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Chemical and biological investigation of the Antarctic red alga delisea pulchra

Santhisree Nandiraju
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Chemical and Biological Investigation of the Antarctic Red Alga *Delisea pulchra*

by

Santhisree Nandiraju

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science Department of Chemistry College of Arts and Sciences University of South Florida

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Edward Turos, Ph.D.
Kirpal Bisht, Ph.D.

Date of Approval:
July 9th, 2004

Keywords: furanones, Pulchralide, dimer, secondary metabolites, antifouling

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DEDICATION

This thesis is dedicated to my beloved mother Mrs. Lakshmi Gouri, who motivated me to pursue this degree and accomplish my goals. I present this work as a token of appreciation and gratitude for all her efforts. I would also like to dedicate this thesis to my sisters for their encouragement and inspiration at all times.
ACKNOWLEDGMENTS

I wish to express my sincerest thanks to my adviser Dr. Bill J. Baker, for his wise counsel, viable guidance and constant encouragement and for ensuring the successful culmination of this thesis.

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I would like to acknowledge my committee members, Dr. Kirpal Bisht and Dr. Edward Turos for their encouragement and assistance. I am thankful to Dr. Baker’s students for their timely help during my research work. Last but not the least I wish to acknowledge my friends and roommates for the lighter moments, I have shared with them.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>[α]</td>
<td>specific rotation = 100α/lc</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>deuterated chloroform</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shifts</td>
</tr>
<tr>
<td>DEPT</td>
<td>distortionless enhancement by polarization transfer</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethylacetate</td>
</tr>
<tr>
<td>ε</td>
<td>the molar extinction coefficient in UV spectroscopy</td>
</tr>
<tr>
<td>gCOSY</td>
<td>gradient correlation spectroscopy</td>
</tr>
<tr>
<td>gHSQC</td>
<td>gradient heteronuclear single quantum correlation</td>
</tr>
<tr>
<td>gHMBC</td>
<td>gradient heteronuclear multiple bond connectivity</td>
</tr>
<tr>
<td>HREIMS</td>
<td>high resolution electron impact mass spectrometry or spectrum</td>
</tr>
<tr>
<td>HRESIMS</td>
<td>high resolution electrospray ionization mass spectrometry</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant</td>
</tr>
<tr>
<td>n_J_CH</td>
<td>n-bond hydrogen to carbon correlation (n = 2, 3 or 4)</td>
</tr>
<tr>
<td>n_J_HH</td>
<td>n-bond hydrogen to hydrogen correlation (n = 2, 3 or 4)</td>
</tr>
<tr>
<td>LREIMS</td>
<td>low resolution electron impact mass spectrometry or spectrum</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>LRESIMS</td>
<td>low resolution electrospray ionization mass spectrometry</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} )</td>
<td>the wavelength at which maximum absorption occurs</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>( m/z )</td>
<td>mass/charge for mass spectrometry</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
</tbody>
</table>
Our interest in the red alga *Delisea pulchra* (= *D. fimbriata*) (Greville) Montagne 1844 (Rhodophyceae, Bonnemaisoniales, Bonnemaisoniaceae) was stimulated by its activity in the bioassays done at Wyeth Pharmaceuticals. Halogenated compounds from *D. pulchra* interfere with Gram-negative bacterial signaling systems, affect the growth of Gram-positive bacteria, inhibit quorum sensing and swarming motility of marine bacteria (inhibit bacterial communication). They also inhibit surface colonization in marine bacteria and exhibit antifouling properties against barnacle larvae and macroalgal gametes.  

Chemical investigation of *D. pulchra* collected near Palmer Station, Antarctica yielded three new dimeric halogenated furanones, pulchralides A-C (41-43), along with previously reported fimbrolide (21), Acetoxyfimbrolide (22), hydroxyfimbrolide (23) and halogenated ketone 40. The reported Compounds were characterized by comparison of their $^1$H and $^{13}$C NMR data with that previously published. Pulchralide A-C were characterized by both 1D ($^1$H NMR, $^{13}$C NMR, DEPT, $^1$H-$^1$H COSY) and 2D (gHMQC, gHMBC) NMR techniques, supported by HREIMS/HRESIMS data. The absolute stereochemistry of Pulchralide A was determined by a single crystal X-ray analysis.
Significant antimicrobial activity was observed in acetoxyfimbrolide (22) and hydroxyfimbrolide (23), where as pulcharlide A (41) and fimbrolide (21) were weakly active.

References

Chapter 1. INTRODUCTION

1.1. Marine Natural Products

If indeed it is true that all life originated in the oceans, some organisms remained in the sea and others escaped onto the land. All have captured our imagination and now in a retro-evolutionary sense, chemists are turning their gaze back from land to their watery origins. This has become possible with the advent of scuba diving equipment and deep sea submersible collection facilities. The recent chromatographic techniques and the wide-spread use of high-field Nuclear Magnetic Resonance (NMR) spectrometers have meant that the complete structural elucidation is now possible on small amounts of material. Because of these developments chemists have all found excitement and adventure in the discovery of secrets of organisms hidden beneath the sea. The oceans cover more than 70% of the earth’s surface and the origin of all forms of life is supposed to have occurred in the sea. Thus it is fair enough to conclude that the biodiversity of the sea could be far greater than any other terrestrial ecosystem. Evidence coming from recent research on marine ecosystems like the deep sea floor and the coral reefs does affirm it suggesting that their biodiversity is much higher than that of tropical rain forests, a terrestrial ecosystem renown for its enormous biodiversity.
Marine natural products are of enormous importance to chemists and pharmacologists, who are keen to discover biologically active lead molecules. Marine natural products chemistry has evolved from being the source of a handful of chemical curiosities to one of the most productive areas of natural products research.\textsuperscript{1,4-9} The advent of sophisticated chromatographic techniques such as High Performance Liquid Chromatography (HPLC), Medium Pressure Liquid Chromatography (MPLC), Counter Current Chromatography (CCC) and gel filtration, high-field NMR techniques such as Heteronuclear Multiple Bond Correlation (HMBC), Heteronuclear Multiple Quantum Correlation (HMQC), Correlation Spectroscopy (COSY) and Nuclear Overhauser Enhancement Spectroscopy (NOESY) and mass spectrometric techniques coupled with X-ray crystallography have led to the structural elucidation of substantially more complex and diverse natural products. This has led to an enhanced interest in the isolation of biologically active natural products. Marine organisms have provided natural product chemists with a rich source of unusual secondary metabolites.\textsuperscript{10} It is the novelty and complexity of the compounds discovered from marine sources that assures the success of research in this area. There are many marine natural products that have no counterparts in the terrestrial world. Oceans are the reservoirs of many complex and unusual secondary metabolites. One of the most striking aspects of the field is its interdisciplinary nature; marine natural products chemists routinely collaborate with industrial pharmacologists, with marine biologists and ecologists whose interest and involvement is essential for meaningful progress in the field.
Many marine organisms are soft bodied and move slowly whereas some of them have a sedentary life style. Moreover, some of them do not have physical armaments like hard external shells or spicules. Such organisms need to defend themselves from predators, as they are vulnerable to predation and competition. Many marine invertebrates such as corals, sponges and ascidians (tunicates) use highly evolved chemical compounds for purposes such as reproduction, communication, and protection against predation, infection, and competition.\(^{11}\) These bioactive chemical compounds may have antibiotic, anti-inflammatory, antiviral, cytotoxic, antitumor or antifungal properties. Some of these marine organisms have the ability to synthesize their own defensive secondary metabolite chemicals via \textit{de novo} biosynthesis. Other organisms have been found to derive their defensive chemicals by a symbiotic relationship or simply from their dietary sources.\(^{12}\) With great biodiversity in the marine realm, marine organisms produce a wide array of secondary metabolites with broad structural diversity.\(^{12,13}\) Therefore, the likelihood of finding a bioactive molecule that would be a potential remedy to dreaded diseases like cancer or AIDS, from the marine realm is far greater. Interest on the part of chemists has been two fold: natural product chemists have probed marine organisms as a source of new and unusual molecules, while synthetic chemists have followed by targeting these novel structures for development of new analogues and new synthetic methodologies and strategies.\(^{14,15}\) More recent studies of marine organisms have focused on their potential applications, particularly to the treatment of human diseases and control of agricultural pests.\(^{16}\) The original literature that has amassed over the years has been reviewed topically by Scheuer.\(^{5-9,15,17-20}\) The task of tracking and cataloging the steady stream of
fascinating new structures has been done by John Faulkner in an annual survey. More natural products have been reported from sponges than from any other marine invertebrate phylum and many of the most promising pharmaceuticals and agents for cell biological research were isolated from sponges. The first isolation of a secondary metabolite from a marine organism was tyrian purple (6,6′-dibromoindigotin) (1) from a marine mollusk, identified in 1901. Tyrian purple was also the first marine natural product to be used for commercial applications.

![Tyrian purple (1)](image)

In the last few decades attention of many natural product drug discovery programs has been focused on the oceans and several drug candidates coming from different phyla of marine organisms have progressed into advanced phases of clinical trials. Metabolites of the phylum porifera account for almost 50% of the natural products reported from marine invertebrates. An unusual acetylinic fatty acid derivative taurospongin A (2) was isolated from an Okinawan sponge, *Hippospongia* sp. which inhibited both DNA polymerase β and HIV reverse transcriptase enzymes.

![Taurospongin A (2)](image)
Discodermalide (3), a polyketide isolated from marine sponge *Discodermia dissolute*, was found to be a potent antitumor agent.\(^{25}\) It induces tubulin polymerization similar to taxol. Structure-activity studies of discodermalide\(^{26}\) resulted in synthetic analogues and derivates, which showed greater promise and versatility than taxol, a current anticancer drug derived from Pacific yew tree *Taxus bravifolia*.\(^{27}\)

\[
\begin{align*}
\text{Discodermalide (3)}
\end{align*}
\]

Papuamine (4), an antifungal pentacyclic alkaloid was isolated from a thin, red encrusting sponge *Haliclona* sp. Papuamine is formally derivable from a C\(_{22}\) unbranched hydrocarbon and 1,3-diaminopropane and inhibits the growth of the fungus *Trichophyton mentagrophytes*.\(^{28}\)

\[
\begin{align*}
Papuamine (4)
\end{align*}
\]

Soft corals also provide an excellent source for bioactive marine natural products. Eleutherobin (5), a novel natural product isolated from an Indopacific marine soft coral is believed to be as potent as taxol.\(^{29}\) It inhibits microtubule depolymerization and thereby prevents division of cancer cells, which is the same mechanism of action as taxol.\(^{30}\)
Eleutherobin (5)

Marine prostaglandins, first discovered in a gorgonian\textsuperscript{31} have been isolated from other invertebrates and from red algae. Punaglandins,\textsuperscript{32} halogenated antitumor eicosanoids, were isolated from the octocoral \textit{Telesto riisei}. Punaglandin 3 (6) is characterized by C-12 oxygen and an unprecedented C-10 chlorine group and inhibits L1210 leukemia cell proliferation with an IC\textsubscript{50} value of 0.02 µg/mL\textsuperscript{32,33}.

Punaglandin 3 (6)

Ecteinascidins, isolated from the colonial ascidian \textit{Ecteinascidia turbinata} were not only cytotoxic but also found to be DNA interactive agents.\textsuperscript{34,35} Ecteinascidin 743 (7) was very selective against breast cancer and melanoma and has advanced into phase II clinical trials.\textsuperscript{36}
Of the alkaloid metabolites reported from tunicates, eudistomins are the most interesting from a chemical point of view. The oxathiazepine-bearing eudistomin C (8), has been isolated from a colonial tunicate *Eudistoma olivaceum*. The oxathiazepine-bearing eudistomins are potent antiviral agents.37

Dolastatin 10 (9) isolated from the sea hare *Dolabella auricularia*, is a short polypeptide containing unique amino acids and showing microtubulin stabilization properties.38 Dolastatins have also been isolated from a nudibranch and a cyanobacterium. Dolastatin analogues are in clinical trials.39
Isolated from the bryozoan Bugula neritina, bryostatin 1 (10) is one of the first drug candidates from the ocean to proceed into clinical trial stages.\textsuperscript{40} Bryostatin 1, a complex polyketide which inhibits protein kinase C and thereby prevents cancer, is in Phase II clinical trails.\textsuperscript{41}
1.2. Antarctic Ecology and Chemistry

A few decades ago it was conventional wisdom that competition and predation among the marine species are most intense in tropical waters\textsuperscript{12} and as a result the chemical ecology of the marine organisms dwelling in the tropical waters received more attention in drug discovery programs.\textsuperscript{12} However, recent research on the organisms of the Antarctic benthos suggests that they are indeed threatened by invertebrate predators and competitors such that they have evolved chemical defenses to ward them off.\textsuperscript{12} The physical environment on the Antarctic benthos has been stable for more than 20 million years\textsuperscript{42}, a period sufficient for the biologically accommodated ecosystem whereby predation and competition are dominant forces determining the species composition and distribution. This would have given ample time to facilitate the evolution of the biogenetic pathways leading to bioactive secondary metabolites.\textsuperscript{13} Also; the continent has been isolated from its lower latitude neighbors even longer. The factors such as physical stability and isolation are important for genetic divergence and this has resulted in high levels of endemism in Antarctica.\textsuperscript{13} An expedition undertaken to study the sea floor of McMurdo Sound Antarctica in 1980 revealed that the benthos under the ice is rich in marine life and is dominated by a dynamic community of sponges, soft corals, molluscs, tunicates and echinoderms.\textsuperscript{42}
Many of these organisms are immobile and cannot move to less densely populated regions if the area they live in becomes over-grown with competitors. Hence, it is apparent that either these sessile organisms do not have predators or that they have some kind of a defense. Studies on bottom-dwellers of Antarctica showed that they do have many predators. These include swarms of the voracious *Paramoera antractica*, a one-centimeter long crustacean resembling a shrimp, and dense populations of sea stars. It can be concluded that these organisms produce defensive chemicals to protect themselves from predators. Among chemical investigations done on Antarctic organisms to date, several bioactive molecules have been characterized. There are nearly 200 different secondary metabolites described from Antarctic organisms. Sponges are the dominant macro-invertebrates found on the Antarctic benthos. The sponge *Dendrilla membranosa* produces defensive chemicals membranolide (11) and 9,11-dihydrogracillin A (12) which showed mild activity against *Bacillus subtilus*.43,44

![Membranolide (11)](image1) ![9,10-dihydrogracillin (12)](image2)

The Antarctic green sponge *Latrunculia apicalis* from McMurdo Sound has been shown to elaborate a series of iminoquinone pigments called discorhabdins typified by discorhabdin C (13).44,45 In addition to significant feeding deterrence activity they are
antibiotic and cytotoxic.\textsuperscript{46} Variolin A (14), an unusual cytotoxic alkaloid, has been
isolated from a bright red sponge \textit{Kirkpartrickia variolosa}.\textsuperscript{47}

\begin{center}
\begin{tabular}{c}
\textbf{Discorhabdin C (13)} & \textbf{Variolin A (14)}
\end{tabular}
\end{center}

\textit{Leucetta leptomorphs}, the Antarctic rubber sponge, produces the acetogenin,
rhapsamine (15), which bears the unusual 1,3-diaminoglycerol group. In addition to
cytotoxicity, rhapsamine has antipredatory activity.\textsuperscript{48}

\begin{center}
\textbf{Rhapsamine (15)}
\end{center}

Sponges of McMurdo Sound Antarctica are subject to predation by sea stars, and it is
considered to be a dominant ecological factor that might drive the production of defense
chemicals. The bright yellow Antarctic sponge, \textit{Isodictiya erinacea}, which lacks physical
defenses (spicules and mucus) is one of the several chemically defended sponges in the
region. Erebusinone (16), a yellow pigment found in the sponge, showed molt inhibition
in crustaceans,\textsuperscript{49} a possible strategy of chemical defense.
Further investigation of *I. erinacea* showed the presence of purines and a nucleoside metabolite erinacin (17), which showed cytotoxicity.\(^{50}\)

\[
\begin{align*}
\text{Erebusinone (16)} & & \text{Erinacin (17)}
\end{align*}
\]

The pteropod *Cliona antarctica* is a shell-less pelagic mollusc which blooms in each austral summer in McMurdo Sound and has an intriguing relationship with the amphipod *Hyperiella dilatata*, where the amphipod positions the mollusc on its dorsum and defends itself from predatory fish utilizing the defense chemicals of the mollusc. A bioassay guided fractionation of the mollusc afforded the feeding deterrent pteroenone (18).\(^{51}\)

\[
\text{Pteroenone (18)}
\]

*Perknaster fuscus* is the major predator of sponges, regulating the abundance of the potentially space-dominating sponge *Mycale acerata*.\(^{42}\) Aqueous extracts from body wall tissues of *P. fuscus* showed cytotoxic activity in a fertilized echinoderm egg assay employing the Antarctic sea urchin *Sterechinus neumayeri*.\(^{52}\) The cytotoxicity likely
results from a novel tetrahydroisoquinoline alkaloid, fuscusine (19) which has been isolated from the tissues of body wall of *P. fuscus*.\textsuperscript{53}

![Fuscusine (19)](image)

The Antarctic nudibranch *Tritoniella belli* sequesters the secondary metabolite chimyl alcohol (20) from its diet, an Antarctic soft coral *Clavularia frankliniana*. Chimyl alcohol (20) was found to cause feeding inhibition in the omnivorous Antarctic sea star *Odontaster validus*.\textsuperscript{54}

![Chimyl alcohol (20)](image)

The benthos of Antarctica has many organisms that need to be investigated for their chemistry and bioactivity. In addition there are many parts of underwater Antarctic Peninsula that have never been explored. Considering the already evident significant variations in chemistry of the species that have been studied from the regions of McMurdo Sound and Palmer Station, a comprehensive chemical and bioactivity investigation of these species in various parts of Antarctica is of considerable interest.
Chapter 2. CHEMICAL INVESTIGATION OF THE ANTARCTIC RED ALGA

DELISEA PULCHRA

2.1. Introduction

Marine macroalgae have been a remarkable source of chemical diversity, responsible for roughly 20% of compounds reported from marine sources. Compounds from macroalgae are characteristic of their biological origin: red algae (Rhodophyceae) produce largely polyhalogenated monoterpenes, sesquiterpenes and acetogenins. Brown algae (Phaeophyceae) produce primarily diterpenes, but are also rich in phlorotannins and known for their prenylated quinones or hydroquinones, and green algae (Chlorophyceae) produce sesqui- and diterpenes and are known for their 1,4-dialdehydes. Marine red alga from the family Bonnemaisoniaceae have been shown to produce a wide range of halogenated metabolites, including butenones, pyranones, acetones, acrylic and acetic acids, octenones and from the genus, Delisea halogenated furanones. Delisea species are somewhat special when compared to many other algal species, in their apparent ability to stave off colonization by common epiphytes and also to be a food source which is generally not preferred by obligate herbivores.
Delisea pulchra (=D. fimbriata) (Figure 1) (Greville) Montagne 1844 (Rhodophyceae, Bonnemaisoniales, Bonnemaisoniacae) has been the focus of prior ecological and bioactivity studies.\textsuperscript{62} Halogenated compounds from \textit{D. pulchra} interfere with Gram-negative bacteria signaling systems, affect the growth of Gram-positive bacteria, inhibit quorum sensing and swarming motility of marine bacteria (inhibit bacterial communication). They also inhibit surface colonization in marine bacteria and exhibit antifouling properties against barnacle larvae and macroalgal gametes.\textsuperscript{63}

Figure 1. The Antarctic red alga \textit{Delisea pulchra} (photograph courtesy: \url{www.seaweed.ie}; © John Huisman).
A series of 2 (5H)-furanones, with structures 21 through 39 were isolated from *D. pulchra* collected over a wide geographic range.⁶⁴
2.2. Extraction and Isolation of Secondary Metabolites

The collection and extraction of *Delisea pulchra* from Palmer Station, Antarctica in 2001, yielded seven compounds, fimbrolide (21), acetoxyfimbrolide (22), hydroxyfimbrolide (23), halogenated ketone 40, pulchralide A (41), pulchralide B (42) and pulchralide C (43). *D. pulchra* (800 g wet) was extracted thrice with 1:1 dichloromethane/methanol to yield 3.5 g of lipophilic extract and then extracted thrice with 1:1 methanol/water to yield 18.8 g of hydrophilic extract (Scheme 1). The lipophilic extract showed a 12 mm zone of inhibition against two Gram-positive bacteria, methicillin-sensitive and -resistant *Staphylococcus aureus* (MRSA and MSSA respectively) and an 8 mm zone of inhibition against a fungus *Candida albicans*. The lipophilic extract was subjected to silica gel flash column chromatography to yield 5 fractions. Fimbrolide (10 mg, 0.0012%) was obtained from Normal Phase (NP) HPLC of fraction 1 (1:99 EtOAc/hexane), halogenated ketone 40 (40 mg, 0.005%) was obtained from NP HPLC of fraction 2 (5:95 EtoAc/Hexane), acetoxyfimbrolide (13 mg, 0.0016%) and two new compounds, pulchralide B (3 mg, 0.00037%) and C (2.5 mg, 0.0003%), were obtained from NP HPLC of fraction 3 (1:9, 12:88, 15:75 EtOAc/hexane). Another new compound, pulchralide A (10 mg, 0.0012%), and hydroxyfimbrolide (14 mg, 0.0017%) were obtained from the NP HPLC of fraction 5 (2:8 EtOAc/hexane).
Scheme 1. Isolation of fimbrolide (21), acetoxyfimbrolide (22), hydroxyfimbrolide (23), halogenated ketone 40, pulchralide A (41), pulchralide B (42) and pulchralide C (43).

**Delisea pulchra**
800 g (Frozen and wet)

1. Extracted with 3X 1:1 CH$_2$Cl$_2$:MeOH, 24 hr each
2. Combine supernatents, filter and concentrate in vacuo

- Lipophilic extract (3.5 g)
- Hydrophilic extract (18.8 g)

NP silica gel column
2.5 cm X 45 cm gradient elution from hexane to EtOAc

- Fraction 1 51.5 mg
- Fraction 2 50 mg
- Fraction 3 500 mg
- Fraction 4 384 mg
- Fraction 5 2.6 g

NP HPLC EtOAc/Hexanes (1:99)
NP HPLC EtOAc/Hexanes (5:95)
NP HPLC EtOAc/hexanes (10:95)
NP HPLC EtOAc/hexanes (15:85)

Fimbrolide (21)  Halogentated Ketone 40  Acetoxyfimbrolide (22)  Pulchralide B (42)  Pulchralide C (43)  Pulchralide A (41)  Hydroxyfimbrolide (23)
2.3. Characterization of Fimbrolide (21)

Compound 21 was the first fraction to elute from NP HPLC of fraction 1 (Scheme 1). Compound 21 was found to be identical with fimbrolide\textsuperscript{65} from the data discussed below. Fimbrolide (21) was obtained as colorless oil and was assigned a molecular formula of C\textsubscript{9}H\textsubscript{10}O\textsubscript{2}Br\textsubscript{2} deduced from LREIMS \textit{m/z} 312/310/308 (1:2:1). The \textsuperscript{1}H NMR spectrum (Figure 2) of fimbrolide in CDCl\textsubscript{3} showed signals at \(\delta\) 6.27 (1H, s) corresponding to an olefinic hydrogen and to an aliphatic chain at \(\delta\) 2.41 (2H, \textit{t}, \(J = 7.5\) Hz), 1.35 (4H, \textit{m}) and 0.93 (3H, \textit{t}, \(J = 6.5\) Hz).

Figure 2. \textsuperscript{1}H NMR spectrum of fimbrolide (21) (500 MHz, CDCl\textsubscript{3}).
The $^{13}$C NMR spectrum (Figure 3) of fimbrolide (21) showed signals corresponding to a lactone carbonyl at $\delta$ 166.3, a tetrasubstituted double bond at $\delta$ 150.2 and 134.1, a trisubstituted, enolic double bond at $\delta$ 130.0 and 91.1 and other signals corresponding to an aliphatic chain at $\delta$ 29.2, 25.2, 22.6 and 13.9. The infrared spectrum gave evidence for a carbonyl at 1787 cm$^{-1}$. The UV $\lambda_{\text{max}}$ of 292 nm (log $\varepsilon$ 4.92) suggested the presence of a conjugated ketone.

![Figure 3. $^{13}$C NMR spectrum of fimbrolide (21) (125 MHz, CDCl$_3$)](image)

The structure of fimbrolide (21) was established by comparing the $^1$H NMR, $^{13}$C NMR, IR, UV, mass spectra and optical rotation values with those of the previously reported compound$^{65}$. For fimbrolide (Figure 4), the reported chemical shifts of the olefinic hydrogen established the configuration of the exocyclic double bond as Z in our isolate.$^{65}$
Figure 4. $^1$H NMR chemical shift values of H-6 in Z and E isomers of fimbrolide (21).\textsuperscript{65}

Fimbrolide (21) was weakly active in antimicrobial assays, with 7 mm hazy zone of inhibition (all antimicrobial assays done at 200µ g/spot) MRSA and MSSA (no inhibition of other microbes tested).
2.4. Characterization of Acetoxyfimbrolide (22)

Compound 22 was eluted from the NP HPLC of fraction 3 as yellow oil (Scheme 1). Compound 22 was found to be identical with acetoxyfimbrolide\textsuperscript{66}, from the spectral and physical data discussed below. Acetoxyfimbrolide (22) was the major halogenated metabolite from our collections of \textit{Delisea pulchra}. LREIMS $m/z$ 370/368/366 (1:2:1) indicated a molecular formula of C$_{11}$H$_{12}$Br$_2$O$_4$. The $^1$H NMR spectrum of acetoxyfimbrolide (Figure 5), showed signals at $\delta$ 2.09 (3H, s) and $\delta$ 5.52 (1H, dd, $J = 7.2$, 7.0 Hz), indicative of an acetoxy group on an aliphatic chain.

![Figure 5. $^1$H NMR spectrum of acetoxyfimbrolide (22) (250 MHz, CDCl$_3$).](image)

22
The $^{13}$C NMR spectrum (Figure 6) of acetoxyfimbrolide (22) also indicated the presence of an acetate function ($\delta$ 170.0 and 20.4) and an $\alpha$-$\beta$-unsaturated $\gamma$-lactone ($\delta$ 163.6, 149.6 and 131.2). From the comparison of $^1$H NMR and $^{13}$C NMR spectra to published spectra$^{66}$ it can be concluded that acetoxyfimbrolide (Figure 7) differs from fimbrolide (21) only in the acetoxy substitution on the butyl chain.

![Figure 6. $^{13}$C NMR spectrum of acetoxyfimbrolide (22) (62.5 MHz, CDCl$_3$).](image)

The $^1$H NMR spectrum of acetoxyfimbrolide (22) showed the bromomethine ($\delta$ 6.38, 1H, s) at a higher field than ($E$)-acetoxyfimbrolide,$^{67}$ indicating that the exocylic double bond has a $Z$ configuration. The absolute configuration of acetoxyfimbrolide, previously reported from X-ray analysis as $1'R$ and $5Z$ was found to match with our isolate because of similar optical rotation value of $[\alpha]_{D}^{25} +15.0$ (c, 0.4 CHCl$_3$)$^{68}$
The IR spectrum of acetoxyfimbrolide (22) showed \( \nu_{\text{max}} \) of 1788 cm\(^{-1}\) (acetate carbonyl) and 1736 cm\(^{-1}\) (lactone carbonyl). Analysis of the UV spectrum, showed \( \lambda_{\text{max}} \) 291 nm (log \( \varepsilon \) 4.94) which is similar to the absorption properties of \( \alpha,\beta \)-unsaturated \( \gamma \)-lactones compounds.\(^{66}\)

From the Palmer Station collections of *Delisea pulchra* antimicrobial activity was greatest in acetoxyfimbrolide, which showed potent activity with 25 and 24 mm zones of inhibition against MRSA and MSSA respectively, a 16 mm zone toward vancomycin-resistant *Enterococci faecium* (VREF) and a 17 mm zone using *Candida albicans*. It was modestly active against permeabilized (a mutant strain with increased permeability to large molecular weight compounds) *Escherichia coli* (9 mm hazy zone).
2.5. Characterization of Hydroxyfimbrolide (23)

The most polar compound isolated in this study was compound 23, obtained from NP HPLC of fraction 5 (Scheme 1) as yellow oil. Compound 23 showed physical and spectral data similar to the reported hydroxyfimbrolide, which is discussed below. LREIMS of hydroxyfimbrolide (23) $m/z$ 328/326/ 324 (1:2:1) supported a molecular formula of $C_9H_{10}O_3Br_2$. The $^1$H and $^{13}$C NMR resonances of hydroxyfimbrolide were very similar to those of acetoxyfimbrolide (22), in particular with reference to the furanone ring and the butyl chain, indicating a closely related structure. The $^1$H NMR spectrum (Figure 8) of hydroxyfimbrolide showed a doublet at a $\delta$ 2.54 (1H, d, $J$ = 9.5 Hz) probably resulting from an unusual coupling of a hydroxyl proton ($D_2O$ exchangeable) to the hydroxy substituted proton. This suggests the presence of a hydroxyl substitution on the butyl chain in hydroxyfimbrolide compared to the acetoxy substitution in acetoxyfimbrolide. Comparison of the $^1$H and $^{13}$C NMR spectral (Figure 8 and 9) data set to that reported for hydroxyl fimbrolide (Figure 10) confirmed their identity. The IR spectrum showed the presence of a carbonyl group at 1736 nm$^{-1}$ ($\gamma$-lactone) and a broad hydroxyl group at 3457 nm$^{-1}$. The UV $\lambda_{\text{max}}$ of 292 nm (log $\varepsilon$ 4.90) demanded that the $\gamma$-lactone function was doubly conjugated.
Figure 8. $^1$H NMR spectrum of hydroxyfimbrolide (23) (250 MHz, CDCl$_3$).

Figure 9. $^{13}$C NMR spectrum of hydroxyfimbrolide (23) (125 MHz, CDCl$_3$).
The assignment of a $Z$ configuration of the double bond in hydroxyfimbrolide (23) was established by consideration of the chemical shift of H-6, which was upfield at $\delta$ 6.38 (1H, s). Hydroxyfimbrolide (23) was found to be $1'R$ and $5Z$ from its $[\alpha]_{25}^{25} +15.0$ ($c$, 0.4 CHCl$_3$), which matched the previously reported value.$^{68}$

![Figure 10. Hydroxyfimbrolide (23).](image)

Hydroxyfimbrolide displayed large zones of inhibition with Gram-positive bacteria (23 mm and 23 mm for MRSA and MSSA respectively), 14 mm for VREF and a 25 mm zone against *Candida albicans*. It was modestly active against *Escherichia coli* (9 mm hazy zone).
2.6. Characterization of Halogenated Ketone 40

NP HPLC of fraction 2 resulted in the isolation of compound 40 (40 mg, 0.005%) as a colorless oil. Compound 40 was found to be identical with the reported\textsuperscript{69} Halogenated ketone from the physical and spectral analysis, which is discussed below. Analysis of mass spectrum showed a molecular formula of C\textsubscript{8}H\textsubscript{11}Br\textsubscript{3}O deduced from LREIMS \textit{m/z} 304/306/308/310 (1:2:2:1). The $^1$H NMR spectrum (Figure 11) of halogenated ketone 40 showed a methylene triplet at $\delta$ 2.79 (2H, t, $J$ = 7.5 Hz), an aliphatic envelope at $\delta$ 1.70-1.33 (6H) and a methyl triplet at $\delta$ 0.94 (3H, t, $J$ = 7.0 Hz).

Figure 11. $^1$H NMR spectrum of halogenated ketone 40 (250 MHz, CDCl\textsubscript{3}).
A signal at δ 197.4 in the $^{13}$C NMR spectrum (Figure 12) of Halogenated ketone 40 has a ketone function and signals at δ 91.2 and 121.9 are indicative of a shielded, tetrasubstituted olefin. A pentyl chain is evident from $^{13}$C NMR resonances at δ 40.7, 31.1, 23.2, 22.4 and 13.9. The infrared spectrum supports the assignment of a carbonyl at 1715 cm$^{-1}$. The UV spectrum showed $\lambda_{\text{max}}$ of 220 nm (log $\varepsilon$ 4.84) and 283 nm (log $\varepsilon$ 4.03).

Figure 12. $^{13}$C NMR spectrum of halogenated ketone 40 (62.5 MHz, CDCl$_3$).
Halogenated ketone 40 (Figure 13) was identified by the matching of physical and spectral data set with those of the previously reported compound.\textsuperscript{69}

Figure 13. Halogenated ketone 40.
2.7. Characterization of Pulchralide A (41)

A second compound, 41, obtained from the polar fraction 5 (Scheme 1) was found to be a new compound from its structural elucidation discussed below. Compound 41 was named after the species name of the red alga *Delisea pulchra* as Pulchralide A. Pulchralide A (41), molecular formula of C_{22}H_{24}Br_{4}O_{8} (HREIMS m/z 734.8260. requires m/z 734.8262), was crystallized from ethanol as colorless needles. Pulchralide A displayed the characteristic aliphatic $^1$H NMR signals (Figure 14) similar to those observed in acetoxyfimbrolide, including the olefinic hydrogen at $\delta$ 5.18 (1H, s), an acetoxy substituted methine at $\delta$ 5.52 (dd, 1H, $J = 6.5, 8.0$ Hz), an acetate methyl at $\delta$ 2.11 (3H, s), signals corresponding to the butyl chain at $\delta$ 1.91, 1.36 and 0.96 (3H, t, $J = 7.5$ Hz). But the olefinic proton is at higher field than in pulchralide A than in acetoxyfimbrolide, where it resonates at $\delta$ 6.38.

The $^{13}$C NMR spectrum (Figure 15) of pulchralide A (41) significantly differed from acetoxyfimbrolide (22). In particular, only two $^{13}$C signals were observed in the olefinic region of pulchralide A, unlike four olefinic signals found in acetoxyfimbrolide ($\delta$ 149.6, 131.2, 130.4, 93.3). It lacked the trisubstituted enolic double bond signals of acetoxyfimbrolide at $\delta$ 130.4. The heteroatom-bearing region suggested there were three heteroatom-bearing sp$^3$ carbons. The complete $^1$H NMR and $^{13}$C NMR assignments can be found in Table 1.
Figure 11. $^1$H NMR spectrum of pulchralide A (41) (500 MHz, CDCl$_3$).

Figure 15. $^{13}$C NMR spectrum of pulchralide A (41) (125 MHz, CDCl$_3$).
The 135° Distortionless Enhancement by Polarization Transfer (DEPT-135) spectrum of pulchralide A (41) (Figure 16) showed a methine at $\delta 39.5$ suggesting the absence of an exocyclic double bond. It also showed the presence of the acetoxy bearing carbon at $\delta 64.6$ as a methine, two methylenes at $\delta 33.8$ and 16.7 and two methyl groups at $\delta 20.9$ and 14.7.

![Figure16. DEPT-135 spectrum of pulchralide A (41) (125 MHz, CDCl₃).](image)

The planar structure was established by 2D NMR techniques. In the $^1$H-$^1$H Correlation Spectroscopy (COSY) spectrum (Figure 17) of pulchralide A (41), cross peaks were observed between H-1' and H-2', H-2' and H-3' and between H-3' and H-4' (Figure 18). Gradient Heteronuclear Multiple Quantum Correlation (gHMQC) spectrum (Figure 19) of pulchralide A showed the correlations of acetate substituted methine proton at $\delta 5.5$ with C-1' ($\delta 68.5$) which suggested the substitution of acetoxy group at C-1' on the butyl
side chain. Cross peaks were also observed between H-6 ($\delta 5.18$) and C-6 ($\delta 43.9$), as well as the acetate methyl Me-8 ($\delta 2.11$) and C-8 ($\delta 20.8$).

Figure 17. gCOSY spectrum of pulchralide A (41) (500 MHz, CDCl$_3$).

Figure 18. $^1$H-$^1$H COSY correlations of pulchralide A (41).
Figure 19. gHMQC spectrum of pulchralide A (41) (500MHz, CDCl₃).

The significant correlations observed in gradient heteronuclear multiple bond correlation spectrum (gHMBC) spectrum (Figure 20) of pulchralide A (41) were between H-1' (δ 5.5) and C-2 to C-4 and C-8 of the lactone ring (Table 1). Correlations were also seen for H-6 to C-5 and C-6. Because gHMBC spectra show only two and three bond correlations ($2J_H$, $3J_H$, correlations), the correlation of H-6 to the C-6 (Figure 21) was the first clue that the metabolite was a dimer. The hypothesis that pulchralide A is a dimer was supported by mass spectral analysis which provided a molecular formula of C$_{22}$H$_{24}$Br$_4$O$_8$ (m/z 732/734/736/738 (1:4:6:4:1), HREIMS 734.8260, C$_{22}$H$_{24}$O$_8^79$Br$_3^{81}$Br requires 734.8262). Thus pulchralide A appears to be a dimer of acetoxyfimbrolide (22) and has not been previously reported.
Figure 20. gHMBC spectrum of pulchralide A (41) (500 MHz, CDCl₃).

Figure 21. KEY HMBC correlations determined in pulchralide A (41).
Table 1. NMR data of pulchralide A (41) (CDCl$_3$ ($^{13}$C, 125 MHz; $^1$H, 500 MHz).

<table>
<thead>
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<th>Position</th>
<th>$^1$H (δ)</th>
<th>$^{13}$C (δ)</th>
<th>gHMBC</th>
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<td>164.9</td>
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<tr>
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<td>3/3a</td>
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The crystallization of the pulchralide A (41) has led to the determination of the absolute stereochemistry of pulchralide A by a single crystal X-ray analysis performed by Dr. Mike Zaworotko at University of South Florida, Tampa. (Figure 22 and 23).

Figure 22. Perspective view of X-ray crystal structure of pulchralide A (41).
To ensure that the pulchralide A (41) is not an artifact, especially of photochemical origin, acetoxyfimbrolide (22) (1 mg), dissolved in chloroform was irradiated with UV and visible light for 24 hrs each. $^1$H NMR spectra of the irradiated acetoxyfimbrolide did not show signs of dimer formation, supporting their biotic origin. Pulchralide A was modestly active in antimicrobial assays against MRSA and MSSA with 7 mm hazy zones of inhibition respectively.
2.8. Characterization of Pulchralide B (42)

Compound 42, obtained from the NP HPLC of fraction 3 (Scheme1) as white solid, was characterized as discussed below and found to be a new compound, which was named as pulchralide B. Pulchralide B (42) demonstrated many of the structural features of pulchralide A (41). The $^1$H NMR spectrum (Figure 24) of pulchralide B lacked the acetoxymethine of pulchralide A, and a new allylic methylene signal was found at $\delta$ 2.43 (3H, t, $J = 7.5$ Hz), analogous to the allylic H-1’ of fimbrolide (21). The $^{13}$C NMR spectrum (Figure 25) of pulchralide B showed only two signals in the olefinic region, similar to pulchralide A, but did not show any signals indicative of an acetoxy group.

![Figure 24. $^1$H NMR spectrum of pulchralide B (42) (500 MHz CDCl$_3$).](image)
The $^{13}$C NMR spectrum (Figure 25) of pulchralide B showed only two signals in the olefinic region, similar to pulchralide A, but did not show any signals indicative of an acetoxy group. The DEPT-135 spectrum (Figure 26) of pulchralide B showed one methine carbon at $\delta$ 44.1, one methyl of the butyl side chain at $\delta$ 13.9 and three methylenes at $\delta$ 29.0, 25.5, and 22.4.

Figure 25. $^{13}$C NMR spectrum of pulchralide B (42) (125 MHz, CDCl$_3$).
Figure 26. DEPT-135 spectrum of pulchralide B (42) (125 MHz CDCl₃).

The planar structure of pulchralide B (42) was deduced by interpretation of 2D NMR spectra. The gHMBC spectrum (Figure 27) of pulchralide B showed correlations of H-6 with C-6 (Figure 28), similar to that in pulchralide A (41) revealing that pulchralide B is also a dimer. The significant correlations (Table 2) observed in pulchralide B are the correlation of H-1' with C-3 indicating the attachment of the butyl side chain to the furanone ring. It also showed cross peaks between H-6 and C-5. Pulchralide B therefore is the symmetrical fimbrolide dimer, an assignment which is fully supported by the COSY and HMBC data sets and confirmed by mass spectral data, (m/z
\(\text{C}_{18}\text{H}_{20}\text{O}_4^{79}\text{Br}_{281}\text{Br}\) requires 538.8891).

Figure 27. gHMBC spectrum of pulchralide B (42) (500 MHz, CDCl\(_3\)).

Figure 28. Key HMBC correlations of pulchralide B (42).
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<th>Position</th>
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<th>$^{13}$C (δ)</th>
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<td>3'/3'a</td>
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Of the two possibilities that pulchralide B (42) is either a C$_2$ dimer or meso dimer (Figure 29), the possibility that it is a meso dimer was ruled out because of its optical rotation of $[\alpha]_{D}^{25} +4.26^\circ$ (c 0.06, CHCl$_3$). Hence pulchralide B is a C$_2$ dimer.

![Figure 29. Pulchralide B C$_2$ dimer and pulchralide B meso dimer.](image)
2.9. Characterization of pulchralide C (43)

Compound 43, was the third compound, to be eluted from NP HPLC of fraction 3 (Scheme 1), whose structural determination as discussed below led to a new compound, named as pulchralide C. Pulchralide C (43), was obtained as white solid and was found to have molecular formula of C_{20}H_{22}O_{6}{^{79}Br}{^{81}Br}_2 (TOFMS m/z 700.8008, requires 700.8007). The $^1$H NMR spectrum (Figure 30) of pulchralide A has signals related to both acetoxylimbrolide (22) and fimbrolide (21). A single acetoxyl methyl group at $\delta$ 2.09 (3H, s) and an acetate substituted proton at $\delta$ 5.49 (1H, dd, $J$ = 6.5, 8.0 Hz), evident in the $^1$H NMR spectrum (Figure 30) confirm the resemblance to acetoxylimbrolide while the signal at $\delta$ 2.38 (1H, t, $J$ = 7.5 Hz) is reminiscent of fimbrolide. The doublets at $\delta$ 5.38 (1H, d, $J$ = 10.0 Hz) and at 5.13 (1H, d, $J$ = 10.0 Hz) are indicative of the presence of olefinic bromo-methines. The $^{13}$C NMR spectrum (Figure 31) of pulchralide C also showed peaks related to acetate group ($\delta$ 170.3 and $\delta$ 20.6), peaks characteristic of two lactone carbonyls (167.0, 165.3) and one acetoxyl substitution at $\delta$ 68.8. The $^1$H and $^{13}$C NMR assignments are illustrated in Table 3.
Figure 30. $^1$H NMR spectrum of pulchralide C (43) (500 MHz, CDCl$_3$).

Figure 31. $^{13}$C NMR spectrum of pulchralide C (43) (125 MHz CDCl$_3$).
The planar structure of pulchralide C (43) was established based on analysis of 2D NMR data. Analysis of the $^1$H-$^1$H COSY spectrum (Figure 32) of pulchralide C (43) illustrated two bromomethine doublets, H-6 and H-6a, at $\delta$ 5.35 and 5.13 respectively, coupled with one another. This significant $^1$H-$^1$H COSY correlation (Figure 33) supports the evidence that pulchralide C is an unsymmetrical dimer. The gHMBC spectrum established a two bond correlation between H-6 and C-6/C-6a supporting the assumption that pulchralide C is an unsymmetrical dimer.

Figure 32. $^1$H-$^1$H COSY spectrum of pulchralide C (43) (500 MHz, CDCl$_3$).
Figure 33. Key $^1$H-$^1$H COSY correlation of pulchralide C (43).

<table>
<thead>
<tr>
<th>Position</th>
<th>$^1$H (δ)</th>
<th>$^{13}$C (δ)</th>
<th>gHMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1'</td>
<td>5.49 (1H, dd, $J = 6.5, 8.0$ Hz)</td>
<td>68.8</td>
<td>C-2, C-3, C-7</td>
</tr>
<tr>
<td>6</td>
<td>5.38 (1H, d, $J = 10.0$ Hz)</td>
<td>47.7</td>
<td>C-5, C-6a</td>
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<tr>
<td>6a</td>
<td>5.13 (1H, d, $J = 10.0$ Hz)</td>
<td>44.4</td>
<td>C-5a, C-6</td>
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<tr>
<td>1'a</td>
<td>2.38 (1H, t, $J = 7.5$ Hz)</td>
<td>33.4</td>
<td>C-3, C-2'a, C-3'a</td>
</tr>
<tr>
<td>8</td>
<td>2.09 (3H, s)</td>
<td>170.3</td>
<td>C-1'</td>
</tr>
<tr>
<td>2'</td>
<td>1.96 (2H, m)</td>
<td>28.9</td>
<td>C-1a, C-3a, C-3</td>
</tr>
<tr>
<td>2'a</td>
<td>1.81 (2H, m)</td>
<td>25.4</td>
<td>C-1’a, C-3’a, C-3a</td>
</tr>
<tr>
<td>3'</td>
<td>1.34 (2H, m)</td>
<td>22.5</td>
<td>C-2a, C-4a, C-1a</td>
</tr>
<tr>
<td>3'a</td>
<td>1.32 (2H, m)</td>
<td>18.7</td>
<td>C-2’a, C-4’a, C-1’a</td>
</tr>
<tr>
<td>4' &amp; 4'a</td>
<td>0.95 (6H, t, $J = 7.0$ Hz)</td>
<td>13.8</td>
<td>C-3a/3’a, C-2a/2’a</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>167.0</td>
<td></td>
</tr>
<tr>
<td>2a</td>
<td>-</td>
<td>165.3</td>
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<tr>
<td>4</td>
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<td>140.9</td>
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<td>4a</td>
<td>-</td>
<td>139.6</td>
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<tr>
<td>3</td>
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<td>135.5</td>
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<tr>
<td>3a</td>
<td>-</td>
<td>134.5</td>
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<tr>
<td>5a</td>
<td>-</td>
<td>88.1</td>
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</tr>
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</table>

The bromomethine protons couple with each other to form doublets with $J = 10$ Hz (δ 5.35) and $J = 10.0$ Hz (δ 5.13). The *trans* coupling constants for protons of a four membered ring like cyclobutane is 2-10. This indicates that these two are *trans* to each
other Figure 34 illustrates the stereochemical assignments of pulchralide C (43) based on the above considerations.

![Figure 34. stereochemical assignments of pulchralide C (43).](image-url)
Chapter 3. DISCUSSION

3.1. Biological Importance of Halogenated Metabolites of Delisea pulchra

Our interest in *Delisea pulchra* is due to the antimicrobial and antifungal activity of the organism we observed in the bioassay of the crude extract performed by Wyeth Pharmaceuticals, Pearl River, NY. Chemical investigation of *D. pulchra* resulted in the isolation of three new dimeric halogenated furanones pulchralide A-C (41-43) along with the previously reported fimbrolide (21), acetoxyfimbrolide (22), hydroxyfimbrolide (23) and halogenated ketone 40. The structures of the new compounds were determined by 1D, 2D NMR and mass spectral data. The absolute stereochemistry of pulchralide A (41) was established by single crystal X-ray crystallography. The structures of known compounds fimbrolide, acetoxy-fimbrolide, hydroxyfimbrolide and halogenated ketone 40 were identified by comparing the physical and spectral data with those reported previously. The absolute stereochemistry of these compounds was already reported on the basis of chemical interconversions and X-ray and CD analyses. Fimbrolide, acetoxyfimbrolide, hydroxyfimbrolide were found have a Z configuration based on the chemical shifts of their respective hydrogen atoms.
The bioassay data (Table 4) indicates that acetoxyfimbrolide (22) and hydroxyfimbrolide (23) have potent antimicrobial activity against two Gram-positive bacteria, MRSA and MSSA and against one Gram-negative bacterium, VREF and mild activity against the fungus *Candida albicans*. Acetoxyfimbrolide and hydroxyfimbrolide showed modest activity against permeablized *Escherichia coli*.

<table>
<thead>
<tr>
<th></th>
<th>MSSA</th>
<th>MRSA</th>
<th>VREF</th>
<th>Permeablized <em>Escherichia coli</em></th>
<th><em>Candida albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetoxyfimbrolide (22)</td>
<td>25</td>
<td>24</td>
<td>16</td>
<td>9:10H</td>
<td>17</td>
</tr>
<tr>
<td>Hydroxyfimbrolide (23)</td>
<td>23</td>
<td>23</td>
<td>14</td>
<td>9H</td>
<td>25</td>
</tr>
<tr>
<td>Fimbrolide (21)</td>
<td>7H</td>
<td>7H</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pulchralide A (41)</td>
<td>7H</td>
<td>6:7H</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
3.2. Origin of Halogenated Metabolites of *Delisea pulchra*

While several dimeric furanones have been described from the genus *Delisea*, two of which bear the central cyclobutane of the pulchralides, pulchralides A and C (41 and 43) are the first such dimers bearing the C-1’ acetoxy group. Pulchralides B (42) and C demonstrated many of the structural features of pulchralide A and C and pulchralide C is the only unsymmetrical member of the group. The cyclobutane dimers, pulchralide A could be derived from acetoxyfimbrolide (22), pulchralide B from hydroxyfimbrolide (23) and pulchralide C from both acetoxy-fimbrolide (22) and fimbrolide (21) by $[\Pi_2^s+\Pi_2^s]$ cycloaddition reactions.\(^{62}\) Molecules which are formed by enzyme mediated reactions are almost invariably optically active.\(^{70}\) Examples of cyclobutane containing dimers are known.\(^{70}\) To ensure that the dimers described here were not artifacts, especially of photochemical origin, the solution of acetoxyfimbrolide was exposed to both UV and visible light for 24 hrs each with no discernable effects on the compound.
3.3. Ecological Importance of Halogenated Furanones of *Delisea pulchra*

Halogenated furanones from *Delisea pulchra* have various ecological and biological functions. The red macroalga (seaweed) *D. pulchra* is relatively free of surface colonization and the knowledge that it produced unusual secondary metabolites, halogenated furanones or fimbrolides prompted an in-depth investigation of the role of these metabolites as inhibitors of surface colonization. These compounds were encapsulated in vesicles in gland cells in the seaweed, providing a mechanism for delivery of the metabolites to the surface of the alga at concentrations which deter a wide range of prokaryote and eukaryote fouling organisms. Inhibition in these studies was by a non-toxic and non-growth inhibitory mechanism and the structural similarities between these algal metabolites and the bacterial signaling molecules, acylated homoserine lactones (AHL’s), led to the hypothesis that furanones act as specific antagonists of AHL regulatory systems. Furanones inhibit AHL-regulated phenotypes in a wide range of Gram-negative bacteria and their specific mode of action has now been demonstrated via both AHL phenotypic bioassays and targeted molecular systems. For example, furanones shut down AHL-regulated swarming in *Serratia liquefaciens*, biofilm development in *Pseudomonas aeruginosa* and bioluminescence in both wild-type *Vibrio fischeri* and luminescence constructs (e.g. in *Escherichia coli* backgrounds). The use of natural products such as furanones, which target specific bacterial signaling and regulatory systems, represents a promising approach to inhibition of biofilm.
Barnacles cause corrosion and make ships heavier and harder to steer. Antifouling paints that contain tin or copper stop barnacles from attaching and leach metals into the sea and kill many non-target organisms. Antifouling furanones made by *Delisea pulchra* blocks bacterial communication systems and prevents bacterial biofilms from developing on its surface. This then stops barnacles from attaching. The seaweed metabolite may be used to replace the toxic chemicals in any environment that can be submerged. Research is now focused on further development of furanone based paints. The geographic variation of halogenated metabolites of *D. pulchra* has also been the subject of study. The large variability in concentrations of furanones, and the absence of any positive relationships between furanones, herbivores and epiphytes, suggest that quantitative variation in furanones in *D. pulchra* is not driven by population-level selection or induction, but is more likely to be a result of small-scale variation in environmental factors such as nutrients, light and genetic differences among individual plants.
Chapter 4. EXPERIMENTAL


Optical rotation was determined on a Rudolph Research Analytical AUTOPOL® IV with a sodium lamp (589 nm) and 0.5 dm cell at 25°C. Infrared spectra were obtained with Nicolet Avatar 320FT-IR in solid state. Ultraviolet-Visible experiments were measured on a Hewlett-Packard 8452A diode array UV/Vis spectrometer. $^1$H and $^{13}$C NMR, HMQC, HMBC and $^1$H-1H COSY spectra were obtained on either a Varian 500 instrument operating at 500 MHz for $^1$H NMR and 125 MHz for $^{13}$C, or a Bruker Avance 250 instrument operating at 250 MHz for $^1$H and 62.5 MHz for $^{13}$C. The $^{13}$C resonance multiplicities were determined by DEPT experiments. $^1$H-$^1$H correlations were determined by using gCOSY experiment optimized coupling constant ($J_{HH}$ of 7 Hz). One bond connectivities ($^1J_{CH}$) of $^1$H-$^{13}$C were determined via the 2D proton-detected gHSQC experiment. The interpulse delays were optimized for average $^1J_{CH}$ of 120 Hz. Two- or three-bond heteronuclear multiple-bonds ($^2J_{CH}/^3J_{CH}$) were recorded via the 2D proton detected gHMBC experiment optimized for a long range coupling constant ($J_{CH}$ of 7 Hz). High resolution mass spectra ESIMS, EI-MS and CIMS were obtained on Micro mass 70-VSE spectrometer at the University of Illinois. HPLC was performed on Waters 510 equipped with a Waters 490E programmable multiwavelength UV detector at 254 nm and
Shimadzu LC-8A equipped with a multisolvent delivery system connected to a Shimadzu SPD-10A UV-VIS tunable absorbance detector and/or an Alltech ELSD 2000 using a YMC-Pack® ODS-AQ C-18 analytical column, a Waters prepLC® (25 mm X 30 cm) C-18 column for reversed phase, or Waters Sphereclone® (250 X 10 mm) for normal phase. Flash column chromatography was performed on EM Science normal phase Silica gel 60 (200 – 400 mesh). Thin layer chromatography (TLC) was carried out using Whatman normal phase silica gel 60Å Partisil® K6F, reversed phase silica gel 60Å Partisil® KC18F, and CNF254s plates 0.25 mm thickness. There were visualized by spraying with 5% phosphomolybdic acid in EtOH or heating and 2 % ninhydrin in BuOH/acetic acid (95:5).

**X-ray Crystal Structure Determination:** Single crystals suitable for x-ray crystallographic analysis were selected following examination under a microscope. Single-crystal x-ray diffraction data for the compounds were collected on a Bruker-AXS SMART APEX/CCD diffractometer using MoKα radiation (λ = 0.7107 Å). Diffracted data were corrected for Lorentz and polarization effects and for absorption using the SADABS73 program. The structure was solved by direct methods and the structure solution and refinement was based on |F|^2. All non-hydrogen atoms were refined with anisotropic displacement parameters whereas hydrogen atoms were placed in calculated positions when possible and given isotropic U values 1.2 times that of the atom to which they are bonded. All crystallographic calculations were conducted with the SHELXTL v.6.174 program package.75
4.2. Bioassay of Pure Compounds

Bioassays for the pure compounds were done at Wyeth Pharmaceuticals, Ford Hospital and in the Marine Natural Products Lab at University of South Florida, using standard disk diffusion method against two Gram-positive bacteria, Methicillin- resistant and -sensitive *Staphylococcus aureus* (MRSA and MSSA respectively) against two Gram-negative bacteria Vancomycin resistant *Escherichia faecium* (VREF) and permeabilized *Escherichia faecium* and against one fungus *Candida albicans*.

**Antibiotic assays:** *In vitro* antimicrobial activities against methicillin-sensitive (MSSA, strain 375) and -resistant (MRSA, strain 310) *Staphylococcus aureus*, vancomycin-resistant *Enterococci faecium* (VREF, strain 379), *E. coli* (strain 442), *E. coli imp* (strain 389, a mutant strain with increased permeability to large molecular weight compounds) and *Candida albicans* (strain 54) were determined by agar diffusion method. Media used were Difco nutrient agar (pH 6.8) for *S. aureus*, LB (Luria-Bertani) agar for *E. faecium* and *E. coli*, and YM agar for *C. albicans*. Assay plates (9”x 9” Sumilon) were prepared by pouring 125 ml volume of agar medium (tempered at 50°C) inoculated with an overnight broth culture of the test organisms (adjusted to approximately 10^6 cells per ml). Sample concentrations of 200 µg in 10 µL aliquots were spotted onto agar surface and the plates were incubated at 37°C for 18h.75
The zones of growth inhibition were measured using a hand-held digital caliper. The zones of growth of inhibition were measured from the edge of the disk to the edge of the clear inhibition zone in mm, respectively. Control disks were treated with solvent alone (MeOH or CHCl₃). The bioassay data for the pure compounds done at Wyeth Pharmaceuticals and at Marine natural products lab at University of South Florida can be found in Table 3.
4.3. Isolation of Secondary Metabolites from Delisea pulchra

**Plant Material.** The algal material was collected at Palmer Station, Antarctica by scuba diving in November – December of 2001. Identifications were made by C. Amsler and K.B. Iken. A voucher specimen is available at the University of Alabama at Birmingham. The red alga *Delisea pulchra* is a reddish brown alga.

The red alga *D. pulchra* (800 g wet) was extracted thrice with 1:1 dichloromethane/methanol to yield 3.5 g of lipophilic, and then extracted thrice with 1:1 methanol/water to yield 18.8 g of hydrophilic extract. The lipophilic extract was subjected to silica gel flash column chromatography to yield 5 fractions. Fimbrolide (21) (10 mg, 0.0012%) was obtained from NP HPLC of fraction 1 (1:99 EtOAc/hexane), halogenated ketone 40 (40 mg, 0.005%) was obtained from NP HPLC of fraction 2 (5:95 EtoAc/Hexane), acetoxyfimbrolide (22) (13 mg, 0.0016%) and two new compounds, pulchralide B (42) (3 mg, 0.00037%) and C (43) (2.5 mg, 0.0003%), were obtained from NP HPLC of fraction 3 (1:9, 12:88, 15:75 EtOAc/hexane). Another new compound, pulchralide A (41) (10 mg, 0.0012%), and hydroxyfimbrolide (23) (14 mg, 0.0017%) were obtained from the NP HPLC of fraction 5 (2:8 EtOAc/hexane).
4.3.1. Spectral Data for Fimbrolide (21)

**Fimbrolide (21):** 10 mg, 0.0012%, colorless oil; \([\alpha]^{25}_D\) +4.5 (c, 0.9 CHCl₃); IR \(\nu_{\text{max}}\) 1787, 1213, 1042 cm\(^{-1}\); UV \(\lambda_{\text{max}}\) 292 nm (log \(\varepsilon\) 4.92); \(^1\)H NMR (250 MHz, CDCl₃) \(\delta\) integration, multiplicity, \(J\) (Hz), assignment): 6.27 (1H, s, H-6), 2.41 (2H, t, 7.5, H-1’), 1.35 (4H, m, H-2’, H-3’), 0.93 (3H, t, 6.5, H-4’); \(^{13}\)C NMR (125 MHz, CDCl₃) \(\delta\) (assignment) 166.3 (C, C-2), 150.2 (C, C-4), 134.1 (C, C-3), 130.0 (C, C-5), 91.1 (C, C-6), 29.2 (CH, C-1’), 25.2 (CH₂, C-2’), 22.6 (CH₂, C-3’), 13.9 (CH₃, C-4’); LREIMS \(m/z\) 312/310/308 (1:2:1) for C₉H₁₀O₂Br₂.
4.3.2. Spectral Data for Acetoxy Fimbrolide (22)

![Chemical Structure of Acetoxy Fimbrolide (22)](image)

**Acetoxyfimbrolide (22):** 13 mg, 0.0016%, yellow oil; $[\alpha]^{25}_D +47.0$ (c, 1.5, CHCl$_3$); IR $\nu_{\text{max}}$ 1788, 1736, 1236, 1092 cm$^{-1}$; UV $\lambda_{\text{max}}$ 291 nm ($\log \varepsilon$ 4.94); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ integration, multiplicity, $J$ (Hz), assignment: 6.38 (1H, s, H-6), 5.52 (1H, t, 7.2, H-1’), 2.09 (3H, s, H-8), 1.83 (2H, m, H-2’), 1.35 (2H, m, H-3’) 0.94 (3H, t, 7.5, H-4’); The $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (multiplicity, assignment) 170.0 (C, C-7), 163.6 (C, C-2), 149.6 (C, C-4), 131.2 (C, C-3), 130.0 (C, C-5), 93.3 (C, C-6), 68.0 (CH, C-1’), 33.5 (CH$_2$, C-2’), 20.4 (CH$_3$, C-8), 18.3 (CH$_2$, C-3’), 13.5 (CH$_3$, C-4’); LREIMS $m/z$ 370/368/366 (1:2:1) for C$_{11}$H$_{12}$Br$_2$O$_4$. 
4.3.3. Spectral Data for Hydroxyfimbrolide (23)

**Hydroxyfimbrolide (23):** 14 mg, 0.0017%, yellow oil; $[\alpha]^{25}_D +15.0$ (c, 0.4 CHCl$_3$); IR $\nu_{\text{max}}$ 3457, 2959, 1736 cm$^{-1}$; UV $\lambda_{\text{max}}$ 292nm (log 4.90); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ integration, multiplicity, $J$ (Hz), assignment): 6.38 (1H, s, H-6), 4.58 (1H, dd, 6.7, 7.0, H-1’), 2.54 (1H, d, 9.5, OH), 1.84 (2H, m, H-2’), 1.42 (2H, m, H-3’), 0.96 (3H, t, 7.5, H-4’). The $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (multiplicity, assignment): 165.5 (C, C-2), 149.9 (C, C-4), 133.6 (C, C-3), 129.7 (C, C-5), 93.7 (C, C-6), 67.7 (CH, C-1’), 38.1 (CH$_2$, C-2’), 18.8 (CH$_2$, C-3’), 13.9 (CH$_3$, C-4’); LREIMS $m/z$ of 328/326/324 (1:2:1) for C$_9$H$_{10}$O$_3$Br$_2$. 

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4.3.4. Spectral Data for Halogenated ketone 40

Halogenated ketone 40: 40 mg, 0.005%, colorless oil; IR $\nu_{\text{max}}$ 1715, 1134,1085 cm$^{-1}$; $\lambda_{\text{max}}$ 220 nm (log $\varepsilon$ 4.84), 283 nm (log $\varepsilon$ 4.03); $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ integration, multiplicity, $J$ (Hz), assignment): 2.79 (t, 2H, 7.5, H-4), (1.70-1.33, 6H, H-5 to H-7), 0.94 (3H, 7.0, H-8); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ (multiplicity, assignment): 197.4 (C, C-3), 91.2 (C, C-2), 121.9 (C, C-1), 40.7(CH$_2$, C-4), 31.1( CH$_2$, C-5), 23.2(CH$_2$, C-6), 22.4 (CH$_2$, C-7), 13.9 (CH$_3$, C-8); LREIMS $m/z$ 304/306/308/310 (1:2:2:1) for C$_8$H$_{11}$Br$_3$O.
4.3.5. Spectral Data for Pulchralide A (41)

**Pulchralide A (41):** Colorless crystals; $[\alpha]^{25}_{D}+6.0$ (c, 0.25, CHCl$_3$); IR $\nu_{\text{max}}$ 1747, 1740, 2962, 1220 cm$^{-1}$; UV $\lambda_{\text{max}}$ 252 nm (log $\varepsilon$ 4.67); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ integration, multiplicity $J$ (Hz), assignment): 5.58 (2H, dd, 6.5, 7.5, H-1’, H-1’a), 5.17 (2H, s, H-6, H-6a), 2.11 (6H, s, H-8, H-8a) 1.90 (4H, m, H-2’, H-2’a), 1.36 (4H, m, H-3’, H-3’a), 0.96 (6H, t, 7.5, H-4’, H-4’a); $^{13}$C NMR (125MHz, CDCl$_3$) $\delta$ (multiplicity, assignment): 170.0 (C, C-7, C-7a), 164.9 (C, C-2, C-2a), 139.7 (C, C-4, C-4a), 134.5 (C, C-3, C-3a), 90.4 (C, C-5, C-5a), 68.5 (CH, C-1’, C-1’a), 43.9 (CH, C-6, C-6a) 33.8 (CH$_2$, C-2’, C-2’a), 20.8 (CH$_3$, C-8, C-8a), 18.6 (CH$_2$, C-3’, C-3’a), 13.8 (CH$_3$, C-4, C-4’a); EIMS $m/z$ 732/734/736/738 (1:4:6:4:1); HREIMS 734.8260 (C$_{22}$H$_{24}$O$_8^{79}$Br$_3^{81}$Br requires 734.8262)
4.3.6. Spectral Data for Pulchralide B (42)

**Pulchralide B (42):** Colorless oil; \([\alpha]_{D}^{25} +4.2\) (c 0.06, CHCl\(_3\)); IR \(v_{\max} \) 1788, 2958 cm\(^{-1}\);

UV \(\lambda_{\max}\) 245 nm (log \(\varepsilon\) 5.20); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) (integration, multiplicity, \(J\) (Hz), assignment): 5.21 (2H, s, H-6, H-6a), 2.43 (2H, t, 7.5, H-1’, H-1’a), 1.56 (4H, m, H-2’, H-2’a), 1.35 (4H, m, H-3’, H-3’a), 0.94 (6H, t, 7.5, H-4’, H-4’a); \(^1^3\)C NMR (125MHz, CDCl\(_3\)) \(\delta\) (multiplicity, assignment): 167.4 (C, C-2, C-2a), 138.2 (C, C-4, C-4a), 137.1 (C, C-3, C-3a), 90.5 (C, C-5, C-5a), 44.1 (CH, C-6, C-6a), 29.0 (CH\(_2\), C-1’, C-1’a), 25.5 (CH\(_2\), C-2’, C-2’a), 22.4 (CH\(_2\), C-3’, C-3’a), 13.9 (CH\(_3\), C-4’, C-4’a); EIMS \(m/z\) 615/617/619/621/623(1:4:6:4:1), [M\(^+\) - H]; HREIMS 538.8894 ([M\(^+\) - Br], C\(_{18}\)H\(_{20}\)O\(_4\)\(^{79}\)Br\(_{2}\)\(^{81}\)Br requires 538.8891).
4.3.7. Spectral Data for Pulchralide C (43)

Pulchralide C (43): Colorless oil $[\alpha]_{25}^{D} +11.6$ (c, 0.06, CHCl₃); IR $\nu_{\text{max}}$ 1786, 1741, 2961, 1234 cm⁻¹; UV $\lambda_{\text{max}}$ 246 nm (log $\varepsilon$ 5.16); $^1$H NMR (500 MHz, CDCl₃) $\delta$ (integration, multiplicity, $J$ (Hz), assignment): 5.49 (1H, t, 7.5, H-1’), 5.35 (1H, d, 10.0, H-6), 5.13 (1H, d, 10.0, H-6a), 2.38 (1H, t, 7.5, H-1’a), 2.09 (3H, s, H-8), 1.89 (4H, m, H-2’, H-2’a), 1.34 (4H, m, H-3’, H-3’a), 0.95 (6H, m, H-4’, H-4’a); $^{13}$C NMR (125MHz, CDCl₃) $\delta$ (multiplicity, assignment): 170.3 (C, C-7), 167.0 (C, C-2), 165.3 (C, C-2a), 140.9 (C, C-4), 139.7 (C, C-4a), 135.5 (C, C-3), 134.5 (C, C-3a), 93.3 (C, C-5), 88.1 (C, C-5a), 68.8 (CH, C-1’), 47.7 (CH, C-6), 44.4 (CH, C-6a), 33.4 (CH₂, C-2’), 28.5 (CH₂, C-2’a), 25.4 (CH₂, C-3’), 22.5 (CH₂, C-3’a), 20.6 (CH₃, C-8), 18.7 (CH₂, C-1), 13.87 (CH₃, C-4’), 13.80 (CH₃,C-4’a); TOFMS $m/z$ 696.8047/698.8026/700.8008/702.7991/704.7977 (1:4:6:4:1, C₂₀H₂₂O₆⁷⁹Br₂⁸¹Br₂Na requires 700.8007).
References


73. SADABS v2.02: Area-Detector Absorption Correction. (1996) Siemens Industrial Automation, Inc.: Madison, WI.


APPENDICES
Figure 35. IR spectrum of fimbrolide (21)

Figure 36. UV spectrum of fimbrolide (21)
Figure 37. LREI Mass spectrum of fimbrolide (23)

Figure 38. IR spectrum of acetoxyfimbrolide (22)
Figure 39. UV spectrum of acetoxyfimbrolide (22)

Figure 40. LREI Mass spectrum of Acetoxyfimbrolide (22)
Figure 41. IR spectrum of hydroxyfimbrolide (23)

Figure 42. UV spectrum of hydroxyfimbrolide (23)
Figure 43. IR spectrum of halogenated ketone 40

Figure 44. UV spectrum of halogenated ketone 40
Figure 45. LREI Mass spectrum of halogenated ketone 40

Figure 46. IR spectrum of pulchralide A (41)
Figure 47. UV spectrum of pulchralide A (41)

Figure 48. LREI Mass spectrum of pulchralide A (41)
Figure 49. gCOSY spectrum of pulchralide B (42) (500 MHz, CDCl₃)

Figure 50. gHMQC spectrum of pulchralide B (42) (500 MHz, CDCl₃)
Figure 51. IR spectrum of pulchralide B (42)

Figure 52. UV spectrum of pulchralide B (42)
Figure 53. LREI Mass spectrum of pulchralide B (42)

Figure 54. gHMQC spectrum of pulchralide C (43) (500 MHz CDCl₃)
Figure 55. gHMBC spectrum of pulchralide C (43) (500 MHz, CDCl₃)

Figure 56. IR spectrum of pulchralide C (43)
Figure 57. UV spectrum of pulchralide C (43)

Figure 58. LRESI Mass spectrum of pulchralide C (43)