

Pyrroloiminoquinone and related metabolites from marine sponges

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Covering: the literature from the first reported isolation of a pyrroloiminoquinone metabolite from a marine sponge in 1986 up to October 1, 2004.

This review presents the structure, biological activity, biosynthetic studies and, where applicable, references to syntheses of 81 marine alkaloids containing either tetra-, hexa- or octa-hydrogenated variants of pyrrolo[4,3,2-*de*]quinoline, pyrrolo[4,3,2-*de*]pyrrolo[2,3-*h*]quinoline and pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline core skeletons. The literature describing the isolation of pyrroloiminoquinones, and related metabolites, from marine sponges is littered with taxonomic inconsistencies and recent efforts to clarify the taxonomy of the sponges that produce this group of metabolites are discussed.

- 1 Introduction
- 2 The taxonomy of marine sponges that produce pyrroloiminoquinone and related metabolites
- 3 Alkaloids containing a pyrrolo[4,3,2-*de*]quinoline skeleton
- 4 Alkaloids containing a pyrrolo[4,3,2-*de*]pyrrolo[2,3-*h*]quinoline skeleton
- 5 Alkaloids containing a pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline skeleton

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1 Introduction

For nearly two decades marine sponges of the families Latrunculiidae (order Poecilosclerida, sub-order Latrunculina *incertae sedis*) and Acarnidae (order Poecilosclerida, sub-order Microciona) have proved to be a prolific source of biologically

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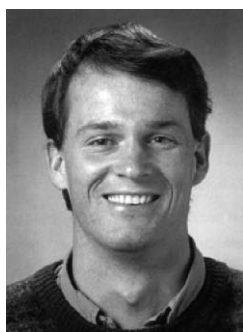
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Edith M. Antunes



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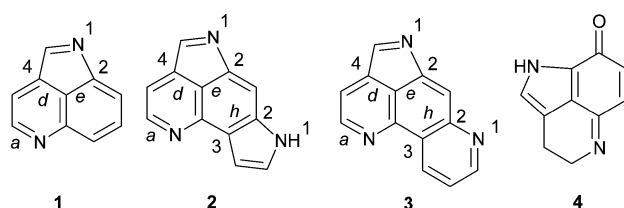


Michael T.
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Toufiek Samaai

active alkaloid metabolites containing either tetra-, hexa- or octa-hydrogenated variants of pyrrolo[4,3,2-*de*]quinoline **1**, pyrrolo[4,3,2-*de*]pyrrolo[2,3-*h*]quinoline **2** and pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline **3** core skeletons.^{1,2} The characteristic pyrroloiminoquinone **4** structural motif dominates in all three core skeletons and provides the name, along with pyrroloquinoline, commonly used to describe the members of this group of secondary metabolites. Marine pyrroloquinoline alkaloids have been previously reviewed as part of a larger review of bioactive marine alkaloids³ and in tandem with pyridoacridine alkaloids from marine sources.⁴ A recent taxonomic revision of a number of the original voucher specimens of the sponges that have yielded some of the published chemistry of pyrroloiminoquinone metabolites^{5–10} and some confusion surrounding the alphabetical ordering of discorhabdins have precipitated this review of the structures, bioactivity and, where applicable, biosynthesis and synthesis of this group of marine natural products.



This review begins with a brief overview of the systematics of the sponge families Latrunculiidae, Acarnidae and Podospongiidae where the information provided by marine natural product chemical studies has helped to partly clarify the phylogenetic relationship between the sponges allocated to these families.^{3,5–10} Following on from the taxonomic discussion, the isolation, structures, biological activity and relevant syntheses of the pyrroloiminoquinone metabolites from the core structural classes, pyrrolo[4,3,2-*de*]quinoline and pyrrolo[4,3,2-*de*]pyrrolo[2,3-*h*]quinoline are presented here in order of increasing structural complexity. Conversely, the pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline core structural class, incorporating the discorhabdins A–V and the closely related priansins A–D and epinardins A–D, is introduced in a chronological sequence reflecting the reported isolation of these alkaloids. The order of presentation of the structures of the discorhabdin, priansin and epinardin series is an attempt to clarify some of the structural overlap and anomalies that surround these metabolites. Syntheses, biosynthetic studies and reports of the subsequent isolation of the ubiquitous discorhabdins, and less common structurally related analogues, follow the historically biased review of the discorhabdin/priansin/epinardin literature.

2 The taxonomy of marine sponges that produce pyrroloiminoquinone and related metabolites

Sponges that produce pyrroloiminoquinone and related metabolites generally possess either silicious discorhabd microscleres (Fig. 1A) aligned vertically on the surface of the ectosome (Fig. 1B) or palmate chelae (Fig. 1C) distributed in various parts of their tissues (family Acarnidae).^{5–9} With the notable exception of the genus *Strongyloidesma*, discorhabd microscleres (which can be further differentiated into aniso, iso, isoconico and isochia forms on closer examination) are found in sponges from the other three genera within the family Latrunculiidae (Scheme 1). Discorhabd microscleres are also superficially similar to the spinorhabd microscleres found in sponges of the family Podospongiidae (Fig. 1D and E). It is not surprising that the presence of an array of these superficially similar microscleres was traditionally used as an easily recognizable, and often hastily assigned taxonomic identifier to combine diverse sponges into a single genus *Latrunculia* (family Latrunculiidae).^{5,7,8} Kelly-Borges and Vacelet¹¹ first drew attention to clear differences in the skeletal architecture and the lack of structural homology in the discorhabd microscleres of sponge genera grouped together in what was then the monophyletic family Latrunculiidae.³ These significant differences necessitated an extensive revision

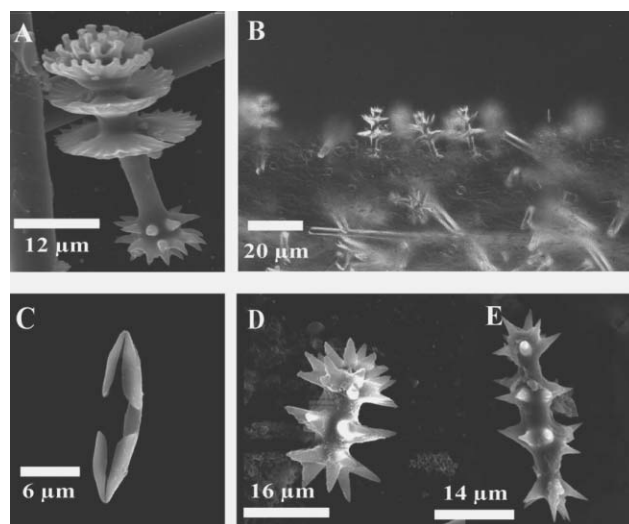
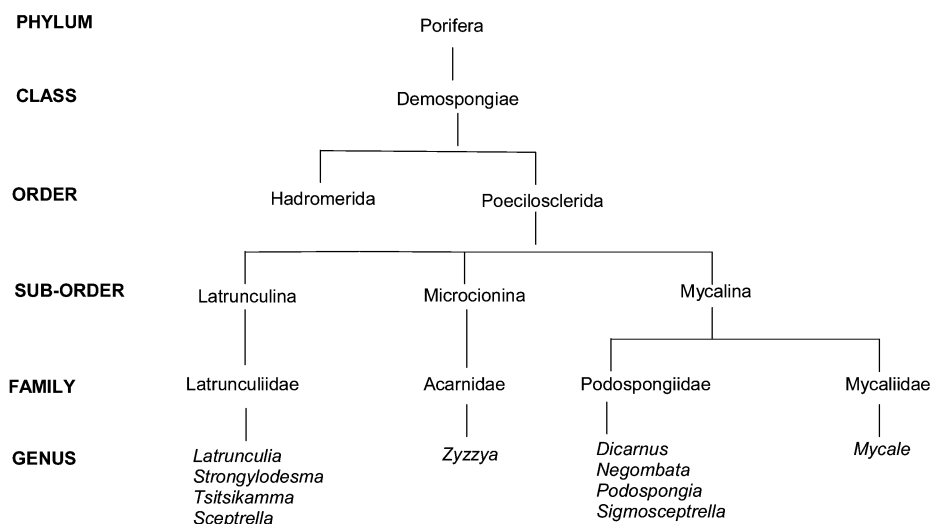
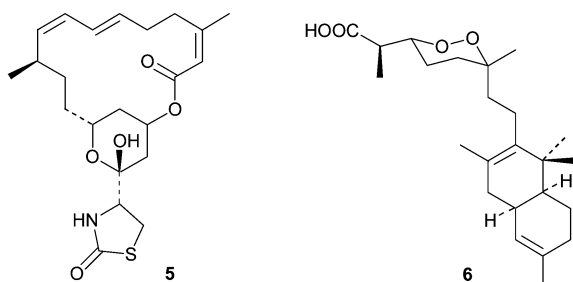


Fig. 1 (A) Anisodiscorhabd from *Latrunculia bocagei* Ridley and Dendy, 1886. (B) Common arrangement of discorhabds on the surface of the ectosome in *Latrunculia* species. (C) A palmate chela, characteristic of sponges assigned to the order Microcionina. (D) and (E) Spinorhabds typically found in sponges of the genera *Sigmosceptrella* and *Negombata*, respectively.



Scheme 1 A suggested phylogenetic relationship between the sponge families Latrunculiidae, Acarnidae, Podospongiidae and Mycaliidae.

of the family Latrunculiidae^{5,7-9} and the taxonomic *status quo* suggests the hypothetical phylogenetic relationship between this family and related families presented in Scheme 1. From an analysis of the plethora of marine natural product investigations of sponges originally loosely grouped together in the sponge family Latrunculiidae, Samaai and Kelly have been able to provide significant chemotaxonomic evidence in support of this phylogenetic revision.^{3,5-9} For example, Indo-Pacific sponges originally identified as *Latrunculia* spp. that produce macrocyclic lactones e.g. latrunculin A **5**,¹² have been re-allocated to the circumtropical genus *Negombata* in the family Podospongiidae while sponges either originally identified as *Latrunculia* or *Prianos* spp., and providing a source of sesterterpene peroxides e.g. trunculin A **6**,¹³ have been re-identified as *Dicarnus* or *Sigmaceptrella* species, also in the family Podospongiidae.^{3,5,6,11}



Sponges of the family Latrunculiidae are ubiquitous in cold water, with diversity centred predominantly in the southern hemisphere.⁸ *Latrunculia* species are relatively abundant in the sub-tidal (< -50m) benthic communities off New Zealand, Antarctica and South Africa.^{8,9} The utilization of definitive chemotaxonomic markers within the family Latrunculiidae can be tenuous given the proposed close biosynthetic relationship between pyrroliminoquinone and related metabolites (Scheme 2). Nonetheless, general chemotaxonomic trends are evident, for example the genera *Latrunculia* (Latrunculiidae) and *Strongyloidesma* (Latrunculiidae) are apparent reservoirs of discorhabdin-type metabolites (see later).

In a comprehensive study of the taxonomic, environmental and chemical variation within the sponge genus *Latrunculia* in New Zealand, Miller *et al.*¹⁴ and Alvarez *et al.*¹⁵ concluded the presence of at least eight species rather than the one to four previously believed. The authors found that there were no single diagnostic characters that could be used to distinguish all eight species and a combination of characteristics was required. The characters studied included allozyme analysis using nine-locus genotypes, spicule morphology and chemical analysis. The eight species of *Latrunculia* displayed fixed or near-fixed genetic differences, indicating that they were reproductively isolated while the discorhabdin alkaloid chemistry produced by the sponges was species specific rather than the result of environmental variation.

Interestingly, a Japanese sponge *Prianos melanos*,¹⁶ a source of the prianosins (synonymous with the discorhabdins – see later) has been re-identified as a species of *Strongyloidesma* (formerly only known from the South Atlantic).^{5,8} Several deep-water Caribbean *Batzella* species¹⁶ that yielded batzelline, secobatzelline and discorhabdin metabolites (see later), are also now recognized as *Strongyloidesma* species.^{5,17} Paradoxically, the genera *Batzella* and *Prianos* have historically both been used as “taxonomic dustbins” which may account for the handful of unrelated species placed in these genera.^{3,16,18} Currently, *Batzella sensu stricta* is placed within the Poecilosclerid family Chondropsidae¹⁹ and *Prianos sensu stricta* is synonymous with the Haplosclerid genus *Haliclona* (*Reniera*) (family Chalinidae).²⁰

Makaluvic acids, damirones and makaluvamines are consistently, but not exclusively, reported from sponges assigned to the genus *Zyzzya* (family Acarnidae, formerly Iophonidae)³ as evidenced by the recent isolation of members of all three of these groups of alkaloids, in addition to discorhabdins,

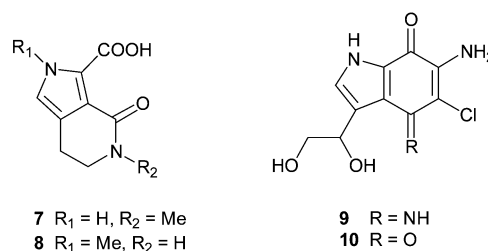
from the South African sponge *Latrunculia bellae*.²¹ During the last decade several major changes in familial classifications within Poecilosclerida have occurred, especially in the transfer of genera and in the reorganization of familial assemblages.^{5-10,19,20} Thus Hooper proposed that the family Iophonidae be renamed Acarnidae as the latter represents the older family name created by Topsent as a family level taxon for *Acarnus*.¹⁰ The original sponge that yielded the damirones, *Damiria* spp. (see later), and the only reference to this species in the chemistry literature, has been re-identified as *Zyzzya fuliginosa*.^{5,16}

The discovery of the tsitsikammamine metabolites (see later) in the new South African genus *Tsitsikamma* (family Latrunculiidae) initially contributed positively to the chemotaxonomic debate as the purported biosynthetic intermediacy of the tsitsikammamine structures between those of the makaluvamines and discorhabdins appeared to be paralleled by the mixture of *Zyzzya* and *Latrunculia* morphological characteristics evident in *T. favus*.^{3,9} However, the absence of tsitsikammamines in a second species of this genus, *T. pedunculata*, recently discovered off South Africa, negates the chemotaxonomic significance originally assigned to these compounds.²¹

There have been no reported natural product studies of the fourth genus, *Sceptrella*, within the family Latrunculiidae and this may be attributed to the difficulty of collecting this sponge from the extreme depths (*ca.* -500 m) where it is known to exist.⁸

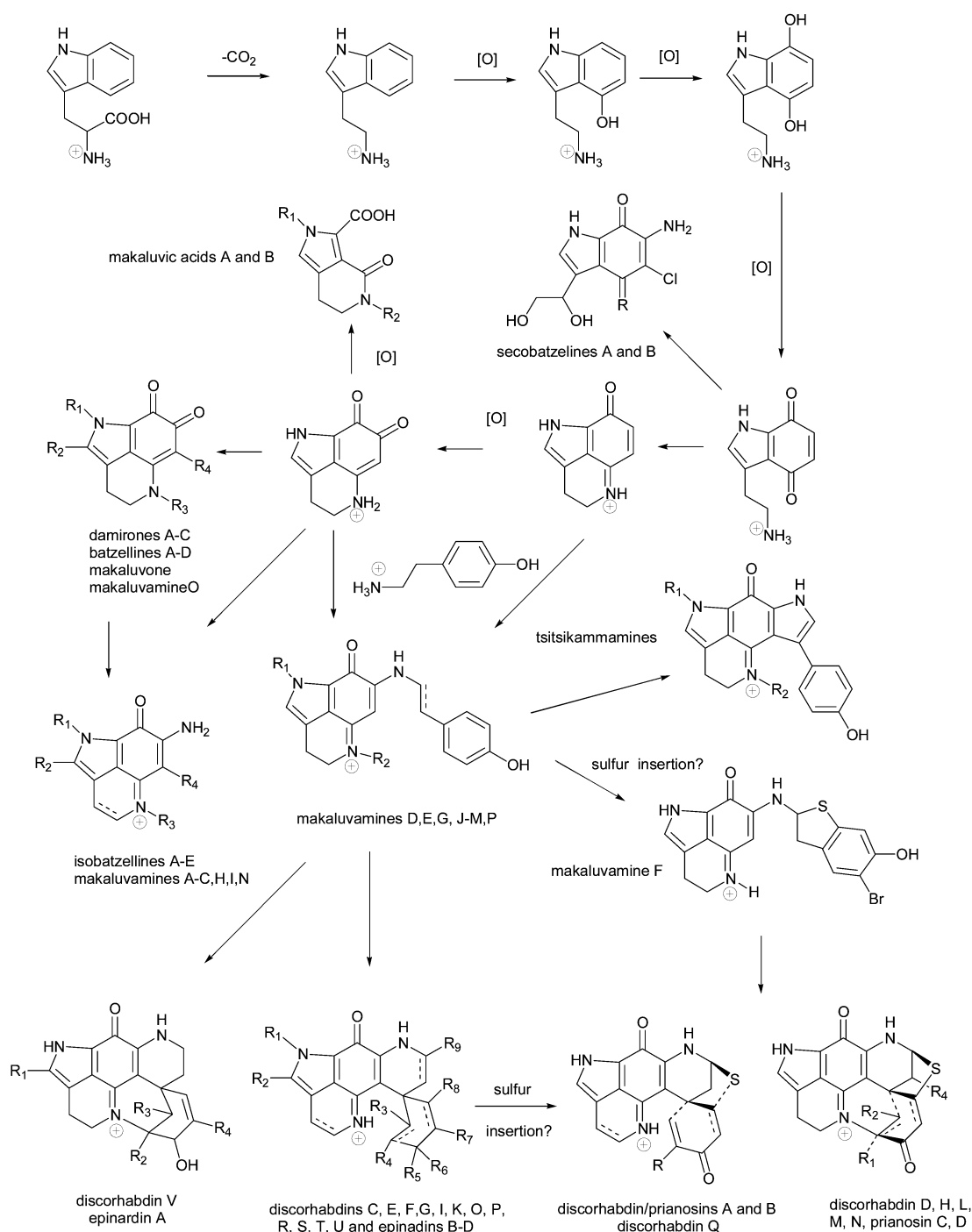
3 Alkaloids containing a pyrrolo[4,3,2-*de*]quinoline skeleton

The mono- and bicyclic makaluvic acids and secobatzellines are ring-opened examples of pyrrolo[4,3,2-*de*]quinolines and are clearly biosynthetically related. Makaluvic acids A **7** and B **8**, isolated from *Zyzzya fuliginosa* (= *fuliginosa*, species name incorrectly spelt in the original paper) collected at Chuuk Atoll, Micronesia, possibly represent oxidative degradation products of tricyclic alkaloids belonging to the batzelline, isobatzelline or makaluvamine families.²² The structure of **7** was confirmed by an X-ray diffraction study. Makaluvic acid A **7** has also recently isolated from the South African sponge *Latrunculia bellae*²¹ and **7** was not cytotoxic in either P388 murine leukaemia²² or HCT-116 human colon tumour cell line screens,²¹ in accordance with the iminoquinone structural requirement necessary for cytotoxicity in the pyrroloiminoquinone alkaloids (see later). Iminoquinone-containing secobatzelline A **9** and the potentially artifactual quinone analogue secobatzelline B **10** were isolated from deep water specimens of *Batzella* sp. (original voucher specimen re-identified as a *Strongyloidesma* species by Samaai),^{5,17} collected in the Caribbean.²³ While **9** inhibited the phosphatase activity of calcineurin and the peptidase activity of CPP32 with IC₅₀ values of 0.55 and 0.02 µg mL⁻¹, quinone **10** was less active. In a similar manner, cytotoxicity towards the P388 and A-549 cell lines was more pronounced in the presence of the iminoquinone moiety (**9**, IC₅₀ 0.06, 0.04 µg mL⁻¹) than the quinone (**10**, IC₅₀ 1.2, 2.9 µg mL⁻¹). No syntheses of makaluvic acids or secobatzellines have been reported to date.



The simplest examples of marine alkaloids that fall within the classification of the tricyclic pyrrolo[4,3,2-*de*]quinoline class are the *ortho*-quinones which include batzellines A–D, damirones A–C, makaluvone and makaluvamine O.

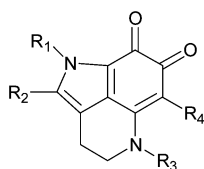
The first examples of alkaloids bearing a tricyclic pyrrolo[4,3,2-*de*]quinoline skeleton isolated from the marine environment



Scheme 2 A putative biosynthetic sequence linking marine pyrroloiminoquinone and related metabolites to a phenylalanine precursor (adapted from Urban *et al.*³ and Lill *et al.*⁸²).

were the batzellines A–C **11–13**, reported in 1989 from a *Batzella* sp. sponge (original voucher specimen re-identified as a *Strongylodesma* species by Samaai),^{5,17} collected at a depth of 120 m off the Grand Bahama Islands.²⁴ The structure of **11** was secured by an X-ray diffraction study, while those of **12** and **13** were established by chemical modification and comparison with **11**. Batzelline D **14**, the *N*-1-desmethyl analogue of batzelline C, was reported more recently from an Abrohlos Island, Indo-West Pacific collection of *Zyzzya fuliginosa*²⁵ while damirones A **15** and B **16** were isolated from a Palauan collection of *Damiria* sp.,²⁶ an Indonesian *Histodermella* sp.²⁷ (both voucher specimens of the *Damiria* and *Histodermella* species have been re-identified as *Z. fuliginosa* by Van Soest²⁸) and more recently from Australian specimens of *Z. fuliginosa*.²⁹ Investigation of a Pohnpeian specimen of *Z. fuliginosa* yielded damirone C **17**.³⁰ An X-

ray diffraction study of damirone A **15** has been reported.³¹ The most recent examples of *ortho*-quinoid containing pyrrolo-quinoline alkaloids are makaluvone **18**, isolated, together with damirone B **16**, from a Fijian collection of *Zyzzya* cf. *marsailis*³² (re-identified as *Z. fuliginosa* by Kelly⁵) and makaluvamine O **19**, the *N*-1-desmethyl analogue of makaluvone, purified from the Jamaican sponge *Smenospongia aurea*.³³ The isolation of **19** from *S. aurea* represents the first report of a pyrroloquinoline metabolite from the sponge family Thorectidae, and the authors, recognizing the chemotaxonomic significance of this discovery, comment that although the voucher specimen is indeed that of *S. aurea*, the very similar gross morphology of *Smenospongia*, *Zyzzya* and *Strongylodesma* species off the coast of Jamaica cannot rule out the possibility that different sponge species were inadvertently mixed together during the field collection.³³



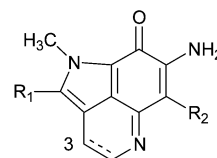
- 11 $R_1 = \text{Me}, R_2 = \text{SMe}, R_3 = \text{H}, R_4 = \text{Cl}$
 12 $R_1 = R_3 = \text{H}, R_2 = \text{SMe}, R_4 = \text{Cl}$
 13 $R_1 = \text{Me}, R_2 = R_3 = \text{H}, R_4 = \text{Cl}$
 14 $R_1 = R_2 = R_3 = \text{H}, R_4 = \text{Cl}$
 15 $R_1 = R_3 = \text{Me}, R_2 = R_4 = \text{H}$
 16 $R_1 = R_2 = R_4 = \text{H}, R_3 = \text{Me}$
 17 $R_1 = R_2 = R_3 = R_4 = \text{H}$
 18 $R_1 = \text{Me}, R_2 = R_3 = \text{H}, R_4 = \text{Br}$
 19 $R_1 = R_2 = R_3 = \text{H}, R_4 = \text{Br}$

Batzelline A **11** exhibited selective cytotoxicity towards A549 (human lung) cells compared to P388 (murine leukaemia), and showed some selectivity for melanoma cell lines in testing at the NCI.³⁴ No activity was observed in *in vivo* testing against A549 tumour xenografts in mice. Batzelline D **14** was inactive in an HIV-1 envelope-mediated cell fusion assay,²⁵ damirone A **15** and B **16** exhibited mild cytotoxicity towards the Ehrlich carcinoma cell line (IC_{50} values of 46.5 and 50 $\mu\text{g mL}^{-1}$ respectively),³¹ while damirone B and makaluvone **18** failed to exhibit cytotoxicity towards the HCT-116 cell line and demonstrated no ability to inhibit the function of the DNA processing enzyme topoisomerase II.³² Makaluvamine O **19** demonstrated moderate activity towards *Plasmodium falciparum* (IC_{50} 0.9 $\mu\text{g mL}^{-1}$) combined with low VERO cell line toxicity.³³

Alkaloids belonging to the batzelline and damirone families have been the target of synthetic efforts, which typically utilize indole or quinoline precursors. Complete syntheses of batzelline A **11**,³⁵ B **12**³⁵ and C **13**^{36–38} and damirone A **15**^{36,39} and B **16**³⁹ have been reported, while formal syntheses, involving new routes to synthetic intermediates, of batzelline C^{40,41} and damirone A^{40,42,43} and B^{40,42,43} have been disclosed. The obviously close biosynthetic relationship between batzelline and damirone *ortho*-quinones and the iminoquinone containing isobatzellines and makaluvamines (see later) has been exploited with alkaline hydrolysis of makaluvamines H and C (see later) affording damirone A and B respectively in high yield.³¹ Diazotization of isobatzelline A (see below) resulted in a low yield of batzelline A **11**.⁴⁴

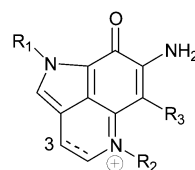
Isobatzellines A–D **20–23** were isolated as bioactive components of a deep water collection of *Batzella* sp. (original voucher specimens HBOM003:00050/51 both re-identified as *Strongyloidesma* species by Samaai),^{5,17} made off the Grand Bahamas.⁴⁴ Structurally they are closely related to the batzelline alkaloids, also isolated from *Batzella* sp. (= *Strongyloidesma* sp.), with replacement of the *ortho*-quinoid functionality by a 7-amino substituted iminoquinone skeleton. The fifth member of the family, isobatzelline E **24**, was isolated from an Indopacific collection of *Zyzzya fuliginosa*.²⁵ In stark contrast to their *ortho*-quinoid analogues, the isobatzelline alkaloids exhibit wide ranging biological activities. Isobatzellines A–D were found to be cytotoxic towards the P388 murine leukaemia cell line with IC_{50} values of 0.42, 2.6, 12.6 and 20 $\mu\text{g mL}^{-1}$ respectively in addition to anti-*Candida albicans* activity with MIC values of 3.1, 25, 50 and 25 $\mu\text{g mL}^{-1}$ respectively.⁴⁴ Isobatzelline C **22**, but not isobatzelline E **24**, was active in an HIV-1 envelope mediated cell fusion assay, however, it was speculated that the activity may have been due to the ability of such compounds to interfere with DNA-processing enzymes (see later under makaluvamines).²⁵ Syntheses of isobatzellines A and B³⁵ and C^{36,38,40,41} have been reported.

The makaluvamines represent a diverse range of structures based upon a 7-amino substituted pyrroloiminoquinone skeleton. Substitution at positions N-1, N-5 and N-9 are common,

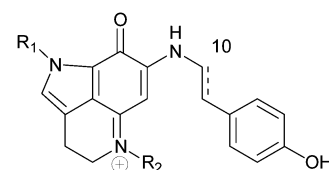


- 20 $R_1 = \text{SMe}, R_2 = \text{Cl}$
 21 $R_1 = \text{SMe}, R_2 = \text{H}$
 22 $R_1 = \text{H}, R_2 = \text{Cl}$
 23 $R_1 = \text{SMe}, R_2 = \text{Cl}, \Delta^3$
 24 $R_1 = \text{H}, R_2 = \text{Cl}, \Delta^3$

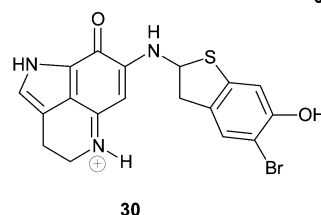
with one example having Δ^3 unsaturation and one example bearing substitution at C-6. The first examples of the series, makaluvamines A–F **25–30**, were reported as cytotoxic pigments of a Fijian collection of the sponge *Zyzzya* cf. *marsailis*³² (= *Z. fuliginosa*, see earlier).⁵ More recently, makaluvamine A **25** has also been reported from harvested plasmodial cells of the myxomycete *Didymium bahiense*, indicating that the biosynthesis of such alkaloids is not limited solely to the marine environment.⁴⁵ The trisubstituted analogue makaluvamine G **31** was isolated from an Indonesian collection of an unidentified *Histodermella* species²⁷ (= *Z. fuliginosa*, see earlier).²⁸ Investigation of Pohnpei (Micronesia) specimens of *Z. fuliginosa* afforded six new makaluvamines H–M **32–37**,³⁰ the same sponge species collected off Australia's Great Barrier Reef recently yielded makaluvamines C, E, G, H and L²⁹ while Philippines and Vanuatu specimens were found to be a source of makaluvamines N **38**⁴⁶ and P **39**⁴⁷ respectively. Makaluvamine O **19**, the only *ortho*-quinone containing makaluvamine, has been discussed earlier.³³



- 25 $R_1 = \text{Me}, R_2 = R_3 = \text{H}$
 26 $R_1 = \text{Me}, R_2 = R_3 = \text{H}, \Delta^3$
 27 $R_1 = R_3 = \text{H}, R_2 = \text{Me}$
 32 $R_1 = R_2 = \text{Me}, R_3 = \text{H}$
 33 $R_1 = R_2 = R_3 = \text{H}$
 38 $R_1 = R_2 = \text{H}, R_3 = \text{Br}$



- 28 $R_1 = R_2 = \text{H}$
 29 $R_1 = \text{Me}, R_2 = \text{H}, \Delta^{10}$
 31 $R_1 = R_2 = \text{Me}, \Delta^{10}$
 34 $R_1 = \text{H}, R_2 = \text{Me}$
 35 $R_1 = \text{Me}, R_2 = \text{H}$
 36 $R_1 = \text{H}, R_2 = \text{Me}, \Delta^{10}$
 37 $R_1 = R_2 = \text{H}, \Delta^{10}$
 39 $R_1 = R_2 = \text{Me}$



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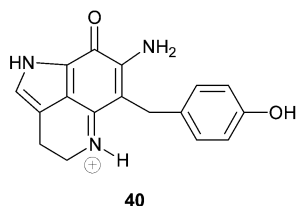
The makaluvamine family of alkaloids typically exhibit cytotoxicity towards tumour cell lines, the mechanism of which is believed to be mediated by inhibition of DNA processing enzymes. Makaluvamines A **25** and C–F **27–30** were found to exhibit cytotoxicity towards the HCT-116 cell line with IC_{50} values of 1.3, 36.2, 17.1, 1.2 and 0.17 μM respectively.³² The HCT-116 cytotoxicity of makaluvamine C **27** is inconsistent with the general trend observed in this series of analogues and the subsequent screening of this compound, isolated recently from the South African sponge *Latrunculia bellae*, against HCT-116 (IC_{50} 1.1 μM) would suggest that the HCT-116 cytotoxicity of makaluvamine C is more significant than originally reported.²¹ Makaluvamines A, C, D, E and F were also shown to inhibit the function of topoisomerase II, to intercalate into DNA and

undergo reductive cleavage of DNA.^{32,48,49} Makaluvamines A and C were found to be more effective *in vivo* against human ovarian ovcar-3 tumours implanted in nude mice [T/C (tumour volume of the treated group/tumour volume of the control group \times 100) 62 and 48% respectively] than P388 murine leukaemia (ILS 0 and 18%).^{32,48} Interestingly, the Δ^3 analogue, makaluvamine B **26**, was not cytotoxic.³² Makaluvamine G **31** exhibited general cytotoxicity to a panel of tumour cell lines (IC_{50} 0.4–0.5 $\mu\text{g mL}^{-1}$), displayed no antifungal or antiviral activity, and while moderate immunomodulatory activity was observed *in vitro*, no efficacy was observed in *in vivo* testing.²⁷ In contrast to other makaluvamine alkaloids, makaluvamine G was found to be a moderate inhibitor of topoisomerase I, but did not significantly inhibit topoisomerase II. RNA, DNA (IC_{50} 15 μM) and protein synthesis (IC_{50} 21 μM) were also inhibited by the alkaloid.²⁷ Utilising a panel of genetically engineered yeast strains, it was concluded that makaluvamines D **28**, G **31**, H **32**, J **34**, K **35**, L **36** and M **37** were inactive against topoisomerase and that makaluvamines I **33** and L **36** were an order of magnitude more cytotoxic towards the HCT-116 cell line than makaluvamines C **27**, G **31**, H **32** and K **35**, and that makaluvamines D **28** and M **37** were the least active.³⁰ Makaluvamines H–M exhibited only mild activity against *Bacillus subtilis*.³⁰ Makaluvamines A, E and K exhibited cytotoxicity towards P388 murine leukaemia cells (IC_{50} 0.4, 0.6 and 0.7 $\mu\text{g mL}^{-1}$ respectively)²² while makaluvamines C and H had IC_{50} values of 26.5 and 12.5 $\mu\text{g mL}^{-1}$ towards Ehrlich carcinoma cells.³¹ Makaluvamine N **38** exhibited cytotoxicity towards the HCT-116 cell line (IC_{50} 0.6 $\mu\text{g mL}^{-1}$) and inhibited the catalytic activity of topoisomerase II,⁴⁶ while makaluvamine P **39** was also cytotoxic to KB cells (64% inhibition at 3.2 $\mu\text{g mL}^{-1}$), inhibited xanthine oxidase (IC_{50} 16.5 μM) and was moderately active in a free-radical scavenging (antioxidant) assay.⁴⁷

Due to their relative structural simplicity and potent biological activities, the makaluvamines represent attractive targets for synthesis. To date there have been reported total or formal syntheses of makaluvamines A,^{36,40,50–52} B,^{40,50,51} C,^{40,42,50,51,53} D,^{40,41,50–52,54–58} E,⁵¹ (racemic) F,^{59,60} I,^{52,58} and K.⁵²

There have been surprisingly few reports of the synthesis and biological evaluation of non-natural makaluvamine-type alkaloids. Three new analogues, in addition to makaluvamines D **28**, and I **33** were synthesized and found to exhibit moderate cytotoxicity (IC_{50} 0.3–5.3 μM) towards the L1210 cell line.⁵⁸ Preparation of lexitropsin⁶¹ and polypyrroleamide⁶² pyrroloiminoquinone hybrids have been reported in an effort to increase cellular uptake and cytotoxic potency.

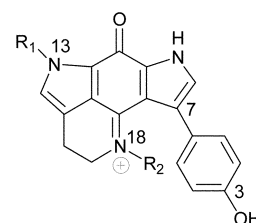
Veitamine **40** is an unusual member of the tricyclic pyrroloiminoquinone alkaloid family in that it bears carbon substitution at C-6.⁶³ Veitamine, isolated from a Fijian collection of *Zyzzya fuliginosa*, was found to exhibit cytotoxicity towards a panel of human tumour cell lines with a mean IC_{50} value of 0.1 $\mu\text{g mL}^{-1}$. The alkaloid was seven times more potent than the structurally related makaluvamine D (IC_{50} 2.0 $\mu\text{g mL}^{-1}$) against the HCT-116 human colon tumour cell line. Veitamine has been synthesized using methodology that relies upon *N*-Boc-directed lithiation for introduction of the C-6 substituent.⁶⁴



4 Alkaloids containing a pyrrolo[4,3,2-de]pyrrolo[2,3-h]quinoline skeleton

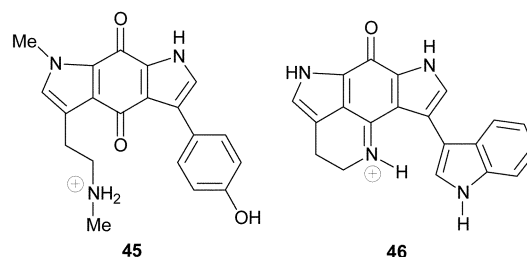
Tsitsikammamines A **41** and B **42** were isolated from the South African latrunculiid sponge *Tsitsikamma favus*.⁶⁵ However, no

tsitsikammamines were found in a second South African member of this genus, *T. pedunculata*.^{9,21} A more detailed investigation of the minor metabolites occurring in extracts of *T. favus* yielded the two novel N-18 oxime analogues **43** and **44** of the tsitsikammamines.²¹ Natural products containing *N*-oxide or *N*-oxime functionalities are uncommon in the marine environment. Several attempts to oxidize **41** and **42** to **43** and **44** were unsuccessful, suggesting that this conversion is of enzymatic origin and is not a result of the isolation protocol.²¹ Interestingly, the N-18 oximes **43** and **44** exhibited significantly reduced cytotoxicity against human colon tumour (HCT-116) cells (IC_{50} 128.2 and 16.5 μM respectively) when compared with their parent compounds, **41** and **42** (IC_{50} 1.4 and 2.4 μM respectively).²¹



- 41** $R_1 = R_2 = \text{H}$
42 $R_1 = \text{Me}, R_2 = \text{H}$
43 $R_1 = \text{H}, R_2 = \text{OH}$
44 $R_1 = \text{Me}, R_2 = \text{OH}$

Recently, a biosynthetically related *seco*-analogue of the tsitsikammamines, zyzzyanone A **45**, possessing a pyrrolo[3,2-*f*]indole-4,8(1*H*, 7*H*)-dione core skeleton, was isolated from an Australian sponge, *Zyzzya fuliginosa*.²⁹ Zyzzyanone A was only moderately cytotoxic (25 $\mu\text{g mL}^{-1}$) to mouse Ehrlich carcinoma cells and fertilized sea urchin eggs, further highlighting the propensity of the intact pyrroloiminoquinone structural motif to enhance cytotoxicity.²⁹



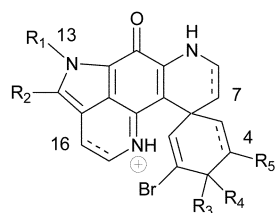
Ironically, the first example of pyrroloiminoquinone metabolites with a pyrrolo[4,3,2-de]pyrrolo[2,3-h]quinoline core skeleton was not obtained from a marine sponge. Wakayin **46** was isolated from a Fijian ascidian, *Clavelina* spp., by Copp *et al.*⁶⁶ and provided the first indication that pyrroloiminoquinone metabolites are not confined to marine sponges, tentatively suggesting a possible symbiont source for these bis-pyrroloiminoquinone metabolites.

5 Alkaloids containing a pyrido[2,3-*h*]pyrrolo[4,3,2-de]quinoline skeleton

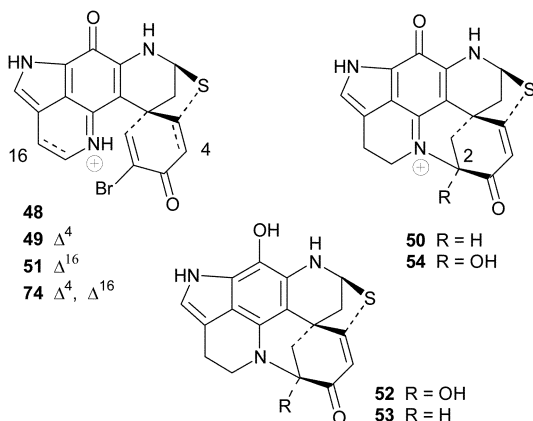
The name discorhabdin is derived from the characteristic discorhabd microsleres (Fig. 1A) used to identify sponges from three of the four genera of the family Latrunculiidae. Pyrroloiminoquinone compounds containing multi-hydrogenated forms of the tetracyclic pyrido[2,3-*h*]pyrrolo[4,3,2-de]quinoline skeleton (also initially referred to as a pyrrolo[1,7]phenanthroline ring system)^{67,68} all possess one of the following additional *spiro*-substituents at C-6; cyclohexanone, cyclohexenol, cyclohexenone and cyclohexadienone. A plethora of different substitution patterns around the six-membered *spiro*-ring, and further cyclization, in the form of a bond linking C-2 and N-18 and a thioether bridge between C-5 and C-8, are not uncommon.

The structure of discorhabdin C **47** was established by X-ray diffraction analysis and was the first structure of the

discorhabdin series of compounds to be published.⁶⁷ Discorhabdin C was isolated from the New Zealand latrunculiid sponge *Latrunculia* du Bocage (later re-identified as *Latrunculia* sp.)⁵ and at the time (1986) the potentially cytotoxic **47** (murine leukaemia L1210 tumour cell line ED₅₀ < 100 ng mL⁻¹) represented a novel class of anti-tumour agents with apparently significant medicinal potential.⁶⁷ Unfortunately, the relatively potent yet non-specific cytotoxicity of the discorhabdins and related metabolites has precluded their further clinical development. The publication of the fully assigned NMR data for **47** in addition to the structures and bioactivity of discorhabdins **A 48** and **B 49** (P388 ED₅₀ 0.03–0.1 µg mL⁻¹) and details of the bioassay guided isolation of these compounds from the *Latrunculia* sponge, followed two years later.⁶⁸ The structure and absolute stereochemistry of prianosin A, isolated from the Okinawan sponge *Prianos melanos*,⁶⁹ (re-identified as a *Strongyloidesma* species by Samaai and Kelly)^{5,7} was published shortly before the structure of discorhabdin **A 48** and ironically, although the structures of prianosin A and discorhabdin A proved to be identical, the latter name has subsequently taken precedence in the literature. Significantly, the X-ray structure of prianosin **A 48** has subsequently provided a configurational model for the stereochemical assignment of related members of the discorhabdin series.³ Not surprisingly, prianosin A exhibited similar cytotoxicity data to those reported for discorhabdins A–C (L1210 and L5178Y IC₅₀ 37 and 14 ng mL⁻¹ respectively).⁶⁹ Prianosin A also induced a ten-fold increase in the release of Ca²⁺ ions from sarcoplasmic reticulum when compared with caffeine in the same assay.⁶⁹



- 47** R₁ = R₂ = H, R₃ + R₄ = O, R₅ = Br, Δ⁴
55 R₁ = R₂ = R₅ = H, R₃ + R₄ = O, Δ⁴
56 R₁ = R₂ = R₅ = H, R₃ + R₄ = O, Δ⁴, Δ¹⁶
57 R₁ = R₂ = R₅ = H, R₃ + R₄ = O, Δ⁷
73 R₁ = Me, R₂ = H, R₃ + R₄ = O, R₅ = Br, Δ⁴
81 R₁ = R₂ = R₃ = H, R₄ = OH, R₅ = Br, Δ⁴
82 R₁ = R₂ = R₃ = H, R₄ = OH, R₅ = Br, Δ⁴, Δ⁷
83 R₁ = H, R₂ = R₅ = Br, R₃ + R₄ = O, Δ⁴
84 R₁ = R₃ = H, R₂ = R₅ = Br, R₄ = OH, Δ⁴
85 R₁ = R₃ = H, R₂ = R₅ = Br, R₄ = OH, Δ⁴, Δ⁷

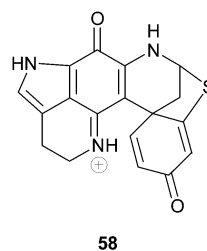


The structures of discorhabdin **D 50**⁷⁰ and prianosins **B–D 51–53**,⁷¹ isolated from the same source sponges that provided the earlier members in their respective series, followed in quick succession. NOE difference spectroscopy provided the relative stereochemistry of **50** while comparison of the CD data of

51–53 with those of discorhabdin/prianosin **A 48** provided the absolute stereochemistry of the latter three compounds. The *p*-aminophenol structure initially proposed for ring C of prianosins **C 52** and **D 53** was inconsistent with the emerging dominance of the ring C iminoquinone structural motif, evident in discorhabdins **A–D** and prianosins **A** and **B**, and the structures of **52** and **53** were later revised to 2-hydroxydiscorhabdin **D 54** and discorhabdin **D 50** respectively.⁷² Prianosin **B 51** is effectively Δ¹⁶-discorhabdin **A** and further exemplified the structural homology between the discorhabdins and prianosins. Although discorhabdin **D 50** exhibited lower *in vitro* activity than discorhabdins **A–C 47–49** against P388 cancer cells, the *in vivo* activity of the former compound (T/C 132% at 20 mg kg⁻¹) was reportedly significant.⁷⁰ Prianosins **B–D** were cytotoxic against murine lymphomas L1210 (0.15–2.0 µg mL⁻¹), L5178Y (0.024–1.8 µg mL⁻¹) and human epidermoid carcinoma KB cell lines (0.46–5 µg mL⁻¹).⁷¹

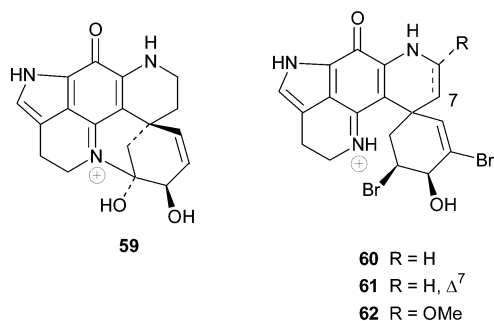
The structures of discorhabdins **E 55** and **F 56** first appeared (without spectral data) in a 1990 general review of the natural products chemistry of southern Pacific shallow water benthic marine invertebrates by Blunt *et al.*⁷³ These structures, along with those of discorhabdins **G–M**, were presented by Munro *et al.* at the New Zealand Institute of Chemistry Conference (December, 1993) and again later at the 37th meeting of the American Society of Pharmacognosy in 1996.³ Spectral data for discorhabdins **F–M** originally isolated from New Zealand sponges have as yet not been published in the mainstream chemical literature, leading to some ambiguities in the discorhabdin nomenclature. The spectral data of discorhabdin **E 55** (although **55** has a single chiral centre at C-6 the compound was found to be present as a racemate) from *Latrunculia* cf. *bocagei* (= *Latrunculia* sp., see earlier) were later reported, together with the structures of a number of semi-synthetic derivatives of discorhabdin **C 47**, prepared as part of a discorhabdin structure–activity relationship study.⁷⁴ In this pioneering study the centrality of the pyrroloiminoquinone functionality to cytotoxicity and the significance of a *spiro*-2,6-dibromocyclohexadienone substituent in enhancing cytotoxicity was established. The apparent selective toxicity of discorhabdin **C 47** to colon and leukaemia cell lines was also noted.⁷⁴

The relative inaccessibility of the original structures and NMR data of discorhabdins **G–M** has led to alphabetical duplication within discorhabdin nomenclature. In 1995, as part of their ongoing extensive investigations of the natural products chemistry of Antarctic marine invertebrates, Baker and co-workers isolated discorhabdin **C 47** from *Latrunculia apicalis* (the ubiquitous Antarctic distribution of *L. bififormis* suggests that this sponge may have been incorrectly identified)⁵ and a new optically active discorhabdin, which they named discorhabdin **G 57**.⁷⁵ The structure of this latter compound differed from the structure **58** originally assigned to discorhabdin **G** by Munro *et al.* and a compound with the structure **58**, recently isolated from a South African latrunculiid sponge *L. bellae*, gave ¹H NMR data identical with those of the original discorhabdin **G**, supplied to Davies-Coleman and co-workers by Munro in 1996.²¹ Compound **58** was accordingly named discorhabdin **G***.²¹ The configuration at C-8 in **58** was not established.

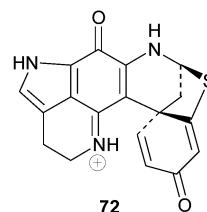
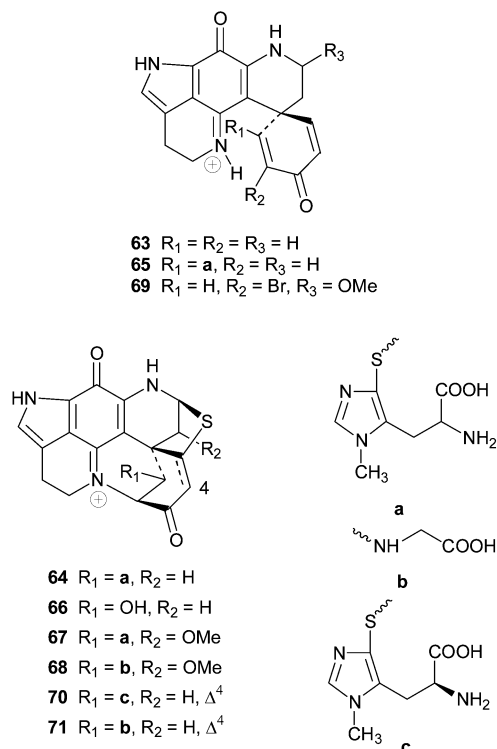


Sub-Antarctic sponges are also a source of discorhabdin-type compounds and the epinardins **A–D 59–62** isolated in 1996 from two species of unidentified sponges dredged from a depth of

200 m off the Crozet archipelago (ca. 2600 km SE of South Africa) possess an allylic alcohol functionality as opposed to the enone system in the *spiro*-six-membered ring common to the discorhabdins.⁷⁶ The relative stereochemistry of these four compounds was established from NMR data. Epinardin C **61** exhibited strong *in vitro* cytotoxicity to doxorubicin-resistant L1210/DX tumour cells (IC_{50} 0.3 $\mu\text{g mL}^{-1}$).⁷⁶



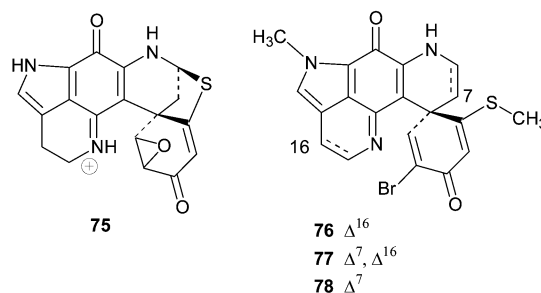
A review of bioactive marine alkaloids by Munro and co-workers published in 2000 has not only shed some light on the original structures of discorhabdins I–M **63–67** but also added two further structures, discorhabdins N and O **68** and **69**, to the growing number of discorhabdins isolated from New Zealand *Latrunculia* sponges.³ Paradoxically, the structure of discorhabdin H **70** (Δ^4 discorhabdin J) was omitted from Munro and co-workers' review. However, the fully assigned spectral data and structure, including determination of an L-configuration for the thiohistidine residue, of **70** (isolated from the South African sponge *Strongylodesma algoensis*) was recently reported by Antunes *et al.*²¹ The spectral data of Δ^4 discorhabdin N **71**, a minor metabolite in the South African sponge *L. bellae*, were also presented by Antunes *et al.*²¹ The relative stereochemistry at the four stereocentres in **71** remains unassigned. The spectral data of discorhabdins "I" **72** and L **66**, isolated from deep water Argentinian specimens of *L. brevis*, were published in 2004 by Reyes *et al.*⁷⁷ Confusingly, the structure of the former compound possesses a thioether bridge linking ring C with C-5 of the *spiro*-ring, which is at variance with the structure of discorhabdin I **63** that appears in Munro and co-workers' review. Discorhabdins "I" and L were both cytotoxic to human colon tumour cells HT-29 (GI_{50} 0.35 and 0.12 μM respectively).⁷⁷



Discorhabdin P **73** (*N*-13-methyldiscorhabdin C), was isolated from a *Batzella* sponge (original voucher specimen re-identified as a *Strongylodesma* species by Samaai¹⁷) collected using a manned submersible from a depth of 141 m off the Great Bahamas bank.⁷⁸ The structure of **73** followed from X-ray diffraction analysis. In common with other discorhabdins, **73** was shown to be cytotoxic to P-338 and A-549 tumour cell lines (IC_{50} 0.03 and 0.4 $\mu\text{g mL}^{-1}$ respectively). Interestingly, **73** was the first discorhabdin alkaloid to be screened for inhibition of calcineurin (an immune system signal transduction enzyme) and the peptidase activity of CPP32 (caspase 3 proteases are an important group of enzymes implicated in apoptosis).⁷⁸ Ironically, the nanomolar inhibition of CaN (IC_{50} 0.55 $\mu\text{g mL}^{-1}$) and CPP32 (IC_{50} 0.55 $\mu\text{g mL}^{-1}$) by **73** was not shared by discorhabdin C **47**, which was inactive in these assays at a concentration of 5 $\mu\text{g mL}^{-1}$.⁷⁸

Discorhabdin Q **74** is effectively 16,17-dehydrodiscorhabdin B and was the dominant discorhabdin isolated from cytotoxic extracts of Australian specimens of *Latrunculia purpurea*, *Zyzzya massalis*, *Z. fuliginosa* and an unidentified *Zyzzya* spp.⁷⁹ Discorhabdin Q exhibited only moderate activity in the NCI's 60 cell line screen (mean panel GI_{50} 0.5 $\mu\text{g mL}^{-1}$) and Boyd and co-workers postulated that Δ^{16} unsaturation reduces cytotoxicity relative to the more common 16,17-saturated members of the discorhabdin series.⁷⁹

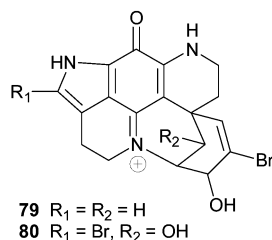
Extracts of a deep water Antarctic *Latrunculia* sponge and a south Australian *Negombata* sp. both yielded discorhabdin B **49** and its closely related *cis*-1,2-epoxy analogue, discorhabdin R **75**.⁸⁰ In light of the clear chemotaxonomic divisions between *Latrunculia* and *Negombata* species (see earlier), it is probable that the latter species was incorrectly identified and is possibly *Latrunculia purpurea* from the widespread distribution of this species off the southern coast of Australia.⁵ Spectroscopic examination of the two sponge extracts that yielded **75** confirmed that this compound was a natural product and not an oxidation artifact of the chromatographic work-up of the extract.⁸⁰ No bioactivity data was reported for this compound.



Three cytotoxic discorhabdins, S, T and U **76–78** were isolated from an unidentified *Batzella* sponge (probably a *Strongylodesma* species, see earlier) collected by a manned submersible from a depth of 141 m off the coast of the Bahamas.⁸¹ NOE data was used to establish the relative stereochemistry at the *spiro*-carbon in **76** and this configuration was extrapolated to the closely related discorhabdin T **77** (Δ^7 discorhabdin S) and discorhabdin U **78** (16,17-dihydrodiscorhabdin S). Discorhabdins S–U **76–78** exhibited *in vitro* cytotoxicity to murine P-388 tumour cells (IC_{50} 3.1, >5, and 0.17 μM respectively), A-549 human lung adenocarcinoma cells (IC_{50} >5, >5 and 0.17 μM respectively) and PANC-1 human pancreatic tumour cells (IC_{50} 2.6, 0.7 and 0.07 μM respectively).⁸¹ The enhanced

cytotoxicity of **78** relative to **76** and **77** against these three cell tumour lines further supports the hypothesis of Boyd and co-workers that cytotoxicity is diminished by Δ^{16} -unsaturation in discorhabdins.⁷⁹

Discorhabdin V **79**, and the closely related 14-bromo-1-hydroxydiscorhabdin V **80**, recently isolated from a South African sponge *Tsitsikamma pedunculata*, exhibited cytotoxicity to HCT-116 human colon tumour cells (1.2 and 12.5 μM respectively).²¹ The relative configurations of the *spiro*-carbon, the oxymethine carbon(s) in the *spiro*-ring and the ring junction linking this ring to N-18 were not established in either compound.



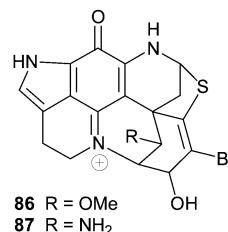
Investigations of the natural product chemistry of latrunculid sponges, containing discorhabdin-type alkaloids, usually inadvertently leads to the re-isolation of known members of the discorhabdin ensemble. Reports of the re-isolation of ubiquitous discorhabdins in addition to new records of the isolation of closely related analogues of these compounds are presented below. Details of the single discorhabdin biosynthetic study are also discussed.

Discorhabdin A **48** has been identified in extracts of *Zyzzya* cf. *marsalis*³² (= *Z. fuliginosa*, see earlier) and more recently, the South African sponge *Strongyloidesma algoensis*²¹ and the Argentinian sponge *Latrunculia brevis*.⁷⁶ The latter sponge also yielded discorhabdin B **49**.⁷⁶ Interestingly, in a comparative screening of 20 pyrroloiminoquinone and related metabolites against the human colon tumour cell line HCT116, **48** was the most cytotoxic (IC_{50} 0.08 μM).²¹

The biosynthesis of discorhabdin B **49** has been investigated utilizing tissue slices of New Zealand collections of *Latrunculia* sp. B.⁸² [$\text{U-}^{14}\text{C}$]-L-phenylalanine was found to be incorporated into the alkaloid, with similar levels of incorporation being experienced for sponge tissue that had been pre-treated with antibiotics and tissue that had not, leading to the conclusion that alkaloid biogenesis was probably a product of the sponge cells and not microbial symbionts. The putative biosynthetic sequence of discorhabdin B from phenylalanine by Munro *et al.* forms the basis of the expanded biosynthetic sequence outlined in Scheme 2 in which recently isolated pyrroloiminoquinone structural classes have been included.

Discorhabdin C **47** is seemingly ubiquitous in New Zealand *Latrunculia* species^{14,15,67,68,70,73,74} and a major metabolite in the Antarctic sponge *L. apicalis* (possibly *L. biformis*, see earlier).⁷⁵ Although **47** has not been isolated from South African latrunculid sponges, a plethora of discorhabdin C analogues have been obtained from *Tsitsikamma favus* and *T. pedunculata*.^{21,65} *T. pedunculata* yielded 3-dihydrodiscorhabdin C **81** previously synthesized *via* reduction of discorhabdin C **47**,^{73,74} and 3-dihydro-7,8-dehydrodiscorhabdin C **82**.²¹ Bromination at C-14 in the left hand hemisphere of the pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline skeleton has only been found in discorhabdin-type metabolites isolated from the genus *Tsitsikamma* and may prove to be a suitable chemotaxonomic marker for this genus. Both 14-bromodiscorhabdin C **83** and 14-bromo-3-dihydrodiscorhabdin C **84** are prevalent in *T. favus* and *T. pedunculata*.^{21,65} A further minor C-14 brominated discorhabdin C metabolite, 14-bromo-3-dihydro-7,8-dehydrodiscorhabdin C **85**, was also isolated from *T. pedunculata*.²¹ All five discorhabdin C analogues, **81–85**, were cytotoxic to HCT-116 tumour cells (IC_{50} 0.32, 0.20, 0.01, 0.65, 0.22 μM respectively).²¹

The South African sponge *Strongyloidesma algoensis* yielded discorhabdin D **50** while two minor discorhabdin D analogues, 1-aminodiscorhabdin D **86** and 1-methoxydiscorhabdin D **87** were isolated from *L. bellae*, a species that co-exists with *S. algoensis* in Algoa Bay, South Africa.²¹ The stereochemistry at the four chiral carbon atoms in **86** and **87** was not established. Both **86** and **87** exhibited cytotoxicity in an HCT-116 cancer cell line screen (IC_{50} 0.12 and 0.23 μM respectively).²¹



Discorhabdins serve as both pigments and chemical defence agents in sponges and a recent investigation of the distribution of the green pigment discorhabdin G **57** in the Antarctic sponge *Latrunculia apicalis* (possibly *L. biformis*, see earlier) revealed that the majority of this compound (>50%) is confined to the ectoderm of the sponge (0–2 mm).⁸³ *L. apicalis* is rarely preyed upon by the dominant Antarctic seastar spongivore, *Perknaster fuscus*, and in an elegant series of seastar tube foot retraction response experiments Baker and co-workers were able to confirm the role of discorhabdin G as a chemical deterrent to predation in *L. apicalis*.⁸³

The complexity of the pyrido[2,3-*h*]pyrrolo[4,3,2-*de*]quinoline skeleton and the universal cytotoxicity associated with alkaloids possessing this core structure have made the discorhabdins attractive synthetic targets. The synthetic challenge provided by these complex metabolites is possibly reflected in the paucity of published discorhabdin syntheses with only the total synthesis of discorhabdins A **48**,^{84,85} C **47**,^{37,40,55,86–88} and E **55**⁸⁶ having been reported.

6 Conclusion

Over the last eighteen years pyrroloiminoquinone and related metabolites have been regularly isolated from sponges generally confined, after recent fairly extensive taxonomic revision,^{5–10} to the two families Latrunculiidae and Acarnidae. The sponges that yielded these metabolites have been collected from both shallow and deep-water habitats stretching from Antarctica to Japan and the Caribbean with the centres of diversity of the family Latrunculiidae, in particular, situated predominantly in the southern hemisphere.⁸ Although there have been substantial investigations of the chemistry of latrunculid sponges collected from the colder water regions off New Zealand,^{3,67,68,70,74} Australia,^{79,80} Antarctica,^{75,80,83} the sub-Antarctic islands⁷⁶ and more recently South Africa,^{21,65} our knowledge of the pyrroloiminoquinone metabolites of South American latrunculid sponges remains limited.⁷⁷ Therefore, it is likely that latrunculid sponges collected from the cold water areas off South America may yield a cohort of new pyrroloiminoquinone metabolites in future studies. No natural product investigations of the deep-water latrunculid genus *Sceptrella* have been reported and chemotaxonomic evidence in support of the inclusion of *Sceptrella* in the family Latrunculiidae is still required. The isolation of makaluvamine A from a terrestrial myxomycete⁴⁵ and wakayin from an ascidian⁶⁶ clearly indicates that pyrroloiminoquinone and related metabolites are neither the sole preserve of marine sponges nor restricted to the marine environment. A microbial source for the pyrroloiminoquinone metabolites isolated from a New Zealand *Latrunculia* species has been tentatively discounted.⁸² However, it may be premature to extrapolate this result to all pyrroloiminoquinone-producing sponges and an investigation of a possible symbiont origin of the bis-pyrroloiminoquinones wakayin and the tsitsikammamines, in particular, would be

useful.²¹ Finally, the complex structures of the discorhabdins and the potent, albeit generally nonspecific, universal cytotoxicity of marine pyrroloiminoquinone metabolites will continue to make these compounds attractive and challenging synthetic targets in the future.

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