SECONDARY METABOLITES FROM SELECTED MARINE ORGANISMS

by

INDURUWA CHARLES PATHIRANA

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES
Department of Chemistry

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA
October 1986

© Induruwa Charles Pathirana, 1986
In presenting this thesis in partial fulfilment of the requirements for an advanced
degree at the University of British Columbia, I agree that the Library shall make it
freely available for reference and study. I further agree that permission for extensive
copying of this thesis for scholarly purposes may be granted by the head of my
department or by his or her representatives. It is understood that copying or
publication of this thesis for financial gain shall not be allowed without my written
permission.

Department of Chemistry

The University of British Columbia
1956 Main Mall
Vancouver, Canada
V6T 1Y3

Date October 1986
ABSTRACT

Marine organisms are known to produce secondary metabolites which have novel structures and are often biologically active. Chemical studies of biologically active metabolites from three different marine organisms led to the discovery of six new compounds and six previously known compounds.

The brown alga *Dictyota binghamiae* is fairly abundant in British Columbia coastal waters. A chemical study of this alga yielded ten diterpenoids of which four are new compounds. All the new compounds, dictyoxide A (66), dictyol G acetate (68), dictyotriol A diacetate (69), and epidictyol B acetate (70) contain a perhydroazulene carbon skeleton first encountered in the algal metabolite pachydictyol A (29). Dictyoxide A (66) appears to be an artifact of isolation. The acetates 68, 69, 70 were found to be antibacterial and antifungal. Six previously known compounds pachydictyol A (29), dictyol C (32), dictyoxide (35), acetyldictyolal (49) and the acetals 61a and 61b were also isolated from this alga.

Chemical studies on an *Agelas* sp. of sponge collected in Sri Lanka yielded the antimicrobial metabolite desbromooroidin (79).

An interesting interaction between the starfish *Dermasterias imbricata* and the sea anemone *Stomphia coccinea* was observed a long time ago. When contacted by the starfish,
the anemone displays an unusual "swimming" response which was, according to other subsequent studies, caused by a single chemical substance in the starfish. A study conducted to elucidate the structure of this starfish metabolite led to the isolation of imbricatine (91), a unique benzyltetrahydroisoquinoline alkaloid. Imbricatine (91) induced *S. coccinea* swimming response at a very low concentration and also exhibited antitumor activity.

Structures of all the new metabolites were determined by spectral analysis, and chemical degradations and chemical interconversions.
TABLE OF CONTENTS

Abstract .................................................. ii
List of figures .............................................. v
List of Schemes ............................................. vii
List of Tables .............................................. viii
List of Appendices ......................................... ix
Acknowledgements ........................................... x
Abbreviations .............................................. xi

I. Introduction ............................................. 1

II. Secondary metabolites from the brown alga
    Dictyota binghamiae ................................ 12
    A. Introduction ........................................ 12
    B. Isolation and structure elucidation ............... 21
    C. Discussion .......................................... 45

III. Secondary metabolites from the Sri Lankan sponge
    Agelas sp. ............................................. 47
    A. Introduction ........................................ 47
    B. Isolation and structure elucidation ............... 52
    C. Discussion .......................................... 59

IV. A secondary metabolite of the starfish
    Dermasterias imbricata that elicits a swimming
    response in the sea anemone
    Stomphia coccinea .................................... 61
    A. Introduction ........................................ 61
    B. Isolation and structure elucidation ............... 66
    C. Discussion .......................................... 104

V. Experimental ........................................... 115

VI. Appendices ............................................. 137

VII. References ........................................... 158
LIST OF FIGURES

1. $^1$H nmr spectrum of dictyol G acetate (68) .................27
2. $^{13}$C nmr spectrum of dictyol G acetate (68) .................28
3. Mass spectrum of dictyol G acetate (68) .........................29
4. $^1$H nmr spectrum of dictyoxide A (66) .........................30
5. $^{13}$C nmr spectrum of dictyoxide A (66) .........................31
6. Mass spectrum of dictyoxide A (66) ..............................32
7. Conformational drawing of dictyoxide A (66) ...................34
8. $^1$H nmr of spectrum of dictyotriol A diacetate (69) .......37
9. SFORD $^{13}$C nmr of spectrum of dictyotriol A diacetate (69) 38
10. Mass spectrum of dictyotriol A diacetate (69) ................39
11. Contour plot of the COSY 2D nmr spectrum of dictyotriol A diacetate (69) ................40
12. $^1$H nmr spectrum of epidictyol B acetate (70) .............41
13. $^{13}$C nmr spectrum of epidictyol B acetate (70) ............42
14. Mass spectrum of epidictyol B acetate (70) ...................43
15. $^1$H nmr spectrum of desbromoaboridin (79) ..................54
16. $^{13}$C nmr spectrum of desbromoaboridin (79) ..................55
17. Infrared spectrum of imbricatine (91) .........................71
18. $^1$H nmr spectrum of imbricatine (91) at r.t. .................72
19. $^1$H nmr spectrum of imbricatine (91) at 100°C ...............73
20. $^{13}$C nmr spectrum of imbricatine (91) ........................74
21. Contour plot of HETCOR 2D nmr spectrum of imbricatine (91) ...........................................75
22. $^1$H nmr spectrum of 92 .......................................76
23. CI mass spectrum of 92 ........................................ 77
24. $^1$H nmr spectrum of 94 at r.t. .............................. 80
25. $^1$H nmr spectrum of 94 at 100°C .......................... 81
26. $^{13}$C nmr spectrum of 94 .................................... 82
27. CI mass spectrum of 94 ........................................ 83
28. Interpretation of CI ms of 94 ................................. 84
29. Contour plot of HETCOR 2D nmr spectrum of 99 ........ 88
30. $^1$H nmr spectrum of 99 ....................................... 89
31. $^{13}$C nmr spectrum of 99 .................................... 90
32. CI mass spectrum of 99 ........................................ 91
33. $^1$H nmr spectrum of 100 .................................... 92
34. $^{13}$C nmr spectrum of 100 .................................. 93
35. CI mass spectrum of 100 ...................................... 94
36. $^1$H nmr spectrum of 102 .................................... 95
37. CI mass spectrum of 102 ...................................... 96
38. Interpretation of CI ms of 102 ............................... 100
39. Interpretation of CI ms of 99 ............................... 101
40. INAPT $^{13}$C nmr spectrum of imbricatine 91 ........... 102
41. INAPT $^{13}$C nmr spectrum of imbricatine 91 ........... 103
LIST OF SCHEMES

1. Chemical conversions of dictyol G acetate (68) ..........26
2. Synthesis of the benzyltetrahydroisoquinolines
   97 and 98 .........................................................87
3. Proposed routes for the biosynthesis of
   benzyltetrahydroisoquinolines in plants .................112
LIST OF TABLES

1. $^1$H nmr data for *dictyota binghamiae* diterpenoids ...........................................25

2. $^1$H nmr data for desbromooroidin (79) and keramadine (80) ......................................57

3. $^{13}$C nmr data for desbromooroidin (79) and keramadine (80) .....................................58

4. $^1$H nmr data for imbricatine (91), 94 and 93 ..............................................69

5. $^{13}$C nmr data for imbricatine (91), 94 and 93 ..............................................70

6. $^1$H nmr data for 102 and 99 .................................................................99
LIST OF APPENDICES

1. $^1$H nmr spectrum of pachydictyol A (29) ...................... 138
2. $^{13}$C nmr spectrum of pachydictyol A (29) ...................... 139
3. Mass spectrum of pachydictyol A (29) ............................ 140
4. $^1$H nmr spectrum of dictyol C (32) ............................. 141
5. Mass spectrum of dictyol C (32) ................................. 142
6. $^1$H nmr spectrum of dictyoide (35) ............................. 143
7. $^{13}$C nmr spectrum of dictyoide (35) ........................... 144
8. Mass spectrum of dictyoide (35) .................................. 145
9. $^1$H nmr spectrum of acetyldictyolal (49) ...................... 146
10. Mass spectrum of acetyldictyolal (49) ......................... 147
11. $^1$H nmr spectrum of acetal 61a ................................. 148
12. Mass spectrum of acetal 61a ....................................... 149
13. $^1$H nmr spectrum of acetal 61b ................................. 150
14. Mass spectrum of acetal 61b ....................................... 151
15. SFOAD $^{13}$C nmr spectrum of desbromooroidin (79) ......... 152
16. $^{13}$C nmr spectrum of imbricatine (91)
   ($^1$H gated decoupled) ............................................ 153
17. ADEPT $^{13}$C nmr spectra of imbricatine (91) ................. 154
18. $^{13}$C nmr spectrum of 94
   ($^1$H gated decoupled) ............................................ 155
19. ADEPT $^{13}$C nmr spectra of 94 ................................. 156
20. APT $^{13}$C nmr spectrum of 99 .................................. 157
ACKNOWLEDGEMENTS

I wish to sincerely thank my research supervisor, Dr. Raymond J. Andersen for his patience, encouragement, meticulous guidance and friendship during the course of this work. It has been a pleasure to work with him.

I am greatly indebted to my wife, Nelun, for her encouragement and unfailing support throughout.

Assistance given by Mike LeBlanc by performing bioassays and collecting specimens is greatfully acknowledged. I thank my colleagues who were helpful in numerous ways. The assistance of the staff of departmental nmr and mass spectrometry laboratories and also of the staff of Bamfield Marine Station is appreciated.
ABBREVIATIONS

AcOH - acetic acid
APT - Attached Proton Test
ADEPT - Automated Distortionless Enhancement by Polarization transfer
calcd. - calculated
CDCl₃ - chloroform-d₄
CI - chemical ionization
COSY - Correlated Spectroscopy
2D - two-dimensional
DMSO-d₆ - dimethyl sulfoxide-d₆
ED - effective dose
EI - electron impact
EtOAc - ethyl acetate
FAB - Fast Atom Bombardment
gc - gas chromatography
gcms - gas chromatography/mass spectrometry
h - hour/s
HETCOR - HETeronuclear CORrelation
hplc - high performance liquid chromatography
hrms - high resolution mass spectrum (electron impact)
i - impurity
ir - infrared
INAPT - Insensitive Nuclei Assigned by Polarization Transfer
MeOH - methanol
MeONa – sodium methoxide

min. – minutes

mp – melting point

ms – mass spectrum (low resolution)

n-BuOH – 1-butanol

^1H nmr – proton nuclear magnetic resonance

^13C nmr – carbon-13 nuclear magnetic resonance

nOe – nuclear Overhauser enhancement

ppm – parts per million

ref. – reference

rel. int. – relative intensity

r.t. – room temperature

s – solvent signal

T/C – test/control

tlc – thin layer chromatography

uv – ultraviolet

w – water signal
To My Parents
I. INTRODUCTION

A. NATURAL PRODUCTS CHEMISTRY

Natural products chemistry (the chemistry of secondary metabolites) has traditionally focused on terrestrial plants and microorganisms. Studies of the extraordinarily large number of plants and animals in the marine environment were not initiated until the last three decades when the advent of SCUBA effectively removed the barrier between man and the shallow ocean.

Classically, the structures of natural products were solved by chemical degradations and interconversions, and they were confirmed by synthesis. Since these techniques required a large amount of material, often only the major metabolites were characterized. Classical structure elucidation was a tedious, time consuming process.

Natural products chemistry has undergone an explosive growth during the past two decades. The rate at which new compounds are discovered is now fast enough to rapidly outdate any compilation of structures. This growth is partly a result of the development of high speed, low cost computers. Computers play a central role in X-ray crystallography, mass spectrometry and Fourier transform nmr, the three most important tools in modern structure elucidation.

X-ray crystallographic analysis is now highly automated
and it routinely provides a fast and accurate method to solve structures of compounds that would be difficult or impossible to handle with other techniques. The structure determination of norhalichondrin A (1) via an X-ray crystallographic analysis of its p-bromophenacylbromide derivative is a recent example.

Mass spectrometry and FT (Fourier transform) nmr have also played a great role in accelerating the pace of structure elucidation. Both these techniques are capable of providing structural information on minute amounts of material. Multipulse, one dimensional and two dimensional, nmr experiments have greatly expanded the information
available to structural chemists. Homonuclear and heteronuclear shift correlations, polarisation transfer, and J-resolved 2D nmr are some of the new techniques that are most useful to natural products chemists. The assignment of all the protons in 11\(\beta\)-hydroxyprogesterone (2) using a variety of one and two dimensional nmr experiments illustrates the application of multipulse nmr to the study of
complex molecules\(^3\).

One cannot, of course, forget the contributions of the advances in separation techniques. The advent of high performance liquid chromatography (hplc) was a remarkable step forward. In particular, the availability of new gels and resins that can be used for the separation of highly polar metabolites has encouraged the study of water soluble compounds. Modern chromatography is in fact so sophisticated that the direct resolution of amino acid enantiomers can now be achieved by thin layer chromatography\(^4\).

The advent of new spectroscopic and separation techniques has given chemists the resources to tackle almost any problem they are faced with. The successful elucidation of the structure of palytoxin (3)\(^5\) illustrates this point. It would not be an exaggeration to state that solving structures such as that of palytoxin (3) would have been unimaginable a few decades ago.

**B. MARINE NATURAL PRODUCTS CHEMISTRY**

The extensive chemical research on marine organisms during the last two decades has brought to light a large number of extraordinary metabolites. In general, many of these metabolites are exclusive to marine organisms and some are isolated only from certain species or group of species. A detailed coverage of the past research can be found in the series of books edited by Scheuer\(^6\) and a series of review
articles by Faulkner.7

The discovery of chemically interesting metabolites with new structures has not only yielded information about nature's molecular architecture but it has also revealed some interesting information about biological interactions taking place in the marine environment. One of the most striking discoveries came from the study of nudibranchs, commonly known as sea slugs. These shell-less, often brightly coloured animals, despite their vulnerability, have almost no known predators. The discovery that metabolites extracted from their skins exhibited fish antifeedent activity led to the hypothesis that they utilized chemical antifeedents to ward off predators. Albicanyl acetate (4)8 and furodysinin (5)8,9 isolated from Cadlina luteomarginata, nakafuran 8 (6) and nakafuran 9 (7) from Hypselodoris godeffroyana and Chromodoris marisdadilus10, and 9-isocyanopupukeanane (8) from Phyllidea varicosa11 are some of the fascinating metabolites that are thought to be defensive substances.

A large portion of the recent marine natural products research has been directed towards marine pharmacology. As a result, a number of marine secondary metabolites have been found to exhibit promising pharmacological properties.

The diterpenes halimadatrial (9)12 and halimadalactone (10)13 isolated from several green algal species of the genus Halimeda show antibacterial and cytotoxic activities. Spatol (11), isolated from the brown alga Spatoglossum schmittii14, and tedanolide (12), isolated from the sponge Tadonia ignis15
are two potent cytotoxins. Agelasines (e.g. agelasine A (13), D (14), E (15)) which show antimicrobial and antispasmodic activities have been isolated from the sponge *Agelas nakamura*. A nucleoside, tubercidin (16), isolated from the marine alga *Hypnea valendiae* was found to be a muscle relaxant. Pettit *et al.* have isolated a family of metabolites, the bryostatins (e.g. bryostatin 1 (17)), which
are potent antitumor agents. The striking red egg masses of the nudibranch *Hexabranchus sanguineus*, though exposed and vulnerable, were observed by Roesner and Scheuer to have a high level of immunity to predators. Prompted by this unusual observation, they isolated ulapualide A (18) and B (19) which both inhibited L1210 leukemia cell proliferation.0.

It is a rather easy task to pick out hundreds of
biologically active compounds similar to the above examples from the marine natural products literature.

Unfortunately, only a small fraction of the many lead compounds have turned out to be successful in more exhaustive drug tests. Rinehart et al. isolated the didemnins from the tunicate *Trididemnum cyanophorum*. These interesting peptides showed very high antitumor activity. The most potent one, didemnin B (20), is now undergoing clinical trials as an antitumor drug (22). A second marine metabolite named manoalide (21) (23) has also reached clinical trials as an antiinflammatory agent, and both compounds undoubtedly have made a major contribution to the recent renewed interest in the search for drugs from the sea.

Another route to drug development exploits the possibility of using natural compounds as templates for synthetic analogs which will have some therapeutic use. The sponge metabolites spongothermidine (22) (24) and spongouridine (23) (25) have led to the synthetic product 24, a powerful antiviral agent used in the treatment of herpes virus (26), and the anticancer drug Ara-C (25) (27) which is used in cancer chemotherapy.

It is evident from past efforts that it is a rather difficult task to discover a metabolite with desirable therapeutic qualities. Clearly the eventual development of a "Drug from the Sea" will require a great deal of patience and perseverance.

This thesis describes the research carried out to
isolate and elucidate the structures of secondary metabolites found in three marine organisms. The structures of the metabolites were determined by interpretation of spectral data, and by chemical degradations and interconversions.

A chemical study of *Dictyota binghamiae*, a brown algal species collected in British Columbia, yielded ten diterpenes of which four are new compounds. A biologically active alkaloid was isolated as the major metabolite from an *Agelas* sp. of sponge collected in Sri Lanka. The starfish *Dermasterias imbricata* was known to induce an unusual "swimming" behavior in the sea anemone *Stomphia coccinea*. It was also known that a single chemical substance in the starfish was responsible for the behavioral response. The structure of the active compound was solved. It turned out to be a benzyltetrahydroisoquinoline alkaloid which has several unusual structural features. The following chapters contain a detailed account of the above mentioned research.
SECONDARY METABOLITES FROM THE BROWN ALGA

Dictyota binghamiae

A. INTRODUCTION

Marine algae have been a very good source of a wide variety of secondary metabolites which are interesting from both chemical and biological points of view. These metabolites range from simple molecules such as the 1,3-dione (26) to complicated macro molecules such as brevitoxin B (27).

Among algal metabolites, the toxins, including the well known saxitoxin (28), are almost exclusively produced by either phytoplankton or blue-green algae. In contrast, algae belonging to other divisions, ie. brown algae, red algae and
green algae, are rarely known to produce secondary metabolites with such drastic biological activities.

Brown algae, are almost exclusively found in the intertidal and subtidal regions of the marine habitat. The distinguishing chemical feature of brown algae in the order Dictyotales is the presence of diterpenoids. Cyclic diterpenoids are the typical metabolites of the members of the family Dictyotaceae. Pachydictyol A (29), reported by Hirschfeld et al. in 1973 from the brown alga *Pachydictyon coriaceum* collected along the Pacific coast of California, was the first member of a group of algal diterpenoids containing the perhydroazulene ring system. A number of oxygenated derivatives of pachydictyol A were subsequently discovered. These include dictyol A (30), B (31), C (32) and D (33) isolated from *Dictyota dichotoma* var. implexa, and dictyol E (34) and dictyoxide (35) from *Dilophus ligulatus*. Almost simultaneously dictyol A and B were
also isolated from a herbivorous sea hare *Aplysia depilans* which was known to feed upon *D. dichotoma*\(^{35}\).

The related metabolites dictyotadiol (36) and dictyol B acetate (37) were reported from *D. dichotoma* collected in the British Channel\(^{35}\), while dictytriol (38), dictyone (39), and the deoxygenated metabolites dictytriene A (40) and B (41) were reported from a Japanese collection of the same alga\(^{37}\). Japanese workers have also reported the isolation of four new compounds, dictyol F (42), epidictyol F (43),
epoxypachydictyol A (44), and methoxydictydiene (45), thought to be an artifact, from *Dictyota dichotoma*. Pachydictyol A epoxide (46) was isolated from *D. flabellata* collected from the Gulf of California.

Metabolites with the xenicane skeleton have been found in Dictyotaceae species belonging to the genera *Dictyota* and *Dilophus*. Xenicin (47), isolated from soft coral *Xenia elongata*, was the first diterpene with this skeleton to be reported.

Dictyodial (48), the first algal metabolite having a xenicane carbon skeleton, was isolated by Finer et al. from *D. crenulata*. This discovery was followed by the isolation of a number of metabolites which had minor structural variations. These include acetyldictyonal (49), hydroxyacetyldictyonal (50), dictyodiacetal (51) and isodictyohemiacetal (52) isolated from *Dictyota dichotoma*, and 4β-hydroxydictyodial (53) isolated from *Dictyota*.
\[ R_1 = \text{CHO} \quad R_2 = \text{H} \]
\[ R_1 = \text{CH}_2\text{OAc} \quad R_2 = \text{H} \]
\[ R_1 = \text{CH}_2\text{OAc} \quad R_2 = \text{OH} \]
\[ R_1 = \text{CHO} \quad R_2 = \text{OH} \]
\[ R = \text{OAc} \]
\[ R = \text{H} \quad 19 \quad R \text{ or } S \]
\[ R = \text{H} \quad 19 \quad S \text{ or } R \]
crenulata. *Dictyota dichotoma* has also yielded dictyofurans T (54) and C (55), while compounds (56), (57), (58) and (59) with lactone formation involving a carbonyl group in the side chain have been reported from *D. proliferans*. The study of minor metabolites of the alga *Pachydictyon coriaceum*, collected along the Japanese coast, yielded acetyldictyol C (60) and the two acetals 61a and 61b which are reported to be artifacts arising from the addition of methanol to dictyodial (48).

Acetylcoriacenone (62) and isoacetylcoriacenone (63) are two more interesting metabolites which show further cyclization of xenicane skeleton, both having a four membered ring fused to the nine membered carbocyclic ring. Both were isolated from *Pachydictyon coriaceum*.

Algae, being a principal component of the marine
ecosystem, experience constant pressure from herbivorous predators. It is not surprising, therefore, that algal metabolites also appear in the chemical studies carried out on herbivorous animals. Dictyol A (30) and B (31) were isolated from both *Dictyota dichotoma* var. *implexa*\(^5\) and the sea hare *Aplysia depilans*\(^8\) by two Italian groups in separate studies. Dictyolactone (64)\(^1\), isolated from *A. depilans* and the hemiacetal of acetoxycrenulide (65) isolated from *A. vaccaria*\(^8\) show minor variations in structure compared to algal metabolites, demonstrating the algal origin of *Aplysia* metabolites.

Many marine organisms are observed to employ a chemical defense mechanisms to ward off predators. Chemical studies of a few algal species have demonstrated the use of this type of defensive strategy against herbivores\(^9\).
The foregoing section includes only selected examples of diterpenoids with perhydroazulene and xenicane skeletons reported from members of Dictyotaceae. Since all the metabolites isolated from *Dictyota binghamiae* in the current study belong to these two families of compounds, a description of the many other types of diterpenes found in Dictyotaceae has not been included.
B. ISOLATION AND STRUCTURE ELUCIDATION

The brown alga *Dictyota binghamiae*, a member of the family Dictyotaceae, is fairly abundant in the subtidal regions of British Columbia coastal waters. We have found, however, that it shows a seasonal variation in abundance. Its availability and the antibacterial activity shown by its crude extracts prompted us to initiate a chemical study of *D. binghamiae*.

The alga was collected in the shallow bays near Dixon Island and Execution Rock in Barkley Sound. Initial collections of the alga were dried in a convection oven at 50°C for 24 h. The dried alga was then powdered in a Wiley mill and extracted with hexane in a Soxhlet extractor. The hexane extract was concentrated *in vacuo* to give a dark oil which was fractionated by step gradient flash chromatography using the following solvent sequence: (i) hexane (ii) hexane/chloroform 1:1 (iii) chloroform (iv) chloroform/ethyl acetate 9:1 (v) chloroform/ethyl acetate 1:1.

It was evident from crude 
$^1$H nmr and thin layer chromatography that the first two fractions contained only fatty compounds, hence, they were not studied further.

The fraction eluted with chloroform looked interesting by tlc and $^1$H nmr examination. Further purification of this fraction by radial tlc on silica gave crude samples of pachydictyol A (29), dictyoxide (35) and dictyoxide A (66). Additional fractionation by reverse phase hplc gave pure
samples of both compounds.

The other two fractions from the flash chromatography were both green oils when concentrated *in vacuo* indicating the presence of pigments. However, tlc revealed two compounds of terpenoid nature which were more polar than the previously isolated compounds. Further studies on these compounds were postponed because of their low concentration.

Later collections of the alga were immersed in methanol immediately after collection. The methanol was decanted from the alga, concentrated *in vacuo*, and partitioned between hexane and water. After drying with anhydrous Na$_2$SO$_4$ and concentration *in vacuo*, the hexane extract was subjected to the previously described step gradient flash chromatography.

The chloroform eluted fractions now contained an additional compound, acetal (61a), which was obtained in pure form from the radial tlc fractionation. Pure acetal (61b) was obtained by radial tlc of the ethyl acetate/chloroform eluted fractions from the flash chromatography. The same flash fractions also yielded crude samples of dictyol G acetate (68), dictyotriol A diacetate (69), dictyol C (32) and a mixture of acetyldictyoyal (48) and epidictyol B acetate (70). Final purification of these compounds was achieved by hplc and preparative tlc.

A number of resonances in the $^1$H nmr spectrum ($\delta$

1.61(s, 3H), 1.69(s, 3H), 1.81(s, 3H), 3.92(br d, $J=8$ Hz, 1H), 4.24(s, 1H), 4.74(s, 1H), 5.12(t, $J=6$ Hz, 1H), 5.38(br s, 1H) ppm, see appendix 1) of one of the pure compounds
(ms: 288.2453, required for C<sub>20</sub>H<sub>32</sub>O 288.2454) indicated that it was pachydictyol A (29). Comparison with an authentic sample ('H nmr, tlc) confirmed this.

Having identified pachydictyol A (29), it was straightforward to identify dictyoxide (35) and dictyol C (32) by comparing their spectral data with the reported values (see appendices 1, 2, 3 for 29; 4, 5 for 32 and 6, 7, 8 for 35). The other previously known compounds acetals 61a (see appendices 11, 12) and 61b (see appendices 13, 14), and acetyldictyolal (49) (see appendices 9, 10) were also identified by comparing their spectral properties to the literature values.

Dictyol G acetate (68) was obtained as an optically active ([α]<sup>22</sup> +50°) colourless oil from preparative reverse phase hplc. High resolution mass spectrometry showed that it had a molecular formula of C<sub>22</sub>H<sub>34</sub>O<sub>3</sub>. Its ir spectrum contained hydroxyl (3500 cm<sup>-1</sup>) and carbonyl (1725 cm<sup>-1</sup>) absorption bands. A three-proton resonance at δ 2.02 ppm in the 'H nmr spectrum and a significant fragment ion at m/z 286 (M<sup>+</sup> -CH<sub>3</sub>CO<sub>2</sub>H) in the mass spectrum, indicated the presence of an acetate ester (see fig. 3). The 'H and <sup>13</sup>C nmr spectra of acetate 68 (see figs. 1 and 2) also clearly indicated that it had the same substituted bicyclic ring system as pachydictyol A (29) (see Table 1) and thus the acetoxy substituent had to be attached to the side chain. A deshielded resonance at δ 5.57 ppm (dt, J=8.4 Hz) in the 'H nmr spectrum of 68, which was not present in the 'H nmr spectrum of 29, could be
assigned to a methine proton α to the acetoxy functionality. The chemical shift of the methine proton implied that it must also be allylic, and a double resonance experiment showed that it was spin coupled to the olefinic proton on C14 (δ 5.12, br d, J=8 Hz). Thus the acetoxy functionality in 68 must be attached to C13. Reduction of dictyol G acetate (68) with lithium in ethylamine gave pachydictyol A (29) which was identical to an authentic sample by tlc and 1H nmr comparison (see Scheme 1). The reaction was stopped before completion to prevent further reduction of products that resulted in a lowered yield.

![Diagram](image)

Dictyoxide A (66) was obtained as an optically active ([α]_D^{22} -5.6°) pale yellow oil that had the molecular formula C_{20}H_{30}O. Its ir spectrum showed an absence of either hydroxyl or carbonyl absorptions and its 13C nmr spectrum (see fig. 5) contained two resonances at δ 79.5 and 68.6 ppm, appropriate for the carbon atoms of an ether functionality.
<table>
<thead>
<tr>
<th>Carbon</th>
<th>Pachydictyol A (29)</th>
<th>Dictyoxide A (66)</th>
<th>Dictyol G acetate (68)</th>
<th>Dictyotriol diacetate (69)</th>
<th>Epidictyol B acetate (70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.67 q, J=10 Hz</td>
<td>2.62 q, J=9 Hz</td>
<td>-</td>
<td>2.79 q, J=10 Hz</td>
<td>2.87 q, J=10 Hz</td>
</tr>
<tr>
<td>2</td>
<td>2.62 m</td>
<td>-</td>
<td>2.49 m</td>
<td>2.49 m</td>
<td>2.25 m</td>
</tr>
<tr>
<td>3</td>
<td>5.33 br s</td>
<td>5.37 br s</td>
<td>5.33 br s</td>
<td>5.34 br s</td>
<td>5.34 br s</td>
</tr>
<tr>
<td>5</td>
<td>2.32 br t</td>
<td>2.75 br t, J=10 Hz</td>
<td>2.33 br t</td>
<td>3.87 dd, J=8, 3 Hz</td>
<td>3.92 dd, J=8, 4 Hz</td>
</tr>
<tr>
<td>6</td>
<td>3.92 br d, J=8 Hz</td>
<td>3.74 dd, J=10, 4 Hz</td>
<td>3.93 br d, J=8 Hz</td>
<td>2.02 dd, J=10, 3 Hz</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5.59 dd, J=6, 2 Hz</td>
<td>5.59 dd, J=6, 2 Hz</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.86 m</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>1.14 q, J=12 Hz</td>
<td>1.93 m</td>
<td>4.92 m</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>-</td>
<td>4.45 ddd, J=12, 8, 4 Hz</td>
<td>5.57 dt, J=8, 4 Hz</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>5.12 br t, J=6 Hz</td>
<td>5.20 br d, J=8 Hz</td>
<td>5.12 br d, J=8 Hz</td>
<td>5.10 t, J=7 Hz</td>
<td>5.10 m</td>
</tr>
<tr>
<td>16</td>
<td>1.61 br s</td>
<td>1.65 br s</td>
<td>1.72 d, J=1, 5 Hz</td>
<td>1.64 br s</td>
<td>1.81 dd, J=1, 5 Hz</td>
</tr>
<tr>
<td>17</td>
<td>1.81 br s</td>
<td>1.79 br s</td>
<td>1.82 d, J=1 Hz</td>
<td>1.82 d, J=1 Hz</td>
<td>5.10 s</td>
</tr>
<tr>
<td>18</td>
<td>4.75 s</td>
<td>4.82 s</td>
<td>4.75 s</td>
<td>5.03 s</td>
<td>5.04</td>
</tr>
<tr>
<td>19</td>
<td>1.00 d, J=7 Hz</td>
<td>0.95 d, J=7 Hz</td>
<td>1.04 d, J=7 Hz</td>
<td>0.94 d, J=7 Hz</td>
<td>0.93 d, J=7 Hz</td>
</tr>
<tr>
<td>20</td>
<td>1.69 br s</td>
<td>1.70 br s</td>
<td>1.74 d, J=1, 5 Hz</td>
<td>1.70 br s</td>
<td>1.69 br s</td>
</tr>
</tbody>
</table>

Chemical Shift, δ ppm
Scheme 1. Chemical conversions of dictyol G acetate (68).
Fig. 1. $^1$H nmr spectrum of dictyol G acetate (68) (400 MHz, CDCl$_3$).
Fig. 2. $^{13}$C nmr spectrum of dictyol G acetate (68) (100.6 MHz, CDCl$_3$).
Fig. 3. Mass spectrum of dictyol G acetate (68).
Fig. 4. $^1$H nmr spectrum of dictyoxide A (66) (400 MHz, CDCl$_3$).
Fig. 5. $^{13}$C nmr spectrum of dictyoxide A (66) (100.6 MHz, CDCl$_3$).
Fig. 6. Mass spectrum of dictyoxide A (66).
Six additional carbon resonances at δ 152.0, 141.0, 133.5, 126.8, 124.9 and 106.8 ppm could be assigned to three olefin functionalities.

Since the molecular formula of 66 requires six sites of unsaturation, it was concluded that 66 must be tricyclic. The 'H nmr spectrum of 66 (see fig. 4) showed methyl resonances at δ 0.95 (d, 3H, J=7 Hz), 1.65 (br s, 3H), 1.79 (br s, 3H) and 1.70 (br s, 3H) ppm that were virtually identical to those observed for the three olefinic and one aliphatic methyl groups in pachydictyol A (29) (see Table 1). In addition, olefinic proton resonances at δ 5.37 (br s, 1H), 4.82 (br s, 1H) and 4.79 (br s, 1H) ppm were similar to those observed for their counterparts on C3 and C18 in 29. The above evidence led to the hypothesis that dictyoxide A (66) had the same carbon skeleton as pachydictyol A (29) but that the alcohol functionality of 29 was replaced by a cyclic ether in 66. It was assumed that one point of attachment of the cyclic ether was C6 and a resonance at δ 3.74 (dd, 1H, J=10.4 Hz) ppm was assigned to the methine proton on that carbon. A downfield resonance at δ 4.45 (ddd, 1H, J=12, 8, 4 Hz) ppm was assigned to a methine proton on the second ether carbon. This methine proton was shown via a double resonance experiment to be spin coupled to the olefinic proton on C14 (δ 5.20 ppm, br d, 1H, J=8 Hz) and hence C13 had to be the second point of attachment of the cyclic ether.

The constitution of dictyoxide A (66) was confirmed by treatment of dictyol G acetate (68) with aqueous acetic acid
which gave a 20% yield of 66 (see Scheme 1) and a very low yield of a second compound which was presumed to have the structure 67. An analysis of the coupling constants observed for H13 and H12a in ether 66 revealed that the tetrahydropyran ring assumed a chair conformation and allowed us to assign the relative configuration at C13. H13 shows a 8 Hz vicinal coupling to H14, a 4 Hz axial-equatorial coupling to H12β and a 12 Hz axial-axial coupling to H12a (see table 1). Therefore H13 must be axial and the vinyl residue must be equatorial. H12a appears as quartet at δ 1.14 ppm with a 12 Hz coupling constant. It must therefore be coupled to H12β with a 12 Hz geminal coupling and to an axial proton on C11. Thus the C19 methyl must be equatorial and cis to the C13 vinyl substituent. Since the relative stereochemistry at C11 in pachydictyol A (29) is known, it can be concluded that dictyoxide A (66) adopts the conformation shown in Fig.7.

Fig. 7. Conformational drawing of dictyoxide A (66).
The epimer 67 could not be adequately characterized due to a very limited sample size, but its $^1$H nmr spectrum was consistent with the proposed structure.

Dictyotriol A diacetate (69) was obtained as an optically active ($[\alpha]_D^{20} +12.5^\circ$) colourless oil that had a molecular formula of $C_{24}H_{36}O_{5}$. Its ir spectrum showed hydroxyl (3450 cm$^{-1}$) and carbonyl (1730 cm$^{-1}$) absorption bands. Two singlet methyl resonances at $\delta$ 2.06 and 2.07 ppm in the $^1$H nmr spectrum of 69 (see fig. 8) indicated that it was a diacetate. The remainder of the $^1$H nmr spectrum suggested that the diacetate 69 had the same carbon skeleton as pachydictyol A (29) and that the $\Delta^3$'4, $\Delta^{10}$'18, $\Delta^{14}$'15 and C6 hydroxy functionalities were intact (see Table 1). Two resonances at $\delta$ 4.92 (m, 1H) and 5.59 (dd, 1H, $J=2,6$ Hz) ppm could be assigned to methine protons $\alpha$ to the acetoxy functionalities. This implied that the acetates were both esters of secondary alcohols, one of them allylic. Irradiation of the allylic methine proton ($\delta$ 5.59 ppm) did not change either the C13 or C14 proton resonances ($\delta$ 5.34 and 5.10 ppm) but it did simplify the H8$\alpha$ resonance to a broad doublet ($\delta$ 1.76 ppm, $J=15$ Hz) and the H8$\beta$ resonance to a doublet of doublets ($\delta$ 1.92 ppm, $J=15,8$ Hz). Decoupling further demonstrated a 1.5 Hz W coupling between H8$\alpha$ and H6, confirming the assignment of the former resonance. The allylic acetate must therefore be on C9 and it was assigned the $\alpha$ configuration because both the multiplicity and chemical shift of the $\alpha$ acetoxy proton were substantially
different than those reported for dictyol B acetate (37) in which the C9 acetate is $\beta$ (12: H9a, $\delta$ 5.16 ppm, t, J=7 Hz). The stereochemistry at C9 was confirmed by demonstrating a difference nOe in H5, H8$\beta$ and H8a when H9 was irradiated.

Placement of the second acetoxy functionality on C12 followed from the fact that it had to be on a non-allylic secondary carbon, which according to decoupling experiments, was not vicinal to the C9 acetate. Further support for the positions of both acetoxy functionalities could be found in a $^1$H 2D nmr COSY experiment (see fig. 11). Clearly visible are the (14/13,13') and (12/13,13') off-diagonal peaks which would be expected if diacetate 69 contains a C12 acetoxy. It is also possible to follow the required coupling sequence from the C9 acetoxy a methine proton to the C6 carbinol methine proton. Thus starting at C9, we find the off-diagonal elements (9/8a), (9/8$\beta$), (7/8$\beta$) and (6/7).

![Diagram]

69 $R$ = OAc

70 $R$ = H

Epidictyol B acetate (70) was obtained as an optically
Fig. 8. $^1H$ nmr spectrum of dictyotriol A diacetate (69) (400 MHz, CDCl$_3$).
Fig. 9. SFORD $^{13}$C nmr spectrum of dictyotriol A diacetate (69) (100.6 MHz, CDCl$_3$).
Fig. 10. Mass spectrum of dictyotriol A diacetate (69).
Fig. 11. Contour plot of the COSY 2D nmr spectrum of dictyotriol A diacetate (69) (400 MHz, CDCl₃).

Off-diagonal responses establishing proton spin-coupling connectivities are labelled with the protons involved, the downfield resonance listed first. Only the connectivities required to demonstrate the positions of the acetate functionalities are shown.
Fig. 12. $^1$H nmr spectrum of epidictyol B acetate (70) (400 MHz, CDCl$_3$).
Fig. 13. $^{13}\text{C}$ nmr spectrum of epidictyol $\beta$ acetate (70) (100.6 MHz, CDCl$_3$).
Fig. 14. Mass spectrum of epidictyol B acetate (70).
active ([α]_D^{22} +43.6°) colourless oil which had a molecular formula of C_{22}H_{34}O_3. Its IR spectrum contained hydroxyl (3500 cm\(^{-1}\)) and carbonyl (1740 cm\(^{-1}\)) absorption bands. A major fragment ion at m/z 286 (M⁺ - CH₃CO₂H) in the mass spectrum (see fig. 14) and a singlet methyl resonance at δ 2.02 ppm in the \(^1\)H nmr spectrum (see fig. 12) indicated the presence of an acetate ester. The \(^1\)H nmr resonances δ 5.59 (dd, 1H, J=6,2 Hz), 5.34 (br s, 1H), 5.10 (m, 1H), 5.10 (br s, 1H) and 5.04 (br s, 1H) ppm of 70 were identical to those of 69 indicating that the acetoxy functionality is attached to C9 (see table 1). The assignment of the \(\alpha\) configuration to the acetate in 70 is paralleled the stereochemical assignment at C9 in 69.
C. DISCUSSION

The secondary metabolites dictyol G acetate (68), dictyotriol A diacetate (69) and epidictyol B acetate (70) represent three new additions to the group of metabolites with perhydroazulene carbon skeletons commonly found in members of family Dictyotaceae. It is interesting to note that all three new compounds contain an acetoxy functionality.

Pachydictyol A (29), dictyol C (32), dictyol F (42) and epidictyol F (43) are reported to have antimicrobial activity while dictyoxide (35), epoxypachydictyol A (44) and methoxydictydiene (45) do not show any antibacterial activity. A standard in-vitro disc bioassay on the three new acetates 68, 69 and 70 showed mild antifungal activity.

'Two diterpenoids isolated from *Dictyota indica* were named dictyotriol A (71) and B (72)\(^5\).'

\[
\text{71: 14 R or S} \\
\text{72: 14 S or R}
\]

The Chinese work on *D. indica*, and our work on *D. binghamiae*\(^5\)\(^2\) appeared almost simultaneously in the literature. It is obvious that the assignment of cis-fused rings for 71 and 72 is an error. When corrected to include trans-fused rings, 71 is the same compound described earlier as dictyotriol (68)\(^3\). (The name dictyotriol B for the C-14 epimer of dictyotriol (68) has the precedence over the name isodictyotriol which appeared later in the literature\(^5\)\(^3\)).
against *Pythium ultimum*, *Rhizoctonia solani*, and *Helminthosporium sativum*. Dictyol G acetate (42) and epidictyol B acetate (70) also showed mild antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. Dictyotriol A diacetate (69) did not show any observable activity against these two bacteria.

Dictyoxide A (66) was found to be present only in the oven-dried samples of *Dictyota binghamiae*. The observed absence of (66) in the methanol extracted alga seems to indicate that it is an artifact formed from dictyol G acetate (68) during oven drying. The two acetals 61a and 61b also appear to be artifacts arising from the reaction of dictyodial (48) with methanol as was reported earlier by Japanese workers.6

It is quite common to find more than one class of diterpenes in a single Dictyotaceae species. This phenomenon is also true for *Dictyota binghamiae* which contains metabolites with both perhydroazulene and xenicane carbon skeletons.
SECONDARY METABOLITES FROM THE SRI LANKAN SPONGE

*Agelas* sp.

A. INTRODUCTION

Sponges (phylum Porifera) are primitive marine invertebrates which are considered to be the simplest multicellular animals. Chemical studies on these animals have revealed that they are a prolific source of secondary metabolites with unusual structures. It is interesting to note that most of these compounds do not have any terrestrial analogs. The majority of sponge metabolites are terpenoids.

![Chemical structure of oroidin (73)](image)

The bromopyrrole alkaloid, oroidin (73), isolated from *Agelas oroides* by Forenza *et al.* in 1971\(^5\) is a typical example of perhaps the best known family of alkaloids from marine sponges. Oroidin (73), has since appeared as the major metabolite of several *Agelas* species. Forenza *et al.* also reported the isolation of bromopyrroles 74, 75, 76 and 77, but their structural proposal for oroidin was controversial and the correct structure was later proposed by Garcia *et al.*
and verified via synthesis\textsuperscript{55}. A recent investigation of the sponge *Agelas sceptrum* (Lamarck) by Walker *et al.* produced a single crystal X-ray diffraction analysis of oroidin (73), confirming the correct structure\textsuperscript{56}. The structure of sceptrin (78), the major antimicrobial constituent of *A. sceptrum*, was also secured by X-ray crystallography. Attempts to prove the hypothesis that 78 is the [2+2] cyclo adduct of desbromooroidin (79) were unsuccessful\textsuperscript{56}. Keramadine (80), which has the Z geometry, was found in a study of an unknown *Agelas* species collected in Japan\textsuperscript{57}. Chevolot *et al.* reported the isolation of midpakamide (81) from the Hawaiian sponge *Agelas mauritiana*\textsuperscript{58}.

\[
\begin{align*}
\text{Br} & \text{Br} \quad \text{Br} & \text{R} \\
\text{N} & \text{H} & \text{CN} & \text{O} \\
\text{74} & \text{75} & \text{76} & \text{77} \\
\text{R} & = \text{NH}_2 & \text{R} & = \text{OH} \\
\text{R} & = \text{O}\text{Me} 
\end{align*}
\]
Metabolites which are biologically related to oroidin (73) have been also isolated from sponges in the family Axinellidae. Sharma and Burkholder isolated monobromophakellin (82) and dibromophakellin (83) from Phakellia flabellata⁵⁹. Sharma et al. later reported the isolation of 84, which shows further cyclisation of the oroidin(73) skeleton, from the same sponge⁶⁰. The brominated
compound 85 has been isolated from *Axinella verrucosa* and *Acanthella aurantiaca*\(^6\), while stevensine (86) has been found in an unidentified sponge\(^6\). The presence of similar metabolites in these sponges of different families indicates a chemical relationship between them and perhaps indicates errors in the taxonomic classifications.

The biological activity exhibited by the metabolites of the 'oroidin group' is noteworthy. All the members of the genus *Agelas* examined in a study of Caribbean sponges by Walker *et al.* were shown to have antimicrobial activity\(^5\). Faulkner, even after studying the metabolites of *Verongia* species for several years, concluded in an earlier report that the most active antibiotic of those sponges had not been described\(^6\). This may be true for *Agelas* species as well.
B. ISOLATION AND STRUCTURE ELUCIDATION

An unidentified Agelas sp. was collected by hand using SCUBA on shallow rocky reefs off Mt. Lavinia and Hikkaduwa, Sri Lanka. Freshly collected animals were immersed in methanol and kept at low temperatures until work up. The aqueous suspension obtained after removing methanol from this extract was extracted sequentially with hexane, chloroform and ethyl acetate. The resulting aqueous phase was freeze-dried. The combined organic extracts were chromatographed on Sephadex LH-20 to obtain pure desbromooroidin (79). LH-20 chromatography of the methanol soluble portion of the freeze-dried aqueous phase gave more of pure 79.

Pure 79 was insoluble in less polar organic solvents but fairly soluble in methanol. This general insolubility sometimes created unexpected problems. When the initial aqueous suspension obtained after removal of methanol from another batch of the sponge collection was extracted with organic solvents, a brown precipitate was obtained. This brown precipitate, upon purification by LH-20 chromatography gave 79.

Desbromooroidin (79) was found to be unstable, and therefore, it was kept away from light and solvents whenever possible. Its IR spectrum showed a broad N-H absorption (3600-2800 cm\(^{-1}\)) and an amide carbonyl stretching band (1680 cm\(^{-1}\)). The UV spectrum of 79 exhibited a maximum at 269 nm,
similar to the metabolites of the oroidin group. The 'H nmr spectrum of 79 in CD$_3$OD was rather simple. Several decoupling experiments revealed the isolated spin system -CH=CH-CH$_2$- [δ 3.97(br d, 2H, J=5 Hz), 6.17(dt, 1H, J=16,5 Hz), 6.23 (d, 1H, J=16 Hz) ppm]. The 16 Hz coupling between the olefinic protons indicated an E configuration for the double bond. The 'H nmr spectrum also contained three aromatic resonances [δ 6.74 (s, 1H), 6.82(d, 1H, J=1.5 Hz), 6.92(d, 1H, J =1.5 Hz) ppm].

The 'H nmr spectrum of 79 in DMSO-d$_6$ (fig. 15) showed, in addition to the previously observed resonances, six exchangeable protons [δ 7.43(br s, 2H), 8.48(t, 1H, J=5 Hz), 11.85(br s, 1H), 12.0(br, 1H), 12.55(br, 1H) ppm]. The methylene protons in DMSO-d$_6$ appeared as a triplet, showing additional coupling to the exchangeable proton at δ 8.48 (t, 1H, J=5 Hz) ppm. This established the subunit -CH=CH-CH$_2$-NH-. The aromatic resonances [δ 6.90(dd, 1H, J=2.5,1.5 Hz), 6.95(dd, 1H, J=2.8,1.5 Hz) ppm] and the exchangeable proton at δ 11.85(br s, 1H) ppm could be assigned to a 2,4-disubstituted pyrrole ring which was indicated by a positive Ehrlich test (red colour). The remaining 'H nmr resonances [δ 7.43(br s, 2H), 12.0(br, 1H), 12.55(br, 1H) ppm] indicated an imidazolinium moiety suggesting the presence of 'oroidin skeleton' in 79.

The electron-impact mass spectrum of 79 did not show a molecular ion and was of no use. A FAB mass spectrum showed molecular ions at m/z 310 (M' +H) and m/z 312 (M' +2+H)
Fig. 15. $^1$H nmr spectrum of desbromooroidin (79) (400 MHz, DMSO-d$_6$).
Fig. 16. $^{13}$C nmr spectrum of desbromooroidin (79) (100.6 MHz, DMSO-$_d_6$).
indicating that 79 had the molecular formula $C_{11}H_{12}N_5OBr$. The $^{13}$C nmr spectrum of 79 was in agreement with this assignment (see fig. 16 and appendix 15). Further support for the proposed structure came from the comparison of spectral data with that of the previously reported metabolite keramadine (80) (see tables 2 and 3).

Mass spectrometry of other fractions obtained from the LH-20 chromatography indicated the presence of oroidin (73) [FAB: $m/z$ 388($M^+H$), 390($M^+2H$), 392($M^+4H$)], sceptrin (78) [FAB: $m/z$ 619($M^+H$), 621($M^+2H$), 623($M^+4H$)], and 4,5-dibromopyrrole-2-carboxamide (75) [EI ms: $m/z$ 266($M^+$), 268($M^+2$), 270($M^+4$)], but the trace amounts isolated were not sufficient for a complete identification of individual compounds. Due to the limited availability of the sponge material, work on these metabolites was not pursued.
Table 2. \(^1\)H nmr data for desbromooroidin (79) and keramadine (80).

<table>
<thead>
<tr>
<th>Position</th>
<th>(79^a)</th>
<th>(80^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.55 br</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>12.00 br</td>
<td>11.96 br s</td>
</tr>
<tr>
<td>4</td>
<td>6.86 br s</td>
<td>7.02 s</td>
</tr>
<tr>
<td>6</td>
<td>6.23 d, (J=16) Hz</td>
<td>6.23 (J=11) Hz</td>
</tr>
<tr>
<td>7</td>
<td>6.17 dt, (J=16,5) Hz</td>
<td>5.81 dt, (J=11,5.6) Hz</td>
</tr>
<tr>
<td>8</td>
<td>3.97 br t, (J=5) Hz</td>
<td>4.01 t, (J=5.6) Hz</td>
</tr>
<tr>
<td>9</td>
<td>8.48 t, (J=5) Hz</td>
<td>8.22 t, (J=5.6) Hz</td>
</tr>
<tr>
<td>12</td>
<td>11.85 br s</td>
<td>11.58 br s</td>
</tr>
<tr>
<td>13</td>
<td>6.95 dd, (J=2.8,1.5) Hz</td>
<td>6.92 dd, (J=2.9,1.5) Hz</td>
</tr>
<tr>
<td>15</td>
<td>6.90 dd, (J=2.5,1.5) Hz</td>
<td>6.80 dd, (J=2.9,1.5) Hz</td>
</tr>
<tr>
<td>N(2)</td>
<td>7.43 br s</td>
<td>7.59 br s</td>
</tr>
<tr>
<td>N(1)CH(_3)</td>
<td>-</td>
<td>3.38 s</td>
</tr>
</tbody>
</table>

\(a\). 400 MHz, DMSO-d\(_6\), \(b\). ref.57
Table 3. $^{13}$C nmr data for desbromooroidin (79) and keramadine (80).

<table>
<thead>
<tr>
<th>Carbon #</th>
<th>79$^a$</th>
<th>80$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>147.6 s</td>
<td>146.9 s</td>
</tr>
<tr>
<td>4</td>
<td>116.4 d$^c$</td>
<td>112.0</td>
</tr>
<tr>
<td>5</td>
<td>124.9 s</td>
<td>123.7 s</td>
</tr>
<tr>
<td>6</td>
<td>127.1 d</td>
<td>133.3 d</td>
</tr>
<tr>
<td>7</td>
<td>110.9 d$^c$</td>
<td>113.8 d</td>
</tr>
<tr>
<td>8</td>
<td>40.0</td>
<td>38.6 t</td>
</tr>
<tr>
<td>10</td>
<td>159.6 s</td>
<td>159.6 s</td>
</tr>
<tr>
<td>11</td>
<td>126.7 s</td>
<td>126.7 s</td>
</tr>
<tr>
<td>13</td>
<td>121.3 d</td>
<td>121.3 s</td>
</tr>
<tr>
<td>14</td>
<td>95.1 s</td>
<td>95.0 s</td>
</tr>
<tr>
<td>15</td>
<td>111.9 d</td>
<td>111.7 d</td>
</tr>
<tr>
<td>N(1)CH$_3$</td>
<td></td>
<td>29.2 q</td>
</tr>
</tbody>
</table>

$^a$ 100.6 MHz, DMSO-$d_6$, assignments are based on comparison, $^b$ ref.57, $^c$ may be interchanged.
C. DISCUSSION

Desbromooroidin (79) is the latest addition to the well known oroidin group of alkaloids found in marine sponges. Members of this family possess both pyrrole and imidazolinium moieties. The natural occurrence of 79 was foreseen by Faulkner et al. when they isolated sceptrin (78) which is conceptually the [2+2] cyclo adduct of 79. Although attempts in Faulkner's laboratory failed to provide any evidence favouring that concept, the natural occurrence of desbromooroidin (79) may be regarded as a favourable indication. However, the question whether desbromooroidin (79) is the biogenetic precursor of 78 still remains to be answered.

Many of the halogenated heterocyclic compounds of the oroidin group have been reported to possess antimicrobial activity. The antimicrobial constituent of *Agelas oroidas* was identified as 4,5-dibromo-2-cyanopyrrole (74). Sceptrin (78) was reported to have considerable antibacterial activity as well as some antifungal activity. The biological activity reported for keramadine (80) is interesting. It was isolated in a study of physiologically active substances of marine sponges by a Japanese group and was discovered to be an antagonist of serotonergic receptors.

Not surprisingly, desbromooroidin (79) was found to exhibit significant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* and antifungal
activity against *Phytophthora ultimum*, *Rhizoctonia solani* and *Helminthosporium sativum*.
III. A SECONDARY METABOLITE OF THE STARFISH

*Dermasterias imbricata*,

THAT ELICITS A SWIMMING RESPONSE IN THE SEA ANEMONE

*Stomphia coccinea*

A.1. INTRODUCTION

Starfishes, or sea stars, are intertidal marine invertebrates belonging to the class Asteroidea of the phylum Echinodermata. These fascinating animals have rather heavy arms which project from a central disc with an imperfect radial symmetry. Most species have 5-6 arms but the number may be as high as 40\(^6\)^5. The arms usually increase in width toward the base. The average diameter of a grown starfish is about 12 to 24 cm but there are some sea stars which are less than 2 cm in diameter. Asteroids are active predators, feeding upon all sorts of invertebrates and even fish\(^6\)^5.

Chemical studies on asteroids have led to the isolation of polyhydroxylated steroids and saponins\(^6\)^6. Saponins were previously known only from a large number of terrestrial plants. However, we now know that saponins are the typical metabolites of echinoderms. The general structure of saponins consists of an aglycone with a carbohydrate side chain. Saponins derived from starfishes are known as asterosaponins and they contain steroidal aglycones.

The presence of saponins in starfish was discovered in 1960 by Yasumoto and Hashimoto\(^6\)^7. In 1965 they isolated the
two major saponins of *Asterias amurensis*. The structure of one of them, asterosaponin A (87), was determined in 1973 by Ikegami *et al.*\(^6\). Thornasteroside A (88) is another example of an asterosaponin which was isolated from the starfish *Acanthaster planci*\(^6\). The presence of a sulfate group at C-3 and the attachment of an oligosaccharide moiety to C-6 of the aglycone are general features of asterosaponins. Sepsitoside A (89) is one of a group of unusual saponins discovered from *Echinaster sapositus*\(^7\). The absence of the sulfate group and the cyclization of the carbohydrate chain between C-3 and C-6 of the aglycone are the distinctly different features possessed by *E. sapositus* saponins.
Holothurins, saponins isolated from sea cucumbers, (for example, holothurin B (90)) are different from asterosaponins. The presence of a steroid aglycone in asterosaponins and a triterpenoid aglycone in holothurins is the basic difference. In both starfishes and sea cucumbers, the saponins are believed responsible for the general toxicity of the animals.

It has been known for a long time that various marine invertebrates such as sea anemones, brittlestars, sea urchins and molluscs display 'avoidance reactions' and 'escape responses' when they are in the presence of, or when they are contacted by, starfishes. According to some studies, this
unusual behavior of prey organisms is caused by asterosaponins\textsuperscript{73}, but it has also been found that some prey organisms react to substances other than asterosaponins\textsuperscript{74}.

2. THE LEATHER STAR, \textit{Dermasterias imbricata}

Biologists long ago discovered an interesting interaction between the Northeast Pacific sea anemone, \textit{Stomphia coccinea}, and the starfish, \textit{Dermasterias imbricata}\textsuperscript{75}. As Ward describes it, "when contacted by the starfish, the sea anemone responds in a striking and extraordinary manner: the anemone releases its basal disc from the substratum and then propels itself through the water by means of a series of whip-like motions"\textsuperscript{76}. He also reported that a single chemical substance was responsible for eliciting the unusual response in the anemone and suggested that the chemical was an aminopolysaccharide. Subsequent work aimed at elucidating the structure of the active metabolite of \textit{Dermasterias imbricata} was carried out by the groups of D. Ross and W. Ayer at the University of Alberta. Using a bioassay guided fractionation scheme they were able to isolate the pure metabolite and to show that it was a sulfur containing, 24-carbon compound\textsuperscript{74, 77}. 

B. ISOLATION AND STRUCTURE ELUCIDATION

In the present study, the starfish *Dermasterias imbricata* was collected from British Columbia coastal waters where it is commonly available. *Dermasterias imbricata* has five arms with an average diameter of about 15 cm in a grown animal. Adult specimens are usually orange-brown in colour.

The animals were immersed in methanol immediately after collection, and usually after 2-3 days, the methanol was decanted and filtered through Celite. The resulting filtrate was concentrated *in vacuo*, dissolved in water, and passed through XAD-4 resin. Imbricatine (91) was adsorbed onto the XAD-4 which was then washed repeatedly with distilled water prior to elution with hot methanol. The golden-yellow eluate was concentrated to dryness *in vacuo* and passed through Biogel P2 using 1% acetic acid in water as the eluent. The collected fractions were monitored by uv spectroscopy, and those with uv absorptions at 283 and 292 nm were pooled together and freeze-dried. The yellow solid obtained was further purified by chromatography on Sephadex LH-20 using methanol/water 4:1 as the eluent to give pure imbricatine (91) as an amorphous white solid. This purification procedure was a modification of the one originally employed by the University of Alberta groups.

Imbricatine (91) is a water-soluble compound whose ir spectrum (see fig. 17) had a broad absorption in the N-H and O-H region (3600-2800 cm⁻¹) and carboxylate stretching bands
at 1630 and 1595 cm\(^{-1}\). The molecular formula of imbricatine (91), \(C_{24}H_{26}N_4O_7S\), was determined from the mass spectra of the parent compound [FAB: 515(M\(^{+}\)+H)] and the dimethylpentaacetyl derivative 92 [FAB: 753(M\(^{+}\)+H); hrms: (M\(^{+}\)-OAcbenzyl) observed 603.1756, required for C\(_{27}\)H\(_{31}\)N\(_4\)O\(_{10}\)S 603.1761]. Its amino acid nature was indicated by a positive colour reaction with ninhydrin\(^2\) and uv absorptions at 283(\(\varepsilon\) 2300) and 292(\(\varepsilon\) 2100) nm.

Eighteen protons attached to carbons were shown by \(^1\)H and \(^{13}\)C ADEPT\(^2\) nmr spectra of imbricatine (91) (see fig.18 and appendix 17). The presence of three -CH-CH\(_2\)- units [\(^1\)H nmr: \(\delta\) 4.67(dd, 1H, \(J=8.4\) Hz), 3.22(dd, 1H, \(J=13.9,4.5\) Hz), 2.85(dd, 1H, \(J=13.9,8.4\) Hz); 3.76(dd, 1H, \(J=12.5,5\) Hz), 4.03(dd, 1H, \(J=16,5\) Hz), 2.71(dd, 1H, \(J=16,12.5\) Hz); 3.60(dd, 1H, \(J=9,5\) Hz), 3.30(dd, 1H, \(J=15,9\) Hz), 3.02(dd, 1H, \(J=15,5\) Hz) ppm] in 91 was established by \(^1\)H nmr decoupling experiments and a HETCOR experiment\(^2\) (a \(^1\)H/\(^{13}\)C nmr correlation experiment, see fig. 21). A 1,4-disubstituted phenyl ring was indicated by \(^1\)H nmr resonances at \(\delta\) 6.71(d, 2H, \(J=8\) Hz) and 7.04(d, 2H, \(J=8\) Hz) ppm. Comparison of the \(^{13}\)C nmr spectrum of 91 (\(\delta\) 170.5, 169.7, 159.7, 156.4, 155.8, 137.8, 137.7, 131.1, 130.5, 126.8, 126.5, 115.3, 112.7, 110.0, 104.0, 54.9, 52.9, 52.3, 37.3, 32.3, 27.2, 25.1 ppm, see fig. 19) with that of tyrosine (\(\delta\) 170.4, 156.9, 130.7, 124.8, 115.6, 53.7, 35.0 ppm) identified a benzylic fragment

\(^2\)(91) gave a brown colour with ninhydrin and that turned bright blue with H\(_2\)SO\(_4\) and heat. A similar colour reaction was observed for N-methylhistidines and tyrosine.
in imbricatine (91), and the the $^{13}$C resonances of 91 at $\delta$ 37.3 (t) and 52.9 (d) ppm were assigned to C-12 and C-1 respectively. An additional $^1$H nmr resonance at $\delta$ 6.42 (s) ppm indicated the presence of a polysubstituted aromatic ring. The $^{13}$C nmr spectrum of (91) also pointed to the presence of two carboxylic acid functionalities [$\delta$ 169.7(s) and 170.5(s) ppm]. A 5-thio-N-methylhistidine fragment in 91 could be identified by comparing $^1$H and $^{13}$C nmr spectral data to the values reported for the symmetrical disulfide 93 (see tables 4 and 5) isolated from echinoderm eggs. 

$$\text{R}=\text{H}$$

$$\text{R}=\text{Ac}\text{ R}=\text{Me}$$
Table 4. \(^1\)H nmr data for imbricatine (91), 94 and 93.

Chemical shift, \(\delta\) ppm

<table>
<thead>
<tr>
<th>Carbon #</th>
<th>91(^a)</th>
<th>94(^a)</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.67 dd, (J=8.4, 4.5) Hz</td>
<td>4.57 dd, (J=8.4) Hz</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>3.76 dd, (J=12.5, 5) Hz</td>
<td>3.20 dd, (J=12.4) Hz</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>4.03 dd, (J=16.5) Hz</td>
<td>2.90 dd, (J=16.4) Hz</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>2.71 dd, (J=12.5, 16) Hz</td>
<td>2.71 dd, (J=12.16) Hz</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>6.42 s</td>
<td>6.22 d, (J=2) Hz</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>3.22 dd, (J=13.9, 4.5) Hz</td>
<td>3.12 dd, (J=14.4) Hz</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>2.85 dd, (J=13.9, 8.4) Hz</td>
<td>2.80 dd, (J=14.8) Hz</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>7.04 d, (J=8) Hz</td>
<td>7.04 d, (J=8) Hz</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>6.71 d, (J=8) Hz</td>
<td>6.70 d, (J=8) Hz</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>7.73 s, 8.73 s*</td>
<td>-</td>
<td>8.95 s*</td>
</tr>
<tr>
<td>6'</td>
<td>3.30 dd, (J=15.9) Hz</td>
<td>-</td>
<td>3.44 d, 2H, (J=7.5) Hz*</td>
</tr>
<tr>
<td>7'</td>
<td>3.60 dd, (J=5.9) Hz</td>
<td>-</td>
<td>4.29 t, (J=7.5) Hz*</td>
</tr>
<tr>
<td>9'</td>
<td>3.66 s, 3.87 s*</td>
<td>-</td>
<td>3.94 s*</td>
</tr>
</tbody>
</table>

\(a\). spectra run in DMSO-d6.

\(*\). chemical shifts in D2O.
Table 5. $^{13}$C nmr data for imbricatine (91), 94 and 93.

Chemical shift, $\delta$ ppm

<table>
<thead>
<tr>
<th>Carbon #</th>
<th>91$^a$</th>
<th>94$^a$</th>
<th>93$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52.9</td>
<td>d</td>
<td>53.0</td>
</tr>
<tr>
<td>3</td>
<td>54.9</td>
<td>d</td>
<td>55.5</td>
</tr>
<tr>
<td>4</td>
<td>27.2</td>
<td>t</td>
<td>30.0</td>
</tr>
<tr>
<td>5</td>
<td>110.0</td>
<td>s</td>
<td>106.0</td>
</tr>
<tr>
<td>6</td>
<td>159.7</td>
<td>s</td>
<td>157.0</td>
</tr>
<tr>
<td>7</td>
<td>104.0</td>
<td>d</td>
<td>100.9</td>
</tr>
<tr>
<td>8</td>
<td>155.8</td>
<td>s</td>
<td>154.7</td>
</tr>
<tr>
<td>9</td>
<td>112.7</td>
<td>s</td>
<td>110.8</td>
</tr>
<tr>
<td>10</td>
<td>137.7</td>
<td>s</td>
<td>136.0</td>
</tr>
<tr>
<td>11</td>
<td>169.7</td>
<td>s</td>
<td>169.2</td>
</tr>
<tr>
<td>12</td>
<td>37.3</td>
<td>t</td>
<td>37.8</td>
</tr>
<tr>
<td>13</td>
<td>126.5</td>
<td>s</td>
<td>126.9</td>
</tr>
<tr>
<td>14</td>
<td>130.5</td>
<td>d</td>
<td>130.2</td>
</tr>
<tr>
<td>15</td>
<td>115.3</td>
<td>d</td>
<td>115.0</td>
</tr>
<tr>
<td>16</td>
<td>156.4</td>
<td>s</td>
<td>156.0</td>
</tr>
<tr>
<td>2'</td>
<td>137.8</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>126.8</td>
<td>s</td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>131.1</td>
<td>s</td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>25.1</td>
<td>t</td>
<td></td>
</tr>
<tr>
<td>7'</td>
<td>52.3</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>8'</td>
<td>170.5</td>
<td>s</td>
<td></td>
</tr>
<tr>
<td>9'</td>
<td>32.3</td>
<td>q</td>
<td></td>
</tr>
</tbody>
</table>

$^a$. spectra run in DMSO-$d_6$

$^*$. Chemical shifts in D$_2$O.
Fig. 17. Infrared spectrum of imbricatine (91) (KBr disc).
Fig. 18. $^1$H nmr spectrum of imbricatine (91) at r.t. (400 MHz, DMSO-d$_6$).
Fig. 19. $^1$H nmr spectrum of imbricatine (91) at 100 C (400 MHz, DMSO-d$_6$).
Fig. 20. $^{13}$C nmr spectrum of imbricatine (91) (75 MHz, DMSO-d$_6$).
Fig. 21. Contour plot of HETCOR 2D nmr spectrum of (91).

Only the $^1H$ and $^{13}C$ nmr chemical shift regions required to demonstrate the C-H correlations of three -CH-CH$_2$- systems were included.
Fig. 22. $^1$H nmr spectrum of 92 (400 MHz, CDCl$_3$).
Fig. 23. CI mass spectrum of 92.
The remaining eight protons required by the molecular formula could be located by a sequence of derivatization reactions. Imbricatine (91) could be methylated (MeOH/HCl, reflux, 2.5h) and acetylated (Ac₂O/pyridine, r.t., 20h) to give the dimethylpentaacetate (92), the ¹H nmr spectrum of which indicated the presence of three phenolic acetates (δ 2.19, 2.29, 2.42 ppm; 3H each), one N alkyl and one N,N dialkyl amide (δ 1.74, 1.91 ppm; 3H each), and two methyl esters (see fig. 22). The above functionalities account for all eight exchangeable protons.

A methine proton resonance at δ 4.73(q, J=8 Hz) ppm showed coupling to a methylene proton resonance at δ 3.18(m) ppm, and a resonance at δ 6.18(d, J=8 Hz) ppm establishing the presence of -CH₂-CH-NH- unit in 92, which in turn required the presence of -CH₂-CH-NH₂ in 91. This unit was assigned to the N-methylhistidine moiety in 91.

Catalytic hydrogenation of 91 (Pd on activated carbon, 24 h) failed to cleave the sulfide linkage. Reduction of imbricatine (91) with Ra-Ni (aqueous MeOH/reflux, 2.5h) did cleave the sulphide bond to give the tetrahydroisoquinoline 94, and N-methylhistidine, which was identical to 3-methylhistidine and different from 1-methylhistidine by tlc comparison. This was supported by gc and gcms comparison of N-trifluoroacetyl, n-butyl ester derivatives of N-methylhistidines⁷⁹. The EI mass spectrum of 94 did not show

---

⁷¹ 1-methylhistidine, Rf = 0.13; 3-methylhistidine, Rf = 0.10 in n-BuOH/AcOH/water 5.5:2:2.5.
a parent ion but the CI mass spectrum (see figs. 27 and 28) showed a parent ion at m/z 316 (M⁺+H) which was consistent with the molecular formula C₁₇H₁₇NO₅ (hrms: (M⁺-OHbenzyl) observed 208.1611, required for C₁₀H₁₀NO₄: 208.1610). The CI mass spectrum also showed fragment ions at m/z 270 (M⁺-CO₂H), 242 (M⁺-NH=CHCO₂H via retro Diels Alder), 208 (M⁺-OHbenzyl) and 164 (M⁺-(OHbenzyl+CO₂)) typical of tetrahydroisoquinolines⁸⁰ (see figs. 27 and 28). 'H nmr resonances at δ 6.70(d, 1H, J=8 Hz) and 7.04(d, 1H, J=8 Hz) ppm required that the benzyl residue in 94 and also in 91, contain a para hydroxyl substituent (see figs. 17, 24). In fact, the 'H and ¹³C nmr data for p-hydroxybenzyl substituent and C-1 are
Fig. 24. $^1H$ nmr spectrum of 94 at r.t. (270 MHz, DMSO-d$_6$).
Fig. 25. $^1$H nmr spectrum of 94 at 100 C (400 MHz, DMSO-$d_6$).
Fig. 26. $^{13}$C nmr spectrum of 94 (75 MHz, DMSO-$d_6$).
Fig. 27. CI mass spectrum of 94.
Fig. 28. Interpretation of CI ms of 94.
virtually identical in both 91 and 94 indicating that this region is least affected in the Ra-Ni reduction (see tables 4 and 5). 'H nmr resonances at $\delta$ 6.08(d, 1H, $J=2$ Hz) and 6.22(d, 1H, $J=2$ Hz) ppm in the spectrum of 94 were assigned to two meta protons on the aromatic ring of the tetrahydroisoquinoline moiety, requiring that the two hydroxyl substituents on this ring also be meta disposed. Imbricatine (91) has only a single proton ($\delta$ 6.42(s) ppm) on the aromatic ring of the isoquinoline moiety, the other non-phenolic position being occupied by the sulphide linkage.

Compound 97 and its trans isomer 98 were synthesized as model compounds for the Ra-Ni reduction product 94. A biogenetic-type synthetic procedure involving the reaction between Dopa methyl ester and epoxide 96 was used to prepare the model compounds 91 (see scheme 2). The cis configuration of the C1 and C3 substituents in 97 was confirmed by the observation of nuclear Overhauser enhancements in the 'H nmr resonances of H3 ($\delta$ 3.48(dd, $J=11.5$ Hz) ppm), H12 ($\delta$3.21(dd, $J=14.4$ Hz) ppm) and H12' ($\delta$ (dd, $J=14.8$ Hz) ppm) when the H1 resonance at $\delta$ 4.06(dd, $J=8.4$ Hz) ppm was irradiated. Both isomers 97 and 98 were converted to their respective CHCl₃ soluble derivatives 99 and 100.

N-Acetylated tetrahydroisoquinolines are known to display a doubling of their 'H nmr resonances. The minor trans isomer 100 showed the presence of two forms in almost equal amounts as determined by 'H nmr (see fig. 33). One form predominates in the case of the major cis isomer 99, the
ratio being 1:8 (see fig. 30). The coupling constants displayed by the protons on the heterocyclic ring of the tetrahydroisoquinoline moiety of the two different forms of 100 were different (for example, in 100 the two resonances for H3: \( \delta 4.63 (dd, J=3.6 \text{ Hz}) \) and \( 4.86 (dd, J=4.8 \text{ Hz}) \), see fig. 33) indicating that doubling of the nmr resonances is due to the existence of two conformers, not to the presence of two acetate rotamers as reported for 101\(^{82}\).

Comparison of the \(^1\)H nmr spectra of the model compound
Scheme 2. Synthesis of the benzyltetrahydroisoquinolines 97 and 98.
Fig. 29. Contour plot of HETCOR 2D nmr spectrum of 99.

Only the $^1$H and $^{13}$C nmr chemical shift regions required to demonstrate the C-H correlations of two -CH-CH$_2$- systems were included.
Fig. 30. $^1$H nmr spectrum of 99 (400 MHz, CDCl$_3$).
Fig. 31. $^{13}$C nmr spectrum of 99 (75 MHz, CDCl$_3$).
Fig. 32. CI mass spectrum of 99.
Fig. 33. $^1$H nmr spectrum of 100 (400 MHz, CDCl$_3$).
Fig. 34. $^{13}$C nmr spectrum of 100 (75 MHz, CDCl$_3$).
Fig. 35. CI mass spectrum of 100.
Fig. 36. $^1$H nmr spectrum of 102 (400 MHz, CDCl$_3$).
Fig. 37. CI mass spectrum of 102.
and its trans isomer 100 to that of the methyltetraacetyl derivative 102 (see table 6, figs. 30, 33 and 36) confirmed the benzyltetrahydroisoquinoline nature of 94 and indicated that the C1 and C3 substituents were cis as shown (see figs. 38 and 39 for interpretations of mass spectra of 99 and 102).

The \(^1\)H nmr resonance assigned to H-3 (\(\delta 3.20\) (dd, \(J=12,4\) Hz) ppm) in 94 showed a 4 Hz coupling to H4e and a 12 Hz coupling to H4a (table 4). This indicated that H3 is axial and the C3 carboxyl substituent is equatorial in 94. Further support for this assignment came from the observed coupling constants for H3 in model compounds 97 (H3: \(\delta 3.48\)(dd, \(J=11.5\) Hz) ppm) and 103 (H3: \(\delta 4.26\)(dd, \(J=11,7\) Hz) ppm)\(^8\).
H4e and H3a are both strongly deshielded in imbricatine (91) relative to their chemical shifts in the Ra-Ni reduction product 94 (table 4). The difference in chemical shifts observed for these two protons in 94 and 91 can best be explained if the thiohistidine sulfide linkage in imbricatine (91) is attached to C5 of the isoquinoline nucleus, which in turn requires that there be a proton meta to it at C7 and hydroxyl substituents at C6 and C8. An INAPT experiment\(^8\), which showed three bond couplings between H1 (δ 4.67 ppm) and both C10 (δ 137.7 ppm) and C8 (δ 155.8 ppm) of imbricatine (91), confirmed this assignment (see fig. 40). A second INAPT experiment showed three bond couplings between H7 (δ 6.42 ppm) and both C5 (δ 110.0 ppm) and C9 (δ 112.7 ppm). It also showed two bond couplings between H7 and both C6 (δ 159.7 ppm) and C8 (δ 155.8 ppm). This result confirmed the placement of a proton on C7 which is ortho to both C6 and C8 which bear hydroxyl substituents and it also confirmed the placement of thiohistidine sulfide linkage on C5 (see fig. 41).
Table 6. \textsuperscript{1}H nmr data for 102 and 99 (400 MHz, CDC1\textsubscript{3}).

Chemical shift, \textit{\delta} ppm

<table>
<thead>
<tr>
<th>Position</th>
<th>102</th>
<th>99</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.01 dd, \textit{\delta}=8.6 Hz</td>
<td>4.73 dd, \textit{\delta}=8.6 Hz</td>
</tr>
<tr>
<td>2</td>
<td>4.48 t, \textit{\delta}=10 Hz</td>
<td>4.49 t, \textit{\delta}=10 Hz</td>
</tr>
<tr>
<td>3</td>
<td>3.25 m</td>
<td>3.21 m</td>
</tr>
<tr>
<td>4</td>
<td>6.93* d, \textit{\delta}=2 Hz</td>
<td>6.67 s*</td>
</tr>
<tr>
<td>5</td>
<td>6.89* d, \textit{\delta}=2 Hz</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>3.28 dd, \textit{\delta}=14.8 Hz</td>
<td>3.28 dd, \textit{\delta}=14.8 Hz</td>
</tr>
<tr>
<td>7</td>
<td>2.88 dd, \textit{\delta}=14.6 Hz</td>
<td>2.88 dd, \textit{\delta}=14.6 Hz</td>
</tr>
<tr>
<td>8</td>
<td>7.29 d, \textit{\delta}=8 Hz</td>
<td>7.15 d, \textit{\delta}=8 Hz</td>
</tr>
<tr>
<td>9</td>
<td>7.04 d, \textit{\delta}=8 Hz</td>
<td>6.84 d, \textit{\delta}=8 Hz</td>
</tr>
<tr>
<td>10</td>
<td>-CH\textsubscript{3}</td>
<td>3.85 s</td>
</tr>
<tr>
<td>11</td>
<td>-OCOCH\textsubscript{3}</td>
<td>3.86 s, 3.80 s</td>
</tr>
<tr>
<td>12</td>
<td>-NCOCH\textsubscript{3}</td>
<td>2.21 s, 2.28 s</td>
</tr>
<tr>
<td>13</td>
<td>-NCOCH\textsubscript{3}</td>
<td>2.30 s, 6H</td>
</tr>
<tr>
<td>14</td>
<td>-NCOCH\textsubscript{3}</td>
<td>1.79 s</td>
</tr>
<tr>
<td>15</td>
<td>-NCOCH\textsubscript{3}</td>
<td>1.85 s</td>
</tr>
</tbody>
</table>

*+, maybe interchanged.
Fig. 39. Interpretation of CI ms of 102.
Fig. 39. Interpretation of CI ms of 99.
Fig. 40. INAPT $^{13}$C nmr spectrum of imbricatine (91) with selective irradiation of H1 (above). $^{13}$C nmr spectrum of imbricatine (91) (below). (75 MHz, DMSO-d$_6$)
Fig. 41. INAPT $^{13}$C nmr spectrum of imbricatine (91) with selective irradiation of H7 (above). $^{13}$C nmr spectrum of imbricatine (91) (below). (75 MHz, DMSO-d$_6$)
C. DISCUSSION

Marine organisms have thus far been a poor source of alkaloids relative to their terrestrial counterparts. Until the late 1960's, it was commonly believed that marine organisms were devoid of alkaloids. Recently, however, a number of alkaloids have been isolated from marine plants and invertebrates.

Because of its extraordinary structural features and also its impact on public health, the marine alkaloid, saxitoxin (28), holds a key place among marine natural products. It is one of the toxins that cause paralytic shellfish poisoning and it is a metabolite of the dinoflagellate *Gonyaulax catenella*. Lingbyatoxin A (104), isolated by Cardellina *et al.* in 1979, represents the first indole alkaloid from a marine source. Since then, a few more indole alkaloids have been isolated from marine species. Most of the simple indoles (e.g. 105) come from marine algae. Tribromoderivative 105 was isolated from the red alga *Laurencia brongniartii*. Bisindoles represented by 106 have been found in the blue-green alga *Rivularia firma*. Tyrian purple (107) is a well known metabolite isolated from various mollusc species. The antineoplastic metabolite aplysinopsin (108), isolated from five *Thorecta* species and *Verongia spengelli*, the cytotoxic compound dendroine (109) isolated from the marine tunicate *dendrodoa grossularia*, and 110 isolated from the sponge *Halichondria melanodocia* are three
examples of simple indole derivatives. Caulerpin (111), the structure of which was recently revised\textsuperscript{93}, has been isolated as the orange-red pigment from several *Caulerpa* species.

A series of linear peptide alkaloids including the celenamides (eg. celenamide A (112)) and clionamide (113) have been isolated from the Pacific sponge *Cliona celata* (Grant)\textsuperscript{94,95}. *Cypridine* luciferin (114) and similar compounds
are responsible for the bioluminescence exhibited by marine animals. The unusual carbazoles (eg. Hyellazole (115)) isolated from the blue green alga *Hyella caespitosa* are, not surprisingly, different from the carbazoles known from terrestrial sources. Flustramine (116) is typical of the tricyclic physostigmine alkaloids that are abundant in the bryozoan *Flustra foliacea*. Phidolopin (117), a purine alkaloid isolated from the bryozoan *Phidolophora pacifica*, contains the rarely found nitro group. It was later observed that many Northeast Pacific bryozoans contained metabolites related to phidolopin (117). Zoanthoxanthin (118) and pseudozoanthoxanthin (119) represent interesting metabolites known as zoanthoxanthins that have been isolated from the Mediterranean Zoanthidae species, *Epizoanthus arenaceus* and *Parazoanthus axinellae*. A novel group of alkaloids, which includes zoanthamine (120), have been isolated from an unknown *Zoanthus* species collected from the coast of India. The structure of the macrocyclic alkaloid ascidiacylamide (121), isolated from an unidentified tunicate collected in Australia, was confirmed recently by synthesis. Malingamides (eg. malingamide A (122)), majusculamides (eg. majusculamide A (123)), and pukeleimides (eg. pukeleimide A (124)) are three interesting classes of compounds isolated from the blue-green alga *Lingbya majuscula*.

The unusual thiohistidine moiety was found in a group of metabolites, adenochromines A-C (125-127) isolated by Prota
et al. from the cephalopod Octopus vulgaris. Later, the disulphide was discovered from the unfertilized eggs of the echinoderm Paracentrotus lividus.

The above examples are a selective sample of the marine alkaloids reported to date. It is not a complete list of all the nitrogenous metabolites known from marine organisms.

Members of the benzyltetrahydroisoquinoline alkaloids have thus far been isolated only from terrestrial plants. Two possible pathways have been postulated for the biosynthesis of benzyltetrahydroisoquinolines. Both involve a condensation reaction of dopamine (see scheme 3). The involvement of dopamine as a precursor leads to products with the C-6 and C-7 oxygen functionality that is found in all naturally occurring benzyltetrahydroisoquinolines (eg. coclaurine, laudanosine). The Dopa carboxyl group is lost in the biogenesis of all known benzyltetrahydroisoquinoline alkaloids. A few tetrahydroisoquinolines containing a carboxyl group at C3 have been isolated. Compound isolated from Mucuna seeds, is an example.

Imbricatine (91) represents the first example of a benzyltetrahydroisoquinoline alkaloid from a marine organism, and it is apparently the first example from a non-plant source. The C3 carboxyl substituent, the C6/C8 hydroxylation pattern, and the thiohistidine substituent represent structural features not previously encountered in this family of alkaloids. Furthermore, the thiohistidine moiety having the methyl group at N-3 is different from the previously
isolated marine N-methylhistidines. The structural similarities between imbricatine (91) and the adenochromines (125-127) is noteworthy. Although there are differences in the substitution pattern, all these compounds contain a phenyl ring with two phenols and a methylthiohistidine substituent. Evidence from model experiments indicated that Dopa is a biogenetic precursor for the adenochromines\(^{109}\).
Scheme 3. Proposed routes for the biosynthesis of benzyltetrahydroisoquinolines in plants. (excerpted from ref. 108)
In principle, a condensation of adenochromines A (125) or B (126) with a suitable substrate could lead to tetrahydroisoquinolines having the traditional C6, C7 hydroxylation pattern. Imbricatine (91), which contains C6, C8 hydroxylation pattern, may be biogenetically derived from a hitherto unknown amino acid 130. In 130 both meta disposed OH functionalities activate C-2'. Therefore, the OH groups in 130 are better situated to promote a condensation reaction leading to benzyltetrahydroisoquinolines than are the hydroxyl groups in Dopa. Is is possible that imbricatine (91) is the first example of a class of benzyl -tetrahydroisoquinolines that are biogenetically derived from
the amino acid 130.

Imbricatine (91) induced *S. coccinea* "swimming" behavior at very low concentrations in laboratory tests. It also displays significant activity in the L1210 (ED <1 µg/ml) and P388 (T/C 139 at 0.5 mg/kg) antineoplastic assays.

*The swimming behavior is very difficult to quantify, however, application of one to two drops of a 1 mg/ml solution of 91 in sea water consistently elicits the response.*
V. EXPERIMENTAL

The $^1$H and $^{13}$C nmr spectra were recorded on Bruker WH-400, Nicolet Oxford 270, Bruker HXS-270, Bruker WP-80 and Varian XL-300 spectrometers. Tetramethylsilane ($\delta=0$ ppm) was employed as an internal standard for $^1$H nmr spectra and CDCl$_3$ ($\delta=77.0$ ppm) or DMSO-d$_6$ ($\delta=39.5$ ppm) were used as both internal standards and solvents for $^{13}$C nmr unless otherwise indicated. Low-resolution electron impact and FAB mass spectra were recorded on an A.E.I. MS-902 spectrometer and high-resolution mass spectra were recorded on an A.E.I. MS-50 spectrometer. CI mass spectra were recorded on a Nermag R 10-10 C spectrometer. Infrared spectra were recorded on Perkin-Elmer 710B and 1710 spectrometers. A Bausch and Lomb Spectronic 2000 spectrometer was used to record uv absorption spectra. Optical rotations were measured on a Perkin-Elmer model 141 polarimeter using a 10 cm cell and a Fisher-Johns apparatus was used to determine melting points which are uncorrected.

Gas chromatography was performed on a Hewlett-Packard 5830A instrument. A flame ionisation detector was used for the detection of peaks. A Perkin-Elmer Series 2 instrument was used for hplc where a Perkin-Elmer LC-55 uv detector and/or a Perkin-Elmer LC-25 refractive index detector were employed for peak detection. A Whatman Magnum-9 Partisil 10 or a Magnum-9 ODS 10 column was used for preparative hplc. The hplc solvents were BDH Omni-solv grade or Fisher hplc.
grade; water was glass distilled. All other chromatography solvents were reagent grade. Merck Silica Gel 60 PF-254 was used for preparative tlc, Merck Silica Gel 230-400 mesh was used for flash chromatography and Merck Silica Gel 60 PF-254 with CaSO₄·1/2 H₂O was used in radial tlc.
Dictyota binghamiae

Collection Data

Dictyota binghamiae was collected by hand using SCUBA at depths of 3 to 5 m in shallow bays off Dixon Island and Execution Rock in Barkley Sound, British Columbia.

Extraction and Chromatographic Separation

Initial collections of the alga were dried in a convection oven at 50°C for 24 h. The dried alga was powdered in a Wiley mill prior to Soxhlet extraction with hexane. Evaporation of hexane extract in vacuo gave a dark green oil (0.02%).

Later collections of the alga were immersed in methanol immediately after the collection. Usually within 2-3 days, the methanol was decanted and the residue was homogenised in an commercial blender with fresh methanol. The combined methanol extracts were concentrated in vacuo to give an aqueous suspension which was exhaustively extracted with hexane. Removal of the hexane in vacuo gave a dark green oil. The collections that were not worked up immediately were stored at low temperature [4-(-5)°C] until used.

The crude hexane extracts were fractionated by step gradient flash chromatography using the following solvent sequence: (i) hexane (ii) hexane/chloroform 1:1
(iii) chloroform  (iv) chloroform/ethyl acetate 9:1  (v) chloroform/ethyl acetate 1:1.

The chloroform eluted components from the flash chromatography were further purified by radial tlc(hexane/ethyl acetate 95:5) to give crude samples of pachydictyol A (29), dictyoxide (35), dictyoxide A (66) and a pure sample of diacetal 61a. Final purification of 29, 35 and 66 was accomplished by reversed phase hplc (29 and 35; water/acetonitrile 20:80, 66; water/ethanol 20:80). The chloroform/ethyl acetate eluted components from the flash chromatography were further purified by radial tlc (hexane/ethyl acetate 85:15) to give a pure sample of diacetal (61b) and crude samples of dictyol G acetate (68), dictyotriol A diacetate (69), dictyol C (32) and a mixture of acetyl-dictyolic (49) and epidictyol B acetate (70). Crude 69 and 68 were purified by reversed phase hplc (water/ethanol 20:80) to give pure compounds. Final purification of 32 was achieved by preparative tlc (acetonitrile/ chloroform 20:80). The mixture of 49 and 70 was separated by preparative tlc (chloroform/ hexane/methanol 48:50:2) and hplc (hexane/ethyl acetate 85:15) to give pure compounds.

Pachydictyol A (29): obtained as a pale yellow oil (ca. 0.013%); ir(CHCl₃): 3300, 3050-2850, 1460, 1400 cm⁻¹; ¹H nmr(400 MHz, CDCl₃): δ 5.33(br s, 1H), 5.12(br t, J=6 Hz, 1H), 4.75(s, 1H), 4.74(br s, 1H), 3.92(br d, J=8 Hz, 1H), 2.67(q, J=10 Hz, 1H), 2.62(m, 1H), 2.49(m, 1H), 2.32(m,
1H), 1.81 (m, 3H), 1.69 (br s, 3H), 1.61 (br s, 3H), 1.00 (d, J=7 Hz, 3H) ppm; $^{13}$C nmr (100.6 MHz, CDCl$_3$): $\delta$ 152.60, 141.48, 131.46, 124.81, 124.02, 107.15, 75.23, 60.58, 47.79, 46.24, 40.54, 35.17, 34.93, 34.01, 25.71, 25.66, 23.61, 17.66, 17.54, 15.80 ppm; ms $m/z$ (rel. int.): 288(M$^+$, 47), 270(19), 255(5), 203(20), 175(13), 159(56), 109(25), 91(32), 69(73), 41(100), 28(93), Exact mass calcd. for C$_{20}$H$_{32}$O: 288.2454; found (hrms): 288.2453.

Dictyol C (32): obtained as a white crystalline compound (ca. 0.005%). m.p. 57 °C; $^1$H nmr (400 MHz, CDCl$_3$): $\delta$ 5.26 (br s, 1H), 5.12 (br t, J=8 Hz, 1H), 3.87 (dd, J=8, 4 Hz, 1H), 2.76 (br t, J=8 Hz, 1H), 2.21 (m, 1H), 2.15 (m, 1H), 2.05 (m, 1H), 1.93 (m, 1H), 1.89 (br dd, J=16, 6 Hz, 1H), 1.82 (br s, 3H), 1.68 (s, 3H), 1.75-1.40 (m, 5H), 1.60 (s, 3H), 1.35-1.10 (m, 3H), 1.20 (s, 3H), 0.98 (d, J=7 Hz, 3H) ppm; ms $m/z$ (rel. int.): 306(M$^+$, 12), 288(77), 270(14), 255(30), 177(50), 81(53), 69(81), 43(100), 41(80). Exact mass calcd. for C$_{20}$H$_{34}$O$_2$: 306.2560; found (hrms): 306.2564.

Dictyoxide (35): obtained as a pale yellow oil (ca. 0.008%). $^1$H nmr (400 MHz, CDCl$_3$): $\delta$ 5.50 (br s, 1H), 5.09 (t, J=8 Hz, 1H), 3.97 (m, 1H), 2.63 (br d, J=12 Hz, 1H), 2.41 (dt, J=12, 6 Hz, 1H), 2.04 (m, 2H), 2.00-1.85 (m, 2H), 1.82-1.72 (m, 1H), 1.78 (br s, 3H), 1.68 (br s, 3H), 1.60 (br s, 3H), 1.65-1.45 (m, 5H), 1.37 (m, 1H), 1.25 (s, 3H), 1.10 (m, 1H), 0.90 (d, J=7 Hz, 3H) ppm; $^{13}$C nmr (100.6 MHz, CDCl$_3$): $\delta$ 141.37, 130.57, 124.87, 123.66,
77.33, 74.19, 63.12, 61.30, 38.79, 38.38, 37.29, 34.51, 29.82, 26.32, 25.55, 22.23, 20.41, 17.63, 16.44, 15.91 ppm; ms m/z (rel. int.): 288(M⁺, 18), 270(3), 207(16), 178(17), 159(14), 121(39), 107(46), 69(50), 55(36), 43(54), 41(64), 28(100). Exact mass calcd. for C₂₀H₃₂O: 288.2454; found (hrms): 288.2465.

**Acetyldictyolal (49):** obtained as a pale yellow oil (ca. 0.007%). uv (CH₃OH): 230.5 nm (ε 6000); [α]D³ -154° (c 0.16, CH₃OH); ir (film): 2930, 2870, 1740, 1680, 1640, 1400, 1380, 1240, 1060 cm⁻¹; 'H nmr (270 MHz, CDCl₃): δ 9.40(d, J=1 Hz, 1H), 6.77(dd, J=4,8 Hz, 1H), 5.37(br d, J=11 Hz, 1H), 5.09(t, J=7 Hz, 1H), 4.6(m, 2H), 3.12(m, 1H), 3.00(br dd, J=10,15 Hz, 1H), 2.76(br t, J=8 Hz, 1H), 2.27(br d, J=10 Hz, 1H), 2.17(m, 1H), 2.00(s, 3H), 2.00-1.60(m, 8H), 1.78(s, 3H), 1.67(s, 3H), 1.57(s, 3H), 0.87(d, J=7 Hz, 3H) ppm; ms m/z (rel. int.): 346(M⁺, 56), 328(53), 303(15), 301(12), 286(13), 109(30), 82(60), 69(76), 55(49), 43(100), 41(90). Exact mass calcd. for C₂₂H₃₄O₃: 346.2509; found (hrms): 346.2517.

**Diacetal 61a:** obtained as a pale yellow oil; 'H nmr (400 MHz, CDCl₃): δ 5.85(d, J=8 Hz, 1H), 5.45(dd, J=3,12 Hz, 1H), 5.16(s, 1H), 5.13(t, J=6 Hz, 1H), 5.08(s, 1H), 3.38(s, 3H), 3.32(s, 3H), 3.07(br dd, J=12,16 Hz, 1H), 2.64(ddd, J=3,8,16 Hz, 1H), 2.27(br s, 1H), 2.21(br d, J=12 Hz, 1H), 2.05-1.85(m, 5H), 1.72(br s, 3H), 1.67(br s, 3H), 1.60(br s, 3H), 1.70-1.55 (m, 2H), 1.22(q, J=8 Hz, 2H), 0.99(d, J=7 Hz, 3H) ppm; ms m/z (rel.
int.): 348(M⁺), 316(14), 284(18), 201(18), 159(57), 145(95), 109(90), 69(85), 41(100). Exact mass calcd. for C₂₂H₃₆O₃: 348.2666; found (hrms): 348.2649.

Diactal 61b: ir (film): 3000-2850, 1480, 1460, 1390, 1120, 960 cm⁻¹; 'H nmr (400 MHz, CDCl₃): δ 5.91(br dd, J=8,2 Hz, 1H), 5.34(br s, 1H), 5.15(s, 1H), 5.14(t, J=6 Hz, 1H), 3.52(s, 3H), 3.35(s, 3H), 3.11(br dd, J=16,12 Hz, 1H), 2.74(ddd, J=16,9,4 Hz, 1H), 2.44(br s, 1H), 2.27(m, 1H), 1.75(br s, 3H), 1.72(br s, 3H), 1.60(s, 3H), 0.95(d, J=7 Hz, 3H) ppm; ms m/z (rel.int.): 348(M⁺,33), 316(71), 284(75), 109(48), 97(100), 69(54), 41(44). Exact mass calcd. for C₂₂H₃₆O₃: 348.2660; found (hrms): 348.2669.

Dictyoxide A (66): obtained as a pale yellow oil (ca.0.010%); [α]D²⁻5.6° (c 0.16, CHCl₃); ir (film): 2980, 2920, 1620, 1520, 1470, 1350, 1240 cm⁻¹; 'H nmr(400 MHz, CDCl₃): δ 5.37(br s, 1H), 5.20(br d, J=8 Hz, 1H), 4.82(s, 1H), 4.79(s, 1H), 4.45(ddd, J=12,8,4 Hz, 1H), 3.74(dd, J=10,4 Hz, 1H), 2.75(br t, J=10 Hz, 1H), 2.62(q, J=9 Hz, 1H), 2.58-2.18(m, 5H), 1.84(m, 1H), 1.79(br s, 3H), 1.70(br s, 3H), 1.65(br s, 3H), 1.70-1.50(m, 3H), 1.14(q, J=12 Hz, 1H), 0.95(d, J=7 Hz, 3H), ppm; ¹³C nmr(100.6 MHz, CDCl₃): δ 151.95, 142.97, 133.46, 126.84, 124.90, 106.81, 79.49, 68.55, 53.59, 46.64, 45.53, 39.67, 34.34, 34.21, 29.45, 25.78, 25.09, 20.21, 18.34, 17.26 ppm; ms m/z (rel.int.): 286(M⁺), 268(23), 177(23), 159(20), 245(20), 120(40), 109(63), 91(40), 82(66), 69(57), 67(54),
43(49), 41(91), 28(100). Exact mass calcd. for C$_{20}$H$_{30}$O: 286.2298; found (hrms): 286.2298.

Dictyol G acetate (68): obtained as a colourless oil (ca.0.015%); [α]$^D_{D}$+50.0° (c 0.13, CHCl$_3$); ir (film): 3500(OH), 3100-2850, 1725(C=O), 1460, 1386, 1250 cm$^{-1}$; $^1$H nmr(400 MHz, CDCl$_3$): δ 5.57(dt, J=4,8 Hz, 1H), 5.33(br s, 1H), 5.12(br s, J=8 Hz, 1H), 4.75(s, 1H), 4.73(s, 1H), 3.93(br d, J=8 Hz, 1H), 2.70-1.45(m, 12H), 2.02(s, 3H), 1.82(d, J=1 Hz, 3H), 1.74(d, J=1.5 Hz, 3H), 1.72(d, J=1.3 Hz, 3H), 1.27(m, 1H), 1.04(d, J=7 Hz, 3H) ppm; $^{13}$C nmr (100.6 MHz, CDCl$_3$): δ 170.48, 152.38, 141.36, 136.33, 124.51, 124.13, 107.21, 74.93, 70.58, 60.47, 48.03, 46.21, 40.45, 40.37, 33.99, 32.11, 25.62, 23.84, 21.25, 18.25, 18.12, 15.82 ppm; ms m/z (rel.int.): 346(M$^+$), 286(30), 268(20), 186(26), 159(21), 145(16), 133(17), 120(35), 109(50), 91(21), 82(100), 69(21), 55(23), 43(40). Exact mass calcd. for C$_{22}$H$_{34}$O$_3$: : 346.2509; found (hrms): 346.2487.

Dictyotriol A diacetate (69): obtained as a colourless oil (ca.0.031%). [α]$^D_{D}$+12.5° (c 0.11, CHCl$_3$); ir (film): 3450(OH), 3050-2850, 1730-1700(C=O), 1450, 1380, 1250 1030, 1000, 980 cm$^{-1}$; $^1$H nmr(400 MHz, CDCl$_3$): δ 5.59(dd, J=2,6 Hz, 1H), 5.34(br s, 1H), 5.10(s, 1H), 5.10(t, J=7 Hz, 1H), 5.03(s, 1H), 4.92(m, 1H), 3.87(dd, J=8,3 Hz, 1H), 2.95(br s, 1H), 2.79(q, J=10 Hz, 1H), 2.49(m, 1H), 2.40-2.15(m, 4H), 2.07(s, 3H), 2.06(s, 3H), 2.02(dd, J=10,3 Hz, 1H), 1.92(dd, J=15,10,2
Hz, 1H), 1.86(m, 1H), 1.82(m, 3H), 1.76(dd, J=15.6 Hz, 1H), 1.70(br s, 3H), 1.64(br s, 3H), 0.94(d, J=7 Hz, 3H) ppm; 13C nmr (100.6 MHz, CDCl3): δ 172.26(s), 170.13(s), 149.95(s), 142.10(s), 134.67(s), 123.84(d), 120.04(d), 113.56(t), 77.00(d), 76.48(d), 74.09(d), 59.85(d), 42.81(d), 37.69(d), 37.32(d), 33.98(t), 28.40(t), 27.94(t), 26.08(q), 21.85(q), 21.53(q), 18.26(q), 16.03(q), 12.62(q) ppm; ms m/z (rel. int.): 404(M+), 344(10), 284(23), 266(8), 157(35), 109(33), 69(42), 43(100). Exact mass calcd. for C24H36O6: 404.2564; found (hrms) 404.2570.

Epidictyol B acetate (70): obtained as a colourless oil (ca. 0.009%). [α]D25 +43.6° (c 0.3, CHCl3); ir (film): 3500(OH), 2950, 2870, 1740, 1460, 1380, 1280-1250 cm⁻¹; 1H nmr (400 MHz, CDCl3): δ 5.59(dd, J=6.2 Hz, 1H), 5.34(br s, 1H), 5.10(m, 1H), 5.10(br s, 1H), 5.04(br s, 1H), 3.92(dd, J=8.4 Hz, 1H), 2.87(q, J=10 Hz, 1H), 2.49(m, 1H), 2.25(m, 2H), 2.09(m, 1H), 2.03(s, 3H), 1.97(m, 2H), 1.90-1.60(m, 2H), 1.81(dd, J=1.5 Hz, 3H), 1.69(br s, 3H), 1.61(br s, 3H), 1.52(m, 2H), 1.20(m, 1H), 0.93(d, J=7 Hz, 3H) ppm; 13C nmr (100.6 MHz, CDCl3): δ 169.83, 149.86, 141.16, 131.61, 124.63, 124.00, 113.02, 76.83, 74.78, 60.91, 42.72, 39.48, 35.04, 34.27, 33.70, 28.04, 25.64, 25.57, 21.44, 17.70, 17.33, 15.61 ppm; ms m/z (rel. int.): 346(M⁺), 286(26), 268(13), 186(18), 109(36), 82(100), 69(68), 43(89), 41(93). Exact mass calcd. for C22H34O3: 346.2509; found (hrms): 346.2502.
Acid Hydrolysis of dictyol G acetate (68)

Dictyol G acetate (68) (5 mg) was dissolved in aqueous acetic acid (30%, 1 ml) containing a few drops of CH₃CN and the reaction mixture was stirred at room temperature for 24 h. It was then freeze-dried and the products were separated by preparative tlc (hexane/ethyl acetate 85:15) to give dictyoxide A (66) (0.9 mg, Rf 0.56) and its epimer (67). Compound 67: ¹H nmr (400 MHz, CDCl₃): δ 1.16(d, J=7 Hz, 1H), 1.68(s, 3H), 1.71(s, 3H), 3.71 (dd, J=9, 4 Hz, 1H), 4.22(ddd, J=12, 8, 4 Hz, 1H), 4.69(br s, 2H), 5.14(br d, J=8 Hz, 1H), 5.35(br s, 1H) ppm.

Li/EtNH₂ reduction of dictyol G acetate (68)

Dictyol G acetate (68) (20 mg) was dissolved in ethylamine (1.5 ml, anhydrous). Freshly cut Li was added in excess and the reaction mixture was stirred for 20 min. The solvent was then evaporated in vacuo and the products, including 7.0 mg of unreacted 68, were separated by tlc (hexane/ethyl acetate 95:5). The product, pachydictyol A (29) (2.9 mg) was identified by ¹H nmr and tlc.
Agelas sp.

Collection Data

The sponge was collected by hand using SCUBA at 10-15 m depths from reefs along the west coast near Colombo, Sri Lanka.

The animals were soaked in methanol immediately after the collection and stored at low temperature [4-(-5)°C] until used.

Extraction and Chromatographic Separation

Methanol was decanted from the collections and the residue was blended in a commercial blender with fresh methanol. The homogenate was filtered and the residue was washed with methanol until the filtrate was colourless. The combined methanol extracts were concentrated in vacuo to obtain an aqueous suspension which could be fractionated by either of the following methods:

(1) The aqueous suspension (from 30 g of sponge, dry weight, after extraction) was extracted with hexane, chloroform, and ethyl acetate separately. The three extracts were combined to give 1.2 g of a brown oil after removal of the solvent. The remaining aqueous suspension was freeze-dried to obtain 4.6 g of pale yellow solid.
The organic extract was chromatographed on Sephadex LH-20 using methanol/chloroform 1:1 as the eluant. Biologically active fractions from this initial separation were rechromatographed on the same column using first methanol/chloroform 4:6 and then methanol/dichloromethane 9:1 as the eluants to give 38 mg of the pure desbromooroidin (79).

The methanol soluble fraction of the freeze-dried material was purified on Sephadex LH-20 with methanol/dichloromethane 9:1 to yield an additional 178 mg of 79.

(2) The aqueous suspension (from 50 g of sponge, dry weight, after extraction) was sequentially extracted with hexane and ethyl acetate. A brown material which precipitated during the extraction was separated from the aqueous suspension by carefully withdrawing the supernatant. The methanol soluble portion of the brown precipitate (2.8 g) was chromatographed on Sephadex LH-20 eluting with methanol to obtain crude 79. This was rechromatographed on the same column using methanol/chloroform 1:1 as the eluant to give 600 mg of pure 79.

Desbromooroidin (79): gave colourless crystals of the hydrochloride, mp 250-260°C dec; uv (CH₃OH): 269.1 nm (ε 18000); ir (film): 3600-2800(br), 1680, 1620, 1570, 1520, 1330 cm⁻¹; 'H nmr (300 MHz, DMSO-d₆): δ 3.97(br t, J = 5 Hz,
2H), 6.17(dt, J=5, 16 Hz, 1H), 6.23(d, J=16 Hz, 1H), 6.86(br s, 1H), 6.90(dd, J=2.5, 1.5 Hz, 1H), 6.95(dd, J=2.8, 1.5 Hz, 1H), 7.43(s, 2H), 8.48(t, J=5 Hz, 1H), 11.85(br s, 1H), 12.00(br, 1H), 12.55(br, 1H) ppm; \(^{13}\text{C nmr}\) (100.6 MHz, DMSO-\(d_6\)): \(\delta\) 159.58(s), 147.60(s), 127.08(d), 126.74(s), 124.93(s), 121.26(d), 116.25(d), 111.91(d), 110.83(d), 95.10(s), 39.95 ppm; ms (FAB) \(m/z\): 310 (M\(^+\)+1), 312 (M\(^+\)+3).
Dermasterias imbricata

Collection Data

*Dermasterias imbricata* was collected by hand using SCUBA (-10 to -20 m) in Barkley Sound and Copper Cove in British Columbia. The animals were immersed in methanol immediately after collection.

Extraction and Chromatographic Separation

The methanol was decanted from the starfish after 2-3 days and the animals (wet weight 8 kg) were re-immersed in fresh methanol to obtain a second extract. The decanted methanol extracts were vacuum filtered through Celite. The filtrate was concentrated *in vacuo* resulting in a brown aqueous suspension which was diluted with water and passed through short columns of XAD-4 which had been equilibrated with water. The first liquid coming through these columns was collected and reapplied to a fresh column of XAD-4. All the XAD-4 columns were washed with water until the eluant was colourless and then with hot methanol. The golden yellow band eluted with methanol was collected and evaporated to dryness *in vacuo*. The resulting brown solid was dissolved in water and chromatographed on a Biogel P2 column using 1% AcOH in water as the eluant. The fractions from this column were monitored by uv spectroscopy (\( \lambda_{\text{max}} \) 283 and 292 nm) and tlc
(Rf 0.29 in n-BuOH/AcOH/water 5.5:2:2.5) for the presence of imbricatine (91). The fractions containing crude imbricatine (91) were combined and freeze-dried to obtain a pale yellow solid which was chromatographed on Sephadex LH-20 using methanol/ water 8:2 as the eluant to give pure imbricatine (91) as a white solid (ca. 0.004%).

Imbricatine (91): ir (KBr disc): 3700-2500(br), 1630, 1595, 1515, 1447, 1397, 1244, 1175, 1080, 840 cm⁻¹; uv (λ): 228(ε 10100), 248(sh, ε 5200), 283(ε 2300), 292(ε 2100) nm; ¹H nmr (400 MHz, DMSO-d₆): δ 7.73(s, 1H), 7.04(d, J=8 Hz, 2H), 6.71(d, J=8 Hz, 2H), 6.42(s, 1H), 4.67(dd, J=4.5, 8.4 Hz, 1H), 4.03(dd, J=5, 16 Hz, 1H), 3.76(dd, J=12.5, 5 Hz, 1H), 3.66(s; 3H), 3.60(dd, J =9, 5 Hz, 1H), 3.30(dd, J=15, 9 Hz, 1H), 3.22(dd, J=13.9, 4.5 Hz, 1H), 3.02(dd, J=15, 5 Hz, 1H), 2.85(dd, J=13.9, 8.4 Hz, 1H), 2.71(dd, J=12.5, 16 Hz, 1H) ppm; ¹³C nmr (75 MHz, DMSO-d₆): δ 170.5(s), 169.7(s), 159.7(s), 156.4(s), 155.8(s), 137.8(d), 137.7(s), 131.1(s), 130.5(d), 126.8(s), 126.5(s), 115.3(d), 112.7(s), 110.00(s), 104.00(d), 54.9(d), 52.9(d), 52.3(d), 37.3(t), 32.3(q), 27.2(t), 25.1(t) ppm; ms (FAB) m/z: 515 [C₂₄H₂₆N₄O₇S⁺]+H.

Preparation of derivative (92)

Imbricatine (91) (9 mg) was dissolved in methanol (saturated with HCl) and refluxed for 2.5 h. The solvent was then evaporated to dryness in vacuo and into that, acetic
anhydride (3 ml) and pyridine (3 ml) were added and the mixture was stirred at room temperature for 20 h. The crude reaction mixture obtained after removal of the solvents in vacuo was separated by reverse phase hplc (CH$_3$CN/water 3:7) to obtain 9 mg of the major product, compound 92.

Compound 92: $^1$H nmr (400 MHz, CDCl$_3$): $\delta$ 7.34 (br s, 1H), 7.29 (d, $J$=8 Hz, 2H), 7.02 (d, $J$=8 Hz, 2H), 6.89 (br s, 1H), 6.15 (br d, $J$=8 Hz, 1H), 5.06 (br t, $J$=6 Hz, 1H), 4.73 (br dd, $J$=8,16 Hz, 1H), 4.64 (br dd, $J$=6,16 Hz, 1H), 4.40 (br dd, $J$=6,12 Hz, 1H), 3.87 (s, 3H), 3.75 (s, 3H), 3.61 (s, 3H), 3.36 (dd, $J$=6,14 Hz, 1H), 3.17 (m, 1H), 3.10 (br d, $J$=16 Hz, 1H), 2.89 (dd, $J$=6.14 Hz, 1H), 2.41 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 1.90 (s, 3H), 1.74 (s, 3H) ppm; ms m/z (rel.int.): 603(4), 561(30), 543(25), 519(24), 501(18), 477(15), 459(10), 394(50), 352(60), 310(100), 268(100), 264(20), 222(55), 208(50), 193(30), 162(20), 127(47), 107(50); ms (FAB, CI) m/z: 753 [C$_{26}$H$_{40}$N$_{4}$O$_{12}$S$^+$+H]; hrms: (M$^+$-OAcbenzyl) observed 603.1756, exact mass calcd. for C$_{27}$H$_{31}$N$_{4}$O$_{10}$S 603.1761.

Analysis of N-methylhistidines by Gas Chromatography

1-Methylhistidine (1 mg) was dried in vacuo at 100°C for 0.5 h, n-BuOH (1.5 ml, saturated with HCl) added and stirred at 100°C for 15 min. The solvent was then evaporated to dryness at 100°C under a stream of nitrogen. Trifluoroacetic anhydride (0.1 ml) and dichloromethane (0.3 ml) were added into this and stirred at 125°C for 5 min. The derivative
obtained after removal of the solvents in vacuo was dissolved in dichloromethane and used for the gc analysis.

3-Methylhistidine and the N-methylhistidine obtained from the Ra-Ni reduction of 91 were treated similarly.

A column packed with SP 2250 coated on 100-120 mesh Supelcoport was used for gc. Initial temperature 125°C; 5 min. hold; rate 5°C/min.; final temperature 225°C.

N-trifluoroacetyl, n-butyl ester of 1-methylhistidine: \( R_t = 26.27 \text{ min.} \); gcms \( m/z \) (rel. int.): 321(\( M^+ \), 10), 220(88), 95(100).
N-trifluoroacetyl, n-butyl ester of 3-methylhistidine: \( R_t = 25.29 \text{ min.} \); gcms \( m/z \) (rel. int.): 321(2), 220(5), 208(16), 95(100).
N-trifluoroacetyl, n-butyl ester of N-methylhisidine from the Ra-Ni reduction of 91: \( R_t = 25.29 \text{ min.} \); gcms \( m/z \) (rel.int.): 220(4), 208(10), 95(100).

Ra-Ni Desulfurization of imbricatine (91)

Imbricatine (91) (16 mg) was dissolved in methanol (15 ml) and water (2 ml). Ra-Ni suspension (0.5 ml) was added and the reaction mixture was refluxed for 2.5 h under nitrogen. The reaction mixture was then vacuum filtered and hot methanol was used to repeatedly wash the residue. The filtrate, after removal of methanol in vacuo, was chromatographed on Sephadex LH-20 using methanol/water 9:1 as the eluant to give crude compound 94 which was further
purified by hplc (methanol/water 20:80) to obtain 8 mg of pure 94. Compound 94: 

**1H nmr (400 MHz, DMSO-d$_6$):**
- δ 7.04 (d, $J=8$ Hz, 2H), 6.71 (d, $J=8$ Hz, 1H), 6.27 (d, $J=2$ Hz, 1H), 6.13 (d, $J=2$ Hz, 1H), 4.57 (dd, $J=4,8$ Hz, 1H), 3.20 (dd, $J=4,12$ Hz, 1H), 3.12 (dd, $J=4,14$ Hz, 1H), 2.90 (dd, $J=16,4$ Hz, 1H), 2.80 (dd, $J=14,8$ Hz, 1H), 2.71 (dd, $J=12,16$ Hz, 1H) ppm; 

**13C nmr (100.6 MHz, DMSO-d$_6$):**
- δ 169.2 (s), 157.0 (s), 156.0 (s), 154.7 (s), 136.0 (s), 130.2 (d), 126.9 (s), 115.0 (d), 110.8 (s), 106.0 (d), 100.9 (d), 55.5 (d), 53.0 (d), 37.8 (t), 30.0 (t) ppm; 

**ms (CI) m/z (rel. int.):**
- 316 (M$^+$H, 1), 272 (0.8), 242 (1), 270 (2), 208 (6), 198 (1), 164 (6), 162 (4), 152 (10), 137 (6), 124 (13), 121 (10), 109 (35), 108 (35), 107 (21); 

hrms: (M$^+-$OHbenzyl) observed 208.1611, exact mass calcd. for C$_{10}$H$_{10}$NO$_2$: 208.1610.

**Preparation of compound 102**

Compound 94 (3 mg) was refluxed with MeOH (saturated with HCl) for 2.5 h. The methanol was evaporated to dryness in vacuo. Acetic anhydride (1 ml) and pyridine (1 ml) were added to the residue and the mixture was stirred at r.t. for 20 h. The crude product obtained after removal of solvents was purified by hplc (EtOAc/hexane 3:2) to give 2 mg of pure 102. Compound 102: obtained as a white solid; 

**1H nmr (400 MHz, CDCl$_3$):**
- δ 7.29 (d, $J=8$ Hz, 2H), 7.04 (d, $J=8$ Hz, 2H), 6.95 (d, $J=2$ Hz, 1H), 6.89 (d, $J=2$ Hz, 1H), 5.06 (dd, $J=8,7$ Hz, 1H), 4.48 (dd, $J=11,9$ Hz, 1H), 3.85 (s, 3H), 3.32 (dd, $J=14,8$ Hz, 2H).
Hz, 1H), 3.25(m, 2H), 2.88(dd, J=14, 7 Hz, 1H), 2.30(s, 3H), 2.21(s, 3H), 1.79(s, 3H) ppm; ms (CI) m/z (rel. int.): 498(M+H, 14), 456(15), 348(56), 306(52), 264(46), 222(52), 162(19), 107(100); hrms: (M+-OAc benzyl) observed 348.1084, exact mass calcd. for C17H18NO7: 348.1083.

Preparation of methyl-3-(4-methoxyphenyl)glycidate 95

Anisaldehyde (5 ml) and methylchloroacetate (5 ml) were added slowly over a period of 2.5 h into a cold solution [0-(-5)°C] of Na (1.5 g) in MeOH (25 ml). The reaction mixture was stirred for another 2.5 h on an ice/water bath and then for 3 h at r.t. It was then poured onto ice-water, filtered and the filtrate was washed several times with ice-cold water to give 7 g of 95 as a pale yellow powder. Compound 95: 1H nmr (270 MHz, DMSO-d6): δ 7.29(d, J=8 Hz, 2H), 6.93(d, J=8 Hz, 2H), 4.10(br s, 1H), 3.79(br s, 1H), 3.78(s, 3H), 3.75(s, 3H) ppm; ms m/z (rel. int.): 208(M+, 10), 192(10), 161(18), 151(54), 135(12), 121(100), 105(9), 91(33), 77(36).

Synthesis of compounds 97 and 98

DL-Dopa methyl ester hydrochloride (580 mg, prepared from DL-Dopa, MeOH/HCl, reflux, 2.5 h) in 10 ml of MeOH was added to a solution of 610 mg of sodium (4-methoxyphenyl)glycidate (96) (prepared by the hydrolysis
of 95 with MeONa) in 15 ml of water. The pH of the solution was adjusted to 4 with AcOH. The reaction mixture was then stirred at 35°C for 36 h.

At the end of this period, methanol was removed in vacuo to obtain an aqueous suspension which was partitioned between EtOAc and 10% HCl. The aqueous layer was next neutralized with K$_2$CO$_3$ and extracted with EtOAc. This EtOAc extract was washed with water, dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo to give a mixture of compounds 97 and 98 which was separated by flash chromatography (EtOAc/CHCl$_3$ 3:2) on silica to obtain 145 mg of 97 and 45 mg of 98.

Compound 97: obtained as a white solid. $^1$H nmr (270 MHz, DMSO-d$_6$): $\delta$ 7.17(d, $J=8$ Hz, 2H), 6.84(d, $J=8$ Hz, 2H), 6.70(s, 1H), 6.43(s, 1H), 4.00(dd, $J=8,4$ Hz, 1H), 3.70(s, 3H), 3.58(s, 3H), 3.48(dd, $J=11,5$ Hz, 1H), 3.21(dd, $J=14,4$ Hz, 1H), 2.71(dd, $J=16,5$ Hz, 1H), 2.56(dd, $J=14,8$ Hz, 1H) ppm; ms (CI) $m/z$ (rel. int.): 344(M$^+$+H, 16), 284(1), 222(100), 162(11), 134(1), 121(13); hrms: observed 343.1403, exact mass calcd. for C$_{17}$H$_{21}$N$_5$: 343.1420.

Compound 98: obtained as a pale yellow oil. $^1$H nmr (400 MHz, DMSO-d$_6$): $\delta$ 7.18(d, $J=8$ Hz, 2H), 6.88(d, $J=8$ Hz, 2H), 4.00(dd, $J=10,4$ Hz, 1H), 3.87(dd, $J=9,5$ Hz, 1H), 3.74(s, 3H), 3.63(s, 3H), 2.86(dd, $J=14,4$ Hz, 1H), 2.79(dd, $J=16,5$ Hz, 1H), 2.74(dd, $J=10,4$ Hz, 1H), 2.64(dd, $J=16,9$ Hz, 1H) ppm.
Preparation of 99

Acetic anhydride (2 ml) and pyridine (2 ml) were added to 25 mg of 97 and stirred at r.t. for 20 h. Pure 99 (38 mg) was obtained after removal of solvent from the reaction mixture.

Compound 99: obtained as a white solid. $^1$H nmr (400 MHz, CDCl$_3$): δ 7.15(d, $J$=8 Hz, 2H), 7.10(s, 1H), 6.84(d, $J$=8 Hz, 2H), 6.67(s, 1H), 4.73(dd, $J$=8,7 Hz, 1H), 4.49(t, $J$=10 Hz, 1H), 3.84(s, 3H), 3.79(s, 3H), 3.27(dd, $J$=14,8 Hz, 1H), 3.21(m, 2H), 2.88(dd, $J$=14,7 Hz, 1H), 2.28(s, 3H), 2.25(s, 3H), 1.85(s, 3H) ppm; $^{13}$C nmr (75 MHz, CDCl$_3$): δ 172.7(s), 170.6(s), 168.1(s), 168.0(s), 158.5(s), 141.2(s), 140.4(s), 135.5(s), 130.6(d), 129.3(s), 122.7(d), 121.7(d), 114.0(d), 61.2(d), 55.4(d), 55.1(q), 52.3(q), 42.5(t), 29.7(t), 21.2(q), 20.4(q), 20.5(q) ppm; ms (CI) m/z (rel. int.): 470(M$^+$+H, 42), 428(42), 348(100), 306(55), 264(64), 222(19), 162(24), 121(36). hrms: (M$^+$-OMebenzyl) 348.1093, exact mass calcd. for C$_{17}$H$_{18}$NO$_7$: 348.1083.

Preparation of 100

Compound 100 was prepared from 20 mg of 98 by the method described for the preparation of 99 from 97. The crude product obtained was purified by hplc (EtOAc/hexane 3:2) to obtain 20 mg of pure 100 as an oil.

Compound 100: $^1$H nmr (400 MHz, CDCl$_3$): δ 6.98(s, 9H),
6.89(s, 1H), 6.87(d, J=8 Hz, 1.8H), 6.79(d, J=8 Hz, 1.8H), 6.70(s, 4H), 6.69(s, .9H), 6.53(s, 1H), 5.36(t, J=5 Hz, 1H), 4.92(t, J=7 Hz, .9H), 4.86(dd, J=8,4 Hz, .9H), 4.63(dd, J=6,3 Hz, 1H), 3.78(s, 2.7H), 3.75, 3H), 3.58(s, 2.7H), 3.51(s, 3H), 3.3(m, 2H), 3.10-2.85(m, 4.4H), 2.43(dd, J=16,6 Hz, 1H), 2.26(s, 2.7H), 2.25(s, 3H), 2.24(s, 2.7H), 2.23(s, 3H), 2.13(s, 3H), 2.06(s, 2.7H) ppm; ms (Cl) m/z (rel. int): 470(M⁺+H, 26), 428(17), 348(100), 306(39), 264(41), 222(13), 162(18), 134(1), 121(23); hrms: (M⁺-OMebenzyl) observed 348.1090, exact mass calcd. for C₁₇H₁₈NO₇ 348.1083.
VI. APPENDICES
Appendix 1. $^1$H nmr spectrum of pachydictyol A (29) (400 MHz, CDCl$_3$).
Appendix 2. $^{13}$C nmr spectrum of pachydictyol A (29) (100.6 MHz, CDCl$_3$).
Appendix 4. $^1$H nmr spectrum of dictyol C (32) (400 MHz, CDCl$_3$).
Appendix 6. $^1H$ nmr spectrum of dictyoxide (35) (400 MHz, CDCl$_3$).
Appendix 7. $^{13}$C nmr spectrum of dictyoxide (35) (100.6 MHz, CDCl$_3$).
Appendix 9. $^1$H nmr spectrum of acetyldictyolal (49) (400 MHz, CDCl$_3$).
Appendix 10. Mass spectrum of acetyldictyolal (49).
Appendix 11. $^1$H nmr spectrum of acetal 61a. (400 MHz, CDCl$_3$).
Appendix 13. $^1$H nmr spectrum of acetal 61b (400 MHz, CDCl$_3$).
Appendix 15. SFORD $^{13}$C nmr spectrum of desbromooroidin (79) (75 MHz, DMSO-d$_6$).
Appendix 16. $^{13}$C nmr spectrum of imbricatine (91) ($^1$H gated decoupled, 75 MHz, DMSO-$d_6$).
Appendix 17. ADEPT $^{13}$C nmr spectra of imbricatine (91) (75 MHz, DMSO-d$_6$).
Appendix 18. $^{13}$C nmr spectrum of (94) ($^1$H gated decoupled, 75 MHz, DMSO-$d_6$).
Appendix 19. ADEPT $^{13}$C nmr spectra of 94 (75 MHz, DMSO-d$_6$).
Appendix 20. APT $^{13}$C nmr spectrum of 99. (75 MHz, CDCl$_3$).
VII. REFERENCES


33. Danise, B.; Minale, L.; Riccio, R.; Amico, V.; Oriente, G.; Piatelli, M.; Tringali, C.; Fattorusso, E.; Magno,
34. Amico, V.; Oriente, G.; Piatelli, M.; Tringali, C.; *Phytochemistry*, 1979, 18, 1895.


72. for examples see: (a) Feder, H. M. *Sci. Amer.*, 1972, 227, 92; (b) Bullock, T. H. *Behaviour*, 1953, 5, 130.


74. ref. 65, p 321.


78. Palumbo, A.; Misuraca, G.; D'Ischica, H.; Donaudy, F.;


100. Tischler, M.; Ayer, S. W.; Andersen, R. J. Comp. Biochem. Physiol., 1986, 84B, 43.


110. Carried out by a) Dr. T Allen, Dept. of Pharmacology, U. of Alberta, and b) Bristol Myers Ltd.