NOVEL SECONDARY METABOLITES FROM SELECTED MARINE INVERTEBRATES

by

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We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
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Department of **CHEMISTRY**

The University of British Columbia
Vancouver, Canada

Date **Aug 02, 91**
Abstract

Chemical studies of a Northeastern Pacific tunicate and three Papua New Guinea sponges have led to the isolation of sixteen new secondary metabolites. The structures of the new compounds were determined by spectroscopic analysis and chemical interconversions. The absolute stereochemistry of imbricatine, a previously reported starfish metabolite, has also been determined.

The northeastern Pacific tunicate *Ritterella rubra* has been found to contain a novel series of aromatic butenolides, rubrolides A-H (149-156). The structures of the rubrolides were solved by the analysis of NMR (^1H, ^13C, COSY, nOe, HETCOR, FLOCK, HMQC and HMBC), MS and IR data combined with chemical interconversions. FLOCK, a new ^1H/13C long-range correlation experiment, played a key role in establishing the rubrolide carbon skeleton. The rubrolides represent the largest family of non-nitrogenous tunicate metabolites. The protein phosphatase inhibitory activity and the potent antibiotic activities of the rubrolides warrant further investigation.

The absolute stereochemistry of imbricatine (179), a compound reported from the starfish *Dermasterias imbricata*, has been determined by comparing the optical properties of its chemical degradation products with those of model compounds. Raney nickel reduction of 179 yielded benzyltetrahydroisoquinoline 188a which was methylated to give 188b. Comparison between the CD spectrum of 188b and those of model compounds 189 and 190 solved the absolute stereochemistry of the tetrahydroisoquinoline fragment of 179. Reductive hydrolysis of 179 followed by oxidation yielded histidine disulphide 182. Comparison of the optical rotation of 182 with the reported value solved the absolute stereochemistry of the histidine fragment of 179. Attempts to study the biogenesis of 179 were unsuccessful.

Examination of three Papua New Guinea sponges resulted in the isolation of eight new compounds. Six new bastadins (211-216) were isolated from *Ianthella basta*. The
structures were elucidated by spectroscopic analysis as well as comparison with the previously reported bastadins. A *Xestospongia* species was found to contain xestospongin E (238), a new metabolite, and a number of known xestospongins. Both the bastadins and the xestospongins possess antibiotic and cytotoxic activities. A symmetrical enyne, callydiyne (247), was isolated from *Callyspongia flammea*. The structure of 247 was determined by spectroscopic studies.
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<td>Ac</td>
<td>acetyl</td>
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<tr>
<td>Ac$_2$O</td>
<td>acetic anhydride</td>
</tr>
<tr>
<td>APT</td>
<td>Attached Proton Test</td>
</tr>
<tr>
<td>BB</td>
<td>Broad Band decoupling</td>
</tr>
<tr>
<td>BIRD</td>
<td>Bilinear Rotation Decoupling</td>
</tr>
<tr>
<td>br</td>
<td>broad</td>
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<tr>
<td>CD</td>
<td>Circular Dichroism</td>
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<td>1D</td>
<td>one-dimensional</td>
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<td>2D</td>
<td>two-dimensional</td>
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<td>DMF</td>
<td>Dimethyl Formamide</td>
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<td>DMSO</td>
<td>Dimethyl Sulphoxide</td>
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HPLC — High Performance Liquid Chromatography
i — NMR resonance due to an impurity (used only in NMR spectra)
IC$_{50}$ — concentration resulting in 50% inhibition
IR — InfraRed
J — scalar coupling constant in hertz
m — multiplet
MS — Mass Spectrometry
$\Delta$M — difference in mass
M$^+$ — parent ion
Me — methyl
MeOH — methanol
MIC — Minimum Inhibitory Concentration
mmu — milimass units
m/z — mass to charge ratio
NMR — Nuclear Magnetic Resonance spectroscopy
nOe — nuclear Overhauser enhancement
PP — Protein Phosphatase
ppm — parts per million
q — quartet
rel. int. — relative intensity
r.t. — room temperature
s — singlet
S — NMR resonance due to the solvent (used only in NMR spectra)
SCUBA — Self-Contained Underwater Breathing Apparatus
sp. — species
t — triplet
T/C — test compared to control
| THF     | — TetraHydroFuran                      |
| TLC     | — Thin Layer Chromatography            |
| UV      | — UltraViolet                          |
| W       | — NMR resonance due to water (used only in NMR spectra) |
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I would like to extend my most sincere gratitude to my research supervisor, Professor Raymond J. Andersen, for his guidance, encouragement and understanding throughout the course of this work. It has been a great pleasure to work with him.

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INTRODUCTION

The world's oceans account for more than 95% of the biosphere and host a large number of living organisms. However, it is only a few decades since natural products chemists realized the importance of the marine environment and began to look at marine organisms as a new source of secondary metabolites. The invention of SCUBA diving techniques and manned research submersibles has significantly extended the accessibility of marine organisms and greatly facilitated the investigation of the secondary metabolites produced by these organisms. As a result, the last decade has witnessed an explosive growth of marine natural products chemistry. Since 1977, about 3,000 new compounds have been isolated from various marine sources including algae, sponges, coelenterates, tunicates, molluscs, echinoderms, bryozoans and microorganisms.

This thesis deals with the isolation and structural studies of new natural products from a number of marine invertebrates. Its theme centers around the biosynthetic novelty, high biomedical potential, and the ecological roles of marine natural products. These three factors constitute the major interests in contemporary marine natural products chemistry research.

Novelty of Marine Natural Products

Due to the uniqueness of the marine environment and the inherent difference between marine invertebrates and terrestrial plants, many marine invertebrates produce biosynthetic products previously unreported from terrestrial sources. Coelenterates alone are responsible for the production of 14 new carbon skeletal classes of terpenoids, not to mention a large number of known terpene skeletons with unique modification patterns.

Sponges, the most primitive multicellular animals, produce a large array of secondary metabolites. Like coelenterates, they synthesize many interesting novel terpenoids. For

* In this thesis, the terms natural product and secondary metabolite are synonymous.
example, a significant number of sesterterpenoids and norsesterterpenoids, rare in non-marine sources, have been isolated from various species.\textsuperscript{8} Two examples are ircinianin (1)\textsuperscript{8c} and wistarin (2),\textsuperscript{8d} two very unusual tetronic acids reported from the sponge \textit{Ircinia wistarii}. Despite the abundant presence of terpenoids, nitrogenous compounds account for over 40\% of all sponge metabolites according to Ireland's survey.\textsuperscript{5} One example is manzamine A (3),\textsuperscript{9,10} a member of a remarkable family of alkaloids isolated from species of \textit{Haliclona}, \textit{Xestospongia} and \textit{Pellina}. It is a very unusual $\beta$-carboline with a complex system of 5-, 6-, 8- and 13-membered rings attached to the C1 position. The biogenesis of the unique ring system is not clear.\textsuperscript{9}

![Structures](image)

**Biomedical Potential**

Over the years, natural products isolated from terrestrial plants and microorganisms have played an important role in human beings' struggle against various diseases. Despite the availability of drugs for treating most diseases, there has been a constant demand for more effective formulations. Moreover, many currently incurable diseases still urgently await effective treatment. Marine organisms, which have been known to be rich in
biologically active compounds, are undoubtedly a promising new source of pharmaceutical agents. Indeed, since the late 1970s, a great number of biologically active natural products have been isolated from marine organisms, some of which have shown significant antitumor, antibacterial and/or antiviral activities and warrant further clinical investigations.\textsuperscript{5,11,12}

For example, the didemnins (4-8), a family of new depsipeptides isolated from the tunicate \textit{Didemnum} sp.,\textsuperscript{13} possess potent antitumor, antiviral and immunosuppressive activities. Didemnin B (5) is strongly inhibitory toward L1210 leukemia cells \textit{in vitro} (IC\textsubscript{50}=2.2 ng/ml) and P388 murine leukemia cells \textit{in vivo} (T/C=200\% at 1 mg/kg). It also has significant \textit{in vivo} activity against some human cell lines such as ovarian, breast and renal carcinomas\textsuperscript{14} as well as B16 melanoma. It is currently in Phase II clinical trials for the treatment of human cancers.\textsuperscript{13a} Despite the structural similarities of the various didemnins, there is a diversity in their biological activities. This feature may allow future modification of the structures to achieve optimum activities.
Chemical Ecology

Why are secondary metabolites produced? This is a question organic chemists frequently ask. There has been increasing evidence\textsuperscript{15} to support the hypothesis that the production of secondary metabolites which may repel or attract other organisms has evolved as one facet of the organisms' strategy for survival.\textsuperscript{16} In the densely populated and hostile marine environment, this appears to be particularly evident.\textsuperscript{12,17,18}

A small number of studies have been carried out to investigate the biological roles of marine natural products and the chemical interaction between some marine organisms. Herbivorous sea hares and carnivorous nudibranchs are the most studied. It has long been known that these shell-less molluscs, that lack physical protection, have few predators.\textsuperscript{19} Studies (reviewed in references 17 and 18) have indicated that they sequester chemicals from dietary sources for the purpose of chemical defense. In some cases, the dietary chemicals are slightly modified in the digestive glands. Aplysin (9) and debromoaplysin (10) are two of the sesquiterpenoids isolated from the sea hare \textit{Aplysia kurodai}\.\textsuperscript{20} Radioisotope labelling studies\textsuperscript{21} clearly demonstrated that 9 and 10 were derived from laurinterol (11) and debromolaurinterol (12), the metabolites of the red alga \textit{Laurencia okamurai}\.\textsuperscript{22}

![Chemical Structures](image)

Chemical studies on nudibranchs\textsuperscript{23} have shown that most of their secondary metabolites can be traced back to their dietary sources such as sponges, corals, bryozoans and tunicates. One example is the nudibranch \textit{Hyposelodoris tricolor},\textsuperscript{23a} which was found
to contain antifeedants furoscalarol (13) and deoxoscalarin (14). The same compounds were also found in the sponge the nudibranch was observed to feed on. Faulkner and Ghiselin\textsuperscript{24} have speculated that the gradual loss of shells in dorid nudibranchs is a result of the development of chemical defense during their evolution from sea snails.

\textbf{Research objectives}

A substantial research effort in the past two decades into the secondary metabolites of marine organisms has resulted in an impressive array of molecular structures, many with desirable biological properties, and greater understanding of marine chemical ecology. However, only a small fraction of marine organisms have been subjected to chemical investigation and in the area of chemical ecology many fundamental questions remain to be answered. Therefore, further studies on the chemistry of marine organisms are still necessary.

The work presented in this thesis involves the investigation of the secondary metabolites of several selected marine invertebrates, with emphasis on the structural and biosynthetic novelty, biomedical potential, and ecological roles of these compounds. Examination of the Northeastern Pacific tunicate \textit{Ritterella rubra} has led to the isolation of a novel series of natural products, rubrolides A-H, which possess moderate phosphatase inhibitory activity and potent antibacterial activities. The studies of three Papua New Guinea sponges have yielded another eight new natural products, namely, six new bastadins from \textit{Ianthella basta}, xestospongin E from \textit{Xestospongia} sp., and callydiyne from \textit{Callyspongia}
flammea. The stereochemistry and the biosynthetic origin of imbricatine, a metabolite of the starfish *Dermasterias imbricata*, have also been investigated. The following chapters will present the isolation and structural studies of rubrolides A-H, the new bastadins, xestospongin E, callydiyne and imbricatine. The biological activities of the rubrolides, the new bastadins and xestospongin E will also be discussed.
I. INTRODUCTION

This chapter deals with the isolation, structure elucidation and the biological activities of rubrolides A-H, a novel series of secondary metabolites from the Northeastern Pacific tunicate *Ritterella rubra*. The isolation of rubrolides A-H is the result of a joint effort between our laboratory and Dr. C. Holmes of the University of Alberta, in search of protein phosphatase inhibitors from marine organisms. In order to put it in perspective, I will first give an introduction to tunicates, secondary metabolites of tunicates and protein phosphatase inhibitors.

1. General Information About Tunicates

Urochordates, commonly known as tunicates in their adult forms, are a group of invertebrate animals belonging to the phylum Chordata. However, they differ significantly from most other chordates because they possess some chordate characteristics, such as a notochord, only at their larval stage. This is why this group of invertebrates have been treated by some biologists as an independent phylum, Urochordata or Tunicata. Tunicates, like sponges, are filter-feeding animals. The basic body structures of most tunicates are vase-shaped (Fig. 1). The body is enclosed within a gelatinous or leathery tunic containing cellulose as one of its structural components. The tunic usually has two openings, an anterior inhalent siphon through which water enters the animal, and an exhalent siphon through which water is expelled.

Tunicates are found in all the world's oceans, either attached to a suitable substratum or free-swimming. Some of the sessile tunicates are solitary and others are colonial. The
majority of the sessile species inhabit shallow waters and the free-swimming tunicates usually occur in the surface waters of the open oceans.

Fig. 1. Generalized diagram of an ascidian showing the main body features.

About 1,500 species of tunicates have been described and they can be categorized into three classes—the Ascidiacea, the Thaliacea and the Larvacea. The ascidians are the most common tunicates, accounting for approximately 95% of all species.

Ascidians, often called sea squirts, are sessile tunicates, living attached to the sea floor, to submerged objects or to other organisms. Their bodies can also be covered by growths of other organisms. The animals grow best in freely flowing cold water where there is no strong wave shock. Solitary forms can grow to over 20 cm in length and colonies can extend for more than 50 cm in diameter. The water current that is drawn through the
ascidian body is involved in ascidian nutrition, respiration, excretion and reproduction. Food particles, such as phytoplankton, are trapped in the mucus of the pharynx. Large quantities of water, up to several thousand times the body volume per day, are passed through by individual ascidians. Some ascidians concentrate trace metals such as vanadium, niobium, titanium and chromium together with iron and some other common metals. In most cases, the metals' functions remain uncertain.

The major predators of ascidians are sea stars, gastropods and some inshore fishes as well as human beings (about half a dozen large species are taken as food in the Orient, Chile and the Mediterranean).

2. Secondary Metabolites of Tunicates

In contrast to algae, sponges and coelenterates, tunicates did not attract much attention from natural products chemists until the 1980s. However, they have proved to be a rich source of novel secondary metabolites with very high biomedical potential. Over 100 secondary metabolites have been isolated from various species of tunicates collected around the world. The overwhelming majority are nitrogenous compounds that include alkaloids and low molecular weight peptides. A great number of these metabolites exhibit cytotoxic, antimicrobial, antiviral and/or enzyme inhibitory activities. In most cases the roles these active compounds play in the tunicates remain unclear. There is evidence suggesting that some act as feeding deterrents to protect the tunicates from predators.

Since the vast majority of the secondary metabolites from tunicates were reported in last two or three years, there has been no updated review on these new metabolites. In order to compare the novel structures of the rubrolides with the other tunicate metabolites, in the following section, I will review the majority of the new metabolites reported from various species of tunicates. Since this is not a comprehensive review, some compounds that have been reported have not been included.
The eudistomins/eudistomidins (15-40) make up the largest single family of natural products isolated from tunicates. This family contains a series of 26 β-carboline derivatives, most of which bear a substituent at the C1 position. Eudistomins A-Q (15-31) have been isolated from the Caribbean colonial tunicate *Eudistoma olivaceum* by Rinehart's group. The same species collected from Bermuda has yielded eudistomins R, S and T (34-36) as well as eudistomins G, H, I and P (19-22). Examination of the New Zealand tunicate *Ritterella sigillinoides* (=*R. pulchra*) also led to the isolation of eudistomins C (27), K (30), and O (18) together with two new members in this family, eudistomin K sulfoxide (33) and debromoeudistomin K (32). The New Zealand group reassigned the stereochemistry of eudistomins C, E, F, K and L (27-31) based on nOe evidence. Very recently the Okinawan tunicate *Eudistoma glaucus* was reported to contain eudistomidins A-D (37-40), four new members of this large eudistomin family (even though they bear new names). Eudistomins D (15), E (28), H (20) and I (21) were also isolated from this tunicate. The eudistomins all possess antiviral and antibacterial activities, but to a widely varying degree. Compounds that have the strongest antiviral activity are those with the oxathiozepine ring (C, E, K, L), e.g., the MIC's of C and E were 5-10 ng/disc.

\[
\begin{align*}
15 & : X=Br \ Y=OH \ Z=H \\
16 & : X=H \ Y=OH \ Z=Br \\
17 & : X=H \ Y=Br \ Z=H \\
18 & : X=H \ Y=H \ Z=Br \\
19 & : X=H \ Y=Br \ Z=H \\
20 & : X=Br \ Y=H \ Z=H \\
21 & : X=H \ Y=H \ Z=H \\
22 & : X=OH \ Y=Br \ Z=H \\
23 & : X=OH \ Y=H \ Z=H \\
24 & : X=Br \\
25 & : X=H \\
26 & : \text{unsolved}
\end{align*}
\]
The Lissoclinum peptides comprise another important family of tunicate metabolites. In total, 17 members, i.e., patellamides A-D (42-45), lissoclinamides 1-6 (46-51), ulicyclamide (52), ulithiacyclamide (53), ulithiacyclamide B (54), preulicyclamide (55), preparpellamide B formate (56), prelissoclinamide-2 (57) and ascidiacyclamide (41), have been reported.
41 \( R^1 = \text{Me} \quad R^2 = \text{Bu}^\text{s} \quad R^3 = \text{Pr}^\text{i} \quad R^4 = \text{Pr}^\text{i} \)

42 \( R^1 = \text{H} \quad R^2 = \text{Bu}^\text{s} \quad R^3 = \text{Pr}^\text{i} \quad R^4 = \text{Pr}^\text{i} \)

43 \( R^1 = \text{Me} \quad R^2 = \text{Bu}^\text{i} \quad R^3 = \text{CH}_2\text{Ph} \quad R^4 = \text{Me} \)

44 \( R^1 = \text{Me} \quad R^2 = \text{Pr}^\text{i} \quad R^3 = \text{CH}_2\text{Ph} \quad R^4 = \text{Me} \)

45 \( R^1 = \text{Me} \quad R^2 = \text{Bu}^\text{s} \quad R^3 = \text{CH}_2\text{Ph} \quad R^4 = \text{Me} \)

46 \( R^1 = \alpha\cdot\text{Bu}^\text{s} \quad R^2 = \beta\cdot\text{Pr}^\text{i} \quad \Delta^9,10 \)

47 \( R^1 = \alpha\cdot\text{Me} \quad R^2 = \alpha\cdot\text{Bu}^\text{s} \)

48 \( R^1 = \beta\cdot\text{Me} \quad R^2 = \alpha\cdot\text{Bu}^\text{s} \)

49 \( R^1 = \beta\cdot\text{CH}_2\text{Ph} \quad R^2 = \alpha\cdot\text{Pr}^\text{i} \)

50 \( R^1 = \beta\cdot\text{CH}_2\text{Ph} \quad R^2 = \alpha\cdot\text{Pr}^\text{i} \quad \Delta^9,10 \)

51 \( R^1 = \alpha\cdot\beta\cdot\text{CH}_2\text{Ph} \quad R^2 = \alpha\cdot\text{Pr}^\text{i} \)

52 \( R^1 = \alpha\cdot\text{Me} \quad R^2 = \beta\cdot\text{Bu}^\text{s} \quad \Delta^9,10 \)

53 \( R = \text{Bu}^\text{i} \)

54 \( R = \text{CH}_2\text{Ph} \)

55
Most members (42-44, 46-48, 52-57) of this large lissoclinum peptide family were isolated by Ireland's group from the tunicate \textit{Lissoclinum patella} collected in Micronesia or the Great Barrier Reef.\textsuperscript{31a} Ascidiacyclamide (41) was found in an unidentified Australian species.\textsuperscript{31c} Patellamide D (45) and lissoclinamides 4-6 (49-51), isolated from the Australian tunicate \textit{Lissoclinum patella}, are the latest members of this family.\textsuperscript{31d} All of these compounds contain at least one thiazole and usually an oxazoline. Recently, patellin-2 (58), a modified cyclic peptide, has been found in \textit{Lissoclinum patella} collected at the Fijian Island of Viti Levu.\textsuperscript{31e} This unique metabolite lacks the characteristic aromatic amino acid units found in the previous \textit{Lissoclinum} peptides and contains two novel threonine residues.
modified as dimethylallyl ethers as well as a thiazoline amino acid. Most members of the Lissoclinum peptide family showed cytotoxicity, with ulithiacyclamide being the most potent against L1210 (IC50=0.1 ug/ml), HeLa (0.1 ug/ml) and CEM (0.01 ug/ml) cell lines in vitro and against P1534J murine leukemia in vivo (T/C 188 at 1 mg/kg).5

*Lissoclinum patella* from the Fiji Island of Ndravuni and Guam, however, yielded a very different array of secondary metabolites, patellazoles A, B, C32a,b and D32c (59-62). The patellazoles represent a new series of macrolides containing a thiazole moiety. They appear to be derived predominantly from the polyketide pathway, but the thiazole very likely originates from an amino acid.32b All patellazoles possess very potent in vitro cytotoxicity (IC50=0.3-10 ng/ml vs. KB cell line). It remains a very interesting question why the chemistry of *Lissoclinum patella* is location dependent. It was suggested that symbiotic algae may be involved in the biosynthesis at some stage(s) of the process.32b,c

![Chemical structure of patellazoles](image)

59 R1=H  R2=H  R3=H  
60 R1=H  R2=H  R3=OH  
61 R1=OH  R2=H  R3=OH  
62 R1=H  R2=OH  R3=OH

Iejimalides A-D (63-66), four macrolides similar to the patellazoles, have been reported from the Okinawan tunicate *Eudistoma cf. rigida*.33a-b The structural similarity between the two families of macrolides lies in the 24-membered lactone ring system. Very
likely, the side chains are also related. The iejimalides also resemble a class of macrolides isolated from nudibranchs and their dietary sponges, such as the ulapualides,\textsuperscript{34a} kabiramides C\textsuperscript{34b} and halichondramide.\textsuperscript{34c} Iejimalides A and B exhibit potent \textit{in vitro} cytotoxicity against L1210 (IC\textsubscript{50}=62 and 32 ng/ml) and L5178Y (IC\textsubscript{50}=22 and 1.0 ng/ml) murine leukemia cell lines. The same tunicate was also found to contain rigidin (67), a novel pyrrolopyrimidine alkaloid.\textsuperscript{33c} Rigidin is the first pyrrolo[2.3-d]pyrimidine type compound to be isolated from a marine source, although there have been precedents isolated from strains of \textit{Streptomyces}.\textsuperscript{33c}

\[
\begin{align*}
63 & : R=H \quad R'=H \\
64 & : R=Me \quad R'=H \\
65 & : R=H \quad R'=SO_3Na \\
66 & : R=Me \quad R'=SO_3Na
\end{align*}
\]
Two macrocyclic compounds, bistratene A (68) and its acetate, bistratene B, have been isolated from the tunicate *Lissoclinum bistratum*. The structures were proposed on the basis of spectroscopic analyses. Both compounds possess potent cytotoxicity.

![Chemical structure of bistratene A](image)

In last three years a large family of polycyclic aromatic alkaloids (69-89) sharing the novel diazatetracyclic ring system (rings A, B, C and D as indicated in the structures) have been isolated from various species of tunicates. The major variation in structure is the substitution pattern on ring A, which, in many cases, is the attachment of an additional heterocyclic ring. Shermilamines A (69) and B (70) are two closely related compounds from a *Tridemnum* species collected in Guam. An unidentified Micronesian tunicate and its predator, the mollusk *Chelynotus semperi*, were found to contain shermilamine B (70) and four new compounds, kuanoniamines A-D (71-74), each of which has an additional thiazole ring attached to the nucleus. Three closely related secondary metabolites, varamines A-B (75-76) and diplamine (77), which are very similar to kuanoniamines B-D, had been previously isolated from the tunicates *Lissoclinum vareau* (varamines) and *Diplosoma sp.* (diplamine), respectively. Examination of the Okinawan tunicate *Cystodytes dellechiajei* resulted in the isolation of three compounds, cystodytins A
(78), B (79) and C (80), each exhibiting potent antineoplastic activity.\textsuperscript{36f} The antileukemic component of another Okinawan tunicate \textit{Didemnum} sp. was found to be ascidemin (81)\textsuperscript{36g} which has a pyridine ring attached to ring A. A \textit{Leptoclinides} species was reported to contain 2-bromoleptoclinidinone (82)\textsuperscript{36h} which is the 2-bromo derivative of ascidemin (81). Another similar compound, 11-hydroxyascididemin (83), was isolated from a \textit{Leptoclinides} species from Micronesia.\textsuperscript{36i} Meridine (84) and its tautomer 85, both of which exhibited strong cytotoxicity, were isolated from the Australian species \textit{Amphicarpa meridiana}.\textsuperscript{50i} The Red Sea tunicate \textit{Eudistoma} sp. is the producer of another four members in this family, segoline (86), isosegoline (87), nor-segoline (88)\textsuperscript{36j} and eilatin (89).\textsuperscript{36k} Segoline and isosegoline each bears an interesting imide group. Eilatin is particularly interesting since it is symmetrical and appears to be quite different from the other members of the family. The structure elucidation of these alkaloids has elegantly demonstrated the power of two dimensional long-range carbon-hydrogen correlation experiments in the determination of complex structures. Since these molecules are highly substituted, structure determination using the traditional methodology would be extremely difficult. Most of these alkaloids have been bioassayed and their potent biological activities warrant further studies. The origins and the biosynthetic pathways leading to this family of alkaloids remain very intriguing questions.\textsuperscript{36f} These compounds may not be biosynthesized by the host tunicates because very similar metabolites have been found in some other unrelated phyla. Examples are amphimedine,\textsuperscript{37a} petrosamine\textsuperscript{37b} and dercitin\textsuperscript{37c} from several sponge species and calliactine from one sea anemone species.\textsuperscript{37d} Symbionts could be responsible for the production of this large family of metabolites,\textsuperscript{36c} but further evidence is required to support this hypothesis.
The didemnins (4-8) are a series of five depsipeptides isolated from the Caribbean tunicate *Trididemnum solidum*. All five compounds share the same macrocyclic skeleton which incorporates the unique hydroxyisovalerylpropionic acid (Hip) and 3S,4R,5S-isostatine (1st) units. The didemnins possess potent antiviral and antitumor activities.
Didemnin B (5) is the most active of the series and has been under investigation as a potential anticancer agent in Phase II clinical trials. Additionally, nordidemnin B (90), a close analogue of didemnin B (5), has also been reported from *T. solidum*.38

![Chemical structure](image)

4 \( R=H, \) \( R'=\text{sec-Bu} \)
5 \( R=L-\text{Lac—L-Pro—}, \) \( R'=\text{sec-Bu} \)
6 \( R=L-\text{Lac—}, \) \( R'=\text{sec-Bu} \)
7 \( R=L-p\text{Glu—(L-Gln)3—L-Lac—L-Pro—}, \) \( R'=\text{sec-Bu} \)
8 \( R=L-p\text{Glu—(L-Gln)2—L-Lac—L-Pro—}, \) \( R'=\text{sec-Bu} \)
90 \( R=L-\text{Lac—L-Pro—}, \) \( R'=\text{CHMe}_2 \)

The blood of the vanadium-sequestering tunicate *Ascidia nigra* is known to contain a high concentration of bright yellow pigments, termed tunichromes, that readily reduce vanadium (V) to vanadium (IV or III) and Fe (III) to Fe (II) *in vivo*.39 Painstaking studies40 of the extremely labile pigments led to the isolation of three tyrosine-derived tripeptides, tunichromes An-1 (91), An-2 (92) and An-3 (93). All isolation procedures were carried out under O\(_2\)-free conditions, using degassed solvents containing an antioxidant. The complexation between the tunichromes and V (III) appears to be
responsible for the stabilization of V (III), which is usually very unstable at the low physiological pH. Examination of an iron-sequestering tunicate *Molgula manhattensis* yielded another two tunichromes, Mm-1 (94) and Mm-2 (95). Like the An tunichromes, Mm-1 and Mm-2 are tripeptides, but one of the tyrosine residues has been replaced by glycine or leucine.

![Image](image.png)

91 $R^1=\text{OH}$  $R^2=\text{OH}$
92 $R^1=\text{H}$  $R^2=\text{OH}$
93 $R^1=\text{H}$  $R^2=\text{H}$

94 $R=\text{H}$
95 $R=\text{iso-Bu}$

The Indian Ocean colonial tunicate *Didemnum chartaceum* contains four new alkaloids, lamellarins E (96), F (97), G (98) and H (99). Lamellarins A-D had been previously isolated from the prosobranch mollusk *Lamellaria* sp., a predator of some colonial tunicates. The isolation of lamellarins E-H (96-99) from a tunicate indicates that *Lamellaria* sp. most likely acquired lamellarins A-D from a similar tunicate food source. Lamellarins appear to be derived from three units of tyrosine.
More than 20 years ago the crude extract of the colonial tunicate *Ecteinascidia turbinata* was reported to exhibit potent *in vivo* antitumor activity. Recently the compounds responsible for the activity were isolated by following bioassay-guided procedures and identified as a remarkable series of tris(tetrahydroisoquinolines), ecteinascidins 729, 743, 745, 759A, 759B and 770. The structures of ecteinascidins 729, 743, 745 and 770 have been assigned as 100, 101, 102 and 103, respectively, based on the analysis of COSY, HETCOR, HMBC and HRFABMS results, while 759A (104) and 759B (105) have been only tentatively assigned as the N-oxides of 101. Despite their structural complexity, it has been suggested that the ecteinascidins are biosynthesized, in a relatively straightforward fashion, by the condensation of carbonyl groups (derived from serine and cysteine) with dopa or dopamine equivalents, perhaps involving the intermediate diketopiperazine 100a. Because of the structural similarities to the microbially derived safracins and saframycins and the sponge-derived renieramycins, it was suggested that symbiotic microorganisms could be the producers of ecteinascidins and experiments to locate a microbial source for the ecteinascidins are in progress. The antitumor activities of the ecteinascidins are very impressive. For example, ecteinascidin 729 (100) exhibited potent *in*
vivo activities against P388 murine leukemia (T/C=214 at 3.8 ug/kg), B16 melanoma (T/C=246 at 10 ug/kg) and colon carcinoma.

Diazonamides A (106) and B (107), two very interesting halogenated macrocyclic peptides, have been isolated from the Philippine colonial tunicate *Diazona chinensis*. The diazonamides represent an entirely new class of halogenated, highly unsaturated cyclic peptides and they are also the first examples of chlorinated metabolites found in tunicates. It was reported that the structural subunits, including derivatives of at least 3 common amino acids, tyrosine, tryptophan and valine, have cyclized in an unprecedented manner to form the extremely rigid skeleton. Diazonamide A is a potent inhibitor of HCT-116 human colon carcinoma and B-16 murine melanoma cell lines, with IC\textsubscript{50} values less than 15 ng/ml. Diazonamide B is less active.
Trididemnum solidum, the tunicate that yielded the five didemnins (4-8), also contains a few pigments, one of which was identified as a new interesting nickel-containing porphynoid named tunichlorin (108). Tunichlorin appears to be closely related to chlorophyll-a and was reported to be the first Ni-containing chlorin ever isolated from a living organism.

The Indo-Pacific tunicate Atapozoa sp. was found to contain two new bipyrrroles, tambjamine E (109) and F (110), and the previously described tambjamine A, which are fish feeding deterrents. The same compounds were also isolated from the tunicate's predators, the nudibranchs Nembrotha spp.. Tambjamines A-D had been previously found in the bryozoan Sessibugula translucens, the nudibranchs Tambja abdere and T. eliora that
feed on the bryozoan, as well as the two nudibranchs' predator, another nudibranch *Roboastra tigris*.46

Four indole compounds, grossularines A (111) and B (112), dendrodoine (113) and imidazolone 114, have been reported from the solitary tunicate *Dendrodoa grossularia*.47 Grossularines (111 and 112)47a were reported to be the first naturally occurring α-carboline compounds (the previously known α-carboline compounds were either pyrolysis products from tobacco distillates or synthetic products). Both grossularines exhibited potent cytotoxicity against human colon and breast tumor lines.47a Dendrodoine (113) is also an interesting compound because it has a S(II)-N bond which is very unusual among natural products.47b

![Chemical structures](image)

The colonial tunicate *polyandrocarpa* sp. collected in the Philippines was reported to contain four new indole-derived metabolites, polyandrocarpamides A-D (115-118).48 It
was suggested that polyandrocarpamide D (118) may be an isolation artifact derived from methanol addition to a carbonyl-containing precursor.

Five very interesting sulphur-rich benzenoids, polycarpamines A-E (119-123), have been isolated from the Indo-Pacific solitary tunicate Polycarpa auzata. Since polycarpamines were found in only one of the P. auzata collections, it was suspected that symbionts may be responsible for the production of these new compounds. It was also suggested that polycarpamines A (119), D (122) and E (123) may be produced by methanol addition to an unknown precursor during the isolation procedure. Polycarpamine B (120) showed significant activity against fungi Saccharomyces cerevisiae and Candida albicans.
A very unusual benzopentathiepin metabolite, varacin (124), has been recently reported from *Lissoclinum vareau* collected in Fiji.\textsuperscript{50} The pentathiepin nature of varacin was established on the basis of FABMS evidence and chemical interconversions. Varacin represents the first naturally occurring benzopentathiepin and also the first natural polysulfide modified from an amino acid.\textsuperscript{50} It exhibits very potent antifungal activity and cytotoxicity against the human colon cancer HCT 116 cell line (IC\textsubscript{50}=0.05 \textmu g/ml).

\[
\begin{align*}
\text{OMe} \\
\text{MeO} \\
\text{NH}_{2} \\
\end{align*}
\]

124

The Australian tunicate *Aplydium pliciferum* was found to contain two new thiazole compounds (125 and 126), and one new imidazole compound (127).\textsuperscript{51} These three compounds appear to be derived from dopamine. Two new iodinated phenethylamine derivatives, 128 and 129, have been reported from an unidentified species in the *Didemnum* genus.\textsuperscript{52} Compounds 128 and 129 represent the first two iodinated secondary metabolites from a tunicate.

\[
\begin{align*}
\text{MeO} & & \text{MeO} & & \text{MeO} \\
\text{HO} & & \text{HO} & & \text{HO} \\
\text{125} & & \text{126} & & \text{127}
\end{align*}
\]
Some long chain aliphatic alkaloids have been reported from a few species of tunicates. For example, the encrusting tunicate *polyandrocarpa* sp. was found to contain four closely related compounds, polyandrocarpiones A-D (130-133), that are responsible for the antimicrobial activity of the crude extract.\(^{53a}\) Each polyandrocarpine has a terminal guanidine group and a \(\gamma\)-lactam ring. Pseudodistomins A (134) and B (135) are two isomeric piperidines with a 13-carbon side chain isolated from the Okinawan tunicate *Pseudodistoma kanoko*.\(^{53b}\) They were reported to be the first piperidine alkaloids from a marine source. Both compounds exhibited cytotoxicity against L1210 murine leukemia and strong inhibitory activity against calmodulin-activated brain phosphodiesterase.
Clavelpictines A (136) and B (the desacetyl derivative of 136), the first quinolizidine derivatives from a tunicate, have been reported very recently from the Bermudian species Clavelina picta. These two compounds are closely related to the pseudodistomines (133-134) and were suggested to be derived from a similar polyketide pathway.

![Structure of 136]

The New Zealand colonial tunicate Pseudodistoma novaezelandiae was found to produce four closely related straight-chain aliphatic amines represented by 137. Compound 137 and the other major metabolite, a stereoisomer of 137 (structure not shown here), were responsible for the cytotoxic and antifungal activities of the tunicate extract. It was suggested that the presence of high levels of these toxic amines in P. novaezelandiae probably accounted for the lack of predation and fouling observed for this tunicate.

![Structure of 137]

Terpenoids are very rare among tunicate secondary metabolites. One of the very few examples is aplidiaspingosine (138), a compound of 22 carbons derived from a diterpenoid. It was suggested that it may be derived from the CoA derivative of a
diterpenoid and serine. Aplidiaspingosine showed a broad spectrum of antimicrobial activities as well as cytotoxicity against KB and L1210 tumor cells.

As mentioned previously, the overwhelming majority of tunicate secondary metabolites are nitrogenous. The 133 new compounds discussed above all possess at least one nitrogen atom. A small number of non-nitrogenous natural products, however, do exist in a few species. One important group are the didemnenones (139-142), four related cyclopentenone derivatives isolated from two didemnid tunicates.\(^5\) Didemnenones A (139) and B (140) are from *Trididemnum cf. cyanophorum* collected in the Bahamas, while didemnenones C (141) and D (142) are from *Didemnum voeltzkowi* collected in Fiji. Compounds 141 and 142 have moderate cytotoxicity against L1210 murine leukemia while 139 and 140 have antibacterial activity against a variety of microorganisms.
Another important group of non-nitrogenous tunicate metabolites are ascidiatrienolides A-C (143-145) isolated from the colonial tunicate *Didemnum candidum*. These novel 9-membered ring lactones appear to be derived from the lipoxygenation of an eicosapolyenoic acid. This is the first report of 9-membered ring lactones among eicosanoids.

![Chemical structures of ascidiatrienolides A-C.](image)

Finally, an interesting unsaturated γ-lactone, lissoclinolide (146), has been recently reported from the tunicate *Lissoclinum patella* which had previously yielded a large number of Lissoclinum peptides and the patellazoles. Lissoclinolide is the only non-nitrogenous secondary metabolite found in the chemically rich *Lissoclinum* genus.

![Chemical structure of lissoclinolide.](image)

The chemical investigation of marine tunicates has become a fast-growing new field in marine natural products chemistry, as attested by the large number of new metabolites reported from tunicates in last three years. One can easily predict that a great number of new biologically active compounds will be discovered from additional species in the next few years since only a small fraction of the 1,500 described species have been subjected to chemical investigation. As more species are examined and more new metabolites are
identified, the origins and the biological roles of tunicate metabolites are expected to become clearer.

3. Phosphatase inhibitors

Enzyme inhibition is one of the most important tools in enzymology. From the study of enzyme inhibitors, biochemists have learned much about the substrate specificity of enzymes, the nature of the active sites and the mechanisms of enzymatic reactions. Enzyme inhibitors also have been used to investigate metabolic pathways in cells. Moreover, enzyme inhibition also plays an important role in medicine because many drugs are basically inhibitors of certain enzymes in malfunctioning or pathogenic cells.

The reversible phosphorylation of proteins is now recognized as a major control mechanism of intracellular events in eukaryotic cells. A variety of biological processes including the transcription and translation of genes, cell division, fertilization and even memory are regulated by the reversible phosphorylation of the functional proteins. The phosphorylation level greatly affects the activities of the proteins. The active form may be the phosphorylated or, in other cases, the unphosphorylated protein. The reversible phosphorylation process of a certain protein is regulated by two types of enzymes; the protein kinase for phosphorylation and the protein phosphatase for dephosphorylation. From studying protein kinases and protein phosphatases, valuable information can be obtained about reversible phosphorylation mechanisms and, therefore, about many biological processes that are regulated by this versatile post-translational modification of proteins.

In contrast to protein kinases, protein phosphatases are not well-understood by biochemists because they are more difficult to characterize. Little attempt had been made to systematically study the large number of phosphatase preparations until 1983, when it was suggested that most of the phosphatases could be categorized into two major classes (PP1 and PP2). The classification was based on the substrate specificity and enzyme sensitivity
towards different inhibitors. Type 2 phosphatases could, in turn, be subclassified into three distinct enzymes, phosphatases 2A, 2B and 2C.

A revolutionary breakthrough in phosphatase research came in 1987 when okadaic acid (147), a diarrhetic shellfish toxin, was found to be a potent and specific inhibitor of phosphatases 1 and 2A, ten times stronger towards PP2A than PP1. Recently, okadaic acid (147) has been widely employed as an extremely powerful probe for the identification of phosphatases and the investigation of biological processes governed by the reversible phosphorylation of functional proteins. Okadaic acid (147) is a polyether fatty acid originally isolated from the marine sponges Halichondria okadai and Halichondria melanodocia. Subsequently, it was found to be produced by several types of marine phytoplankton and to be concentrated in the marine sponges and shellfish that feed on the phytoplankton. The discovery of the potent phosphatase inhibitory activity of okadaic acid has prompted the search for new inhibitors of phosphatases. As a result, another toxin, calyculin A (148) from the sponge Discodermia calyx, was also shown to be a potent inhibitor of PP1 and PP2A. It inhibits PP2A with similar potency to okadaic acid, but inhibits PP1 with 10- to 100-fold greater potency.

\[
\text{147}
\]
Cohen, Holmes and Tsukitani,\textsuperscript{60} some of the leading workers in this field, put it in this way in 1990: "there was clearly no way of predicting a year ago that the study of protein phosphatases and the analysis of the phosphorylation systems would be revolutionized by a toxin extracted from marine sponges. The okadaic acid story is yet another example of the way in which progress in science can frequently come from the most unlikely directions."

In our laboratory, we have recently initiated a collaboration with Dr. Charles Holmes of the University of Alberta searching for new phosphatase inhibitors from various Northeastern Pacific marine organisms. Bioassays have uncovered the phosphatase inhibitory activity in the crude extracts of several tunicates. Our work on one species, \textit{Ritterella rubra}, has given promising results. Bioassay-guided isolation procedures have led to the discovery of a novel series of aromatic compounds, which we have given the trivial names rubrolides A-H. Rubrolides A-H are responsible for the moderate phosphatase
inhibitory activity and the strong antibiotic activity of the crude extract. In this chapter, the isolation, structure elucidation and biological activities of the rubrolides will be presented.
B. RESULTS AND DISCUSSION

1. Taxonomy and Description of *Ritterella rubra*

*Ritterella rubra* belongs to the Family Polyclinidae in the Order Enterogona according to Abbott and Trason's classification (Fig. 2). These colonial animals are found in shaded caves in low intertidal and adjacent subtidal zones and on rocks exposed to moderate surf. This species was originally known from Monterey Bay, California. Austin later found it in the Queen Charlotte Islands region. Colonies are normally composed of numerous scarlet to crimson, round-topped lobes up to 3 cm high and 1.5 cm in diameter. It was described by Abbott as "a very attractive and distinctive species".25

The *Ritterella rubra* specimens (collection #: QC-22-5-90-63) (Fig. 3) that are dealt with in this chapter were collected at Anthony Island in the Queen Charlotte Islands chain (Fig. 4) in the Northeastern Pacific. Colonies of 8-9 individuals were found on exposed rocks 3-5 m below the surface. The lobes were approximately 3 cm high and the colonies were about 5 cm in diameter. The chemical investigation of *Ritterella rubra* was prompted by the phosphatase inhibitory and antibacterial activities of the crude methanol extracts which were discovered in our routine bioassay-screening programs.
Fig. 2. Phylogenetic classification of *Ritterella rubra*. 

Phylum: 

Subphylum: Vertebrata
Urochordata Cephalochordata

Class: Thaliacea Ascidiacea Larvacea

Order: Enterogona Pleurobranchia

Suborder: Aplausobranchia Phlebobranchia

Family: Clavelinidae Didemnidae Polyclinidae (=Synoicidae) Polycitoridae

Genus: Polyclinum Aplidium *Ritterella* Synoicum Euherdmania

Species: *R. pulchra* *R. rubra* *R. aequalisiphonis*
Fig. 3. *Ritterella rubra*
Fig. 4. Collection site of *Ritterella rubra*
2. Isolation of The Rubrolides (149-156)

The frozen tunicate specimens (400 g) were immersed in methanol (1 litre), defrosted and homogenized in a Waring blender. The bright-red methanol extract of *Ritterella rubra* was filtered and concentrated *in vacuo* to yield an aqueous suspension, which was diluted to 300 ml with water and partitioned against hexanes (400 ml × 3), dichloromethane (400 ml × 3) and ethyl acetate (400 ml × 3) in a separatory funnel. Bioassays indicated that the active metabolites were concentrated in the dichloromethane and ethyl acetate fractions. ¹H NMR experiments indicated that both fractions contained similar aromatic compounds. Therefore, the dichloromethane and ethyl acetate soluble materials were combined and chromatographed on Sephadex LH20 (eluent: MeOH) to give seven major fractions containing rubrolides G/H, F, E, D, B, C and A in sequence. Three fractions containing rubrolides A, B and C were independently rechromatographed on Sephadex LH20 using a solvent system of EtOAc/MeOH/H₂O (40:10:4) to afford pure rubrolides A (149) (132 mg), B (150) (64 mg) and C (151) (48 mg). Rubrolide F (154) (3 mg), less polar than the other rubrolides, was purified by preparative TLC (silica gel, 10:1 CH₂Cl₂/MeOH). The other three crude mixtures containing rubrolides D (152), E (153) and G (155)/H (156) were acetylated and the resulting acetates (161 (4 mg), 162 (3 mg), 163 (8 mg) and 164 (11 mg)) were purified by silica-gel preparative TLC and normal phase HPLC.
3. Structure Elucidation of The Rubrolides

149  \(X=Y=\text{Br}, \ Z=R=R'=\text{H}\)
150  \(X=Y=\text{Br}, \ Z=\text{Cl}, \ R=R'=\text{H}\)
151  \(X=Z=R=R'=\text{H}, \ Y=\text{Br}\)
152  \(X=\text{Br}, \ Y=Z=R=R'=\text{H}\)
153  \(X=Y=Z=R=R'=\text{H}\)
154  \(X=Y=Z=R=\text{H}, \ R'=\text{Me}\)
157  \(X=Y=\text{Br}, \ Z=\text{H}, \ R=R'=\text{Me}\)
158  \(X=Y=\text{Br}, \ Z=\text{H}, \ R=R'=\text{Ac}\)
159  \(X=Y=\text{Br}, \ Z=\text{Cl}, \ R=R'=\text{Ac}\)
160  \(X=Z=\text{H}, \ Y=\text{Br}, \ R=R'=\text{Ac}\)
161  \(X=\text{Br}, \ Y=Z=\text{H}, \ R=R'=\text{Ac}\)
162  \(X=Y=Z=\text{H}, \ R=R'=\text{Ac}\)
155  \(Z=\text{H}, \ R=\text{H}\)
156  \(Z=\text{Cl}, \ R=\text{H}\)
163  \(Z=\text{H}, \ R=\text{Ac}\)
164  \(Z=\text{Cl}, \ R=\text{Ac}\)
Rubrolide A (149):

![Rubrolide A structure]

149 R=H; 157 R=Me; 158 R=Ac

Rubrolide A (149) was obtained as a red amorphous solid that gave a parent ion in the EIHRMS at m/z 595.7118 Da, appropriate for a molecular formula of C\(_{17}\)H\(_8\)O\(_4\)\(_{79}\)Br\(_2\)\(_{81}\)Br\(_2\) (\(\Delta M\ -0.1\) mmu). The \(^{13}\)C NMR spectrum of 149 (Fig. 6) contained only thirteen resonances, indicating that there was some element(s) of symmetry in the molecule. The \(^1\)H NMR spectrum of 149 (in DMSO-d\(_6\), Fig. 5) contained only five resonances, all singlets, four of which were in the aromatic/olefinic region (\(\delta\) 6.0-8.5 ppm). The final resonance, a broad singlet accounting for two protons, had a chemical shift of \(\delta\) 10.52 ppm. Integration indicated that the two singlets resonating at \(\delta\) 7.78 and 8.05 ppm accounted for two protons each and the other two singlets at \(\delta\) 6.35 and 6.63 ppm each accounted for one proton. The broad singlet at \(\delta\) 10.52 ppm was exchangable and it was replaced by two acetyl signals (\(\delta\) 2.40 and 2.45 ppm, see Table 2) upon acetylation and by two methoxyl signals (\(\delta\) 3.98 and 3.92 ppm) upon methylation with CH\(_3\)I, suggesting that these two protons were phenolic. A HETCOR experiment (Fig. 8) revealed that the two sets of protons at \(\delta\) 7.78 and 8.05 ppm were attached to relatively deshielded \(sp^2\) carbons at \(\delta\)
Fig. 5. $^1$H NMR spectrum of rubrolide A (149) (400 MHz, DMSO-d$_6$)
Table 1. $^1$H and $^{13}$C NMR Data for Rubrolide A (149) (DMSO-d$_6$)

<table>
<thead>
<tr>
<th>position No.</th>
<th>$^1$H (400 MHz)</th>
<th>COSY$^a$ (long-range)</th>
<th>nOes$^b$</th>
<th>$^{13}$C (50 MHz)</th>
<th>FLOCK$^c$</th>
<th>JC/H (Hz)</th>
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<td></td>
<td></td>
<td>167.76</td>
<td>H2</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>6.63, s</td>
<td>H5</td>
<td>H2'/H6'</td>
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<td></td>
<td>185.2*</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>154.96</td>
<td>H2, H5, H2'/H6'</td>
<td>≈4; 4; 4</td>
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<td></td>
<td></td>
<td>146.58</td>
<td>H2, H5</td>
<td>9.7; 4.8</td>
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<td>H2, H2''/H6''</td>
<td>H2'/H6', H2''/H6''</td>
<td>110.17</td>
<td>H2''/H6''</td>
<td>160.9*; 4.4</td>
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<td>H2, H5</td>
<td></td>
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<td>H6'/H2'</td>
<td>168.6*; 7.5</td>
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<tr>
<td>3'/5'</td>
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<td></td>
<td></td>
<td>112.16</td>
<td>H2'/H6'</td>
<td>2.7</td>
</tr>
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<td>4'</td>
<td></td>
<td></td>
<td></td>
<td>152.77</td>
<td>H2'/H6'</td>
<td>7.6</td>
</tr>
<tr>
<td>1''</td>
<td></td>
<td></td>
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<td>127.57</td>
<td></td>
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</tr>
<tr>
<td>2''/6''</td>
<td>8.05, s</td>
<td>H5</td>
<td>H5</td>
<td>134.22</td>
<td>H5, H6''/H2''</td>
<td>168.1*; 6.3; 7.0</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>111.87</td>
<td>H2''/H6''</td>
<td>2.6</td>
</tr>
<tr>
<td>4''</td>
<td></td>
<td></td>
<td></td>
<td>151.44</td>
<td>H2''/H6''</td>
<td>7.8</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Correlations to the proton in $^1$H column;

$^b$ Proton in $^1$H column irradiated;

$^c$ Correlations to the carbon in $^{13}$C column;

* One bond C-H coupling constants; the others are long-range.
132.45 and 134.22 ppm, respectively. A gated decoupled $^{13}$C NMR experiment* (Fig. 7) showed that both of these carbon signals were methines, suggesting that each resonance accounted for two equivalent sp$^2$ carbons because each proton signal integrated for two protons.

A FLOCK** experiment (long-range C-H correlation) (Fig. 9) revealed that the two aromatic signals ($\delta$ 7.78 and 8.05 ppm) were attributed to two independent phenyl ring systems. FLOCK correlations were observed from H2'/H6' ($\delta$ 7.78 ppm) to C3'/C5' ($\delta$ 112.16 ppm), C4' ($\delta$ 152.77 ppm) and C6'/C2' ($\delta$ 132.45 ppm). The observation of both one-bond (HETCOR) and long-range (FLOCK) correlations between the proton resonance at $\delta$ 7.78 ppm (H2'/H6') and the carbon resonance at $\delta$ 132.45 ppm (C6'/C2') suggested the presence of two equivalent methine carbons situated meta to each other (i.e. 2', 6') on a symmetrically substituted phenyl ring. The gated decoupled $^{13}$C NMR experiment (Fig. 7), which showed the carbon signal at $\delta$ 132.45 ppm to be a dd (J=168.6, 7.5 Hz), also provided evidence for this symmetrical phenyl ring system. The chemical shifts of C3'/C5' and C4' ($\delta$ 112.16 and 152.77 ppm, respectively) suggested that this phenyl ring was hydroxylated at C4' and dibromonated at C3'/C5' (calculated $\delta$'s: 112.0 (C3'/C5') and 152.2 (C4') ppm). All these data unambiguously assigned the proton resonances at $\delta$ 7.78 (H2'/H6') and 10.52 (4'-OH) ppm and the carbon resonances at $\delta$ 112.16 (C3'/C5'), 132.45 (C2'/C6') and 152.77 (C4') ppm to a 3,5-dibromo-4-hydroxyphenyl ring system. A

* This gated decoupling experiment gives a fully proton-coupled $^{13}$C spectrum without losing nOe enhancement. This is achieved by turning on the broadband decoupler during the preparation period to build up nOes and turning off the decoupler during the acquisition period to observe the proton coupling.

** FLOCK is a new version of the long range carbon-proton correlation experiment designed by Professor W. Reynolds of the University of Toronto in 1989. Three BIRD pulse sequences, from which the name FLOCK derives, were incorporated into this experiment to fully suppress one-bond correlations. According to our experience, this new long-range heteronuclear correlation experiment works very effectively as long as a sufficient sample size is available. All possible 2- or 3-bond correlations are observed as expected. For details, see reference 69.
Fig. 8. 2D HETCOR spectrum of rubrolide A (149) (75 MHz, DMSO-d$_6$)
Fig. 9. 2D FLOCK spectrum of rubrolide A (149) (75 MHz, DMSO-d$_6$)
Fig. 10. Low resolution EI mass spectrum of rubrolide A (149)
similar set of arguments led to the assignment of the proton resonances at δ 8.05 (H2''/H6'') and 10.52 ppm and the carbon resonances at δ 134.22 (C2''/6''), 111.87 (C3''/5'') and 151.44 (C4'') ppm to the second 3,5-dibromo-4-hydroxyphenyl residue in 149. Two of the $^{13}$C NMR resonances in the spectrum of 149 (δ 123.59: C1' and 127.57: C1'' ppm) had chemical shifts appropriate for the C1 carbons of these two 3,5-dibromo-4-hydroxyphenyl groups.

The two phenyl ring systems accounted for C$_{12}$H$_{6}$O$_{4}$Br$_{4}$. Therefore, the remaining fragment of rubrolide A (149) had to account for an elemental composition of C$_{5}$H$_{2}$O$_{2}$ and four degrees of unsaturation. Both the IR ($v_{\text{max}}$=1734 cm$^{-1}$) and $^{13}$C NMR (δ 167.76 ppm) data of 149 indicated that an ester group was present in the molecule. The carbonyl stretch in the IR spectrum of rubrolide A dimethylether (157) was 1758 cm$^{-1}$, suggesting that the ester group in rubrolide A (149) was a γ-lactone. The HETCOR experiment (Fig. 8) showed that the protons at δ 6.35 and 6.63 ppm were attached to the carbons at δ 114.57 and 110.17 ppm, respectively. These two =C-H's could be assigned to two trisubstituted double bonds due to the absence of any significant J-coupling between the two olefinic signals. Each of the remaining two olefinic carbon signals (δ 146.58, 154.96 ppm) had to be the other olefinic carbon in each of these two double bonds. Since long range coupling was observed between the two olefinic protons even though both were sharp singlets in the $^1$H NMR, the two double bonds had to be connected to form a diene.

\[ \text{C$_{5}$H$_{2}$O$_{2}$:} \]
Since a FLOCK correlation was observed between the proton resonance at δ 6.63 ppm (H2) and the ester carbon at δ 167.76 ppm (C1), the ester carbon had to be attached to C2 (δ 114.57 ppm). ¹H nOe experiments (Fig. 11) indicated that the phenyl rings were attached to C3 and C5 of the C₅H₂O₂ fragment, respectively. Irradiation of H2'/H6' (δ 7.78 ppm) induced nOes in both of the olefinic protons (δ 6.35: H5; 6.63: H2 ppm), while irradiation of H2''/H6'' (δ 8.05 ppm) induced an nOe only in the olefinic proton at δ 6.35 ppm (H5). On the other hand, an nOe was observed only in H2'/H6' when δ 6.63 ppm (H2) was irradiated, while nOes were observed in both H2'/H6' and H2''/H6'' when δ 6.35 ppm (H5) was irradiated. Therefore, the phenyl ring (B ring) with H2'/H6' (δ 7.78 ppm) was attached to C3, while the other ring (C ring) which had H2''/H6'' (δ 8.05 ppm) was attached to C5. The final site of unsaturation required by the molecular formula was achieved by connecting the alkoxy oxygen with C4 to form an α,β-unsaturated γ-lactone. Therefore, the structure of rubrolide A (149) could be assembled as:

![Diagram of rubrolide A (149)](image)

All of the other available data were completely consistent with this structure (149). For example, in the long-range COSY spectrum of rubrolide A (149), the proton at δ 6.35 ppm (H5) correlated to H2''/H6'' but not to H2'/H6' because it is situated at the benzylic position of ring C but not of ring B. Most importantly, all of the FLOCK long-range C-H correlations (Fig. 12) were fully in agreement with this structure. The three-bond
correlations between $H_2'/6'$ ($\delta$ 7.78) and $C_3$ ($\delta$ 154.96) and between $H_2$ ($\delta$ 6.63) and $C_1'$ ($\delta$ 123.57) fixed the attachment of one 3,5-dibromo-4-hydroxyphenyl residue (ring B) to $C_3$; and the three-bond correlations between $H_2''/6''$ ($\delta$ 8.05) and $C_5$ ($\delta$ 110.17) and between $H_5$ ($\delta$ 6.35) and $C_2''/C_6''$ ($\delta$.134.22) fixed the attachment of the second 3,5-dibromo-4-hydroxyphenyl residue (ring C) to $C_5$. Additional FLOCK correlations between each of the $H_2$ ($\delta$ 6.63) and $H_5$ ($\delta$ 6.35) resonances and both of the carbon resonances at $\delta$ 154.96 ($C_3$) and 146.58 ($C_4$) ppm supported the placement of $H_2$ and $H_5$ on the terminal carbons of a conjugated diene.

![Fig. 12. FLOCK correlations of rubrolide A (149)](image)

The gated decoupled $^{13}$C NMR spectrum of rubrolide A (Fig. 7) was consistent with the above FLOCK results (Fig. 12). $C_1$ was a doublet with $J=8.5$ Hz due to the coupling with $H_2$. This $J$ value was nearly identical with that in a typical $\alpha,\beta$-unsaturated $\gamma$-lactone ring ($J_{H_2-C_1}$=9 Hz), thus confirming the presence of the lactone system in the molecule. The carbon resonance at $\delta$ 134.22 ppm ($C_2''/C_6''$) was a dt ($J=168.1, 7.0$ Hz) due to the one bond coupling to $H_2''/H_6''$ and three-bond couplings to both $H_5$ and $H_6''/H_2''$. $C_1'$ ($\delta$ 123.59 ppm) was a doublet ($J=2.5$ Hz) due to the three bond coupling to $H_2$. $C_4$ was observed as a dd because of the long-range couplings to $H_2$ and $H_5$. $C_1''$ was a singlet, explaining why no FLOCK correlations were observed for $C_1''$. Therefore, the gated
decoupling experiment confirmed the reliability of the FLOCK technique. All of the possible 2- or 3-bond correlations were observed!

Further support for the structure of rubrolide A \( (149) \) came from the similarity of its \(^{13}\text{C} \) NMR chemical shifts (C1: 167.76; C2: 114.57; C3: 154.96; C4: 146.58; C5: 110.17 ppm) to those published for the synthetic compound \( 165 \) (δ 168.5: C1; 114.3: C2 or C5; 158.6: C3; 147.6: C4; 113.6: C2 or C5).\(^{71}\)

![](image)

The \(^1\text{H} \) NMR, \(^{13}\text{C} \) NMR, nOe, long-range COSY and HMBC\(^{72}\) (inverse-detected long-range carbon-proton correlation experiment) data (Table 2) collected for rubrolide A diacetate \( (158) \) were all in complete agreement with the proposed structure of rubrolide A \( (149) \).

An alternative structure, the pyrone \( 166 \), was also consistent with the \(^1\text{H} \) NMR and nOe results. However, it was ruled out on the basis of the following evidence:

![Diagram of 166]
Table 2. $^1$H and $^{13}$C NMR Data for Rubrolide A Diacetate (158) (CDCl$_3$)

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<th>position</th>
<th>$^1$H (400 MHz)</th>
<th>COSY$^a$ (long-range)</th>
<th>nOes$^b$</th>
<th>$^{13}$C (125 MHz)</th>
<th>HMBC$^c$</th>
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<tbody>
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<td>No.</td>
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</tr>
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<td>1</td>
<td></td>
<td></td>
<td></td>
<td>167.0$^d$</td>
<td>H2</td>
</tr>
<tr>
<td>2</td>
<td>6.30, s</td>
<td>H5</td>
<td>H2'/H6'</td>
<td>117.1</td>
<td>H2, H5, H2'/H6'</td>
</tr>
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<td>3</td>
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<td>154.7</td>
<td>H2, H5</td>
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<td>148.5</td>
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</tr>
<tr>
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<td>5.96, s</td>
<td>H2, H2'/H6&quot;</td>
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<td>H2'/H6'</td>
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<td>H5, H6'/H2&quot;</td>
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<td>167.0$^d$, 20.5</td>
<td></td>
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</tbody>
</table>

$^a$ Correlations to the proton in $^1$H column;
$^b$ Proton in $^1$H column irradiated;
$^c$ Correlations to the carbon in $^{13}$C column;
$^d$ Assignments may be interchanged.
1) The carbonyl chemical shift of pyrone systems is very constant, ca. $\delta$ 162 ppm, regardless of the substitution pattern of the pyrone ring. In rubrolide A, the carbonyl resonance was at $\delta$ 167.76 ppm;

2) The IR stretch of the carbonyl in the pyrone ring of the synthetic compound 4,6-diphenyl-\(\alpha\)-pyrone is 1720 cm\(^{-1}\). In rubrolide A, the carbonyl stretch was at 1734 cm\(^{-1}\); and in rubrolide A dimethylether (157), the carbonyl stretch was at 1758 cm\(^{-1}\);

3) The coupling constant between H3 and H5 in a pyrone system is normally ca. 2 Hz. In rubrolide A, each proton was a singlet; also, in the long-range COSY spectrum of rubrolide A (149), the proton at $\delta$ 6.35 ppm (H5) showed long-range coupling to H2''/H6'' but not to H2'/H6';

4) In contrast to pyrones, rubrolide A dimethylether (157) failed to undergo a Diels-Alder reaction with acetylene dicarboxylate;

5) Several FLOCK correlations were inconsistent with the pyrone structure (166). The most improbable was the correlation between H2'/H6' ($\delta$ 7.78 ppm) and the carbon at $\delta$ 154.96 ppm appropriate for C6 in the pyrone system (166). This would represent a very unlikely 5-bond correlation.
UV Studies on Rubrolide A (149)

The color of the rubrolide A (149) solution was found to be dependent on the solvent and the pH of the solution. The neutral methanol solution was yellow. The color intensified upon basification but disappeared upon acidification. This observation was consistent with the following UV results:

UV spectra (Fig. 13) were recorded for rubrolide A in MeOH, 0.1 N NaOH/MeOH and 0.1 N HCl/MeOH. The spectrum of the MeOH solution showed three major absorption bands, i.e., λ 439 nm (ε 14,000), 355 nm (19,000) and 257 nm (23,000). In 0.1 N NaOH/MeOH, the band at 355 nm disappeared and the band at 439 nm significantly intensified (ε 34,000). In contrast, when recorded in 0.1N HCl/MeOH, the band at 355 nm significantly intensified (ε 26,000) and the band at 439 nm disappeared.

This pH dependence of the UV spectrum of rubrolide A can be easily explained by a reversible protonation/deprotonation process (Scheme 1). The absorption band at 439 nm is attributed to the deprotonated form and the band at 355 nm is attributed to the protonated form. Changes in solution pH caused the shifts of equilibrium. As a result, in a basic solution only the fully deprotonated phenolate (149c) existed while in an acidic solution only the phenol form (149) existed. Therefore, rubrolide A is a pH indicator.

In solvents like acetone, DMSO, DMF and pyridine, rubrolide A (149) was bright red. The pH dependence of the acetone solution UV spectrum was very similar to that of the MeOH solution except that the longer wavelength band (phenolate band) experienced a bathochromic shift to 472 nm.
Fig. 13. UV spectra of rubrolide A (149) in
(a) ——— MeOH; (b) ——- 0.1N NaOH/MeOH; (c) ———- 0.1N HCl/MeOH
Scheme 1. Protonation/deprotonation equilibrium of rubrolide A (149)
Rubrolide B (150):

![Chemical Structure of Rubrolide B](image)

In nearly every aspect, rubrolide B (150) was very similar to rubrolide A (149). It was obtained as a red amorphous solid that gave a parent ion in the EIHRMS at m/z 629.6726 Da corresponding to a molecular formula of C_{17}H_{17}O_{4}^{79}Br_{2}^{81}Br_{2}^{35}Cl (ΔM -0.3 mmu). The elemental composition of rubrolide B (150) differed from that of rubrolide A (149) simply by the replacement of one hydrogen atom by a chlorine atom, suggesting that rubrolide B (150) was a monochloro derivative of rubrolide A (149). The $^1$H NMR spectrum of rubrolide B (Fig. 14) was nearly identical with that of rubrolide A except for the absence of the resonance that could be assigned to H2. The absence of H2 was confirmed by both the nOe and long-range COSY results (Table 3). When the olefinic proton at δ 6.32 ppm was irradiated, both of the aromatic signals at δ 7.73 ppm (H2'/H6') and 8.05 ppm (H2''/H6'') were enhanced, indicating that the only olefinic proton (δ 6.32 ppm) in rubrolide B was H5. In the long-range COSY spectrum of rubrolide B (150), H5 was coupled only to the singlet at δ 8.05 ppm attributed to H2''/H6''. Therefore, it was concluded that rubrolide B (150) was the 2-chloro derivative of rubrolide A (149).

Both the FLOCK (Fig. 16) and gated decoupling experiments were in complete agreement with the proposed structure 150. No FLOCK correlations were observed for Cl (δ 163.40 ppm) and Cl' (δ 121.05 ppm) due to the absence of H2. Indeed, both Cl and
Fig. 14. $^{1}H$ NMR spectrum of rubrolide B (150) (400 MHz, DMSO-$d_6$)
Fig. 15. $^{13}$C NMR spectrum of rubrolide B (150) (50 MHz, DMSO-d$_6$)
Fig. 16. 2D FLOCK spectrum of rubrolide B (150) (75 MHz, DMSO-d$_6$)
Table 3. $^1$H and $^{13}$C NMR Data for Rubrolide B (150) (DMSO-d$_6$)

<table>
<thead>
<tr>
<th>position No.</th>
<th>$^1$H (400 MHz)</th>
<th>COSY$^a$ (long-range)</th>
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<th>$^{13}$C (50 MHz)</th>
<th>FLOCK$^c$.</th>
<th>JC/H (Hz)</th>
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</tr>
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<td>H5, H2'/H6'</td>
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<td>H2''/H6''</td>
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<td></td>
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<td></td>
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<td>H2''/H6''</td>
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</table>

$^a$ Correlations to the proton in $^1$H column;  
$^b$ Proton in $^1$H column irradiated;  
$^c$ Correlations to the carbon in $^{13}$C column;  
* One bond C-H coupling constants; the others are long-range.
Cl' appeared as singlets in the gated decoupled $^{13}$C spectrum. Just as in rubrolide A, H5 ($\delta$ 6.35 ppm) showed FLOCK correlations to C3 ($\delta$ 147.25 ppm), C4 ($\delta$ 145.19 ppm) and C2"/C6" ($\delta$ 134.33 ppm); H2'/H6' ($\delta$ 7.73 ppm) showed FLOCK correlations to C3 ($\delta$ 147.25 ppm), C6'/C2' ($\delta$ 132.86 ppm), C3'/C5' ($\delta$ 112.08 ppm) and C4' ($\delta$ 152.78 ppm); and H2"/H6" ($\delta$ 8.05 ppm) showed FLOCK correlations to C5 ($\delta$ 111.41 ppm), C6"/C2" ($\delta$ 134.33 ppm), C3"/C5" ($\delta$ 111.97 ppm) and C4" ($\delta$ 151.88 ppm).

The carbon chemical shifts of C4, C5 and the two phenyl rings (B and C) in rubrolide B (150) (Fig. 15) were nearly identical with those in rubrolide A. The carbon signals ($\delta$ 163.40, 117.96 and 147.25 ppm) assigned to C1, C2 and C3, however, were noticeably different from their counterparts in rubrolide A ($\delta$ 167.76, 114.57 and 154.96 ppm). This was attributed to the chlorination at C2. In an $\alpha,\beta$-unsaturated carboxylic acid derivative, chlorination at C2 usually causes a deshielding effect on C2 (+8 ppm) and shielding effects on C1 and C3.

The $^1$H NMR, $^{13}$C NMR, nOe, long-range COSY and HMBC results (Table 5) of rubrolide B diacetate (159) were completely consistent with the proposed structure of rubrolide B (150).
Rubrolide C (151):

Rubrolide C (151) was obtained as a red amorphous solid that gave a parent ion in the EIHRMS at m/z 437.8928 Da corresponding to a molecular formula of C_{17}H_{10}O_{4}Br^{2}Br (ΔM 0.1 mmu). The ¹H NMR spectrum (Fig. 17) of 151 showed a close correspondence to the ¹H NMR spectrum (Fig. 5) of rubrolide A (149), except that the two-proton singlet resonance assigned to H2'/H6' (δ 7.78, s) in 149 was replaced by a pair of mutually coupled two proton doublets (δ 6.93, d, J = 8.5 Hz and 7.50, d, J = 8.5 Hz). This ¹H NMR evidence indicated that the 3,5-dibromo-4-hydroxyphenyl residue (ring B) attached to C3 in 149 was replaced by a 4-hydroxyphenyl residue at C3 in rubrolide C (151). Both long-range COSY and nOe difference experiments confirmed the placement of a 4-hydroxyphenyl residue (ring B) at C3. NOe enhancements were induced in both H2 (δ 6.42, s) and H5 (δ 6.37, s) when the H2'H6' resonance (δ 7.50, d, J=8.5 Hz) was irradiated and there was no correlation between the H2'H6' (δ 7.50, d, J=8.5 Hz) and H5 resonances (δ 6.37, s) in the long-range COSY.

As expected, the ¹³C chemical shifts of rubrolide C (151) (Fig. 18) were nearly identical with those in rubrolide A (149) except for the 4-hydroxyphenyl residue (ring B). The FLOCK experiment (Fig. 20) showed all of the expected long-range carbon-proton correlations: H2 (δ 6.42) was correlated to C1 (δ 168.27) and C4 (δ 146.31); H5 was
Fig. 17. $^1$H NMR spectrum of rubrolide C (151) (400 MHz, DMSO-d$_6$)
Fig. 18. $^{13}$C NMR spectrum of rubrolide C (151) (50 MHz, DMSO-d$_6$)
Fig. 19. 2D HETCOR spectrum of rubrolide C (151) (75 MHz, DMSO-d₆)
Fig. 20. 2D FLICK spectrum of rubricide C (151) 75 MHz DMSO-d6.
Table 4. $^1$H and $^{13}$C NMR Data for Rubrolide C (151) (DMSO-d$_6$)

<table>
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<tr>
<th>position No.</th>
<th>$^1$H (400 MHz)</th>
<th>COSY$^a$</th>
<th>nOes$^b$</th>
<th>$^{13}$C (50 MHz)</th>
<th>FLOCK$^c$</th>
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<td>152.82</td>
<td>H2''/H6''</td>
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$^a$ Correlations to the proton in $^1$H column; *Observed only in long-range COSY.

$^b$ Proton in $^1$H column irradiated;

$^c$ Correlations to the carbon in $^{13}$C column.
Table 5. $^1$H and $^{13}$C NMR Data for Rubrolides B Diacetate (159) and Rubrolide C Diacetate (160) (CDCl$_3$)

<table>
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<th>position No.</th>
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<th>Rubrolide C diacetate (160)</th>
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<tbody>
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<td>147.0$^b$</td>
<td>H2'/H6', H2''/H6''</td>
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<td>4</td>
<td>146.8$^b$</td>
<td>H2'/H6', H2''/H6''</td>
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<td>7.28, d, J=8.5 Hz H2'/H6'</td>
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<td>133.5</td>
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$^a$ Proton in $^1$H column irradiated;

$^b$, $^c$ Assignments may be interchanged.
correlated to C3 (δ 157.71) and C2"/C6" (δ 134.16); H2'/H6' were correlated to C3 (δ 157.71), C6'/C2' (δ 130.48) and C4' (δ 159.90); H3'/H5' were correlated to C1' (δ 120.28) and C5'/C3' (δ 115.89); and H2"/H6" (δ 8.05) were correlated to C5 (δ 110.56), C6"/C2" (δ 134.16), C3"/C5" (δ 112.38) and C4" (δ 152.82).

The 1H NMR, 13C NMR, nOe, long-range COSY and HMBC results (Table 5) of rubrolide C diacetate (160) were completely consistent with the proposed structure of rubrolide C (151).

Rubrolide D (152):

Rubrolide D (152) was isolated as its diacetate 161. A parent ion was observed at m/z 521.9132 Da appropriate for a molecular formula of C21H14O679Br81Br (ΔM 0.6 mmu) in the EIHRMS of 161. The 1H NMR spectrum (Fig. 21) of 161 revealed that the proton singlet assigned to ring C in rubrolide A (δ 8.05 ppm) was replaced by two mutually coupled doublets (δ 7.85, d, J=8.7 Hz; δ 7.16, d, J=8.7 Hz), indicating that rubrolide D (152) differed from rubrolide A (149) only in the replacement of the 3,5-dibromo-4-hydroxyphenyl residue attached to C5 (ring C) in 149 with a 4-hydroxyphenyl residue in 152.
Fig. 21. $^1$H NMR spectrum of rubrolide D diacetate (161) (400 MHz, CDCl$_3$)
Fig. 22. $^{13}$C NMR spectrum of rubrolide D diacetate (161) (125 MHz, CDCl$_3$)
Both the nOe and long-range COSY experiments (Table 6) on rubrolide D diacetate (161) confirmed this structure. Irradiation of the doublet at δ 7.85 ppm (H2''/H6'') induced nOes in both H3''/H5'' (δ 7.16 ppm) and H5 (δ 6.11 ppm), but not in H2 (δ 6.25 ppm); irradiation of H5 (δ 6.11 ppm) induced nOes in both H2''/H6'' (δ 7.85 ppm) and H2'/H6' (δ 7.69 ppm). In the long-range COSY spectrum, the doublet at δ 7.85 ppm (H2''/H6'') was found to be coupled to H5 (δ 6.11 ppm), confirming the attachment of the 4-hydroxyphenyl ring (ring C) to C5.

Long-range carbon-proton correlations (Table 6) observed in the HMBC spectrum of rubrolide D diacetate (161) were in full agreement with the proposed structure (152) for rubrolide D. Correlations from H2 (δ 6.25 ppm) to both C1 (δ 167.74 ppm) and C4 (δ 147.12 ppm) and from H5 (δ 6.11 ppm) to C3 (δ 154.91 ppm), C4 (δ 147.12 ppm) and C2''/C6'' (δ 132.18 or 132.11 ppm, indistinguishable due to the limited resolution in the carbon domain) allowed the assignment of the carbons in the central fragment (ring A). H2'/H6' (δ 7.69 ppm) correlated to C6'/C2' (δ 132.11 or 132.18 ppm), C3'/C5' (δ 118.72 ppm), C4' (δ 147.98 ppm) and C3 (δ 154.91 ppm), confirming the attachment of the 3,5-dibromo-4-acetoxyphenyl ring (ring B) to C3. H2''/H6'' (δ 7.85 ppm) correlated to C6''/C2'', C4'' (δ 151.56 ppm) and C5 (δ 113.18 ppm), confirming the attachment of the 4-acetoxyphenyl ring (ring C) to C5. The correlation between H3''/H5'' (δ 7.16 ppm) and the carbon at δ 130.23 ppm identified C1'.
Table 6. \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR Data for Rubrolide D Diacetate (161) (in CDCl\(_3\))

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<th>nOes(^b)</th>
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<td>H2'/H6'</td>
<td></td>
<td>147.98</td>
<td>H2'/H6'</td>
</tr>
<tr>
<td>4'</td>
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<td>130.23</td>
<td>H3''/H5''</td>
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<td>1''</td>
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<td>132.11(^d)</td>
<td>H5, H6''/H2''</td>
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<tr>
<td>2''/6''</td>
<td>7.85, d, J=8.7 Hz</td>
<td>H5*, H3''/H5''</td>
<td>H5, H3''/H5''</td>
<td>122.17</td>
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<tr>
<td>3''/5''</td>
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<td>H2''/H6''</td>
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<td>151.56</td>
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<td>167.00, 20.51</td>
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<td>4'-OAc</td>
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<td>4''-OAC</td>
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</table>

\(^a\) Correlations to the proton in \( ^1\text{H} \) column; * Observed only in long-range COSY.
\(^b\) Proton in \( ^1\text{H} \) column irradiated;
\(^c\) Correlations to the carbon in \( ^{13}\text{C} \) column;
\(^d\) Assignments may be interchanged.
Rubrolide E (153):

![Rubrolide E structural formula]

Rubrolide E (153) was isolated as its diacetate 162, a colorless solid that gave a parent ion at m/z 364.0945 Da in the EIHRMS, appropriate for a molecular formula of C_{21}H_{16}O_{6} (ΔM -0.2 mmu). The ^1H NMR spectrum (Fig. 23) of rubrolide E diacetate (162) was nearly identical with that of rubrolide D diacetate (161) except that the singlet assigned to H2'/H6' was replaced by a set of ortho coupled doublets at δ 7.53 (d, J=8.6 Hz) and 7.27 (d, J=8.6 Hz) ppm, indicating that like ring C, ring B was also a 4-acetoxyphenyl residue in 162. Therefore, rubrolide E (153) was the tetra-debromo derivative of rubrolide A (149).

Both nOe (Table 7) and long-range COSY (Fig. 24) experiments performed on rubrolide E diacetate (162) supported this structure. Irradiation of the new doublet at δ 7.53 ppm induced nOes in both H2 (δ 6.21 ppm) and H5 (δ 6.16 ppm) as well as in the other new doublet at δ 7.27 ppm, confirming that these two new doublets were attributed to the H2'/H6' and H3'/H5' protons in ring B. Irradiations of the other protons in rubrolide E diacetate induced nOes in all of the expected protons. In the long-range COSY spectrum (Fig. 24), H5 (δ 6.16 ppm) was correlated to H2 (δ 6.21 ppm) and H2''/H6'' (δ 7.83, d, J=8.7 Hz); the H2''/H6'' resonance was correlated to H3''/H5'' (δ 7.14, d, J=8.7 Hz); the
Fig. 23. $^1$H NMR spectrum of rubrolide E diacetate (162) (400 MHz, CDCl$_3$)
Fig. 24. 2D COSY spectrum of rubrolide E diacetate (162) (400 MHz, CDCl₃)
Fig. 25. $^{13}$C NMR spectrum of rubrolide E diacetate (162) (125 MHz, CDCl₃)
### Table 7. $^1$H and $^{13}$C NMR Data for Rubrolide E Diacetate (162) (CDCl$_3$) and Rubrolide F (154) (CD$_3$OD)

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<th>position No.</th>
<th>$^1$H (400 MHz)</th>
<th>COSY*</th>
<th>nOes$^b$</th>
<th>$^{13}$C (125 MHz)</th>
<th>HMBC$^c$</th>
<th>$^1$H (400 MHz)</th>
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<tr>
<td>2</td>
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<td>157.75</td>
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<tr>
<td>4</td>
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<td></td>
<td>147.78</td>
<td>H2, H5</td>
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<td>H2*, H2''<em>/H6''</em></td>
<td>H2'/H6', H2''/H6''</td>
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<td>H2''/H6''</td>
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<td>1'</td>
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<td>127.90</td>
<td>H3'/H5'</td>
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<td>H3'/H5'</td>
<td>H2, H5, H3'/H5'</td>
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<td>H6'/H2'</td>
<td>7.42, d, J=8.7 Hz</td>
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<td>152.34</td>
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<td>130.60</td>
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<td>2&quot;/6&quot;</td>
<td>7.83, d, J=8.7</td>
<td>H5*, H3''/H5''</td>
<td>H5, H3''/H5&quot;</td>
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<td>H2''/H6&quot;</td>
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<td></td>
<td></td>
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<td>151.27</td>
<td>H2''/H6&quot;</td>
<td></td>
</tr>
<tr>
<td>4'-OAc</td>
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<td></td>
<td>169.15$^d$ 21.12</td>
<td></td>
<td>OMe: 3.85, s, 3H</td>
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<tr>
<td>4&quot;-OAc or OMe</td>
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<td></td>
<td>169.10$^d$ 21.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

*a Correlations to the proton in $^1$H column; * Observed only in long-range COSY.

*b Proton in $^1$H column irradiated; * Correlations to the carbon in $^{13}$C column;

d Assignments may be interchangable.
H2'/H6' (δ 7.53, d, J=8.7 Hz) resonance was only coupled to the doublet at δ 7.27 (J=8.6 Hz, H3'/H5').

HMBC correlations (Table 7) observed for rubrolide E diacetate (162) were in full agreement with the proposed structure. H2 (δ 6.21 ppm) showed correlations to C1 (δ 168.51 ppm), C3 (δ 157.75 ppm) and C4 (δ 147.78 ppm). H5 (δ 6.15 ppm) showed correlations to C3, C4 and C2''/C6'' (δ 132.00 ppm). The correlation between H2''/H6'' (δ 7.83 ppm) and C5 (δ 112.84 ppm) as well as the one between H5 and C2''/C6'' confirmed the attachment of one of the 4-acetoxyphenyl residues to C5. Both H2''/H6'' (δ 7.83 ppm) and H3''/H5'' (δ 7.14 ppm) showed the expected correlations to the appropriate carbons in ring C. The correlation between H2'/H6' (δ 7.53 ppm) and C3 (δ 157.75 ppm) confirmed the attachment of the other 4-acetoxyphenyl residue (ring B) to C3. The correlations from H2'/H6' to both C6'/C2' (δ 129.73 ppm) and C4 (δ 152.34 ppm), and from H3'/H5' (δ 7.27 ppm) to C1' (δ 127.90 ppm) furnished the assignment of the ring B carbons.

Rubrolide F (154):

Rubrolide F (154) was obtained as a colorless solid that gave a parent ion in the EIHRMS at m/z 294.0885 Da, appropriate for a molecular formula of C\textsubscript{18}H\textsubscript{14}O\textsubscript{4} (ΔM -0.7 mmu). The \textsuperscript{1}H NMR spectrum (Fig. 26) of rubrolide F showed that it was a monomethyl ether of rubrolide E (153). NOe difference experiments revealed that the methoxy residue (δ
Fig. 26. $^1$H NMR spectrum of rubrolide F (118) (400 MHz, CD$_3$OD)
3.83 ppm, s, 3H) in 154 was located at C4' of ring C. Irradiation of the singlet at δ 6.38 ppm induced nOes in both of the relatively deshielded doublets at δ 7.78 and 7.36 ppm (H2'/H6' and H2''/H6''), identifying this olefinic singlet as H5. An nOe in H5 was observed when the doublet at δ 7.78 ppm was irradiated, indicating that this doublet was H2''/H6''. The δ 7.78 ppm resonance was ortho coupled to H3''/H5'' (δ 6.97, d, J=8.9 Hz). Irradiation of H3''/H5'' induced an nOe in the methyl singlet at δ 3.83 ppm, and thus the methyl ether was located at the C4'' of ring C.

Rubrolide G (155):

Rubrolide G (155) was isolated as its triacetate 163, a colorless solid that gave a parent ion in the EIHRMS at m/z 739.7532 Da corresponding to a molecular formula of C23H16O879Br281Br2 (ΔM -0.9 mmu). Unlike rubrolides A-F (149-154), which did not have any aliphatic protons in the molecules, rubrolide G triacetate (163) had an aliphatic methylene group in the molecule as indicated by its 1H NMR spectrum (Fig. 27) (AB system: δ 3.55, d, J=14.2 Hz; δ 3.08, d, J=14.2 Hz). There were three signals in the downfield region: a one-proton singlet at δ 6.33 ppm that could be assigned to H2 or H5, a two-proton singlet at δ 7.71 ppm, and a broad two-proton singlet at δ 7.22 ppm that
Fig. 27. $^1$H NMR spectrum of rubrolide G triacetate (163) (400 MHz, CDCl$_3$)
Fig. 28. $^{13}$C NMR spectrum of rubrolide G triacetate (163) (125 MHz, CDCl$_3$)
sharpened at 50°C. The third acetyl observed in the spectrum was at δ 2.18 ppm, indicating that it was not a phenol acetate.

Irradiations of the methylene protons induced nOes (Table 8) in both the singlet at δ 7.71 ppm and the broad signal at δ 7.22 ppm, indicating that the methylene was located at C5. NOes were observed to both this methylene and the olefinic proton at δ 6.33 ppm when δ 7.71 ppm was irradiated, therefore identifying the δ 7.71 ppm peak as H2'/H6' and the signal at δ 6.33 ppm as H2. The nOe results as well as the correlation observed in a high temperature long-range COSY experiment between the methylene and the singlet at δ 7.22 ppm assigned the broad signal at δ 7.22 ppm to H2''/H6''.

The 13C NMR spectrum (Fig. 28) of rubrolide G triacetate (163) revealed the presence of a methylene carbon (δ 41.97 ppm) and an acetal carbon (δ 105.46 ppm)* as well as the carbons that could be easily assigned to two 3,5-dibromo-4-acetoxyphenyl rings and an α,β-unsaturated ester system based on the comparison with the 13C NMR spectrum of rubrolide A diacetate (158). HMBC (Fig. 29) correlations to the acetal carbon (δ 105.46 ppm) from both H2 (δ 6.33 ppm) and the C5 methylene protons (δ 3.55, 3.08 ppm) located the acetal at C4. Therefore, rubrolide G (155) simply was the 4,5-hydration version of rubrolide A (149). This was confirmed by further HMBC correlations of rubrolide G triacetate (163): H2 also showed correlations to C1 (δ 167.60 ppm), C3 (δ 158.55 ppm) and C1' (δ 129.33 ppm); the methylene protons also correlated to C3, C1'' (δ 132.26 ppm) and C2''/C6'' (δ 134.18 ppm), the latter two correlations confirmed the attachment of one 3,5-dibromo-4-acetoxyphenyl residue (ring C) at C5; and the correlation from H2'/H6' (δ 7.71 ppm) to C3 (δ 158.55 ppm) confirmed the attachment of the second 3,5-dibromo-4-acetoxyphenyl ring (ring B) to C3, while the correlations to C6'/C2' (δ 131.06 ppm),

* The 13C chemical shift of the acetal carbon in rubrolide G triacetate (163) was nearly identical with that (105.3 ppm) of the model compound, citricolic acid (169),75 which also had the α, β-unsaturated γ-lactone system with an acetal at the γ-position.
Fig. 29. 2D HMBC spectrum of rubrolide G triacetate (163) (CDCl₃)
Fig. 30. Low resolution EI mass spectrum of rubrolide G triacetate (163)
Table 8. $^1$H and $^{13}$C NMR Data for Rubrolide G Triacetate (163) and Rubrolide H Triacetate (164) (CDCl₃)

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<th>$^1$H (400 MHz)</th>
<th>COSY$^a$ (long range)</th>
<th>nOes$^b$</th>
<th>$^{13}$C (125 MHz)</th>
<th>HMBC$^c$</th>
<th>$^1$H (400 MHz)</th>
<th>$^{13}$C (125 MHz)</th>
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<td>H2</td>
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<td>124.66</td>
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<td>158.55</td>
<td>H2, H5, H2'/H6'</td>
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<td>2.40, s, 3H</td>
<td>167.66,$^e$ 20.46$^f$</td>
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</table>

$^a$ Correlations to the proton in $^1$H column; $^b$ Proton in $^1$H column irradiated; $^c$ Correlations to the carbon in $^{13}$C column; $^d$ Sharpened at 50°C. $^e,f$ Assignments may be interchanged.
C3'/C5' (δ 119.30 ppm) and C4' (δ 148.85 ppm) identified the carbons in ring B. The remaining two unassigned carbons at δ 117.58 and 146.02 ppm were therefore attributed to C3''/C5'' and C4'', respectively. The expected correlations from H2''/H6'' (δ 7.22 ppm) to C3''/C5'' (δ 117.58 ppm) and C4'' (δ 146.02 ppm) were not observed due to the broadening of this proton signal. The broadening was attributed to hindered rotation about the C5-C1'' bond caused by the presence of the C4-acetoxyl group.

Further support for the proposed structure of 155 came from the trapping of the ring-opened tautomer (155a) to form the methyl ester 167 when a crude mixture of rubrolides G (155) and H (156) was treated with diazomethane (Scheme 2). Another product obtained in this reaction was compound 168, a diazomethane addition product. The spectral data obtained for compounds 167 and 168 (see Experimental) were in complete agreement with the structures shown. It was reported that the model compound 169, citricolic acid, also existed in two tautomeric forms similar to 155 and 155a.\(^{75}\)

The HREIMS fragmentation patterns of rubrolide G triacetate (163) provided additional support for the proposed structure. For example, the base peak at m/z 264.8680 Da, appropriate for C7H5O79Br-81Br (ΔM -0.9 mmu), was attributed to the 3,5-dibromo-4-hydroxybenzyl fragment which was never observed in the EIMS spectra of rubrolides A-F (149-154) and their corresponding acetates.
Scheme 2. Methylation of rubrolide G (155)

169
Rubrolide H (156):

Rubrolide H (156) was isolated as its triacetate 164, a colorless solid that gave a parent ion in the EIHRMS at m/z 773.7158 Da, appropriate for a molecular formula of C_{23}H_{15}O_8^{79}Br_2^{81}Br_2^{35}Cl (ΔM 0.6 mmu). The elemental composition of 164 differed from that of 163 simply by the replacement of one hydrogen atom by a chlorine atom, suggesting that rubrolide H triacetate (164) was a monochloro derivative of rubrolide G triacetate (163). Examination of the 1H NMR spectrum (Fig. 31) of 164 showed that it was nearly identical with the 1H NMR spectrum (Fig. 27) of 163 except for the absence of the H2 resonance, indicating that rubrolide H triacetate (164) was the 2-chloro derivative of rubrolide G triacetate (163). Like rubrolide G triacetate (163), rubrolide H triacetate (164) also showed a broad signal (δ 7.22 ppm, H2"/H6") in the 1H NMR spectrum which sharpened at 50°C.

Both nOe and long-range COSY results of rubrolide H triacetate (164) supported the proposed structure. Irradiation of H2'/H6' (δ 7.87 ppm) induced nOes only in the methylene protons (H5a,b: δ 3.48, d, J=14.3 Hz; 3.05, d, J=14.3 Hz), providing evidence for the absence of H2. In the long-range COSY spectrum recorded at 50°C, couplings were observed between the methylene protons (H5a,b: δ 3.48, 3.05 ppm) and the singlet at δ 7.22
Fig. 31. $^1$H NMR spectrum of rubrolide H triacetate (164) (400 MHz, CDCl$_3$)
Fig. 32. $^{13}$C NMR spectrum of rubrolide H triacetate (164) (125 MHz, CDCl$_3$)
ppm (H2"/H6"), confirming the attachment of a 3,5-dibromo-4-acetoxyphenyl ring (ring C) to C5.

The 13C NMR chemical shifts (Fig. 32) of rubrolide H triacetate (164) were nearly identical with those of rubrolide G triacetate (163) except for C1 (δ 163.33 ppm), C2 (δ 124.66 ppm) and C3 (δ 147.52 ppm). The upfield shifts observed for C1 (-4.3 ppm) and C3 (-11.0 ppm) and the downfield shift observed for C2 (+4.8 ppm) were consistent with the chlorination at C2. A similar effect was also observed in rubrolide B (150).

4. Biological Activities of Rubrolides A-C (149-151)

Rubrolides A-C (149-151) have been subjected to phosphatase inhibition, antibacterial, antifungal and cytotoxicity bioassays. Some of the observed biological activities are presented in Table 9. The phosphatase inhibitory activity was moderate but selective, stronger against phosphatase 2A than against phosphatase 1. The antibacterial activities were very significant. It is interesting that rubrolides A-C (149-151) were quite potent against several human pathogens (Methicillin resistant Staphylococcus aureus, Enterococcus sp., Pneumococcus) that lack effective clinical antibiotics. Both the antifungal (vs. Candida albicans) and the cytotoxic (vs. murine leukemia L1210 cell lines) activities were negligible.
<table>
<thead>
<tr>
<th>Bioassay</th>
<th>Specificity</th>
<th>Rubrolide A (149)</th>
<th>Rubrolide B (150)</th>
<th>Rubrolide C (151)</th>
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</thead>
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<td>Phosphatase inhibition (IC₅₀: µM)</td>
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<td>155</td>
<td>630</td>
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<tr>
<td></td>
<td>PP2A</td>
<td>195</td>
<td>60</td>
<td>190</td>
</tr>
<tr>
<td>Antibacterial activity (MIC: µg/disc)</td>
<td><em>Staphylococcus aureus</em></td>
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<td>10.9</td>
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<td><em>Bacillus subtilis</em></td>
<td>8.2</td>
<td>2.1</td>
<td>10.9</td>
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<tr>
<td>Antibacterial activity (MIC: µg/ml)</td>
<td>Methicillin resistant <em>Staphylococcus aureus</em></td>
<td>30-60</td>
<td>&lt;30*</td>
<td>&lt;30*</td>
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<tr>
<td></td>
<td><em>Enterococcus sp.</em></td>
<td>30-60</td>
<td>&lt;30*</td>
<td>&lt;30*</td>
</tr>
<tr>
<td></td>
<td><em>Pneumococcus</em></td>
<td>30-60</td>
<td>&lt;30*</td>
<td>&lt;30*</td>
</tr>
<tr>
<td>Cytotoxicity (IC₅₀: µg/ml)</td>
<td>Murine Leukemia L1210</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 30 µg/ml was the lowest concentration tested and it was clear that rubrolides B and C were still very active at this level.
C. CONCLUSIONS

The rubrolides (149-156) represent a novel class of marine secondary metabolites which contain a butenolide skeleton. A few natural products from terrestrial plants and microorganisms are structurally related to the rubrolides. The butenolides 170-172, metabolites of the fungus Aspergillus terreus, have a carbon skeleton similar to that of the rubrolides. The significant difference between the two families of metabolites is the presence of a carboxyl group at C4 and a hydroxyl group at C2 as well as the absence of the C4-C5 exocyclic double bond in the three fungal metabolites 170-172. Biosynthetic studies utilizing radioisotope-labelled precursors have demonstrated that the butenolides 170-172 are biosynthesized in the fungus Aspergillus terreus from two molecules of phenylalanine (Scheme 3). The key reaction in this biosynthetic pathway involves the coupling of two molecules of 3-(4'-hydroxyphenyl)pyruvic acid methyl ester (173a), which is derived from phenylalanine via oxidation and methylation, to form the γ-lactone ring.

Based on the structural similarities of the rubrolides to the butenolides 170-172, we propose that the rubrolides are also derived from phenylalanine or tyrosine via a similar biosynthetic pathway (Scheme 4). The Aldol condensation reaction between two molecules of 3-(4'-hydroxy)phenylpyruvic acid (173) gives the intermediate 174, which in turn can be
Scheme 3. Biosynthesis of butenolides 170-172 by *Aspergillus terreus*
Scheme 4. Proposed biosynthetic pathway for rubrolides (149-156)
converted to the γ-keto-α,β-unsaturated acid 175 via an oxidative decarboxylation at C4 and reduction-dehydration at C2. Cyclization of 175 yields the key intermediate, lactone 176. Halogenation of this intermediate, 176, leads to the formation of rubrolides G (155) and H (156). Intermediate 176 can also readily undergo dehydration to yield rubrolide E (153), which in turn can be methylated to form rubrolide F (154). Halogenation of rubrolide E (153) leads to the formation of rubrolides A, B, C and D (149-152). The isolation of rubrolides G and H along with rubrolides A-F provides evidence for the involvement of the intermediate 176 in the biosynthetic pathway.

Compound 177, a secondary metabolite isolated from the heartwood of the African medicinal plant *Pericopsis elata*,\(^7\) has a carbon skeleton identical with that of the rubrolides. However, it lacks the C4-C5 double bond and has a *meta* hydroxylation pattern in the B ring, which indicates that ring B is likely not derived from phenylalanine or tyrosine. The abundance of flavanoids and related compounds in the same plant, especially the close structural resemblance between 177 and the co-existing chalcone derivative 178, suggests that this new butenolide, 177, is probably derived from flavanoid biosynthesis. Therefore, despite the striking structural similarity between 177 and the rubrolides, they appear to be biosynthesized through different pathways.

![Diagram of 177 and 178](image)

At the beginning of this project, we were expecting to encounter nitrogenous natural products from the tunicate *Ritterella rubra*. The isolation of the non-nitrogenous rubrolides...
Rubrolides A-H (149-156) represent the largest group among the small minority of non-nitrogenous tunicate secondary metabolites.

A number of brominated secondary metabolites, i.e., several eudistomins (15-40),27-30 shermilamine A (69),36a 2-bromoleptoclinidinone (82)36e and diazonamide B (107),45 have been reported from tunicates. Iodinated compounds, e.g., 116,48 128 and 129,52 have also been encountered previously. Chlorinated tunicate metabolites, however, remained unknown until very recently (1991). Diazonamides A (106) and B (107),45 the first two chlorinated tunicate compounds, were reported during the preparation of this thesis. Rubrolides B (150) and H (156) are also among the first examples of chlorinated natural products from tunicates.

*R. sigillinoides* (=*R. pulchra*) is the only other species in the genus *Ritterella* that has been subjected to chemical investigation.29 The major secondary metabolites isolated from this species are several β-carboline compounds, namely, eudistomins C (27), K (30), O (18), eudistomin K sulfoxide (33) and debromoeudistomin K (32), most of which are brominated. *Ritterella rubra* is the first Northeast Pacific marine tunicate from which novel secondary metabolites have been isolated.

In conclusion, our chemical investigation of the Northeast Pacific tunicate *Ritterella rubra* has led to the isolation and identification of a novel class of natural products, rubrolides A-H (149-156). Rubrolides are the metabolites responsible for the phosphatase inhibitory and antibiotic activities found in the crude tunicate extract. It is interesting that the chemical structures of rubrolides differ distinctively from those of the existing phosphatase inhibitors (e.g., 147 and 148). This information may be valuable for understanding the mechanisms of phosphatase inhibition. Moreover, the rubrolides possess potent antibacterial activity against several human pathogens that urgently need effective antibiotics. This property and the low cytotoxicity of the rubrolides are very promising signs for potential clinical antibiotics. The antibiotic properties of the rubrolides warrant further study.
D. EXPERIMENTAL

1. General:

All $^1$H NMR spectra were recorded on a Brucker WH-400 spectrometer. Chemical shifts are reported in ppm downfield from the tetramethylsilane resonance with either internal tetramethylsilane ($\delta$ 0.00 ppm) or the solvent residual peak (DMSO-$d_6$: $\delta$ 2.50 ppm; CDCl$_3$: $\delta$ 7.26 ppm; CD$_3$OD: $\delta$ 3.20 ppm) as the reference. Coupling constants are reported in hertz. The $^{13}$C NMR spectra were recorded either at 50 MHz on a Brucker AC200, at 75 MHz on a Varian XL300, or at 125 MHz on a Brucker AMX-500 spectrometer. Solvent peaks were used as internal standards: DMSO-$d_6$: $\delta$ 39.5 ppm; CDCl$_3$: $\delta$ 77.0 ppm. All COSY, long-range COSY and nOe difference NMR experiments were carried out on a Brucker WH-400 spectrometer using standard parameters. The HETCOR and FLOCK experiments were performed on a Varian XL-300 spectrometer. In the FLOCK experiments, either 32 or 64 increments were recorded with delays optimized for 2- or 3-bond carbon-proton coupling (ca. 7.5 Hz). The inverse-detected HMQC (one-bond) and HMBC (2- or 3-bond) experiments were carried out on a Brucker AMX-500 spectrometer (500 MHz for $^1$H and 125 MHz for $^{13}$C).

Low resolution electron impact mass spectra were recorded on a Kratos AEI-59 mass spectrometer and the high resolution mass spectra were recorded on a Kratos AEI-50 mass spectrometer. Infrared spectra were recorded on a Perkin-Elmer 1600 FT spectrometer. Ultraviolet-visible absorption spectra were recorded on a Bausch-Lomb Spectronic-2000 spectrophotometer.

Gel permeation chromatography was carried out using Sephadex LH20 resin. Normal phase column chromatography was carried out on Merck silica gel G60 (230-400 mesh). Normal phase thin layer chromatography was performed on Merck Type 5554 silica gel plates (aluminum backed). All solvents used were either analytical or BDH Omnisolve grade. High performance liquid chromatography was performed on a Perkin-Elmer Series 2
instrument using a Perkin-Elmer LC-55 spectrophotometer as the detector. All solvents used for HPLC were BDH Omnisolve grade.

Protein phosphatase assays were carried out by Dr. C. Holmes of the University of Alberta using the procedure reported in the literature (reference 60 and the references therein). Cytotoxicity assays were carried out by Dr. T. M. Allen of the University of Alberta using the murine leukemia cell line. Antibacterial assays against Methicillin resistant Staphylococcus aureas, Enterococcus sp., and Pneumococcus were performed by Dr. M. Kelly of the University of British Columbia using liquid cultures. Antibacterial assays against Staphylococcus aureas and Bacillus subtilis and antifungal assays against Candida albicans and Pseudomonas aeruginosa were performed by Mr. M. LeBlonc of the University of British Columbia using the disc diffusion method.

2. Sample Collection Data:

The bright red Ritterella rubra specimens (collection #: QC-22-5-90-63) were collected by hand using SCUBA near Anthony Island, Queen Charlotte Islands, B.C., at -3 m to -5 m in May 1990 and kept frozen until workup.

3. Isolation and Purification:

The frozen sample (400 g) was immersed and thawed in 1L methanol, homogenized in a Waring blender and extracted at r.t. for two days. The methanol extract was filtered and concentrated in vacuo to give a red aqueous suspension which was then diluted to 300 mL and partitioned sequentially against hexanes (400 mL x 3), dichloromethane (400 mL x 3) and ethyl acetate (400 mL x 3). All three organic solutions were dried over anhydrous sodium sulphate and evaporated in vacuo. $^1$H NMR experiments indicated that both the dichloromethane and the ethyl acetate solubles contained similar aromatic compounds. The two fractions were thus combined and chromatographed on Sephadex LH20 (methanol as the eluent) to give seven major fractions containing rubrolides G/H, F, E, D, B, C and A in
Rubrolides A, B and C were independently rechromatographed on Sephadex LH20 (40:10:4 EtOAc/MeOH/H2O as the eluent) to afford pure rubrolides A (149) (132 mg), B (150) (68 mg) and C (151) (48 mg). Fractionation on TLC (1:1 EtOAc/hexane) yielded 3 mg pure rubrolide F (154). Samples of each of the impure rubrolides D (152), E (153) and G (155)/H (156) were acetylated with Ac2O/pyridine (5 h at r.t.) and purified on TLC (silica gel, 1% MeOH/CH2Cl2) followed by HPLC (silica gel, 25% EtOAc/hexane) to give pure rubrolides D (4 mg), E (3 mg), G (8 mg) and H (11 mg) in their respective acetylated forms.
Rubrolide A (149):

A red (from acetone or DMSO) or yellow (from methanol or water) amorphous solid; UV (MeOH), $\lambda_{\text{max}}$: 439 ($\varepsilon$ 14,000), 355 (19,000), 324 (17,000 sh), 257 (23,000) nm; UV (0.1N NaOH/MeOH), $\lambda_{\text{max}}$: 439 ($\varepsilon$ 34,000), 337 (6,000), 321 (85,00), 266 (21,000) nm; UV (0.1N HCl/MeOH), $\lambda_{\text{max}}$: 355 ($\varepsilon$ 26,000), 253 (19,000) nm; IR (film), $\nu_{\text{max}}$: 1734, 1717, 1654, 1559, 1508, 1474, 1458, 1219, 732 cm$^{-1}$; $^1$H NMR (DMSO-d$_6$, 400 MHz), see Table 1; $^{13}$C NMR (DMSO-d$_6$, 50 MHz), see Table 1; EIMS, $m/z$ (relative intensity): 600 (16.9), 598 (65.2), 596 (100.0), 594 (67.1), 592 (17.4), 540 (2.3), 538 (1.6), 520 (6.1), 518 (19.1), 516 (19.5), 514 (6.8), 463 (7.0), 461 (19.8), 459 (19.5), 457 (6.5), 440 (2.0), 438 (5.0), 436 (4.7), 434 (1.5), 410 (6.4), 408 (13.6), 406 (6.5), 381 (6.9), 294 (16.8), 292 (28.6), 290 (14.5), 279 (14.5), 204 (16.2), 185 (27.9); HREIMS: 597.7104 (M$^+$: C$_{17}$H$_8$O$_4$Br$_{81}$Br$_3$, $\Delta M$=0.4 mmu); Biological activities: see Table 9.
Rubrolide B (150):

\[
\begin{align*}
\text{Cl} & \quad \text{O} \\
\text{Br} & \quad \text{O} \\
\text{Br} & \quad \text{Br} \\
\text{OH} & \quad \text{Br} \\
\text{Br} & \quad \text{OH} \\
\end{align*}
\]

A red (from acetone or DMSO) or yellow (from methanol or water) amorphous solid; \(^1\)H NMR (DMSO-d\textsubscript{6}, 400 MHz), see Table 3; \(^1^3\)C NMR (DMSO-d\textsubscript{6}, 50 MHz), see Table 3; EIMS, \textit{m/z} (relative intensity): 634 (30.0), 632 (81.5), 630 (100.0), 628 (60.7), 626 (14.8), 600 (0.3), 598 (1.8), 596 (3.0), 594 (2.0), 592 (0.3), 552 (8.5), 550 (6.3), 541 (12.6), 539 (18.6), 537 (12.6), 535 (3.7), 518 (3.2), 516 (4.7), 497 (3.9), 495 (7.7), 493 (6.0), 444 (6.1), 442 (7.9), 440 (10.8), 438 (15.4), 436 (8.6), 310 (7.3), 294 (19.4), 292 (31.1), 279 (21.5), 262 (6.1), 185 (38.1), 183 (38.5), 134 (18.0), 121 (26.0), 82 (71.0), 80 (70.3), 43 (32.9), 32 (56.7); HREIMS: 629.6726 (M\textsuperscript{+}: C\textsubscript{17}H\textsubscript{7}O\textsubscript{4}\textsuperscript{79}Br\textsubscript{2}\textsuperscript{81}Br\textsuperscript{35}Cl, \Delta M=0.3 \text{ mmu}); Biological activities: see Table 9.

Rubrolide C (151):

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{Br} & \quad \text{Br} \\
\text{OH} & \quad \text{Br} \\
\text{OH} & \quad \text{Br} \\
\end{align*}
\]
A red (from acetone or DMSO) or yellow (from methanol or water) amorphous solid; 
$^1$H NMR (DMSO-d$_6$, 400 MHz), see Table 4; $^{13}$C NMR (DMSO-d$_6$, 50 MHz), see Table 4; EIMS, $m/z$ (relative intensity): 440 (21.8), 438 (46.0), 436 (22.1), 384 (2.8), 382 (5.7), 380 (2.8), 303 (23.9), 301 (24.6), 294 (12.6), 292 (25.2), 290 (13.1), 278 (5.8), 264 (6.4), 262 (5.6), 250 (10.3), 222 (7.1), 185 (23.2), 183 (22.6), 165 (16.1), 154 (35.0), 32 (100); HREIMS: 437.8928 (M$^+$: C$_{17}$H$_{10}$O$_4$H$_7$Br$_{81}$Br, ΔM=0.1 mmu); Biological activities: see Table 9.

**Rubrolide F (154):**

![Rubrolide F](image)

Obtained as a yellow solid; IR (CH$_2$Cl$_2$), $\nu_{\text{max}}$: 2927, 2856, 1750, 1603, 1510, 1420, 1172, 1086, 1031, 896, 832 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz), see Table 7; EIMS, $m/z$ (relative intensity): 295 (25.4), 294 (100.0), 279 (5.1), 266 (4.4), 223 (13.2), 165 (4.9), 148 (42.2), 133 (21.8), 120 (23.9), 77 (19.6); HREIMS: 294.0885 (M$^+$: C$_{18}$H$_{14}$O$_4$, ΔM=-0.7 mmu), 279.0667 (M$^+$-CH$_3$: C$_{17}$H$_{11}$O$_4$, ΔM=0.9 mmu).

**Methylation of Rubrolide A (149):**

Rubrolide A (3 mg) was dissolved in 5 ml dry dimethylformamide. To this red solution were added 1 ml methyl iodide (through a basic alumina plug that was flamed and cooled under nitrogen) and 0.5 g anhydrous K$_2$CO$_3$. The reaction mixture was stirred at r.t.
for 2 h until the red color completely disappeared. After removal of the K$_2$CO$_3$ via filtration and evaporation of DMF *in vacuo*, the mixture was purified on TLC (silica gel, 1:1 EtOAc/hexane) to give 3 mg rubrolide A dimethylether (157) as a colorless solid. \textsuperscript{1}H NMR (CDCl$_3$, 400 MHz) $\delta$ 7.96 (s, 2H), 7.62 (s, 2H), 6.25 (s, 1H), 5.92 (s, 1H), 3.98 (s, 3H), 3.92 (s, 3H) ppm; EIMS, $m/z$ (relative intensity): 628 (17.3), 626 (63.6), 624 (98.6), 622 (66.2), 620 (16.4), 613 (6.8), 611 (24.9), 609 (37.0), 607 (24.7), 605 (6.5), 548 (5.0), 546 (14.6), 544 (15.0), 542 (5.8), 533 (1.8), 531 (6.0), 529 (6.3), 527 (2.5), 468 (4.0), 466 (9.1), 464 (5.3), 438 (3.8), 436 (6.8), 434 (3.7), 423 (2.4), 421 (5.1), 419 (2.7), 293 (50.7), 291 (100.0), 289 (49.8), 277 (10.4), 232 (14.3), 218 (19.8), 82 (43.0), 80 (44.0), 32 (64.0).

**Acetylation of the Rubrolides**

Each crude rubrolide sample was individually dissolved in 1 ml dry pyridine. To this red solution was added 1 ml dry acetic anhydride. The color immediately changed to greenish. The reaction mixture was stirred at r.t. for 5 h and then evaporated to dryness *in vacuo*. Chromatography on TLC (silica gel, 1% MeOH/CH$_2$Cl$_2$) followed by HPLC (silica gel, 25% EtOAc/hexane) afforded the corresponding acetate.

**Rubrolide A Diacetate (158):**

Obtained as a colorless solid; IR (CH$_2$Cl$_2$), $\nu_{\text{max}}$: 1784, 1772, 1544, 1455, 1371, 1181, 1010, 850 cm$^{-1}$; \textsuperscript{1}H NMR (CDCl$_3$, 400 MHz), see Table 2; \textsuperscript{13}C NMR (CDCl$_3$, 125 MHz), see Table 2; EIMS, $m/z$ (relative intensity): 642 (9.2), 640 (34.6), 638 (50.6), 636 (35.3), 634 (9.3), 600 (10.4), 598 (37.7), 596 (54.6), 594 (38.8), 592 (9.6), 518 (1.9), 516 (2.7), 459 (7.1), 457 (2.2), 408 (5.6), 291 (11.7), 43 (100.0); HREIMS: 637.7226 (M$^+$-C$_2$H$_2$O: C$_{19}$H$_{10}$O$_5$$^{79}$Br$_2$$^{81}$Br$_2$, $\Delta$M=0.2 mmu), 595.7125 (M$^+$-2C$_2$H$_2$O: C$_{17}$H$_8$O$_4$$^{79}$Br$_2$$^{81}$Br$_2$, $\Delta$M=0.6 mmu).
Rubrolide B Diacetate (159):

Obtained as a colorless solid; IR (CH₂Cl₂), vₓmax: 1790, 1544, 1455, 1371, 1182, 1008, 898 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz), see Table 5; ¹³C NMR (CDCl₃, 125 MHz), see Table 5; EIMS, m/z (relative intensity): 718 (0.6), 716 (1.3), 714 (1.5), 712 (0.9), 676 (16.9), 674 (42.5), 672 (60.0), 670 (31.2), 668 (7.7), 636 (4.7), 634 (18.8), 632 (46.6), 630 (58.8), 628 (35.4), 626 (8.5), 598 (2.3), 596 (3.7), 594 (3.4), 593 (3.2), 591 (2.6), 541 (2.3), 539 (3.9), 537 (92.6), 291 (10.1), 289 (4.7), 235 (2.1), 183 (5.7), 121 (3.1), 80 (6.2), 43 (100.0); HREIMS: 713.6925 (M⁺: C₂₁H₁₁O₆⁷⁹Br₂₈¹Br₂₃⁵Cl, ΔM=-1.5 mmu or C₂₁H₁₁O₆⁷⁹Br₃₈¹Br₃⁷Cl, ΔM=-0.6 mmu), 671.6840 (M⁺-C₂H₂O: C₁₉H₉O₅⁷⁹Br₂₈¹Br₂₃⁵Cl, ΔM=0.5 mmu or C₁₉H₉O₅⁷⁹Br₃₈¹Br₃⁷Cl, ΔM=1.5 mmu), 629.6710 (M⁺- 2C₂H₂O: C₁₇H₇O₄⁷⁹Br₂₈¹Br₂₃⁵Cl, ΔM=-1.9 mmu or C₁₇H₇O₄⁷⁹Br₃₈¹Br₃⁷Cl, ΔM=-0.9 mmu).

Rubrolide C Diacetate (160):

Obtained as a colorless solid; IR (CH₂Cl₂), vₓmax: 2925, 2854, 1775, 1503, 1458, 1371, 1213, 1200, 1180, 1168, 1065, 912 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz), see Table 5; ¹³C NMR (CDCl₃, 125 MHz), see Table 5; EIMS, m/z (relative intensity): 482 (20.9), 480 (41.5), 478 (20.3), 440 (36.3), 438 (74.8), 436 (410), 384 (4.0), 382 (7.4), 380 (4.1), 336 (16.3), 303 (22.2), 301 (24.1), 294 (39.0), 292 (23.7), 223 (12.5), 185 (17.6), 183 (17.9), 165 (20.2), 148 (17.3), 118 (32.1), 43 (100.0); HREIMS: 479.9024 (M⁺-C₂H₂O: C₁₉H₁₂O₅⁷⁹Br₈¹Br, ΔM=-0.9 mmu), 437.8923 (M⁺- 2C₂H₂O: C₁₇H₁₀O₄⁷⁹Br₈¹Br, ΔM=-0.4 mmu).
Rubrolide D Diacetate (161):

Obtained as a colorless solid; IR (CH₂Cl₂), vmax: 1766, 1506, 1455, 1370, 1217, 1198, 1183, 1169, 946 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz), see Table 6; ¹³C NMR (CDCl₃, 125 MHz), see Table 6; EIMS, m/z (relative intensity): 524 (3.6), 522 (7.1), 520 (3.7), 482 (37.6), 480 (74.6), 478 (37.2), 440 (51.0), 438 (100.0), 436 (51.7), 382 (2.4), 358 (3.1), 330 (2.2), 301 (5.3), 274 (5.0), 250 (17.6), 221 (7.7), 134 (36.7), 43 (91.0), 32 (81.4); HREIMS: 521.9132 (M⁺: C₂₁H₁₄O₆⁷⁹Br⁸¹Br, ΔM=-0.7 mmu), 479.9039 (M⁺-C₂H₂O: C₁₉H₁₂O₅⁷⁹Br⁸¹Br, ΔM=0.6 mmu), 437.8925 (M⁺- 2C₂H₂O: C₁₇H₁₀O₄⁷⁹Br⁸¹Br, ΔM=-0.2 mmu).

Rubrolide E Diacetate (162):
Obtained as a colorless solid; IR (CH$_2$Cl$_2$), $v_{\text{max}}$: 2927, 2854, 1764, 1609, 1501, 1370, 1201, 1166, 1085, 1016, 960, 915 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz), see Table 7; $^{13}$C NMR (CDCl$_3$, 125 MHz), see Table 7; EIMS, m/z (relative intensity): 364 (15.0), 322 (72.3), 280 (100.0), 252 (5.2), 224 (7.4), 134 (38.1), 133 (35.2), 77 (18.3), 43 (87.8); HREIMS: 364.0945 (M$^+$: C$_{21}$H$_{16}$O$_6$, $\Delta M$=-0.2 mmu), 322.0840 (M$^+$-C$_2$H$_2$O: C$_{19}$H$_{14}$O$_5$, $\Delta M$=-0.2 mmu), 280.0728 (M$^+$-2C$_2$H$_2$O: C$_{17}$H$_{12}$O$_4$, $\Delta M$=-0.8 mmu).

**Rubrolide G Triacetate (163):**

\[
\begin{align*}
\text{Br} & \quad \text{OAc} \\
\text{AcO}^- & \quad \text{Br}
\end{align*}
\]

Obtained as a colorless solid; IR (CH$_2$Cl$_2$), $v_{\text{max}}$: 1778, 1459, 1370, 1183, 1034, 909 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz), see Table 8; $^{13}$C NMR (CDCl$_3$, 125 MHz), see Table 8; EIMS, m/z (relative intensity): 702 (3.8), 700 (13.4), 698 (19.7), 696 (13.2), 694 (3.3), 660 (1.2), 658 (4.1), 656 (6.2), 654 (4.3), 652 (1.3), 642 (19.5), 640 (58.7), 638 (78.9), 636 (55.2), 634 (13.0), 600 (21.1), 598 (58.0), 596 (75.4), 594 (47.7), 592 (11.6), 482 (16.9), 480 (34.7), 478 (17.5), 440 (20.2), 438 (40.3), 436 (21.2), 349 (11.4), 334 (10.0), 332 (15.6), 330 (7.9), 310 (38.7), 308 (76.9), 306 (39.8), 278 (12.4), 276 (24.2), 274 (13.0), 267 (53.3), 265 (100.0), 263 (53.0), 185 (12.4), 43 (29.9); HREIMS: 739.7532 (M$^+$: C$_{23}$H$_{16}$O$_8$Br$_2$, $\Delta M$=-0.9 mmu), 697.7441 (M$^+$-C$_2$H$_2$O: C$_{21}$H$_{14}$O$_7$Br$_2$, $\Delta M$=0.5 mmu), 637.7214 (M$^+$ - C$_2$H$_2$O - AcOH: 113
Rubrolide H Triacetate (164):

Obtained as a colorless solid; IR (CH$_2$Cl$_2$), $v_{\text{max}}$: 1798, 1776, 1543, 1459, 1371, 1182, 1066, 992, 908, cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz), see Table 8; $^{13}$C NMR (CDCl$_3$, 125 MHz), see Table 8; EIMS, $m/z$ (relative intensity): 776 (0.4), 774 (0.4), 740 (0.6), 738 (6.4), 736 (25.2), 734 (52.2), 732 (100.0), 730 (37.6), 692 (14.4), 690 (15.5), 688 (8.2), 686 (1.9), 634 (27.3), 632 (61.6), 630 (72.1), 628 (943.0), 626 (10.6), 384 (6.5), 382 (8.1), 312 (18.1), 310 (72.5), 308 (100.0), 306 (45.0), 267 (56.4), 265 (76.0), 263 (35.0), 185 (11.1), 80 (20.4), 43 (37.6), 32 (87.9); HREIMS: 773.7158 (M$^+$: C$_{23}$H$_{15}$O$_8$Br$_2$, AM=0.6 mmu or C$_{23}$H$_{15}$O$_8$Br$_3$, AM=1.5 mmu), 731.7051 (M$^+$-C$_2$H$_2$O: C$_{21}$H$_{13}$O$_7$Br$_2$, AM=0.6 mmu or C$_{21}$H$_{13}$O$_7$Br$_3$, AM=1.6 mmu), 671.6839 (M$^+$ - C$_2$H$_2$O - AcOH: C$_{19}$H$_9$O$_5$Br$_2$, AM=0.6 mmu or C$_{17}$H$_7$O$_4$Br$_3$, AM=1.6 mmu), 307.8867 (C$_9$H$_8$O$_2$Br$_2$, AM=0.2 mmu).
Methylation of Rubrolide G (155):

An acetone solution of crude rubrolide G (=1 mg in 0.3 ml) was mixed with an ethereal solution of CH₂N₂ (0.2 mmol in 5 ml) and stirred at r.t. for 15 h. After evaporation of the solvent, the mixture was chromatographed on TLC (silica gel, 1% MeOH/CH₂Cl₂) followed by HPLC (silica gel, 25% EtOAc/hexane) to give two pure compounds, trimethyl rubrolide G (167) (=0.5 mg) and the diazomethane addition product 168 (=0.5 mg). Further treatment with CH₂N₂ following the same procedure converted trimethyl rubrolide G (167) into the same addition product 168. trimethyl rubrolide G (167): colorless solid; ¹H NMR (CDCl₃, 400 MHz) δ 7.44 (s, 2H), 7.34 (s, 2H), 6.13 (s, 1H), 3.91 (s, 3H), 3.88 (s, 2H), 3.86 (s, 3H), 3.82 (s, 3H) ppm; IR (CH₂Cl₂), v max: 2926, 2854, 1719, 1712, 1655, 1560, 1177, 993 cm⁻¹; EIMS m/z (relative intensity): 660 (1.4), 658 (4.0), 656 (5.8), 654 (3.8), 652 (1.5), 628 (5.6), 626 (16.3), 624 (22.1), 622 (14.1), 620 (3.6), 379 (43.4), 377 (86.0), 375 (43.0), 351 (21.1), 349 (34.0), 291 (19.3), 281 (15.2), 279 (30.0), 277 (18.8), 270 (11.8), 268 (12.3), 183 (5.3), 154 (8.2). Compound 168: colorless solid; IR (CH₂Cl₂), v max: 2949, 2923, 1736, 1729, 1605, 1472, 1423, 1211, 1177, 991, 909 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.60 (s, 2H), 7.22 (s, 2H), 5.05 (dd, J=18.3, 4.2 Hz), 4.61 (s, J=18.2, 8.6 Hz), 4.15 (d, J=17.1 Hz), 3.97 (d, J=17.1 Hz), 3.90 (s, 3H), 3.85 (s, 3H), 3.71 (s, 3H), 3.14 (dd, J=8.6, 4.2 Hz) ppm; EIMS m/z (relative intensity): 700 (0.3), 698 (0.5), 696 (0.4), 694 (0.1), 674 (4.3), 672 (16.3), 670
(24.9), 668 (17.3), 666 (5.0), 615 (2.2), 613 (8.1), 611 (12.6), 609 (8.6), 607 (2.6), 393 (25.7), 391 (51.1), 389 (25.5), 365 (16.6), 363 (33.0), 361 (19.4), 284 (30.1), 282 (32.7), 281 (19.9), 279 (40.2), 277 (21.3), 225 (9.5), 223 (8.2), 203 (15.1).
II. STEREOCHEMICAL AND BIOSYNTHETIC STUDIES OF IMBRICATINE, A METABOLITE OF THE STARFISH *DERMASTERIAS IMBRICATA*

A. INTRODUCTION

Some species of sessile anemones are known\textsuperscript{78-82} to be capable of detecting starfish predators and attempting to detach and escape. One of the best known examples is the interaction between the sea anemone *Stomphia coccinea* and the starfish *Dermasterias imbricata*, commonly known as the leather star. The escape response of *S. coccinea* (Fig. 33\textsuperscript{82}) when approached by *D. imbricata* includes the following sequence of behaviors:\textsuperscript{81} (1) retraction of the tentacles and closure of the oral disk, (2) elongation of the column, (3) opening of the oral disk and inflation of the tentacles, (4) flexions of the column, and (5) detachment of the pedal disk from the substratum and flexions of the column (swimming).

Studies have demonstrated that certain chemicals released by the sea stars are responsible for inducing the responses in the sea anemones. However, the chemicals involved remain unknown in most cases. Studies on the interaction between *S. coccinea* and *D. imbricata* by Singer and Ayer\textsuperscript{83a-b} led to the isolation of a water-soluble compound, named imbricatine (179), that was responsible for this specific interaction. Imbricatine (179) was isolated from the methanol extract of *D. imbricata* by following an anemone detachment and swimming bioassay. Subsequently, Pathirana and Andersen\textsuperscript{84} re-isolated the compound utilizing a different chromatographic procedure. Quantitative bioassays\textsuperscript{81} demonstrated that imbricatine was very active at causing the detachment and swimming response in *Stomphia coccinea*, the minimal effective concentration being $2 \times 10^{-7}$M.
Fig. 33. Response of Stomphia coccinea to Dermasterias imbricata
Spectroscopic and chemical studies by Pathirana and Andersen\textsuperscript{84} elucidated the general structure and the partial relative stereochemistry of imbricatine (179), a benzyltetrahydroisoquinoline alkaloid with a thiohistidine substituent and a C3 carboxyl substitution. Imbricatine also has an unprecedented \textit{meta} C6/C8 hydroxylation pattern in the isoquinoline nucleus.

Imbricatine represents the only example of a benzyltetrahydroisoquinoline alkaloid to be isolated from a nonplant source. On the other hand, the thiohistidine fragment has been encountered in a few secondary metabolites previously isolated from marine invertebrates. Examples are the ovothiols (181-183) and the disulphides 184 and 185 from the eggs of several marine echinoderms\textsuperscript{85*} and adenochromines A and B (186-187), novel amino acid units of the peptide adenochrome isolated from an octopus.\textsuperscript{86} The ovothiols have been synthesized.\textsuperscript{87}

\begin{itemize}
  \item 181 R=H R=H
  \item 182 R=Me R=H
  \item 183 R=Me R=Me
\end{itemize}

* The original structures of ovothiols were assigned as 1-methyl-5-thiohistidine derivatives in ref. 85a-b. The incorrect assignments were caused by two conflicting nomenclature systems for the imidazole ring of histidine. Organic chemists generally designate the side chain as C4 and the adjacent nitrogen as N-3 and biochemists designate the side chain as C5 and the adjacent nitrogen as N-1. The original workers (organic chemists of course, ref. 85a-b) used the commercial 1-methylhistidine, which later turned out to be named according to the biochemical system, as a model compound in their study. This confusion of nomenclature apparently led to the misassignment of the methyl position in the imidazole ring of the ovothiols. For a clarification, see ref. 85d.
184 $R=H$
185 $R=Me$

186 $Z=H$ $X=Y=R$
187 $X=H$ $Y=Z=R$
1. The Absolute Stereochemistry of Imbricatine

In Pathirana and Andersen's study, the C1/C3 relative stereochemistry in imbricatine (179) was assigned to be the cis configuration based on a $^1$H NMR study of two model compounds. The absolute configurations of all three chiral centres, however, were undetermined.

One of the goals of the present project was to determine the absolute stereochemistry of imbricatine (179). The general strategy was to cleave the sulphur linkage in the molecule to liberate the methylhistidine fragment and the benzyltetrahydroisoquinoline fragment and to study the two fragments separately. Raney nickel reduction liberated the benzyltetrahydroisoquinoline 188a but, unfortunately, failed to yield 3-methylhistidine in a sufficient yield for determining its stereochemistry. Therefore, reductive hydrolysis with red P/HI had to be employed to obtain a good yield of 3-methyl-5-thiolhistidine (181). Compound 181 could be oxidized into its corresponding disulphide. Comparison of the optical rotation of this disulfide with the reported value for 184 (L configuration) would settle the absolute configuration of the thiohistidine fragment in imbricatine.

The absolute stereochemistry of the benzyltetrahydroisoquinoline 188a was expected to be determined by comparing the circular dichroism spectrum of its methyl ester 188b with that of the model compound 189 with known stereochemistry.
Fig. 34. $^1$H NMR spectrum of imbricatine (179) (400 MHz, DMSO-d$_6$)
Fig. 35. $^1$H NMR spectrum of dimethylpentaacetylimbricatine (180) (400 MHz, CDCl$_3$)
2. The Biosynthetic Origin of Imbricatine (179)

The second goal of this project was to study the biogenesis of imbricatine (179). This was prompted by the unique structural features of this benzyltetrahydroisoquinoline.

Most of the naturally occurring benzyltetrahydroisoquinolines are ortho hydroxylated at C6/C7, while the rest at C7/C8 or C6/C7/C8. Radioisotope-labelling studies have demonstrated that they are the coupling products (Scheme 5) of dihydroxyphenylalanine (DOPA) and para-hydroxyphenylacetaldehyde (or an equivalent), both of which are derived from tyrosine, a product of the well-known shikimic acid biosynthetic pathway.

![Scheme 5. Biosynthesis of benzyltetrahydroisoquinoline alkaloids](image)

Imbricatine (179), however, has the unprecedented C6/C8 meta hydroxylation pattern. It is likely to be derived from the coupling between the hypothetical unknown amino acid, 6,8-dihydroxyphenylalanine (191), and a para-hydroxyphenylacetaldehyde equivalent, which is, as in the normal pathway, derived from tyrosine.
The *meta* hydroxylation pattern of 191 is very unusual. Compound 191 could be derived from phenylalanine through a highly unusual oxidation pathway leading to the *meta* hydroxylation pattern. It is also possible that 191 is not biosynthesized from tyrosine/phenylalanine (i.e., via the common shikimic acid biosynthetic pathway), but rather, from acetate through the polyketide pathway, the products of which usually have a characteristic 1,3-oxygenation pattern. If this is the case, imbricatine (179) will be the first benzyltetrahydroisoquinoline whose nucleus is biosynthesized through a mixed pathway involving both the polyketide pathway and the shikimic acid pathway. We have proposed a plausible pathway, as shown in Scheme 6, using acetate and aspartic acid as precursors. Aspartic acid can be produced through the citric acid metabolism cycle. Addition of three molecules of acetate to aspartic acid leads to the formation of the polyketide amino acid 192. Cyclization of 192 followed by the loss of the terminal carboxyl group (not the amino acid carboxyl) affords the key intermediate 191, which in turn couples with 4-hydroxyphenylacetaldehyde (or an equivalent) to form the benzyltetrahydroisoquinoline fragment (188a) of imbricatine. Additionally, the thiohistidine substituent on the tetrahydroisoquinoline nucleus very likely is derived from histidine.

In this project, we intended to find evidence to support these hypotheses on the biosynthetic origins of imbricatine by incubating *D. imbricata* with $^3$H-labelled precursors.
Scheme 6. Proposed biosynthetic pathway for the benzyltetrahydroisoquinoline part (188a) of imbricatine (179)
B. RESULTS AND DISCUSSION

1. The Absolute Stereochemistry of Imbricatine (179)

1.1 The Benzyltetrahydroisoquinoline Fragment

The benzyltetrahydroisoquinoline $188a$ was obtained by refluxing imbricatine (179) in MeOH/H$_2$O with Raney nickel followed by purification on Sephadex LH20 and reverse-phase silica. Compound $188a$ gave a parent ion (M$^+$ + H) in the CIMS at m/z 316 Da and fragment ions at m/z 270 (M$^+$ - CO$_2$H), 242 (M$^+$ - NH=CHCO$_2$H via a retro Diels-Alder), 208 (M$^+$ - HO-benzyl) and 164 (M$^+$ - (HO-benzyl + CO$_2$)) Da characteristic of benzyltetrahydroisoquinolines with a carboxylic acid substituent at C3. The EIHRMS spectrum of $188a$ failed to show a parent ion; however, an intense fragment ion at m/z 208.1611 Da (C$_{10}$H$_{10}$NO$_4$ ΔM +0.1 mmu), which was assigned to a M$^+$ - (HO-benzyl) fragment ion, was consistent with a molecular formula of C$_{17}$H$_{17}$NO$_5$ for the intact molecule.

The carbon resonances and the proton resonances (Fig. 36) of $188a$ were nearly identical with their counterparts in the benzyltetrahydroisoquinoline fragment of imbricatine (see Table 10). Only 15 resonances were observed in the $^{13}$C NMR spectrum of $188a$, indicating that the molecule contained some element of symmetry. $^1$H NMR (Fig. 36) resonances at δ 6.70 (d, J=8.4 Hz, 2H) and 7.04 (d, J=8.4 Hz, 2H) and $^{13}$C NMR resonances at δ 126.9 (C), 130.2 (2 × CH), 115.0 (2 × CH) and 156.0 (C) were assigned to the symmetrical 4-hydroxyphenyl ring in the benzyl residue of $188a$. Additional deshielded $^1$H resonances at δ 6.12 (d, J=2.0 Hz) and 6.21 (d, J=2.0 Hz) were assigned to protons on the aromatic ring of the tetrahydroisoquinoline moiety. The magnitude of the scalar coupling between these protons (2.0 Hz) showed that they were meta to each other and their chemical shifts suggested that the other substituents on the ring were a pair of meta hydroxyls. The
Fig. 36. $^1$H NMR spectrum of tetrahydroisoquinoline 188a (400 MHz, DMSO-d$_6$)
Fig. 37. $^1$H NMR spectrum of methylester 188b (400 MHz, acetone-$d_6$)
Table 10. NMR data for 179, 188a and 184

<table>
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<tr>
<th>Position</th>
<th>179 (DMSO-d$_6$)</th>
<th>188a (DMSO-d$_6$)</th>
<th>184 (D$_2$O)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$^1$H (400 MHz)</td>
<td>$^{13}$C (75MHz)</td>
<td>$^1$H (400 MHz)</td>
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<td>137.7, s</td>
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<td>169.7, s</td>
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<td>2.78, dd, 14.8, 9.0</td>
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<td>3.66, s; 3.87, s$^a$</td>
<td>32.3, q</td>
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$^a$ recorded in D$_2$O
remaining resonances in the $^1$H NMR spectrum of 188a (Fig. 36) were assigned to the two CH-CH$_2$ fragments ($\delta$ 4.55, dd, $J=9.0, 4.0$ Hz, H1; $\delta$ 3.20, dd, $J=14.8, 4.0$ Hz, H12a; $\delta$ 2.78, dd, $J=14.8, 9.0$ Hz, H12b; $\delta$ 3.15, dd, $J=12.1, 3.8$ Hz, H3; $\delta$ 2.87, dd, $J=16.4, 4.3$ Hz, H4$_{eq}$; $\delta$ 2.70, dd, $J=16.4, 12.5$ Hz, H4$_{ax}$) in the reduced ring and the benzyl substituent of the isoquinoline nucleus on the basis of double resonance experiments. The cis relative stereochemistry of 188a was determined by an nOe experiment on its methyl ester 188b. Since the chemical shifts of H3 and H12a of 188b were only 0.01 ppm apart when recorded in acetone-d$_6$ (Fig. 37), the nOe experiment was conducted in ca. 10% C$_6$D$_6$/acetone-d$_6$ which gave a satisfactory dispersion (H3: $\delta$ 3.42 ppm; H12a: $\delta$ 3.48 ppm). Irradiation of H1 ($\delta$ 4.45 ppm) induced nOes in both H3 and H12a, indicating that H1 and H3 were cis.

The trans product 193a, formed from the epimerization of 188a at C1, could be isolated as a minor product from the Raney nickel reduction mixture if the reaction was allowed to reflux much longer than the normal time and a large excess of catalyst was used. The trans relative stereochemistry of 193a was determined by an nOe experiment performed on its methyl ester of 193b. The absence of nOe between H1 ($\delta$ 4.22, dd, $J=10.0, 2.5$ Hz) and H3 ($\delta$ 4.01, dd, $J=10.5, 4.5$ Hz) suggested they were trans with each other. The other expected product of the Raney nickel reaction, histidine, did not survive the reaction condition. This was confirmed by refluxing authentic histidine under the same conditions.

\[ \text{HO} \quad \text{NH} \quad \text{COOR} \]

\[ \text{OH} \quad \text{OH} \quad \text{R=H; 193b  R=Me} \]
The two model compounds 189 and 190 were synthesized from the optically pure L-DOPA methylester and 4-methoxylphenylglycidate through a biomimetic reaction (Scheme 7). The ratio between the two diastereomers was 3.2:1, consistent with an earlier study on the Pictet-Spengler reaction of 3,4-dimethoxylphenylglycidate. The NMR data (Table 11) of both 189 and 190, their carbon chemical shifts in particular, were very similar to that of 188a. The relative stereochemistries of both 189 and 190 were determined by nOe experiments (400 MHz, DMSO-d$_6$). In 189, nOes were observed in H3 ($\delta$ 3.48, dd, J=11.2, 3.8 Hz) and H12a ($\delta$ 3.22, dd, J=13.4, 3.2 Hz) when H1 ($\delta$ 4.06, dd, J=9.1, 3.2 Hz) was irradiated, while in 190, irradiation of H1 ($\delta$ 4.00, dd, J=10.0, 3.6 Hz) only gave an nOe in H12a ($\delta$ 2.85, dd, J=13.7, 3.6 Hz).

Circular dichroism spectra (Fig. 42, 43) were recorded for imbricatine (179), the methylester 188b of the Raney nickel reduction product 188a, the diastereomer 193b of compound 188b, and the two model compounds 189 and 190, all in 0.1N HCl/MeOH. For each compound, three major Cotton effects were observed, i.e., in the long UV region (290-300 nm), the middle range UV region (230-240 nm) and the short UV region (200-220 nm). The three Cotton effects observed for compound 188b had the opposite signs of those observed for the cis model compound 189. This was also the case for compound 193b and the trans model compound 190. This suggested that compound 188b and the model compound 189 had the opposite configurations at C1/C3, and so did compound 193b and the model compound 190. Since the cis model compound 189 had the known absolute stereochemistry (1S, 3S), the CD spectra provided unambiguous evidence for the 1R, 3R absolute configuration for compound 188b. Moreover, the Cotton effects observed for imbricatine (179) in the long UV and the middle UV regions (the short UV region of imbricatine was obscured by the histidine chromophore) had the same signs as those of
Scheme 7. Preparation of benzyltetrahydroisoquinolines 189 and 190
Fig. 39. $^{13}$C BB and APT NMR spectra of model compound 189 (75 MHz, DMSO-$d_6$)
Fig. 40. $^1$H NMR spectrum of model compound 190 (400 MHz, acetone-\text{d}_6)$
Fig. 41. $^{13}$C BB and APT NMR spectra of model compound 190 (75 MHz, DMSO-d$_6$)
Table 11. NMR data\textsuperscript{a} for 188b, 193b, 189 and 190

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<th>193b \textsuperscript{1H}</th>
<th>189 \textsuperscript{1H}</th>
<th>13C</th>
<th>190 \textsuperscript{1H}</th>
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<td>2.71, dd, 13.4, 7.8</td>
<td>2.68, dd, 13.5, 10.0</td>
<td>2.63, dd, 13.5, 9.3</td>
<td>41.18, t</td>
<td>2.83, dd, 13.7, 10.1</td>
<td>41.46, t</td>
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<tr>
<td>13</td>
<td>3.43, dd, 13.5, 3.0</td>
<td>3.14, dd, 13.5, 2.5</td>
<td>3.31, dd, 13.5, 3.4</td>
<td></td>
<td>2.97, dd, 13.7, 3.7</td>
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<td>14/18</td>
<td>7.02, d, 8.6</td>
<td>7.09, d, 8.5</td>
<td>7.23, d, 8.6</td>
<td>130.39, d</td>
<td>7.22, d, 8.5</td>
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<td>15/17</td>
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<td>6.78, d, 8.5</td>
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<td>113.70, d</td>
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<td>16-OMe</td>
<td></td>
<td></td>
<td>3.78, s, 3H</td>
<td>55.43\textsuperscript{b}, q</td>
<td>3.78, s, 3H</td>
<td>54.98\textsuperscript{d}, q</td>
</tr>
<tr>
<td>CO\textsubscript{2}Me</td>
<td>3.68, s, 3H</td>
<td>3.70, s, 3H</td>
<td>3.66, s, 3H</td>
<td>56.70\textsuperscript{b}, q</td>
<td>3.66, s, 3H</td>
<td>55.41\textsuperscript{d}, q</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Recorded at 400 MHz in acetone-d\textsubscript{6} for \textsuperscript{1H}, at 75 MHz in DMSO-d\textsubscript{6} for \textsuperscript{13C};

\textsuperscript{b-d}Assignments may be interchanged within the same column.
Fig. 42. CD spectra recorded in 0.1N HCl/MeOH.

(— — —) cis model compound 189 (0.1g/l); (———) trans model compound 190 (0.2g/l);

(— — —) methyl ester 188b of the Raney nickel reduction product (0.15g/l); (— — —) 193b
Fig. 43. CD spectra recorded in 0.1N HCl/MeOH.

(—) Raney nickel reduction product 188a (0.34g/l); (-------) imbricatine (179) (0.5g/l)
compound 188b (Fig. 43), indicating that the same 1R, 3R stereochemistry was retained during the Raney nickel reduction and the methylation reactions. Therefore, the absolute stereochemistry of the tetrahydroisoquinoline part of imbricatine (179) was assigned 1R, 3R as shown in structure 179.

Circular dichroism has been used for three decades or so in the determination of stereochemistry of organic molecules and has been proved to be relatively reliable compared to other empirical methods. The absolute configurations of a large number of tetrahydroisoquinolines have been solved using this method. In the case of imbricatine, the difference between compound 188b and the two model compounds 189 and 190 is very small and it doesn't seem significant enough to affect the conformations of the molecules. Therefore, the absolute stereochemistry of the benzyltetrahydroisoquinoline part of imbricatine determined by CD is expected to be reliable.

1.2 The Histidine Fragment

Our original plan was to isolate 3-methylhistidine from the Raney nickel reduction mixture and to compare its optical rotation with the optically pure authentic sample. Since methylhistidine could not be isolated in appreciable yield, an alternative degradation reaction had to be employed to cleave the sulphur linkage of imbricatine. Reductive hydrolysis (Scheme 8) with red phosphorus in 57% hydroiodic acid (reflux, 48 h) successfully liberated ovothiol A (181), a previously reported natural product. Ovothiol A was unstable and prone to air oxidation. Treatment with a few crystals of I2 in aqueous HCl converted it into the corresponding disulphide 184, which is also a known natural product. The spectral data for 184 obtained from imbricatine was consistent with the literature values and also very similar to those in the thiohistidine part in imbricatine (see Table 10). The 1H NMR spectrum (Fig. 44) showed a CH-CH2 system (8 4.20, t, J=8 Hz;
Fig. 44. $^1$H NMR spectrum of disulphide 184 (300 MHz, D$_2$O)
δ 3.35, dd, J=16, 8 Hz; δ 3.30, dd, J=16, 8 Hz), a methyl (δ 3.85, s; NMe), and a very deshielded aromatic proton (δ 8.95, s) attributed to H2 in the imidazole ring.

![Scheme 8. Preparation of disulphide 184 from imbricatine (179)](image)

The optical rotation of disulphide 184 was recorded in 0.1 N aqueous HCl and found to be +67°. Comparison with the literature value (+76°, in 0.1N HCl)85a-b indicated the configuration was L. Therefore, the absolute configuration of the methylhistidine residue of imbricatine was assigned 7'S (L).

In conclusion, the absolute stereochemistry of imbricatine (179) was determined to be 1R, 3R, 7'S.

2. Biosynthetic Studies on Imbricatine (179)

In order to test the proposed biosynthetic scheme (Scheme 6) for imbricatine, three ^3H-labelled potential precursors, L-(2,3-^3H)aspartic acid, L-(3,5-^3H)tyrosine and L-(2,5-^3H)histidine, were planned to be administered into live D. imbricata individuals. Among these three compounds, L-histidine seemed to be the one that would take the least number of steps to be incorporated into imbricatine. Therefore, it was the first compound we decided to inject into the animals.
250 μci L-(2,5-3H)histidine (in 2 ml 2% aqueous EtOH) was injected, in equal amounts, into two animals that were kept in a non-circulating sea water aquarium (20 litre). The aquarium was cooled with running water and well aerated. The animals were incubated for 24 h with the L-(2,5-3H)histidine and then extracted with MeOH. Fractionation of the MeOH extract on XAD-4 resin, Biogel-P2 and Sephadex LH20 (see Experimental) afforded pure imbricatine (179) (ca. 10 mg). Methylation with HCl/MeOH followed by acetylation with Ac2O/pyridine converted 179 into dimethylpentaacetyltrimbricatine (180), which was purified by preparative TLC and HPLC. The radioactivity of 180 was counted on a liquid scintillation counter and found to be only ca. 10% higher than the background (HPLC baseline collection) level. Therefore, the incorporation rate of L-histidine into imbricatine was not significant enough to be considered positive.

In a subsequent incubation experiment, L-(3,5-3H)tyrosine was injected into two animals. After a similar workup, no significant radioactivity was found in 180. The negative incorporation results do not necessarily indicate that L-histidine and L-tyrosine are not biosynthetic precursors of imbricatine (179). There are several factors that likely contributed to the negative results: 1) unlike in microorganisms and some plants, extremely low incorporation rate of precursors has been a common problem in animals. 2) very little is known about the biology of imbricatine production, i.e., its function in D. imbricata, in which part(s) of the body it is synthesized, what the turnover rate is, whether it is released constantly or upon stimulation, etc. Lack of this knowledge limited the optimization of the injection/incubation conditions. For example, details such as injection
position and incubation period need further studying. 3) use of a non-circulating water system prohibited longer incubation time because of the water quality deterioration leading to death of the starfishes. These problems have to be solved before any future injection study will be successful.
C. EXPERIMENTAL

Isolation of imbricatine (179):

Freshly collected Dermasterias imbricata was immersed in MeOH for 2 days. The red MeOH extract was decanted, filtered through celite and concentrated in vacuo to give a brown aqueous suspension which was diluted with water and passed through an XAD-4 resin column. The column was washed with water and eluted with hot MeOH to give a brown eluate. Fractionation of this eluate on Biogel-P2 (1%AcOH/H₂O) afforded crude imbricatine. The final purification of imbricatine was achieved by fractionation on either (1) Sephadex LH20 (2:8 H₂O/MeOH), or (2) Iatrobead silica gel (10:7:3 MeOH/CH₂Cl₂/H₂O) followed by XAD-4 (washed with water and eluted with hot methanol). Pure imbricatine (179) was obtained as a pale yellow amorphous solid (ca. 6 mg/animal). ¹H and ¹³C NMR, see Table 10.

Derivatization of imbricatine (179):

10 mg imbricatine (179) was dissolved in 10 ml MeOH that was pre-saturated with HCl gas and refluxed for 2.5 h. After the removal of solvent in vacuo, the methylation mixture was acetylated with Ac₂O/pyridine for 12 h. The solvent was removed in vacuo and the reaction mixture was purified on preparative TLC (silica gel, 10:1 CH₂Cl₂/MeOH) followed by HPLC (silica gel, 4% MeOH/CH₂Cl₂) to give 10 mg dimethylpentaacetylimbricatine (180). 180: ¹H NMR (400 MHz, CDCl₃), δ 7.34 (s, H2'), 7.29 (d, J=8.4 Hz; H14/H18), 7.09 (d, J=8.4 Hz; H15/H17), 6.89 (s, H7), 6.19 (d, J=7.8 Hz; 7'-NH), 5.06 (dd, J=7.6, 5.8 Hz; H1), 4.74 (q, J=7.3 Hz; H7'), 4.64 (dd, J=15.7, 5.9 Hz; H4a), 4.39 (dd, J=12.8, 5.9 Hz; H3), 3.87 (s, 3H), 3.75 (s, 3H), 3.61 (s, 3H), 3.35 (dd, J=13.6, 7.6 Hz; H12a), 3.17 (d, J=7.2 Hz; H6'), 3.10 (br d, J=15.3 Hz; H4b), 2.88
(dd, J=13.6, 5.8 Hz; H12b), 2.41 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 1.90 (s, 3H), 1.73 (s, 3H) ppm.

**Raney nickel reduction of imbricatine (179):**

Imbricatine (179) (16 mg, in 2 ml H2O/MeOH) was refluxed with 0.5 ml of Ra-Ni suspension for 2.5 h under N2. The reaction mixture was vacuum filtered and the Ra-Ni residue was washed with hot MeOH. The combined filtrate, after removal of MeOH under vacuum, was chromatographed on Sephadex LH20 using 9:1 MeOH/H2O as the eluent to give crude compound 188a which was further purified on reverse phase HPLC (20:80 MeOH/H2O) to obtain pure 188a (8 mg). 188a: 1H NMR (400 MHz, DMSO-d6), see Table 10; 13C NMR (75 MHz, DMSO-d6), see Table 10; CIMS m/z : 316 (M+ + H), 272, 242, 270, 208, 198, 164, 162, 152, 137, 124, 121, 109, 108, 107; EIHRMS m/z: 208.1611 (M+ - HOBenzyl: C10H10NO4, ΔM=+0.1 mmu).

**Methylation of the Raney nickel reduction product 188a:**

188a (8 mg) was dissolved in 10 ml MeOH saturated with hydrogen chloride gas and refluxed under nitrogen for 2.5 h. After the removal of solvent, the reaction mixture was purified on TLC plates (silica, 4:1 EtOAc/CH2Cl2) to afford 8 mg of the methyl ester 188b. 1H NMR, see Table 11; CIMS m/z (rel. int.): 330 (M+ + H, 100), 212 (24.5), 107 (13.9), 85 (34.0).

**Preparation of methyl (4-methoxyphenyl)glycidate:**

Anisaldehyde (5 ml) and methylchloroacetate (5 ml) were added slowly over a period of 2.5 h into a cold solution of Na (1.5 g) in MeOH (25 ml). The reaction mixture was stirred for another 2.5 h on an ice bath and then for 3 h at r.t. It was then poured into ice-water and filtered. The filtrate was washed with cold water to give 7 g methyl (4-
methoxyphenyl)glycidate. $^1$H NMR (300 MHz, DMSO-d$_6$), $\delta$ 7.29 (d, J=8 Hz, 2H), 6.93 (d, J=8 Hz, 2H), 4.10 (br s, 1H), 3.79 (br s, 1H), 3.75 (s, 3H) ppm; EIMS m/z (relative intensity): 208 (10), 192 (10), 161 (18), 151 (54), 135 (12), 121 (100), 105 (9).

Synthesis of the model compounds 189 and 190:

Methyl-L-dopa (580 mg) in 10 ml MeOH was added to a solution of sodium (4-methoxyphenyl) glycidate (prepared from methyl (4-methoxyphenyl)glycidate) in 15 ml water. The mixture was brought to pH4 with AcOH and stirred at 35 °C for 36 h. At the end of the reaction period, the mixture was concentrated in vacuo to yield an aqueous suspension which was partitioned between EtOAc (15 ml) and 10% HCl (15 ml). The aqueous layer was neutralized with K$_2$CO$_3$ and extracted with EtOAc (15 ml x 3). The EtOAc solution was washed with water, dried over Na$_2$SO$_4$, and concentrated in vacuo to afford a brown residue which was chromatographed on normal phase HPLC (3:2 EtOAc/CHCl$_3$) to give 189 (145 mg) and 190 (45 mg). Compound 189: obtained as a white solid; $^1$H NMR (400 MHz, acetone-d$_6$), see Table 11; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 7.19 (d, J=8.6 Hz, H14/H18), 6.86 (d, J=8.6 Hz, H15/H17), 6.74 (s, IH, H8), 6.48 (s, 1H, H5), 4.06 (J=9.1, 3.2 Hz, H1), 3.73 (s, 16-OMe), 3.63 (s, COOMe), 3.48 (dd, J=11.2, 3.8 Hz, H3), 3.22 (dd, J=13.4, 3.2 Hz, H12a), 2.71 (dd, J=15.2, 3.7 Hz, H$_{4eq}$), 2.61 (dd, J=15.2, 11.2 Hz, H$_{4ax}$), 2.56 (dd, J=13.4, 9.1 Hz, H12b) ppm; $^{13}$C NMR (75 MHz, DMSO-d$_6$): see Table 11; EIMS m/z (rel. int): 343 (0.2), 342 (3.2), 340 (3.9), 324 (1.7), 284 (2.1), 282 (4.4), 280 (2.5), 222 (100.0), 162 (60.8), 134 (3.2), 121 (15.8); EIHRMS: m/z: 343.1415 (M$^+$: C$_{19}$H$_{21}$NO$_5$, $\Delta$M=–0.5 mmu), 222.0758 (M$^+$-MeObenzenyl: C$_{11}$H$_{12}$NO$_4$, $\Delta$M=–0.9 mmu), 162.0557 (M$^+$-MeObenzenyl-COOMe-H: C$_9$H$_8$NO$_2$, $\Delta$M=0.2 mmu). Compound 190: obtained as a white solid; $^1$H NMR (400 MHz, acetone-d$_6$), see Table 11; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 7.18 (d, J=8.5 Hz, H14/H18), 6.87 (d, J=8.5 Hz, H15/H17), 4.00 (dd, J=10.0, 3.6 Hz, H1), 3.87 (dd,
J=9.0, 4.7 Hz, H3), 3.74 (s, 16-OMe), 3.62 (s, COOMe), 2.85 (dd, J=13.7, 3.6 Hz, H12a), 2.78 (dd, J=15.5, 4.7 Hz, H4eq), 2.74 (dd, J=13.7, 10.0 Hz, H12b), 2.63 (dd, J=15.5, 9.1 Hz, H4ax) ppm; $^{13}$C NMR (75 MHz, DMSO-d$_6$): see Table 11; EIMS m/z (rel. int): 343 (0.1), 342 (0.4), 341 (1.6), 340 (2.1), 326 (0.3), 324 (1.0), 284 (2.3), 282 (3.1), 280 (2.6), 278 (1.6), 264 (1.7), 236 (7.4), 222 (89.5), 162 (100.0), 134 (13.1), 121 (23.6), 77 (24.1), 31 (50.4); EIHRMS: 343.1412 (M$^+$: C$_{19}$H$_{21}$NO$_5$, $\Delta$M=-0.8 mmu), 222.0763 (M$^+$-MeObenzenyl: C$_{11}$H$_{12}$NO$_4$, $\Delta$M=-0.3 mmu), 162.0554 (M$^+$-MeObenzenyl-COOME-H: C$_9$H$_8$NO$_2$, $\Delta$M=-0.1 mmu).

**Measurement of circular dichroism spectra:**

Each sample was dissolved in 0.1N HCl/MeOH and its CD spectrum was measured on a JASCO 700 spectrophotopolarimeter with an 1 mm cell.

**Reductive hydrolysis of imbricatine (179):**

Red phosphorus (150 mg) was added to a solution of imbricatine (179) (100 mg) in 57% aqueous HI and the reaction mixture was refluxed under N$_2$ for 48 h. At the end of this period, the reaction mixture was filtered to remove the solid phosphorus and the filtrate was evaporated under vacuum. The residue was taken up with 5 ml aqueous HCl (pH2) and chromatographed on Sephadex LH20 using 1:1 MeOH/H$_2$O as an eluent to give pure 3-methyl-5-thiohistidine (181) (34 mg).

**Oxidation of thiohistidine 181 to the Disulfide 184:**

To a solution of histidine thiol 181 (17 mg) in 2 ml 0.1 N aqueous HCl was added a few crystals of I$_2$. The reaction mixture was stirred at room temperature for 24 h, diluted with 4 ml water, extracted with CHCl$_3$ to remove I$_2$, and then chromatographed on Sephadex LH20 (eluent: 1:1 MeOH/H$_2$O) to give 15 mg disulphide 184 as a white solid.
\(^{1}\text{H} \text{NMR} (300 \text{ MHz}, \text{D}_2\text{O}): \delta 8.78 (s, 1\text{H}), 4.20 (t, J=8 \text{ Hz}, 1\text{H}), 3.85 (s, 3\text{H}), 3.30 (\text{dd}, J=15, 8 \text{ Hz}, 1\text{H}), 3.35 (\text{dd}, J=15, 8 \text{ Hz}, 1\text{H}) \text{ ppm.}

**Measurement of the optical rotation of disulfide 184:**

Disulfide 184 (10 mg) was dissolved in 1.0 ml 0.1 N aqueous HCl and the optical rotation was measured using a 1 decimeter cell. The specific rotation \([\alpha]_{D}^{20}\) was found to be +67°.

**Biosynthetic studies of imbricatine (179):**

Two freshly collected *D. imbricata* specimens were maintained in a 20-litre sea water aquarium. The sea water was non-circulating and well aerated. The water temperature was controlled at ca. 15°C by cooling the aquarium with running water. 250 \(\mu\text{Ci} \ L-(2,5-^{3}\text{H})\text{histidine (in 2 ml 2\% aqueous ethanol) was injected in equal amounts into the two starfish specimens. The animals were incubated for 24 h and then extracted in 1-litre methanol for 48 h. The majority (2/3) of the radioactivity was found in the aquarium sea water. The sea water, however, was found not to contain significant a amount of imbricatine. Normal workup of the methanol extract of the starfish led to the isolation of ca.10 mg imbricatine, which, after methylation and acetylation, yielded crude dimethylpentaacetyltrimbricatine (180). Purification by normal phase TLC (1:10 MeOH/CH\(_2\)Cl\(_2\)) followed by HPLC (4\% MeOH/CH\(_2\)Cl\(_2\)) afforded 8 mg dimethylpentaacetyltrimbricatine (180). The radioactivity of 180 was counted on a liquid scintillation counter and was found only 10\% higher than that of the HPLC baseline fraction. Therefore, the incorporation rate of histidine into imbricatine was too low to be considered positive.

In a following trial, 3,5-\(^{3}\text{H}\)-tyrosine was injected into two specimens and the animals were incubated under similar conditions for 48 h. Imbricatine was isolated and derivatized
following the normal protocol. The purified dimethylpentaacetyllimbricatine (180) was not radioactive.
III. NEW METABOLITES
FROM THREE PAPUA NEW GUINEA SPONGES*

GENERAL INTRODUCTION

This chapter presents the results of three individual, but related, projects, each involving the study of the secondary metabolites of a Papua New Guinea sponge. Before each project is discussed, a general introduction to sponges and their chemistry will be given.

1. General Information About Sponges2,7

Sponges, the most primitive multicellular animals, constitute the large phylum Porifera. There are over 10,000 different species, the majority (ca. 99%) of which are marine. These sessile animals have a body structure very different from any other invertebrates. They lack any true tissues or organs and the cells are highly independent. It's not surprising that their animal nature was not recognized until 1765.

Sponges are filter-feeding animals and they obtain nutrition by pumping a large volume of water through their bodies at low pressure. The basic structure of a sponge, a collection of cells constructed around a system of canals and chambers which are connected to the exterior through numerous small pores, is well designed for this purpose. The sponge skeleton is relatively complex and provides a supporting framework for the living cells of the animal. The skeleton may consist of calcareous or siliceous spicules, a fibrous protein material (spongin), or a combination of siliceous spicules and spongin fibers. The shape of

* All three sponges were identified by Dr. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam, P. O. Box 4766, 1009 AT, Amsterdam, The Netherlands. Voucher samples are deposited at Zoological Museum of Amsterdam.

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the spicules is highly diversified and can provide information for the identification of species. The basic sponge model (Fig. 45a), from which all others can be derived, is a vase-like structure consisting of a central cavity surrounded by a poriferous wall. A water current is drawn into the body through the pores and expelled through a large opening (osculum) on the top of the vase. The water current supplies the animal with oxygen and food particles and takes away waste materials. Many sponges have body walls with complex folding patterns to increase filtering efficiency (Fig. 45c,d).

The body forms of sponges are extremely varied and depend on the type of surfaces they are attached to and the amount of water movement. A particular species may assume different appearances in different environments.

Sponges are common in all of the world's seas. The majority inhabit shallow waters while some exist at great depths. Most of these sessile animals are attached to suitable substrata such as rocks, hard-shelled animals and seaweeds. Sponges also harbour many symbiotic microorganisms such as algae and bacteria. Their major food sources include planktonic organisms and organic detritus.

The phylum Porifera includes four distinct classes, Calcarea, Hexactinellida, Demospongiae and Sclerospongiae. Demospongiae is the largest class and it accounts for over 95% of all sponge species. All of the sponge species dealt with in this thesis belong to this large class.
Fig. 45. Sponge structure. (a) Basic sponge model; (b) Sponge with simply folded body wall; (c) and (d) Sponge with more specialized functions.
2. Sponge Metabolites

Being sessile organisms and lacking apparent physical protection, sponges are vulnerable to predation in the hostile marine environment. As a result, sponges rely heavily on chemical defenses to deter numerous predators ranging from invertebrate gastropods to vertebrate fishes. Over the last 500 million years of evolution, sponges have been under constant selective pressure to develop their chemical defenses. Many species, especially encrusting and soft-bodied ones, have gradually built up sophisticated biosynthetic systems to produce biologically active secondary metabolites.

In the last 15 years or so, there has been a worldwide interest in biologically active metabolites obtained from various species of sponges. As a consequence, a large number of sponges have been subjected to chemical investigation and over 1,000 new compounds, accounting for about one half of the total number of known marine natural products, have been isolated and identified. Sponge metabolites have a great diversity of chemical structures which include terpenoids, polycyclic aromatics, peptides, etc. Many sponge metabolites possess previously unencountered skeletons. The novel chemical structures have expanded our knowledge of secondary metabolism. Many of the sponge metabolites possess biological activities representing important biomedical potential.

Sponges are filter-feeding animals and the majority contain symbiotic microorganisms, which always raises some uncertainty concerning the true origin of sponge metabolites. In spite of this complication, the majority of metabolites isolated from sponges are believed to be produced by the sponges themselves. For example, the bromotyrosine-derived metabolites of verongiid sponges are widely believed to be biosynthesized by these sponges due to the extensive occurrence of bromotyrosine derivatives in verongiid sponges. In some exceptional cases, such as Dysidea herbacea, there is circumstantial evidence to suggest that metabolites obtained from sponge extracts are actually produced by symbionts.
Tropical sponges have been found to be particularly rich in secondary metabolites. In 1986 and 1988, we made two expeditions to Papua New Guinea for the purpose of collecting marine organisms. The chemical investigation of several sponges from these collections has led to the isolation and identification of a number of new secondary metabolites. In the following sections of this chapter, the isolation and structure elucidation of six new bastadins from *Ianthella basta*, xestospong in E from *Xestospongia* sp., and callydiyne from *Callyspongia flammea* will be presented.
IIIa. BASTADINS-8,-9,-10,-13,-14,-15 FROM IANTHELLA BASTA

A. INTRODUCTION

1. Taxonomy And Description of Ianthella basta (Pallas, 1766)

*Ianthella basta* belongs to the family Ianthellida within the order Verongidae according to Berquist's classification scheme (Fig. 46). Prior to 1978, the verongiid sponges had been treated as a family within the sponge order Dictyoceratida. The order Dictyoceratida, along with the order Dendroceratida, encompasses sponges lacking any mineral spicules but possessing spongin fiber skeletons. The two orders had also been grouped as a large order, Keratosa, for some time. There were, however, many biological and physiological factors suggesting that the verongiid sponges should be separated from the typical dictyoceratid sponges. In 1978, based on these biological factors and the new evidence provided by marine natural products chemists, Berquist finally raised the verongiid sponges to the ordinal level to form an independent order Verongida. The chemotaxonomic evidence was that all verongiid sponges contain bromotyrosine-derived metabolites and lack terpenes, which are commonly found in dictyoceratid sponges, and many contain sterols with the novel aplystane skeleton. All of the verongiid sponges possess characteristic pigments that follow a rapid oxidation sequence after death, changing from bright colors through navy blue to purple or purple-black.

*Ianthella basta* occurs in tropical waters, as large folded-fan-shaped sheets (Fig. 47). The color may be yellow, orange, red, purple, or blue. The *Ianthella basta* specimens dealt with in this chapter were collected on exposed reefs at 10-15 m below the surface off Madang, Papua New Guinea, in April 1986 and November 1988.
Phylum: **Porifera**

Class: Calcarea Hexactinellida **Demospongiae** Sclerospongiae

Subclass: Homoscleromorpha **Ceratinomorpha** Tetratinomorpha

Order: Halichondrida Poecilosclerida Nepheliospongia Haplosclerida

Family: Aplysinidae **Ianthellidae** Aplysinellidae (Verongidae)

Genus: **Ianthella**

Species: *I. flabelliformis* *I. basta* *I. ardis*

Fig. 46. Phylogenetic classification of *Ianthella basta*\(^91\)
Fig. 47. *Ianthella basta*
2. Secondary Metabolites of The Verongida

A large number of secondary metabolites have been reported from the verongiid sponges. Verongiid metabolites are almost exclusively bromotyrosine-derived and their presence has become an important taxonomic marker for the verongiid sponges. Since there have been several recent reviews on verongiid metabolites, I will discuss only some highlights and a few of the latest examples in this section.

The number of bromotyrosine units in verongiid metabolites ranges from one to four. In several metabolites the bromophenyl ring remains intact. In most cases, however, the bromophenyl rings have been transformed into cyclohexadiene derivatives through oxidation. In one case, the phenyl ring has even been expanded to a seven-membered dihydro oxepine ring. In the metabolites that incorporate two or more tyrosine units, the tyrosine units usually are connected through an amide bond either directly or via a smaller unit derived from a simple amino acid. Direct linkages between the two phenyl rings through an oxidative phenolic coupling are also common.

About two dozen verongiid metabolites contain only one bromotyrosine unit. Two simple examples are the dibromophenethylammonium salt 194 and its close analogue, aplysamine-1 (195), from two Aplysina species. Both compounds have an intact bromophenyl ring and they apparently arise directly from a dibromotyrosine unit.

The majority of the secondary metabolites isolated from verongiid sponges incorporate two tyrosine units in the structures. For example, araplysillins I-II (196-197) are two
metabolites recently isolated from the sponge *Psammaplysilla arabica*. Each araplysillin contains a spirocyclohexadienylisoxazoline system as well as one dibromotyromine unit in the molecule. Both compounds displayed some antibacterial activity and inhibitory activity against porcine brain Na⁺/K⁺ ATPase.

Another two metabolites with two tyrosine units are Psammaplins A and D (198-199) found in the Tongan sponge *Psammaplysilla purpurea* and an unidentified sponge collected in Guam. The two bromotyrosine units are connected by a fragment that was suggested to be derived from a rearranged cysteine. The stereochemistries of the oximes were determined according to the chemical shifts of the adjacent methylene carbons.
There have been very few verongiid metabolites derived from three tyrosine units. Two examples are fistularin-3 (200) isolated from the sponge *Aplysina (Verongia)* fistularis\(^{99a}\) and 11,19-dideoxyfistularin-3 (201) from the Australian species *Pseudoceratina durissima*\(^{99b}\)

![Chemical structures](image)

200 \(R=\text{OH}\)

201 \(R=\text{H}\)

Perhaps the most remarkable secondary metabolites isolated from verongiid sponges are the bastadins, each incorporating four units of bromotyrosine. The first seven bastadins (202-208) were found in the Australian sponge *Ianthella basta*\(^{100}\). Bastadins-1, -2 and -3 (202-204) are acyclic and arise from a single oxidative phenolic coupling reaction. The structures of these three bastadins were solved by extensive NMR, MS and chemical degradation studies. Bastadins-4, -5, -6, and -7 (205-208) each possess a 28-membered macrocyclic ring, termed 13,32-dioxa-4,22-diazabastarane, which is apparently derived from two oxidative coupling reactions. The structures of bastadins-4 and -5 (205-206) were determined by X-ray diffraction analysis. The structures of bastadins 6-7 (207-208) were based on the NMR and MS results coupled with the assumption that all of the macrocyclic bastadins share the same 13,32-dioxa-4,22-diazabastarane skeleton. All seven bastadins exhibit potent antimicrobial activity against Gram positive bacteria. Bastadins-1, -2 and -3
have been synthesized through a biomimetic coupling between a dibromotyrosine derivative and a 3-bromotyramine moiety.\textsuperscript{101a} Bastadin-6 dimethylether has also been synthesized from its acyclic analogue, bastadin-2 trimethylether, through an oxidative phenolic coupling.\textsuperscript{101b}

\begin{align*}
\text{202} & \quad X=H \\
\text{203} & \quad X=Br
\end{align*}

\begin{align*}
\text{204} & \\
\text{205} & \quad X=H, \text{ trans } \Delta^{5,6} \\
\text{206} & \quad X=H \\
\text{207} & \quad X=Br \\
\text{208} & \quad X=Br, \text{ trans } \Delta^{5,6} \\
\end{align*}

13,32-dioxa-4,22-diazabastarane
Bastadin-12 (209) has recently been reported from *Ianthella basta*. This compound represents the first bastadin that possesses the new 13,32-dioxa-4,22-diazaisobastarane skeleton rather than the more common 13,32-dioxa-4,22-diazabastarane skeleton. The new skeleton in bastadin-12 (209) was established unambiguously on the basis of long-range $^1\text{H}/^{13}\text{C}$ correlations.
Our chemical investigation of the *Ianthella basta* samples collected in Papua New Guinea has led to the isolation of six new bastadins, bastadins-8, -9, -10, -13, -14 and -15 along with the previously reported bastadins-4, -5, -6 and -7. In the following section, the isolation, structure elucidation and biological activity of the new bastadins will be presented.
B. RESULTS AND DISCUSSION

1. Isolation of The Bastadins

Specimens of *I. basta* (collection #: PNG-4-20-5-31) were collected by hand using SCUBA on reefs near Madang, Papua New Guinea. Freshly collected sponge was immediately frozen on site using dry ice and kept frozen until examined. Thawed sponge was homogenized in a Waring blender with methanol and extracted at room temperature for two days. The methanol extract was concentrated *in vacuo* and the resulting residue was suspended in water and sequentially extracted with hexanes, dichloromethane and ethyl acetate. Both the dichloromethane and ethyl acetate soluble fractions contained mixtures of bastadins. Separation of the mixtures was accomplished by sequential application of Sephadex LH20 (MeOH/CH₂Cl₂ 1:1), silica gel centrifugal (CH₂Cl₂/MeOH 40:1), and normal phase high performance liquid (hexane/EtOAc 1:1) chromatographies to give pure samples of the known bastadins -4 (205), -5 (206), -6 (207) and -7 (208) and the new bastadins-8 (211), -9 (214) -10 (213), -13 (212), -14 (215) and -15 (216). The previously described bastadins-4 (205), -5 (206), -6 (207) and -7 (208) were identified by comparing their spectral data to the literature values.100
2. Structure Elucidation of Bastadins-8 (211), -9 (214), -10 (213), -13 (212), -14 (215) and -15 (216)

Bastadin-8 (211):

Bastadin-8 (211) was isolated as a white amorphous solid. A preliminary examination of the $^1$H NMR data obtained for 211 (Table 13) indicated that the new metabolite was closely related to the previously described bastadin-4 (205). Since bastadin-4 (205) had been most fully characterized as its tetramethylether (210), bastadin-8 (211) was converted to its tetramethylether 217 by reaction with methyl iodide and potassium carbonate in DMF. The EIMS of bastadin-8 tetramethylether (217) (Fig. 48) showed a parent ion cluster at m/z 1086/1088/1090/1092/1094/1096 Da, indicating the
Fig. 48. EIMS of bastadin-8 tetramethylether (217)
Fig. 49. $^1$H NMR spectrum of bastadin-8 tetramethylether (217) (400 MHz, CDCl$_3$)
Fig. 50. $^{13}$C BB and APT NMR spectra of bastadin-8 tetramethylene (217) (75 MHz, CDCl$_3$)
The presence of five bromine atoms in the molecule. Its molecular formula was determined to be $\text{C}_{38}\text{H}_{35}\text{N}_4\text{O}_9\text{Br}_5$ by EiHRMS (1089.8282, $\text{C}_{38}\text{H}_{35}\text{N}_4\text{O}_9\text{Br}_3 \Delta M - 0.2$ mmu).

Fig. 51. $^1$H NMR comparison between bastadin-8 tetramethylether (217) and bastadin-4 tetramethylether (210)
The aromatic regions of the $^1$H and $^{13}$C NMR spectra of the tetramethyl derivative 217 (Fig. 49, 50) were very similar to those of bastadin-4 tetramethylether (210) (Fig. 51). $^1$H decoupling and COSY experiments performed on 217 clearly identified the spin systems for the four aromatic rings, the chemical shifts of which were nearly identical with those in bastadin-4 tetramethylether (210) (Fig. 51).

It was apparent from the $^1$H NMR data (Fig. 49) that bastadin-8 tetramethylether (217) lacked the C5/C6 double bond found in bastadin-4 tetramethylether (210). The aliphatic region in the $^1$H NMR of 217 differed from that of bastadin-4 tetramethylether (210) by the presence of a triplet at $\delta$ 4.86 ppm assigned to a carbinol methine and a broad exchangable singlet at $\delta$ 2.90 ppm. These observations, coupled with the fact that the molecular formula of bastadin-8 tetramethylether (217) differed from that of bastadin-4 tetramethylether (210) by the extra elements of H$_2$O, suggested that bastadin-4 (205) was simply the dehydration product of bastadin-8 (211). This was also consistent with the IR spectrum of bastadin-8 tetramethylether (217) which showed a broad absorption peak at 3402 cm$^{-1}$ attributed to a hydroxyl group.

The structure of bastadin-4 (205), with a 13,32-dioxa-4,22-diazabastarane skeleton, was originally solved unambiguously by X-ray diffraction analysis. Since the aromatic fragments in bastadin-8 tetramethylether (217) had $^1$H NMR chemical shifts virtually identical with those in bastadin-4 tetramethylether (210), it was assumed that bastadin-8 tetramethylether (217) possessed the same 13,32-dioxa-4,22-diazabastarane skeleton. Another possible structure for bastadin-8 was 211a with the alternate 13,32-dioxa-4,22-diazaisobastarane skeleton. It was reported that bastadin-12 (209), with the new 13,32-dioxa-4,22-diazaisobastarane skeleton, had significantly different $^1$H NMR chemical shifts than its counterpart bastadin-9 (214) with the 13,32-dioxa-4,22-diazabastarane skeleton. Therefore, based on the fact that the $^1$H NMR data of bastadin-8 tetramethylether (217) was
nearly identical with that of bastadin-4 tetramethylether (210) (Fig. 51), the correct structure of bastadin-8 was assigned to be 211 with the 13,32-dioxa-4,22-diazabastarane skeleton.

Extensive NMR studies on bastadin-8 tetramethylether (217) including $^1$H decoupling, nOe difference, COSY and long-range COSY supported the proposed structure for bastadin-8 (211). The $^1$H decoupling and COSY experiments performed on bastadin-8 tetramethylether (217) revealed two aliphatic fragments as shown below. Additionally, the aliphatic region in the $^1$H NMR also contained signals attributed to two isolated methylenes: a two-proton singlet at $\delta$ 3.69 ppm and an AB system at $\delta$ 3.77 (d, 13.2 Hz) and $\delta$ 3.82 (d, 13.2 Hz) ppm.
A series of nOe difference experiments (Fig. 52) as well as a long-range COSY experiment (Fig. 53) established the connectivities between the four aromatic rings and the four aliphatic moieties. Irradiation of the carbinol methine at δ 4.86 ppm induced nOes in the two ortho protons (δ 7.70, d, 2.0 Hz; δ 7.27, dd, 8.4, 2.0 Hz) on phenyl ring D (Fig. 52), indicating that the carbinol methine was located at C6, the benzylic position of ring D. NOes were also observed to the alcohol proton (δ 2.90) and the proton at δ 3.38 ppm (H5). Irradiation of H20 (δ 2.74 ppm) induced NOes in the ortho protons (δ 7.14, d, 1.9 Hz; δ 6.65, d, 1.9 Hz) of ring A and H21/H21' (δ 3.46, m; δ 3.55, m). The AB system at δ 3.77, 3.84 ppm was found to be the benzylic methylene of phenyl ring B and the other isolated methylene at δ 3.69 ppm was attached to ring C according to the nOe results (Fig. 52). The long-range COSY correlations observed between the benzylic protons and the corresponding phenyl rings also confirmed these connectivities (Fig. 53).

![Diagram](image)

**Fig. 52** NOe results of bastadin-8 tetramethylether (217)

Further support for the structure of bastadin-8 (211) came from the EIMS (Fig. 48) of bastadin-8 tetramethylether (217), which contained a prominent fragment ion cluster at m/z
Fig. 53. Long-range COSY spectrum of bastadin-8 tetramethylether (217) (400 MHz, CDCl₃)
Table 12. $^1$H NMR data for bastadin-8 tetramethylether (217) and bastadin-4 tetramethylether (210) (400 MHz, CDCl$_3$)

<table>
<thead>
<tr>
<th>position</th>
<th>(210) $^1$H NMR</th>
<th>(217) $^1$H NMR</th>
<th>nOe$^a$</th>
<th>COSY$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.68 (s)</td>
<td>3.69 (s)</td>
<td>H36, H38</td>
<td>H36, H38</td>
</tr>
<tr>
<td>4</td>
<td>8.32 (d, 11.2 Hz)</td>
<td>6.86 (t, 6.1 Hz)</td>
<td>H5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.38 (dd, 11.2, 14.7)</td>
<td>3.38 (m), 3.70 (m)</td>
<td>H4, H6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.18 (d, 14.7 Hz)</td>
<td>4.86 (t, 6.0 Hz)</td>
<td>H8, H12, H5</td>
<td>H5</td>
</tr>
<tr>
<td>8</td>
<td>7.60 (d, 2.0 Hz)</td>
<td>7.70 (d, 2.0 Hz)</td>
<td>H12</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>6.99 (d, 8.4 Hz)</td>
<td>6.97 (d, 8.4 Hz)</td>
<td>H12</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>7.39 (dd, 2.0, 8.4 Hz)</td>
<td>7.27 (dd, 2.0, 8.4)</td>
<td>H8, H11</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>7.13 (d, 1.9 Hz)</td>
<td>7.14 (d, 1.9 Hz)</td>
<td>H19, H20*</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>6.65 (d, 1.9 Hz)</td>
<td>6.65 (d, 1.9 Hz)</td>
<td>H17, H20*</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2.81 (t, 5.8 Hz)</td>
<td>2.74 (m)</td>
<td>H17, H19, H21</td>
<td>H19*</td>
</tr>
<tr>
<td>21</td>
<td>3.57 (q, 6.0 Hz)</td>
<td>3.55 (m), 3.46 (m)</td>
<td>H20, H22</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>6.59 (t, 6.2 Hz)</td>
<td>6.75 (t, 6.1 Hz)</td>
<td>H21</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3.73 (s)</td>
<td>3.77 (d, 13.2 Hz), 3.84 (d, 13.2 Hz)</td>
<td>H27/H31</td>
<td>H27/H31</td>
</tr>
<tr>
<td>27</td>
<td>7.50 (s)</td>
<td>7.53 (s)</td>
<td>H25</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>7.50 (s)</td>
<td>7.53 (s)</td>
<td>H25</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>7.11 (d, 1.9 Hz)</td>
<td>7.16 (d, 1.9 Hz)</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>6.25 (d, 1.9 Hz)</td>
<td>6.28 (d, 1.9 Hz)</td>
<td>H1</td>
<td></td>
</tr>
<tr>
<td>6-OH</td>
<td></td>
<td>2.90 (br s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCH$_3$'s</td>
<td>4.01 (s, 12H)</td>
<td>4.02 (s), 4.01 (s), 3.98 (s), 3.70 (s)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Proton in Position column irradiated;
$^b$ Correlations to the proton in Position column;
* Observed only in long-range COSY.
Table 13. $^1$H NMR data (400 MHz) * for bastadins-8 (211), -13 (212) and -10 (213)

<table>
<thead>
<tr>
<th>position</th>
<th>bastadin-8 (211)</th>
<th>bastadin-13 (212)</th>
<th>bastadin-10 (213)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.80 (d, 12.9 Hz), 3.88 (d, 12.9 Hz)</td>
<td>3.72 (d, 12.9 Hz), 3.78 (d, 12.9 Hz)</td>
<td>3.69 (d, 12.9 Hz), 3.65 (d, 12.9 Hz)</td>
</tr>
<tr>
<td>4</td>
<td>7.28 (t, 6.1 Hz)</td>
<td>7.14 (t, 6.1 Hz)</td>
<td>6.97 (t, 6.1 Hz)</td>
</tr>
<tr>
<td>5</td>
<td>3.46 (m), 3.55 (m)</td>
<td>3.41 (m), 3.63 (m)</td>
<td>3.41 (m), 3.52 (m)</td>
</tr>
<tr>
<td>6</td>
<td>4.82 (t, 6.1 Hz)</td>
<td>4.80 (t, 6.1 Hz)</td>
<td>4.75 (t, 5.4 Hz)</td>
</tr>
<tr>
<td>8</td>
<td>7.70 (d, 2.0 Hz)</td>
<td>7.58 (br s)</td>
<td>7.55 (d, 1.8 Hz)</td>
</tr>
<tr>
<td>11</td>
<td>6.97 (d, 8.3 Hz)</td>
<td></td>
<td>6.86 (d, 8.4 Hz)</td>
</tr>
<tr>
<td>12</td>
<td>7.21 (dd, 2.0, 8.3)</td>
<td>7.58 (br s)</td>
<td>7.10 (dd, 1.8, 8.4)</td>
</tr>
<tr>
<td>17</td>
<td>7.08 (d, 1.8 Hz)</td>
<td>7.03 (d, 1.8 Hz)</td>
<td>7.00 (d, 1.8 Hz)</td>
</tr>
<tr>
<td>19</td>
<td>6.33 (d, 1.8 Hz)</td>
<td>6.13 (d, 1.8 Hz)</td>
<td>6.32 (d, 1.8 Hz)</td>
</tr>
<tr>
<td>20</td>
<td>2.70 (m)</td>
<td>2.66 (m)</td>
<td>2.59 (t, 6.1 Hz)</td>
</tr>
<tr>
<td>21</td>
<td>3.40 (m)</td>
<td>3.36 (m)</td>
<td>3.31 (q, 6.1)</td>
</tr>
<tr>
<td>22</td>
<td>7.04 (t, 6.1 Hz)</td>
<td>7.08 (t, 6.1 Hz)</td>
<td>6.92 (t, 6.1 Hz)</td>
</tr>
<tr>
<td>25</td>
<td>3.69 (d, 12.6 Hz), 3.60 (d, 12.6 Hz)</td>
<td>3.89 (d, 13.0 Hz), 3.81 (d, 13.0 Hz)</td>
<td>3.75 (d, 13.7 Hz), 3.82 (d, 13.7 Hz)</td>
</tr>
<tr>
<td>27</td>
<td>7.47 (s)</td>
<td>7.52 (d, 1.8 Hz)</td>
<td>7.43 (d, 1.8 Hz)</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>6.78 (d, 8.4 Hz)</td>
<td>6.75 (d, 8.4 Hz)</td>
</tr>
<tr>
<td>31</td>
<td>7.47 (s)</td>
<td>7.14 (dd, 2.0, 8.4)</td>
<td>7.06 (dd, 1.9, 8.4)</td>
</tr>
<tr>
<td>36</td>
<td>7.16 (d, 1.8 Hz)</td>
<td>7.26 (d, 1.8 Hz)</td>
<td>7.17 (d, 1.7 Hz)</td>
</tr>
<tr>
<td>38</td>
<td>6.50 (d, 1.8 Hz)</td>
<td>6.60 (d, 1.8 Hz)</td>
<td>6.53 (d, 1.7 Hz)</td>
</tr>
<tr>
<td>Ar-OH's</td>
<td>10.50 (br s)</td>
<td>10.73 (br s)</td>
<td>10.45 (br s)</td>
</tr>
<tr>
<td>C=N-OH's</td>
<td>11.65 (br s)</td>
<td>11.27 (br s)</td>
<td>11.22 (br s)</td>
</tr>
</tbody>
</table>

*recorded in CDCl₃ with one-drop DMSO-d₆.
512/514/516/518 Da (EIHRMS: 513.8370, C_{17}H_{11}N_{2}O_{2}^{79}Br_{2}^{81}Br, \Delta M -0.9 \text{ mmu}), attributed to the major mass spectral fragmentation pathway (Scheme 9) typical for the bastadins. The cleavage labelled aa', which gives information on the total number of bromine atoms in rings B and C, usually leads to one of the most intense fragment ions in the electron impact mass spectra of this family of compounds. Therefore, the EIMS of bastadin-8 tetramethylether (217) was consistent with the assigned structure.

The CIMS spectrum of bastadin-8 tetramethylether (217) was very simple, with the parent ion cluster being very intense. It is worth noting that the highest mass observed was the M^+ + Br cluster around m/z 1172 Da. A similar mass cluster due to the addition of a bromine was also observed in the CIMS of bastadin-4 tetramethylether (210).

Scheme 9. Major EIMS fragmentation pathway of the bastadins\textsuperscript{100}

The stereochemistries of the two oxime groups in the molecule were determined to be $E$ (trans) in both cases based on the $^{13}$C NMR evidence (Fig. 50). The chemical shifts of C1 and C25 in bastadin-8 tetramethylether (217, in CDCl$_3$) were 28.6 and 29.1 ppm, consistent with those observed for the psammaplins (198-199),\textsuperscript{98a-c} which also possess $E$ (trans) oxime groups, and significantly different from that (δ 35.7 ppm) of the methylene adjacent to the $Z$ (cis) oxime in 199.\textsuperscript{98c}

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Bastadin-13 (212):

Bastadin-13 (212), isolated as a white amorphous solid, was likewise converted to its tetramethyl ether 218 for characterization. Bastadin-13 tetramethylether (218) showed a parent ion cluster at m/z 1086/1088/1090/1092/1094/1096 Da in the EIHRMS, appropriate for a molecular formula (1091.8294 C38H35N4O9Br2, AM 3.0 mmu) isomeric with bastadin-8 tetramethylether (217). The similarity of the ¹H and ¹³C NMR data of bastadin-13 tetramethylether (218) (Fig. 54-56) and bastadin-8 tetramethylether (217) indicated that they had the same 6-hydroxy-13,32-dioxa-4,22-diazabastarane macrocyclic skeleton. A very broad two-proton resonance appeared in the aromatic region (δ 7.65) of the ¹H NMR spectrum (Fig. 54) of bastadin-13 tetramethylether (218) recorded in CDCl₃ at room temperature. Recording the ¹H NMR spectrum at -30°C converted this broad signal into a pair of sharp meta-coupled resonances at δ 7.43 (d, J=1.8 Hz) and 7.81 (d, J=1.8 Hz).

The ¹H NMR spectrum of bastadin-13 tetramethylether (212) recorded in CDCl₃ (Fig. 54) did not give adequate dispersion in the aliphatic region. Therefore, most of the ¹H NMR experiments were carried out in 2:1 C₆D₆/CDCl₃ (Fig. 55). The ¹H NMR decoupling and COSY experiments readily identified the four aromatic rings and the two
Fig. 54. \(^1\)H NMR spectrum of bastadin-13 tetramethylether (218) (400 MHz, CDCl\(_3\))
Fig. 55. $^1$H NMR spectrum of bastadin-13 tetramethylether (218) (400 MHz, 2:1 C$_6$D$_6$/CDCl$_3$)
Fig. 56. $^{13}$C BB and APT NMR spectra of bastadin-13 tetramethylether (218) (75 MHz, CDCl$_3$)
aliphatic spin systems. Additionally, the two singlets attributed to the C1 and C25 methylenes were at $\delta$ 3.73 and 3.69 ppm.

NOe difference and long-range COSY results (Table 14, Fig. 51) established the connectivities between the four aliphatic moieties and the four aromatic rings. Irradiation of the carbinol methine resonance ($\delta$ 4.37 ppm) in 218 induced an NOe in the broad two-proton aromatic resonance at $\delta$ 7.45 ppm. When the same experiment was repeated at -30°C in CDC13, NOes were again observed from this carbinol methine ($\delta$ 4.86 at -30°C in CDC13) in these two aromatic protons which became two sharp doublets at low temperature. Therefore, the two-carbon aliphatic fragment containing the benzylic hydroxyl was attached to the above aromatic ring. Only a 9,11-dibromo ring D would have the symmetry and the ethylamine side-chain attachment required to explain both the coalescence and NOe results described above. Cooling the sample apparently slows the rotation of the D ring about the C6/C7 and C10/O13 bond axes preventing coalescence of the H8 and H12 NMR signals. Irradiation of H20 ($\delta$ 2.19 ppm at r.t. in 2:1 C6D6/CDC13) induced NOes in H17 and H19 ($\delta$ 6.86, 6.30 ppm). Long-range COSY correlations were observed between H25 ($\delta$ 3.73
Table 14. $^1$H NMR data (400 MHz) for bastadin-13 tetramethylether (218)

<table>
<thead>
<tr>
<th>position</th>
<th>in CDCl$_3$</th>
<th>in 1:2 CDCl$_3$/C$_6$D$_6$</th>
<th>nOe$^a$</th>
<th>COSY$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.80 (s)</td>
<td>3.69 (s)</td>
<td>H36*, H38*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7.07 (t, 6.1 Hz)</td>
<td>6.70 (t, 6.1 Hz)</td>
<td>H5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.38 (m)</td>
<td>2.94 (m), 3.32 (m)</td>
<td>H4, H6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4.86 (dd, 3.2, 7.5 Hz)</td>
<td>4.37 (br d, 7.3 Hz)</td>
<td>H8/H12, H5, 6-OH</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.65 (br s); 7.81 (d, 1.8)**</td>
<td>7.30-7.60 (br s)</td>
<td>H5</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>7.65 (br s); 7.43 (d, 1.8)**</td>
<td>7.30-7.60 (br s)</td>
<td>H19, H20*</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>7.08 (d, 1.9 Hz)</td>
<td>6.86 (d, 1.9 Hz)</td>
<td>H19, H20*</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>6.15 (d, 1.9 Hz)</td>
<td>6.30 (d, 1.9 Hz)</td>
<td>H17, H20*</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>2.69 (m)</td>
<td>2.19 (m)</td>
<td>H17, H19, H21</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>3.38 (m)</td>
<td>3.10 (m), 2.94 (m)</td>
<td>H20, H22</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>6.73 (t, 6.1 Hz)</td>
<td>6.26 (t, 6.0 Hz)</td>
<td>H21</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3.84 (d, 13.0), 3.78 (d, 13.0 Hz)</td>
<td>3.73 (s)</td>
<td>H27*, H31*</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>7.52 (d, 2.0 Hz)</td>
<td>7.60 (d, 2.0 Hz)</td>
<td>H31, H25*</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>6.65 (d, 8.4 Hz)</td>
<td>6.64 (d, 8.4 Hz)</td>
<td>H31</td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>7.14 (dd, 2.0, 8.4 Hz)</td>
<td>7.13 (dd, 2.0,8.4 Hz)</td>
<td>H27, H25*</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>7.29 (d, 2.0 Hz)</td>
<td>7.28 (d, 2.0 Hz)</td>
<td>H38, H1*</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>6.70 (d, 2.0 Hz)</td>
<td>6.78 (d, 2.0 Hz)</td>
<td>H36, H1*</td>
<td></td>
</tr>
<tr>
<td>6-OH</td>
<td>3.21 (br s)</td>
<td>2.70 (br s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OCH$_3$'s</td>
<td>4.05 (s), 4.01 (s), 3.89 (s), 3.87 (s)</td>
<td>4.00 (s), 3.80 (s), 3.65 (s), 3.58 (s)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Proton in Position column irradiated;

$^b$ Correlations to the proton in Position column;

* Observed only in long-range COSY;

** Recorded at -30°C.
ppm) and both H27 (δ 7.60, d, 2.0) and H31 (δ 7.13, dd, 8.4, 2.0), indicating that ring B in bastadin-13 tetramethylether (218) was only trisubstituted and, therefore, lacked the C30 bromine substituent found in tetramethyl bastadin-8 (217). Long-range COSY correlations (Table 14, Fig. 57) were also observed between H1 (δ 3.69 ppm) and both H36 and H38 (δ 7.28, 6.78 ppm).

Fig. 57. Selected nOe and long-range COSY correlations for bastadin-13 tetramethylether (218)

Based on the above information, it was concluded that bastadin-13 (212) is a close analogue of bastadin-8 (211), with a dibrominated ring D and a monobrominated ring B.
Fig. 58. Long-range COSY spectrum of bastadin-13 tetramethylene (218) (400 MHz, 2:1 C₆D₆/CDCl₃)
The structure of bastadin-13 (212) was further supported by the EIMS of bastadin-13 tetramethylether (218). A prominent fragment ion cluster at m/z 434/436/438 Da (1:2:1) in the EIMS of 218, resulting from the general aa' cleavage shown in Scheme 9, was consistent with the bromination pattern of B and C rings indicated by the $^1$H NMR data. The $^{13}$C NMR chemical shifts of C25 and C1 (δ 28.58, 28.99 ppm) of 218 (Fig. 56) indicated that the two oximes in bastadin-13 had E (trans) configurations.

Additionally, it is interesting to notice that all the nOes observed for bastadin-13 tetramethylether (218) at -30°C were negative. This was attributed to slow molecular tumbling at the low temperature.
Bastadin-10 (213):

\[
\begin{align*}
R &= H; & R &= \text{Me} \\
213 & \text{R=H;} & 219 & \text{R=Me} \\
(\text{H NMR assignments for 213})
\end{align*}
\]

Bastadin-10 (213) was isolated as a white amorphous solid. Its molecular formula was \(C_{34}H_{28}N_4O_9Br_4\) as suggested by the EIMS of its tetramethylether (219) (\(C_{38}H_{36}N_4O_9Br_4: \text{m/z 1008/1010/1012/1014/1016 Da}\)). The \(^1\text{H NMR} \) spectrum of 213 (Fig. 59) was nearly identical with that of bastadin-8 (211) except for the signals for ring B (Table 13). Ring B in bastadin-10 is 1,2,4-trisubstituted as demonstrated by the \(^1\text{H NMR} \) data (\(\delta 7.43, \text{d, J=1.8 Hz; 6.75, d, J=8.4 Hz; 7.06, dd, J=8.4, 1.8 Hz}\)). Since the molecular formula of bastadin-10 differed from that of bastadin-8 by replacement of a bromine with a hydrogen, its structure was assigned as the ring B debromo analogue of bastadin-8. The bromination pattern of ring B in bastadin-10 was therefore identical with that of ring B in bastadin-13 (212); the \(^1\text{H NMR} \) resonances assigned to ring B protons were nearly identical in these two compounds (Table 13). The observation of the mass
Fig. 59. $^1$H NMR spectrum of bastadin-10 (213) (400 MHz, CDCl$_3$/1-drop DMSO-d$_6$)
cluster in the EIMS of bastadin-10 tetramethylether (219) attributed to the "B+C" fragment (Scheme 9) was also in agreement with the proposed structure.

A series nOe experiments provided further support for the structure of bastadin-10 (213). Irradiation of H25 (δ 3.75, d, J=13.7 Hz; 3.82, d, J=13.7 Hz) induced nOes in H27 (δ 7.43, d, J=1.8 Hz) and H31 (δ 7.06, dd, J=8.4, 1.8 Hz) which was ortho coupled to H30 (δ 6.75, d, J=8.4 Hz). This result unambiguously identified the 1,2,4-trisubstitution pattern in ring B. As expected, nOes were also observed from H1 (δ 3.69, 3.65 ppm) to H36/H38 (δ 7.17, 6.53 ppm) of ring C, from H6 (δ 4.75 ppm) to H8/H12 (δ 7.55, 7.10 ppm) of ring D, and from H20 (δ 2.59 ppm) to H17/H19 (δ 7.00, 6.32 ppm) of ring A.

Bastadin-9 (214):

214  R=H;  220  R=Me

(1H NMR assignments for 214)

Bastadin-9 (214) was isolated as a white amorphous solid. Its molecular formula was determined to be C34H28N4O8Br4 based on the CIMS of its tetramethylether (220). Both the 13C (Experimental) and 1H (Fig. 60) NMR spectra of 214 indicated the absence of the 6-OH group in the molecule. 1H decoupling experiments clearly identified the four aromatic
Fig. 60. 1H NMR spectrum of bastadin-9 (214) (400 MHz, CDCl3/drop DMSO-d6)
Table 15. $^1$H NMR data (400 MHz)* for bastadins-9 (214), -14 (215) and-15 (216)

<table>
<thead>
<tr>
<th>position</th>
<th>bastadin-9 (214)</th>
<th>bastadin-14 (215)</th>
<th>bastadin-15 (216)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.57 (s)</td>
<td>3.61 (s)</td>
<td>3.68 (s)</td>
</tr>
<tr>
<td>4</td>
<td>7.19 (t, 6.1 Hz)</td>
<td>7.13 (t, 5.7)</td>
<td>7.14 (t, 6.1 Hz)</td>
</tr>
<tr>
<td>5</td>
<td>3.46 (q, 6.1 Hz)</td>
<td>3.45 (q, 6.0)</td>
<td>3.50 (q, 6.1 Hz)</td>
</tr>
<tr>
<td>6</td>
<td>2.71 (t, 6.1 Hz)</td>
<td>2.76 (t, 5.8)</td>
<td>2.81 (t, 6.1 Hz)</td>
</tr>
<tr>
<td>8</td>
<td>7.34 (d, 2.0 Hz)</td>
<td>7.31 (s)</td>
<td>7.42 (d, 1.9 Hz)</td>
</tr>
<tr>
<td>11</td>
<td>6.81 (d, 8.4 Hz)</td>
<td></td>
<td>6.89 (d, 8.4 Hz)</td>
</tr>
<tr>
<td>12</td>
<td>6.91 (dd, 2.0, 8.4 Hz)</td>
<td>7.31 (s)</td>
<td>7.03 (dd, 1.9, 8.4 Hz)</td>
</tr>
<tr>
<td>16</td>
<td>6.87 (d, 8.4 Hz)</td>
<td>6.89 (d, 8.1 Hz)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>6.72 (dd, 2.0, 8.4 Hz)</td>
<td>6.70 (dd, 1.9, 8.1 Hz)</td>
<td>7.05 (d, 2.0 Hz)</td>
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<tr>
<td>19</td>
<td>6.44 (d, 2.0 Hz)</td>
<td>6.12 (d, 1.9 Hz)</td>
<td>6.41 (d, 2.0 Hz)</td>
</tr>
<tr>
<td>20</td>
<td>2.62 (t, 6.1 Hz)</td>
<td>2.64 (t, 6.1 Hz)</td>
<td>2.65 (t, 6.1 Hz)</td>
</tr>
<tr>
<td>21</td>
<td>3.30 (q, 6.1 Hz)</td>
<td>3.27 (q, 6.0 Hz)</td>
<td>3.34 (q, 6.1 Hz)</td>
</tr>
<tr>
<td>22</td>
<td>6.61 (t, 6.2 Hz)</td>
<td>6.60 (t, 6.1 Hz)</td>
<td>6.68 (t, 6.1 Hz)</td>
</tr>
<tr>
<td>25</td>
<td>3.79 (s)</td>
<td>3.81 (s)</td>
<td>3.88 (s)</td>
</tr>
<tr>
<td>27</td>
<td>7.36 (s)</td>
<td>7.35 (s)</td>
<td>7.48 (d, 1.9 Hz)</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td>6.93 (d, 8.2 Hz)</td>
</tr>
<tr>
<td>31</td>
<td>7.36 (s)</td>
<td>7.35 (s)</td>
<td>7.13 (dd, 1.9, 8.2 Hz)</td>
</tr>
<tr>
<td>36</td>
<td>7.08 (d, 1.9 Hz)</td>
<td>7.11 (d, 1.8 Hz)</td>
<td>7.19 (d, 1.9 Hz)</td>
</tr>
<tr>
<td>38</td>
<td>6.21 (d, 1.9 Hz)</td>
<td>6.24 (d, 1.8 Hz)</td>
<td>6.48 (d, 1.9 Hz)</td>
</tr>
<tr>
<td>Ar-OH's</td>
<td>9.75 (br s)</td>
<td>9.73 (br s)</td>
<td>9.88 (br s)</td>
</tr>
<tr>
<td>C=N-OH's</td>
<td>12.10 (br s)</td>
<td>11.49 (br s)</td>
<td>11.32 (br s)</td>
</tr>
</tbody>
</table>

*recorded in CDCl3 with one-drop DMSO-d$_6$. 

192
rings and the aliphatic spin systems (Table 15). These spin systems were identical with those in bastadin-12 (209) but the chemical shifts were significantly different,\textsuperscript{102} indicating that bastadin-9 possessed the common 13,32-dioxa-4,22-diazabastarane skeleton rather than the 13,32-dioxa-4,22-diazaobastarane skeleton found in bastadin-12 (209). A series of nOe experiments involving irradiation of the four benzylic methylenes fully established the connectivities between the four aliphatic moieties with the four aromatic rings. Irradiation of H6 (δ 2.71 ppm) induced nOes in H8 (7.34, d, J=2.0 Hz) and H12 (δ 6.91, dd, J=8.4, 2.0 Hz), indicating that ring D was 1,2,4-trisubstituted. NOes from H20 (δ 2.62) to H19 (δ 6.44, d, J=2.0 Hz) and H17 (δ 6.72, dd, 8.4, 2.0) which ortho coupled to H16 (δ 6.87, d, 8.4) revealed the absence of a bromine atom at C16. The nOe from H25 (δ 3.79) to the two-proton singlet at δ 7.36 ppm suggested that ring B was symmetrically dibrominated at C28/C30. As expected, nOes were also observed from H1 (δ 3.57) to both H36 (δ 7.08) and H38 (δ 6.21) of ring C.
Bastadin-14 (215):

\[
\begin{array}{c}
\text{215 } R=H; \quad \text{221 } R=Me \\
(1^H \text{NMR assignments for 215})
\end{array}
\]

Bastadin-14 (215) was isolated as a white amorphous solid. Its molecular formula was determined to be C_{34}H_{27}N_{4}O_{8}Br_{5} based on the EIMS of its tetramethylether (221) (C_{38}H_{35}N_{4}O_{8}Br_{5}: m/z 1070/1072/1074/1076/1078/1080 Da). It differed from that of bastadin-9 (214) by replacement of a hydrogen with a bromine. The $^1$H NMR spectrum (Fig. 61) of bastadin-14 (215) was virtually identical with that of bastadin-9 except for the resonances attributed to ring D. In contrast to bastadin-9, which had a monobrominated D ring, bastadin-14 had a dibrominated D ring as indicated by the $^1$H NMR data (\(\delta 7.31, s; 2H\)). NOe experiments involving irradiations of the four benzylic methylenes established the connectivities between the four aromatic rings and their side chains. The nOe from H6 to the two-proton singlet at \(\delta 7.31 \text{ ppm}\) confirmed the dibromination pattern of ring D. The nOes observed from the other three benzylic methylenes were in complete agreement with the proposed structure of bastadin-14.
Fig. 61. $^1$H NMR spectrum of bastadin-14 (215) (400 MHz, CDCl$_3$/1-drop DMSO-d$_6$)
The observation of the mass cluster at m/z 512/514/516/518 (1:3:3:1) Da in the EIMS of 221 arising from the aa' general fragmentation (Scheme 9) was also consistent with the substitution patterns of ring B and ring C.

Bastadin-15 (216):

Bastadin-15 (216) was isolated as a white amorphous solid. Its molecular formula was determined to be C_{34}H_{28}N_{4}O_{8}Br_{4} based on the EIMS of its tetramethylether (222). It differed from that of bastadin-14 by replacement of a bromine with a hydrogen. $^1$H NMR decoupling experiments readily identified the spin systems assigned to the four phenyl rings in the molecule, the chemical shifts of which (Table 15) were virtually identical with those in bastadin-10 (213) (Table 13). Bastadin-15 (216), however, lacked the C6 hydroxyl as indicated by the $^1$H NMR (Fig. 62). This was also consistent with the fact that the molecular formula of bastadin-15 differed from that of bastadin-10 by the absence of one oxygen atom. Therefore, bastadin-15 (216) is the C6 deoxy analogue of bastadin-10.
Fig. 62. $^1$H NMR spectrum of bastadin-15 (216) (400 MHz, CDCl$_3$/1-drop DMSO-d$_6$)
(213). The nOes observed from the four benzylic methylenes to the corresponding benzene rings were in full agreement with this proposed structure.
C. CONCLUSIONS

Our chemical investigation of the Papua New Guinea sponge *Ianthella basta* has led to the isolation of bastadins-8 (211), -9 (214), -10 (213), -13 (212), -14 (215), and -15 (216), six new members of the bastadin family. Bastadins-8, -10 and -13 are particularly interesting because they are the first bastadins that bear an aliphatic hydroxyl group. The previously reported bastadins-4 and -7, which have a C5/C6 trans double bond, are very likely the dehydration products of bastadins-8 and -10. The benzylic hydroxylation pattern had been previously encountered in a few other verongiid sponge metabolites such as fistularin-3 (200). Bastadins-9 and -14 are very unique not only among the bastadins but also among the verongiid sponge metabolites, since they represent the only examples that incorporate a nonbrominated tyrosine unit (ring A). We believe the bastadins are biosynthesized by the sponge *Ianthella basta* instead of any symbiotic microorganism, because, as mentioned earlier in this chapter, bromotyrosine-derived metabolites are very typical for verongiid sponges.

Similar to bastadins-1 to -7, the six new bastadins also possess strong antibacterial activity. Our cytotoxicity assay indicated that all bastadins are active in vitro against the L1210 murine leukemia cell line (IC50<5 ug/ml).

Initially we reported\(^\text{103}\) the six new bastadins at the 72nd Canadian Chemical Conference and Exhibition (Victoria, B. C., June 1989) as bastadins-8, -9, -10, -11, -12 and -13. While our paper on bastadins-8 and -13 (Bastadin-13 was named bastadin-9 in that paper) was in press (*J. Nat. Prod.*, 1990, 53, 1441),\(^\text{104}\) Schmitz and co-workers\(^\text{105}\) reported the isolation of bastadins-8, -9, -10 and -11 from *Ianthella basta* collected from Guam. Shortly after our publication, Capon and co-workers\(^\text{102}\) reported bastadin-12 (209), the first bastadin to have the new 13,32-dioxa-4,22-diazaisobastarane rather than the more common 13,32-dioxa-4,22-diazabastarane ring system. In order to avoid confusion, we have renumbered the six bastadins we isolated in accordance with the currently accepted
system. Up to the present, there are fifteen bastadins in total, all of which are from *Ianthella basta* collected from various locations (Great Barrier Reef, Guam and Papua New Guinea).
IIIb. XESTOSPONGIN E FROM XESTOSPONGIA SP.

A. INTRODUCTION

1. Taxonomy and Description of Xestospongia

Sponges of the genus *Xestospongia* belong to the class Demospongiae. There are a number of classification systems at the Order and Family levels. According to Berquist's system,92 *Xestospongia* belongs to the family Nepheliospongiidae within the order Nepheliospongida. The Nepheliospongiidae family also includes two other genera, *Petrosia* and *Strongylophora*. The genus *Xestospongia* occurs throughout tropical waters and in the Antarctic and north Pacific oceans. This genus is comprised of approximately 30 species.

According to van Soest,106 the *Xestospongia* species (sample #: PNG-4-20-5-47) we obtained from Papua New Guinea is possibly a new species closely related to *Xestospongia exigua* (Kirkpatrick, 1900). This PNG *Xestospongia* species is a brown cake sponge with erect columns and its surface is not smooth as in *Xestospongia exigua*, but irregular.
2. Secondary Metabolites of the *Xestospongia* Sponges

A variety of secondary metabolites have been reported from some *Xestospongia* species. These compounds include steroids with novel side chains, polyacetylenic compounds, polycyclic aromatic quinones, alkaloids and triterpenoids.

Examples of the steroids are xestosterol (223)\textsuperscript{107} and mutasterol (224)\textsuperscript{108} from *Xestospongia muta* and xestospongesterol (225)\textsuperscript{109} from an unidentified *Xestospongia* species. All of these new steroids share a common nucleus found in cholesterol and they all possess unusual side-chain structures.

![Steroids](image)

Over a dozen polyacetylenic compounds, many of which are brominated, have been isolated from *Xestospongia* species.\textsuperscript{110} One example is 18-bromoocatodeca-9(E),17(E)-dien-7,15-diynoic acid (226) isolated from the Australian sponge *Xestospongia testudinaria*.\textsuperscript{110b} The same compound, along with five other similar unsaturated fatty acids (C\textsubscript{18}, C\textsubscript{16} and C\textsubscript{9}), has also been reported from an unidentified Red Sea *Xestospongia* species.\textsuperscript{110c}
A number of pentacyclic aromatic quinones have been isolated from *Xestospongia exigua* collected in Hawaii\textsuperscript{111a} and *Xestospongia sapra* collected in Okinawa.\textsuperscript{111b} The structure of halenaquinone (227) from *Xestospongia exigua* was determined by X-ray diffraction analysis.\textsuperscript{111a} All these compounds share the same pentacyclic carbon skeleton.

![Image of molecular structure](image)

Novel alkaloids have been isolated from several *Xestospongia* species. The Australian sponge *Xestospongia exigua* was found to contain xestospongins A-D (228-231), a new family of macrocyclic bis-1-oxa-quinolizidines.\textsuperscript{112} The structure of xestospongin C (230) was determined by X-ray diffraction analysis. Xestospongins possess \textit{in vivo} vasodilative activity. An unidentified Okinawan *Xestospongia* species was reported to contain manzamines E and F (232-233),\textsuperscript{113a} the latest members of the remarkable manzamine family. Manzamines A-D had been previously isolated from the sponges *Haliclona* sp.\textsuperscript{9}, \textsuperscript{113b} and *Pellina* sp.\textsuperscript{10} Manzamine F (233) was shown\textsuperscript{113a} to be identical with the previously described keramamine B, the structure of which had been misassigned.\textsuperscript{10} A few *Xestospongia* species\textsuperscript{114} have been found to contain a number of long chain alkaloids, as exemplified by xestomine A (234) isolated from *Xestospongia wiedenmayeri*.\textsuperscript{114b} Renierol (235) has been reported from *Xestospongia caycedoi* but was suggested to be of
microbial origin since similar compounds had been previously isolated from another sponge and a microorganism. 115
A remarkable series of triterpenoid glycosides, represented by xestovanin A (236), have been isolated from the Northeastern Pacific sponge Xestospongia vaniila. Each compound contains a triterpenoid aglycon and two or three hexose units attached to the side chains. It was suggested that all these related compounds were biosynthesized from a common acyclic precursor derived from squalene. The same sponge was also found to contain three degraded triterpenoid metabolites such as xestenone (237).

In the following section, the result of our study on the secondary metabolites of an unidentified Papua New Guinea Xestospongia species will be presented.
B. RESULTS AND DISCUSSION

1. Isolation of The Secondary Metabolites of *Xestospongia* sp.

The specimens of *Xestospongia* sp. (collection #: PNG-4-20-5-47) were collected on reefs off Madang, Papua New Guinea, in April 1986 and kept frozen until workup. The frozen sponge material (370g) was defrosted in MeOH (1000 ml), homogenized in a Waring blender, and extracted for two days. The crude MeOH extract was filtered and concentrated *in vacuo* to give an aqueous suspension which was sequentially partitioned against hexanes, methylene chloride and ethyl acetate. The methylene chloride soluble material was found to contain cytotoxic and antimicrobial compounds. The TLC (silica gel) of this fraction showed tailing spots that could be visualized in an iodine chamber. Repeated chromatography on Sephadex LH-20 (1:1 MeOH/CH₂Cl₂; 2:1:1 hexane/CH₂Cl₂/MeOH) led to the isolation of crude xestospongin E (238) as well as a mixture of previously described xestospongins. Crude xestospongin E was dissolved in aqueous HCl and extracted with EtOAc to remove the non-alkaloidal impurities. The aqueous solution was basified with ammonium hydroxide and extracted with EtOAc to yield pure xestospongin E (238) (48 mg). Xestospongin B (229) was isolated from the LH-20 fraction containing the previously described xestospongins by repeated chromatography on silica gel (0-10% MeOH/CH₂Cl₂). The structure of xestospongin B (229) was identified by comparing its NMR data with the published values.¹¹²
2. Structure Elucidation of Xestospongin E (238)

Xestospongin E (238) was obtained as a white amorphous solid. Its EIHRMS showed an intense parent ion at m/z 478.3768 Da appropriate for a molecular formula C_{28}H_{50}N_{2}O_{4} (ΔM=-0.2 mmu) with 5 degrees of unsaturation. Its $^{13}$C NMR spectrum (Fig. 64) contained only 14 resonances (11 × CH$_2$, 2 × CH, 1 × C), suggesting that the molecule was symmetrical. The absence of resonances that could be attributed to sp$^2$ carbons in the $^{13}$C NMR spectrum indicated that the molecule was pentacyclic. A comparison between the NMR data of 238 and those of xestospongin B (229) (Table 17) readily identified the 1-oxa-quinolizidine nature of this new metabolite.

The $^1$H NMR spectrum (Fig. 63) of xestospongin E (238) contained eight resonances between δ 4.3 and 1.7 ppm. The upfield region between δ 1.7 and 1.1 ppm was poorly dispersed, indicating the presence of a long aliphatic chain. $^1$H NMR decoupling and COSY (Fig. 65) experiments performed on xestospongin E identified the coupling system attributed to the 1-oxa-quinolizidine skeleton as shown below:
Fig. 63. $^1$H NMR spectrum of xestospong E (238) (400 MHz, acetone-d$_6$)
Fig. 64. $^{13}$C NMR BB and APT spectra of xestospong E (238) (75 MHz, CDCl$_3$)
Fig. 65. 2D COSY spectrum of testospongicin E (238) (400 MHz, acetone-d6)
Since the $^{13}$C NMR chemical shifts of the above fragment (ring A and ring B) in xestospongine E (238) were essentially identical with those of the ring A and ring B in xestospongine B (229) (Table 17), the substitution pattern of this fragment had to be the same as that of rings A and B in xestospongine B. Therefore, both C2 and C9 were each connected to a six-carbon straight chain and C9 also had a hydroxyl substituent. Since xestospongine E (238) was symmetrical, the other ends of the six-carbon chains had to be attached to a second identical 1-oxa-quinolizidine system. As a result, the gross structure of xestospongine E was assigned to be two identical 1-oxa-quinolizidine fragments linked together by two 6-carbon chains as shown in structure 238. This structure was in agreement with the $^{13}$C NMR spectrum (Fig. 64) and the molecular formula of xestospongine E. The C2 symmetry explained why only 14 resonances were observed in the $^{13}$C NMR spectrum.

The relative stereochemistry of xestospongine E (238) was established on the basis of a series of nOe experiments (Fig. 66) combined with some IR and COSY evidence. The nOes from H10 (4.23 ppm) to both H2 (3.69 ppm) and H4β (3.25 ppm) suggested that all three hydrogens were axial in B ring. Therefore, the side chain at C2 was equatorial and so was the C9-10 bond with respect to ring B. The absence of the characteristic Bohlmann bands*

*Trans-fused quinolizidines/l-oxaquinolizidines exhibit characteristic IR bands (Bohlmann bands) at ca. 2800 and 2750 cm$^{-1}$ while cis-fused compounds lack these bands. This IR property has been widely used to identify the relative stereochemistry of quinolizidines/l-oxaquinolizidines.
at 2800 and 2750 cm\(^{-1}\) in the IR spectrum of xestospongin E (238) revealed the cis configuration of the 1-oxa-quinolizidine system. Therefore, the C6-N5 bond had an axial orientation at N5 with respect to ring B. That H10 was equatorial with respect to ring A was also consistent with the W-couplings observed between H10 and both H6\(\beta\) and H8\(\beta\) in the COSY experiment (Fig. 65). Based on the fact that C2, N5 and C10 in xestospongin E (238) had the same relative stereochemistries as their counterparts in xestospongin B (229) and that these two compounds had virtually identical \(^{13}\)C NMR chemical shifts for rings A and B, the hydroxyl at C9 in 238 was assigned to be axial (\(\beta\)) as in xestospongin B (229).

Fig. 66. Selected nOe results for xestospongin E (238)
Table 16. Partial $^1$H NMR data for xestospongins E (238)

(400 MHz, acetone-d$_6$)

<table>
<thead>
<tr>
<th>H No.</th>
<th>$^1$H δ (ppm)</th>
<th>COSY</th>
<th>nOe</th>
</tr>
</thead>
<tbody>
<tr>
<td>2β</td>
<td>3.69, td, J=11.0, 2.0 Hz</td>
<td>H3α, 3β</td>
<td>H10β, H4β</td>
</tr>
<tr>
<td>3α</td>
<td>1.80, m</td>
<td>H3β, 2α, 2β, 4α, 4β</td>
<td>H3β, H4α, H6α</td>
</tr>
<tr>
<td>3β</td>
<td>1.25, m</td>
<td>H3α, 2α, 2β, 4α, 4β</td>
<td></td>
</tr>
<tr>
<td>4α</td>
<td>3.05, dd, J=13.7, 3.7 Hz</td>
<td>H4β, 3α, 3β</td>
<td>H4β, H6β</td>
</tr>
<tr>
<td>4β</td>
<td>3.25, td, J=13.7, 3.6 Hz</td>
<td>H4α, 3α, 3β</td>
<td>H10β, H4α</td>
</tr>
<tr>
<td>6α</td>
<td>3.12, td, J=13.0, 2.3 Hz</td>
<td>H6β, 7α, 7β</td>
<td>H6β, H8α</td>
</tr>
<tr>
<td>6β</td>
<td>2.50, br dd, J=10.6, 1.9 Hz</td>
<td>H6α, 7α, 7β, 10β</td>
<td>H6α</td>
</tr>
<tr>
<td>7α</td>
<td>1.96, dt, J=13.3, 3.5 Hz</td>
<td>H7β, 6α, 6β, 8α, 8β</td>
<td></td>
</tr>
<tr>
<td>7β</td>
<td>1.50, m</td>
<td>H7α, 6α, 6β, 8α, 8β</td>
<td></td>
</tr>
<tr>
<td>8α</td>
<td>1.50, m</td>
<td>H8β, 7α, 7β</td>
<td></td>
</tr>
<tr>
<td>8β</td>
<td>1.35, m</td>
<td>H8α, 7α, 7β, 10β</td>
<td></td>
</tr>
<tr>
<td>10β</td>
<td>4.23, br s</td>
<td>H8β, 6β</td>
<td>H2β, H4β</td>
</tr>
</tbody>
</table>

Table 17. Partial $^{13}$C NMR data for xestospongins E (238) and B (229)

(75 MHz, CDCl$_3$)

<table>
<thead>
<tr>
<th>Carbon No.</th>
<th>xestospongins E (238), δ</th>
<th>xestospongins B (229), δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>76.6</td>
<td>76.3</td>
</tr>
<tr>
<td>3</td>
<td>25.9</td>
<td>26.3</td>
</tr>
<tr>
<td>4</td>
<td>52.2</td>
<td>52.7</td>
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<tr>
<td>6</td>
<td>44.4</td>
<td>44.7</td>
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<td>7</td>
<td>20.4</td>
<td>21.4</td>
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<td>8</td>
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<td>9</td>
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<td>70.6</td>
</tr>
<tr>
<td>10</td>
<td>90.4</td>
<td>91.1</td>
</tr>
</tbody>
</table>

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3. Biological Activities of Xestospongin E (238)

Xestospongin E (238) exhibited strong *in vitro* cytotoxicity against the L1210 murine leukemia cell line (IC₅₀<1 µg/ml). Its antibacterial activity (vs. *Staphylococcus aureus* and *Bacillus subtilis*) and antifungal activity (vs. *Candida albicans* and *Pseudomonas aeruginosa*) were very potent, with MIC's less than 1 µg/disc.
CONCLUSIONS

Our chemical study of the Papua New Guinea sponge *Xestospongia* sp. has demonstrated that its major metabolites are the xestospongins, a family of macrocyclic bis-1-oxa-quinolizidine alkaloids. Xestospongin E (238) is a new compound while the others have been reported previously. The xestospongins are responsible for the cytotoxicity and the potent antibacterial activity of the crude extract of this sponge.

This work was initially reported\textsuperscript{103} at the 72nd Canadian Conference and Exhibition (Victoria, June 1989). Shortly after that, Kitagawa's group reported\textsuperscript{118} a series of xestospongin alkaloids, namely, araguspongines B-J, from the Okinawan sponge *Xestospongia* sp.. In fact, araguspongine D is simply a 3:7 mixture of (+)-xestospongin A (228) and (-)-xestospongin A. Araguspongines B (239) and E are diastereomers of xestospongin A (228) resulting from different spatial orientations of the N5/N5' lone-pair orbitals. Araguspongines F-J (240-243) are analogues of xestospongin B (229). Moreover, araguspongine C is identical with the xestospongin E (238) we have reported.

\[
\begin{align*}
&239 \\
&240 \quad R^1=\alpha-\text{Me}, \quad R^2=H \\
&241 \quad R^1=\beta-\text{Me}, \quad R^2=H \\
&242 \quad R^1=\beta-\text{Me}, \quad R^2=\alpha-\text{Me} \\
&243 \quad R^1=\beta-\text{Me}, \quad R^2=\beta-\text{Me}
\end{align*}
\]
PART IIIc. CALLYDIYNE FROM CALLYSPONGIA FLAMMEA DESQUEYROX

A. INTRODUCTION

According to Berquist's classification scheme,\(^{92}\) *Callyspongia flammea* is a member of the sponge family Callyspongiidae within the order Haplosclerida. The *Callyspongia* sponges have massive spongin fibre skeletons with some spicule reinforcement.

We have examined the secondary metabolites of *Callyspongia flammea* collected from Papua New Guinea and discovered that the major secondary metabolite was a new diacetylene, to which we have given the trivial name callydiyne. The isolation and structure elucidation of callydiyne will be presented in this section. To the best of our knowledge, this is the first study on the secondary metabolites of any Callyspongiidae sponges.

Polyacetylenes, with both odd and even carbon numbers, have been reported from sponges.\(^{3c}\) *Reniera fulva* was found to contain five new diacetylenes as exemplified by compound 244.\(^{119}\) Six 22-carbon polyacetylenes, represented by compound 245, have been isolated from a Red Sea species of the genus *Siphonochalina*.\(^{120}\) *Siphonodiol*, a 23-carbon polyacetylene, has been isolated from *Siphonochalina truncata*.\(^{121}\) Five very long chain polyacetylenes (C$_{46}$, C$_{49}$, C$_{52}$ and C$_{55}$; e.g., 246) have been obtained from the sponge *Petrosia ficiformis* and its predator, the nudibranch *Peltodoris atromaculata*.\(^{122}\) Due to the close structural similarity, the five compounds were isolated as two mixtures. Only general formulas were assigned to these polyacetylenes.

\[ \text{Diagram of 244} \]

244

216
245

\[ \text{OH} \]

\[ \text{HC} = \text{CCHCH} = \text{CHCH}_2 - \text{R}_1 - \text{CH}_2 \text{CH} = \text{CHC} = \text{CHC} = \text{CCH}_2 - \text{R}_2 - \text{CH}_2 \text{CH} = \text{CHCHC} = \text{CH} \]

\[ \text{OH} \quad \text{OH} \quad \text{OH} \]

246: \( R_1 + R_2 = C_{28}H_{50} \)
B. RESULTS AND DISCUSSION

1. Isolation of Callydiyne (247)

Specimens of Callyspongia flammea (collection #: PNG-8-26-11-66; pinkish vase-shaped sponge) were collected in 1988 on reefs off Madang, Papua New Guinea, and kept frozen until workup. Thawed sponge material was homogenized in a Waring blender with MeOH and extracted at r.t. for two days. The MeOH extract was filtered and concentrated in vacuo to yield an aqueous suspension which was diluted with water and partitioned against hexanes, methylene chloride and ethyl acetate. The hexanes fraction was sequentially chromatographed on Sephadex LH20 (eluent: 1:1 MeOH/CH₂Cl₂) and silica gel (eluent: hexane) to afford pure callydiyne (247) (41 mg).

2. Structure Elucidation of Callydiyne (247)

Callydiyne (247) was obtained as a colorless oil. The EIHRMS showed a parent ion at m/z 214.1713 Da appropriate for a molecular formula C₁₆H₂₂ (ΔM=-0.9 mmu) with six degrees of unsaturation. Its \(^{13}\)C NMR spectrum (Fig. 68) contained only eight resonances, suggesting that the molecule was symmetrical. IR bands at 3302 and 2097 cm\(^{-1}\) indicated that there was a terminal triple bond in the molecule. This was supported by both the \(^1\)H and \(^{13}\)C NMR data of callydiyne (\(^{1}\)H δ 3.06; \(^{13}\)C δ 81.14, 80.52 ppm) (Table 18).

Besides the terminal alkyne group, callydiyne also possessed a 1,2-disubstituted double bond (\(^{1}\)H δ 5.99, dtd, J=10.6, 7.5, 0.8 Hz; δ 5.44, ddt, J=10.6, 2.3, 1.4 Hz; \(^{13}\)C δ 107.95 (d), 146.15 (d) ppm) and four methylenes (\(^{1}\)H δ 2.32, 2H; 1.40, 2H; 1.30, 4H;
Fig. 67. 1H NMR spectrum of caldydine (247) (400 MHz, CDCl₃)
Fig. 68. $^{13}$C NMR BB and APT spectra of callydiyne (247) (75 MHz, CDCl$_3$)
Fig. 69. 2D COSY spectrum of callydyne (247) (400 MHz, CDCl₃)
$^{13}$C $\delta$ 30.20 (t), 29.28 (t), 28.67 (t), 29.09 (t) ppm) as demonstrated by the $^1$H (Fig. 67) and $^{13}$C NMR (Fig. 68) spectra. The COSY spectrum (Fig. 69) showed correlations that linked the terminal alkyne to the disubstituted double bond which in turn was connected to at least three contiguous methylenes ($\delta$ 2.32, qd, J=7.4, 1.4 Hz; $\delta$ 1.40, m; $\delta$ 1.30 ppm). The remaining methylene ($\delta$ 1.30 ppm) had to be connected to the end of this three carbon aliphatic chain to form the C$_8$H$_{11}$ fragment shown in Fig. 70. Since callydiyne was symmetrical, it had to be a dimer of this C$_8$H$_{11}$ fragment.

![Diagram of callydiyne](image)

**Fig. 70.** COSY correlations of callydiyne (247)

<table>
<thead>
<tr>
<th>position#</th>
<th>$^1$H $\delta$ (400 MHz)</th>
<th>COSY</th>
<th>nOe</th>
<th>$^1$H $\delta$ (75 MHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.06, dd, 2.3, 0.8</td>
<td>H3, H4, H5</td>
<td></td>
<td>81.14</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>80.52</td>
</tr>
<tr>
<td>3</td>
<td>5.44, ddt, 10.6, 2.3, 1.4</td>
<td>H1, H4, H5</td>
<td>H4</td>
<td>107.95</td>
</tr>
<tr>
<td>4</td>
<td>5.99, dt, 10.6, 7.5, 0.8</td>
<td>H1, H3, H5</td>
<td>H3, H5</td>
<td>146.15</td>
</tr>
<tr>
<td>5</td>
<td>2.32, qd, 7.4, 1.4</td>
<td>H1, H3, H4</td>
<td>H4, H6</td>
<td>30.20</td>
</tr>
<tr>
<td>6</td>
<td>1.40, m</td>
<td>H5, H7</td>
<td></td>
<td>29.28*</td>
</tr>
<tr>
<td>7</td>
<td>1.30, m</td>
<td>H6</td>
<td></td>
<td>28.67*</td>
</tr>
<tr>
<td>8</td>
<td>1.30, m</td>
<td></td>
<td></td>
<td>29.09*</td>
</tr>
</tbody>
</table>

*interchangable.
An nOe observed between the two olefinic protons (δ 5.99, 5.44 ppm) as well as the coupling constant between the two protons (J=10.6 Hz) established the cis configuration of the double bond. An additional point that should be mentioned is that, in contrast to normal methines, the terminal alkyne carbon of callydiyne (247) appeared as a positive signal in the APT spectrum (Fig. 68). This was attributed to the large C-H coupling constant typical for sp carbons (J=250 Hz, compared to ca.125 Hz for sp$^3$ carbons$^{123}$).
EXPERIMENTAL

General

See the Experimental section of chapter I.

IIIa. New Bastadins From *Ianthella basta*

Collection of *Ianthella basta* and isolation of the bastadins:

The fan-shaped sponge (ca. 60 × 100 cm) (collection #: PNG-4-20-5-31) was collected by hand using SCUBA on reefs 10-15 m below surface off Madang, Papua New Guinea, in April of 1986 and November of 1988. The samples were frozen with dry ice immediately after collection and preserved at -20°C until workup. Thawed sponge (275 g) was homogenized in a Waring blender with 1 liter of methanol and extracted for two days. Filtration of the methanol extract followed by evaporation under vacuum at r.t. afforded a red aqueous suspension which was diluted with water to 200 ml and sequentially partitioned with hexanes (300 ml × 3), dichloromethane (300 ml × 3) and ethyl acetate (300 ml × 3). The three organic soluble fractions were individually dried over anhydrous sodium sulphate and concentrated under vacuum. TLC and ¹H NMR indicated that both the dichloromethane soluble fraction (750 mg) and the ethyl acetate fraction (400 mg) contained bastadins.

The dichloromethane soluble material (750 mg) was chromatographed on Sephadex LH20 using 1:1 MeOH/CH₂Cl₂ as an eluent, to yield four fractions monitored by TLC (silica, 10:1 CH₂Cl₂/MeOH). The first fraction contained mainly fat, carotenoids and steroids, whereas the following ones were found to contain bastadins. The second fraction was chromatographed on preparative radial TLC using 40:1 CH₂Cl₂/MeOH and final purification on normal phase HPLC using 1:1 hexane/EtOAc as the solvent system gave pure bastadins-5 (206, 19 mg), -6 (207, 30 mg), -14 (215, 4 mg) and -15 (216, 3 mg). The third fraction was purified by the same procedure and it gave bastadins-8 (211, 11 mg), -9
The fourth fraction was methylated with CH$_3$I and anhydrous K$_2$CO$_3$ in DMF and fractionated on preparative radial TLC using 1:9 hexane/CH$_2$Cl$_2$ as an eluent to give 20 mg of bastadin-4 tetramethylether and 12 mg of bastadin-7 tetramethylether.

**Bastadins-8 (211):**

Obtained as a white amorphous solid; $^1$H NMR (400 MHz, CDCl$_3$) see Table 13; $^1$H NMR (400 MHz, DMSO-d$_6$) δ 11.90 (s), 11.73 (s), 9.94 (s), 9.70 (s), 7.97 (t, J=6.0 Hz), 7.73 (t, J=5.4 Hz), 7.68 (d, J=1.8 Hz), 7.58 (s, 2H), 7.25 (dd, J=8.4, 1.8 Hz), 7.14 (d, J=1.9 Hz), 7.06 (d, J=1.8 Hz), 6.94 (d, J=8.4 Hz), 6.59 (d, J=1.9 Hz), 6.23 (d, J=1.9 Hz), 4.63 (br d, J=6.4 Hz), 3.60 (s), 3.55 (s), 3.40 (m), 3.00 (m), 2.67 (t, 6.2) ppm.

**Bastadin-13 (212):**

Obtained as a white amorphous solid; $^1$H NMR (400 MHz, CDCl$_3$) see Table 13; $^1$H NMR (400 MHz, DMSO-d$_6$) δ 11.85 (s), 11.82 (s), 9.89 (s), 9.82 (s), 7.90 (t, J=5.4 Hz), 7.80 (t, J=5.2 Hz), 7.70 (br s, 2H), 7.52 (br s), 7.18 (br s, 2H), 7.05 (br s), 6.78 (br d, J=8.4 Hz), 6.47 (br s), 6.14 (br s), 5.79 (d, J=4.6 Hz), 4.67 (t, J=4.0 Hz), 3.73 (d, J=13.2 Hz), 3.68 (d, J=13.2 Hz), 3.63 (d, J=13.2 Hz), 3.60 (d, J=13.2 Hz), 3.20 (m), 2.61 (t, J=6.8 Hz) ppm.

**Methylation of bastadins-8 (211) and -13 (212):**

Bastadin-8 (211, 11 mg) was stirred with CH$_3$I (0.5 ml, passed through a pre-flamed basic alumina plug) and anhydrous K$_2$CO$_3$ (0.3 g) in DMF (5 ml) at r.t. for 18 h. The solvent was removed under vacuum to give a yellow residue which was suspended in CH$_2$Cl$_2$ and filtered. Purification of the CH$_2$Cl$_2$ soluble on normal phase HPLC using 6:4 hexane/EtOAc as the solvent system gave 10 mg of bastadin-8 tetramethylether (217).
Treatment of bastadin-13 (212, 10 mg) in the same procedure gave 9 mg of bastadin-13 tetramethylether (218).

**Bastadin-8 tetramethylether (217):**

Obtained as a white amorphous solid; IR (film) $v_{\text{max}}$: 3402, 2974, 2932, 2825, 1669, 1487 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) see Table 12; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 28.63 (t), 29.06 (t), 35.02 (t), 39.65 (t), 47.14 (t), 60.96 (q), 61.26 (q), 62.82 (q), 63.34 (q), 72.43 (d), 113.92 (d), 114.60 (s), 117.86 (d), 117.94 (d), 117.99 (s), 118.04 (s), 120.13 (d), 126.55 (d), 127.47 (d), 127.93 (d), 131.14 (d), 132.72 (s), 133.74 (d), 135.57 (s), 136.39 (d), 139.20 (s), 144.44 (s), 146.40 (s), 146.71 (s), 149.93 (s), 150.16 (s), 150.28 (s), 150.79 (s), 152.36 (s), 162.13 (s), 163.33 (s) ppm; EIMS m/z (rel. int.): 1096 (0.4), 1094 (2.7), 1092 (5.8), 1090 (7.1), 1088 (4.7), 1086 (1.7), 1078 (2.6), 1076 (10.6), 1074 (19.3), 1072 (18.1), 1070 (9.4), 1068 (2.0), 1065 (4.8), 1063 (18.5), 1061 (33.1), 1059 (34.0), 1057 (17.5), 1055 (4.5), 1047 (2.5), 1045 (7.1), 1043 (11.2), 1041 (10.5), 1039 (5.1), 1037 (1.6), 1033 (2.3), 1031 (3.7), 1029 (4.2), 1027 (3.6), 1025 (2.5), 1023 (1.3), 1015 (2.8), 1013 (4.7), 1011 (5.5), 1009 (3.5), 1007 (1.6), 682 (0.7), 680 (2.2), 678 (2.3), 676 (1.3), 651 (1.8), 649 (5.5), 647 (5.3), 645 (1.9), 606 (0.5), 604 (0.8), 602 (0.9), 593 (0.9), 591 (2.4), 590 (1.9), 589 (2.3), 574 (10.1), 572 (10.8), 570 (4.6), 518 (19.3), 516 (53.4), 514 (56.8), 512 (23.8), 456 (12.5), 454 (16.6), 452 (15.2), 450 (7.8), 422 (15.1), 420 (26.7), 418 (13.9), 410 (14.9), 408 (11.5), 397 (12.6), 395 (13.1), 393 (7.2), 356 (18.0), 354 (26.9), 240 (5.3), 238 (5.4), 236 (3.0), 212 (7.6), 210 (4.5), 208 (2.8), 169 (18.7), 82 (37.0), 80 (37.6), 44 (39.3), 31 (91.9), 29 (100.0); HREIMS m/z 1089.8282 (C$_{38}$H$_{35}$N$_4$O$_9$^{79}Br$_3^{81}$Br$_2$, $\Delta M=-0.2$ mmu); CIMS m/z (showed an extra Br) 1176 (15.2), 1174 (15.8), 1172 (26.0), 1170 (20.3), 1168 (4.4), 1096 (34.8), 1094 (85.4), 1092 (100.0), 1090 (84.8), 1088 (27.9), 1086 (2.5).
Bastadin-13 tetramethylether (218):

Obtained as a white amorphous solid; IR (film) $v_{\text{max}}$: 3327, 2932, 2857, 1669, 1487 cm$^{-1}$; $^1$H NMR see Table 14; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 28.58 (t), 28.99 (t), 34.77 (t), 39.59 (t), 47.31 (t), 61.08 (q), 61.16 (q), 63.17 (q), 63.17 (q), 72.26 (d), 112.72 (d), 113.38 (s), 117.87 (s), 118.37 (s), 118.54 (d), 119.60 (d), 126.57 (d), 129.24 (d), 129.71 (d), 130.63 (d), 133.07 (s), 133.15 (s), 134.22 (d), 135.35 (s), 142.10 (s), 144.46 (s), 146.83 (s), 147.49 (s), 149.52 (s), 150.17 (s), 150.74 (s), 151.05 (s), 151.81 (s), 162.26 (s), 163.63 (s) ppm; EIMS $m/z$ (rel. int.): 1096 (0.5), 1094 (2.0), 1092 (4.1), 1090 (4.2), 1088 (2.4), 1086 (0.7), 1078 (1.0), 1076 (3.7), 1074 (7.2), 1072 (6.5), 1070 (3.4), 1068 (0.8), 1065 (1.1), 1063 (2.3), 1061 (5.6), 1059 (5.3), 1057 (3.0), 1055 (0.8), 1047 (1.1), 1045 (3.2), 1043 (5.6), 1041 (5.0), 1039 (2.9), 1037 (0.7), 1015 (1.3), 1013 (2.2), 1011 (2.7), 1009 (2.1), 1007 (0.9), 648 (0.4), 632 (0.8), 630 (0.6), 602 (3.1), 600 (5.7), 598 (2.8), 592 (1.2), 590 (1.6), 571 (8.0), 569 (13.9), 567 (7.6), 551 (2.0), 549 (5.1), 547 (4.9), 545 (2.3), 536 (2.5), 534 (3.6), 532 (4.6), 530 (3.3), 512 (2.3), 511 (3.2), 438 (21.1), 436 (40.4), 434 (20.8), 412 (12.9), 411 (14.1), 410 (19.6), 409 (14.0), 408 (10.0), 342 (20.5), 340 (18.3), 318 (5.6), 316 (13.8), 314 (10.4), 276 (22.5), 261 (8.8), 169 (9.2), 115 (9.4), 82 (41.5), 80 (42.1), 31 (73.6), 29 (100.0); HREIMS: 1089.8312 (C$_{38}$H$_{35}$N$_4$O$_9^{79}$Br$_3^{81}$Br$_2$, $\Delta M=2.8$ mmu); CIMS (showed one extra Br): 1178 (12.7), 1176 (37.3), 1174 (72.2), 1172 (92.9), 1170 (56.4), 1168 (21.8), 1166 (1.2), 1096 (21.0), 1094 (52.0), 1092 (74.2), 1090 (54.0)), 1088 (22.6), 1086 (2.4).

Bastadin-10 (213):

Obtained as a white solid; IR (film) $v_{\text{max}}$: 3210, 2925, 1662, 1576, 1529, 1486, 1424, 1283, 1235, 1180, 1045, 1023, 989. 823, 762, 715 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$/DMSO-d$_6$) see Table 13; $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 11.81 (s), 11.80 (s), 9.79 (s), 9.74 (s), 7.90 (t, $J=6.2$ Hz), 7.74 (t, $J=5.6$ Hz), 7.64 (d, $J=1.8$ Hz), 7.50 (d, $J=1.9$ Hz), 7.23 (dd, $J=8.4, 1.8$ Hz), 7.16 (d, $J=1.8$ Hz), 7.12 (d, $J=1.8$ Hz), 7.09 (dd,
J=8.4, 1.8 Hz), 6.92 (d, J=8.4 Hz), 6.76 (d, J=8.4 Hz), 6.48 (d, J=1.9 Hz), 6.47 (d, J=1.8 Hz), 4.61 (dd, J=7.8, 4.1 Hz), 3.65 (d, J=13.6 Hz), 3.63 (d, J=13.6 Hz), 3.30 (s), 3.10 (m), 2.61 (t, J=7.1 Hz) ppm.

Bastadin-9 (214):

Obtained as a white solid; IR (film) v_max: 3212, 2928, 1660, 1590, 1516, 1488, 1453, 1426, 1362, 1282, 1248, 1045, 1023, 990, 821 cm⁻¹; ¹H NMR (400 MHz, CDCl₃ with 1 drop DMSO-d₆) see Table 15; ¹H NMR (400 MHz, DMSO-d₆) δ 11.90 (s, 1H), 11.64 (s, 1H), 9.92 (s, 1H), 9.33 (s, 1H), 7.96 (t, J=6.0 Hz, 1H), 7.92 (t, J=6.1 Hz, 1H), 7.58 (s, 2H), 7.48 (d, J=1.7 Hz, 1H), 7.11 (dd, J=1.7, 8.4 Hz, 1H), 7.06 (d, J=1.6 Hz, 1H), 6.86 (d, J=8.4 Hz, 1H), 6.81 (d, J=8.4 Hz, 1H), 6.75 (dd, J=1.7, 8.4 Hz, 1H), 6.72 (d, J=1.7 Hz, 1H), 6.23 (d, J=1.6 Hz, 1H), 3.55 (s, 2H), 3.53 (s, 2H), 3.40 (q, J=6.1 Hz, 2H), 3.21 (q, J=6.1 Hz, 2H), 2.67 (t, J=6.1 Hz, 2H), 2.62 (t, J=6.1 Hz, 2H) ppm; ¹³C NMR (75 MHz, CD₃OD) δ 28.61 (t), 29.61 (t), 35.57 (t), 35.71 (t), 41.44 (t), 42.05 (t), 110.84 (d), 114.48 (d), 114.89 (s), 117.81 (d), 118.87 (s), 120.11 (d), 121.18 (d), 121.97 (s), 125.94 (d), 128.36 (d), 129.83 (s), 129.87 (s), 130.67 (d), 132.17 (s), 134.75 (d), 134.93 (d), 137.47 (s), 139.05 (s), 140.15 (s), 145.73 (s), 146.24 (s), 147.74 (s), 148.41 (s), 151.84 (s), 152.76 (s), 153.89 (s), 156.70 (s), 165.56 (s) ppm.

Bastadin-14 (215):

Obtained as a white solid; IR (film) v_max: 3200, 1660, 1519, 1498, 1454, 1426, 1282, 1249, 1023, 990, 821 cm⁻¹; ¹H NMR (CDCl₃/DMSO-d₆) see Table 15; ¹H NMR (400 MHz, DMSO-d₆) δ 11.92 (s), 11.70 (s), 10.02 (br s), 9.29 (s), 8.10 (t, J=6.8 Hz), 7.99 (t, J=5.6 Hz), 7.67 (s, 2H), 7.65 (s, 2H), 7.15 (d, J=1.8 Hz), 6.90 (d, J=8.4 Hz), 6.74 (dd, J=8.4, 1.8 Hz), 6.25 (d, J=1.8 Hz), 6.22 (d, J=1.8 Hz), 3.76 (s, 2H), 3.63 (s, 2H), 2.79 (t, J=5.3 Hz), 2.74 (t, J=6.9 Hz) ppm; EIMS for its tetramethylether (238) m/z (rel. int.): 1080 (1.0), 1078 (4.2), 1076 (7.5), 1074 (8.1), 1072 (3.8), 1070 (0.9), 1064...
(0.9), 1062 (1.7), 1060 (1.8), 1058 (0.9), 1049 (1.7), 1047 (5.2), 1045 (8.5), 1043 (7.2),
1041 (3.2), 1039 (0.7), 1019 (0.6), 1017 (1.5), 1015 (2.7), 1013 (2.9), 1011 (1.8), 1009
(0.7), 984 (2.1), 982 (6.0), 980 (8.2), 978 (5.1), 976 (1.6), 954 (1.5), 952 (3.5), 950
(4.9), 948 (3.6), 946 (1.3), 872 (0.7), 870 (1.1), 679 (0.4), 677 (0.3), 666 (0.4), 665
(0.3), 664 (0.4), 636 (0.9), 634 (1.2), 632 (0.9), 593 (2.4), 591 (6.6), 589 (6.5), 587
(2.5), 518 (17.2), 516 (48.3), 514 (49.3), 512 (17.4), 501 (6.9), 499 (10.5), 497 (10.1),
456 (6.8), 454 (12.8), 452 (7.3), 442 (9.7), 440 (19.1), 438 (16.2), 436 (15.0), 422
(11.7), 420 (20.9), 418 (11.6), 410 (18.7), 408 (12.3), 360 (14.4), 358 (14.9), 356
(18.5), 354 (18.1), 316 (29.9), 314 (27.9).

Bastadin-15 (216):

Obtained as a white solid; IR (film) \( \nu_{\text{max}} \): 3199, 1663, 1572, 1529, 1486, 1425,
1283, 1235, 1046, 1023, 989, 821 cm\(^{-1}\); \(^1\)H NMR see Table 15.
**IIIb. Xestospongin E From *Xestospongia* sp.**

**Isolation of xestospongins**

The sponge specimens (collection #: PNG-4-20-5-47) were collected on reefs off Madang, Papua New Guinea, in April 1986 and kept frozen until workup. The frozen sponge material (370 g) was defrosted in MeOH (1000 ml), homogenized in a Waring blender and extracted for two days. The crude MeOH extract was filtered and concentrated in vacuo to give an aqueous suspension which was diluted to 300 ml and partitioned against hexanes (300 ml x 3), methylene chloride (300 ml x 3) and ethyl acetate (300 ml x 3) in a separatory funnel. The methylene chloride soluble material was found to contain cytotoxic and antimicrobial compounds. The potent antimicrobial activity was used to guide the fractionation of this mixture. Repeated chromatographies on Sephadex LH-20 (1:1 MeOH/CH₂Cl₂; 2:1:1 hexane/CH₂Cl₂/MeOH) led to the isolation of crude xestospongion E as well as a mixture of previously described xestospongins. Crude xestospongion E was dissolved in 2N aqueous HCl and extracted with EtOAc to remove the non-alkaloidal impurities. The aqueous solution was basified with ammonium hydroxide and extracted with EtOAc to yield pure xestospongion E (238) (48 mg). Xestospongion B was isolated from the LH-20 fraction containing the previously described xestospongins by repeated chromatographies on silica gel (0-10% MeOH/CH₂Cl₂).

**Xestospongion E (238):**

Obtained as a white amorphous solid; IR (film) ν<sub>max</sub>: 3394, 2929, 2854, 1656, 1466, 1405, 1133, 1100, 1021 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 4.23 (br s), 3.63 (br t, J=9 Hz), 3.28 (br t, J=10 Hz), 3.12 (br t, J=9 Hz), 3.08 (dd, J=12, 4 Hz), 2.53 (br d, J=10 Hz), 1.98 (m), 1.83 (qd, J=12, 4 Hz), 1.7-1.1 (m, 16 resonances) ppm; ¹H NMR (400 MHz, acetone-d₆), see Table 16; ¹³C NMR (75 MHz, CDCl₃) δ 90.4 (d), 76.6 (d), 70.8 (s), 52.2 (t), 44.4 (t), 38.7 (t), 36.1 (t), 32.0 (t), 31.6 (t), 29.6 (t), 25.9 (t), 24.9 (t), 230
22.6 (t), 20.5 (t) ppm; EIMS m/z (relative intensity): 478 (100), 462 (69.7), 460 (67.4), 450 (18.5), 435 (15.9), 421 (20.3), 406 (30.6), 390 (12.5), 326 (35.0), 213 (49.9), 112 (40.1), 84 (41.1), 36 (95.2).
IIIc. Callydiyne From Callyspongia flammea

Collection and isolation:
Specimens of Callyspongia flammea were collected by hand using SCUBA on reefs off Madang, Papua New Guinea, in November 1988 and kept frozen until workup. Thawed sponge material (195 g) was homogenized in a Waring blender with MeOH (1000 ml) and extracted at r.t. for two days. The MeOH extract was filtered and concentrated in vacuo to yield an aqueous suspension which was diluted with water to 300 ml and partitioned against hexanes (300 ml × 3), methylene chloride (300 ml × 3) and ethyl acetate (300 ml × 3). The hexanes fraction was sequentially chromatographed on Sephadex LH-20 (eluent: 1:1 MeOH/CH₂Cl₂) and silica gel (vacuum flash column; eluent: hexane) to afford pure callydiyne (247) (41 mg).

Callydiyne (247):
Obtained as a colorless oil; IR (neat) ν\text{max}: 3302, 3022, 2926, 2855, 2097, 1698, 1616, 1463, 1441, 1216 cm⁻¹; \(^1\)H NMR, see Table 18; \(^{13}\)C NMR, see Table 18; EIMS m/z (relative intensity): 214 (0.9), 199 (1), 185 (20), 171 (7), 157 (11), 143 (31), 129 (64), 117 (82), 91 (100), 79 (77), 77 (58), 67 (59), 65 (66).
REFERENCES


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73. (a) P. J. Brogden, C. D. Gabbutt and J. D. Hepworth, in "Comprehensive Heterocyclic Chemistry", A. J. Boulton, A. McKillop, Eds.; Pergamon: Toronto,


90. For reviews, see (a) G. C. Barrett in "Techniques of Chemistry", Vol. IV, Chapter 8, Wiley-Interscience, New York; (b) P. M. Scopes, in "Progress in the Chemistry of Organic Natural Products", W. Herz, H. Grisebach, G. W. Kirby and Ch.


94. S. A. Morris, Ph. D. Dissertation, Department of Chemistry, University of British Columbia, Vancouver, Canada; 1990.


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106. Personal communication between R. W. M. Van Soest and M. LeBlonc of University of British Columbia.


