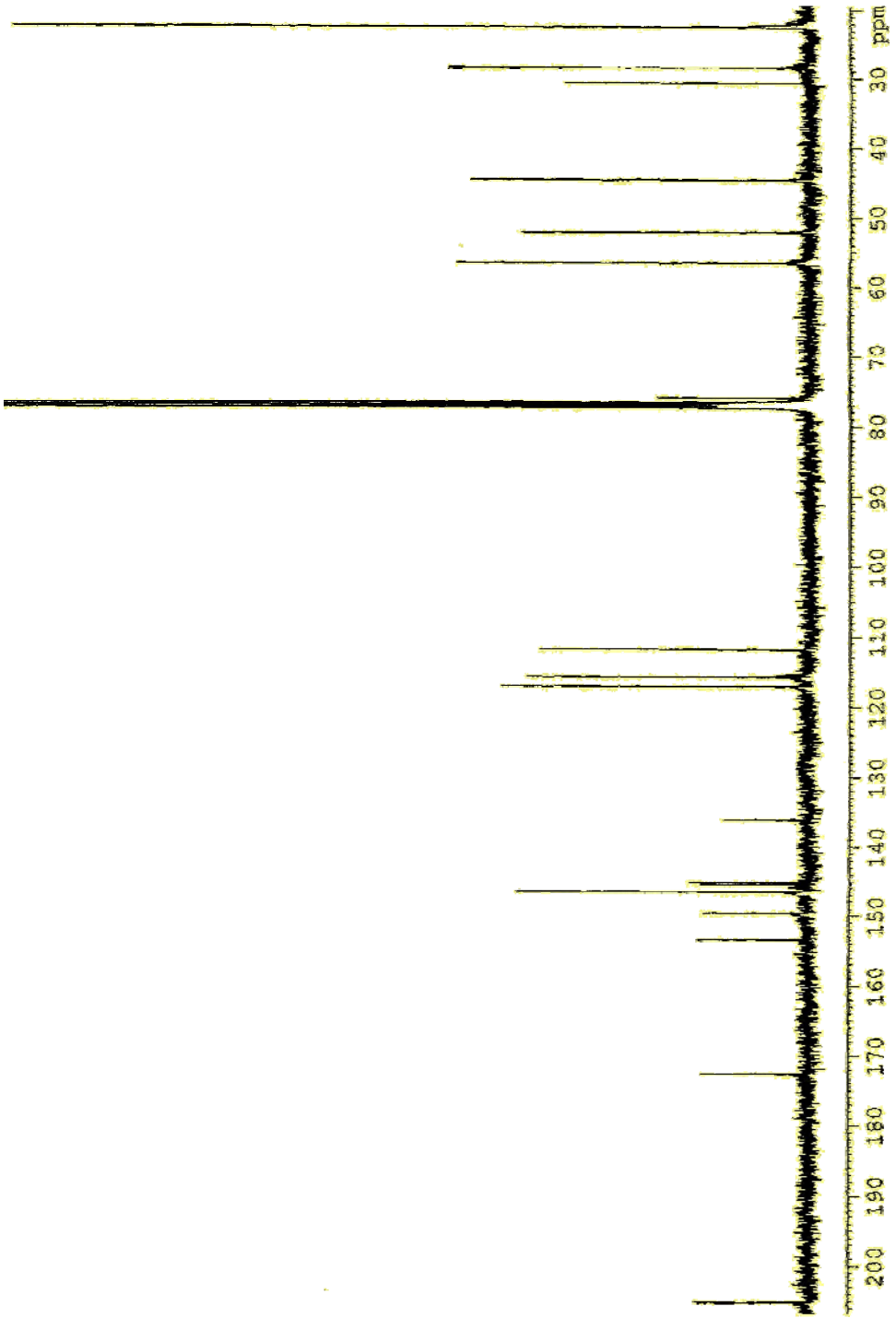
Appendix One: NMR Spectra of Pterocellin H

A 1.1: ^{13}C NMR Spectrum of pterocellin H recorded in $CDCl_3$

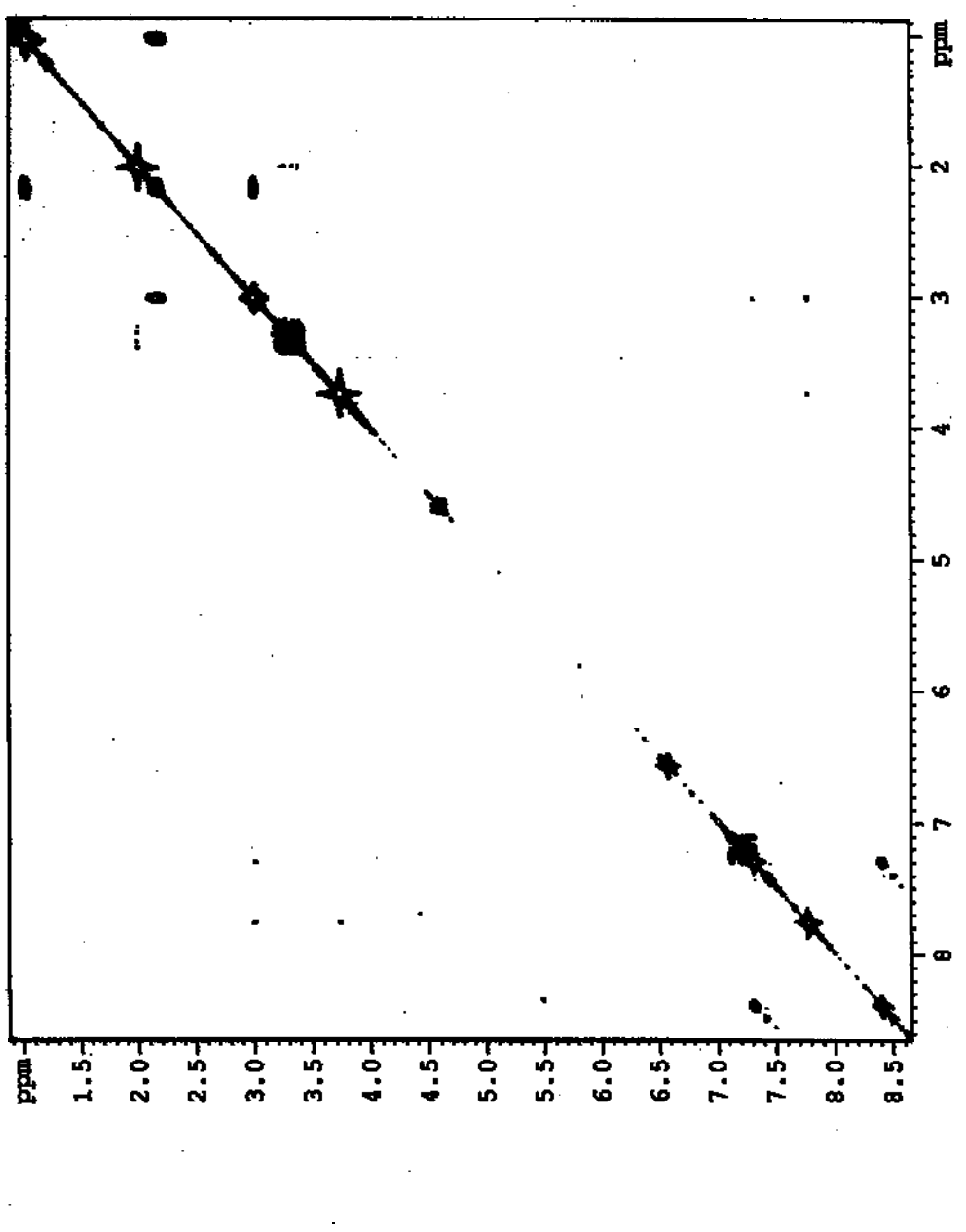
A 1.2: COSY NMR spectrum of pterocellin H recorded in $CDCl_3$

A 1.3: HMBC NMR spectrum of pterocellin H recorded in $CDCl_3$

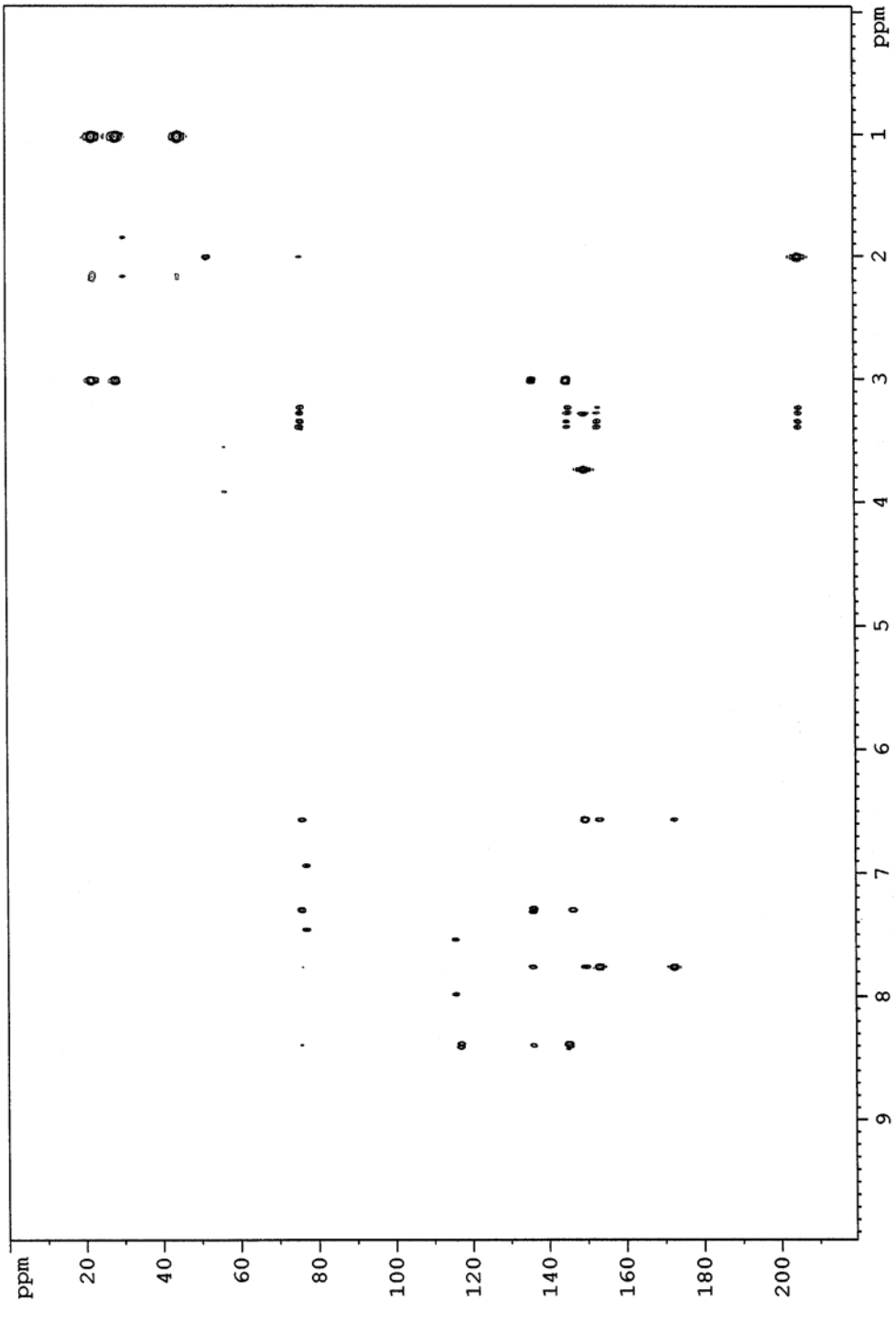
A.1.4: HSQC NMR spectrum of pterocellin H recorded in $CDCl_3$



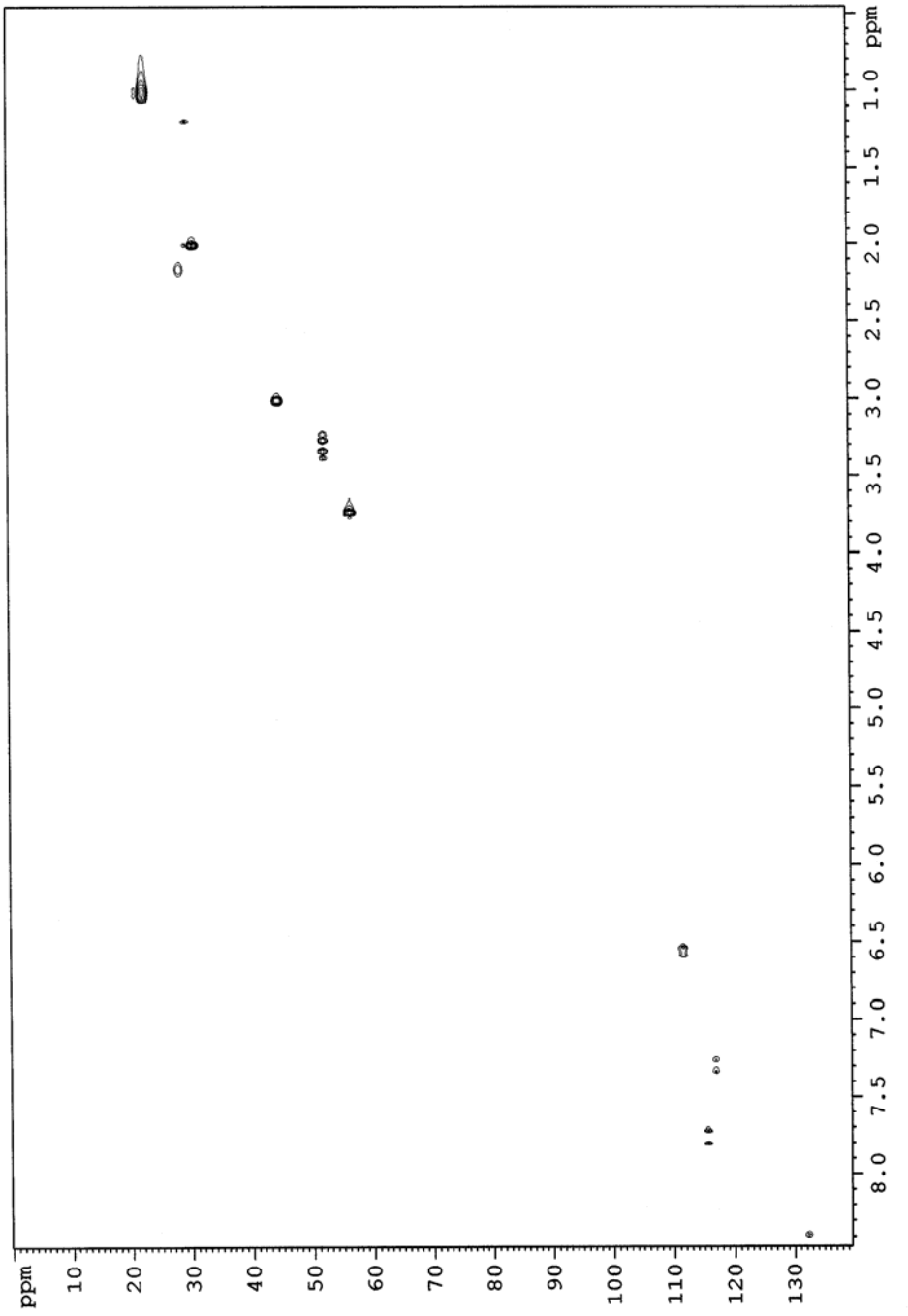
A 1.1: ^{13}C NMR Spectrum of pterocellin H recorded in $CDCl_3$



A 1.2: COSY NMR spectrum of pterocellin H recorded in $CDCl_3$



A 1.3: HMBC NMR spectrum of pterocellin H recorded in $CDCl_3$



A 1.4: HSQC NMR spectrum of pterocellin H recorded in $CDCl_3$

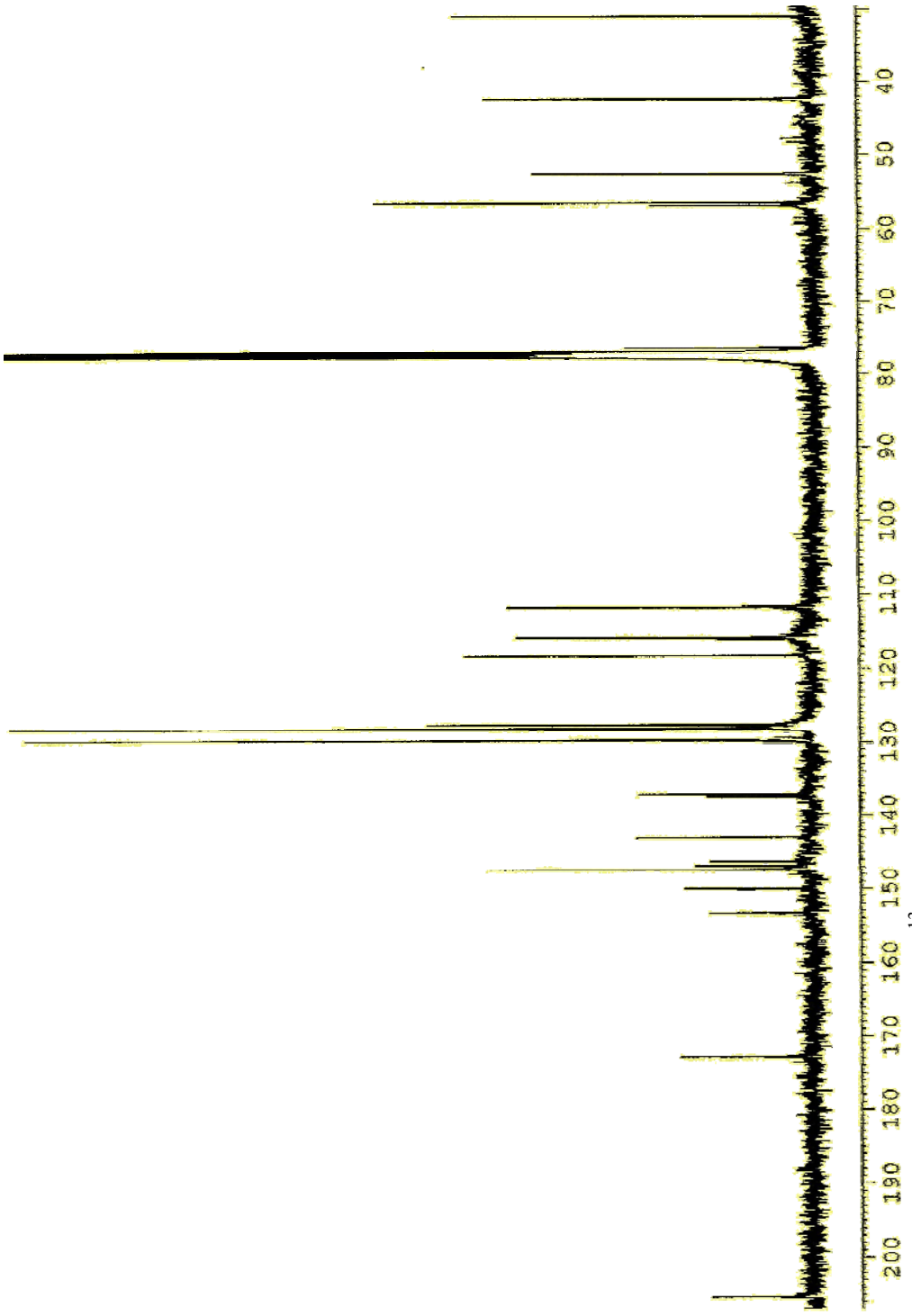
Appendix Two: NMR Spectra of Pterocellin I

A 2.1: ^{13}C NMR spectrum of pterocellin I recorded in $CDCl_3$

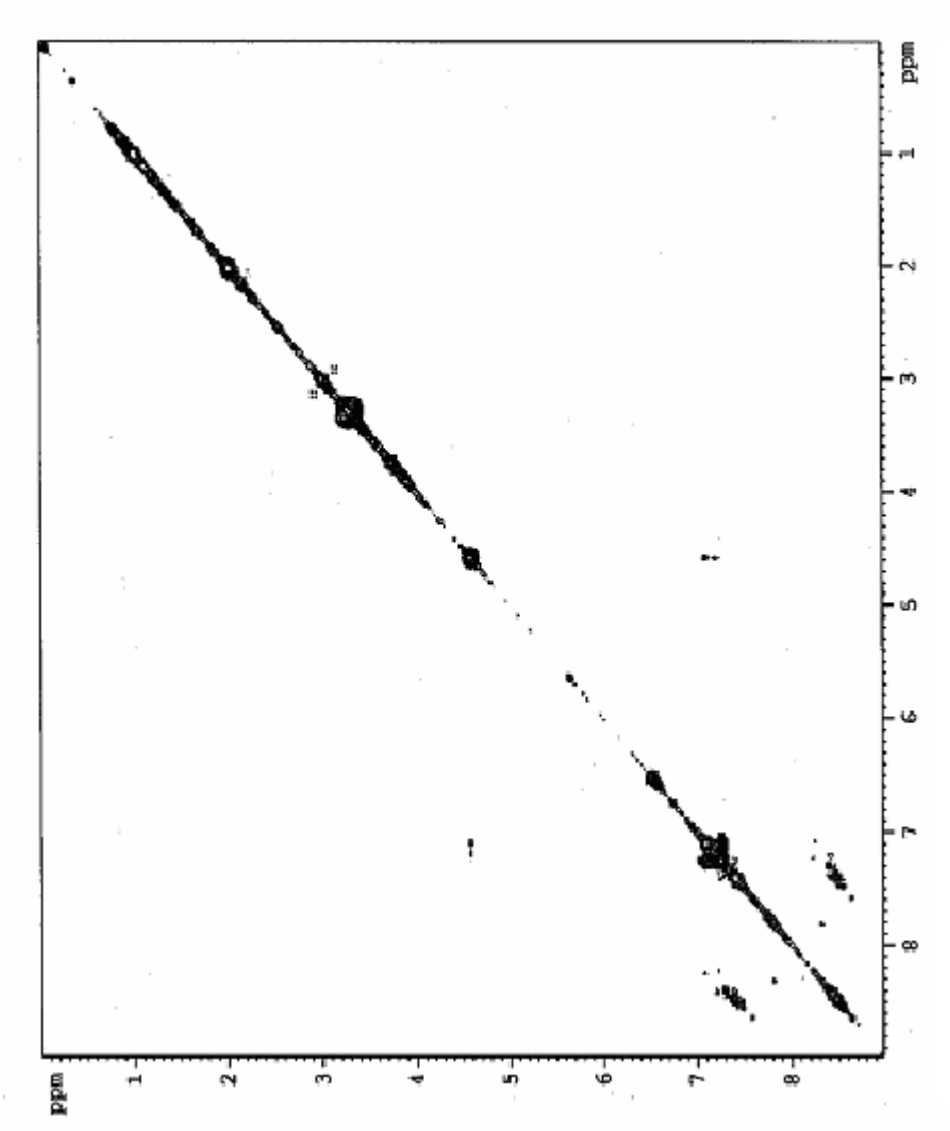
A 2.2: COSY NMR spectrum of pterocellin I recorded in $CDCl_3$

A 2.3: HMBC NMR spectrum of pterocellin I recorded in $CDCl_3$

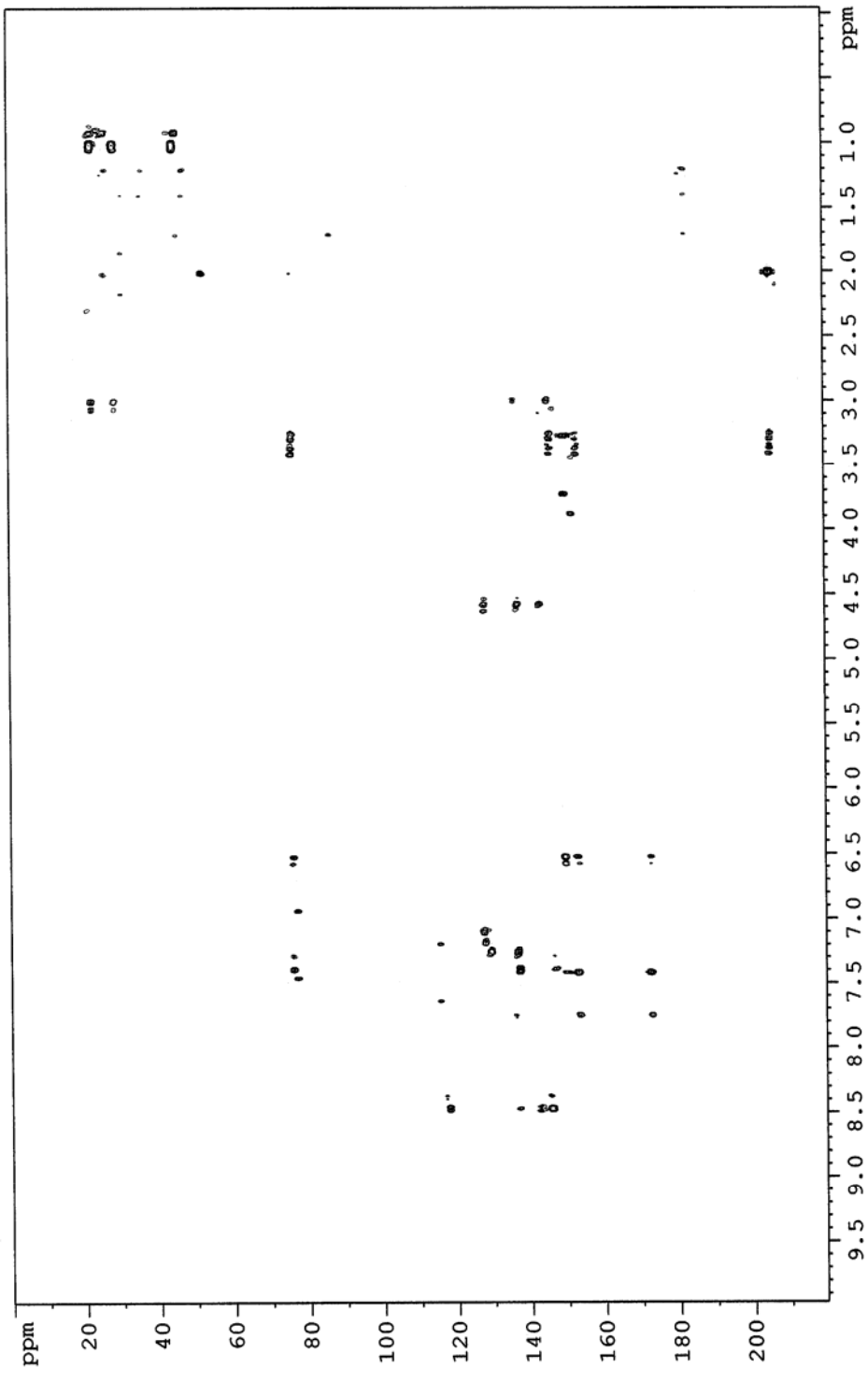
A 2.4: HSQC NMR spectrum of pterocellin I recorded in $CDCl_3$



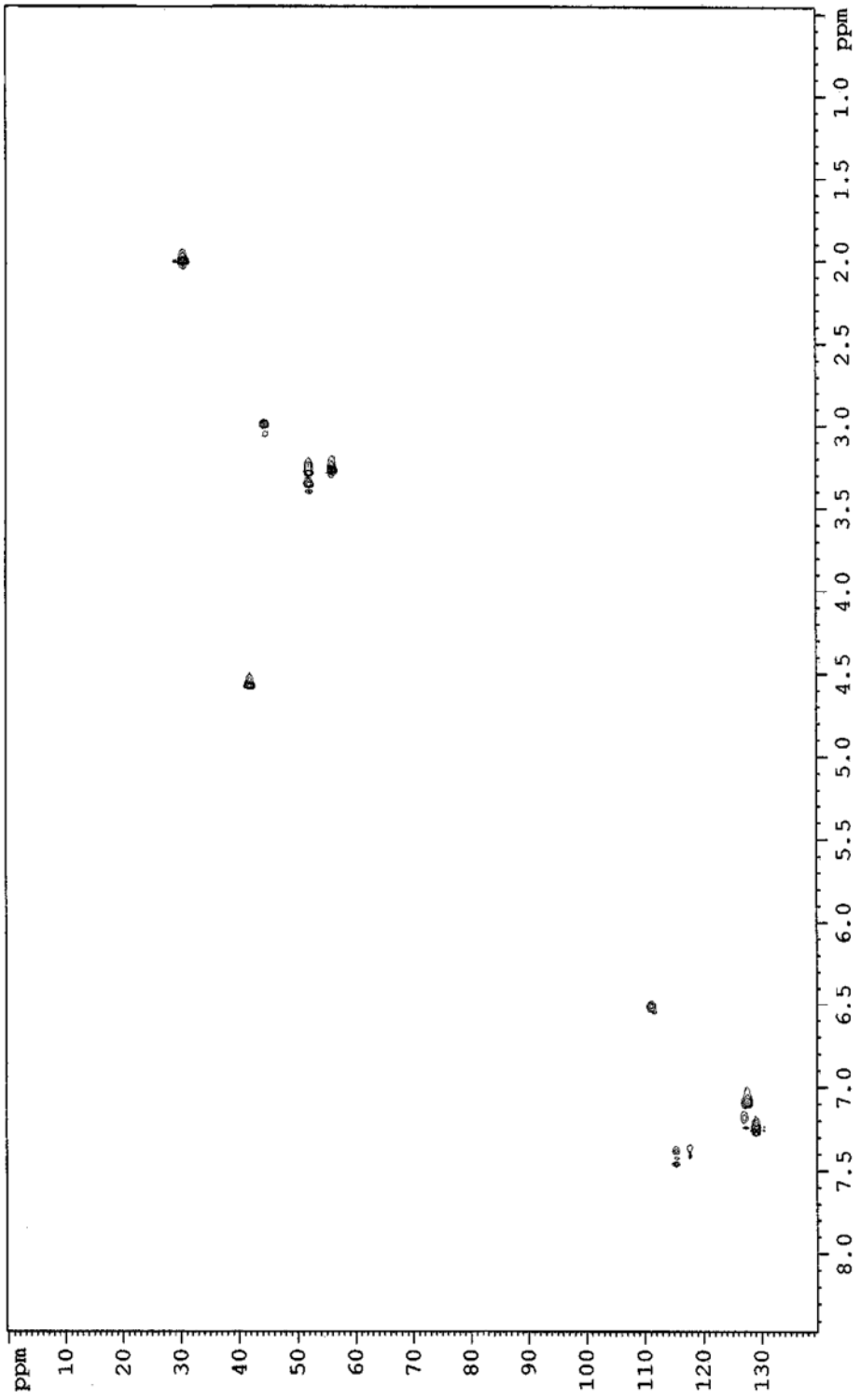
A 2.1: ^{13}C NMR spectrum of pterocellin I recorded in $CDCl_3$



A 2.2: COSY NMR spectrum of pterocellin I recorded in $CDCl_3$



A 2.3: HMBC NMR spectrum of pterocellin I recorded in $CDCl_3$



A 2.4: HSQC NMR spectrum of pterocellin I recorded in $CDCl_3$

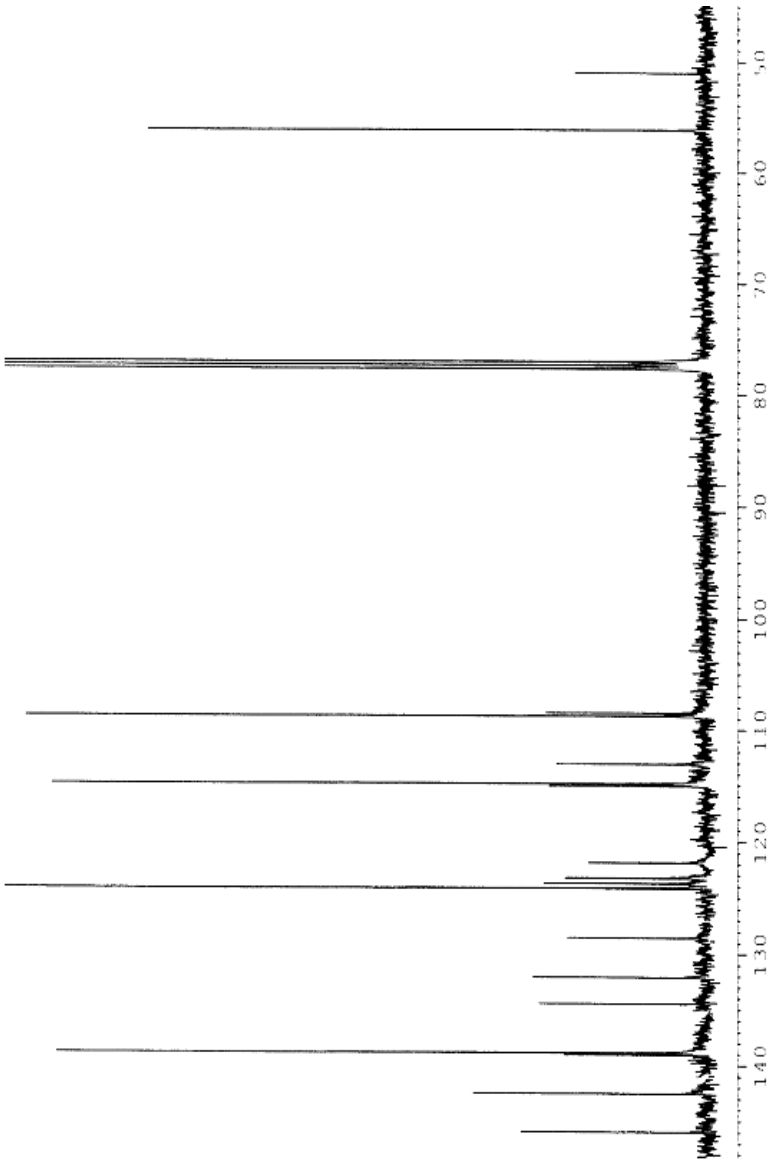
Appendix Three: NMR Spectra of 1-Methyl-5-bromo-8-methoxy-β-carboline (**190**)

A 3.1: ^{13}C NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$

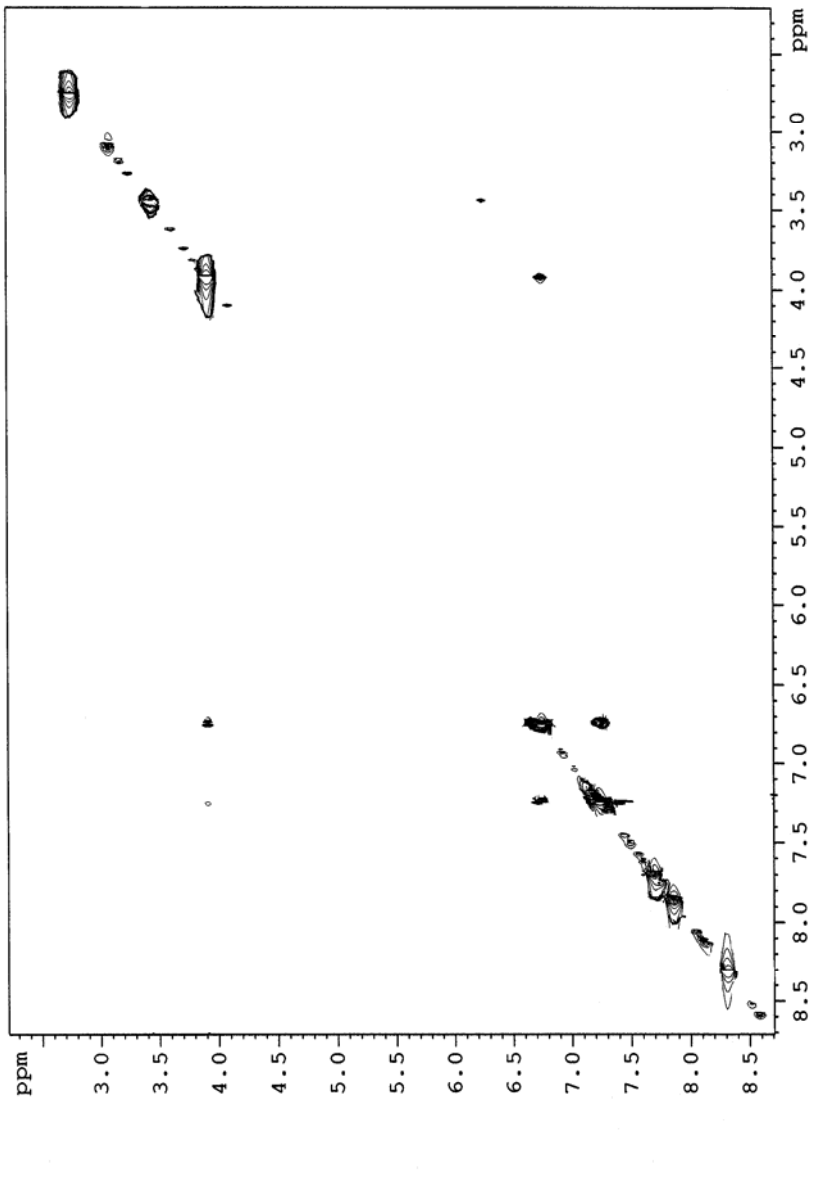
A 3.2: NOESY NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$

A 3.3: HSQC NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$

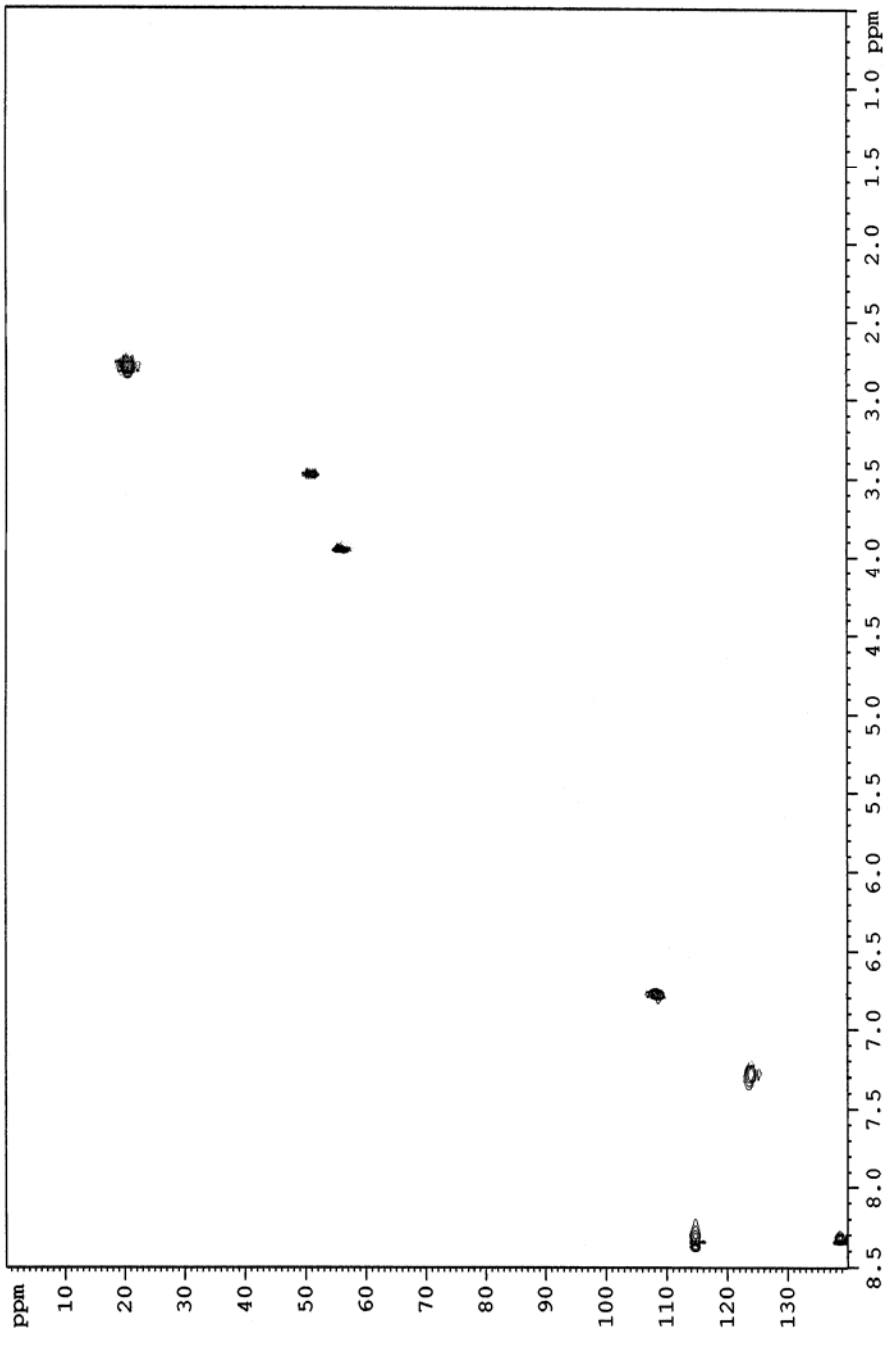
A 3.4: HMBC NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$



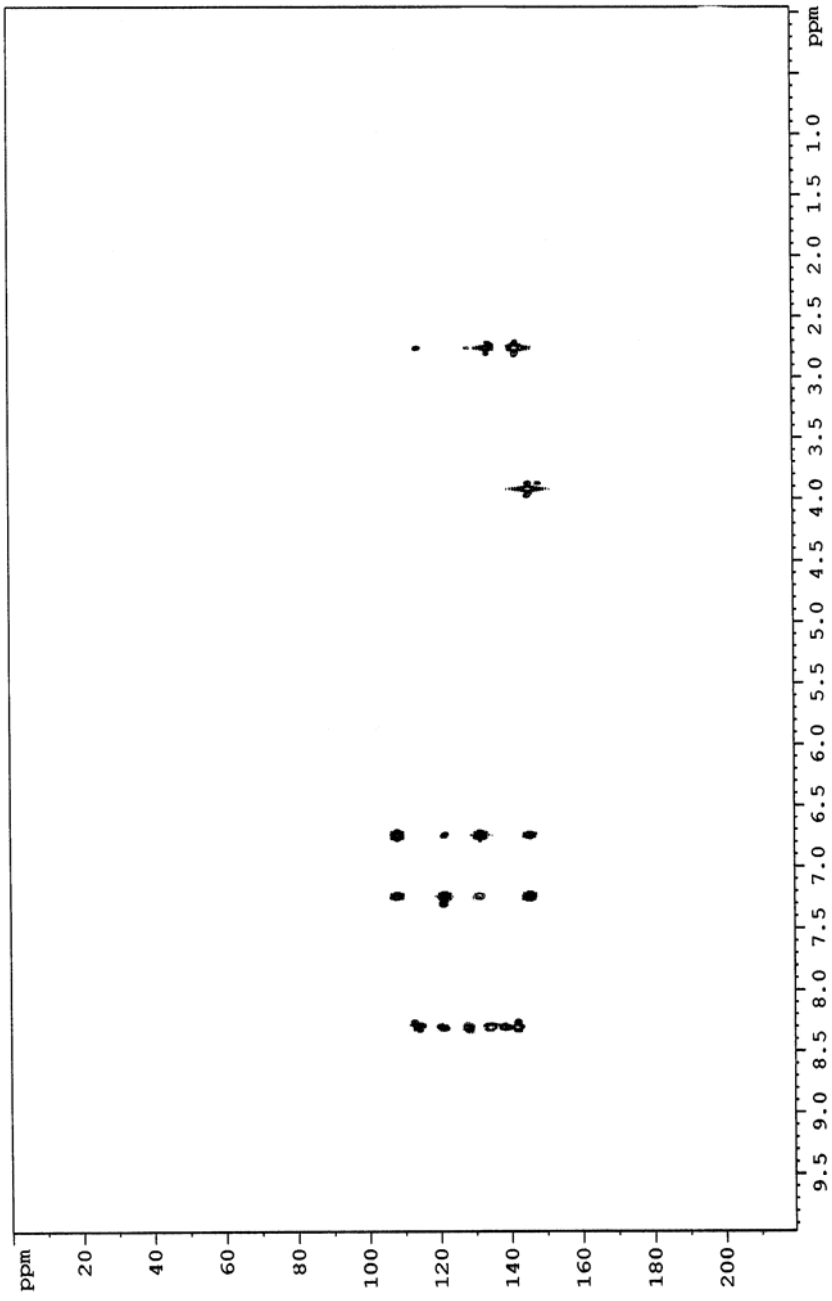
A 3.1: ^{13}C NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$



A 3.2: NOESY NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$



A 3.3: HSQC NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$



A 3.4: HMBC NMR spectrum of 1-methyl-5-bromo-8-methoxy-β-carboline (**190**) in $CDCl_3$

Appendix Four: Biological Assay Results from Survey (Chapter Three)

A 4.1: P388 and antiviral/cytotoxicity results for marine samples collected in 2005.

A 4.2: Antimicrobial and antifungal results for marine samples collected in 2005.

A 4.1: P388 and antiviral/cytotoxicity results for marine samples collected in 2005.[a]

Sample	P388	HSV[b]	HCyt[b]	PV1[b]	PCyt[b]
05-TS1-01	> 125000	ND	ND	ND	ND
05-TS1-02	> 125000	ND	ND	ND	ND
05-TS1-03	> 125000	ND	ND	ND	ND
05-TS1-04	121357	ND	ND	ND	ND
05-TT1-01	> 125000	ND	ND	ND	ND
05-TT1-02	> 125000	ND	ND	ND	ND
05-TT1-03	92827	?*	2+	ND	ND
05-TT1-04	> 125000	ND	ND	ND	ND
05-TT1-05	> 125000	ND	ND	?*	+,8
05-TT1-06	61181	?*	2+,6	?*	+,6
05-MC1-01	> 125000	?*	2+,6	?*	+,6
05-MC1-02	> 125000	ND	ND	ND	ND
05-MC1-03	> 125000	ND	ND	ND	ND
05-MC1-04	> 125000	ND	ND	ND	ND
05-MC1-05	> 125000	ND	ND	ND	ND
05-MC1-06	> 125000	ND	ND	ND	ND
05-MC1-07	> 125000	ND	ND	ND	ND
05-OB1-01	> 125000	ND	ND	ND	ND
05-OB1-02	> 125000	ND	ND	3+	+*,7
05-OB1-03	117797	ND	ND	ND	ND
05-OB1-04	> 125000	ND	ND	ND	ND
05-OB1-05	> 125000	ND	ND	ND	ND
05-OB1-06	> 125000	ND	ND	ND	ND
05-OB1-07	> 125000	ND	ND	ND	ND
05-HC1-01	> 125000	ND	ND	ND	ND
05-HC1-02	> 125000	ND	ND	ND	ND
05-HC1-03	> 125000	ND	ND	ND	ND
05-HC1-04	> 125000	ND	ND	ND	ND
05-HC1-05	> 125000	ND	ND	ND	ND
05-HC1-06	> 125000	ND	ND	ND	ND
05-HC1-07	> 125000	ND	ND	ND	ND
05-OI1-01	> 125000	ND	ND	ND	ND
05-OI1-02	39141	?*	2+,8	?*	3+,8
05-OI1-03	> 125000	ND	ND	ND	ND
05-OI1-05	> 125000	ND	ND	ND	ND
05-OI1-06	> 125000	ND	ND	ND	ND
05-OI1-07	> 125000	ND	ND	ND	ND
05-OI1-08	> 125000	ND	ND	ND	ND
05-OI1-09	114341	?	4+,6	?	4+,6
05-OI1-10	114341	ND	ND	ND	ND
05-OI1-11	110986	ND	ND	ND	ND
05-OI1-12	61181	ND	ND	ND	ND
05-OI1-13	> 125000	ND	ND	ND	ND
05-DP1-01	> 125000	ND	ND	ND	ND
05-DP1-02	> 125000	ND	ND	ND	ND

Sample	P388	HSV[b]	HCyt[b]	PV1[b]	PCyt[b]
05-DP1-03	> 125000	ND	ND	ND	ND
05-DP1-04	77639	ND	ND	ND	ND
05-DP1-05	> 125000	ND	ND	ND	ND
05-DP1-06	> 125000	ND	ND	ND	ND
05-DP1-07	> 125000	ND	ND	ND	ND
05-DP1-08	> 125000	ND	ND	ND	ND
05-DP1-09	> 125000	ND	ND	ND	ND
05-DP1-10	> 125000	ND	ND	ND	ND
05-DP1-11	> 125000	ND	ND	ND	ND
05-DP1-12	> 125000	?	4+,6	?	4+,6
05-DP1-13	> 125000	?	4+,6	?	4+,6
05-DP1-14	> 125000	ND	ND	ND	ND
05-DP1-15	> 125000	ND	ND	ND	ND
05-DP1-17	> 125000	ND	ND	ND	ND
05-DP1-18	68920	ND	ND	ND	ND
05-DP1-19	> 125000	ND	ND	ND	ND
05-HR1-01	> 125000	ND	ND	ND	ND
05-HR1-02	82403	ND	ND	ND	ND
05-HR1-03	> 125000	ND	ND	ND	ND
05-HR1-04	> 125000	ND	ND	ND	ND
05-HR1-05	> 125000	ND	ND	ND	ND
05-HR1-06	65196	ND	ND	ND	ND
05-HR1-07	> 125000	ND	ND	ND	ND
05-HR1-08	> 125000	ND	ND	ND	ND
05-HR1-09	> 125000	ND	ND	ND	ND
05-HR1-10	> 125000	ND	ND	ND	ND
05-HR1-11	> 125000	ND	ND	ND	ND
05-SA1-01	> 125000	ND	ND	ND	ND
05-SA1-02	71004	ND	ND	ND	ND
05-SA1-03	> 125000	ND	ND	ND	ND
05-SA1-04	121357	ND	ND	ND	ND
05-SA1-05	121357	ND	ND	ND	ND
05-SA1-06	75361	ND	ND	ND	ND
05-SA1-07	> 125000	ND	ND	ND	ND
05-SA1-08	> 125000	ND	ND	ND	ND
05-SA1-09	> 125000	ND	ND	ND	ND
05-SA1-10	52717	ND	ND	ND	ND
05-SA1-11	77639	ND	ND	ND	ND
05-SA1-12	> 125000	ND	ND	ND	ND

[a] refer to Section 3.2 for details of assays and Tables 3.1 and 3.2 for explaination of results

[b] all antiviral/cytotoxicity assays at 400 μg/well

A 4.2: Antimicrobial and antifungal results for marine samples collected in 2005.[a]

Sample	E coli[b]	B sub[c]	P aer[d]	C alb[e]	T ment[f]	C res[g]
05-TS1-01	ND[h]	1	ND	ND	ND	ND
05-TS1-02	ND	1	ND	ND	ND	ND
05-TS1-03	ND	ND	ND	ND	ND	ND
05-TS1-04	ND	ND	ND	ND	ND	ND
05-TT1-01	ND	ND	ND	ND	ND	ND
05-TT1-02	ND	2	ND	ND	ND	ND
05-TT1-03	ND	1	ND	ND	ND	ND
05-TT1-04	ND	1	ND	ND	ND	ND
05-TT1-05	ND	ND	ND	ND	ND	ND
05-TT1-06	ND	1	ND	ND	1	ND
05-MC1-01	ND	1	ND	ND	ND	ND
05-MC1-02	ND	1	ND	ND	ND	ND
05-MC1-03	ND	1	ND	ND	ND	ND
05-MC1-04	ND	1	ND	ND	ND	ND
05-MC1-05	ND	1	ND	ND	ND	ND
05-MC1-06	ND	ND	ND	ND	ND	ND
05-MC1-07	ND	ND	ND	ND	ND	ND
05-OB1-01	ND	1	ND	ND	ND	ND
05-OB1-02	ND	2	ND	ND	ND	ND
05-OB1-03	ND	ND	ND	ND	ND	ND
05-OB1-04	ND	ND	ND	ND	ND	ND
05-OB1-05	ND	ND	ND	ND	ND	ND
05-OB1-06	ND	ND	ND	ND	ND	ND
05-OB1-07	ND	ND	ND	ND	ND	ND
05-HC1-01	ND	2	ND	ND	ND	ND
05-HC1-02	ND	2	ND	ND	ND	ND
05-HC1-03	ND	2	ND	ND	ND	ND
05-HC1-04	ND	ND	ND	ND	ND	ND
05-HC1-05	ND	ND	ND	ND	ND	ND
05-HC1-06	ND	ND	ND	ND	ND	ND
05-HC1-07	ND	ND	ND	ND	ND	ND
05-OI1-01	ND	ND	ND	ND	ND	ND
05-OI1-02	ND	1	ND	1	1	ND
05-OI1-03	ND	ND	ND	ND	ND	ND
05-OI1-05	ND	ND	ND	ND	ND	ND
05-OI1-06	ND	ND	ND	ND	ND	ND
05-OI1-07	ND	ND	ND	ND	ND	ND
05-OI1-08	ND	ND	ND	ND	ND	ND
05-OI1-09	ND	ND	ND	ND	ND	ND
05-OI1-10	ND	ND	ND	ND	ND	ND
05-OI1-11	ND	ND	ND	ND	ND	ND
05-OI1-12	ND	ND	ND	ND	1	ND
05-OI1-13	ND	ND	ND	ND	1	ND
05-DP1-01	ND	ND	ND	ND	ND	ND
05-DP1-02	ND	ND	ND	ND	3	ND
05-DP1-03	ND	ND	ND	ND	3	ND

Sample	E coli[b]	B sub[c]	P aer[d]	C alb[e]	T ment[f]	C res[g]
05-DP1-04	ND	ND	ND	ND	3	ND
05-DP1-05	ND	ND	ND	ND	ND	ND
05-DP1-06	ND	ND	ND	ND	ND	ND
05-DP1-07	ND	ND	ND	5	ND	ND
05-DP1-08	ND	ND	ND	ND	1	ND
05-DP1-09	ND	ND	ND	ND	ND	ND
05-DP1-10	ND	ND	ND	ND	ND	ND
05-DP1-11	ND	ND	ND	ND	ND	ND
05-DP1-12	ND	ND	ND	ND	ND	ND
05-DP1-13	ND	ND	ND	ND	ND	ND
05-DP1-14	ND	ND	ND	ND	ND	ND
05-DP1-15	ND	ND	ND	ND	ND	ND
05-DP1-17	ND	ND	ND	ND	ND	ND
05-DP1-18	ND	ND	ND	ND	ND	ND
05-DP1-19	ND	ND	ND	ND	ND	ND
05-HR1-01	ND	ND	ND	ND	ND	ND
05-HR1-02	ND	3	ND	ND	ND	ND
05-HR1-03	ND	ND	ND	ND	ND	ND
05-HR1-04	ND	ND	ND	ND	ND	ND
05-HR1-05	ND	ND	ND	ND	ND	ND
05-HR1-06	ND	ND	ND	ND	ND	ND
05-HR1-07	ND	2	ND	ND	ND	ND
05-HR1-08	ND	ND	ND	ND	ND	ND
05-HR1-09	ND	ND	ND	ND	ND	ND
05-HR1-10	ND	ND	ND	ND	ND	ND
05-HR1-11	ND	ND	ND	ND	ND	ND
05-SA1-01	ND	ND	ND	ND	ND	ND
05-SA1-02	ND	ND	ND	ND	ND	ND
05-SA1-03	ND	ND	ND	ND	ND	ND
05-SA1-04	ND	ND	ND	ND	ND	ND
05-SA1-05	ND	ND	ND	ND	ND	ND
05-SA1-06	ND	3	ND	ND	ND	ND
05-SA1-07	ND	ND	ND	ND	ND	ND
05-SA1-08	ND	ND	ND	ND	ND	ND
05-SA1-09	ND	ND	ND	ND	ND	ND
05-SA1-10	ND	1	ND	ND	ND	ND
05-SA1-11	ND	5	ND	ND	ND	ND
05-SA1-12	ND	ND	ND	3	ND	ND

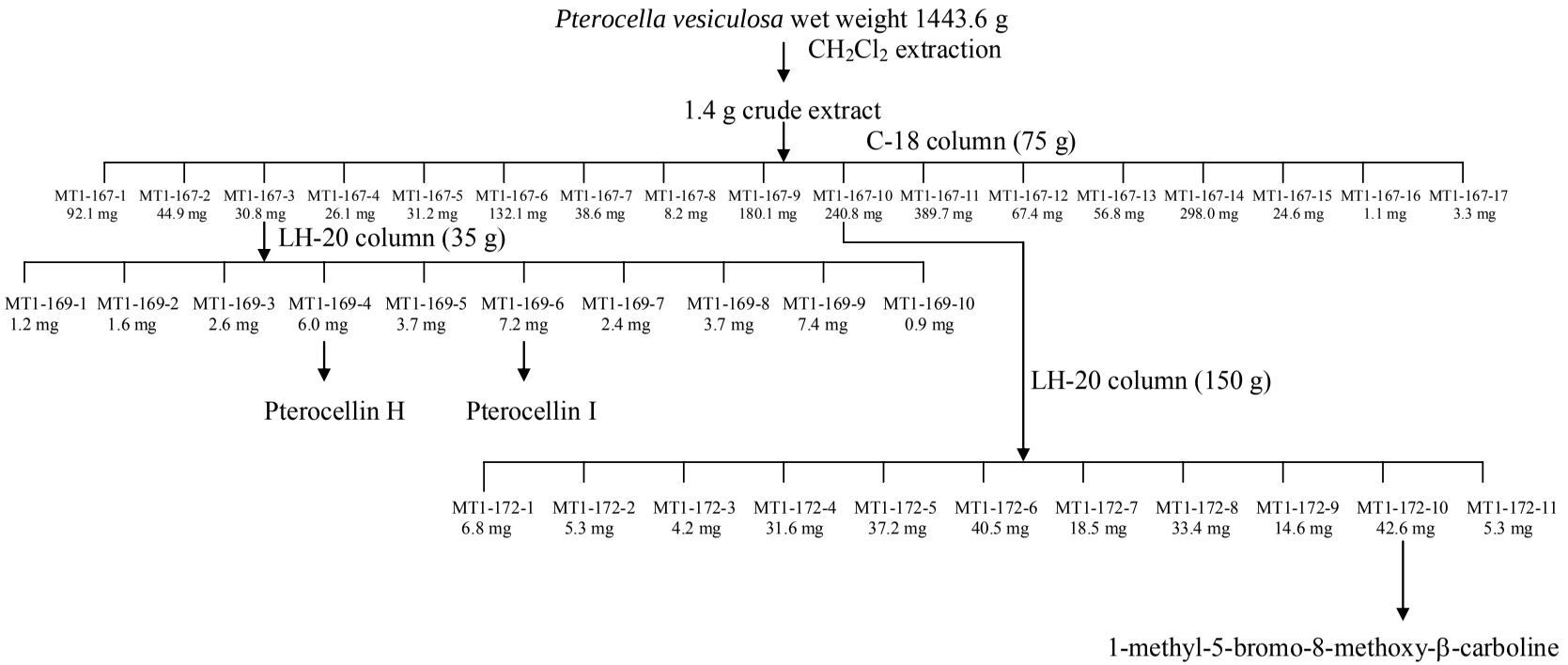
[a] refer to Section 3.2 for details of assays, all the assays run at 400 μg/disk.
[b] *Escherichia coli,* [c] *Bacillus subtilis,* [d] *Pseudomonas aeruginosa,* [e] *Candida albicans,* [f] *Trichophyton mentagrophytes,* [g] *Cladosporium resinae*
[h] indicates no death

Appendix Five: Separation trees

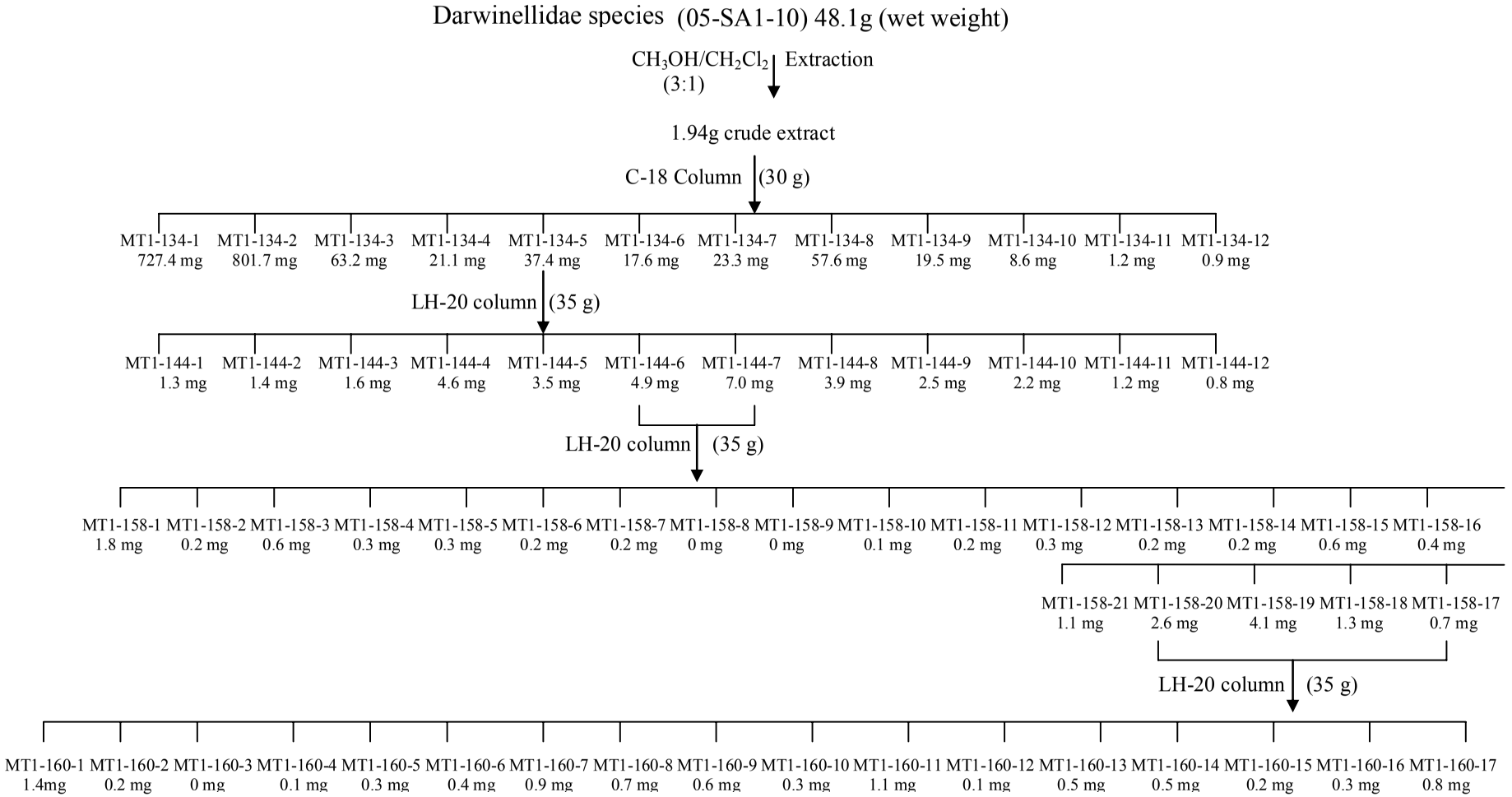
A 5.1: Separation tree for the extract of *Pterocella vesiculosa*

A 5.2: Separation tree for the extract of the Darwinellidae species. (05-SA1-10)

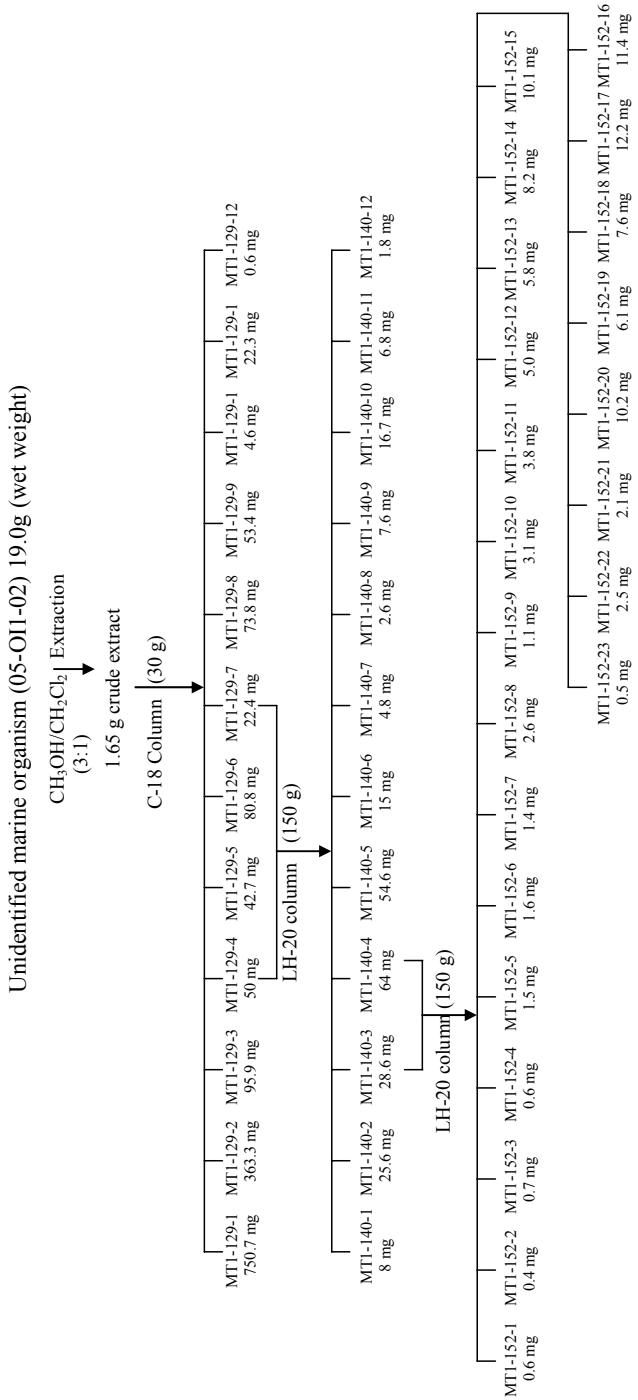
A 5.3: Separation tree for the extract of an unidentified marine organism (05-OI1-02)



A.5.1: Separation tree for the extract of *Pterocella vesiculosa*



A.5.2: Separation tree for the extract of the Darwinellidae species (05-SA1-10).



A 5.3: Separation tree for an unidentified marine organism (05-OI1-02).

References

(1) Prinsep, M. R. *Chem. NZ.* **2000**, *81*, 37-44.

(2) Hanson, J. R. *Natural Products the Secondary Metabolites*; The Royal Society of Chemistry: Cambridge, 2003.

(3) Donia, M.; Hamann, M. T. *Lancet Infect. Dis.* **2003**, *3*, 338-348.

(4) Scheuer, P. J. *Science* **1990**, *248*, 173-177.

(5) Pawlik, J. R. *Chem. Rev.* **1993**, *93*, 1911-1922.

(6) Haefner, B. *Drug Discov. Today* **2003**, *8*, 536-544.

(7) Flam, F. *Science* **1994**, *266*, 1324-1325.

(8) Brusca, R. G.; Brusca, G. J. *Invertebrates*; 2nd ed.; Sinauer Associates Incorporated Publishers: Massachusets, 2003.

(9) Ryland, J. S. *Bryozoans*; Hutchinson and Company Limited: London, 1970.

(10) Christophersen, C. *Acta Chem. Scand.* **1985**, *B39*, 517-529.

(11) Hickman, C. H. *Biology of the Invertebrates*; The C. V. Mosby Company: Saint Louis, 1973.

(12) Dorit, R. F.; Walker, W. F.; Barnes, R. D. *Zoology*; Saunders College Publishing: Philadelphia, 1991.

(13) Kerr, R. G.; Kohl, A. C.; Boehnlein, J. M. Bioactoive Compounds from Bryozoans, in "Recent Advances in Marine Biotechnology Volume 6: "Bio-Organic Compounds: Chemistry and Biomedical Applications". M. Fingerman and R. Nagabhushanam (Eds), Science Publishers Inc, New Hampshire, U.S.A. pp.159-164 (2001).

(14) M. R. Prinsep, Novel Alkaloids from Marine Bryozoans, in "Recent Advances in Marine Biotechnology Volume 6: "Bio-Organic Compounds: Chemistry and Biomedical Applications". M. Fingerman and R. Nagabhushanam (Eds), Science Publishers Inc, New Hampshire, U.S.A. pp.165-186 (2001).

(15) Faulkner, D. J. *Nat. Prod. Rep.* **1986**, *3*, 1-33.

(16) Faulkner, D. J. *Nat. Prod. Rep.* **1987**, *4*, 539-576.

(17) Faulkner, D. J. *Nat. Prod. Rep.* **1988**, *5*, 613-663.

(18) Faulkner, D. J. *Nat. Prod. Rep.* **1990**, *7*, 269-309.

(19) Faulkner, D. J. *Nat. Prod. Rep.* **1991**, *8*, 97-147.

(20) Faulkner, D. J. *Nat. Prod. Rep.* **1992**, *9*, 323-364.

(21) Faulkner, D. J. *Nat. Prod. Rep.* **1993**, *10*, 497-539.

(22) Faulkner, D. J. *Nat. Prod. Rep.* **1994**, *11*, 355-394.

(23) Faulkner, D. J. *Nat. Prod. Rep.* **1995**, *12*, 223-269.

(24) Faulkner, D. J. *Nat. Prod. Rep.* **1996**, *13*, 75-125.

(25) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259-302.

(26) Faulkner, D. J. *Nat. Prod. Rep.* **1998**, *15*, 113-158.

(27) Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155-198.

(28) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7-55.

(29) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1-49.

(30) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1-48.

(31) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1-48.

(32) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2004**, *21*, 1-49.

(33) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2005**, *22*, 15-61.

(34) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2006**, *23*, 26-78.

(35) Blunt, J. W.; Copp, B. R.; Wan-Ping, H.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2007**, *24*, 31-86.

(36) Pettit, G. R. *Prog. Ch. Org. Nat. Prod.* **1991**, *57*, 153-195.

(37) Pettit, G. R.; Herald, C. L.; Kamano, Y.; Gust, D.; Aoyagi, R. *J. Nat. Prod.* **1983**, *46*, 528-531.

(38) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tozawa, M. *Can. J. Chem.* **1985**, *63*, 1204-1208.

(39) Kageyama, M.; Tamura, T.; Nantz, M. H.; Roberts, J. C.; Somfai, P.; Whritenour, D. C.; Masamune, S. *J. Am. Chem. Soc.* **1990**, *112*, 7407-7408.

(40) Pettit, G. R.; Gao, F.; Blumberg, P. M.; Herald, C. L.; Coll, J. C.; Kamano, Y.; Lewin, N. E.; Schmidt, J. M.; Chapuis, J. C. *J. Nat. Prod.* **1996**, *59*, 286-289.

(41) Pettit, G. R.; Gao, F.; Sengupta, D.; Coll, J. C.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Van Camp, J. R.; Rudloe, J. J.; Nieman, R. A. *Tetrahedron* **1991**, *47*, 3601-3610.

(42) Mutter, R.; Wills, M. *Bioorg. Med. Chem.* **2000**, *8*, 1841-1860.

(43) Pettit, G. R.; Kamano, Y.; Aoyagi, R.; Herald, C. L.; Doubek, D. L.; Schmidt, J. M.; Rudloe, J. J. *Tetrahedron* **1985**, *41*, 985-994.

(44) Lin, H.; Yao, X.; Yi, Y.; Li, X.; Wu, H. *Zhongguo Haiyang Yaowu* **1998**, *17* 1-6.

(45) Lopanik, N.; Gustafson, K. R.; Lindquist, N. *J. Nat. Prod.* **2004**, *67*, 1412-1414.

(46) Evans, D. A.; Carter, P. H.; Carreira, E. M.; Prunet, J. A.; Charette, A. B.; Lautens, M. *Angew. Chem.* **1998**, *37*, 2354-2359.

(47) Ohmori, K.; Ogawa, Y.; Obitsu, T.; Ishikawa, Y.; Nishiyama, S.; Yamamura, S. *Angew. Chem.* **2000**, *39*, 2290-2294.

(48) Pettit, G. R.; Herald, C. L.; Doubek, D. L.; Herald, D. L.; Clardy, J.; Arnold, E. *J. Am. Chem. Soc.* **1982**, *104*, 6846-6848.

(49) Clamp, A.; Jayson, G. C. *Anti-cancer drug.* **2002**, *13*, 673-683.

(50) Nezhat, F.; Walder, S.; Muggia, F.; Mandeli, J.; Goldberg, G.; Rahaman, J.; Runowicz, C.; Murgo, A. J.; Gardner, G. J. *Gynecol. Oncol.* **2004**, *93*, 144-148.

(51) Newman, D. "Old, New, Borrowed and Blue. Marine-Derived Drugs, Past, Present and Future", *Manapro XII*, Queenstown, New Zealand, 2007.

(52) National Cancer Institute, www.cancer.gov/drugs., 2007.

(53) Pettit, G. R.; Gao, F.; Herald, D. L.; Blumberg, P. M.; Lewin, N. E.; Nieman, R. A. *J. Am. Chem. Soc.* **1991**, *113*, 6693-6695.

(54) Lopanik, N.; Lindquist, N.; Targett, N. *Oecologia* **2004**, *139*, 131-139.

(55) Kamano, Y.; Kotake, A.; Hashima, H.; Hayakawa, I.; Hiraide, H.; Zhang, H.-P.; Kizu, H.; Komiyama, K.; Hayashi, M.; Pettit, G. R. *Collect. Czech. Chem. Commun.* **1999**, *64*, 1147-1153.

(56) Kamano, Y.; Zhang, H.-P.; Ichihara, Y.; Kizu, H.; Komiyama, K.; Pettit, G. R. *Tetrahedron Lett.* **1995**, *36*, 2783-2784.

(57) Zhang, H.-P.; Kamano, Y.; Ichihara, Y.; Kizu, H.; Komiyama, K.; ltokawa, H.; Pettit, G. R. *Tetrahedron* **1995**, *51*, 5523-5528.

(58) Montanari, A. M.; Fenical, W.; Lindquist, N.; Lee, A. Y.; Clardy, J. *Tetrahedron* **1996**, *52*, 5371-5380.

(59) Carte, B.; Faulkner, D. J. *J. Org. Chem.* **1983**, *48*, 2314-2318.

(60) Borah, S.; Melvin, M. S.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **1998**, *120*, 4557-4562.

(61) Lindquist, N.; Fenical, W. *Experientia* **1991**, *47*, 504-506.

(62) Blackman, A. J.; Li, C. *Aust. J. Chem.* **1994**, *47*, 1625-1629.

(63) Matsunaga, S.; Fusetani, N.; Hashimoto, K. *Experientia* **1986**, *42*, 84.

(64) Chevolot, L.; Chevolot, A.-M.; Gajhede, M.; Larsen, C.; Anthoni, U.; Christophersen, C. *J. Am. Chem. Soc.* **1985**, *107*, 4542-4543.

(65) Anthoni, U.; Bock, K.; Chevolot, L.; Larsen, C.; Nielsen, P. H.; Christophersen, C. *J. Org. Chem.* **1987**, *52*, 5639-5639.

(66) Anthoni, U.; Chevolot, L.; Larsen, C.; Nielsen, P. H.; Christophersen, C. *J. Org. Chem.* **1987**, *52*, 4709-4712.

(67) Sun, C.; Lin, X.; Weinreb, S. M. *J. Org. Chem.* **2006**, *71*, 3159-3166.

(68) Rahbaek, L.; Anthoni, U.; Christophersen, C.; Nielsen, P. H.; Petersen, B. O. *J. Org. Chem.* **1996**, *61*, 887-889.

(69) Rahbaek, L.; Christophersen, C. *J. Nat. Prod.* **1997**, *60*, 175-177.

(70) Blackman, A. J.; Matthews, D. J. *Heterocycles* **1985**, *23*, 2829-2833.

(71) Blackman, A. J.; Green, R. D. *Aust. J. Chem.* **1987**, *40*, 1655-1662.

(72) Blackman, A. J.; Eldershaw, T. P. D.; Garland, S. M. *Aust. J. Chem.* **1993**, *46*, 401-405.

(73) Morris, B. D.; Prinsep, M. R. *J. Nat. Prod.* **1999**, *62*, 688-693.

(74) Lee, N.-K.; Fenical, W.; Lindquist, N. *J. Nat. Prod.* **1997**, *60*, 697-699.

(75) Morris, B. D.; Prinsep, M. R. *J. Org. Chem.* **1998**, *63*, 9545-9547.

(76) Yu, C.-M.; Wright, J. L. C. *J. Nat. Prod.* **1995**, *58*, 1978-1982.

(77) Milanowski, D. J.; Gustafson, K. R.; Kelley, J. A.; McMahon, J. B. *J. Nat. Prod.* **2004**, *67*, 70-73.

(78) Wipf, P.; Joo, B.; Nguyen, T.; Lazo, J. S. *Org. Biomol. Chem.* **2004**, *2*, 2173-2174.

(79) Rinehart, K. L.; Holt, T. G.; Fregeau, N. L.; Keifer, P. A.; Wilson, J. R.; Perun, T. J., Jr; Sakai, R.; Thompson, A. G.; Stroh, J. G.; Shield, L. S.; Seigler, D. S.; Li, L. H.; Martin, D. G.; Grimmelikhuijzen, C. J. P. *J. Nat. Prod.* **1990**, *53*, 771-792.

(80) Blackman, A. J.; Matthews, D. J.; Narkowicz, C. K. *J. Nat. Prod.* **1987**, *50*, 494-496.

(81) Prinsep, M. R.; Blunt, J. W.; Munro, M. H. G. *J. Nat. Prod.* **1991**, *54*, 1068-1076.

(82) Harwood, D. T.; Urban, S.; Blunt, J. W.; Munro, M. H. G. *Nat. Prod. Res.* **2003**, *17* 15-19.

(83) Kon-ya, K.; Shimidzu, N.; Adachi, K.; Miki, W. *Fisheries Sci.* **1994**, *60*, 773-775.

(84) Choi, Y.; Park, A.; Schmitz, F. J.; Altena, I. *J. Nat. Prod.* **1993**, *56*, 1431-1433.

(85) O'Malley, M. M.; Damkaci, F.; Kelly, T. R. *Org. Lett.* **2006**, *8*, 2651-2652.

(86) Yao, B.; Prinsep, M. R.; Nicholson, B. K.; Gordon, D. P. *J. Nat. Prod.* **2003**, *66*, 1074-1077.

(87) Prinsep, M. R. **2006**, Unpublished Research.

(88) Jeong, S.-J.; Higuchi, R.; Miyamoto, T.; Ono, M.; Kuwano, M.; Mawatari, S. F. *J. Nat. Prod.* **2002**, *65*, 1344-1345.

(89) Blackman, A. J.; Hambley, T. W.; Picker, K.; Taylor, W. C.; Thirasasana, N. *Tetrahedron Lett.* **1987**, *28*, 5561-5562.

(90) Liu, Y.; McWhorter, W. W., Jr. *J. Am. Chem. Soc.* **2003**, *125*, 4240-4252.

(91) Eisenbarth, S.; Gehling, M.; Harder, A.; Steffan, B. *Tetrahedron* **2002**, *58*, 8461-8464.

(92) Ayer, S. W.; Andersen, R. J.; He, C.; Clardy, J. *J. Org. Chem.* **1984**, *49*, 3869-3870.

(93) Peters, L.; Wright, A. D.; Krick, A.; Konig, G. M. *J. Chem. Ecol.* **2004**, *30*, 1165-1181.

(94) Carle, J. S.; Christophersen, C. *J. Am. Chem. Soc.* **1979**, *101*, 4012-4013.

(95) Carle, J. S.; Christophersen, C. *J. Org. Chem.* **1980**, *45*, 1586-1589.

(96) Sjoblom, T.; Bohlin, L.; Christophersen, C. *Acta. Pharm. Suec.* **1983**, *20*, 415-418.

(97) Carle, J. S.; Christophersen, C. *J. Org. Chem.* **1981**, *46*, 3440-3443.

(98) Wulff, P.; Carle, J. S.; Christophersen, C. *Comp. Biochem. Physiol.* **1982**, *71B*, 525-526.

(99) Wright, J. L. C. *J. Nat. Prod.* **1984**, *47*, 893-895.

(100) Laycock, M. V.; Wright, J. L. C.; Findlay, J. A.; Patil, A. D. *Can. J. Chem.* **1986**, *64*, 1312-1316.

(101) Lysek, N.; Rachor, E.; Lindel, T. *J. Bioscience.* **2002**, *57*, 1056-1061.

(102) Keil, P.; Nielsen, E. G.; Anthoni, U.; Christophersen, C. *Acta Chem. Scand.* **1986**, *B40*, 555-558.

(103) Holst, P. B.; Anthoni, U.; Christophersen, C.; Nielsen, P. H. *J. Nat. Prod.* **1994**, *57*, 997-1000.

(104) Peters, L.; Konig, G. M.; Terlau, H.; Wright, A. D. *J. Nat. Prod.* **2002**, *65*, 1633-1637.

(105) Morales-Ríos, M. S.; Sua´rez-Castillo, O. R.; Trujillo-Serrato, J. J.; Joseph-Nathan, P. *J. Org. Chem.* **2001**, *66*, 1186-1192.

(106) Rinehart, K. L., Jr.; Cheng, J.-F.; Lee, J.-S. Patent US 94-198444, 1996.

(107) Beutler, J. A.; Cardellina, J. H.; Prather, T.; Shoemaker, R. H.; Boyd, M. R.; Snader, K. M. *J. Nat. Prod.* **1993**, *56*, 1825-1826.

(108) Kinzer, K. F.; Cardellina, J. H. *Tetrahedron Lett.* **1987**, *28*, 925-926.

(109) Badre, A.; Boulanger, A.; Abou-Mansour, E.; Banaigs, B.; Combaut, G.; Francisco, C. *J. Nat. Prod.* **1993**, *57*, 528-33.

(110) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Mascal, M.; Holt, T. G.; Shield, L. S.; Lafargue, F. *J. Am. Chem. Soc.* **1987**, *109*, 3378-3387.

(111) Schupp, P.; Poehner, T.; Edrada, R.; Ebel, R.; Berg, A.; Wray, J.; Proksch, P. *J. Nat. Prod.* **2003**, *66*, 272-275.

(112) Davies, R. A.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **1998**, *61*, 959-960.

(113) Blunt, J. W.; Lake, R. J.; Munro, M. H. G.; Toyokuni, T. *Tetrahedron Lett.* **1987**, *28*, 1825-1826.

(114) Lake, R. J.; Breenan, M. M.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *Tetrahedron Lett.* **1988**, *29*, 2255-2256.

(115) Lake, R. J.; Blunt, J. W.; Munro, M. H. G. *Aust. J. Chem.* **1989**, *42*, 1201-1206.

(116) Kobayashi, J.; Cheng, J.; Ohta, T.; Nozoe, S.; Ohizumi, Y.; Sasaki, T. *J. Org. Chem.* **1990**, *55*, 3666-3670.

(117) Aiello, A.; Fattorusso, E.; Magno, S.; Mayol, L. *Tetrahedron* **1987**, *43*, 5929-5932.

(118) Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 1526-1528.

(119) Alley, S. R., The University of Waikato, *Masters Thesis*, 1995.

(120) de Araújo-Júnior, V. T.; da Silva, M. S.; da-Cunha, E. V. L.; Agra, M. F.; da Silva-Filho, R. N.; Barbosa-Filho, J. M.; Braz-Filho, R. *Pharma. Biol.* **2004**, *42*, 62-67.

(121) Sheldrick, G. M. In *Program for solution of crystal structures*; University of Göttingen: Germany, 1997.

(122) Sheldrick, G. M. In *Program for the refinement of crystal structures*; University of Göttingen: Germany, 1997.

(123) Ishida, J.; Wang, H. K.; Bastow, K. F.; Hu, C. Q.; Lee, K. H. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3319-3324.

(124) Karuso, P.; Bergquist, P. R.; Cambie, R. C.; Buckleton, J. S.; Clark, G. R.; Rickard, C. E. F. *Aust. J. Chem.* **1986**, *39*, 1643-1653.

(125) Bobzin, S. C.; Faulkner, D. J. *J. Org. Chem.* **1989**, *54*, 5727-5731.

(126) Bobzin, S. C.; Faulkner, D. J. *J. Nat. Prod.* **1991**, *54*, 225-232.

(127) Molinski, T. F.; Faulkner, D. J. *J. Org. Chem.* **1987**, *52*, 296-298.

(128) Ankisetty, S.; Amsler, C. D.; McClintock, J. B.; Baker, B. J. *J. Nat. Prod.* **2004**, *67*, 1172-1174.

(129) Rudi, A.; Kashman, Y. *J. Nat. Prod.* **1992**, *55*, 1408-1414.

(130) Kerr, R. G.; Vicchiarelli, R.; Kerr, S. S. *J. Nat. Prod.* **1999**, *62*, 468-470.

(131) Bergquist, P. R.; Hofheinz, W.; Oesterhelt, G. *Biochem. Syst. Ecol.* **1980**, *8*, 423-435.

(132) Gerst, N.; Ruan, B.; Pang, J.; Wilson, W. K.; Schroepfer, G. J., Jr *J. Lipid Res.* **1997**, *38*, 1685-1701.

(133) Bergquist, P. R.; Lavis, A.; Cambie, R. C. *Biochem. Syst. Ecol.* **1986**, *14*, 105-112.

(134) Kondo, H.; Ishiwatari, R.; Yamamoto, S.; Uemura, H. *Nagasaki Daigaku Kyoikugakubu Shizen Kagaku Kenkyu Hokoku* **1991**, *44*, 57-75.