View Article Online / Journal Homepage / Table of Contents for this issue

REVIEW

NPR
www.rsc.org/npr

Investigations of the marine flora and fauna of the Islands of Palau

D. John Faulkner,[a] David J. Newman *†[b] and Gordon M. Cragg[b]
[a] *Scripps Oceanographic Institution, La Jolla, CA 92093, USA*
[b] *Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, NCI-Frederick, Frederick, MD 21702, USA*

Received (in Cambridge, UK) 3rd September 2003
First published as an Advance Article on the web 23rd December 2003

Covering: 1970s to July 2003

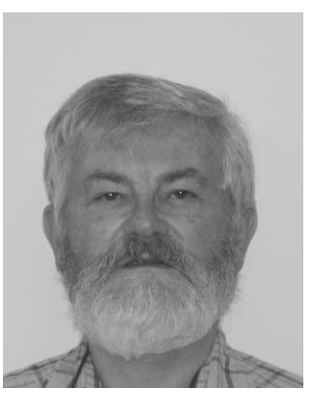
The Islands of Palau have proven to be an excellent source of bioactive marine natural products primarily as a result of the systematic studies from the late 1970s by the research groups of Scheuer at the University of Hawaii, Faulkner at the Scripps Oceanographic Institution/University of California at San Diego, and Paul at the University of Guam. Their efforts were materially aided by the excellent facilities provided by the Government of Palau and for the last 10 years, those of the NCI's shallow water collection contractor, the Coral Reef Research Foundation. This review covers the structures and biological activities where noted, of the multitudinous marine-derived natural products isolated from the marine flora and fauna of this nation and demonstrates the enormous variety of novel structures elaborated by these organisms.

† *Address for correspondence*: Natural Products Branch, NCI, PO Box B, Frederick, MD 21702, USA. E-mail: dn22a@nih.gov

D. John Faulkner, born in England in 1942, received his BSc and PhD degrees from Imperial College, London, where he studied synthetic organic chemistry under the guidance of Sir Derek Barton. He received postdoctoral training from R. B. Woodward at Harvard University and W. S. Johnson at Stanford University before joining the faculty of the Scripps Institution of Oceanography, University of California at San Diego, in 1968. Recognizing the need to do something more marine, he began a new career in marine natural products chemistry. When John died in November 2002 he was Professor of Marine Chemistry.

D. John Faulkner

David Newman was born in Grays, Essex, UK, and received his D. Phil. in microbial chemistry from the University of Sussex in 1968. Following two years of postdoctoral studies on the structure of electron transport proteins at the University of Georgia, USA, he worked for a number of US-based pharmaceutical companies in natural products discovery programs, and joined the Natural Products Branch of the NCI in 1991. His scientific interests are in the discovery and history of novel natural products as drug leads in the anti-infective and cancer areas. In conjunction with Gordon Cragg, he has established collaborations between the National Cancer Institute and organizations in many countries promoting drug discovery from their natural resources. He is responsible for the marine and microbial collection programs of the NCI and in concert with Gordon Cragg (Chief, NPB), for the NCI's Open and Active Repository programs. He is both an UK Chartered Chemist and an UK Chartered Biologist and has published over 70 papers related to these interests.



David Newman

Gordon Cragg

Gordon Cragg was born in Cape Town, South Africa, and obtained his D. Phil. from Oxford University in organic chemistry in 1963. After two years of postdoctoral research in natural products chemistry at the University of California, Los Angeles, he returned to South Africa where he held several academic positions before returning to the United States in 1979 to join the Cancer Research Institute at Arizona State University. In 1985, he moved to the National Cancer Institute in Bethesda, Maryland, and was appointed Chief of the Natural Products Branch in 1989. His major interests lie in the discovery of novel natural product agents for the treatment of cancer and AIDS. In 1991 he was awarded the National Institutes of Health Merit Award for his contributions to the development of the anticancer drug, taxol, and in 1998 he was elected President of the American Society of Pharmacognosy. He has established collaborations between the National Cancer Institute and organizations in many countries promoting drug discovery from their natural resources. He has published over 100 papers related to these interests.

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

DOI: 10.1039/b300664f

This journal is © The Royal Society of Chemistry 2004

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

1 Introduction

With the untimely death of John Faulkner in November of 2002, following complications from cardiac surgery, the marine natural products world in particular and chemistry in general, lost one of its greatest exponents of the arcane art of structural determination of the extremely complex and unusual compounds elaborated by marine-dwelling organisms.

John published approximately 300 scientific papers in the field of marine natural products chemistry and related topics and was instrumental, together with his colleagues at The Scripps Institute of Oceanography, where he spent all of his time after post-doctoral studies at Harvard and Stanford, in demonstrating the exquisite chemistry that marine organisms of all types elaborate as mechanisms of attack and defence.

Although not able to dive due to medical restrictions, he was an avid snorkeller and collector and was able to gather around him a large number of very talented graduate students, post-doctoral fellows, collaborators (in particular, Bob Jacobs at the University of California, Santa Barbara) and colleagues who collected marine organisms in a variety of places in the Pacific, but notably in the Islands of Palau.

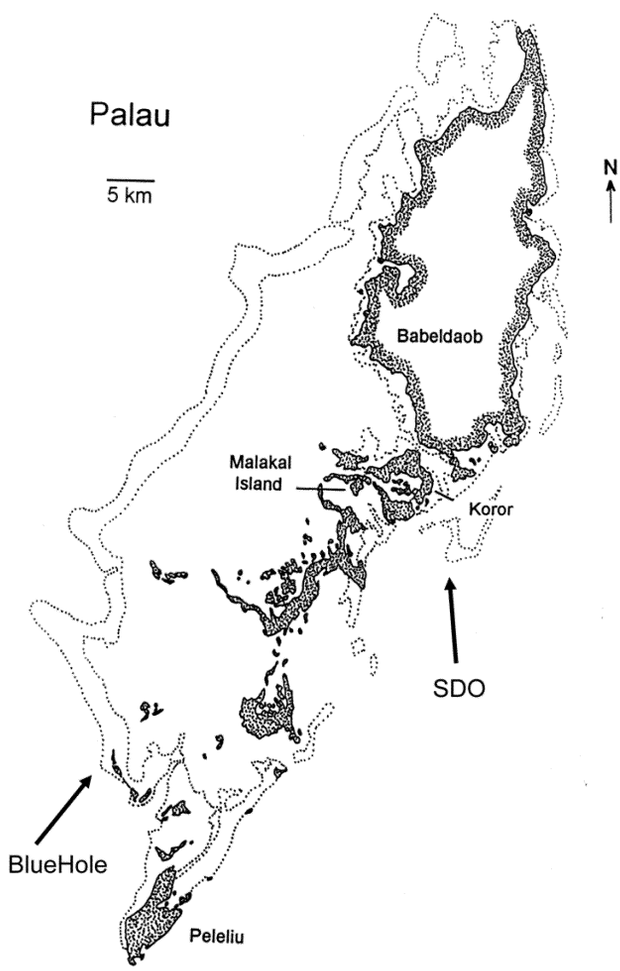
This review is based on a report that John initially started with a Palauan undergraduate student, Jason Kurtei, who spent a few months with John as part of a Scripps Undergraduate Research Fellowship Program in La Jolla in 1997, and was then further developed by John but never published.

With the express consent of Meryl Faulkner, John's widow, we have taken his original manuscript, extended it to July of 2003, shortened some of the entries, extended others, and then updated and included organisms that John did not cover in the original. We have tried to keep the same general format that was used in the original report, a short series of history and highlights and then what is effectively an annotated bibliography organized by phylum/organism, where structures are shown and biological activities are reported and commented on where necessary.

We (D. J. N. & G. M. C.) should emphasize from herein that any errors are ours, not his.

2 The Islands of Palau

The Islands of Palau (also known as the Republic of Belau) are found between 7° and 8° N Latitude and 133° to 134° E Longitude (*cf.* Fig. 1). Originally known as the Western Caroline Islands, following their discovery by Spain in the 1500s, there was no formal incorporation into a "Spanish Empire" until 1885, when the Pope confirmed Spain's rights to the islands. In the 300 years prior to this, both English and German seafarers had established first trading posts and then coaling stations. Following the Spanish–American war, in 1899 the Carolines were sold to Imperial Germany. In 1914, when Japan declared war on Germany, the German possessions in Micronesia came under Japanese control and then they continued under Japanese control as a League of Nations mandate, finally becoming fortified islands until the end of WWII. Following WWII and a very convoluted series of associations with Micronesia and the USA, Palau became an independent nation on October 1st, 1994 as the Republic of Belau. For further information, the reader should consult the excellent history of Palau by Mandy Etpison, the daughter-in-law of the third president of Belau.[1]



SDO = Short Drop Off; 07 15.96 N, 134 31.28 E

Blue Hole; 07 08.10 N, 134 13.90 E

Fig. 1 The Islands of Palau (pen and ink drawing courtesy of Dr and Mrs Patrick Colin, CRRF).

Over the early part of the 20th Century, a variety of German and Japanese scientists had begun to investigate the flora and fauna of the islands, but it was really with the advent of SCUBA apparatus and the establishment of a relatively stable form of government after the 1960s that marine biologists and

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

marine natural product chemists began to systematically study the fauna of these islands, and from 1979, John was one of these investigators.

3 Why investigate Palauan waters?

The scientific rationale is quite simple. Palau has the greatest diversity of marine habitats and organisms that can be packed into such a small geographical area. Although this sentence was originally written in 1997, the publication in 2002 by Hooper *et al.*, confirmed this comment as in their report, the authors demonstrated that Palau, like the Great Barrier Reef in Australia, is a "biodiversity hotspot" with over 73% of the reported sponges demonstrating endemism when compared with other areas of the Pacific.[2]

Add to this the availability of excellent diving resources, freezer facilities, and most of all, knowledgeable guides and you have an almost perfect location for the collection of marine specimens. Palau is served by reliable and frequent air transportation so that samples can be transported back to major research facilities in good condition. The laboratories of the Marine Mariculture Demonstration Center (MMDC) in the past and now those at the Coral Reef Research Foundation (CRRF, the current Collection Contractor for the NCI's shallow water collection programme) have allowed researchers to do more than just collect specimens – they can get their research started while still in a position to refine their collections for later study.

3.1 Palau's microenvironments lead to increased biodiversity

Most tourists visit Palau to dive along the precipitous reef walls that provide spectacular vistas of brightly colored fishes and the occasional thrill of encountering sharks, rays or turtles. While they are vaguely aware of brightly colored invertebrates packed along the vertical surfaces of these underwater cliffs, they seldom stop to wonder at the numbers of different plants and invertebrates to be found and give no thought at all to the distribution of the sessile inhabitants of the reef. To most divers, invertebrates are the background against which fish are photographed.

Scientists see this panorama in a very different light. They notice that the community of sessile invertebrates changes somewhat with depth but often more depending on exposure to direct sunlight. The inhabitants of a pass or channel between reefs are different from those on the outer or inner faces of a reef due to changes in water flow and turbidity. Caves and tunnels provide a different set of inhabitants, which is often comprised of animals that would normally exist at much greater depths. Mud flats, mangrove swamps, shallow reefs, coral rubble and even manmade docks all provide different contributions to the overall marine biodiversity of an area. When you add to this catalog of microenvironments and their occupants, the unique communities that are found in the marine lakes of Palau, you have a paradise for scientists seeking a broad diversity of marine invertebrates. Palau, which is located not far from the presumed center of coral reef biodiversity, appears to have captured more than its fair share of invertebrate species, in particular, sponges.[2]

4 A brief history of marine natural product research in Palau

Marine natural product research started in the 1950s largely as a result of the availability of SCUBA but it did not really become a separate scientific discipline until about 1970, when it became an integral part of the search for new pharmaceutical agents. The search for "Drugs from the Sea" has been and still is the driving force behind the funding of research on the chemistry of marine organisms. To a lesser extent, funding agencies have also supported basic research on the chemical ecology of marine ecosystems, which concentrates on demonstrating how marine plants and invertebrates have benefited from producing toxic or deterrent chemicals.

As far as we have been able to determine, the first groups to systematically collect marine organisms in Palau for chemical studies were those of Paul Scheuer from Hawaii, the Suntory Institute from Japan, and the Faulkner group from Scripps, who were housed at MMDC in the late 1970s and early 1980s. In 1979, Bill Fenical (from Scripps) led a research expedition to Palau aboard the R/V Alpha Helix but the collection of organisms was not the major activity of the expedition. The early studies led to the discovery of a number of anti-inflammatory agents, the most promising of which was manoalide. The US National Cancer Institute has had a long association with Palau, indirectly as a result of Bob Pettit's (Arizona State University) collections in the 1980s and now directly, as they support the professional collecting activities of the Coral Reef Research Foundation, who are based in Koror. Japanese scientists and ships have visited Palau quite frequently over the past 20 years but their activities have resulted in relatively few publications. For the past fifteen years, the majority of US supported natural product research from the waters of Palau has been reported by Paul Scheuer from Hawaii, Valerie Paul from Guam, and John Faulkner from Scripps.

5 Overview of therapeutic areas

5.1 Anti-inflammatory compounds

Collections from Palau played a key role in the US government's Sea Grant funded research to discover new anti-inflammatory agents from marine organisms. In particular, the discovery that manoalide **123** from the sponge *Luffariella variabilis* inhibited the enzyme phospholipase A_2 (PLA_2), which plays a key role in inflammatory conditions such as arthritis, allowed researchers to study the role of PLA_2 in inflammation as well as providing the stimulus for further research to discover new agents. Despite initial expectations and hopes, manoalide was eventually dropped as a drug candidate but only after human trials had proved it to be ineffective, though there are still derivatives in preclinical and clinical studies.

Manoalide is a major constituent of the sponge *Luffariella variabilis* that is one of the most common sponges on the reefs around Palau. The structure of manoalide was published in 1980 by Paul Scheuer's group, but without any biological activity testing. In the meantime Faulkner's group had isolated manoalide and supplied it to Robert Jacobs at the University of California, Santa Barbara, where it was first observed to be a pain-killer and was subsequently found to interrupt the biochemical sequence responsible for pain caused by inflammatory conditions such as insect stings, arthritis, burns, *etc.* In animal models, it was more effective than the best anti-inflammatory agents then available.

Since it was a novel agent, why did it not progress further? There were several reasons, some scientific and others more practical. The best scientific reason was that manoalide did not interact with the enzyme PLA_2 in the usual manner. Rather than directly inhibiting the enzyme by binding at the active site, thus blocking its enzymatic activity, manoalide reacted with the surface of the enzyme and prevented the enzyme from moving across membranes. Thus it was neither a direct competitor nor an allosteric inhibitor. It also had other side effects as PLA_2 was not the only enzyme that it bound to the surface of. Thus it was not an ideal drug candidate. In addition, since Scheuer had published the structure before any biological activity was identified, the natural product could not be patented, though a "use patent" could be awarded.

Allergan Pharmaceuticals embarked on a program to synthesize compounds that resembled manoalide, in the hope that a synthetic compound would be better than manoalide and to a certain extent they succeeded. As part of their development strategy, Allergan decided to perform a clinical

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

trial to determine whether manoalide could be used topically for the treatment of psoriasis but that trial was not successful. Although the unsuccessful clinical trial sealed the fate of manoalide as a pharmaceutical, it has continued to be used as a biochemical reagent to block the action of phospholipase A_2 and is essentially a standard reagent for this purpose.

A second anti-inflammatory agent resulted from a collaboration between academic scientists and a small biotechnology start-up company, OsteoArthritis Sciences Inc., and was again sponsored by Sea Grant. It was found that debromohymenialdisine (DBH) **164**, which is a major metabolite of the sponge *Stylotella aurantium*, which is very common in the shallow waters of Palau, could be successfully used to treat osteoarthritis in laboratory animals. Progress in the development of DBH appeared to be going very well when the company was suddenly closed down.

One problem in the further development of this agent is the justifiable position by the University of California that any developer must accept an obligation to share benefits arising from the marketing of DBH with the country of origin, which in this case is Palau. This is also the case with any agent identified by investigators using any of the materials collected by CRRF as part of their NCI collection contract. DBH has been reported from other sources and under the NCI programme, some benefit would have to be paid even if made by total synthesis. The ultimate fate of this agent/patent is unknown.

Although manoalide and DBH represented the best opportunities to date for development of a pharmaceutical product from Palauan marine specimens, they are by no means the only chemicals to be studied in depth by pharmacologists and molecular biologists. Palauan specimens have provided more than fifty compounds that have been studied to some level or another as potential anti-inflammatory agents by academic and industrial pharmacologists. The contributions of these studies to basic science have been extremely valuable and cannot be overlooked.

5.2 Studies of anti-cancer agents

Many of the metabolites reported from Palauan specimens are cytotoxic in as much as they kill cancer cells in tissue culture (*in vitro*), but very few of these compounds have proved successful in animal models (*in vivo*). The underlying problem is that many cytotoxins are toxic both to normal cells and cancer cells and would therefore be as damaging to the patient as to the patient's tumour. Therefore, scientists seek compounds that show selective toxicity toward the cancer cells. It is important when reading the annotated bibliography to differentiate between compounds that show real promise for the treatment of cancer (*in vivo* activity) and those that are reported as cytotoxic (*in vitro* activity).

There are compounds from sponges collected in Palau that have undergone preliminary *in vivo* testing in animal models but, at present, no compounds from Palauan marine organisms have been/are in clinical trials as anti-cancer agents, though the number of novel structures being reported, particularly from cyanophytes (see below in the annotated bibliography), bodes well for the future.

5.3 Contributions to cellular biology

During the past few years, many cellular biologists have realized the value of marine natural products as biological probes that can be used to inhibit specific enzymes and thereby discover the inner workings of cells. They have gone from using pure compounds that were (sometimes) identified in pharmaceutical screens to the testing of extracts of marine organisms in assays developed to study specific organelles or processes that are essential to cellular organization. Two successes have been recorded in this area, ilimaquinone **98** and adociasulfate-2 **170**.

Ilimaquinone, which was first isolated from the Palauan sponge *Hippospongia metachromia* by Paul Scheuer's group, causes the Golgi apparatus to break down into small particles in the same manner that it does during cell division. Collaborative research between the Faulkner laboratory at Scripps and the group of Vivek Malhotra at UCSD Biology Department has led to a better understanding of how the Golgi maintains its essential 3-dimensional structure within the cell.

Collaboration between the Faulkner laboratory and the group of Larry Goldstein at the UCSD Pharmacology Department led to the discovery of the first inhibitor of motor proteins, which are used to transport peptides along a network of microtubules from the Golgi to their final destination. The inhibitor, adociasulfate-2 (AS-2), was obtained from a *Haliclona* (aka *Adocia*) species of sponge that was collected in Palau.

6 Studies on the role of chemistry in symbiosis

This is a new area of study that has clearly benefited from the ability to do laboratory research in Palau. Carole Bewley was able to separate symbiotic microorganisms from sponge cells while in Palau.[3] On her return to Scripps, she performed chemical analyses on the cell fractions and was able to demonstrate that the biologically active constituents of the sponge *Theonella swinhoei* were in fact produced by symbiotic microorganisms. Further research by Eric Schmidt has resulted in identification of one of the symbiotic microbes using DNA analysis after demonstrating that the microbe can grow in medium supplemented with a piece of the host sponge but not in pure culture medium (*pers. comm.*). Further work by Christine Salomon on the localization of dercitamide[4] and cyclic peptides[5] are also examples of this approach. These and others are discussed under the specific organism headings below. This essentially new approach to the problem of finding out how and why symbiotic associations occur could not have been contemplated without the proximity of laboratory facilities to the collecting site.

7 Organisms (organized by Phylum, Class, Order and alphabetized by Genus/species)

In these subsequent sections, the common names of most of the dive sites, their Palauan names and the GPS coordinates are shown in Fig. 1 (the Islands themselves), Fig. 2 (common dive sites, frequently on the Eastern side of the larger islands) and in Table 1. All geographic information and maps were provided by Dr and Mrs Patrick Colin of the Coral Reef Research Foundation.

7.1 Chordata, Ascidacea, Enterogona

Aplidium longithorax

This tunicate was collected in Palau in 1994, identified by Dr F. Monniot, Museum National d'Histoire Naturelle, Paris and a voucher specimen was deposited at the University of Oklahoma (#1-PA-94).

Chemistry/bioactivity: longithorols A **1** and B **2** were isolated as their pentaacetates because of stability problems and are the first examples of hydroquinones from this chemical class. They are easily converted to the corresponding quinones by exposure to air and thus no bioactivities have been reported for longithorols A and B.[6]

Didemnum rubeum

The red encrusting colonial ascidian *Didemnum rubeum*, collected from the walls of Lighthouse Channel, was identified by Dr F. Monniot and has been deposited at the Museum National d'Histoire Naturelle (registry # MN1 N A2 Did C376).

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

Table 1 Selected dive sites in Palau: their proper Palauan names, the names used in this review and GPS coordinates

Site	Proper Palauan name	Review names	Latitude	Longitude
1	Lighthouse Channel (Toachel ra Kesebekuu)	Lighthouse Channel	07 17.03N	134 27.82E
2	Ngel Channel (Toachel ra Ngel)	Ngell Channel	07 18.48N	134 28.12E
3	Short Drop Off (Uchelbeluu Reef)	Short Drop Off or Agulpelu Reef	07 15.96N	134 31.28E
4	Bab el Lukes Reef	Babelukes Reef	07 17.58N	134 30.59E
5	Goby Lake	Big Goby Marine Lake	07 18.76N	134 30.10E
6	Iwayama Bay	Iwayama Bay, Arumizu Bay, Nikko Bay	07 19.01N	134 29.83E
7	Hotel Nikko Dock	Hotel Nikko Dock	07 20.25N	134 29.60E
8	Kaibakku Lake	Kaibakku Marine Lake	07 19.47N	134 29.42E
9	Turtle Island (Ucheliungs)	Turtle Island	07 18.56N	134 30.10E
10	Risong Lake	Risong Marine Lake	07 18.74N	134 28.04E
11	Siaes Tunnel	Siaes Tunnel	07 18.76N	134 13.50E
12	Ulong Channel (Ngerumekaul)	Ulong Channel (Aulong Channel)	07 16.99N	134 14.71E
13	Ngemelis Drop Off	Ngemelis Drop Off	07 06.95N	134 14.44E
14	Wonder Channel	Wonder Channel	07 10.83N	134 21.67E
15	Ngeruktabel Island	Urukthapel Island	none[a]	none
16	Ngeruktabel Lake	Ngeruktebel Marine Lake	07 16.80N	134 25.92E
17	Beduliaes	Peduliaes Headland	07 20.20N	134 25.70E

[a] Very large island, cannot give a specific GPS coordinate.

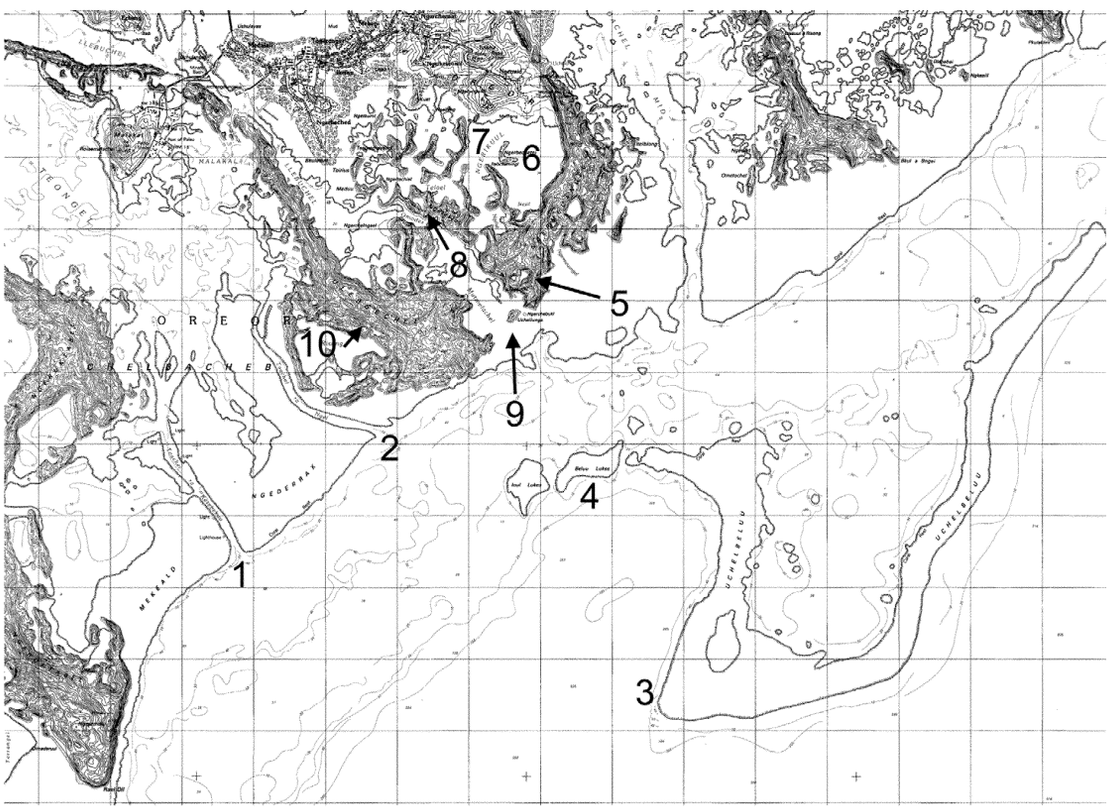
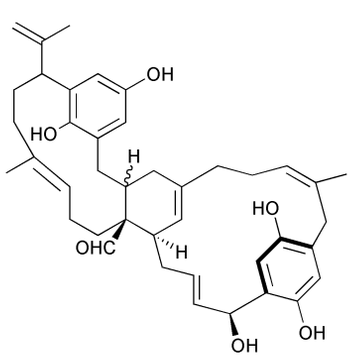
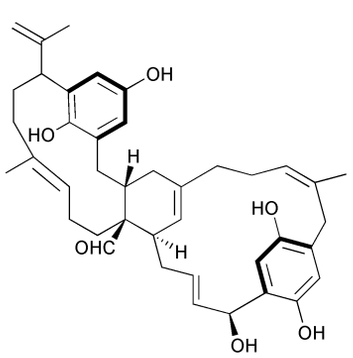


Fig. 2 Selected dive sites in Palau.



1

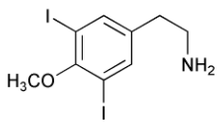


2

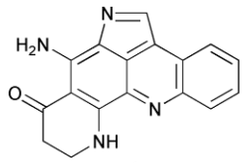
Chemistry/bioactivity: *Didemnum rubeum* contained large amounts of 3,5-diiodo-4-methoxyphenyl-ethylamine **3** and plakinidine D **4**.[7] Plakinidine D **4** was also obtained from an Indonesian *Didemnum* sp.[7] and the isolation of both **3** and **4** was simultaneously reported from the same species by Ford and Davidson[8] from samples collected in the Marshall Islands. The

View Article Online

only reported activity was an *in vitro* study showing cytotoxicity of **4** against HCT-116 at 5 μg mL^{-1}.



3



4

Didemnum sp

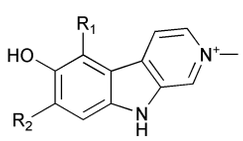
A magenta colored colonial tunicate was found in a bay off Ngell Channel on Auluptagel and collections were made in 1979 and 1993. Currently, it is still an undescribed species (F. Monniot, *pers. comm.*) with a voucher specimen deposited in the Museum National d'Histoire Naturelle, Paris (registration # MNHN Az DidC 266).

Chemistry/bioactivity: the first sample to be studied had been stored in methanol for about 10 years and when extracted gave two terpenoids, didemnaketals A and B **5**.[9] However, when a freshly collected specimen was investigated, only didemnaketal C **6** was isolated, which led to the conclusion that didemnaketals A and B were artifacts of the storage and extraction processes.[10] The full stereochemistry of didemnaketal C **6** has not yet been elucidated. Didemnaketals A and B inhibited the enzyme, HIV-1 protease[9] and though several protease inhibitors are now available, the didemnaketals were not considered drug candidates because the ester groups are too easily hydrolyzed. However, although these compounds may well be isolation artifacts, their basic structural motif has been used as the starting point for simplified analogues that appear to inhibit HIV protease by an unusual mechanism, inhibition of dimerization of the inactive HIV monomers to give the active dimeric HIV protease albeit with the most active, **7** having a high *in vitro* K_i of 2.1 μM.[11]

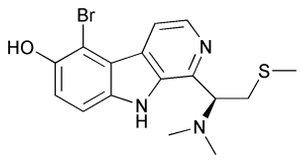
Eudistoma gilboverde

This particular example of *Eudistoma gilboverde* was collected in the Siaes Tunnel by the NCI collection contractor, the Coral Reef Research Foundation, identified by Dr F. Monniot and a voucher was deposited at the Smithsonian Institution (# 0CDN5058).

Chemistry/bioactivity: the organic extract of this ascidian was cytotoxic in the NCI 60 cell line panel and from this material, three new β-carboline alkaloids, 2-methyleudistomin D **8**, 2-methyleudistomin J **9** and 14-methyleudistomin C **10** were isolated and identified by following their bioactivities against four cell lines. In addition to these new derivatives, six known eudistomins were also found, eudistomins C, D, E, J, K and L. Of the three new compounds, 14-methyleudistomin C exhibited the most potent *in vitro* activity, with IC_{50} values of < 1 μg mL^{-1} against the test lines.[12]



8; R_1 = Br, R_2 = H
9; R_1 = H, R_2 = Br



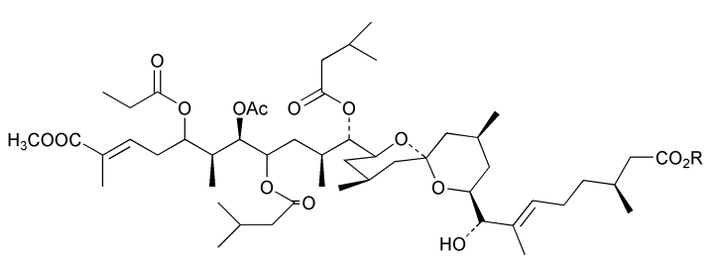
10

Lissoclinum patella

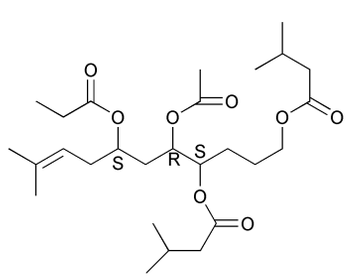
The colonial ascidian *Lissoclinum patella* is commonly found in shallow water around Koror and elsewhere in Palau. It is generally described as being off-white with green striations that are caused by the presence of the symbiotic cyanobacterium, *Prochloron* sp., which has been studied extensively by Lewin and colleagues and recently, some evidence of a form of protective symbiosis between the ascidian and the *Prochloron* sp. has been demonstrated in the case of the Palauan organism by workers with the Japanese Marine Biotechnology Institute, who reported a "sunscreen effect" by mycosporine-like amino acids produced by the ascidian.[13]

Chemistry/bioactivity: Palauan specimens of *L. patella* are the source of ulicyclamide **11** and ulithiacyclamide **12**,[14] patellamides A–C **13**–**15**,[15] preulithicyclamide **16**,[16] and several other closely related cyclic peptides.[17,18] Most of the cyclic peptides are cytotoxic to various degrees with ulithiacyclamide **12** being the most potent.[15] This compound also inhibits the Macrophage Scavenger Receptor, resulting in inhibition of the progress of atherosclerotic lesions, with an IC_{50} of 98 nM.[16]

Cellular location studies: there had been considerable discussion in the literature as to whether these peptides were produced (at least in part) by the cyanophyte but variable results were reported. In 2002, Salomon and Faulkner, using the on-site facilities of the Coral Reef Research Foundation on Koror, were able to carefully dissect examples of *L. patella* and subsequently show that the peptides were distributed through-



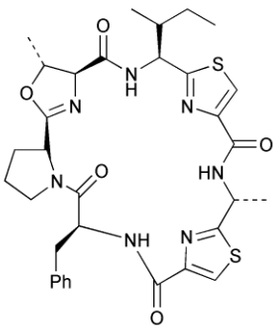
5; R = CH_3
6; R = $CH_2CH_2SO_3Na$



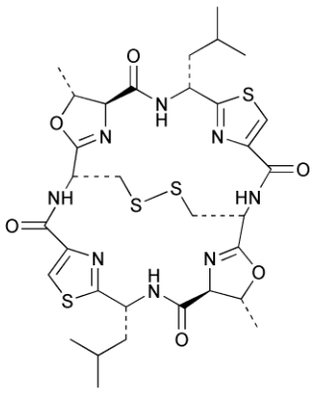
7

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online



11



12

out the tunic of the ascidian and were not found in any detectable quantity in intact *Prochloron* cells.[5]

These findings do not demonstrate the source of the peptides but do open up a series of questions as to how peptides could migrate from the cyanophyte into the lower tunic.

Lissoclinum voeltzkowi

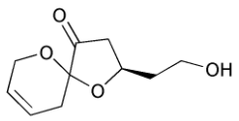
This encrusting grey ascidian was collected from sea grass blades in shallow waters in the lagoon at Palau. The identification was by Dr F. Monniot and a voucher specimen has been deposited at the Museum National d'Histoire Naturelle in Paris (registry # MNHN A2 Lis 138).

Chemistry/bioactivity: an unusual spiroketal, lissoketal **17**, was isolated from this organism but no bioactivity data was reported.[19]

7.2 Cnidaria, Alcyonaria, Alcyonacea

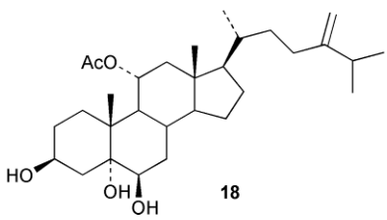
Lobophytum cf. *pauciflorum*

This soft coral, *Lobophytum* cf. *pauciflorum* was collected at Short Drop Off in 1995 and a voucher specimen was deposited at SIO (collection # 95–095).



17

Chemistry/bioactivity: two new sterols, 11 α-acetoxy-24-methylenecholesta-3β,5α,6β-triol **18** and 11α-acetoxy-24*R*-methylcholesta-3β,5α,6β-triol were isolated from the soft coral. No bioactivity data was reported by the authors.[20]

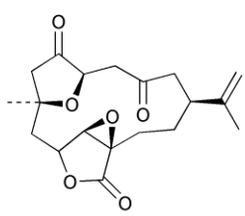


18

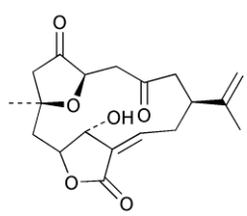
Sinularia inelegans

This specimen of the soft coral *S. inelegans* (PSC-217) was collected during the 1979 cruise of the R/V Alpha Helix.

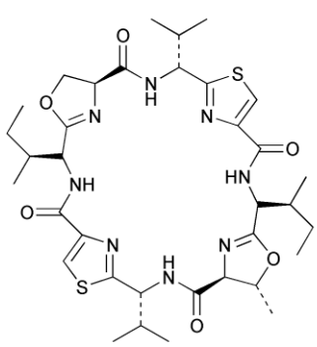
Chemistry/bioactivity: *S. inelegans* contained two new norcembrane diterpenes **19** and **20** with the structure of **19** being determined by X-ray crystallography. No bioactivity data was reported by the authors.[21]



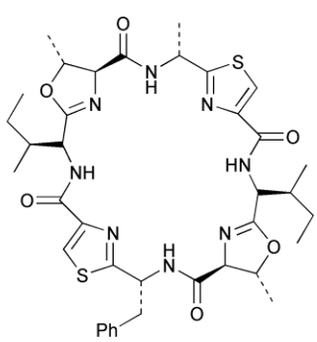
19



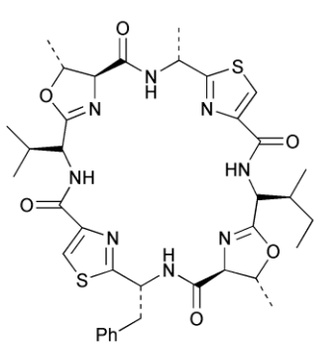
20



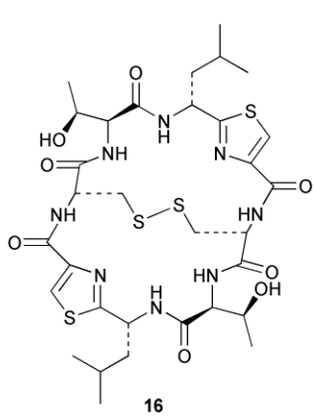
13



14



15



16

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

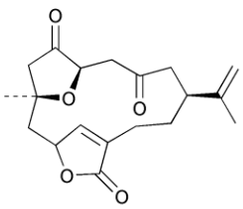
View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

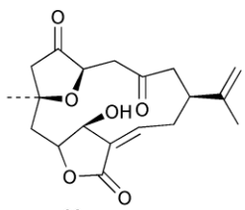
Sinularia numerosa

As with *S. inelegans*, this specimen of the soft coral *S. numerosa* (PSC-187) was also collected during the 1979 cruise of the R/V Alpha Helix.

Chemistry/bioactivity: *S. numerosa* contained one new norcembrane diterpene **21** and the known norcembenolide **22**.[22] As with **19** above, the structure of **21** was determined by X-ray crystallography.[21]



21

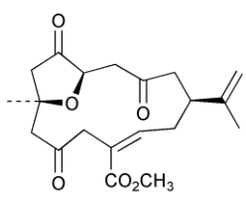


22

Sinularia querciformis

This specimen of the soft coral *S. querciformis* (PSC-30) was another collected during the 1979 cruise of the R/V Alpha Helix.

Chemistry/bioactivity: *S. querciformis* contained the known norcembenolide **22** and one new norcembrane diterpene **23**, but no bioactivity information was presented.[21]



23

***Sinularia* sp. A**

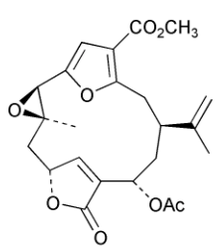
As above, this specimen of a new soft coral, *Sinularia* sp. (PSC-159) was also collected during the 1979 cruise of the R/V Alpha Helix.

Chemistry/bioactivity: the *Sinularia* sp. contained the new norcembrane diterpene **21** but no bioactivity information was presented.[21]

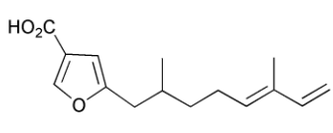
***Sinularia* sp. B**

This unidentified specimen of *Sinularia* sp. was collected at Aulong Island in 1991.

Chemistry/bioactivity: this *Sinularia* sp. contained 13α-hydroxypukalide **24**, first isolated from *S. polydactyla* by Bowden *et al.*[23] and the known[24] furanosesquiterpene acid **25**.[25] 13α-hydroxypukalide was reported to inhibit settlement of the blue mussel *Mytilus edulis* at 0.1 μg mL^{-1}.



24

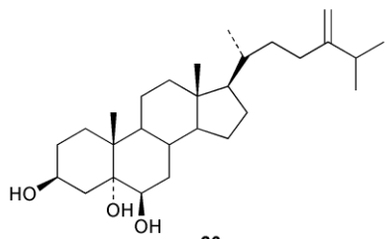


25

***Sinularia* sp. C**

This unidentified specimen of *Sinularia* sp. was collected in 1991 during a cruise of the R/V Sogen Maru.

Chemistry/bioactivity: this *Sinularia* sp. contained five related steroidal triols (*e.g.* **26**) 24-methylenecholesta-3β,5α,6β-triol, which inhibited settlement of the blue mussel *Mytilus edulis* at a dose of 700 μg mL^{-1}.[26]



26

7.3 Cyanophycota, Cyanophyceae, Nostocales

Lyngbya majuscula

This sample was collected from shallow water in the lagoon close to Big Goby Marine Lake in 1995 and contained 90–95% *L. majuscula* with minor amounts of *Phormidium* sp. or *Schizothrix* sp. A voucher sample (95–079) is maintained at the Scripps Institute of Oceanography.

Chemistry/bioactivity: following methanolic extraction and subsequent ethyl acetate partitioning, the ethyl acetate soluble material was fractionated following activity in an HIV-integrase inhibition assay. This led to the isolation and identification of the known cyclic peptide, dolastatin 3 **27** as an inhibitor of the integrase with an IC_{50} value of 5 mM. What was of significant interest was the discovery that this agent and other similar molecules, appear to bind to plastic wells and pipette tips. Thus concentrations delivered are probably not those reported. Two other peptides that appeared to have little to no activity in this assay were also isolated and purified, homodolastatin 3 **28** and kororamide **29**. In addition to these peptides, the known cytotoxic metabolites, aplysiatoxin **30**, debromoaplysiatoxin **31** and oscillotoxin **32** were also isolated and purified.[27]

***Lyngbya* sp. (I)**

This sample was collected in 1999 at the Short Drop Off in Palau and a voucher was stored at the University of Guam (# NIH 309).

Chemistry/bioactivity: the lipophilic extract of this material was cytotoxic to KB and LoVo cell lines and following conventional isolation techniques, two very cytotoxic peptides were purified. One was apratoxin A **33** previously reported from a *Lyngbya* sp. collected in Apra Harbor, Guam and the other, apratoxin C **34** was novel. In both cases, their activities (IC_{50} values) against KB and LoVo were at the sub-nanomolar level.[28]

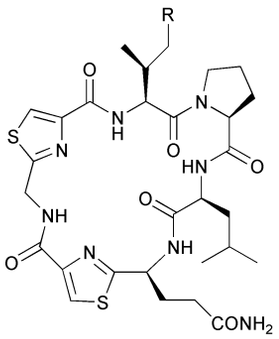
***Lyngbya* sp. (II)**

This sample was a combination of smaller collections made at various dive sites (1999–2000) in Palau and then a major collection was made in 2000 in Ulong Channel. These samples together with the one referred to above (NIH 309) were the source for the chemistry described below. Vouchers are stored for all at the University of Guam marine station.

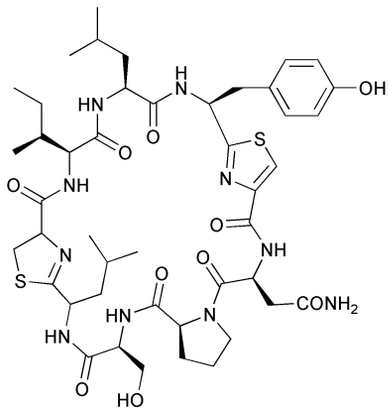
Chemistry/bioactivity: six closely related cyclic peptides, ulongamides A–F **35**–**40** were isolated from these samples of *Lyngbya*. Unlike the apratoxins with which they share the same host, and which have cytotoxic activity at the sub-nanomolar level, five of the six compounds (A–E) are cytotoxic (IC_{50} values) to KB/LoVo at 1–5 μM, whereas the sixth, (F), the only one without an aromatic side-chain, is not active below 10 μM.[29]

View Article Online

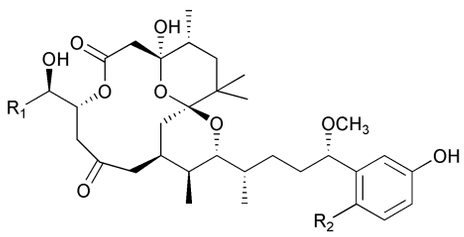
Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.



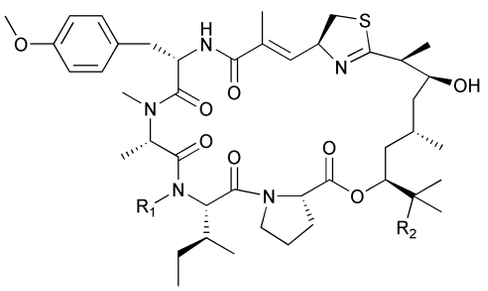
27; R = H
28; R = CH_3



29



30; R_1 = CH_3, R_2 = Br
31; R_1 = CH_3, R_2 = H
32; R_1 = R_2 = H



33; R_1 = R_2 = CH_3
34; R_1 = H, R_2 = CH_3

Lyngbya sp. (III)

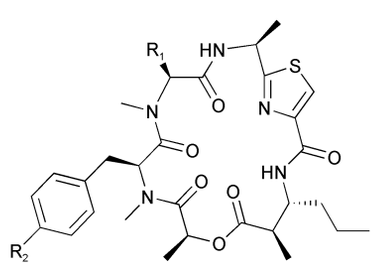
This sample of *Lyngbya* was the large collection from the Ulong Channel referred to above and a voucher sample was deposited at the University of Hawaii.

Chemistry/bioactivity: in addition to the apratoxins, ulongamides and lyngbyapeptins, this collection also yielded an unusual brominated glycosidic macrolide that was named lyngbyaloside B **41** due to its close similarity to the previously reported lyngbyaloside **42**. It is also a structural analogue of the recently reported lyngbouilloside **43** that was isolated by Gerwick's group from the cyanophyte, *L. bouillonii* and on re-examination of the voucher specimen, this producing organism matches the description for *L. bouillonii*. Lyngbyaloside was weakly cytotoxic against KB (IC_{50} of 4.3 μM) and roughly four-fold less active *versus* LoVo.[30]

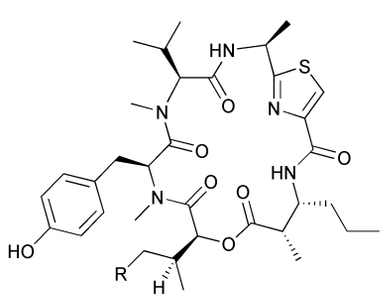
Lyngbya sp. (IV)

Further investigations of the same collections as in I to III above demonstrated, yet again, the immense biosynthetic potential of these cyanophytes.

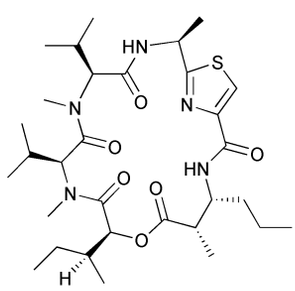
Chemistry/bioactivity: all isolations were followed using cytotoxicity against either KB or LoVo cells as the initial



35; R_1 = *i*-Pr; R_2 = H
36; R_1 = *i*-Pr; R_2 = OH
37; R_1 = Bn; R_2 = OH



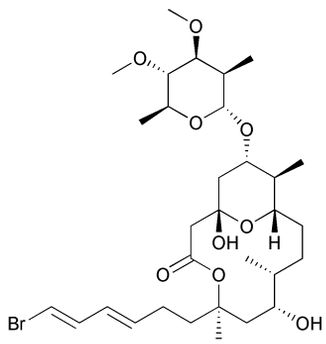
38; R = H
39; R = CH_3



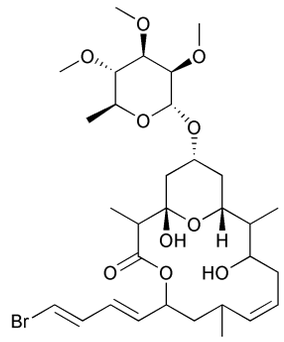
40

View Article Online

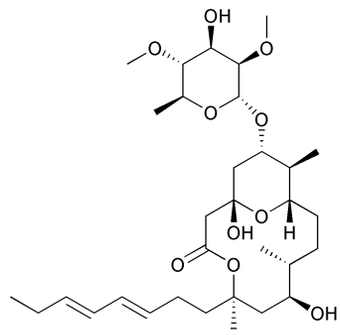
Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.



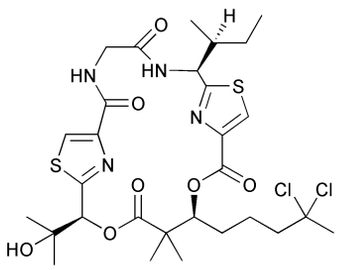
41



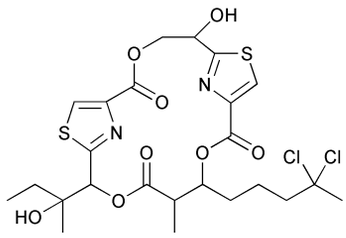
42



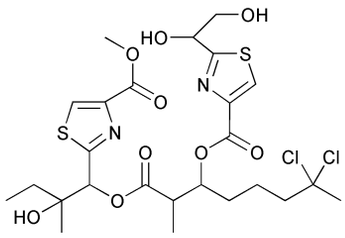
43



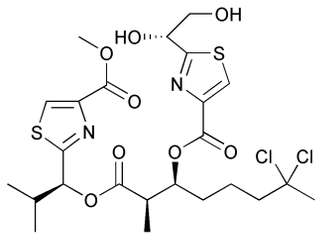
44



45



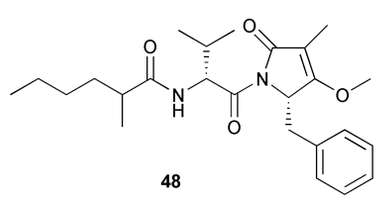
46



47

indicator. Whilst investigating the apratoxin producers from Guam, lyngbyabellin A **44** was found.[31] This compound, together with a weaker (IC_{50} values of 2–5 μM) cytotoxic analogue, named lyngbyabellin C **45** were isolated from the Short Drop Off collection. What is of interest is that during the isolation of the latter compound, a methanolysis of one of the ring ester groups occurred, giving rise to a linear compound that was named as homohydroxydollabellin **46** because of its resemblance to the *D. auricularia* metabolite, dollabellin **47** reported by Yamada's group in 1995.[32] Since in the Palauan case, the linear compound had effectively the same cytotoxicity as the cyclic precursor, and methanol was used extensively in the isolation of dollabellin, it is probable that this compound is an artifact of the isolation protocol and thus demonstrates that the actual source should be searched for once the material has been isolated from its host.

From the "mixed collection" another quite cytotoxic material (IC_{50}'s in the 0.4 to 1 μM range) was obtained. It was not encountered in any of the Guamanian collections and in only the one Palauan sample. This was the mixed amide-imide compound that was given the trivial name of palau'imide **48**.[31]

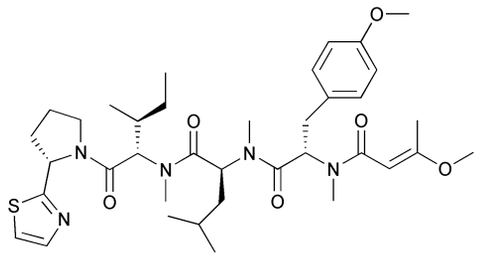


48

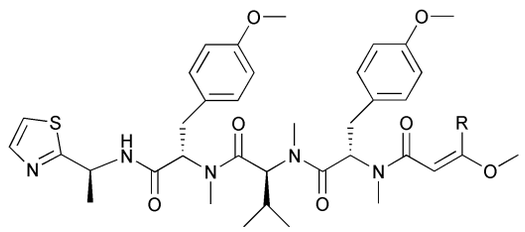
Finally, from the work-up of all of these compounds, two non-cytotoxic tetrapeptides related to the known lyngbyapeptin A **49**, lyngbyapeptins B **50** and C **51** were isolated and purified from side-cuts. These latter two were not reported from any of the apratoxin-containing Guamanian samples, nor was the former compound found in the Palauan samples.[31]

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.



49



50; R = CH_3
51; R = CH_2CH_3

Symploca sp. (I)

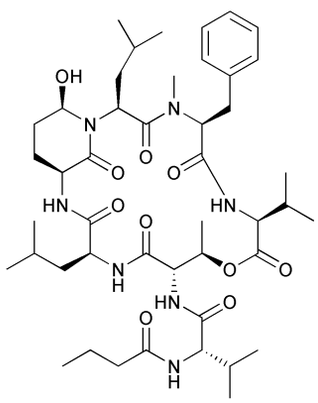
This sample of *Symploca* was collected in 1999 at Short Drop Off in Palau and a voucher was deposited at the University of Guam (#NIH 304).

Chemistry/bioactivity: the lipophilic extract of this material demonstrated potent solid tumour selectivity and following bioactivity-driven isolation, the linear peptide tasiamide **52** (now tasiamide A) was isolated and purified demonstrating cytotoxicity against both KB and LoVo cells in the μg mL^{-1} range.[33] This linear peptide's closest match in cyanobacterial metabolites is malevamide A which shares approximately half of the sequence. Very recently, further workup of the aqueous extract of this same collection by following bioactivity against the KB cell line yielded the linear peptide tasiamide B **53** containing the unusual amino acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, that had previously been reported as a component of protease inhibitors from *Candida* and *Streptomyces* spp.[34]

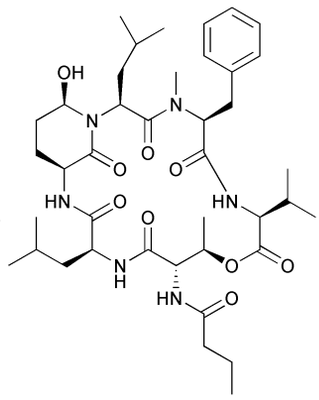
Symploca sp. (II)

A second collection from the same general area (Short Drop Off) was made in 2000 and a larger sample was obtained with the voucher sample being deposited at the Smithsonian Marine Station in Fort Pierce, FL (# NIH 304).

Chemistry/bioactivity: in contrast to tasiamide, the two



54



55

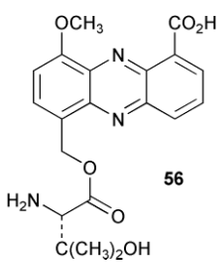
cytotoxic peptides that were purified from this preparation were in the hydrophilic fraction. These two closely related cyclic peptides were named tasipeptin A **54** and tasipeptin B **55** and demonstrated activity against KB cells with IC_{50} concentrations at the 1 μM level.[35]

7.4 Miscellaneous bacteria/fungi

Pelagiobacter variabilis

This marine bacterium was isolated from the macro-alga *Pocockiella variegata* collected in Palau and then cultured in Difco marine broth rather than in one of the more esoteric culture media.

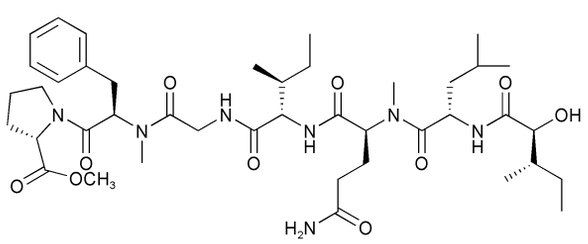
Chemistry/bioactivity: the culture filtrate contained the known compound griseoluteic acid and three new phenazine derivatives, pelagiomicins A **56**–C. Of these, pelagiomicin A was reported to exhibit antimicrobial and antitumour activity.[36]



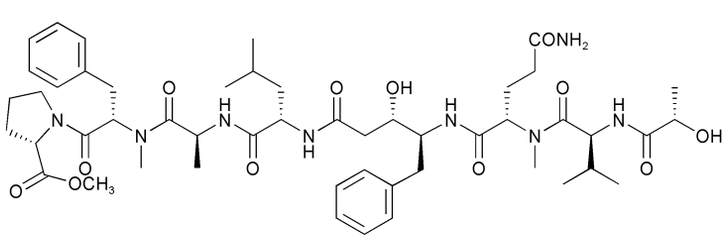
56

Pseudoalteromonas sp. F-420

This marine bacterium was isolated from the green alga *Halimeda* sp. collected in shallow water off Koror.



52

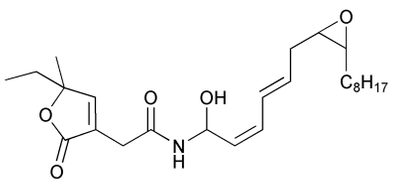


53

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

Chemistry/bioactivity: following isolation and subsequent fermentation in Difco marine broth, this bacterium, *Pseudoalteromonas* sp. F-420, produced a new lipid metabolite, korormicin **57** that was active against marine (halophilic) Gram-negative bacteria, but was not active against marine (halophilic) Gram-positive bacteria and terrestrial microorganisms irrespective of Gram type.[37] This molecule might serve as a method of defining such Gram-negative marine microbes.

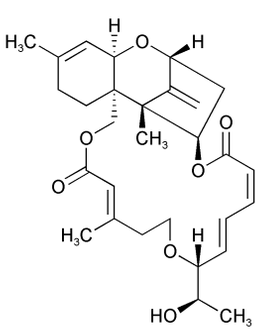


57

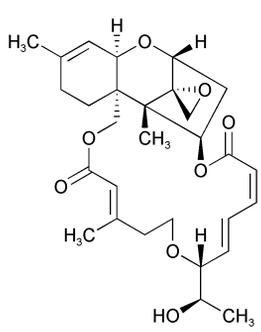
Myrothecium roridum

This filamentous fungus was isolated from a small sample of woody material collected from the bottom of a coral reef at a depth of 5 m in 1998 during a cruise by the training vessel T/V Umitaka-maru.

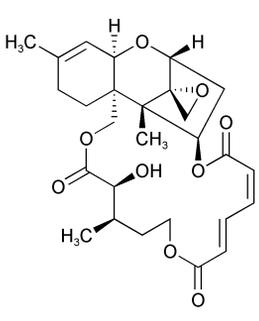
Chemistry/bioactivity: the fungus was cultivated in half-strength potato-dextrose medium in seawater and the materials isolated from an acetone/ethyl acetate treatment/extraction following their bioactivity against HL-60 and L1210 tumour lines. One new trichothecene, 12,13-deoxyroridin E **58** and three previously described trichothecenes, roridin E **59**, verrucarin A **60** and verrucarin J **61** were also isolated. The novel derivative of roridin E was about 80 fold less active than the parent epoxy-containing roridin E.[38]



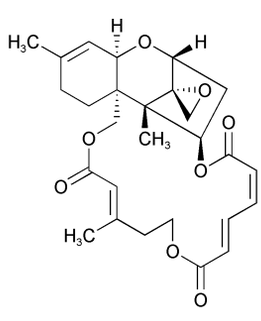
58



59



60



61

Microbial Phylogenies

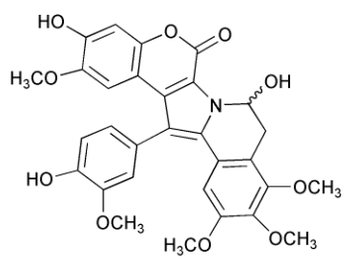
The invertebrates and waters around Palau are laden with as yet undescribed microbes. As an example, in 2001, Suzuki *et al.* from the Kyowa Hakko Kogyo company published an article that utilized sequence information from five microbes isolated from Palauan sponges and algae that permitted the identification of a completely new taxon of bacteria, the genus *Tenacibaculum*.[39]

7.5 Mollusca

***Lamellaria* sp**

This *Lamellaria* sp. is a black-colored prosobranch mollusc that feeds on ascidians and may well be nocturnal. Six specimens were collected during a night dive at Peduliaes Headland at the entrance to Malakal Harbor and a few others have been sighted in relatively shallow water.

Chemistry/bioactivity: lamellarins A **62**–D were isolated from specimens of *Lamellaria* sp. and the structure of **62** was determined by X-ray crystallography.[40] Subsequently, the same compounds were isolated from the ascidian upon which the mollusc was feeding. These compounds inhibited the cell division of fertilized sea urchin eggs.



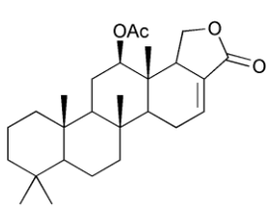
62

7.6 Mollusca, Gastropoda, Nudibranchia

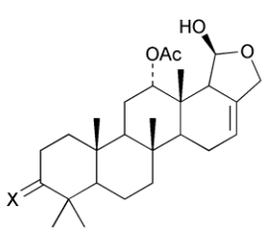
Chromodoris funerea

This black and white striped dorid nudibranch was collected in the shallow waters of Iwayama Bay near the Nikko Hotel and also from the Kaibakku Marine Lake.

Chemistry/chemical ecology: the specimens from the marine lake contained 12-epi-scalarin **63**, deoxoscalarin **64**, luffariellins C **65** and D **66**, and 3-ketodeoxoscalarin **67**, which are all considered to be metabolites of dictyoceratid sponges. In contrast, the specimens from Iwayama Bay contained furodysin **68**, furodysinin **69**, and their singlet oxygen oxidation products, and the pentabrominated biphenyl ether **70**, all of which have been found in species of *Dysidea* from the same location.[41,42] Dorid nudibranchs are known to sequester allelochemicals from their sponge diets and use them for their own defense.



63



64; X = H_2
67; X = O

From this study one may conclude that the ability of *C. funerea*, and dorid nudibranchs in general, to distinguish allelochemicals from other metabolites is not dependent on the nudibranch being adapted to a specific food source.

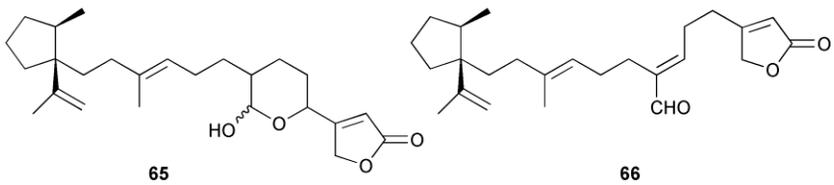
7.7 Porifera

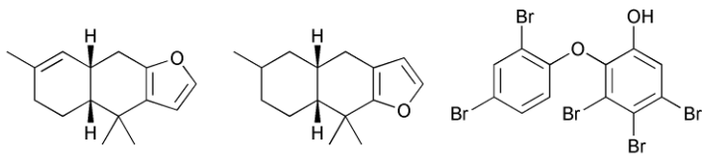
Unidentified sponge or mixture of sponges

The sponge, or sponges, were collected by the Suntory Institute for Biomedical Research prior to 1986, but cannot be identified as though the paper mentions *Halichondria* sp. and *Dictyoceratida* sp., since the latter is the name of an order of sponges, any identification is suspect.

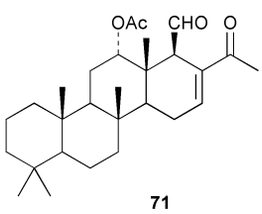
Chemistry/bioactivity: the specimen(s) contained six scalarin-type sesterterpenes (*e.g.* **71**),[43] three of which had been

View Article Online





described previously from an Australian *Lendenfeldia* sp.[44] and it was reported that 24-methylscalaradial **71** inhibited platelet aggregation with an IC_{50} of 0.5 μg mL^{-1}.



7.8 Porifera, Demospongiae, Agelascida

Agelas sp

A massive orange sponge that is fairly common on the reefs of Palau (the specimen studied was from Argulpelu Reef). Van Soest[45] considered this specimen to be *A. mauritiana* and also considered the Japanese sponge *A. nakamurai* to be the same as *A. mauritiana*. Chemical studies certainly support these proposals.

Chemistry/bioactivity: the *Agelas* sp. contained agelines A **72** and B **73**, and agelasidine A **74** in good yields but they were difficult to separate.[46] Agelines A and B are quaternary 9-methyladenine salts of diterpenes that are unstable in basic conditions whereas agelasidine A is a taurocyamine derivative of a sesquiterpene. Compounds **72** and **73** were also reported from *A. nakamurai*. Compounds **72** and **73** were antimicrobial and ichthyotoxic, and although the crude extract of the sponge was cytotoxic, neither of these compounds was active. Related compounds from *A. mauritiana* and *A. nakamurai* were reported to be cytotoxic and to inhibit the enzymatic activity of Na/K ATPase. There are no known uses and the instability of agelines A and B and related salts makes them unlikely candidates for commercial development.

7.9 Porifera, Demospongiae, Astrophorida

Asteropus sarasinosum

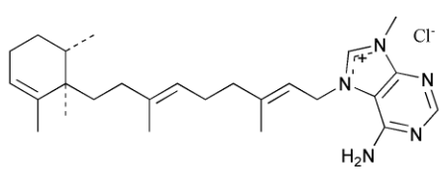
This massive sponge is found growing out of sandy substrates. Although collected in Koror Harbor, it is probably the same sponge as the *Asteropus* sp. collected in Guam and Chuuk.

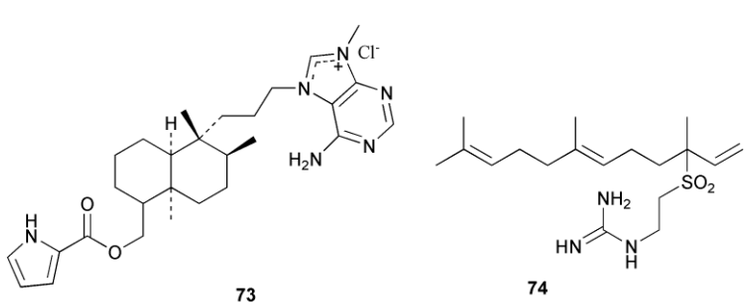
Chemistry/bioactivity: a total of nine closely related saponins called sarasinosides A_1, **75**, A_2, A_3, B_1, B_2, B_3, C_1, C_2, and C_3 were isolated.[47,48] These saponins consist of 4 or 5 sugar units, two of which are amino-sugars, linked to a 14-*nor*-lanostane triterpenoid and are somewhat similar in structure to the saponins found in sea cucumbers. Like all saponins, the sarasinosides are ichthyotoxic (the saponins are thought to cause hemolysis of gill tissues) and are cytotoxic due to their action on cell membranes, where they cause irreversible non-specific lysis.

Jaspis sp. (aka *Dorypleres splendens*)

This orange sponge was collected in a marine lake in Palau and in Fiji. It appears to be fairly common throughout Palau. In other parts of the Pacific, this sponge has been collected under a variety of names, including *Jaspis splendens*. The identification as *Dorypleres splendens* was made by Dr Michelle Kelly(-Borges) working with the materials collected for a large-scale jaspamide isolation project involving the NCI collection contractor, the Coral Reef Research Foundation in Palau and Yap.

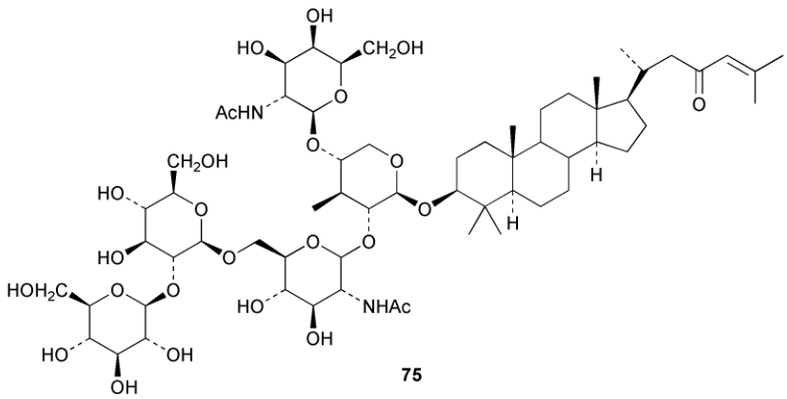
Chemistry/bioactivity: this *Jaspis* sp. contains jaspamide **76** as the major metabolite[49] which is also known as jasplakinolide when isolated from a Fijian-derived sample of *Jaspis johnstoni*.[50] Modified jaspamides have been reported from the sponge *Jaspis splendens* collected in Vanuatu.[51] Jaspamide was initially reported to be a potent insecticide and antifungal agent[49,50] but subsequent work by NCI scientists demonstrated





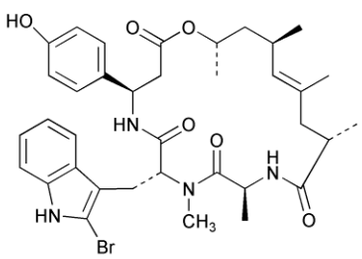
Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online



75

that it induces actin polymerization *in vitro* leading to disruption of the actin skeleton.[52,53] However, in spite of many attempts at NCI, no reproducible *in vivo* antitumour activity could be demonstrated due to the therapeutic index being very close to unity. In this respect, it is similar to the effects seen with other actin inhibitors such as the latrunculins and cytochalasins.



76

The question as to the actual source of these molecules is open to debate as very similar molecules, the geodiamolides, were first reported[54] from a *Geodia* sp. in 1987 and from other taxonomically distant sponges in following years,[55] Similar molecules, the chondramides, with an 18 membered macrolide ring, were reported from a terrestrial myxobacteria, by the Reichenbach group in 1995,[56] with actin stabilizing activity of these compounds being reported in 1998.[57]

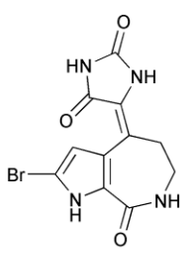
Thus, is the sponge the actual source of the jaspamides or merely a host (commensal or co-metabolic) to microbes that produce these peptides?

7.10 Porifera, Demospongiae, Axinellida

Axinella sp

This sponge is described as having a brownish-yellow exterior of irregular mass and it must be fairly abundant as over 200 kg of sponge was collected by Pettit's group, an amount normally considered excessive by most investigators.

Chemistry/bioactivity: the first metabolites to be reported from this sponge were hymenialdesine, debromohymenialdesine (see *Stylotella aurantium)* and axinohydantoin **77**, which possibly resulted from hydrolysis of hymenialdesine.[58] Bioassay-guided fractionation using a leukemia cell line resulted in the isolation of very small quantities of halichondrin B **78** and



77

homohalichondrin B, which had previously been obtained from a Japanese *Halichondria* sp., and three new cyclic peptides, axinastatins 1 **79**, 2 and 3.[59,60] Although discovered using an anti-leukemia assay, the axinastatins were cytotoxic against several tumour cell lines including representative ovarian, CNS, renal, lung, colon and melanoma lines. Following the syntheses a few years later,[61,62] the original report of the bioactivity of the axinastatins was questioned when it was found that the synthetic compounds had only a fraction of cytotoxicity claimed for the natural products, but the reason for the discrepancy is not known. Finally, a synthetic derivative of halichondrin B is currently in Phase I clinical trials for cancer.

Raspailia (Raspaxilla) sp

Raspailia (Raspaxilla) sp. is a small orange sweet-smelling sponge that was collected from the outer side of the reef west of Koror with a voucher specimen deposited in the SIO Benthic Invertebrate Collection (# P-1151).

Chemistry/bioactivity: the sponge contained two homologous cyclic hemiketals **80** and **81** but without any reported bioactivity.[63]

7.11 Porifera, Demospongiae, Dendroceratida

Chelonaplysilla sp

This small deep purple dendritic sponge was collected from a marine lake on Kaibaku Island in 1981 and 1990. Though originally considered to be a *Dendrilla* sp., it was subsequently identified in 1990 as a *Chelonaplysilla* sp.

Chemistry/bioactivity: after reidentification of the sponge, four alkaloids, chelonin A **82**, chelonin B **83**, bromochelonin B **84** and chelonin C **85** were reported as metabolites of *Chelonaplysilla* sp.[64] Compounds **82**–**84** exhibited antimicrobial activity against *Bacillus subtilis* and in addition, chelonin A **82** demonstrated *in vivo* antiinflammmatory activity.

Dendrilla sp

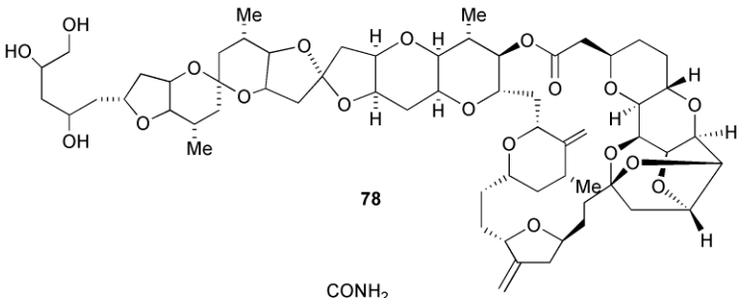
This small, deep purple dendritic sponge of the genus *Dendrilla* was collected from a marine lake on Kaibakku Island in Iwayama Bay in both 1981 and 1988. Subsequently, this particular sponge was reclassified as a *Chelonaplysilla* sp. (*cf* above).

Chemistry/bioactivity: the 1981 collection contained dehydroambliol A **86**, previously described from *Dysidea amblia*,[65] norrisolide **87**, a known metabolite of the spongivorous dorid nudibranch *Chromodoris norrisi*,[66] and four new compounds, 1-bromo-8-ketoambliol A acetate **88** and dendrillolides A **89** B, and C **90**.[67] The 1988 collection yielded four additional compounds, dendrillolides D **91** and E **92**, 12-desacetoxypolyraphin A **93** and 12-desacetoxyshahamin C **94**, together with a reassessment of the structure of dendrillolides A and B, where dendrillolide A should have the original structure assigned to dendrillolide B with the structure of dendrillolide B being currently unknown.[68,69] No bioactivity was recorded

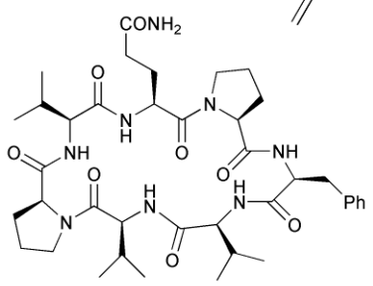
Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online

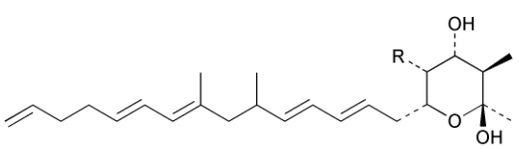
Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.



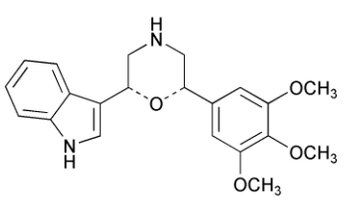
78



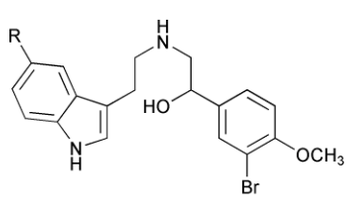
79



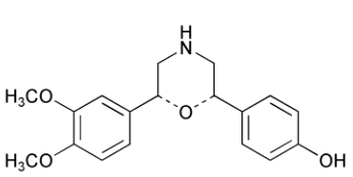
80; R = H
81; R = CH_3



82



83; R = H
84; R = Br



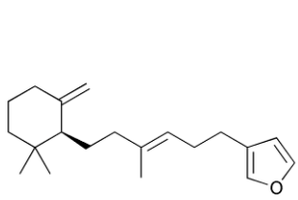
85

originally but recently Blackburn found that dendrillolide A **89** causes vesiculation of Golgi membranes (C. Blackburn, *pers. comm.*).

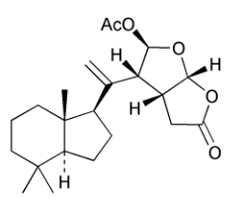
Igernella sp

The sample of *Igernella* sp. was collected from the fringing reef on the Southeastern side of Palau.

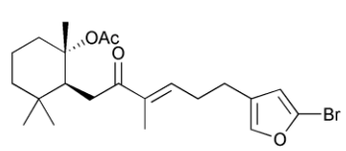
Chemistry/bioactivity: this sample contained the known compound halisulfate 3 **95**, which exhibited modest antimicrobial activity and had previously been reported from a Californian halichondrid sponge,[70] together with the new sesterterpene igemellin **96**.[71]



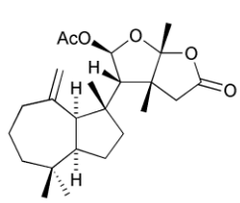
86



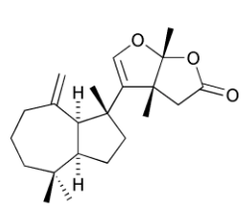
87



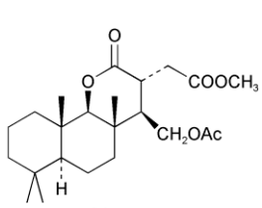
88



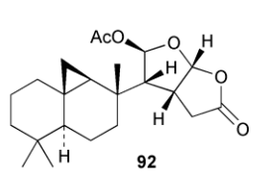
89



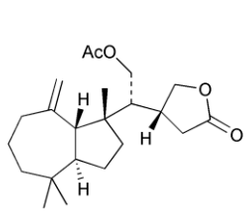
90



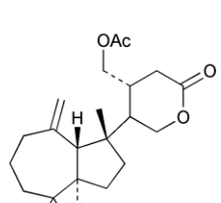
91



92



93



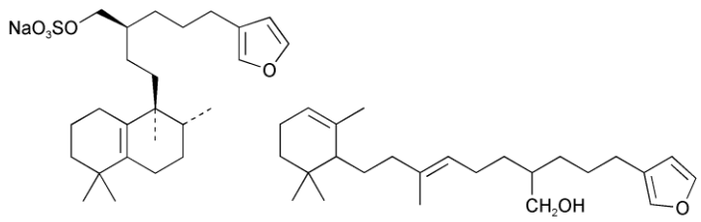
94

7.12 Porifera, Demospongiae, Dictyoceratida

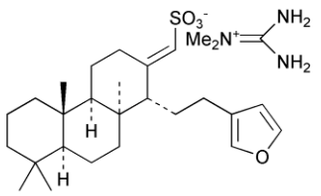
Coscinoderma sp

The *Coscinoderma* sp. (originally called *Ircinia* sp.) was found in both Fiji and Palau.

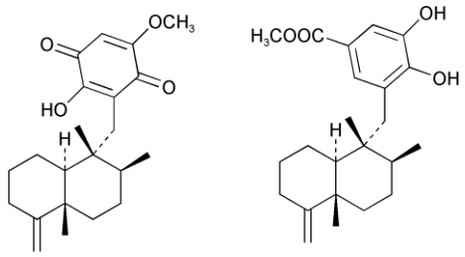
View Article Online



95 96



97



98 99

Chemistry/bioactivity: the major metabolite in *Coscinoderma* sp. was suvanine **97**, the structure of which was corrected as a result of studies of the Palauan specimen.[72] Suvanine **97** facilitates neuromuscular transmission in indirectly stimulated rat hemidiaphram preparations (Jacobs, unpublished data), is an acetylcholinesterase inhibitor and Kimura *et al.*[73] reported that they had isolated it by following serine protease inhibitory activity from a sample of *Coscinoderma mathewsi* collected in Pohnpei. They found that the activity against trypsin and thrombin was independent of the counter-ion, thus the known activity of the guanidine moiety was not the responsible factor.

Dactylospongia sp

A small specimen of this sponge of the genus *Dactylospongia* was collected at a depth of 15 metres at Bairakaseru Reef in 1985.

Chemistry/bioactivity: the sponge contained ilimaquinone **98** (*cf. Hippospongia metachromia)*, dictoceratidin A **99**, which had previously been isolated from a *Hippospongia* sp.[74] and five new compounds, dactylospongenones A–D **100**–**103** and dictoceratidin C **104**.[75] No bioactivity was recorded for the new compounds although the crude extract of the sponge was antimicrobial, probably due to the presence of ilimaquinone.

Dysidea sp. (general comments)

Sponges of the genus *Dysidea* are very common in Palau, where they are often found in shallow water in lagoons and near the tops of the reefs. At least three different *Dysidea* species can usually be seen near the landing dock at the Hotel Nikko. The taxonomy of the *Dysidea* species in Palau is somewhat confused because the same species may take on different growth forms in response to environmental factors. Chemotaxonomy is unlikely to be helpful because the chemical compounds may well be produced by symbiotic cyanobacteria or by a "co-metabolic process" between the cyanophyte and the host organism

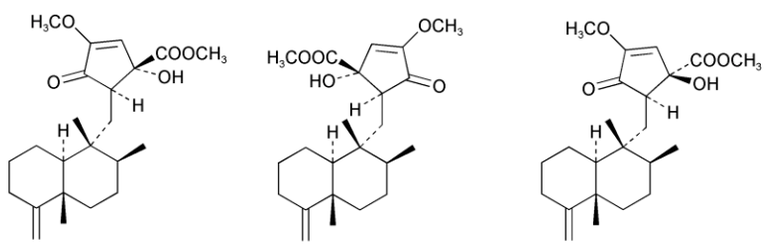
Dysidea chlorea

This sponge was collected near the Hotel Nikko Dock and was separated with difficulty from other *Dysidea* species.

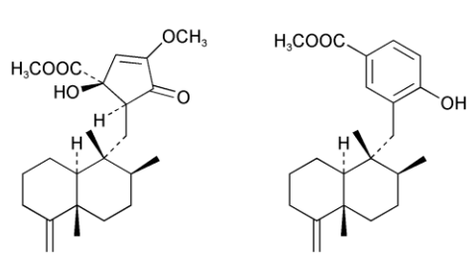
Chemistry/bioactivity: *D. chlorea* contained only 2-(2′,4′-dibromophenoxy)-4,6-dibromophenol **105** which demonstrated strong antimicrobial activity.[76]

Dysidea herbacea

This is probably the most common species of *Dysidea* in the shallow waters of Palau and it can always be found across from the dock of the Hotel Nikko. Collections were also made from Kaibaku marine lake and from the reef-top at Ngemelis drop-off, where it overgrows a red coralline algae (*Jania* sp.). The sponge contains symbiotic cyanobacteria *(Oscillatoria spongeliae)* to the extent of about 50% of the mass – this is very



100 101 102



103 104

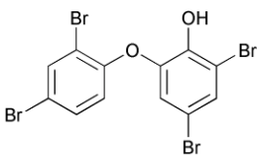
Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online

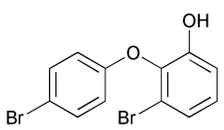
Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

unusual among sponges other than *Dysidea* spp. *D. herbacea* from Palau is illustration #204 in Colin and Arneson.[77]

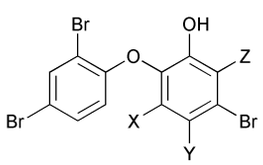
Chemistry/bioactivity: in 1972, one of the first studies of marine sponges from a secondary metabolite aspect was performed on *Dysidea herbacea* from Palau. In this study, Sharma and Vig[78] reported the isolation of two antimicrobial agents, 2-(4′-bromophenoxy)-3-bromophenol **106** and 2-(2′,4′-dibromophenoxy)-3,4,5-tribromophenol **107**. In a later study in 1981, Carte and Faulkner[76] also reported the isolation of **107** together with 2-(2′,4′-dibromophenoxy)-4,5,6-tribromophenol **108** and 2-(2′,4′-dibromophenoxy)-3,5-dibromophenol **109** from the Hotel Nikko samples.



105

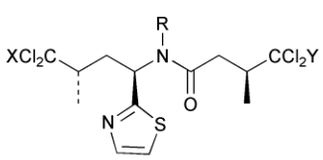


106



107; X = Y = Br
108; X = H, Y = Z = Br
109; X = Br, Y = Z = H

In contrast, the Kaibakku Marine Lake specimen contained 10-dechloro-*N*-methyl-dysideathiazole **110**, 9,10-didechloro-*N*-methyldysideathiazole **111**, 10-dechloro-dysideathiazole **112**, and dysideathiazole **113** and the Ngemelis specimen yielded dysideapyrrolidone and **111**.[79]



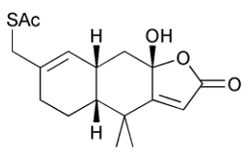
110; R = CH_3, X = H, Y = Cl
111; R = CH_3, X = Y = H
112; R = X = H, Y = Cl
113; R = H, X = Y = Cl

Symbiosis studies: using a specimen of *D. herbacea* from the Hotel Nikko Dock site, Unson *et al.*[80] demonstrated that 2-(2′,4′-dibromophenoxy)-4,6-dibromophenol **105** was localized in, and presumably produced by, the cyanobacterium *Oscillatoria spongeliae.* Crystals of **105** were also found just beneath the surface of the sponge: it is assumed that the cyanobacteria release such large quantities of **105** (which is essentially insoluble in water), that it crystallizes on exposure to seawater.

Dysidea sp

This unidentified species of *Dysidea* was collected in 1981 at a depth of 30 m along the outer reef due west of Malakal Harbor.

Chemistry/bioactivity: 15-acetylthiofurodysinin lactone **114** was isolated as a result of bioassay-driven isolation as a partial agonist using a human lung LTB_4 receptor-binding assay linked to calcium mobilization.[81] Although the scientists at then Smith Kline & French, now Glaxo SmithKline, performed QSAR studies these were never published.



114

Another example of this genus was also collected in 1981 at a depth of 20 m at Short Drop Off and was subsequently stored in methanol for 15 years prior to work-up. A voucher specimen was deposited in the SIO Benthic Invertebrate Collection (#P 1172).

Chemistry/bioactivity: three new dolabellane diterpenes **115**–**117** and an unstable 14-membered macrolide, arenolide **118** were isolated from this sponge. All demonstrated modest cytotoxicity, but the isolation of these classes of compounds from this genus was previously unknown. Extensive reinspection of the sponge sample demonstrated that it was an example of a *Dysidea* sp. and did not appear to contain any gorgonian or brown algal contaminants/symbionts. A possible answer is that this particular sponge had adsorbed materials from its surroundings, a scenario that has been proposed to account for the presence of red algal metabolites in sponges.[82]

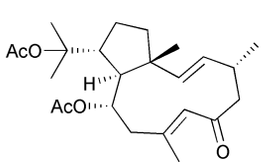
Fenestraspongia sp

This sponge was collected near Urakthapel Island but due to the condition of the voucher specimen of the sponge, it could not be further identified.

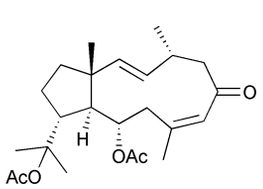
Chemistry/bioactivity: the sponge contained a 6 : 4 mixture of ilimaquinone **98** (see *Dactylospongia* sp.) and 5-epi-ilimaquinone[83] **119**, with the latter compound inhibiting fish-feeding at 5 pg mg^{-1} of pellet.

Hippospongia metachromia

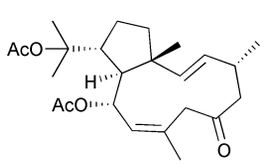
This "dirty" yellow sponge is found commonly on the reefs around Palau and throughout the Indo-Pacific with a repre-



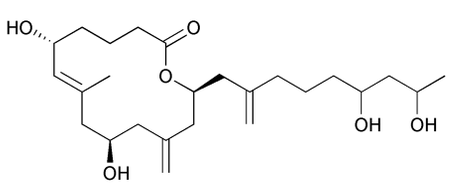
115



116

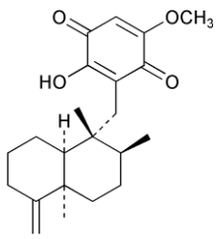


117



118

View Article Online



119

sentative photograph of the sponge in Colin and Arneson[77] (entry 192).

Chemistry/bioactivity: the merosesquiterpene ilimaquinone **98** was first isolated from a specimen of *H. metachromia* from Hawaii[84] whereas the bioactivity studies referred to below used ilimaquinone isolated from Palauan specimens of *H. metachromia* and *Dactylospongia elegans*. Ilimaquinone is generally regarded as a nuisance compound because it is active in too many bioassays. It has been patented for use against cancer and HIV but it is unlikely to be used in treating humans because of its non-specific activity. However, as a result of demonstrating that ilimaquinone causes reversible breakdown of the Golgi membranes and disruption of the microtubule network in normal rat kidney cells,[85] it is now being used as a biochemical probe to study the dynamics of Golgi membranes and their role in sorting, modifying and distributing proteins.

Luffariella sp

Studies on two specimens of *Luffariella* sp. from Palau have been reported. The first specimen (from 1985) was collected at Short Drop Off. The second specimen (from 1995) was collected in shallow water at "Tee" marine lake. In both cases, the specimens had a fine tertiary fibre network that is typical of the genus *Luffariella*.

Chemistry/bioactivity: the first specimen (from 1985) contained luffariellolide **120** as a major constituent comprising 14.5% by dry weight[86] and luffolide **121** as a minor metabolite.[87] In contrast, the second specimen (from 1995) contained three 9,11-secosterols, luffasterols A **122**–C, as well as manoalide **123** and secomanoalide **124**.[88] Both **120** and **121** inhibited PLA_2 and were anti-inflammatory agents whilst luffasterol A **122** demonstrated activity in the NCI's 60 cell line panel.

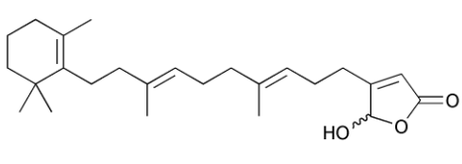
Luffariella variabilis

This sponge has a dark grey to black exterior with a cream to orange interior. It can be differentiated from other sponges of similar exterior appearance because the fibres are usually orange in color. The sponge can be massive or encrusting and has many different shapes. It is sometimes fouled by other sponges and/or tunicates and is one of the 10 most common sponges on the reefs around Palau. In 1981 it was estimated to be the third most common sponge between 10 and 20 m depth at the Short Drop Off. This sponge has also been reported from Guam and the Philippines.

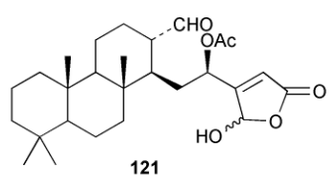
Chemistry/bioactivity: Scheuer and coworkers[89,90] reported the isolation of manoalide **123**, secomanoalide **124** and (*E*)- and (*Z*)-neomanoalides, but did not report their important anti-inflammatory activity. After examining a number of specimens of *L. variabilis*, Kernan *et al.*[91] found that although most individuals contained manoalide and secomanoalide, some contained luffariellins A **125** and B **126** and others contained mixtures of all four compounds. Further research on *L. variabilis* from Palau[92] resulted in the discovery of luffalactone **127** and (4*E*,6*E*)-dehydromanoalide.

Although manoalide was first reported as an antimicrobial agent, its most important activity was as an anti-inflammatory agent. The groups of Jacobs[93–96] and Dennis[97,98] independently established that manoalide inhibits the enzyme phospholipase A_2, which is involved in the initial step of the inflammatory response. Since manoalide was present at a level of about 1% dry weight from a common sponge, it was regarded as a good candidate for drug development and its use as an anti-inflammatory agent was patented by the University of California. It was tested by Allergan Corp. as a topical treatment for psoriasis but the formulation used did not allow sufficient drug to pass through the skin and subsequently Allergan continued work on synthetic compounds based on the manoalide structure. All subsequent trials were not performed with the natural product.

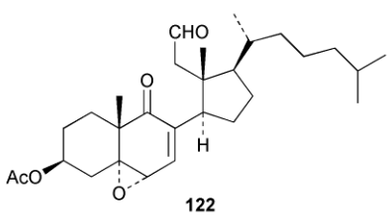
The apparent failure of manoalide as a potential drug did not discourage additional research on details of its mechanism as a phospholipase A_2 (PLA_2) inhibitor.[96,99–102] PLA_2 is the enzyme responsible for the hydrolysis of membrane-bound phospholipids to release arachidonic acid that is subsequently converted into the prostaglandins and leukotrienes that ultimately cause the pain experienced in bee stings, psoriasis, arthritis and other inflammatory conditions. It was shown that manoalide undergoes an irreversible chemical reaction with lysine



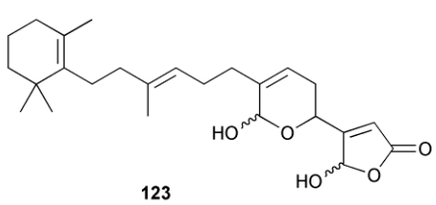
120



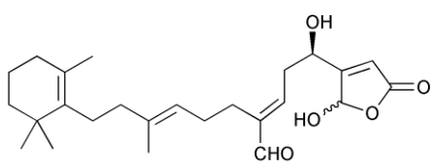
121



122



123

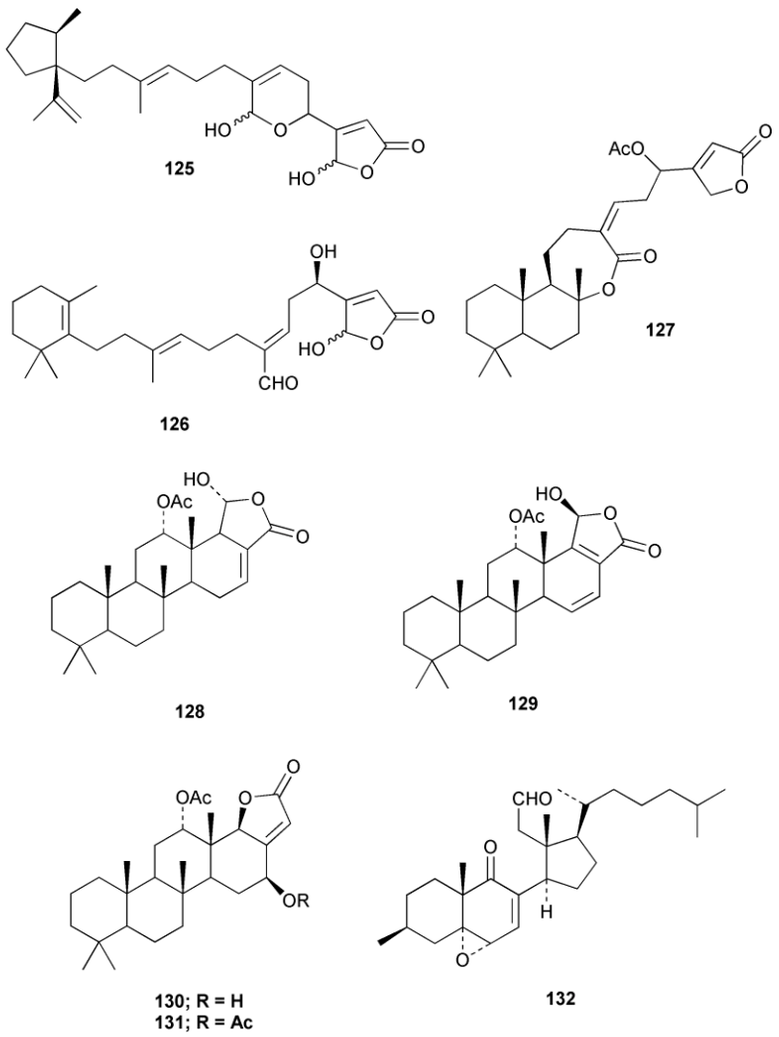


124

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.



units on the interfacial binding site on PLA_2 and thus prevents the correct binding between PLA_2 and membrane-bound phospholipids.[103]

Manoalide is now widely used as a biochemical reagent to inhibit PLA_2, although at higher concentrations it inhibits other lysine-rich enzymes. Interestingly, manoalide also reacts with the lysine residues in sponge fibers to form an orange complex that stains the fibers. The biological activity of luffariellin A **125** is identical to that of manoalide **123**, while secomanoalide **124** and luffariellin B **126** are less active. A structure–activity study of manoalide and its derivatives has been reported[104] together with a review of the work through 1992.[105] As mentioned earlier, there are derivatives based on the manoalide pharmacophore that are still in different research phases in industry.

Spongia matamata

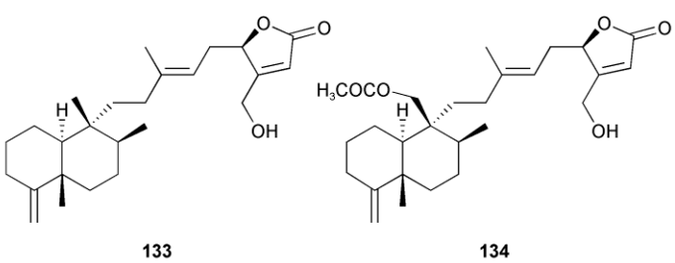
This specimen of *S. matamata* de Laubenfels was collected at Risong Marine Lake in 1995 and a voucher specimen was deposited in the SIO Benthic Invertebrate collection (#P-1165).

Chemistry/bioactivity: *S. matamata* contained scalarin **128**, 12α-acetoxy-19β-hydroxyscalara-15,17-dien-20,19-olide **129**, 12α-acetoxy-16β-hydroxyscalarolbutenolide **130**, 12α,16β-diacetoxyscalarolbutenolide **131**, and a new 9,11-secosterol, 3β-hydroxy-5α,6α-epoxy-9-oxo-9,11-seco-5α-cholest-7-en-11-al **132**.[106] Unpublished research by Dr Robert Jacobs and co-workers (UC Santa Barbara) suggested that **129** exhibited significant anti-inflammatory activity.

Thorectandra sp

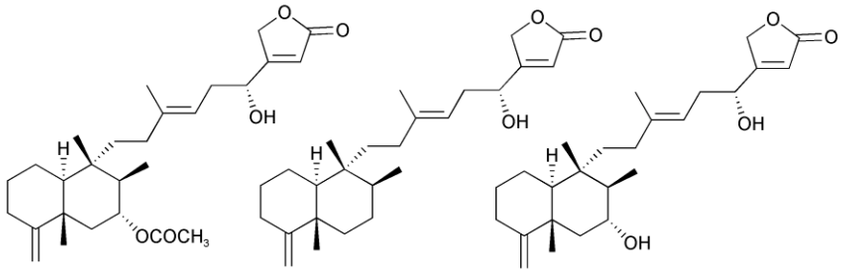
This sponge was collected in Palau by the Coral Reef Research Foundation at 10 m depth and a voucher specimen is held at the Smithsonian Institution (# 0CDN5079).

Chemistry/bioactivity: the crude organic extract of this sponge demonstrated activity against breast and melanoma lines and following bioactivity-driven isolations, five new sesterterpenes, thorectandrols A–E **133**–**137** were reported.[107,108] In addition, the known compounds luffarin R **138**, luffarin V **139** and palauolide **140** and palauolol **141** were also isolated. Both **140** and **141** were shown to have IC_{50} values in the range of 1–100 μg mL^{-1} depending upon cell line. The original reports of

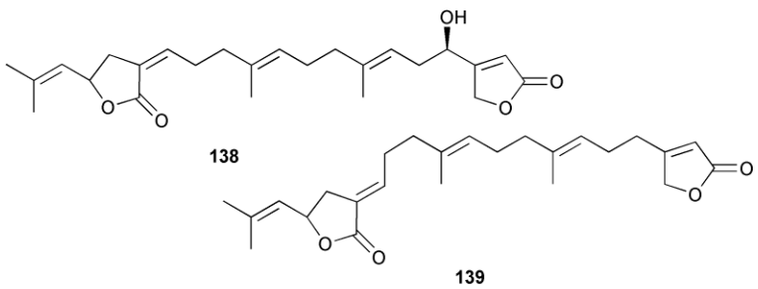


View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

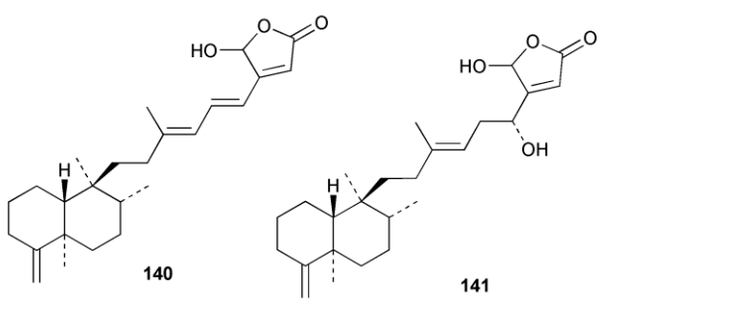


135 136 137

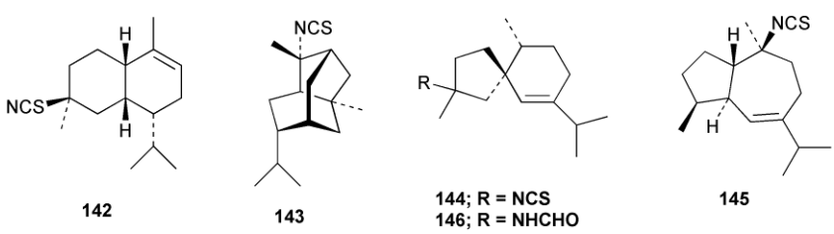


138

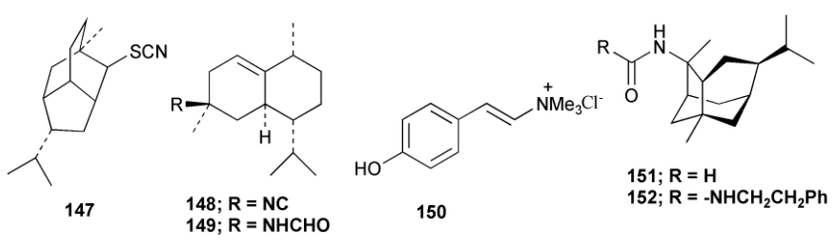
139



140 141



142 143 144; R = NCS 146; R = NHCHO 145



147 148; R = NC 149; R = NHCHO 150 151; R = H 152; R = -NHCH$_2$CH$_2$Ph

the latter two agents did not report this type of activity (*vide infra*).

7.13 Porifera, Demospongiae, Halichondrida

Axinyssa aplysinoides (= Trachyopsis aplysinoides)

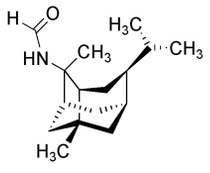
This fairly common peach colored sponge was collected in and around Malakal harbor in 1985 and 1993 and was also reported from Pohnpei.

Chemistry/bioactivity: this sponge (previously called *T. aplysinoides)* was the source of the first naturally occurring sesquiterpene thiocyanate **142** plus three sesquiterpene isothiocyanates **143**–**145** and a formamide **146**.[109] Subsequent studies of *A. aplysinoides* uncovered another thiocyanate **147**,[110] an isonitrile **148**, a formamide **149**, a tyrosine derivative **150**, two unrelated diterpenes, neoverrucosan-5β-ol and homoverrucosan-5β-ol[111] and the formamide **151** and urea **152**,[112] with the structures of **141** and **151** being determined by X-ray analysis. Although sesquiterpene isonitriles, formamides and isothiocyanates are believed to be feeding deterrents, no such studies with this particular group of compounds have been reported. Though the crude extract of another sample of this sponge exhibited DNA damaging activity, the active constituent(s) was/were not reported but two novel nitrogenous sesterterpenes were isolated and purified, 2-(formylamino)-trachyopsane **153** and *N*-phenethyl-*N*-2-trachyopsanylurea **154**.[112]

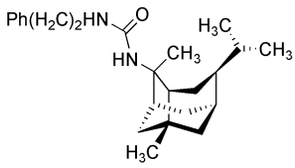
Halichondria* cf. *lendenfeldi

Halichondria cf. lendenfeldi is a dark grey-green hispid rope sponge that was collected in a bay next to the entrance to the Ngell Channel.

View Article Online

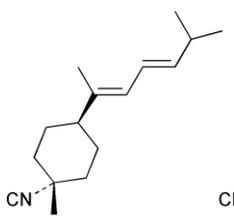


153

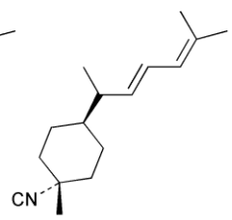


154

Chemistry/bioactivity: *Halichondria* cf. *lendenfeldi* contained two sesquiterpene isonitriles, 3-isocyanotheonellin **155** and 3-isocyanobisabolane-8,10-diene **156** and the corresponding formamides, 3-formamidotheonellin and 3-formamidobisabolane-8,10-diene.[113] No bioactivity data was determined as the sponge was being studied as a possible food source for nudibranchs of the genus *Phyllida*.



155

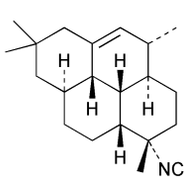


156

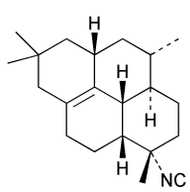
Halichondria sp

A black sponge of the genus *Halichondria* was collected in "Mini Marine Lake" on the east side of Urukthapel due south of MMDC with a voucher specimen deposited in the SIO Benthic Invertebrate Collection (# PI 116).

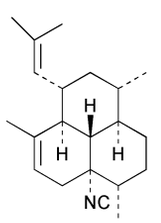
Chemistry/bioactivity: this sponge contained four diterpene isonitriles, 7-isocyano-1-cycloamphilectene **157**, 7-isocyano-11-cycloamphilectene **158**, 8-isocyano-10,14-amphilectadiene **159**, and 8-isocyano-1(12)-cycloamphilectene **160**, that were identified by X-ray analyses.[114] As with other isonitriles, compounds **159** and **160** were mildly antimicrobial against Gram positive bacteria.



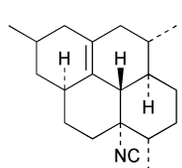
157



158



159

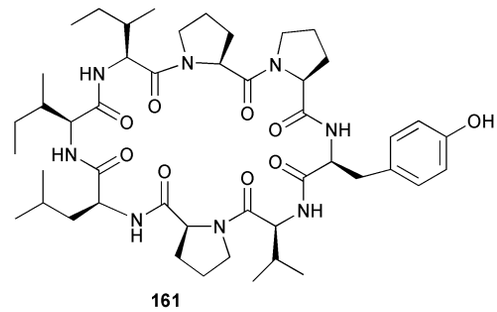


160

Hymeniacidon sp

The specimen of *Hymeniacidon* sp. was collected in 1979 near Long Island and after initial screening, a larger (250 kg) sample was collected in 1985.

Chemistry/bioactivity: the cyclic peptide hymenistatin 1 **161** was obtained in very low yield after an extensive bioassay guided fractionation.[115] Owing to the low yield, hymenistatin 1 was subsequently synthesized.[116] The compound was isolated by following P388 activity and provided a 30% life extension against this murine leukemia.

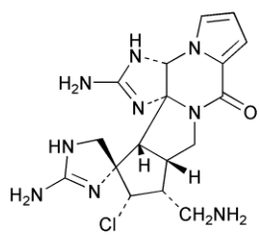


161

Stylotella agminata (renamed *S. aurantium*, 1998)

Specimens of *S. agminata* were collected in 1977 and 1991 near Wonder Channel. In 1998, this sponge was reclassified as *S. aurantium.*[117]

Chemistry/bioactivity: the bisguanidine alkaloid palau'amine **162** was isolated from the 1991 collection of *S. agminata*[118] with five additional related metabolites being reported by workers from the same group in 1998 when the taxonomy was revized.[117] Palau'amine is relatively non-toxic in mice but is cytotoxic to P-388 and A549 cells. It is also antimicrobial and antifungal and showed promise in an immunomodulatory assay. It should be noted that the dust from the dried sponge caused a powerful allergic reaction in man, manifested as shortness of breath for 4 hours.



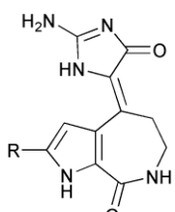
162

There have been several syntheses of this alkaloid in the past but recently, synthetic routes to palau'amine precursors *via* construction of the spirocyclic core,[119] *via* formation of triazacyclopent(*cd*)-pentalenes[120] or *via* modifications of a phakellin synthesis[121] have been reported. These have the potential to construct variations on the structures by synthetic methodologies that could lead to determination of SAR characteristics.

Stylotella aurantium

This golden orange colored sponge is one of the most common sponges found in the shallow waters of the lagoon in Palau. It is illustrated in Colin and Arneson[77] (entry 107).

Chemistry/bioactivity: *S. aurantium* is a good source of hymenialdisine **163** and debromohymenialdisine **164**, compounds that were first described from *Axinella verrucosa* and *Acanthella auriantica* by Cimino *et al.*,[122] and also from *Hymeniacidon aldis*,[123] and *Phakellia flabellata*.[124] In addition, the 10E-geometrical isomers were reported as minor constituents of the sponge in 1996.[125] Debromohymenialdisine (DBH)



163; R = Br
164; R = H

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

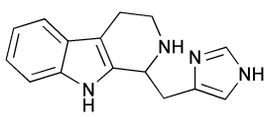
has been identified as a potent anti-inflammatory agent, which led to its being patented for the treatment of rheumatoid arthritis by SmithKline Beecham (SKB, now Glaxo SmithKline) and for osteoarthritis (by OsteoArthritis Sciences Inc., now defunct) and as a protein kinase C inhibitor by SKB. At least two separate syntheses have been published.[126,127]

7.14 Porifera, Demospongiae, Haplosclerida

Order *Haplosclerida*

This sponge was collected by hand in 1993 at a depth of 50 m near the "Blue Hole" in Palau, and was identified by Dr M. Kelly(-Borges), with a voucher sample being deposited at the Natural History Museum in London (# BMNH 1996.9.17.1).

Chemistry/bioactivity: using a bioassay for cathepsin K (a cysteine protease implicated as playing a role in osteoporosis), a new tryptamine derived alkaloid, haploscleridamine **165** was isolated and demonstrated activity in the assay with an IC_{50} value of 26 μM.[128]

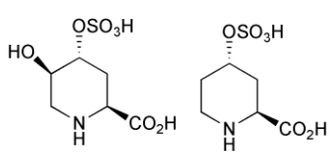


165

Cribochalina olemda

This material was collected in Palauan waters by scientists from the Suntory Institute but no physical collection data were given in the paper. Taxonomic identification was made by Dr J. Hooper of the Queensland Museum and a voucher sample (QMG306418) was deposited there.

Chemistry/bioactivity: using the aqueous extract of the sponge and following activity by inhibition of ^{3}H-CGP39653 binding to *N*-methyl-D-aspartic acid (NMDA) receptors from rat brain, a novel amino acid named cribonic acid **166** that demonstrated good activity (IC_{50} value of 83 nM) was isolated and purified. This compound was also active *in vivo* in mice following direct intracerebroventricular injection and gave an estimated ED_{50} of 29 pM per mouse.[129] What is also of interest is that the des-hydroxy compound **167** was also isolated from two non-Palauan Micronesian sponges, *A. carteri* and *S. aurantium* but was not found in the Palauan sample. This compound was almost as active as the hydroxyl analogue but what is of particular note is that this compound **167** was first reported from a phytochemical study of the legume *Pletophorum africanum*,[130] thus the actual source of the agent may be non-invertebrate.



166 **167**

Haliclona sp. (Red)

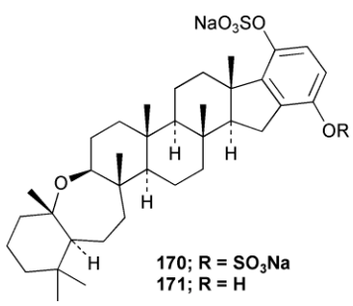
This is a bright red encrusting sponge that is found on reef walls, especially along the south wall of Lighthouse Channel. The sponge resembles the description of *Haliclona hornelli* given by Dendy in 1916, but no definitive identification has been made (van Soest, *pers. comm.*). There is a photograph of what is probably the same sponge in Colin and Arneson[77] but there it is identified as an *Adocia* sp. (entry 122). In Pohnpei the same sponge apparently occurs as part of a two-sponge association with a choristid sponge.

Chemistry/bioactivity: the sponge contains good quantities of two isomeric alkaloids, haliclonadiamine **168** and papua'-amine **169**, which were first described from a specimen of *Haliclona* sp. from Papua New Guinea[131] occurring in a ratio of approximately 2 : 1 in the Palauan specimen with the structure of haliclonadiamine being confirmed by X-ray crystallographic analysis of the corresponding diacetate.[132] There was some confusion about the physical and spectral properties of the compounds because they can form both mono- and di-hydrochloride salts, but there have been several syntheses of these alkaloids reported. Both haliclonadiamine **168** and papua'-amine **169** inhibited *Candida albicans, Bacillus subtilis* and *Staphylococcus aureus* at 1–5 μg.disc^{-1}. Papua'amine also demonstrated reasonably effective antifungal activity against the dermatophyte, *Trichophyton mentagrophytes* at 10 μg.disc^{-1}. In addition, recent work has demonstrated that papua'amine **169** caused vesiculation of the Golgi membranes (see *Hippospongia metachromia*) and work is still continuing on this aspect.

Haliclona (aka *Adocia*)

This pale lilac colored rope sponge was collected at Turtle Island basin and though originally considered to be an *Adocia* sp. it is now placed in the genus *Haliclona.*

Chemistry/bioactivity: *Haliclona* (aka *Adocia)* sp. contains six hexaprenyl hydroquinone sulfates exemplified by adociasulfates 2 **170** and 6 **171**. Adociasulfate 2 and the corresponding mono-sulfate, adociasulfate 6, are the first known natural product inhibitors of kinesin motor proteins, which are responsible for the transport of chemicals along microtubules within the cell,[133,134] with the only other published inhibitors being a synthetic small molecule from a chemical genetics program (monastrol), nucleotide analogues or organic dyestuffs.[135]

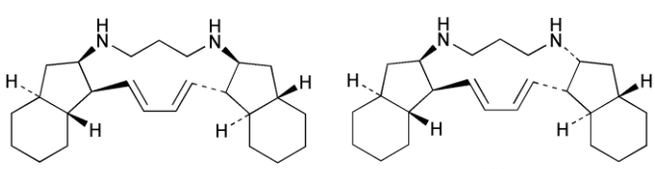


170; R = SO_3Na
171; R = H

Haliclona sp

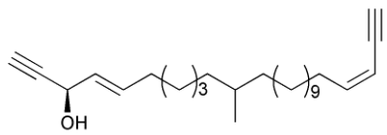
This specimen of a *Haliclona* sp. was collected at a depth of 18–20 m on the fringing reef and a voucher specimen has been deposited at the SIO Benthic Invertebrate Collection (registry # P1160).

Chemistry/bioactivity: the *Haliclona* sp. contained three new acetylenes, (3*R*,4*E*,23*Z*)-3-hydroxy-11-methylhexacosa-4,23-diene-1,25-diyne **172**, (3*Z*,23*Z*)-methylhexacosa-3,23-diene-1,25-diyne, and (3*Z*)-14-methyldocosa-3-en-1-yne, but no bioactivity assays were performed.[136]



168 **169**

View Article Online

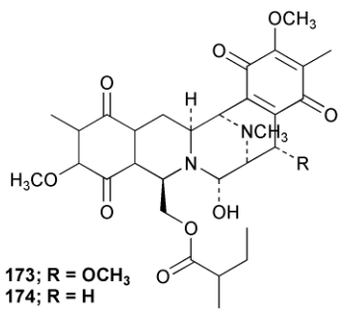


172

***Reniera* sp**

This small purple sponge was collected in 1987 from a small marine lake on Urukthapel Island, which subsequently has almost filled with silt. On further inspection it proved to be the same sponge that had previously been collected on the Pacific coast of Mexico.

Chemistry/bioactivity: the *Reniera* sp. from Palau contained two new alkaloids, renieramycins E **173** and F **174** which were potent antimicrobial agents but unstable.[137] The base structure of these materials is very close to that of the saframycins from a terrestrial streptomycete and to safracin B from a marine pseudomonad.



173; R = OCH_3
174; R = H

7.15 Porifera, Demospongiae, Homosclerophorida

Plakortis* aff. *angulospiculatus

Specimens of *Plakortis* aff., *angulospiculatus* were collected from a reef on the Southwestern side of Palau in 1993 and at Siaes Tunnel in 1996. The Palauan specimens most closely resemble *P. angulospiculatus*, which is a Caribbean sponge.

Chemistry/bioactivity: both specimens of *P.* aff. *agulospiculatus* contained the cyclic peroxide **175** while the 1993 specimen also contained the cyclic peroxide **176**, which indicated that this earlier specimen was probably contaminated with a closely related *Plakortis* species, which was subsequently found among the Palau collections made in 1997. In addition to these cyclic peroxides, a series of related furans was also isolated. Both peroxides inhibited the proliferation of *Leishmania mexicana* promastigotes with **175** having an LD_{50} of 0.29 μg mL^{-1} and peroxide **176** being approximately three-fold less effective with an LD_{50} of 1.00 μg mL^{-1}.[138] What may be of interest is that a number of marine-derived cyclic peroxides have been reported to demonstrate anti-malarial activity as well.

Plakortis lita

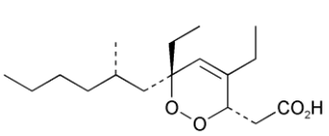
This sponge was collected off Koror Island at depths from 0–10 m in 1995 and 1997, being frozen following collection and maintained frozen until extracted.

Chemistry/bioactivity: the extracts yielded the known compound, plakotenin **177**, its sodium salt **178**, *homo*-plakotenin as both the free acid **179** and sodium salt **180** and the sodium salt of *nor*-plakotenin **181**. Of these five compounds, plakotenin, its sodium salt and *homo*-plakotenin were found to inhibit the proliferation of rheumatoid synovial fibroblasts by 36 to 77% at a concentration of 1 μg mL^{-1} in an assay performed at Smith-Kline Beecham.[139]

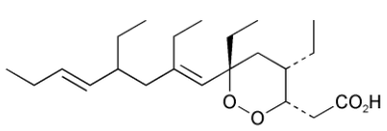
Plakortis nigra

This sponge was collected by hand in Palau at ~120 m using a mixed gas rebreathing apparatus and was deposited in the SIO Benthic Invertebrate Collection (# P1181). The taxonomic identification was by Dr J. Hooper of the Queensland Museum.

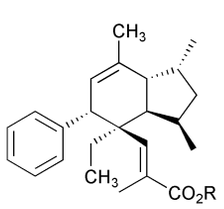
Chemistry/bioactivity: using activity against the human colon tumour cell line HCT-116 as the assay, eight new



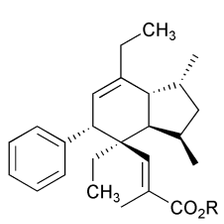
175



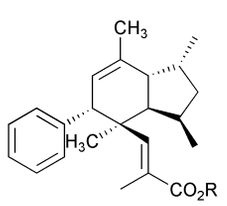
176



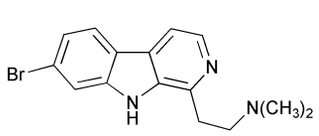
177; R = H
178; R = Na



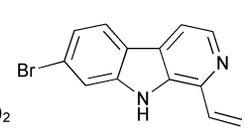
179; R = H
180; R = Na



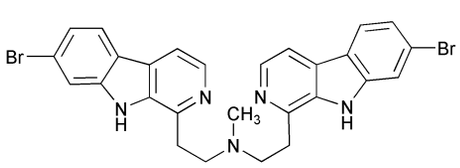
181; R = Na



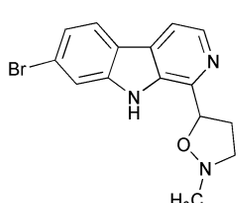
182



183



184

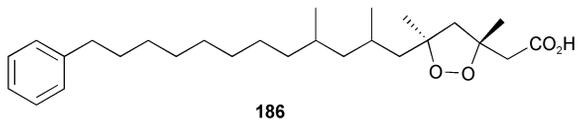


185

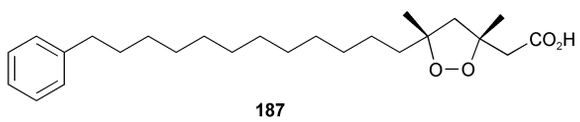
Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online

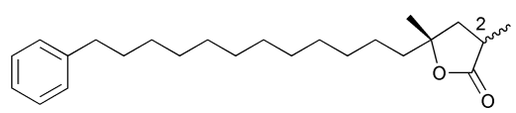
compounds were isolated and purified, of which the first seven demonstrated activities in the 0.4–15 μM range for IC_{50} values.[140] The compounds were plakortamines A–D **182**–**185**, epiplakinic acids G **186** and H **187** and two related γ-lactones, (2*S**,4*R**)-2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone **188** and (2*R**,4*R**)-2,4-dimethyl-4-hydroxy-16-phenylhexadecanoic acid 1,4-lactone **189**.



186



187



188; 2-alpha-CH_3
189; 2-beta-CH_3

7.16 Porifera, Demospongiae, Lithistida

Theonella swinhoei

Specimens of *T. swinhoei* were collected at depths of 20–30 m on the inside of Babelukes Reef and a representative photograph is shown in Colin and Arneson (entry 46).[77]

Chemistry/bioactivity: the Palauan *T swinhoei* contains theonellasterol **190**,[141] the cytotoxic macrolide swinholide A **191**, and the cyclic peptide theopalauamide **192**.[142] Swinholide A was reported to demonstrate strong antitumour activity[143] whilst theopalauamide demonstrated antifungal activity.

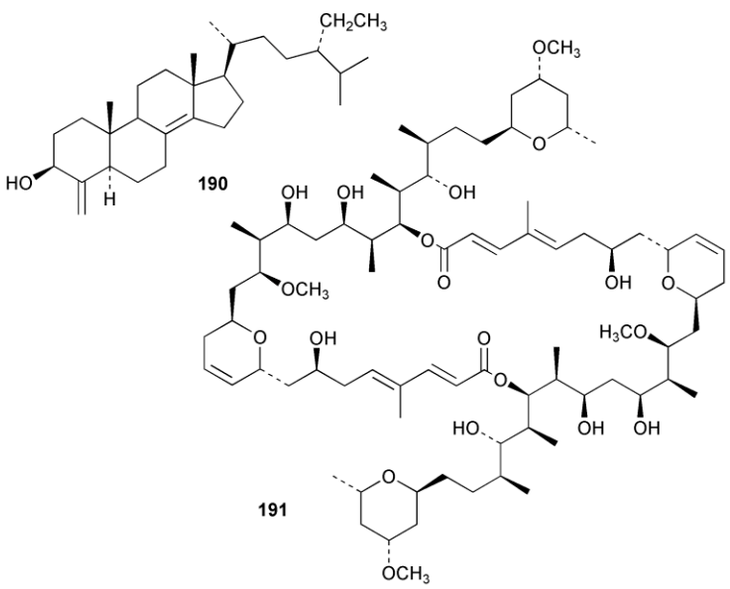
Symbiosis studies: using novel techniques including fixation of the sponge cellular contents and subsequent HPLC and NMR studies on the isolated cells, Bewley *et al.*[3] demonstrated that both **191** and **192** were shown to be associated with symbiotic unicellular bacteria and filamentous fungi, respectively. Identification of the microbes has not been formally reported as yet.

7.17 Porifera, Demospongiae, Petrosida

Oceanapia saggitaria

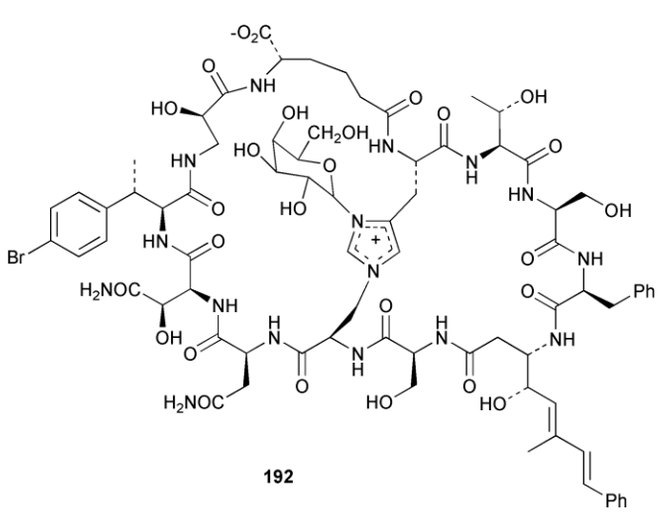
A specimen of *O. sagittaria* was collected in relatively shallow water at Ngeruktebel Marine Lake in 1995 and has been deposited in the SIO Benthic Invertebrate Collection (P1163).

Chemistry/bioactivity: the major metabolite of *O. sagittaria* is dercitamide **193**, which was first isolated from a deep-water sample of a *Stelletta* sp.[144] and also from a tunicate of the genus *Cystodytes*.[145] In addition to dercitamide, whilst working on the cellular location of that compound, the minor metabolite sagitol **194** was isolated and identified.[146] Dercitamide was reported to be a cytotoxic and immunosuppressive agent[144] and very similar compounds of the same pyridoacridine class were



190

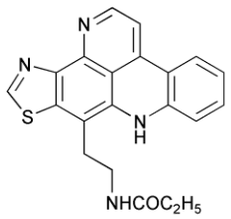
191



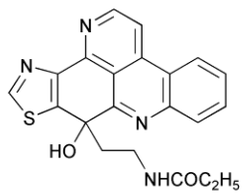
192

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online



193



194

found to be topoisomerase II inhibitors. In contrast, sagitol was inactive in cytotoxicity assays.

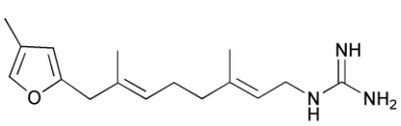
Cellular location studies: since dercitamide (also known as kuanoniamine C) was found in both sponges and ascidians, it had been proposed that the compound was produced by a symbiotic microorganism. Studies using confocal microscopy to detect the natural fluorescence of **193**, following cell separation techniques and chemical analysis, showed that it was localized exclusively in bacteria-free sponge inclusional cells, and was probably not produced by extracellular bacteria and then transferred to the sponge cells.[4]

Finally, there is a recent paper by Skyler and Heathcock describing the "Family Tree" of the pyridinoacridine metabolites that should be consulted for its predictions as to as yet undiscovered/unsynthesized compounds of this general structural class.[147]

Siphonodictyon sp

Siphonodictyon sp. is a white colored sponge that burrows into live coral heads leaving only the oscular chimney protruding. It is not very common and was occasionally found on the western fringing reef of Palau.

Chemistry/bioactivity: this *Siphonodictyon* sp. contained relatively large amounts of siphonodictidine **195**, an unusual guanidinosesquiterpene that inhibits photosynthesis and respiration in *Acropora formosa* causing death of the coral polyps.[148] The siphonodictidine appears to be released in a mucous secretion that inhibits the growth of coral polyps around the oscular chimney.

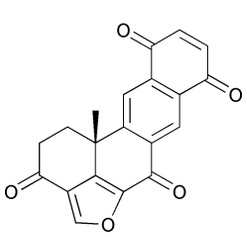


195

Xestospongia exigua

Xestospongia exigua was collected in 1977 and 1981 by members of the Scheuer group and was identified by Bergquist, but no details of the collection are currently available.

Chemistry/bioactivity: the structure of halenaquinone **196** was determined by single crystal X-ray analysis,[149] and the compound exhibited modest *in vitro* activity *versus S. aureus* and *B. subtilis*.

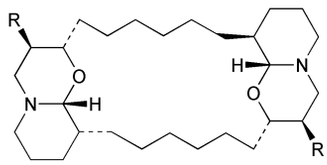


196

Xestospongia sp

This specimen of *Xestospongia* sp. was collected in 1995 at a depth of 20 m from the fringing reef at Palau and a voucher was deposited in the SIO Benthic Invertebrate Collection (P-1168).

Chemistry/bioactivity: the major metabolite isolated from this sponge was araguspongine C **197**, which had previously been described from an Okinawan specimen of *Xestospongia* sp. by Kobayashi *et al.*[150] who reported that it exhibited vasodilative properties in an isolated rat artery model, together with a new minor metabolite, 3β,3β′-dimethylxestospongine C **198**.[151]



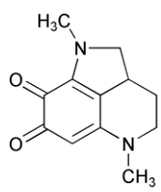
197; R = H
198; R = CH_3

7.18 Porifera, Demospongiae, Poecilosclerida

Damiria sp

This black encrusting sponge, which was collected at Ngemelis drop-off at a depth of 20 m, has since been reclassified as *Zyzzya fuliginosa*.[152]

Chemistry/Bioactivity: two pyrroloquinones, damirones A **199** and B, were isolated from this sponge but no bioactivity was reported.



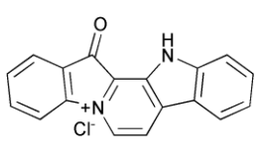
199

7.19 Porifera, Demospongiae, Verongida

Fascaplysinopsis sp

This sponge was collected at Ngemelis drop-off in 1995 and is probably the same sponge that was studied in 1980 from a R/V Alpha Helix cruise where many sponge samples were combined due to a freezer failure.

Chemistry/bioactivity: a compound called palauolide **140** was obtained from the 1980 (mixed) collection of sponges.[153] In contrast, from the 1985 collection palauolol **141** was found to be the major metabolite of the of *Fascaplysinopsis* sp. and was shown to dehydrate to obtain palauolide.[154] This sample also contained the known metabolite fascaplysin **200**, which had been reported in 1988 from a Fijian collection of *Fascaplysinopsis reticulata*.[155] Both **140** and **141** inhibit phospholipase A_2 and antimicrobial activity was reported for **140** and **200**.



200

8 Conclusion

The work that is presented above is just an example of the chemical, biochemical, microbiological and marine biological riches that are present in one atoll in the Central South Pacific Ocean. As a result of the geographic location and the ease of access, together with the support of the Government of the Republic of Belau, scientists have been permitted to investigate the waters of this country.

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

View Article Online

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.

Due to the presence of the NCI's shallow water collection contractor, the Coral Reef Research Foundation in Palau for the last 10 years, Palau has what is probably the best inventory of taxonomically identified marine fauna of any country when expressed on a square kilometre basis. Even with that, there are still immense areas that have not been investigated, particularly in the microbial environment.

One has only to look at the vastly different chemical entities that have been found from what is effectively less than 3 kilograms of cyanophytes (*cf Lyngbya* and *Symploca* above), to realize the immensity of the scientific investigations yet to come and the potential for discovery of novel pharmaceutical agents and biological probes is definitely proven by the work listed above.

The Government of Belau is justifiably reticent in awarding research collection permits to non-citizens for such studies and it was one of John's proudest accomplishments that he held one of the first permits awarded. The foresight of the Government of Belau in giving John such a collection permit is amply demonstrated by the extensive investigations performed by his group over the period 1979–2002 as shown in this review.

This work will be continued both by his students from over the years and by others at Scripps and other universities/institutions, who, like John, have the best interests of Palau at heart, as any samples from their collections that may ultimately be commercialized will have as part of any development programme, a requirement that Palau must benefit.

9 References

1 M. Etpison, *Palau, Portrait of Paradise*, NECO Marine Corp., Palau, 1997.
2 J. N. A. Hooper, J. A. Kennedy and R. J. Quinn, *Biodiv. Conserv.*, 2002, **11**, 851.
3 C. A. Bewley, N. D. Holland and D. J. Faulkner, *Experientia*, 1996, **52**, 716.
4 C. E. Salomon, T. Deerinck, M. H. Ellisman and D. J. Faulkner, *Mar. Biol.*, 2001, **139**, 313.
5 C. E. Salomon and D. J. Faulkner, *J. Nat. Prod.*, 2002, **65**, 689.
6 X. Fu, M. L. G. Ferreira and F. J. Schmitz, *J. Nat. Prod.*, 1999, **62**, 1306.
7 C. J. Smith, D. A. Venables, C. M. Ireland, C. Hopmann, C. E. Salomon, D. J. Faulkner, J. Jompa and A. Tahir, *J. Nat. Prod.*, 1997, **60**, 1048.
8 P. W. Ford and B. S. Davidson, *J. Nat. Prod.*, 1997, **60**, 1051.
9 B. C. M. Potts, D. J. Faulkner, J. A. Chan, G. C. Simolike, P. Offen, M. Hemling and T. A. Francis, *J. Am. Chem. Soc.*, 1991, **113**, 6321.
10 J. Pika and D. J. Faulkner, *Nat. Prod. Lett.*, 1995, **7**, 291.
11 X. Fan, G. R. Flentke and D. H. Rich, *J. Am. Chem. Soc.*, 1998, **120**, 8893.
12 M. A. Rashid, K. R. Gustafson and M. R. Boyd, *J. Nat. Prod.*, 2001, **64**, 1454.
13 M. L. Dionisio-Sese, M. Ishikura, T. Maruyama and S. Miyachi, *Mar. Biol.*, 1997, **128**, 455.
14 C. Ireland and P. J. Scheuer, *J. Am. Chem. Soc.*, 1980, **102**, 5688.
15 C. M. Ireland, A. D. Durso, Jr., R. A. Newman and M. P. Hacker, *J. Org. Chem.*, 1982, **47**, 1807.
16 A. D. Patil, A. J. Freyer, L. Killmer, C. Chambers and R. K. Johnson, *Nat. Prod. Lett.*, 1997, **9**, 181.
17 J. M. Wasylyk, J. E. Biskupiak, C. E. Costello and C. M. Ireland, *J. Org. Chem.*, 1983, **48**, 4445.
18 D. F. Sesin, S. J. Gaskell and C. M. Ireland, *Bull. Soc. Chim. Belg.*, 1986, **95**, 853.
19 C. Hopmann and D. J. Faulkner, *Tetrahedron Lett.*, 1997, **38**, 169.
20 Q. Lu and D. J. Faulkner, *Nat. Prod. Lett.*, 1997, **10**, 231.
21 A. Sato, W. Fenical, Q. Zheng and J. Clardy, *Tetrahedron Lett.*, 1985, **41**, 4303.
22 B. F. Bowden, J. C. Coll, S. J. Mitchell, J. Mulden and G. J. Stokie, *Aust. J. Chem.*, 1978, **31**, 2049.
23 B. F. Bowden, J. C. Coll and A. D. Wright, *Aust. J. Chem.*, 1989, **42**, 757.
24 B. F. Bowden, J. C. Coll, E. D. de Silva, M. S. L. de Costa, P. J. Djura, M. Mahendran and D. M. Tapiolas, *Aust. J. Chem.*, 1983, **36**, 371.
25 S. Mizobuchi, K. Kon-ya, K. Adachi, M. Sakai and W. Miki, *Fish. Sci.*, 1994, **60**, 345.
26 S. Mizobuchi, K. Adachi and W. Miki, *Fish. Sci.*, 1996, **62**, 98.
27 S. S. Mitchell, D. J. Faulkner, K. Rubins and F. D. Bushman, *J. Nat. Prod.*, 2000, **63**, 279.
28 H. Luesch, W. Y. Yoshida, R. E. Moore and V. J. Paul, *Bioorg. Med. Chem.*, 2002, **10**, 1973.
29 H. Luesch, P. G. Williams, W. Y. Yoshida, R. E. Moore and V. J. Paul, *J. Nat. Prod.*, 2002, **65**, 996.
30 H. Luesch, W. Y. Yoshida, G. G. Harrigan, J. P. Doom, R. E. Moore and V. J. Paul, *J. Nat. Prod.*, 2002, **65**, 1945.
31 H. Luesch, W. Y. Yoshida, R. E. Moore and V. J. Paul, *Tetrahedron*, 2002, **56**, 7959.
32 H. Sone, T. Kondo, M. Kiryu, H. Ishiwata, M. Ojika and K. Yamada, *J. Org. Chem.*, 1995, **60**, 4774.
33 P. G. Williams, W. Y. Yoshida, R. E. Moore and V. J. Paul, *J. Nat. Prod.*, 2002, **65**, 1336.
34 P. G. Williams, W. Y. Yoshida, R. E. Moore and V. J. Paul, *J. Nat. Prod.*, 2003, **66**, 1006.
35 P. G. Williams, W. Y. Yoshida, R. E. Moore and V. J. Paul, *J. Nat. Prod.*, 2003, **66**, 620.
36 N. Imamura, M. Nishijima, T. Takadera, K. Adachi, M. Sakai and H. Sano, *J. Antibiot.*, 1997, **50**, 8.
37 K. Yoshikawa, T. Takadera, K. Adachi, M. Nishijima and H. Sano, *J. Antibiot.*, 1997, **50**, 949.
38 M. Namikoshi, K. Akano, S. Meguro, I. Kasuga, Y. Mine, T. Takahashi and H. Kobayashi, *J. Nat. Prod.*, 2001, **64**, 396.
39 M. Suzuki, Y. Nakagawa, S. Harayama and S. Yamamoto, *Int. J. Syst. Evol. Microbiol.*, 2001, **51**, 1639.
40 R. J. Andersen, D. J. Faulkner, C.-h. He, G. D. Van Duyne and J. Clardy, *J. Am. Chem. Soc.*, 1985, **107**, 5492.
41 B. Carte, M. R. Kernan, E. B. Barrabee, D. J. Faulkner, G. K. Matsumoto and J. Clardy, *J. Org. Chem.*, 1986, **51**, 3528.
42 M. R. Kernan, E. B. Barrabee and D. J. Faulkner, *Comp. Biochem. Physiol., B: Biochem. Mol. Biol.*, 1988, **89**, 275.
43 M. Nakagawa, Y. Hamamoto, M. lshihama, S. Hamasaki and M. Endo, *Tetrahedron Lett.*, 1987, **28**, 431.
44 R. Kazlauskas, P. T. Murphy and R. J. Wells, *Aust. J. Chem.*, 1982, **35**, 51.
45 J. C. Braekman, D. Daloze, C. Soller and R. W. M. Van Soest, *Biochem. Syst. Ecol.*, 1992, **20**, 417.
46 R. J. Capon and D. J. Faulkner, *J. Am. Chem. Soc.*, 1984, **106**, 1819.
47 L. Kitagawa, M. Kobayashi, Y. Okamoto, M. Yoshikawa and Y. Hamamoto, *Chem. Pharm. Bull.*, 1987, **35**, 5036.
48 M. Kobayashi, Y. Okamoto and I. Kitagawa, *Chem. Pharm. Bull.*, 1991, **39**, 2867.
49 T. M. Zabriskie, J. A. Klocke, C. M. Ireland, A. H. Marcus, T. F. Molinski, D. J. Faulkner, C. Xu and J. Clardy, *J. Am. Chem. Soc.*, 1986, **108**, 3123.
50 P. Crews, L. V. Manes and M. Boehler, *Tetrahedron Lett.*, 1986, **27**, 2797.
51 A. Zampella, C. Giannini, C. Debitus, C. Roussakis and M. V. D'Auria, *J. Nat. Prod.*, 1999, **62**, 332.
52 M. R. Bubb, A. M. Senderowicz, E. A. Sausville, K. L. Duncan and E. D. Korn, *J. Biol. Chem.*, 1994, **269**, 14869.
53 A. M. Senderowicz, G. Kaur, E. Sainz, C. Laing, W. D. Inman, J. Rodriguez, P. Crews, L. Malspeis and M. R. Grever, *J. Nat. Can. Inst.*, 1995, **87**, 46.
54 W. R. Chan, W. F. Tinto, P. S. Manchand and L. J. Todaro, *J. Org. Chem.*, 1987, **52**, 3091.
55 J. E. Coleman, R. W. M. Van Soest and R. J. Andersen, *J. Nat. Prod.*, 1999, **62**, 1137.
56 B. Kunze, R. Jansen, F. Sasse, G. Hofle and H. Reichenbach, *J. Antibiot.*, 1995, **48**, 1262.
57 F. Sasse, B. Kunze, T. M. A. Gronewold and H. Reichenbach, *J. Nat. Can. Inst.*, 1998, **90**, 1559.
58 G. R. Pettit, C. L. Herald, J. E. Leet, R. Gupta, D. E. Schaufelberger, R. B. Bates, P. J. Clelow, D. L. Doubek, K. P. Manfredi, K. Riltzler, J. M. Schmidt, L. P. Tackett, F. B. Ward, M. Bruck and F. Camou, *Can. J. Chem.*, 1990, **68**, 1621.
59 G. R. Pettit, C. L. Herald, M. R. Boyd, J. E. Leet, C. Dufresne, D. L. Doubek, J. M. Schmidt, R. L. Cemy, J. N. A. Hooper and K. Riltzler, *J. Med. Chem.*, 1991, **34**, 3339.
60 G. R. Pettit, F. Gao, R. L. Cerny, D. L. Doubek, L. P. Tackett, J. M. Schmidt and J.-C. Chapius, *J. Med. Chem.*, 1994, **37**, 1165.
61 G. R. Pettit, J. W. Holman and G. M. Boland, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2411.
62 O. Mechnich and H. Kessler, *Tetrahedron Lett.*, 1996, **37**, 5355.
63 C. M. Cerda-Garcia-Rojas and D. J. Faulkner, *Tetrahedron Lett.*, 1995, **51**, 1087.
64 S. C. Bobzin and D. J. Faulkner, *J. Org. Chem.*, 1991, **56**, 4403.
65 R. Walker and D. Faulkner, *J. Org. Chem.*, 1981, **46**, 1098.
66 J. E. Hocklowski, D. J. Faulkner, G. K. Matsumoto and J. Clardy, *J. Org. Chem.*, 1983, **48**, 1141.
67 B. Sullivan and D. J. Faulkner, *J. Org. Chem.*, 1984, **49**, 3204.

View Article Online

68 T. F. Molinski, D. J. Faulkner, H. Cun-heng, G. D. Van Duyne and J. Clardy, *J. Org. Chem.*, 1986, **51**, 4564.
69 S. C. Bobzin and D. J. Faulkner, *J. Org. Chem.*, 1989, **54**, 5727.
70 M. R. Kernan and D. J. Faulkner, *J. Org. Chem.*, 1988, **53**, 4574.
71 G. Liu, J. Pika and D. J. Faulkner, *Nat. Prod. Lett.*, 1995, **7**, 297.
72 L. V. Manes, P. Crews, M. R. Kernan, D. J. Faulkner, F. R. Fronczek and R. D. Gandour, *J. Org. Chem.*, 1988, **53**, 570.
73 J. Kimura, E. Ishizuka, Y. Nakao, W. Y. Yoshida, P. J. Scheuer and M. Kelly-Borges, *J. Nat. Prod.*, 1998, **61**, 248.
74 H. Nakamura, S. Deng, J. Kobayashi, Y. Ohizumi and Y. Hirata, *Tetrahedron Lett.*, 1986, **42**, 4197.
75 D. M. Kushlan, D. J. Faulkner, L. Parkanyi and J. Clardy, *Tetrahedron Lett.*, 1989, **45**, 3307.
76 B. Carte and D. J. Faulkner, *Tetrahedron Lett.*, 1981, **37**, 2335.
77 P. L. Colin and C. Arneson, *Tropical Pacific Invertebrates*, Coral Reef Press, Beverly Hills, 1995.
78 G. M. Sharma and B. Vig, *Tetrahedron Lett.*, 1972, 1715.
79 M. D. Unson, C. B. Rose, D. J. Faulkner, L. S. Brinen, J. Rios Steiner and J. Clardy, *J. Org. Chem.*, 1993, **58**, 6336.
80 M. D. Unson, N. D. Holland and D. J. Faulkner, *Mar. Biol.*, 1994, **119**, 1.
81 B. Carte, S. Mong, B. Poehland, H. Sarau, J. W. Westley and D. J. Faulkner, *Tetrahedron Lett.*, 1989, **30**, 2725.
82 Q. Lu and D. J. Faulkner, *J. Nat. Prod.*, 1998, **61**, 1096.
83 B. Carte, C. B. Rose and D. J. Faulkner, *J. Org. Chem.*, 1985, **50**, 2785.
84 R. T. Luibrand, T. R. Erdman, J. J. Vollmer, P. J. Scheuer, J. Finer and J. Clardy, *Tetrahedron Lett.*, 1979, **35**, 609.
85 P. A. Takizawa, J. K. Yucel, B. Viet, D. J. Faulkner, T. Deerinck, G. Soto, M. Ellisman and V. Malhotra, *Cell*, 1993, **73**, 1079.
86 K. F. Albizati, T. Holman, D. J. Faulkner, K. B. Glaser and R. S. Jacobs, *Experientia*, 1987, **43**, 949.
87 M. R. Kernan, D. J. Faulkner, L. Parkanyi, J. Clardy, M. S. de Carvalho and R. S. Jacobs, *Experientia*, 1989, **45**, 388.
88 M. V. R. Reddy, M. K. Harper and D. J. Faulkner, *J. Nat. Prod.*, 1997, **60**, 41.
89 E. D. de Silva and P. J. Scheuer, *Tetrahedron Lett.*, 1980, **21**, 1611.
90 E. D. de Silva and P. J. Scheuer, *Tetrahedron Lett.*, 1980, **21**, 3147.
91 M. R. Kernan, D. J. Faulkner and R. S. Jacobs, *J. Org. Chem.*, 1987, **52**, 3081.
92 B. C. M. Potts, R. J. Capon and D. J. Faulkner, *J. Org. Chem.*, 1992, **57**, 2965.
93 J. C. de Freitas, L. A. Blankmeier and R. S. Jacobs, *Experientia*, 1984, **40**, 864.
94 R. S. Jacobs, P. Culver, R. Langdon, T. O'Brien and S. White, *Tetrahedron Lett.*, 1985, **41**, 981.
95 K. B. Glaser and R. S. Jacobs, *Biochem. Pharmacol.*, 1986, **35**, 449.
96 K. B. Glaser and R. S. Jacobs, *Biochem. Pharmacol.*, 1987, **36**, 2079.
97 D. Lombardo and E. A. Dennis, *J. Biol. Chem.*, 1985, **260**, 7234.
98 R. A. Deems, D. Lombardo, B. P. Morgan, E. D. Mihelich and E. A. Dennis, *Biochem. Biophys. Acta*, 1987, **917**, 258.
99 C. F. Bennett, S. Mong, M. A. Clarke, L. I. Kruse and S. T. Crooke, *Biochem. Pharmacol.*, 1987, **36**, 733.
100 P. B. Jacobson, L. A. Marshall, A. Sung and R. S. Jacobs, *Biochem. Pharmacol.*, 1990, **39**, 1557.
101 L. J. Reynolds, E. D. Mihelich and E. A. Dennis, *J. Biol. Chem.*, 1991, **266**, 16512.
102 A. R. Ortiz, M. T. Pisabarro and F. Gago, *J. Med. Chem.*, 1993, **36**, 1866.
103 B. C. M. Potts, D. J. Faulkner, M. S. de Carvahlo and R. S. Jacobs, *J. Am. Chem. Soc.*, 1992, **114**, 5093.
104 K. B. Glaser, M. S. de Carvalho, R. S. Jacobs, M. R. Kernan and D. J. Faulkner, *Mol. Pharmacol.*, 1989, **36**, 782.
105 B. C. M. Potts, D. J. Faulkner and R. S. Jacobs, *J. Nat. Prod.*, 1992, **55**, 1701.
106 Q. Lu and D. J. Faulkner, *J. Nat. Prod.*, 1997, **60**, 195.
107 R. D. Charan, T. C. McKee and M. R. Boyd, *J. Nat. Prod.*, 2001, **64**, 661.
108 R. D. Charan, T. C. McKee and M. R. Boyd, *J. Nat. Prod.*, 2002, **65**, 492.
109 H. He, D. J. Faulkner, J. S. Shumsky, K. Hong and J. Clardy, *J. Org. Chem.*, 1989, **54**, 2511.
110 H. He, J. Salvo, R. F. Catalos and D. J. Faulkner, *J. Org. Chem.*, 1992, **57**, 3191.
111 R. S. Compagnone and D. J. Faulkner, *J. Nat. Prod.*, 1995, **58**, 145.
112 A. D. Patil, A. J. Freyer, R. Reichwein, M. F. Bean, L. Faucette, R. K. Johnson, R. C. Haltiwanger and D. S. Eggleston, *J. Nat. Prod.*, 1997, **60**, 507.
113 K. E. Kassuhlke, C. M. Potts and D. J. Faulkner, *J. Org. Chem.*, 1991, **56**, 3747.
114 T. F. Molinski, D. J. Faulkner, G. D. Van Duyne and J. Clardy, *J. Org. Chem.*, 1987, **52**, 3334.
115 G. R. Pettit, P. J. Clewlow, C. Dufresne, D. J. Doubek, R. L. Cerny and K. Ratzler, *Can. J. Chem.*, 1990, **68**, 708.
116 R. K. Konat, D. F. Mierke, H. Kessler, B. Kutscher, M. Bernd and R. Voegeli, *Helv. Chim. Acta*, 1993, **76**, 1649.
117 R. B. Kinnel, H.-P. Gehrken, R. Swali, G. Skoropowski and P. J. Scheuer, *J. Org. Chem.*, 1998, **63**, 3281.
118 R. B. Kinnel, H.-P. Gehrken and P. J. Scheuer, *J. Am. Chem. Soc.*, 1993, **115**, 3376.
119 A. S. Dilley and D. Romo, *Org. Lett.*, 2001, **3**, 1535.
120 G. Belanger, F.-T. Hong, L. E. Overman, B. N. Rogers, J. E. Tellew and W. C. Trenkle, *J. Org. Chem.*, 2002, **67**, 7880.
121 K. G. Poullennec and D. Romo, *J. Am. Chem. Soc.*, 2003, **125**, 6344.
122 G. Cimino, S. De Rosa, S. De Stefano, L. Mazzarella, R. Puliti and G. Sodano, *Tetrahedron Lett.*, 1982, **23**, 767.
123 I. Kitagawa, M. Kobayashi, K. Kitanaka, M. Kido and Y. Kyogoku, *Chem. Pharm. Bull.*, 1983, **31**, 2321.
124 G. W. Sharma, J. S. Buyer and M. W. Pomerantz, *J. Chem. Soc., Chem. Commun.*, 1980, 435.
125 D. H. Williams and D. J. Faulkner, *Nat. Prod. Lett.*, 1996, **9**, 57.
126 H. Annoura and T. Tatsuoka, *Tetrahedron Lett.*, 1995, **36**, 413.
127 Y. Xu, K. Yakushijin and D. A. Home, *J. Org. Chem.*, 1997, **62**, 456.
128 A. D. Patil, A. J. Freyer, B. Carte, P. B. Taylor, R. K. Johnson and D. J. Faulkner, *J. Nat. Prod.*, 2002, **65**, 628.
129 R. Sakai, H. Matsubara, K. Shimamoto, M. Jimbo, H. Kamiya and M. Namikoshi, *J. Nat. Prod.*, 2003, **66**, 784.
130 S. V. Evans, T. K. M. Shing, R. T. Aplin, L. E. Fellows and G. W. J. Fleet, *Phytochemistry*, 1985, **24**, 2593.
131 B. J. Baker, P. J. Scheuer and J. N. Shoolery, *J. Am. Chem. Soc.*, 1988, **110**, 965.
132 E. Fahy, T. F. Molinski, M. K. Harper, B. W. Sullivan, D. J. Faulkner, L. Parkanyi and J. Clardy, *Tetrahedron Lett.*, 1988, **29**, 3427.
133 C. L. Blackburn, C. Hopmann, R. Sakowicz, M. S. Berdelis, L. S. B. Goldstein and D. J. Faulkner, *J. Org. Chem.*, 1999, **64**, 5564.
134 R. Sakowicz, M. S. Berdelis, K. Ray, C. L. Blackburn, C. Hopmann, D. J. Faulkner and L. S. B. Goldstein, *Science*, 1998, **280**, 292.
135 D. J. Newman, G. M. Cragg, S. Holbeck and E. A. Sausville, *Curr. Cancer Drug Targs.*, 2002, **2**, 279.
136 D. H. Williams and D. J. Faulkner, *J. Nat. Prod.*, 1996, **59**, 1099.
137 H. He and D. J. Faulkner, *J. Org. Chem.*, 1989, **54**, 5822.
138 R. S. Compagnone, I. C. Pisa, H. R. Rangel, F. Dagger, A. I. Suarez, M. V. R. Reddy and D. J. Faulkner, *Tetrahedron Lett.*, 1998, **54**, 3057.
139 A. Qureshi, C. S. Stevenson, C. A. Albert, R. S. Jacobs and D. J. Faulkner, *J. Nat. Prod.*, 1999, **62**, 1205.
140 J. S. Sandler, P. L. Colin, J. N. A. Hooper and D. J. Faulkner, *J. Nat. Prod.*, 2002, **65**, 1256.
141 E. Kho, D. K. Imagawa, M. Rohmer, Y. Kashman and C. Djerassi, *J. Org. Chem.*, 1981, **46**, 1836.
142 E. W. Schmidt, C. A. Bewley and D. J. Faulkner, *J. Org. Chem.*, 1998, **63**, 1254.
143 M. Kobayashi, K. Kawazoe, T. Okamoto, T. Sasaki and I. Kitagawa, *Chem. Pharm. Bull.*, 1994, **42**, 19.
144 G. P. Gunawardana, S. Khomoto and N. S. Burres, *Tetrahedron Lett.*, 1989, **30**, 4359.
145 G. P. Gunawardana, F. E. Koehn, A. Y. Lee, J. Clardy, H. He and D. J. Faulkner, *J. Org. Chem.*, 1992, **57**, 1523.
146 C. E. Salomon and D. J. Faulkner, *Tetrahedron Lett.*, 1996, **37**, 9147.
147 D. Skyler and C. H. Heathcock, *J. Nat. Prod.*, 2002, **65**, 1573.
148 B. Sullivan, D. J. Faulkner and L. Webb, *Science*, 1983, **221**, 1175.
149 D. M. Roll, P. J. Scheuer, G. K. Matsumoto and J. Clardy, *J. Am. Chem. Soc.*, 1983, **105**, 6177.
150 M. Kobayashi, K. Kawazoe and I. Kitagawa, *Chem. Pharm. Bull.*, 1989, **37**, 1676.
151 M. V. R. Reddy and D. J. Faulkner, *Nat. Prod. Lett.*, 1997, **11**, 53.
152 R. W. M. van Soest, S. Zea and M. Kielman, *Bijdragen Dierkunde*, 1994, **64**, 163.
153 B. Sullivan and D. J. Faulkner, *Tetrahedron Lett.*, 1982, **23**, 907.
154 E. W. Schmidt and D. J. Faulkner, *Tetrahedron Lett.*, 1996, **37**, 3951.
155 D. M. Roll, C. M. Ireland, H. S. M. Lu and J. Clardy, *J. Org. Chem.*, 1988, **53**, 3276.

Published on 23 December 2003. Downloaded on 29/10/2014 09:51:10.