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Chemical and Bioactivity Studies on metabolites from Three Fijian Marine Sponges

A thesis submitted to the University of the South Pacific in partial fulfillment of the requirements for the degree of

Master of Science in Chemistry

By
Sachin Singh (BSc)
University of the South Pacific

February 2005
Declaration

I declare that this submission is a result of my own investigation and that, to the best of my knowledge, it contains no material written by another author or previously reported, nor any material that has been submitted as a diploma or any form of another degree at any university or institute of higher learning, except where due acknowledgement is credited in the text.

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University of the South Pacific

Date submitted
Dedication

To my parents Arjun Singh and Sushila Wati who have been very supportive throughout my research and to my sister Roshila, without whom this thesis would not have been possible.
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List of abbreviations

\(^{13}\text{C-NMR}\) Carbon 13 nuclear magnetic resonance

\(^1\text{H-1H COSY}\) \(^1\text{H}-^1\text{H}\) Correlation spectroscopy

\(^1\text{H-NMR}\) Proton nuclear magnetic resonance

2D-NMR Two dimensional nuclear magnetic resonance spectroscopy

bs Broad singlet

\(\text{CyLD}_{99}\) Lethal dose required to kill 99% of cells

\(\text{CyT}\) Titre volume for cytotoxicity test.

(Increase in titre volume = decrease in concentration)

d Doublet

dd Doublet of doublets

\(\text{DEPT-135}\) Distortionless enhancement by polarisation transfer-135

dt Doublet of triplets

ESIMS Electrospray ionisation mass spectroscopy

FT-ICR MS Fourier transform ion cyclotron resonance mass spectroscopy

FTIR Fourier transform infrared spectroscopy

HMBC Heteronuclear multiple bond correlation

HMQC Heteronuclear multiple-quantum coherence

HREIMS High resolution electron ionisation mass spectroscopy

m Multiplet

mult Multiplicity

\(\text{NeLD}_{99}\) Lethal dose required to kill 99% of nematode population

\(\text{NeT}\) Titre volume required for nematode toxicity test.
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<td>nOe</td>
<td>Nuclear overhauser effect</td>
</tr>
<tr>
<td>ppm</td>
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</tr>
<tr>
<td>q</td>
<td>Quartet</td>
</tr>
<tr>
<td>s</td>
<td>Singlet</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>t</td>
<td>Triplet</td>
</tr>
<tr>
<td>UV</td>
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Abstract

Eight metabolites have been isolated from three Fijian marine sponges (*Spongosorites* sp, *Stelleta splendens* and *Styliissa massa*).

A novel polyketide, Spongosoritin A, was isolated from *Spongosorites* sp. This compound was biologically inactive against all bioassays conducted.

From *Stelleta splendens* the known cyclodepsipeptide jaspamide/jasplakinolide, was isolated. Jaspamide exhibited excellent antihelminthic properties against the parasitic nematode *Haemonchus contortus* (NeT=64, NeLD$_{99}$=2.6\(\mu\)g/mL) and cytotoxicity (CyT=1024, CyLD$_{99}$= 0.16\(\mu\)g/mL).

*Styliissa massa* yielded six known metabolites: Bromopyrrole-imidazoles oroidin and sceptrin, Hexabromodiphenyl ether, taurine, zooanemonin and trigonelline. Moderate antihelminthic properties were shown by Oroidin (NeT=8, NeLD$_{99}$=15\(\mu\)g/mL), taurine (NeT=4, NeLD$_{99}$=42\(\mu\)g/mL), zooanemonin (NeT=4, NeLD$_{99}$=50\(\mu\)g/mL).

Hexabromodiphenyl ether (NeT=16, NeLD$_{99}$=8.9\(\mu\)g/mL) and trigonelline (NeT=16, NeLD$_{99}$=3.4\(\mu\)g/mL) showed good antihelminthic activity. Sceptrin was inactive against bioassays conducted.

Structural elucidation was made by spectroscopic methods (1H-NMR, 13C-NMR, 1H-1H COSY NMR, HMBC, HMQC, ESIMS, FTIR) and using MarinLit database.
1.0 Introduction

1.1 Introduction

1.1.1 Historical uses of Natural Products

Man has harnessed drugs from nature for thousands of years. Extracts from animal or plant sources have been used as medicines, poisons and for recreational purposes. The benefits of these have been enormous. Natural products have been the basis of ancient practices of the Ayuverda in India and in Chinese medicine for centuries. The plant and animal extracts contained healing properties that cured various ailments. Monks of the Benedictine order applied *Papaver somniferum* to treat pain and anaesthetize patients. The active ingredient was discovered to be morphine and was first isolated in 1806 (Grabley and Thiericke 2000). In the 17th century the bark of the Peruvian *Cinchona* tree was imported to Europe, where its extract was used to treat malaria. Its active ingredient, quinine was first isolated in 1820. This was later replaced by synthetic derivatives.

Salicin, the active principle in willow-tree bark was used by the Greeks and Romans since 400 BC for its antipyretic and analgesic properties. The acetyl derivative of salicylic acid (more active degradation product of salicin), aspirin, is now one of the most common drugs used in modern day medicine (Grabley and Thiericke 2000).

The discovery of penicillin by Alexander Fleming in 1928 added a new dimension to Natural Product Chemistry. While plants had been the main source of Natural Product drugs, microorganisms were now being looked at with growing interest. Unlike plants,
microbes could be cultured to provide almost unlimited supply of new raw material for
drugs (at moderate costs).

1.1.2 The Marine Environment

The Natural Products Chemist began to look towards the ocean depths in search for
useful, natural compounds. The harsh, competitive Marine environment was home to
many species, which employed biochemical warfare frequently as a means of survival.
Consequently, a whole new range of exciting chemistry and potential biologically active
compounds was available. An added bonus was that many invertebrates had symbiotic
relationships with microorganisms, which was sometimes responsible for the production
of chemical compound of interest. Most of the chemicals found have been highly toxic, a
direct result of trying to survive in the highly competitive marine environment.

Brevetoxin B is a neurotoxin produced by the dinoflagellate *Ptychodiscus brevis*
(Grabley and Thiericke 2000) and is associated with the Red Tide catastrophe’s that
occurs along coastlines causing death of marine life and human poisoning. Other marine
natural products exhibiting toxicity are being tested clinically for anticancer treatment.
Didemnin B, a cyclic peptide was first isolated from the ascidian *Trididemnum solidum* (Rinehart et. al. 1981) and became the first marine metabolite to reach human clinical trials. Didemnin B had anticancer, antiviral as well as immunosuppressant abilities. It was withdrawn from clinical trials after the compound proved to be too toxic.

Dehydrodidemnin B, isolated from another ascidian *Aplidium albicans*, exhibited superior anticancer activity to didemnin B (Sakai et. al. 1996). This compound can be prepared by oxidation of didemnin B or by total synthesis and is undergoing clinical trials.
Bryostatin 1, a macrocyclic metabolite was first isolated from the bryozoan *Bugula neritina* (Pettit et. al. 1982). It was until recently undergoing clinical trials for anticancer treatment. Wender et. al. (1998) synthesized a simplified analog that retains the bioactivity of bryostatin 1.

![Bryostatin 1](image)

1.1.3 Agrochemicals

Both agricultural and pharmaceutical chemistry are vital tools, on the basis of which modern society exists. One gives rise to an abundant food supply while the other provides a healthy mind and body. On the agricultural scene, industrial companies focused on
producing synthetic agrochemicals. The success of these synthetic chemicals (particularly pesticides) accelerated developments in synthetic chemistry. In the pharmaceutical industry synthetic compounds began to quickly replace natural product therapy. Soon problems with the use of synthetic pesticides surfaced. Chlorinated hydrocarbons, such as DDT, began causing problems in the food chain. DDT had been directly linked to the production of thin eggshells in wild birds. Methyl bromide, a popular fumigant and soil sterilant was discovered in groundwater. Methyl bromide causes sterility in human males exposed to it and contributes to the ozone hole at the polar icecaps. The shift to biologically active natural product remedies had major advantages. They were target specific, had high specific activity and were biodegradable (Cutler and Cutler 1999).

Insecticides developed from the natural product template pyrethrin became commercially available in the late 1980s. Cyclopenol, a benzodiazepine from the fungus *Penicillium cyclopium* (Cutler and Cutler 1999) is active against *Phytophthora infestans*, which is responsible for the potato late blight.

\[
\text{Cyclopenol}
\]
Ivermectin, a broad-spectrum antihelminthic is a modified version of avermectins, a group of compounds produced naturally by *Streptomyces avermitilis* (Grabley and Thiericke 2000; Cutler and Cutler 1999).

**Ivermectin**

Mixture of B₁₄: \( R = \text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3 \)

and  \( B₁₆: R = \text{CH}(\text{CH}_3)_2 \)

### 1.1.4 Drug discovery Strategies

Due to the amount of investment involved in researching natural products, it is no longer viable to research “blindly”. Today a chemical screening strategy is in place at most institutions where natural product research is undertaken. Crude extracts taken from test samples are screened for potential bioactivity. Once a “hit” (Crude showing positive
bioactivity) is obtained further research is conducted to identify and isolate the bioactive metabolite (Koch et. al. 2000). This reduces the guesswork involved and increases the chances of finding a biologically active compound.

Bioactivity studies are conducted either at each stage of fractionation or only on the isolated pure metabolite. Bioassay guided fractionation tends to take much longer and is a little more costly but the more active “hits” usually yield the metabolites of interest to the Natural Products Chemist (Faulkner 2000).

1.1.5 Limitations

The biggest drawback of marine natural product chemistry is the insufficient supply of material. To obtain grams of a pure metabolite, hundreds of kilograms of animal material are required. This cannot be obtained without severely affecting the marine ecosystem. Clinical trials are limited due to this reason.

Other problems include getting permission to collect biological specimens and access to collection sites.
1.2 Present Study

The main objectives of this project were to:

- Isolate biologically active secondary metabolites from Marine sources, primarily targeting metabolites with antihelminthic properties. Secondary targets were cytotoxic, antifungal and antibacterial metabolites.
- Isolate a novel compound.
- Isolate a compound previously undiscovered in the particular species of sponges being investigated.

1.2.1 The Project: Nematocides from marine sources

Nematodes or Roundworms (Phylum Nematoda) are the most abundant of metazoan animals. They inhabit almost every substrate, geographical area and multicellular organism. Over 12,000 species have been described, however, biologists believe more unidentified species exist. Although some are free-living freshwater, marine and terrestrial species most are parasitic (Nybakken 1996). The parasitic forms are best known and are of great medical and agricultural significance. Unlike microbial pathogens, nematodes are larger, multicellular organisms. As a result their biochemical processes are different from microorganisms. Therefore most antihelminthic agents (Agosta 1996) tend to be chemically different from drugs used to combat bacteria and fungi.
Marine Natural Products group (MNP) at Melbourne University primarily targets the parasitic nematode *Haemonchus contortus*. This particular nematode is a parasite of ruminants and is a major pest to livestock. The MNP group is dedicated to finding antihelminthic agents from marine sources to combat nematodes affecting the agricultural industry. Some success has been obtained in the past with the discovery of phoriospongin A (Capon et al. 2002) obtained from the sponges *Phoriospongia* sp and *Callyspongia bilamellata*. A nematocidal depsipeptide (LD$_{99}$ 8.3 µg/ml), onnamide F (Vuong et al. 2001) and its methyl ester (LD$_{99}$ 5.2 µg/ml, LD$_{99}$ 2.6 µg/ml) was obtained from the sponge *Trachycladus laevispirulifer*, and thiocyanatin A (a dithiocyanate LD$_{99}$ 1.3 µg/ml) from the sponge *Oceanapia* sp (Capon et al. 2001).

![Phoriospongin A](image-url)
1.2.2 Phylum Porifera

Sponges (Phylum Porifera) have been a rich source of biologically active, structurally novel natural products. The most primitive of multicellular organisms, they specialise in
being sessile. This characteristic, in conjunction with their relative abundance in shallow waters, make sponges an easy target for natural product chemists.

Sponges exhibit a wide variety of colours depending on the species. This makes them rather conspicuous to predators. Other problems arise from harmful microorganisms ingested while filter feeding and from fungal growth. To combat these problems sponges have resorted to chemical warfare as a defence mechanism.

Chemical warfare is not the only reason that chemicals are produced. Reproduction, growth and signalling all involve chemistry.

These chemicals are sought after by Natural Products Chemists in the hope of finding a miracle drug to cure the many ailments that afflicts human health and quality of life.

1.2.3 Bioactive Metabolites from Fijian Sponges

Fiji boasts the third largest reef system in the world. Most of this is undocumented in terms of fauna. Natural products chemists have shown some interest in sponges from Fiji. One of the more interesting classes of bioactive metabolites reported from Fijian sponges are bengamides (Quinoa et. al. 1986). Bengamides have exhibited a variety of biological activity and are currently undergoing clinical trials.

Three Fijian marine sponges: Spongosorites sp, Stylissa massa and Stelleta splendens were chemically investigated in an effort to discover new bioactive metabolites.
1.2.4 Genus *Spongosorites*

Sponges of the genus *Spongosorites* (Demospongiae, Halichondriidae) are generally found in deep marine waters in the Indo-Pacific region. These sponges have received somewhat limited attention from Marine natural products chemists, with only one major structural class of bioactive metabolites, the bis-indole alkaloids having been discovered.

1.2.4.1 Alkaloids

Topsentin and its analogues are a series of bis-indole alkaloids that were isolated from *Topsentia genitrix* (Bartik et. al. 1987), *Spongosorites* sp. (Murray et. al. 1995; Sakemi and Sun 1991; Wright et. al. 1992) and *Hexadella* sp. (Morris and Andersen 1988) sponges.

Topsentin, bromotopsentin and a related dihydro compound were isolated from a Caribbean sponge of the genus *Spongosorites* and were active as antiviral and cytotoxic (P388 leukemia cell line) agents (Tsujii and Rinehart 1988). Nortopsentins A, B and C were isolated from the deep-sea sponge, *Spongosorites ruetzleri* and exhibited cytotoxic and antifungal properties (Sakemi and Sun 1991).
1. $R_1^1 = H$, $R_2^2 = OH$ (Topsentin)
2. $R_1^1 = Br$, $R_2^2 = OH$ (Bromotopsentin)
3. $R_1^1 = OH$, $R_2^2 = H$ (Isotopsentin)
4. $R_1^1 = R_2^2 = OH$ (Hydroxytopsentin)
5. $R_1^1 = R_2^2 = H$ (Deoxytopsentin)

4,5-Dihydro-6”- deoxybromotopsentin

Nortopsentins

A $R_1 = R_2 = Br$
B $R_1 = Br$, $R_2 = H$
C $R_1 = H$, $R_2 = Br$
Researchers at the Harbor Branch Oceanographic Institution, along with Dr Robert Jacobs at UC Santa Barbara, demonstrated that topsentins are potent mediators of both immunogenic and neurogenic inflammation (Faulkner 2000). At the 9th International Symposium on Marine Natural Products (Townsville, July 1998), Dr Jacobs and associates reported synthesising a simple bis-indole derivative possessing excellent anti-inflammatory activity.

Dragmacidins

The dragmacidin series of bisindolyl piperazines are a new class of marine natural products. The first Dragmacidin was isolated in 1988 from the deepwater marine sponge, *Dragmacidon* sp. It was the first bisindolyl marine alkaloid to contain an unoxidized piperazine ring, and exhibited a wide range of biological activity. (Kohmoto et. al.1988).

All members of the series contain a central piperazine ring and two indole units.

Wright et.al. reported dragmacidin D in 1992, from a *Spongosorites* sp of sponge.

Dragmacidin D was found to inhibit growth of feline leukemia virus and P388 and A549 tumor cell lines. It also exhibited activity against fungal pathogens *Cryptococcus neoformans* and *Candida albicans*. In 1998 Capon et. al. isolated dragmacidin D and E from a southern Australian sponge of the same genus. Dragmacidin E was found to be a potent inhibitor of serine-threonine protein phosphatases.
Methylated Purine Base

Methylated purine bases have been isolated from both marine and terrestrial sources. Many of these have exhibited a wide range of biological activity. An example of this structure class, 1, 9-dimethylhypoxanthine was isolated from a *Spongosorites* sp obtained from Southern Australia. This was found to be biologically inactive (Capon et. al. 2000).
1.2.5 Genus *Stelleta*

The major natural metabolites reported from different species of the genus *Stelleta* belong to the classes of terpenes, terpenoids and alkaloids.

1.2.5.1 Terpenes

All Triterpenoids reported from genus *Stelleta*, had isomalbaricane skeletons. In 1982, McCabe et. al. reported a biolocally inactive triterpenoid pigment. Su et. al. (1994) reported stelletin A, a biologically active triterpenoid pigment. Subsequent researchers reported stelletins C, D, E, F and G (McCormick et. al. 1996). All these stelletins were found to be cytotoxic.
Globostellatic Acids A-D were isolated from *S. Globostelleta* (Ryu et. al. 1996). These exhibited antitumour properties.

\[
\text{Globostellatic Acid A}
\]

\[
\text{Globostellatic acid B}
\]

\[
\text{Globostellatic acid C, } R = \text{Ac}
\]

\[
\text{Globostellatic acid D, } R = \text{H}
\]
Stelletadine A, an acylated bisguanidine sesquiterpene alkaloid was reported (Tsukamoto et. al. 1996) to induce metamorphosis of ascidian larvae.

Tsukamoto et. al. (1999b) reported bistelletadines A and B, dimers of the sesquiterpene stelletadines.

A  \( R=H \)
B  \( R=(CH_3)_2C=CHCH_2- \)

*Bistellettadines*
1.2.5.2 Alkaloids

A large number of alkaloids have been reported from the genus *Stelletta*.

Fused ring alkaloids isolated from sponges of genus *Stelletta* were cytotoxic acridine alkaloids, nordercitin, dercitamine and dercitamide [Gunawardana et al. 1989].

Also reported were indolizidine alkaloids, stellettamide A (Hirota et al. 1990) and B (Shin et al. 1997).

\[
\text{Nordercitin}\quad R = N(CH_3)_2 \\
\text{Dercitamine}\quad R = NHCH_3 \\
\text{Dercitamide}\quad R = NHCOC_2H_5 \\
\]

\[
\text{Stelletamide } A
\]
Fusetani et. al. (1994) reported a family of pyridine alkaloids, cyclostellettamines A-F from the sponge *S. maxima*. Cyclostellettamines were found to inhibit the muscarinic acetylcholine binding receptors, which play a role in memory and learning.

A. \(m=1, n=1\)
B. \(m=1, n=2\)
C. \(m=2, n=2\)
D. \(m=1, n=3\)
E. \(m=2, n=3\)
F. \(m=3, n=3\)

*Cyclostellettamines*
Stellettazole A, B and C were found to be antibacterial. Stellettazole A (Tsukamoto et. al 1999a), B and C have all been classified as alkaloids (Matsunaga et. al. 1999).

Stellettazole A

Stellettazole B

Stellettazole C
1.2.6 Genus *Stylotella*

Sponges of the genus *Stylotella* (Demospongiae, Dictyonellidae) have been found to contain cyclopeptides, as well as terpenes and alkaloids.

1.2.6.1 Cyclopeptides

Two cycloheptapeptides were isolated from sponges of the genus *Stylotella*. Stylostatin 1 (Pettit et. al. 1993) has antitumour properties whereas stylopeptide 1 is biologically inactive (Pettit et. al. 1995).

![Stylostatin 1](image)
1.2.6.2 Terpenes

Stylotelline, belonging to a rare class of sesquiterpene isocyanides (Pais et. al. 1987) and stylotellanes, a group of dichloroimines (Simpson et. al. 1997) were isolated from two different sponges of the genus *Stylotella*. The former was found to have antibiotic and antitumour activity while the latter was inactive.
1.2.6.3 Alkaloids

An important family of C$_{17}$N$_{9}$ bioactive bisguanidine alkaloids was isolated from palau’an sponges, *S. aurantium* and *S. agminata*. Palau’amine (Kinnel et. al. 1998) is composed of six contiguous rings with an unbroken chain of eight chiral centres. In 1995 Kato et. al. reported styloguanidines (isopalau’amines) which also belong to this family and were isolated from the same genus of sponge. Palau’amines and its congeners exhibit a wide range of biological activity which includes antibiotic, immunosuppressive and antitumour activity.

![Diagram of Palau’amines](image)

1. $R_1 = R_2 = R_3 = H$
2. $R_1 = R_2 = H, R_3 = \text{Ac}$
3. $R_1 = H, R_2 = \text{Br}, R_3 = H$
4. $R_1 = R_2 = \text{Br}, R_3 = H$

*Palau’amines*

![Diagram of Styloguanidines](image)

5. $R_1 = R_2 = H$
6. $R_1 = H, R_2 = \text{Br}$
7. $R_1 = R_2 = \text{Br}$

*Styloguanidines*
Also found were a range of known ‘nuisance’ compounds: sceptrin, oroidin, dibromophakellin, hymenin, hymenidin, hymenialdisine, and debromohymenialdisine (Patil et al. 1997; Williams and Faulkner 1996). These compounds are found in a large number of sponges of the class Demospongiae. Of these, debromohymenialdisine or DBH has been patented as a Protein Kinase C inhibitor and more recently as a treatment for osteoarthritis (Faulkner 2000). There is still considerable interest in commercial development of DBH as it can easily be synthesized (Xu et. al. 1997).

Debromohymenialdisine
2.0 Experimental

2.1 General Experimental Procedures (Capon et. al. 2003; Capon per comm. 2002)

High performance liquid chromatography (HPLC) was performed using either a Waters 600 solvent delivery system equipped with a Waters 2700 sample manager and Waters 996 photodiode array detector, or a Waters 2790 separations module equipped with Waters 996 photodiode array detector, Alltech 500 evaporative light scattering detector with low temperature adapter, and Waters Fraction Collector II. Both systems operated under PC control running Waters Millenium software operating through Microsoft NT. Size exclusion chromatography was performed using sealed columns packed with Sephadex LH-20 or Sephadex G-10. Fractions generated on the open columns were collected on an ISCO Retriever IV fraction collector. Sealed columns were all connected to an ISCO Tris peristaltic pump fitted with an ISCO UA-60 UV detector and an ISCO UA-500 fraction collector through a column switch.

$^1$H and $^{13}$C as well as the 2-D NMR experiments were performed on either a Varian Unity 400 MHz or a Varian Inova 400 MHz spectrometer in the solvents indicated. All spectra were referenced to residual 1H signals in deuterated solvents. ESIMS were acquired using a Waters 2790 separations module equipped with a Micromass ZMD mass spectrometer at the cone voltage indicated and recorded using Masslynx 3.5 operating through Microsoft NT. High resolution ESI measurement were recorded on a Bruker
BioApex 47e FT-ICR MS (FTMS) fitted with an analytical electrospray at a capillary voltage of 100-150 eV.

Chiral optical rotation measurements ([α]_D) were recorded at room temperature on a Jasco Dip-1000 digital polarimeter in a 100 mm x 2 mm cell. Ultraviolet (UV) absorption spectra were obtained using a Hitachi Model 150-20 double beam spectrophotometer, while infrared (IR) spectra were acquired using a Bio-rad FTS 165 FT-IR spectrometer under PC control running Bio-rad Win-IR software. Solvents indicated were spectroscopic grade solvents.

**Bioassays**

Bioassays were performed by Microbial Screening Technologies Pty Ltd., using published procedures (Gill et al. 1995). Screening was done for nematocides, cytotoxic agents, Protox (procaryotes) and Eutox (Eucaryotes).

**2.2 General Methodology**

**2.2.1 Collection and Identification**

*Spongosorites* sp (USP ID 55-087) was collected on 10th March, 2001 at a depth of 5-15m(18°20.788S; 177°59.751E) from Filis Delight. This sponge was identified by Lisa Goudie (Museum of Victoria, Nov 2002). This has been registered at Museum of Victoria (Registration No.MVF83540).
Stylissa massa (USP ID 55-033) was collected on 9\textsuperscript{th} November, 2000 at a depth of 1.5m (18°20.788S; 177°59.751E) from Suva Barrier Reef. Dr John Hooper (Queensland Museum, Apr 2003) identified this sponge. This has been registered at Queensland Museum (Registration No.QMG319998). *Stylissa massa* was previously known as *Stylotella aurantium*.

*Stelletta splendens* (USP ID 55-029) was collected on 9\textsuperscript{th} November, 2000 at a depth of 5-15m (18°20.788S; 177°59.751E) from Denise Patch. Dr John Hooper (Queensland Museum, Apr 2003) identified this sponge. This has been registered at Queensland Museum (Registration No.QMG20000). *Stelletta splendens* was previously known as *Dorypleres splendens*.

A voucher specimen of all three sponges is being kept at Cold Room 6, Department of Chemistry, University of the South Pacific.

### 2.2.2 Extraction and Isolation

The samples of *Spongororites* sp, *Stelleta splendens* and *Stylissa massa* were collected and stored in 95\% Ethanol. For each of the samples stored in EtOH, one third of the solvent was decanted, the bottle refilled with fresh EtOH and stored for future use. The EtOH extracts were dried \textit{in vacuo}. Thus *Spongororites* gave 65.4mg of crude extract. The masses of *Stelleta splendens* (524.4mg) and *Stylissa massa* (1621.8mg) were obtained and recorded.
The crude extracts were extracted with DCM (3 x 10mL) followed by BuOH (30mL) and H2O (30mL). The BuOH and H2O were placed in a separating funnel, shaken and left to stand until two distinct layers formed. The layers were collected separately. All extracts were dried in vacuo and the mass obtained.

2.2.2.1 *Spongosorites* sp

Three fractions were generated: DCM soluble (24.1 mg, 36.3%), BuOH soluble (6.2 mg, 9.5%) and H2O soluble (35.1 mg, 57.5%). The DCM solubles (CyT =256, CyLD_{99} =0.65) and BuOH solubles (CyT =128, CyLD_{99} =1.70) exhibited bioactivity. The bioactive solubles (BuOH and DCM) displayed similar HNMR and HPLC characteristics and were thus combined. The combined fraction was fractionated further on silica SPE. Material eluting from 20-40% EtOAc in Petroleum spirit was subjected to semipreparative HPLC (gradient 80-100% MeCN in H2O @ 2mL/min), to yield a biologically inactive pure compound (2.2mg, 3.4%) which was later identified as Spongosoritin A.

2.2.2.1.1 Spongosoritin A

Numbering shown on structure (Page 43-44).

\[[\alpha]_D \quad -148.1^0 \quad (c \ 1.54, \text{MeOH}); \quad -111.0^0 \quad (c \ 1.11, \text{CH}_2\text{Cl}_2)\]
IR (CHCl₃) cm⁻¹: 1699, 1674, 1625, 1461, and 1434.

UV (MeOH)λ_max: 272.5 nm

¹H-NMR (400MHz, CDCl₃): "δ = 0.75(t, H-17); 0.93(t, H-12); 1.12(t, H-14); 1.16 (m, H-16a); 1.36(m, H-16b); 1.40(s, H-15); 1.76(m, H-7a); 1.76(m, H-8); 1.96(m, H-7b); 1.96(m, H-11); 2.10(q, H-13); 3.70(s, OMe); 4.80(bs, H-4); 5.02(dd, H-9); 5.23(dt, H-10); 6.21(s, H-5).

¹³C-NMR (100MHz, CDCl₃): "δ = 11.3(C-17); 11.7(C-14); 13.9(C-12); 18.5(C-13); 25.6(C-11); 26.3(C-15); 29.4(C-16); 40.1(C-8); 45.0(C-7); 50.5(OMe); 83.9(C-4); 95.1(C-6); 132.2(C-10); 133.8(C-9); 138.2(C-3); 141.7(C-5); 166.9(C-1); 171.5(C-2);

¹³C-NMR (DEPT 135, CDCl₃): "δ = 11.3(C-17, CH₃); 11.7(C-14, CH₃); 13.9(C-12, CH₃); 18.5(C-13, CH₂); 25.6(C-11, CH₂); 26.3(C-15, CH₃); 29.4(C-16, CH₂); 40.1(C-8, CH); 45.0(C-7, CH₂); 83.9(C-4, CH); 50.5(OMe, CH₃); 132.2(C-10, CH); 133.8(C-9, CH); 141.7(C-5, CH).

¹H-¹H COSY δ= 6.21: 4.80, 2.10(H5: H4, H13); 5.23: 5.02, 1.96 (H-10: H-9, H-11); 5.02: 1.76, 1.76, 1.96, 1.96, 5.23(H-7a, H-8, H-7b, H-11, H-10); 4.80: 6.21(H-4:H-5); 2.10: 6.21,1.12(H-13: H-5, H-14); 1.96: 1.76, 1.76, 5.02(H-7b:H-7a, H-8, H-9); 1.96: 5.23, 0.93(H-11: H-10, H-12); 1.76; ,1.76, 1.96 (H-7a: H-8, H-7b); 1.76: 1.76, 1.96, 5.02,
1.16, 1.36 (H-8: H-7a, H-7b, H-9, H-16a, H-16b); 1.36: 1.76, 1.16, 0.75 (H-16b: H-8, H-16a, H-17); 1.16: 1.76, 0.75, 1.36 (H-16a: H-8, H-17, H-16b); 1.12: 2.10 (H-14: H-13); 0.93: 1.96 (H-12: H-11); 0.75: 1.16, 1.36 (H-17: H-16a, H-16b).

\textbf{HMQC} \delta = 11.3: 0.75(C-17); 11.7:1.12(C-14); 13.9: 0.93(C-12); 18.5:2.10(C-13); 25.6: 1.96(C-11); 26.3: 1.40(C-15); 29.4: 1.16, 1.36(C-16); 40.1: 1.76(C-8); 45.0: 1.76, 1.96(C-7); 50.5: 3.70(OMe); 83.9: 4.80(C-4); 132.2: 5.23(C-10); 133.8: 5.02(C-9); 141.9: 6.21(C-5).

\textbf{HMBC} \delta = 4.80: 171.5, 138.2 (H-4: C-2, C-3); 6.21: 171.5, 138.2, 95.1, 18.5 (H-4: C-2, C-3, C-6, C-13); 1.76: 40.1, 133.8 (H-7a: C-8, C-9); 1.96: 141.7, 95.1, 40.1, 29.4 (H-7b: C-5, C-6, C-8, C-16); 1.76: 133.8 (H-8: C-9); 5.02: 40.1, 132.2, 25.6 (H-9: C-8, C-10, C-11); 5.23: 40.1, 133.8, 25.6, 13.9 (H-10: C-8, C-9, C-11, C-12); 1.96: 132.2, 13.9 (H-11: C-10, C-12); 0.93: 132.2, 25.6 (H-12: C-10, C-11); 2.10: 138.2, 141.7, 11.7 (H-13: C-3, C-5, C-14); 1.12: 138.2, 18.5 (H-14: C-3, C-13); 1.40: 141.7, 95.1, 45.0 (H-15: C-5, C-6, C-7); 1.16: 40.1 (H-16a: C-8); 1.36: 40.1 (H-16b: C-8); 0.75: 40.1, 29.4 (H-17: C-8, C-16); 3.70: 166.9(OMe: C-1).

\textbf{nOe:} 4.80: 2.10, 3.70 (H-4: H-13, OMe); 6.21: 4.80, 2.10, 1.12 (H-5: H-4, H-13, H-14); 3.70: 4.80, 1.12 (OMe: H-4, H-14).

\textbf{ESI (+) ms:} 293 [M+H]^+, 315 [M+Na]^+

\textbf{ESI (+) HRMS:} m/z 315.19124 with an error of -3.8ppm, for $\text{C}_{18}\text{H}_{28}\text{O}_{3}\text{Na}$. 
Molecular Formula: C_{18}H_{28}O_{3}

2.2.2.2 Stelleta splendens

The crude was partitioned into DCM solubles (16.5mg, 3.3%), BuOH solubles (70.9mg, 13.5%) and H_{2}O solubles (437mg, 83.3%). The bioactive DCM and BuOH solubles revealed identical HNMR and HPLC characteristics and were combined.

Solvent trituration of the combined fraction was conducted using the following solvents (in order): hexane, EtOAc, DCM and MeOH (30 mL each). EtOAc fraction yielded Jaspamide (13.6mg, 2.6%) as colourless oil.

Marfey’s Analysis was carried out to confirm stereochemistry as that published in literature (Fujii et. al. 1997).

2.2.2.2.1 Marfey's Analysis

Used to determine D and L isomers of common amino acids.

Acid Hydrolysis

Samples to be analysed (100 μg) were dissolved in 6N HCl (0.5 mL) and stirred for 24 hrs at 110°C. The resulting solution was dried in vacuo and redissolved in H_{2}O (50 μL).
Marfey's Reaction

50μL of the acid hydrolystate (or authentic amino acid standard) was added to 20μL of 1M sodium bicarbonate and 100μL of 1% 1-Fluoro-2,4-dinitrophenyl-5-L-alaninamide(1-FDAA) in acetone. The resulting mixture was stirred at 37°C for 60 minutes. The solution was then neutralized with 1N HCl and diluted with 810 μL of acetonitrile.

HPLC analysis

Column: Phenomex Luna C_{18}(2) 4.6mm x 150mm

Flow rate: 1 mL/min

Gradient: linear 45 min [15% B to 45% B] in A.

A: 0.1M aqueous NH₄Ac adjusted to pH 3 with Trifluoroacetic acid (TFA)

B: MeCN

Detection: PDA with selective monitoring at 340 nm

2.2.2.2 Jaspamide/Jasplakinolide

Numbering shown on structure (Page 48).

[ɑ]_D +30.4 (0.93, CH₂Cl₂)

[ɑ]_D +20.7 (0.97, CH₃OH)
$^1$HNMR (CDCl$_3$, 400MHz): $\delta$ = 0.72(3H, d, H-39); 0.80(3H, d, H-20); 1.02(3H, d, H-21); 1.09(3H, d, H-18); 1.27(2H, m, H-7); 1.53(3H, s, H-19); 2.13(1H, m, H-6); 2.60(2H, ddd, H-2, H-10); 2.95(3H, s, H-38); 3.32(2H, m, H-28); 4.70(3H, m, H-5, H-8, H-16); 5.22(1H, m, H-11); 5.75(1H, dd, H-14); 6.60(NH, d, H-17); 6.65(2H, d, H-24, H-26); 6.90(2H, d, H-23, H-27); 7.15(1H, m, H-35); 7.20(1H, s, H-36); 7.50(1H, m, H-33); 8.50 (NH, s, H-29).

$^{13}$CNMR (CDCl$_3$, 100MHz): $\delta$= 17.7(C-39); 18.5(C-19); 19.0(C-21); 20.3(C-18); 21.9(C-20); 23.3(C-28); 29.2(C-6); 30.8(C-38); 39.7(C-10); 40.1(C-2); 40.7(C-3); 43.3(C-7); 45.9(C-16); 48.9(C-11); 55.5(C-14); 70.7(C-8); 109.0(C-30); 110.6(C-36); 115.5(C-24, C-26, C-31); 118.1(C-33); 120.1(C-35); 122.4(C-34); 127.2(C-23, C-27); 127.8(C-5); 131.3(C-4, C32); 133.6(C-22); 136.1(C-37); 155.6(C-25); 168.9(C-15); 170.8(C-13); 174.3 (C-9); 175.1(C-1).

**HPLC retention times for Marfey's Analysis**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>13.10</td>
</tr>
<tr>
<td>D-Alanine</td>
<td>18.11</td>
</tr>
<tr>
<td>Jaspamide (Marfey's deriv.)</td>
<td>13.60, 37.38, 39.83</td>
</tr>
</tbody>
</table>

**ESI (+) ms** $= 709.26$ [M+H]$^+$

**ESI (-) ms** $= 707.26$ [M-H]$^+$

**Molecular Formula**: C$_{36}$H$_{45}$N$_4$O$_6$Br
This was determined by using Mass Spec data to conduct a search on Marinlit database. A match was made with known compounds. Spectroscopic data matched well with that of known compound, Jaspamide (Zabriskie et al 1986; Crews et al 1986).

### 2.2.2.3 *Stylissa massa*

The Crude (1621.8mg) was partitioned into DCM solubles (95mg, 5.9%), BuOH solubles (208.6mg, 15.2%) and H2O solubles (1298.4, 80.1%).

BuOH solubles were fractionated further by C18 Solid Phase Extraction to obtain 11 fractions. The fractions (each 10mL) were eluted in order with the following solvent mixture (H2O: MeOH): 1 (Insoluble residue in 80:20), 2-3 (80:20), 4-5(60:40), 6-7 (40:60), 8-9 (20:80), 10-11 (100% MeOH).

Based on HNMR and ESI (±) MS data, a hexabromodiphenyl ether (Fr 1-2, 58.3mg) eluted early from the SPE, followed by known bromo sponge metabolites oroidin (Fr 4, 3.3mg) and Sceptrin (Fr 11, 2.6mg). Fraction 3 was subjected to semipreparative C18 HPLC (gradient) to yield Trigonelline (2.0mg).

Part of the H2O solubles (256mg) were fractionated further on a G10 Sephadex column using H2O as the eluent. 60 fractions were collected at a flowrate of 2.7mL/min with each fraction volume being 8mL respectively. Zooanemonin (24.1mg) eluted early (Fr 10-13), followed by taurine (35.7mg, Fr 20-25) and additional trigonelline (58.2mg, Fr 32-43).
2.2.2.3.1 Oroidin

$^1$H-NMR (400MHz,CDCl$_3$): $\delta = 4.02$ (H-8: d, 2H), 6.03 (H-9: dt, 1H), 6.24 (H-10: d, 1H), 6.74 (H-12: s, 1H), 6.80 (H-4: s, 1H).

ESI(+)MS = 390 [M+H]

Molecular Formula: C$_{11}$H$_{13}$N$_5$OBr$_2$ (MarinLit)

A search on marinlit database using Mass Spectrometry data found a match with the known compound, Oroidin. Spectroscopic data (experimental) matched that of literature (Garcia et al 1973; Lindel et al 2000).

2.2.2.3.2 Sceptrin (Dimer of Debromooroidin/Hymenidin)

$^1$H-NMR (400MHz,CDCl$_3$): $\delta = 2.29$ (H-8: s, 1H), 3.43 (H-9: s, 2H), 6.40 (H-4: s, 1H), 6.70 (H-14/16: s, 1H), 6.90 (H-16/14: s, 1H)

ESI(+)MS = 619 [M+H], 621[M+2+H], 623[M+4+H]

Molecular Formula: C$_{22}$H$_{26}$N$_{10}$O$_2$Br$_2$ (Marinlit)

A search on marinlit database using Mass Spectrometry data found a match with the known compound, Sceptrin. Spectroscopic data (experimental) matched with that of literature (Walker et al 1981).
2.2.2.3.3 Trigonelline

$^1$H-NMR (400MHz,CDCl$_3$): $\delta$ = 4.28(H8: s, 3H), 7.82(t), 8.70(d), 9.01(s) [4H].

ESI(+)MS = 138 [M+H]

Molecular Formula:
A search on marinlit database using Mass Spectrometry data found a match with the
known compound, Trigonelline.

2.2.2.3.4 Zooanemonin

$^1$H-NMR (400MHz,CDCl$_3$): $\delta$ = 3.46(H-6/7: s, 3H), 3.58(H-6/7: s, 3H), 3.70(H-8: s, 2H), 7.06(H-5: s, 1H), 8.40(H-2: s, 1H).

ESI(+)MS = 155 [M+H]$^+$

Molecular Formula: C$_7$H$_{10}$N$_2$O$_2$ (Marinlit)
A search on marinlit database using Mass Spectrometry data found a match with the
known compound, Zooanemonin. Spectroscopic data (experimental) matched that of
literature (Hattori et al 2001).
2.2.2.3.5 Hexabromodiphenyl ether

$^{1}$H-NMR (400MHz, CDCl$_3$): $\delta = 6.50$(H-4':s), 7.37(H-5':s)

ESI(+)MS = 670 [M]$, 672[M+2]-, 674[M+4]$

Molecular Formula: C$_{12}$H$_4$O$_3$Br$_6$ (Marinlit)


2.2.2.3.6 Taurine

$^{1}$H-NMR (400MHz, CDCl$_3$): $\delta$ = 3.11(t), 3.30(t)

Molecular Formula: C$_2$H$_7$NO$_3$S

A search on marinlit database using Mass Spectrometry data found a match with the known compound, Taurine. Comparison of experimentally obtained data was made with spectroscopic data obtained from an authentic standard of Taurine, with results matching perfectly.
3.0 Discussion

3.1 Polyketide

The structure elucidation of a novel polyketide, Spongosoritin A, from the sponge *Spongosorites* sp is described below.

3.1.1 Spongosoritin A

This compound was isolated as a clear oil.

It was assigned the Molecular formula C$_{18}$H$_{28}$O$_{3}$ by High Resolution Mass Spectrometry ( (M+Na, m/z= 315.1924). Five double bond equivalents were calculated from the molecular formula.

H to C assignments was made on the basis of DEPT 135 and HMQC experiments (Refer Table 1).

From the $^1$HNMR and $^{13}$CNMR the following were deduced:

1. Six olefinic carbons were present together with 3 vinylic protons ($\delta_C$=132.2, 133.8, 95.1, 141.7, 171.5, 138.2; $\delta_H$=5.23, 5.02, 6.21)

2. Three primary methyls ($\delta_C$=11.3,11.7,13.9 ; $\delta_H$=0.75, 1.12, 0.93)

3. One deshielded tertiary methyl group ($\delta_C$=29.4, $\delta_H$=1.24)

4. One methoxyl group ($\delta_C$=50.5, $\delta_H$=3.70)

5. One methine substituted by two oxygens ($\delta_C$=83.8, $\delta_H$=4.80).
6. One ester carbonyl ($\delta_{C=}$ 166.9).

The C-C connectivity was deduced using $^1$H-$^1$H COSY and HMBC (see Table 1).

The following moiety was deduced from $^1$H-$^1$H COSY:

![Fig 1. $^1$H-$^1$H COSY correlations](image)

From HMBC (Refer Table 1) this moiety was further elucidated and two possible partial structures were deduced:
Comparing the molecular formula of the tentative partial structures (Fig 2) with the molecular formula for the compound showed that C₃H₄O₃ were still unassigned. This consisted of a methine (δ_C=83.8, δ_H=4.80), methoxyl group (δ_C=50.5, δ_H=3.70), and an ester carbonyl (δ_C= 166.9); deduced previously from NMR data. Drawing possible structures showed that one of the oxygen atoms on the methine was from the methoxyl group while the other was part of an ester. The carbonyl present is actually an ester as no other C (apart from the ones mentioned) shows a downfield shift associated with having oxygen attached. This formed the moiety:
When combining the two moieties deduced (Fig 1 and Fig 2), comparison of the molecular unsaturation number to the count of unsaturation sites indicated that one additional ring was present which could contain the ester. This ring was either 6-membered or 5-membered.

Combining this data gave four possible structures:

\[ R_1 = \text{-CH}_2\text{CH(C}_2\text{H}_5\text{)CH=CHC}_2\text{H}_5 \]

\[ R_2 = \text{-CH} = \text{C(CH}_3\text{)CH}_2\text{CH(C}_2\text{H}_3\text{)CH=CHC}_2\text{H}_5 \]

\[ \text{(i)} \]

\[ \text{(ii)} \]

\[ \text{(iii)} \]

\[ \text{(iv)} \]

\[ R_1 = \text{-CH}_2\text{CH(C}_2\text{H}_5\text{)CH=CHC}_2\text{H}_5 \]

\[ R_2 = \text{-CH} = \text{C(CH}_3\text{)CH}_2\text{CH(C}_2\text{H}_3\text{)CH=CHC}_2\text{H}_5 \]

*Fig. 4*
Nuclear Overhauser Effect (nOe) experiments showed enhancements at H4 ($\delta_H=4.80$), H13 ($\delta_H=2.10$), H14 ($\delta_H=1.12$) when H5 ($\delta_H=6.21$) was irradiated, OMe ($\delta_H=3.70$), H13 ($\delta_H=2.10$) when H4 ($\delta_H=4.80$) was irradiated and H4 ($\delta_H=4.80$), H14 (1.12) when OMe ($\delta_H=3.70$) was irradiated. This meant that C4 was attached to C3 (Thus enhancing H13 and OMe but not affecting H15).

As H15 was unaffected by any of the above nOe experiments the number of possible structures were now limited to two (Structures: ii and iii).

Relooking at HMBC correlations (H to C) for H15 showed that neighbouring carbons were C5, C6 and C7, which supported structure (iii).

Hence the structure was tentatively determined as $1_a$ and was assigned the trivial name, Spongiosoritin A.
While this structure matched with most of the spectroscopic data there were still irregularities:

1) Olefinic sp\(^2\) carbons do not usually occur at 95 ppm (C6), especially in a trisubstituted double bond as proposed in 1\(_a\).

2) Acetal carbons (C4) don’t usually occur at 84 ppm.

An alternative to 1\(_a\), is a lower homologue of a polyketide sponge metabolite 2, reported by Faulkner et al in 1980. The spectroscopic data for 2 agree closely with those of Spongosoritin A.

In Spongosoritin A (1\(_b\)) the C6 ethyl substituent in 2 is replaced by a methyl substituent. The IR spectrum of ester 2 contained a complex group of bands at 1710, 1690 and 1640 cm\(^{-1}\). This compared relatively well to Spongosoritin A, which had IR bands at 1699, 1674 and 1625 cm\(^{-1}\).

Faulkner deduced the structure of ester 2 by \(^1\)H-NMR, \(^{13}\)C-NMR and ozonolysis of the ester 2.
In comparing the two possible structures for Spongosoritin A: 1\textsubscript{a} and 1\textsubscript{b}; C7 to C12 and C16, C17 moieties were the same in both structures.

The notable differences between 1\textsubscript{a} and 1\textsubscript{b} were the carbon appearing at 95 ppm (C6), was not an sp\textsuperscript{2} hybridized carbon but a tertiary sp\textsuperscript{3} carbon attached to an oxygen atom and the acetal carbon (C4) in 1\textsubscript{a} was an sp\textsuperscript{2} hybridized carbon forming an alkene bond (C2) in 1\textsubscript{b}. Even with this rearrangement, the 2D-NMR data matches with the new structure proposed.

Again there are some doubts, as tertiary sp\textsuperscript{3} hybridised carbons even those with a single oxygen substituent, do not occur easily at 95 ppm and olefinic carbons very rarely occur at 84 ppm. Both structures: 1\textsubscript{a} and 1\textsubscript{b} correspond to most of the spectroscopic data including the 2D-NMR data. Both structures are tentative at this stage as neither possibility can be ruled.

Unfortunately spectroscopic data available is insufficient to conclusively determine the final structure. Further analysis is required to discard one of the possible structures.

Although the crude fraction showed cytotoxic activity (CyT= 256, LD\textsubscript{99}= 0.65) Spongosoritin A was completely inactive against all bioassays. As this was a bioassay-guided fractionation, this compound has either degraded or needs to work in tandem with another compound to be biologically active.
Table 1: NMR data for Spongosoritin A

<table>
<thead>
<tr>
<th>Atom #</th>
<th>$^{13}$C NMR (ppm)</th>
<th>$^1$H NMR (ppm)</th>
<th>Type of C (HMQC, DEPT-135)</th>
<th>$^1$H-$^1$H COSY</th>
<th>HMBC (H to C Correlations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>166.9</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>171.5</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>138.2</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>83.9</td>
<td>4.80 (bs)</td>
<td>CH H5</td>
<td></td>
<td>C2, C3</td>
</tr>
<tr>
<td>5</td>
<td>141.7</td>
<td>6.21 (s)</td>
<td>CH H4, H13</td>
<td></td>
<td>C2, C3, C6, C13</td>
</tr>
<tr>
<td>6</td>
<td>95.1</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>45.0</td>
<td>1.76 (m)</td>
<td>CH H8, H9</td>
<td></td>
<td>C8, C9, C5, C6, C8, C16</td>
</tr>
<tr>
<td>7b</td>
<td></td>
<td>1.96 (m)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>40.1</td>
<td>1.76 (m)</td>
<td>CH H7a, H7b, H9, H11, H16a, H16b</td>
<td>C9</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>133.8</td>
<td>5.02 (dd)</td>
<td>CH H7b, H8, H10, H11</td>
<td></td>
<td>C8, C10, C11</td>
</tr>
<tr>
<td>10</td>
<td>132.2</td>
<td>5.23 (dt)</td>
<td>CH H9, H11</td>
<td></td>
<td>C8, C9, C11, C12</td>
</tr>
<tr>
<td>11</td>
<td>25.6</td>
<td>1.96 (m)</td>
<td>CH2 H8, H9, H10, H12</td>
<td></td>
<td>C10, C12</td>
</tr>
<tr>
<td>12</td>
<td>13.9</td>
<td>0.93 (t)</td>
<td>CH3 H11</td>
<td></td>
<td>C10, C11</td>
</tr>
<tr>
<td>13</td>
<td>18.5</td>
<td>2.10 (q)</td>
<td>CH2 H5, H14</td>
<td></td>
<td>C3, C5, C14</td>
</tr>
<tr>
<td>14</td>
<td>11.7</td>
<td>1.12 (t)</td>
<td>CH3 H13</td>
<td></td>
<td>C3, C13</td>
</tr>
<tr>
<td>15</td>
<td>26.3</td>
<td>1.40 (s)</td>
<td>CH3</td>
<td></td>
<td>C5, C6, C7</td>
</tr>
<tr>
<td>16a</td>
<td>29.4</td>
<td>1.16(m, 1H)</td>
<td>CH2 H8, H16b, H17</td>
<td></td>
<td>C8, C8</td>
</tr>
<tr>
<td>16b</td>
<td></td>
<td>1.36(m, 1H)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>11.3</td>
<td>0.75 (t, 3H)</td>
<td>CH3 H16a, H16b</td>
<td></td>
<td>C8, C16</td>
</tr>
<tr>
<td>OMe</td>
<td>50.5</td>
<td>3.70 (s, 3H)</td>
<td>CH3</td>
<td></td>
<td>C1</td>
</tr>
</tbody>
</table>

Assignments supported by HMQC and Dept 135 experiments.
Numbering follows Structure $\text{I}_a$.
Changes to numbering $\text{I}_a=\text{I}_b$: C2=C3, C3=C4, C4=C2. All other assignments are identical.
3.2 Depsipeptide

A known depsipeptide was isolated from the sponge *Stelleta splendens*. The structure of this depsipeptide was elucidated by the spectroscopic methods described below.

### 3.2.1 Jaspamide/Jasplakinolide

The molecular formula was determined as C₃₆H₄₅O₆N₄Br ([M]⁺=709) by its ESIMS, ¹H-NMR and ¹³C-NMR. The ¹³C-NMR showed signals for four carbonyl groups including one ester (δ_c= 175.1, 174.4, 170.5, 168.9, 70.7). ¹H-NMR showed signals for a p-substituted benzene ring [δ_H=6.90(d), 6.65(d), 6.65(d), 6.90(d)], o-substituted benzene ring [δ_H= 7.50(m), 7.15(m), 7.20(s)] and a tri-substituted alkene(δ_H=4.70).

A search on Marinlit Database using the mass number ([M]+ 709) found a match with the known marine compound, Jaspamide/Jasplakinolide (Zabriskie et al 1986; Crews et al 1986). Spectroscopic data were compared and a near perfect match was obtained (refer to Table 2).

As an additional confirmation test Marfey’s analysis was carried out. Jaspamide is composed of three amino acids: L-alanine, N-methyltryptophan (2-bromoabrine) and β-tyrosine. Acid hydrolysis and subsequent HPLC analysis showed that there were three amino acids present. One of these was confirmed to be L-alanine by using authentic standards of L and D-alanine. The other two amino acids are very rare and authentic standards of these were unavailable for comparison.
Hence the structure was determined to be jaspamide/Jasplakinolide (3).
Table 2: Comparison of NMR data for jaspamide (jasplakinolide)

<table>
<thead>
<tr>
<th></th>
<th>Jaspamide (Zabriskie et. al. 1986)</th>
<th>Jasplakinolide (Crews et. al. 1986)</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>¹³C (ppm)  ¹H (mult)  ¹³C (ppm)  ¹H(mult)  ¹³C (ppm)  ¹H(mult)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>175.1  175.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>40.1  2.50(m)  40  2.50(m)  40.1  2.60(ddd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>40.7  2.38(dd)  1.89(d)  41.1  2.39  1.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>131.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>127.8  4.75(d)  128.3  4.74(m)  127.8  4.70(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>29.2  2.23(m)  29.3  2.28(m)  29.2  2.13(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>43.3  1.32(m)  43.7  1.30(m)  43.3  1.27(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>70.8  4.62(m)  70.7  4.62(m)  70.7  4.70(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>174.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>39.7  2.65(dd)  2.65(dd)  40.4  2.65  2.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>49  5.26(dd)  49.2  5.27(m)  48.9  5.22(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>7.65(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>170.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>55.5  5.85(dd)  55.7  5.85(dd)  55.5  5.75(dd)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>168.9  174.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>45.8  4.75(m)  46.1  4.74(m)  45.9  4.70(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>6.63(bs)  6.70(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>20.3  1.12(d)  20.4  1.11(d)  20.3  1.09(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>18.5  1.56(s)  18.5  1.55(s)  18.5  1.53(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>21.9  0.81(d)  21.9  0.80(d)  21.9  0.80(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>19  1.05(d)  19.2  1.05(d)  19  1.02(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>133.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>127.1  6.94(d)  127.4  6.93(d)  127.2  6.90(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>115.6  6.66(d)  115.7  6.70(d)  115.5  6.65(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>155.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>115.6  6.66(d)  115.7  6.70(d)  115.5  6.65(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>127.1  6.94(d)  127.4  6.93(d)  127.2  6.90(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>23.2  3.38(dd)  3.24(dd)  23.4  3.37  3.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>8.70(br s)  9.20(s)  8.50(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>109</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>111.1  110.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>131.3  127.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>118.1  7.24(d)  118.3  7.53(d)  118.1  7.50(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>122.3  7.13(dd)  120.4  7.09(t)  122.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>120.9  7.10(dd)  122.5  7.11(t)  120.1  7.15(m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>110.6  7.56(br d)  110.6  7.21(d)  110.6  7.20(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>136.1  136.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>30.8  2.98(s)  30.9  2.95(s)  30.8  2.95(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>17.8  0.70(d)  17.9  0.66(d)  17.7  0.72(d)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Jaspamide exhibited antihelminthic properties against the parasitic nematode, *H. contortus*, (NeT=64, LD$_{90}$=2.6), as well as cytotoxicity (CyT=1024, LD$_{90}$=0.16). This is a known compound and its biological activity has been well documented in literature.

Jaspamide/ jasplakinolide is a cyclic depsipeptide that has been isolated exclusively from marine sponges. It was discovered independently by two different research groups. Zabriskie and associates (1986) reported Jaspamide, derived from the *Jaspis* sp collected from Suva harbour in Fiji and from a marine lake in Palau. They found that the compound was a potent insecticide, with activity against *Heliothis virescens* (LC$_{50}$ 4ppm).

Crews et. al. (1986) reported the cyclic depsipeptide as Jasplakinolide. This compound was derived from a *Jaspis sp* of sponge from Beqa lagoon in Fiji. This research group found that jaspamide possessed antihelminthic properties (*in vitro* ED$_{50}$< 1 μg/ml against the nematode *Nippostrongylus braziliensis*) as well as cytotoxicity against larynx epithelial carcinoma (0.32 μg/ml) and a human embryonic lung cell line (0.01 μg/ml). Both groups found that jaspamide had potent activity against the fungus *Candida albicans* but was inactive against a variety of gram positive and gram negative bacteria.

Clinical trials on jaspamide were discontinued after the compound proved to be too toxic (Faulkner 2000). However, further research on jaspamide continued. Studies showed that the drug possessed *in vitro* cytotoxicity to HT-29 cells (Crews et. al. 1994) as well as
antiproliferative activity in the NCI-60 cell line screen. It was most effective against a number of tumour derived cell lines, human prostrate carcinoma (Crews et. al. 1994) and myeloid leukemia (Fabian et. al. 1995). Odaka et. al. (2000) attempted to explain the mode of action regarding the antiproliferative activity of jaspamide. This group found that Jaspamide induces cell death via apoptosis. They also reported that transformed cells were more susceptible to jaspamide induced apoptosis than normal non-transformed cell. In summary, jaspamide possessed excellent anticancer properties.

Jaspamide’s biosynthetic origin is as follows. It is a mixed polyketide consisting of three amino acids: (R)-2-bromoabrine (N-methyltryptophan), (R)-β-tyrosine and (S)-Alanine and a propionate unit (Zabriskie et. al. 1986; Crews et. al. 1986). The former two amino acids are exceptionally rare.

3.3 Bromopyrrole-imidazole alkaloids

The following known bromopyrroles were isolated from the marine sponge Stylissa massa. Structures of these were elucidated by spectroscopic methods outlined below.

3.3.1 Oroidin

Molecular mass of this compound was found to be 389 ([M⁻]). The [M+H]⁺: [M+2+H]⁺: [M+4+H]⁺ peak height ratios were 1:2:1, showing presence of 2 Br atoms. The ¹H-NMR showed presence of 2 vinylic protons [δ_H= 6.03(1H, dt), 6.24(1H, s)] and 2 allylic protons [δ_H= 4.02(2H, d)]. From this the following moiety was deduced: -CH₂CH=CH-.
A search on Marinlit database found a close match to the known marine natural product Oroidin. The $^1$H-NMR data of the literature (Lindel et. al. 2000) matched (as shown in table 3) that of the experimental obtained data. Thus the structure was concluded to be that of oroidin(4).

Table 3: comparison of literature and experimental $^1$H-NMR of Oroidin

<table>
<thead>
<tr>
<th>Literature (Lindel et. al. 2000) $^1$H NMR(δ, CDCl$_3$)</th>
<th>Experimental $^1$H NMR(δ, CDCl$_3$) {ppm}</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-isomer (ppm)</td>
<td>Z-isomer (ppm)</td>
</tr>
<tr>
<td>4.04 (dd, 2H)</td>
<td>4.07 (dd, 2H)</td>
</tr>
<tr>
<td>6.01 (dt, 1H)</td>
<td>5.75 (dt, 1H)</td>
</tr>
<tr>
<td>6.30 (d, 1H)</td>
<td>6.20 (d, 1H)</td>
</tr>
<tr>
<td>6.74 (s, 1H)</td>
<td>6.81 (s, 1H)</td>
</tr>
<tr>
<td>6.82 (s, 1H)</td>
<td>6.82 (s, 1H)</td>
</tr>
</tbody>
</table>

Oroidin showed no biological activity against the bioassays conducted.
Oroidin is a major metabolite of several species of the Genus *Agelas*. It was first isolated from *Agelas oroide* in 1971 (Forenza et. al.) but an error was made in deducing its structure. Garcia et al proposed a revised structure in 1973. It functions as a secondary defense metabolite acting as a feeding deterrent against several predatory fish (Chanas et. al. 1996). Van Soest and Richelle-Maurer (2000) found that the concentration of oroidin (as well as sceptrin) increased in sponge cells after damage to sponges and during confrontation (stony corals placed near sponge substrate). Oroidin has displayed antimuscarinic inhibition activity, inhibiting acetylcholine receptors (Rosa et. al. 1992).

Oroidin is the precursor molecule for over 50 bioactive pyrrole-imidazole alkaloids. The C_{11}N_{5} skeleton of oroidin forms part of many derivatives through cyclization, dimerisation, isomerisation of the double bond and/or oxidation/reduction. This includes compounds like the Palau’aamines, isophakellin, sceptrin, hymenidin and hymenidialdisine.

In nature, six modes of oroidin cyclization have been identified (Lindel 1999; Fattorusso and Taglialetela-Scafati 2000). These have been classified according to their linkage formation (see Table 4).
Table 4: Cyclization modes of the Oroidin skeleton.

<table>
<thead>
<tr>
<th></th>
<th>Linkage formation</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>C4/C10</td>
<td>Hymenialdisine (Kitagawa et al. 1983)</td>
</tr>
<tr>
<td>3</td>
<td>N1/C12 + N7/C12</td>
<td>Dibromoagelasponge (Fedoreyev et. al. 1989)</td>
</tr>
<tr>
<td>4</td>
<td>N1/C12 + N7/C11</td>
<td>Dibromophakellin (Sharma and Magdoff-Fairchild 1977)</td>
</tr>
<tr>
<td>5</td>
<td>C4/C12 + N7/C11</td>
<td>Dibromoisophakellin (Fedoreyev et. al. 1986)</td>
</tr>
<tr>
<td>6</td>
<td>N1/C9 + C8/C12</td>
<td>Agelastatin (D’Ambrosio et. al. 1993)</td>
</tr>
<tr>
<td>7</td>
<td>N1/C9</td>
<td>Cycloooridin (Fattorusso and Tagliatela-Scafati 2000)</td>
</tr>
</tbody>
</table>
Fig 5: Six modes of cyclization of the Oroidin skeleton.
3.3.2 Sceptrin

Molecular mass of this compound was found to be 622. The [M]+: [M+2]+: [M+4]+ peak height ratios were 1:2:1, showing the presence of 2 Br atoms. The $^1$H-NMR revealed the following:

a) 3 aromatic proton signals [$\delta_{H}= 6.90(s), 6.46(s), 6.77(s)$].

b) CH$_2$-CH-CH- functionality [$\delta_{H}= 3.43(d), 2.30(dt), 2.94(d)$].

The lack of proton signals suggested that the compound was symmetrical. A search on MarinLit database came up with a close match with the known bromopyrrole-imidazole, sceptrin (5).

Spectroscopic data matched well with that published in literature (Walker et. al. 1981).
Table 5: Comparison of Literature and Experimental 1H-NMR of Sceptrin

<table>
<thead>
<tr>
<th>Literature 1H NMR[Walker et. al. 1981] (δ,Me₂SO-d₆)</th>
<th>Experimental 1H NMR(δ,CDCl₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.29 (br s, 1H)</td>
<td>2.29 (s, 1H)</td>
</tr>
<tr>
<td>3.10 (d, 1H)</td>
<td>*</td>
</tr>
<tr>
<td>3.42 (br s, 2H)</td>
<td>3.43 (s, 2H)</td>
</tr>
<tr>
<td>6.66 (s, 1H)</td>
<td>6.40 (s,1H)</td>
</tr>
<tr>
<td>6.97 (s, 1H)</td>
<td>6.70 (s, 1H)</td>
</tr>
<tr>
<td>6.99 (s, 1H)</td>
<td>6.90 (s, 1H)</td>
</tr>
<tr>
<td>7.33 (br s, 2H)</td>
<td>*</td>
</tr>
<tr>
<td>8.59 (br t, 1H)</td>
<td>*</td>
</tr>
</tbody>
</table>

*H corresponding to N-H bonds

Sceptrin showed no biological activity against the bioassays conducted.

Walker et. al. (1981) reported sceptrin from the sponge *Agelas sceptrum*. It functions as a chemical defense metabolite, acting as a feeding deterrent against predatory fish (Chanas et. al. 1996; Assmann et. al. 2000). Concentrations of sceptrin and oroidin increase in sponge cells after damage and during confrontation with another species (Van Soest and Richelle-Maurer 2000).

Sceptrin and its analogues have potent antibacterial/antifungal activity (Bernan et. al. 1993), anti-muscarinic (Rosa et. al. 1992) and antihistaminic activity (Cafieri et. al. 1997).

Sceptrin is formed via a head-to-head [2+2] cycloaddition of debromoooroidin. The reaction was believed to be photochemical in nature. Hao and associates (2001) refuted
this, arguing that if the reaction was photochemically driven, then oroidin (which is achiral) should yield sceptrin as a racemic mixture. Sceptrin is a chiral compound suggesting that the reaction is in fact an enzyme catalyzed one. If this were the case, then Sceptrin would be the first example of a biological [2+2] cycloaddition or pericyclic reaction.

One of the possibilities proposed by Hao et. al. (2001) involves a polar conjugate addition and not a pericyclic reaction.

Fig 6: Possible polar Mechanism. Two Sequential conjugate additions catalyzed by protonation of one debromo-oroidin molecule.
3.4 Polybrominated diphenyl ether

Polybrominated Diphenyl ethers (PBDEs) are a class of chemicals that are commercially used as flame-retardants. Products based on penta-, octa- and deca- BDEs are added to plastics used in electrical appliances as well as building materials and transport. Hexa-BDEs are present in small amounts in both penta- (4-8%) and octa- BDEs (10-12%) based flame-retardants.

PBDEs are ecologically of concern as pollutants. Similar to Polychlorinated biphenyls, PBDEs are a threat to wildlife and humans. They are persistent, lipophilic and are able to bioaccumulate. Many PBDEs can travel long distances from its origin in the environment. When combusted many PBDEs form Polybrominated dibenzodioxins (PBDDs) and Polybrominated dibenzofurans (PBDFs) which have similar toxicity and environmental impact as well known pollutants, Polychlorinated dibenzofurans (PCDFs) and Polychlorinated dibenzodioxins (PCDFs). PBDEs are known to disrupt human endocrine systems.

(van Esch, International Programme on Chemical Safety)

A known polybrominated diphenyl ether (PBDE) was isolated from the marine sponge *Stylissa massa*. The structure of this was elucidated from the spectroscopic methods mentioned below.
3.4.1 Hexabromodiphenyl ether

Hexabromodiphenyl ether was assigned the molecular formula $\text{C}_{12}\text{H}_4\text{O}_3\text{Br}_6$ on the basis of ESI (-) MS ($[\text{M}]^+ = 670$) and $^1\text{H}$ NMR. The $[\text{M}]^+ : [\text{M}+2]^+ : [\text{M}+4]^+ : [\text{M}+6]^+ : [\text{M}+8]^+ : [\text{M}+10]^+ : [\text{M}+12]^+$ peaks had peak height ratios of 1:6:15:20:15:6:1. This showed presence of 6 bromine atoms. There were only two signals in the $1\text{H}$-NMR. Both were from aromatic protons $\delta_\text{H} 7.37(\text{s})$ and 6.50(\text{s}). DBE was calculated to be 8. Therefore structure was composed of two benzene rings substituted with 6 Br atoms and 2 protons. The two rings were either linked directly or by an oxygen atom. The unassigned oxygen and two protons were in the form of two hydroxyl groups. Therefore the aromatic rings were linked via the oxygen atom as an ether link.

Substitution pattern of Br atoms on the aromatic rings was deduced by comparing spectroscopic data with that of known hexabromodiphenyl ether (Faulkner and Carte 1981, see Table 6).

<table>
<thead>
<tr>
<th>Literature (Carté and Faulkner 1981) {ppm}</th>
<th>Experimental (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.37</td>
<td>7.37</td>
</tr>
<tr>
<td>6.50</td>
<td>6.50</td>
</tr>
</tbody>
</table>

The structure was found to be 2-(3’, 5’-dibromo-2’-hydroxyphenoxy)-3,4,5,6-tetrabromophenol.
Hexabromodiphenyl ether exhibited antihelminthic properties against the parasitic nematode, *H. contortus*, (NeT=16, LD$_{99}$=21).

Faulkner and Carte first reported 2- (3'-5'-dibromo-2'-hydroxyphenoxy)-3,4,5,6-tetrabromophenol in 1981, from the sponge *Phyllospongia foliascens*. It was isolated as an inseparable mixture with 2-(3’-5’-dibromo-2’-hydroxyphenoxy)-3,5,6-tribromophenol. There was no mention of biological activity.

### 3.5 Aminosulfonic acid

The isolation and structure elucidation of the known aminosulfonic acid, taurine, from the marine sponge *Stylissa massa* by spectroscopic methods are outlined below.

#### 3.5.1 Taurine

This compound was obtained as a clear gum. The molecular mass was established by
ESI (-) MS ([M]+ = 125). The 1H NMR revealed only two proton signals (3.10[t], 3.29[t]), that corresponded to the moiety: -CH₂-CH₂-. A search on Marinlit database found a match with the common marine metabolite, taurine (C₂H₇NO₃S). Comparison was made with spectroscopic data obtained from an authentic sample of taurine. This data matched perfectly with experimental data.

Taurine exhibited antihelminthic activity against the parasitic nematode, *H. contortus* (NeT=4, LD₉₀= 42).

Taurine or 2-aminoethanesulfonic acid was first discovered in ox bile in 1827. It was not until 1975 that its significance in human nutrition was realized (Birdsall 1998). Taurine is a semi-essential amino acid. It is not utilized in protein synthesis and is found in either free form or as simple peptides. Unlike other amino acids, taurine contains a sulfonic acid moiety instead of a carboxylic acid. It is one of the most abundant free amino acids found in human tissue including skeletal and cardiac muscle, and the brain (Huxtable 1992).

Taurine is synthesized from methionine and cysteine. Jacobsen and Smith (1968) demonstrated five pathways of taurine synthesis from methionine. The most common pathway is methionine – cysteine – cystein - sulfuric acid - hypotaurine- taurine. In the human body there are three known pathways of taurine synthesis from cysteine. All three
pathways require the active coenzyme form (pyridoxal-5’-phosphate (P5P) of vitamin B6 (Shin and Linkswiler 1974).

Taurine plays an important role in several human physiological functions. Two major roles are:

Conjugation of bile acids (Birdsall 1998)
To solubilize at the pH levels inside the human body bile acids need to be conjugated through peptide linkages with either glycine or taurine to form bile salts. Taurine-conjugated acids increase the excretion of cholesterol. Bile acids help to absorb lipids and fat-soluble vitamins.

Detoxification
Taurine acts as an antioxidant by neutralizing hypochlorous acid (a potent oxidising agent). Taurine has a sulfonic acid group in place of a carboxylic acid. Instead of forming an aldehyde from hypochlorous acid, a relatively stable chloroamine compound is produced, thus protecting the body from toxic effects of aldehyde release that can cause DNA damage (Kozumbo et. al. 1992).

Other functions include membrane stabilization, osmoregulation and modulation of cellular calcium levels (Birdsall 1998).
Taurine has been used clinically to treat several conditions including cardiovascular diseases, epilepsy and other seizure disorders (Fariello et. al. 1985), Alzheimer’s disease (Tomaszewski et. al. 1982; Csernansky et. al. 1996), hepatic disorders (Matsuyama et. al. 1983) and cystic fibrosis (Smith et. al. 1991; Carrasco et. al. 1990). Acamprosate (an analogue of taurine) is used in treatment of alcoholism (Wilde and Wagstaff 1997; Sass et. al. 1996; Whitworth et. al. 1996; Paille et. al. 1995; Bara et. al. 1995; Guiet-Bara 1995).

Most sponges contain hypotaurine. Taurine is also present in high quantities. Ackermann and List (1959) reported trimethyltaurine from Geodia sp while Bergquist and Hartman (1969) reported taurocyamine in many species of Hadromerida, Spirophorida and Choristida. More recently Fattorusso and Taglialatela-Scafati (2000) reported taurodispacamide A, an antihistaminic bromo-pyrrole alkaloid containing a taurine moiety, from Agelas oroides sponge.

![Taurodispacamide A](image-url)
3.6 Nicotinic acid

The isolation and structure elucidation of the known nicotinic acid analogue, trigonelline from the marine sponge, *Stylissa massa*, by spectroscopic methods are outlined below.

### 3.6.1 Trigonelline (*N*-methyl nicotinic acid)

This compound was obtained as a yellow gum. The molecular formula, C<sub>7</sub>H<sub>7</sub>NO<sub>2</sub>, was established by ESI (+) MS and <sup>1</sup>H-NMR. The <sup>1</sup>H-NMR revealed the following functionalities:

a) 1 primary methyl [δ<sub>H</sub> = 4.21(s)] unattached to any neighbouring protons and

b) A 6-membered m-substituted aromatic ring [δ<sub>H</sub> = 7.92(t), 8.68(d), 8.98(s): Integrating for 4H]. This ring was deduced to be a pyridine ring (from the molecular formula and <sup>1</sup>H-NMR).

A search on Marinlit database showed a close match to the natural product, trigonelline (8).
Trigonelline exhibited antihelminthic properties against the nematode, *H. contortus* (NeT= 16, LD$_{99}$= 3.4).

Trigonelline’s presence has been detected in various terrestrial plants (mainly legumes and coffee beans) and in several marine organisms.

In plants, trigonelline (TRG) acts as a natural hormone, controlling plant growth by inducing G2 arrest in root and shoot meristems (Evans et al. 1979; Evans and Tramontano 1981). This was proposed after Evans and Tramontano (1981) found that by adding TRG to plants, it replaced cotyledons (which contained natural TRG) in promoting G2 arrest. The proportion of G2 arrested cells were indirectly related to TRG levels in plants. TRG induces defense metabolism in plants and accumulation of secondary defense metabolites (Berglund 1994). It also serves as an osmoregulator (Tramontano and Jouve 1997), achieving this feat by increasing the in vitro thermal and salt stability of pyruvate kinase (Shomerilan et al. 1991).

TRG acts as a reusable storage form of the vitamin, niacin (nicotinic acid) and can re-enter the nicotinamide metabolic pathway by demethylation (Minorsky 2002). In coffee beans, the roasting process degrades TRG into volatile flavoured compounds that give coffee its distinct smell (Saldana 1997). TRG is thought to be of nutritional importance to humans. It enhances the performance of the Central nervous system, secretion of bile and the intestine (Saldana 1997). Fenugreek (*Trigonella foenugreca*) is an herb that is used widely throughout Asia and Southern Europe. It is used as a natural cure for
diabetes, migraines, allergies, elevated cholesterol and constipation (Shapiro and Gong 2002). TRG is a major metabolite of this plant and has a hypoglycemic effect on humans (Mishkinsky et. al. 1967). In Fiji the seeds are known as methi, and are used as a spice. Fenugreek is also a vital component of several commercially available breast enlargement products.

Presence of TRG has been detected in several marine organisms including shellfish. Trigonelline has antifouling activity against cyprids of the barnacle, Balanus amphitrite (Miki et. al. 1996).

3.7 Pyridinium salt

The isolation and structure elucidation of the known pyridinium salt, zooanemonin, from the marine sponge Stylissa massa by spectroscopic methods are outlined below.

3.7.1 Zooanemonin

This compound was isolated as a white powder. The molecular formula, C₇H₁₀N₂O₂, was established from the ESI (+) MS and 1H-NMR. The 1H-NMR revealed the following functionalities:

a) two primary methyls (δ_H= 3.46[s], 3.58[s])

b) And two aromatic protons (δ_H= 8.40[s], 7.06[s]).

c) One methine attached to a carboxyl group (δ_H= 3.70[s])
The lack of carbons meant the ring was heterocyclic. A search on MarinLit found a close match to the known alkaloid, zooanemonin (9).

Zooanemonin showed moderate antihelmintic activity against the nematode *H. contortus* (NeT = 4, LD$_{99}$ = 50).

Zooanemonin has been isolated from the marine sponge *Protophlitaspongia aga* as an antifouling substance against the barnacle *Balanus amphitrite* (Hattori et al. 2001) and as a constituent of the Chilean marine invertebrate *Antholoba achates* (Gonzalez et al. 1984). Fouling organisms cause serious problems by settling on ships' hulls, and other marine infrastructures. Organotin compounds are currently used as antifoulants but cause too many environmental problems. Many sessile marine organisms produce antifouling compounds as a secondary defense metabolites. Natural compounds tend to exhibit a lower toxicity than substances in current use or are more environmentally friendly.
4.0 Conclusion

Eight natural products have been isolated from three different species of Fijian marine sponges.

A novel polyketide, Spongosoritin A, was isolated from *Spongosorites* sp. Spongosoritin A exhibited no bioactivity in all bioassays conducted.

*Stelleta splendens* yielded the known cyclodepsipeptide, jaspamide/jasplakinolide. Jasplamid proved to be both cytotoxic and antihelminthic.

*Stylissa massa* yielded six known metabolites: hexabromodiphenyl ether, oroidin, sceptrin, trigonelline, taurine and zooanemonin. All these compounds with the exception of sceptrin showed, moderated to good, antihelminthic activity.

Unfortunately a new potential agrochemical was not discovered in this project. However a new compound, although biologically inactive, has been discovered and its structure determined. Marine invertebrates are still an excellent source for potential agrochemicals and pharmaceuticals and continued research may uncover a veritable goldmine in terms of biologically active compounds and interesting chemistry.
5.0 References


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Appendix

Spectroscopic data for Spongortin A, a novel polyketide from the Fijian marine sponge

*Spongisorites* sp.