STRUCTURAL AND SYNTHETIC INVESTIGATIONS OF SOUTH AFRICAN MARINE NATURAL PRODUCTS

THESIS

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LIST OF ABBREVIATIONS

CIMS	Chemical Ionisation Mass Spectrometry
COSY	^{1}H-^{1}H-homonuclear Correlation spectroscopy
DEPT	Distortionless Enhancement by Polarisation Transfer
EIMS	Electron Impact Mass Spectrometry
ESMS	Electrospray Mass Spectrometry
FABMS	Fast Atom Bombardment Mass Spectrometry
GC-MS	Gas Chromatography-Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
HOHAHA	Homonuclear Hartmann Hahn
HPLC	High Performance Liquid Chromatography
HPLC-ESMS	High Performance Liquid Chromatography-Electrospray Mass Spectrometry
HRCIMS	High Resolution Chemical Ionisation Mass Spectrometry
HREIMS	High Resolution Electron Impact Mass Spectrometry
HRFABMS	High Resolution Fast Atom Bombardment Mass Spectrometry
IR	Infrared
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Enhancement
NOEDS	Nuclear Overhauser Enhancement Difference Spectroscopy
NOESY	Nuclear Overhauser Enhancement Spectroscopy
ROESY	Rotating Frame Nuclear Overhauser and Exchange Spectroscopy
SCUBA	Self Contained Underwater Breathing Apparatus
TLC	Thin Layer Chromatography
UV	Ultra Violet
br	broad (used in conjunction with s, d, or t)
d	doublet
eV	electron Volt
Fn	fraction

m	multiplet
q	quartet
s	singlet
t	triplet

DIBAH	Diisobutylaluminium hydride
DMSO	Dimethyl sulfoxide
LAH	Lithium aluminium hydride
PPA	Polyphosphoric acid
PTSA	*para*-Toluene sulfonic acid
Ra Ni	Raney nickel
TFA	Trifluoroacetic acid

ABSTRACT

A chemical investigation of six different marine invertebrates, collected along the South African coastline, resulted in the isolation and structural elucidation of fifteen previously undescribed secondary metabolites along with seven known compounds. The structures of the new metabolites were determined by a combination of spectroscopic and chemical methods.

The endemic false limpet *Siphonaria capensis* was shown to contain two unusual polypropionate metabolites capensinone **(162)** and capensifuranone **(163)** as well as 2,4,6,8-tetramethyl-2-undecenoic acid **(164)** and the known polypropionates (*E*)- and (*Z*)-siphonarienfuranone **(149** and **161)**. Capensinone is the first example of a marine polypropionate containing a cyclopentenone moiety.

An investigation of the endemic South African soft coral *Pieterfaurea unilobata* yielded six new, highly oxygenated, pregnadiene sterols **(180-185)** and the known metabolite **(169)**. Compounds **180-185** are the first pregnadienes obtained from the marine environment containing a C-7 substituent. An alternative procedure for the quick assignment of the absolute configuration at C-3 in this series of compounds was proposed.

A comparison of the pyrroloiminoquinone alkaloids of three undescribed latrunculid sponges resulted in the isolation of 3-dihydrodiscorhabdin C **(243)**, 3-dihydrodiscorhabdin B **(244)**, discorhabdin H **(197)** and the previously reported alkaloids discorhabdin A **(189)** and discorhabdin D **(192)**. While all three sponges were found to be morphologically different they all contained discorhabdin A as the major metabolite and discorhabdin H as one of their minor metabolites. It was found that a feature common to most of the South African latrunculid sponges is the reduction of the C-3 carbonyl group in some of the minor metabolites.

The indole alkaloids, dilemmaones A-C **(261-263)**, containing an unusual cyclopentanone-indole skeleton, were isolated in trace amounts by bioassay guided fractionation of an extract obtained from a mixed collection of sponges collected near

Cape Town. In an attempt to acquire more of these novel compounds for further investigation of their biological activity, several synthetic strategies towards their total synthesis were explored. A key feature of these approaches was the exploitation of the regioselective Gassman's *ortho*-alkylation procedure for the introduction of an aromatic methyl substituent.

Chapter One

INTRODUCTION

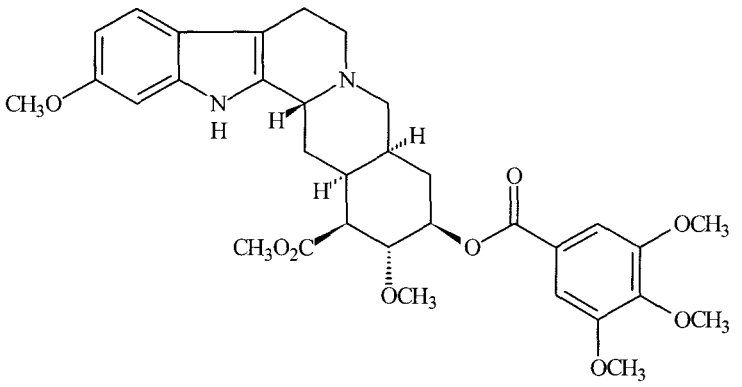
1.1 WHAT ARE NATURAL PRODUCTS?

Primary metabolites (sugars, amino acids, nucleotides) are organic substances produced by all living organisms and are essential for the survival of the organism. Natural products or secondary metabolites, on the other hand, are organic compounds of natural origin that are unique to one organism, or common to a small number of closely related organisms. Although not apparently essential for the day to day existence of the individual, secondary metabolites often play a key role in the long term survival of the species. Various biological roles, such as feeding deterrence, sex attractants, *etc.* have only been established for a small proportion of the many thousands of natural products isolated and identified from both marine and terrestrial microorganisms, plants and invertebrates. Williams *et al.*[1] have reviewed several hypotheses which attempt to explain, in fundamental terms, why these organisms produce natural products and conclude that the great diversity and biological activity of natural products may confer an evolutionary advantage to the producing organisms, by allowing them to communicate information to potential mates, competitors and predators.

1.2 WHY STUDY NATURAL PRODUCTS?

The origin of natural product chemistry can be traced back to the earliest investigations of plants for food and medicine. Nature has been the original source of drugs to treat various ailments. A number of compendia (*e.g.* the Ayurveda, 6000 BC), describing various herbal mixtures as medicinal agents, have been compiled through the centuries. As the knowledge of chemistry evolved during the 19th and early 20th centuries, the active principles of herbal mixtures were isolated and then formulated into various dosage forms. For example, the Indian plant, *Rauwolfia serpentina*, has been used for thousands of years to treat hypertension and insanity.[2] Half a century ago scientists in the Ciba laboratories isolated and identified the main active constituent, reserpine **(1)**, responsible for most of the sedative and hypotensive properties of the plant. Ciba subsequently marketed reserpine

("Serpasil") in 1953 as a hypotensive drug.[3] Reserpine is only one of the many examples of drugs from natural products which abound in the literature.



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Plants and microorganisms are still a very important source of new drugs. Today, some 25% of all pharmaceutical sales are drugs derived from plant natural products, and an additional 12% are based on microbially produced drugs.[4] The structural diversity of natural products make them a valuable source of novel lead compounds with activity against newly discovered therapeutic targets.[5] Furthermore, natural products often occur as families of related molecules so that it is often possible to obtain structure-activity information from the investigation of naturally occurring homologues of a bioactive natural product. In addition, the bioactivity of lead compounds, found from the screening of natural products, can be optimized by synthetic chemistry or by the application of combinatorial approaches to drug discovery. Since only a fraction of the world's biodiversity has been tested for pharmacological activity, it can be assumed that natural products will continue to offer new leads for novel therapeutic targets into the next millenium.

Apart from their importance in the drug discovery process, natural products are also important in other areas of study. For example, natural products chemistry has been, and still is, central to the continuing development of organic chemistry - new synthetic methodologies are being developed as synthetic chemists "battle" to synthesize the complicated chemical structures produced by nature.[6] Furthermore, the importance of natural products in mediating interactions between organisms is also slowly beginning to

be appreciated. Thus, the study of natural products and their involvement in chemical ecology will increase our understanding of the ecosystems that surround us.

1.3 WHY STUDY MARINE NATURAL PRODUCTS?

Marine natural products have received increasing attention from chemists and pharmacologists during the last three to four decades. A number of reviews describing the chemistry, ecology and biomedical potential of marine natural products have been published during this period.[7] It has been estimated that marine organisms form approximately half of the total global biodiversity.[8] This, combined with the fact that only an estimated 10 000 compounds from marine organisms are known, compared to about 200 000 from terrestrial sources,[7,9] suggest that more novel compounds await discovery from the organisms that inhabit the worlds oceans.

Oceans cover the majority of the earth's surface and provide a habitat for over 500 000 species of plants, animals and countless microorganisms.[10] It is not surprising that marine organisms, which are adapted to environments completely different from those of terrestrial species, will have evolved different secondary metabolic pathways. One of the most obvious differences between terrestrial and marine natural products is the frequent incorporation of halogens, particularly bromine, into the latter. This is clearly an adaptation to the marine environment which contains *ca* 19 g L^{-1} of chloride and 65 mg L^{-1} of bromide.[10]

Many marine invertebrates (sponges, soft corals, sea squirts, *etc*) are immobile, brightly coloured, soft-bodied and lack physical protection in the form of spines or shells. In the absence of any physical protection or an immune system many of these organisms have evolved chemical defense mechanisms, incorporating secondary metabolites, in order to ensure their survival.[11-13]

1.4 SOUTH AFRICAN MARINE NATURAL PRODUCTS

Southern Africa has two major currents sweeping its coasts: the Agulhas flows down the east coast, and the Benguela up the west coast (Figure 1.1). The Agulhas brings warm water from the tropics to the Mozambique/Kwazulu-Natal coast while the occasional upwelling of cold water from Port St Johns to Cape Point results in the latter region experiencing cooler coastal waters. The Benguela current which runs parallel to the west coast is cold and the contrast between the two currents manifests itself in the different marine *fauna* and *flora* found on the two coasts.[14] The subtropical east coast from southern Mozambique to Port St Johns in the Transkei is inhabited largely by circumtropical marine organisms. However, few tropical species occur along the warm temperate south east coast, stretching from Port St Johns to Cape Point, and this region is characterized by a high level of endemism.[14] Day *et al.*[15] reported that approximately 66% of the benthic invertebrate species occurring in False Bay are South African endemics. The colder water of the west coast from Cape Point to Walvis Bay is dominated by kelp beds.

With the exception of soft corals[16] and nudibranchs,[17] South African marine invertebrates are generally not well described. In the last two decades a number of research groups have studied the marine natural product chemistry of South African marine organisms. Unfortunately, apart from the "new chemistry" that has been published, very little information regarding the number and distribution of species investigated, or the prevalence of "known metabolites", has been reported. Known metabolites, and thus the organisms producing them, are generally not considered worth publishing unless they occur with new metabolites. This information can provide very important clues regarding the evolution of similar metabolic pathways in different organisms.[18]

The following review covers the marine natural products isolated (both "known" and "new" metabolites) from South African marine invertebrates. Only reports where a structure has been unambiguously determined are included. Therefore the pioneering work at the University of Cape Town (1985 - 1988),[19] which yielded several partial structures, has been excluded. Soft corals and sponges are the two main groups of South African

marine invertebrates that have been studied by marine natural product chemists. Fewer reports describe the chemistry of South African molluscs, ascidians and algae. As far as possible, the metabolites presented in the following review are grouped according to their structural class within the phyla in which they occur.

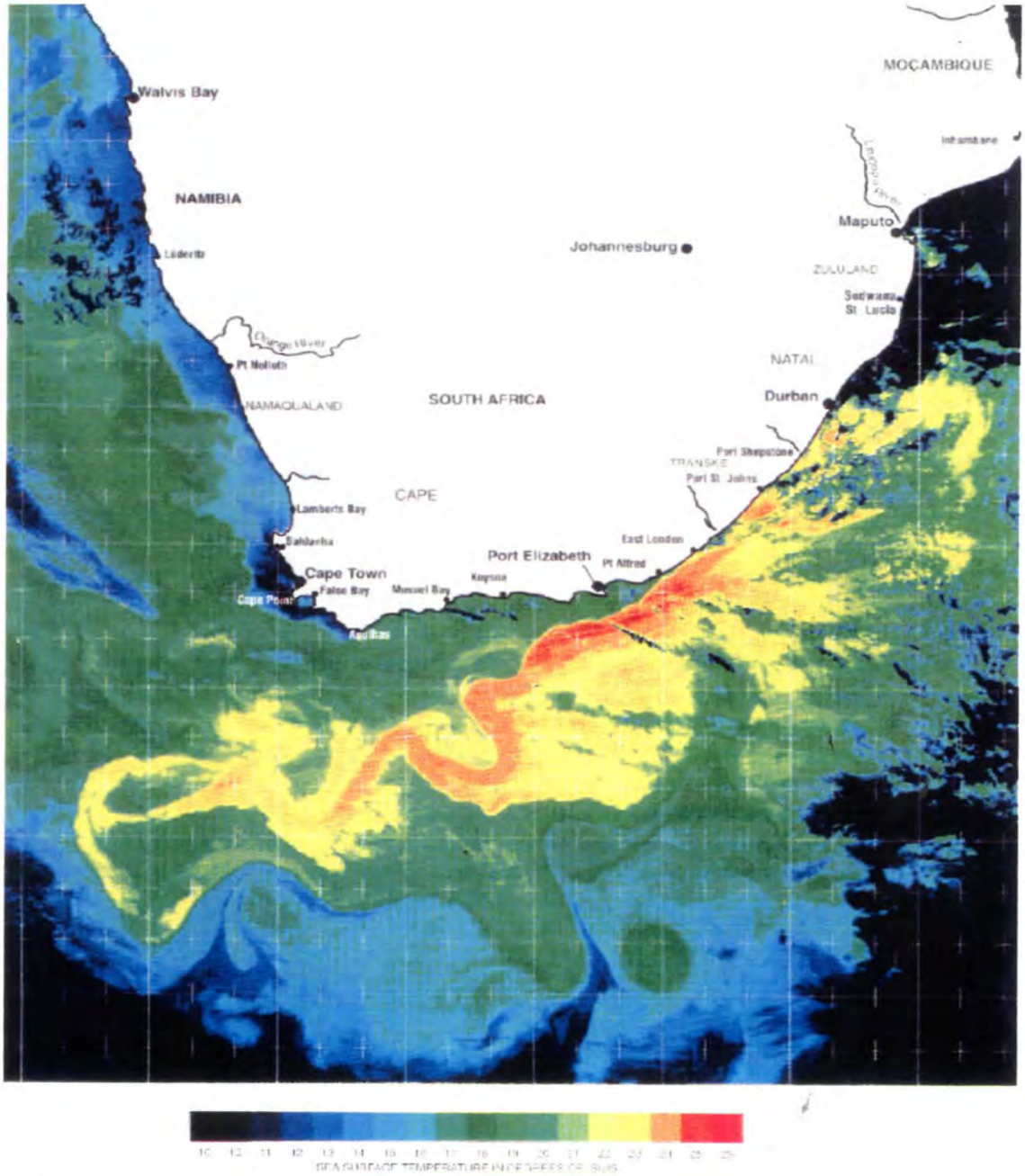


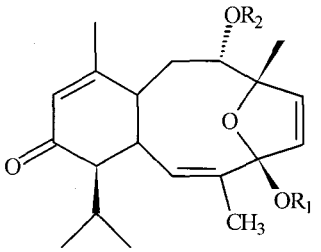
Figure 1.1 Satellite photograph of the coast of Southern Africa (reproduced with permission of Prof. G. Branch)[14]

1.4.1 Soft corals

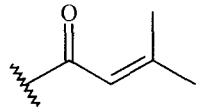
The term "soft coral" refers to members of the order Alcyonacea (Phylum Cnidaria, Class Anthozoa, Subclass Octocoralia). Soft corals have no internal skeleton but often possess calcareous spicules within their body tissues which contribute to the support and maintenance of large colonies. Most octocorals are filter feeders and frequently contain symbiotic zooxanthellae (microscopic unicellular plants). Soft corals are rich in nutritionally important substances and should serve as a valuable food source to marine predators. However, the incidence of predation on these invertebrates is low. Secondary metabolites produced by soft corals are known to play a number of ecological roles, including feeding deterrence, in the lives of these organisms.[12,13,20]

The Kwazulu-Natal reefs are dominated by soft corals which are estimated to represent approximately 60-70% of the biomass of Sodwana Bay.[16] The most common octocoral genera occurring off South Africa are *Sinularia*, *Sarcophyton*, and *Lobophyton*. Other less common genera are *Dendronephthya*, *Nephthea*, *Alcyonium*, *Clavularia, Cladiella, Anthelia, Rumphella, Echinogorgia,* and *Leptogorgia*.[16]

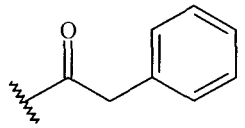
Faulkner *et al.*[21] screened marine invertebrates collected from the Coffee Bay region of the Transkei coast for general bioactivity and found a disappointing incidence of activity from the collection of 90 sponges, tunicates and soft corals. A secondary screening of the ^{1}H NMR spectra of the crude extracts led to the investigation of the soft coral, *Alcyonium valdivae*. Five diterpene esters, valdivones A **(2)** and B **(3)**, their corresponding methoxy ketals **(4)** and **(5)**, and dihydrovaldivone A **(6)**, were isolated and identified by standard spectroscopic methods. The methyl ketals, **4** and **5**, were considered to be artifacts of the isolation process which involved extraction of the soft coral with methanol. Valdivones A and B inhibit chemically-induced inflammation in the mouse ear assay, but were inactive against a standard panel of bacteria and fungi. The valdivones are structurally related to the sarcodictyins, [*e.g.* sarcodictyins A **(7)** and B **(8)**].[22]



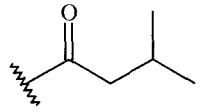
a =



b =



c =



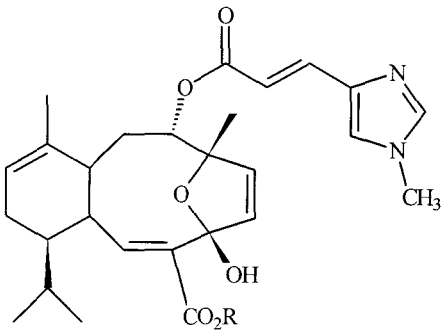
2 R_1 = H, R_2 = **a**

3 R_1 = H, R_2 = **b**

4 R_1 = CH_3, R_2 = **a**

5 R_1 = CH_3, R_2 = **b**

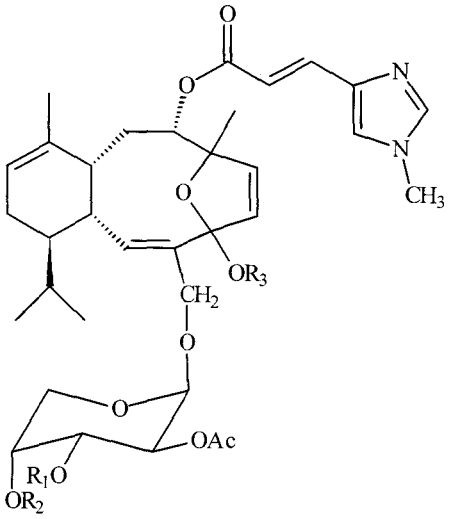
6 R_1 = H, R_2 = **c**



7 R = Me

8 R = Et

The soft coral *Eleutherobia aurea*, collected from Sodwana Bay in Kwazulu-Natal, contained sarcodictyin A and two related glycosides, eleuthosides A **(9)** and B **(10)**.[23] The structure determination of all three compounds was based mainly on one- and two-dimensional NMR spectroscopy and mass spectrometry data. The eleuthosides are closely related to the potent cancer cell inhibitor, eleutherobin **(11)** (IC_{50} 10-15 nM),[24] which mimics paclitaxel by stabilizing microtubules. The sarcodictyins,[22] which also possess paclitaxel-like microtubule-stabilizing properties, are much less toxic (IC_{50} 400-900 nM). Eleutherobin, the eleuthosides and the sarcodictyins have been synthesized by K.C. Nicolaou's group at The Scripps Research Institute.[25,26]



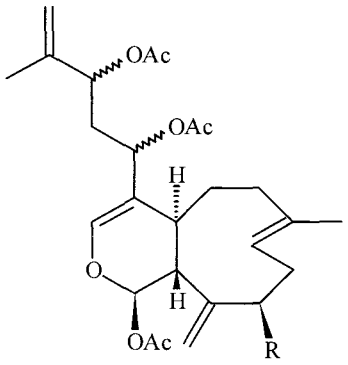
9 R_1 = Ac, R_2 = R_3 = H

10 R_1 = R_3 = H, R_2 = Ac,

11 R_1 = R_2 = H, R_3 = CH_3

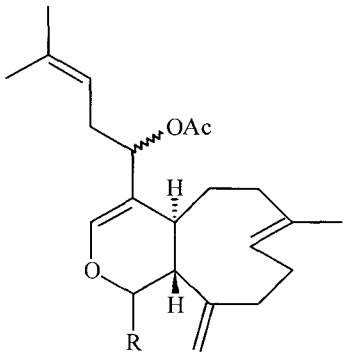
Interestingly, specimens of *Eleutherobia aurea*, collected from Aliwal Shoal (approximately 500 km south of Sodwana Bay, Figure 1.1) off the southern Kwazulu-Natal coast, did not contain any eleuthosides.[27] Instead they yielded the known xenicane diterpenoids zahavin A **(12)**[28] and 9-deacetoxy-14,15-deepoxyxeniculin **(13)**[29] and the two new diterpenoids 7,8-epoxyzahavin A **(14)** and xeniolide C **(15)**.[27] The structures of these compounds were determined by standard spectroscopic methods and comparison of spectral data with literature values. Compounds **12-14** showed anti-inflammatory activity by inhibiting superoxide production in rabbit neutrophils.

Zahavins A and B **(16)** were previously isolated from the soft coral *Alcyonium aureum* (reclassifed as *E. aurea*) from Sodwana Bay.[28,30] *Anthelia glauca*, which was collected with *A. auream*, yielded a single new xenicane diterpenoid, antheliatin **(17)**. The structures of **12**, **16** and **17** were determined by NMR methods with X-ray diffraction studies confirming the structure of **17**. All three compounds were cytotoxic ($IC_{50} \leq 1$ μg mL^{-1}) towards P-388 mouse leukemia, A-549 human lung carcinoma, MEL-28 human melanoma, and HT-29 human colon carcinoma.



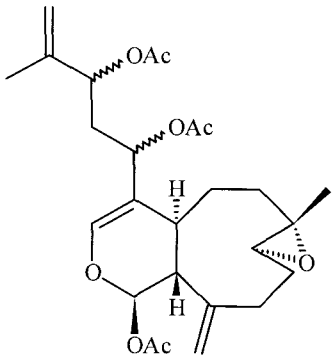
12 R = H

16 R = OH



13 R = β-OAc

18 R = α-OAc



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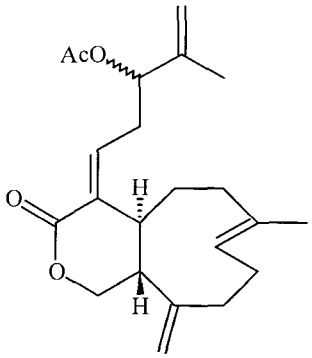

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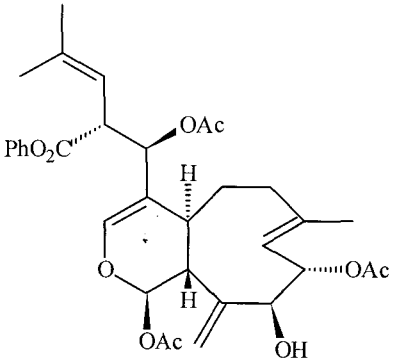
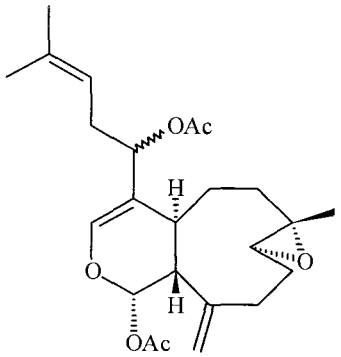


17

Ethyl acetate extracts of two phenotypic colour variants of the endemic South African soft coral, *Capnella thyrsoidea*, collected in the Tsitsikamma Marine Reserve, yielded four new xenicane diterpenes, the tsitsixenicins A-D **(18-21)**, together with the known steroid 5α-pregna-1,20-dien-3-one **(22)**[31] and two new pregnadiene steroids, 16β-hydroxy-5α-pregna-1,20-dien-3-one 16-acetate **(23)** and 3α,16β-dihydroxy-5α-pregna-1,20-diene 3,16-diacetate **(24)**.[32] Standard spectroscopic methods were used to determine the

structures and stereochemistry of these compounds. Tsitsixenicins A-D showed significant inhibition (> 80%) of superoxide production in rabbit neutrophils at a concentration of 12.5 μg mL^{-1}. Unfortunately the instability of the tsitsixenicins precluded further investigation of their anti-inflammatory activities.



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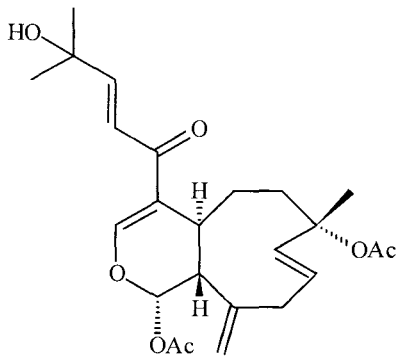

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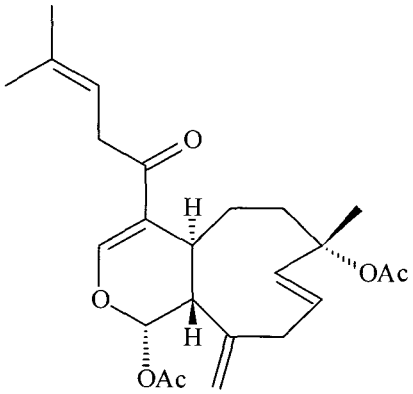

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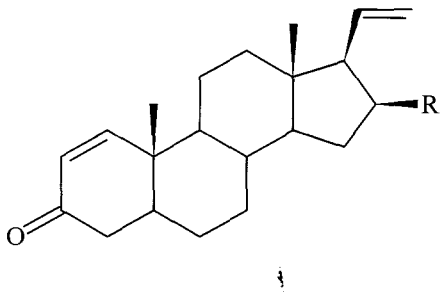
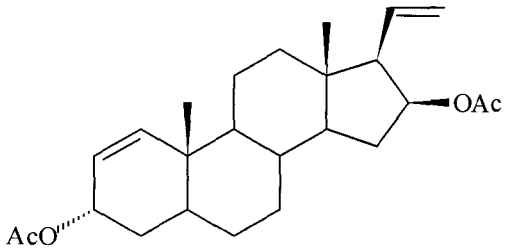


22 R = H

23 R = OAc



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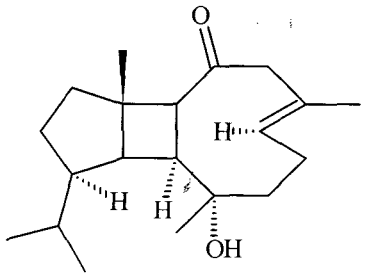
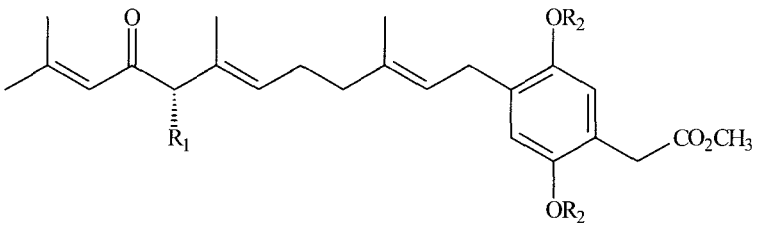


25

The new cytotoxic diterpene, sarcoglane **(25)**, was isolated from the Sodwana Bay soft coral *Sarcophytum glaucum* and its structure determined by spectral methods.[33] Compound **25** was found to inhibit cell division in fertilized sea urchin eggs.

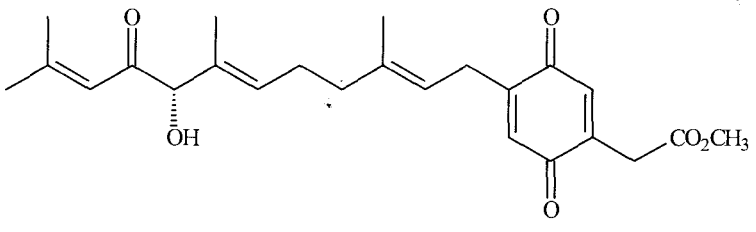
A series of three sesquiterpene hydroquinones, rietone **(26)**, 8'-acetoxyrietone **(27)** and 8'-desoxyrietone **(28)** were isolated from the endemic South African soft coral *Alcyonium fauri*, collected near Port Alfred on the south-east coast.[34] The structures of compounds **26-28** were determined by standard spectroscopic methods, while the absolute stereochemistry of **26** was determined by the modified Mosher's method[35] of its oxidation product, the quinone **(29)**. Rietone showed activity (IC_{50} 9.32 μM) in the NCI's CEM cell line screen designed to detect anti-HIV activity.



26 R_1 = OH, R_2 = H

27 R_1 = OAc, R_2 = H

28 R_1 = R_2 = H



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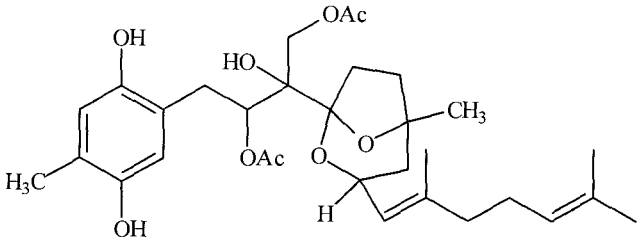
The cytotoxic extracts of two Kwazulu-Natal soft corals, *Sinularia dura* (Alcyoniidae) and *Nephthea* sp. (Nephtheidae) yielded four new polyprenylhydroquinones **(30-33)**.[36] The crude EtOAc extract of *S. dura* collected by Schleyer in Sodwana Bay gave the new unstable tetraprenyltoluquinol derivative sindurol **(30)** and the known cembranoid, epoxypukalide **(34)**.[37] The *Nephthea* sp., also collected in Sodwana Bay, contained the new substituted D-arabinose glycoside, nephthoside **(31)**, and two related compounds,

(32) and **(33)**. The structures of these compounds were determined by extensive one- and two-dimensional NMR studies, mass spectrometry and chemical transformations.[36] Compounds **30-33** were cytotoxic to P-388 mouse leukemia cells with IC_{50} values of 1.2 - 2.0 μg mL^{-1}.



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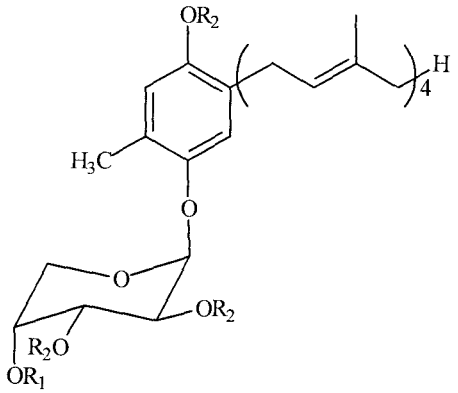
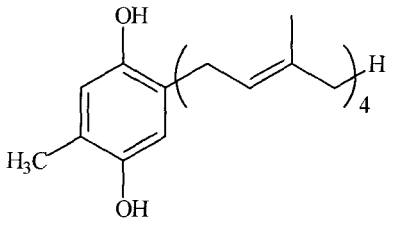


31 $R_1 = R_2 = H$

33 R_1 = Ac, $R_2 = H$



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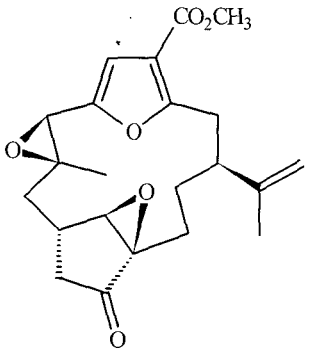
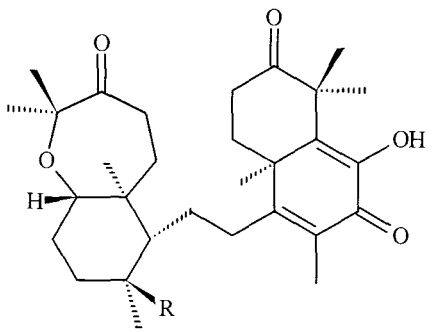


34

1.4.2 Sponges

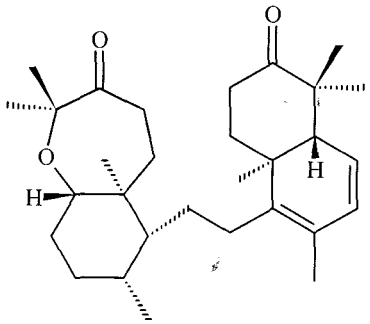
Sponges (Phylum Porifera) are primitive, aquatic, sessile animals which lack a mouth, digestive tract or other conventional organs. They consist of a few types of cells, forming tissues that are either supported by a skeleton of collagen or spongin, a network of calcium carbonate or silica spicules, or a combination of these structural features. Because of their prevalence, and their ability to biosynthesize a variety of natural product structural classes, sponges have become the dominant source for the discovery of biologically active marine natural products.[4] Although sponges are conspicuous on rocky reefs, particularly in temperate areas, the southern African species are poorly described and many remain unnamed.[14]

In continuation of their studies of the chemistry of invertebrates from Sodwana Bay, Kashman's group investigated the purple-brown fan sponge *Axinella weltneri* (Axinellidae). Extracts of this sponge yielded the nine triterpenoids, the sodwanones A-I **(35-43)**.[38-40] The structure of sodwanone A was unambiguously determined by X-ray analysis while the structures of sodwanones B-I followed from comparisons of their spectral data with those of sodwanone A. Sodwanones G-I showed cytotoxicity against cancer cell lines P-388, A-549, HT-29 and MEL-28 with IC_{50} values ranging from 0.02-20 μM. The sodwanones are related to the previously reported sipholenols[41] and the raspacionins [*e.g.* raspacionin A, **(44)**].[42-44]



35 R = OH

36 R = H



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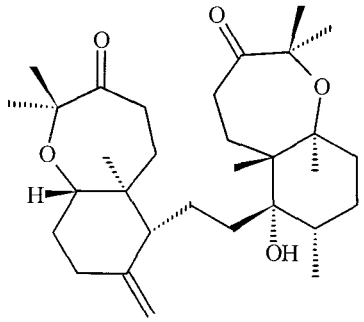

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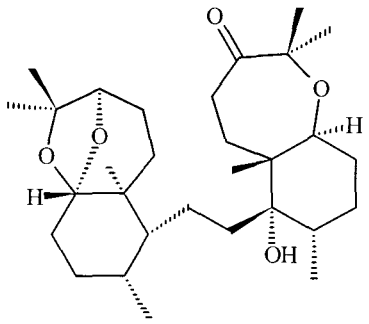

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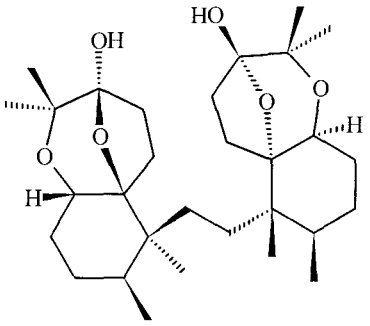

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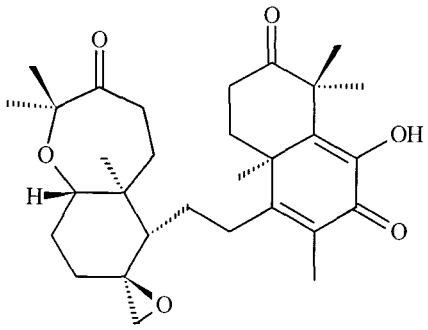
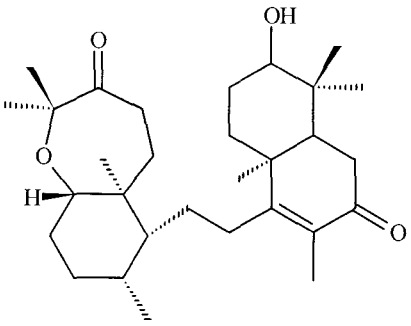


41



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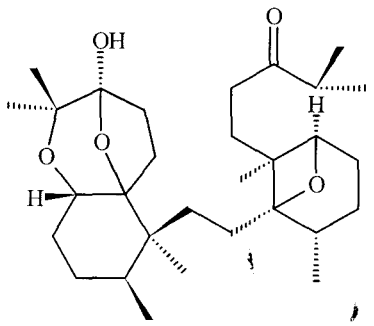
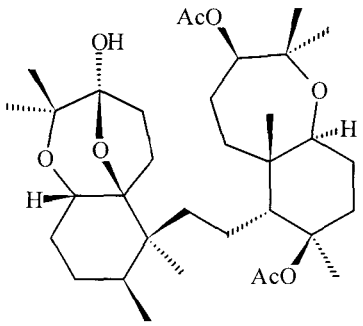
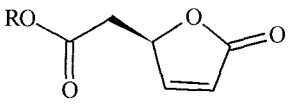


43



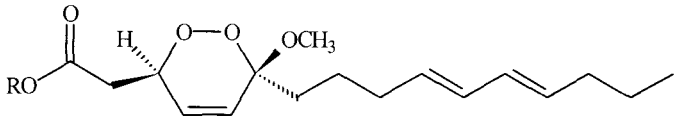
44

The sponge *Plakortis simplex* (Plakinidae), also collected in Sodwana Bay, contained 2-oxo-2,5-dihydrofuran-5-acetic acid **(45)** and four new cyclic peroxide-containing polyketides **(46-49)**.[45] The structures of these compounds were determined by comparison of their spectral data with those of the known metabolites methyl 5-butenolidyl acetate **(50)** and xestin A **(51)**.[46] The carboxylic acid derivatives, compounds **45** and **46**, were very unstable and were purified by reversed-phase chromatography. Compound **48** was not isolated, but was converted to the methyl ester **(49)** which is also a metabolite of the sponge. Compounds **47** and **49** were cytotoxic to P-388 murine leukemia cells ($IC_{50} < 0.1$ μg mL^{-1}). Although *Plakortis simplex* is widely distributed in tropical and subtropical seas, this is the first reported occurrence of this sponge off South Africa.



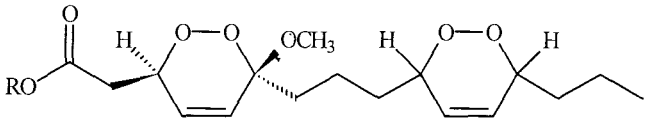
45 R = H

50 R = CH_3



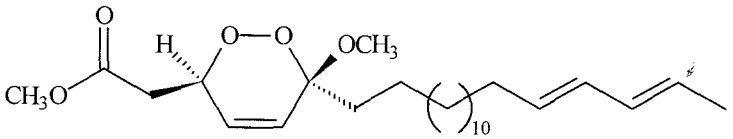
46 R = H

47 R = CH_3



48 R = H

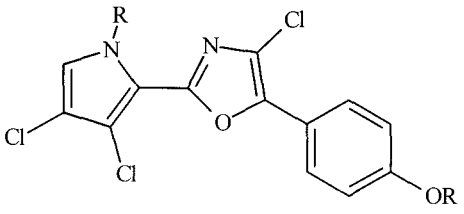
49 R = CH_3



51

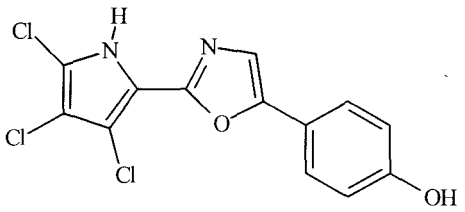
The novel chlorinated phenylpyrrolyloxazoles, phorbazoles A-D **(52-55)**, were isolated from the sponge *Phorbas* aff. *clathrata* collected in Sodwana Bay.[47] The structure of

phorbazole A was determined by X-ray crystallography of its dimethyl derivative **(56)** while the structures of compounds **53-55** were assigned from one- and two-dimensional NMR data.

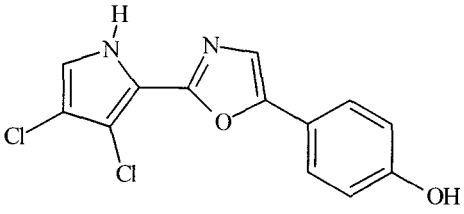


52 R = H

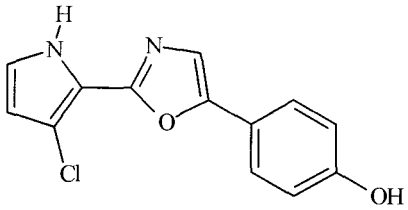
56 R = CH_3



53

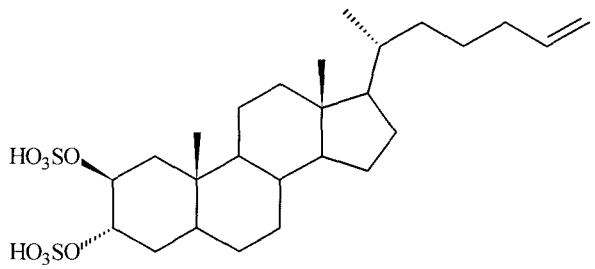


54



55

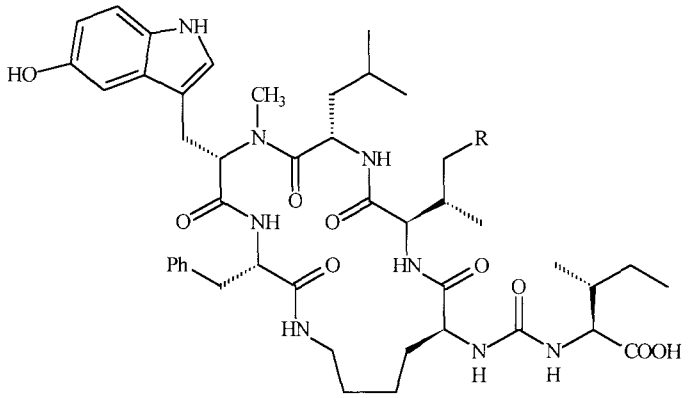
Extracts of an undescribed species of a *Pachastrella* sponge collected off Aliwal Shoal by Carte *et al.* gave the sterol halistanol disulphate B (**57**).[48] The structure of **57** was determined largely by NMR and this compound showed significant inhibition (IC_{50} 2.1 μM) of endothelin converting enzyme (ECE).[48]



57

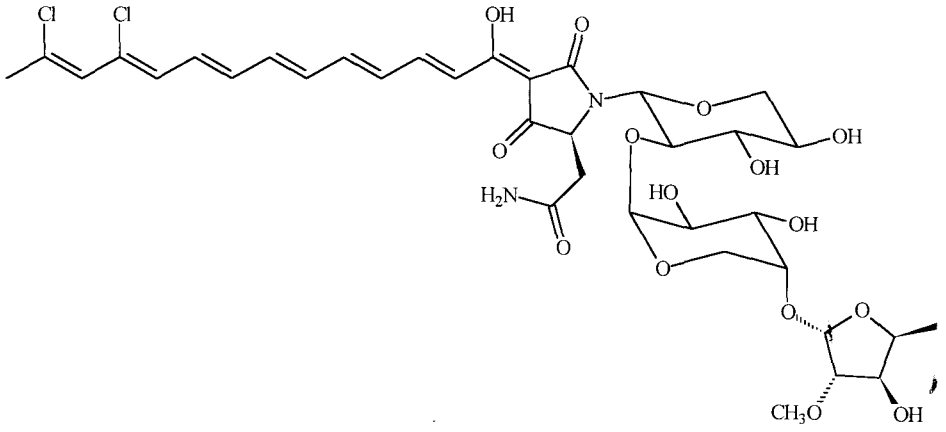
The cyclic peptides, mozamides A **(58)** and B **(59)**, together with the known metabolite aurantoside A **(60)**, were obtained from a theonellid sponge collected off southern

Mozambique.[49] The structures and absolute configurations of **58** and **59** were determined by spectroscopic methods and chiral GC-MS analysis of the amino acids resulting from acid hydrolysis of these two compounds.



58 R = H

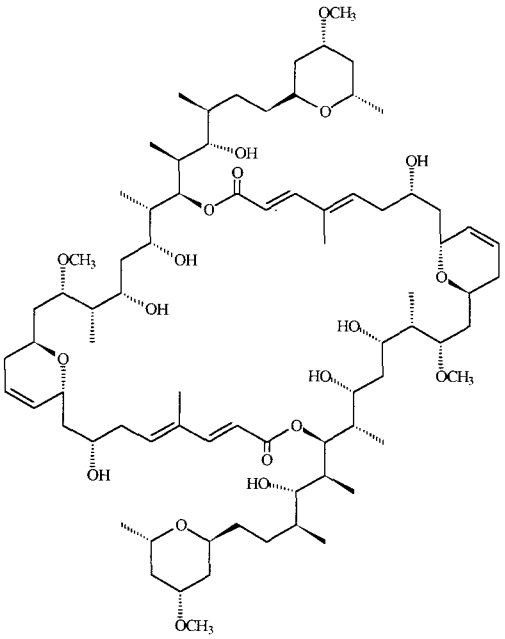
59 R = CH_3



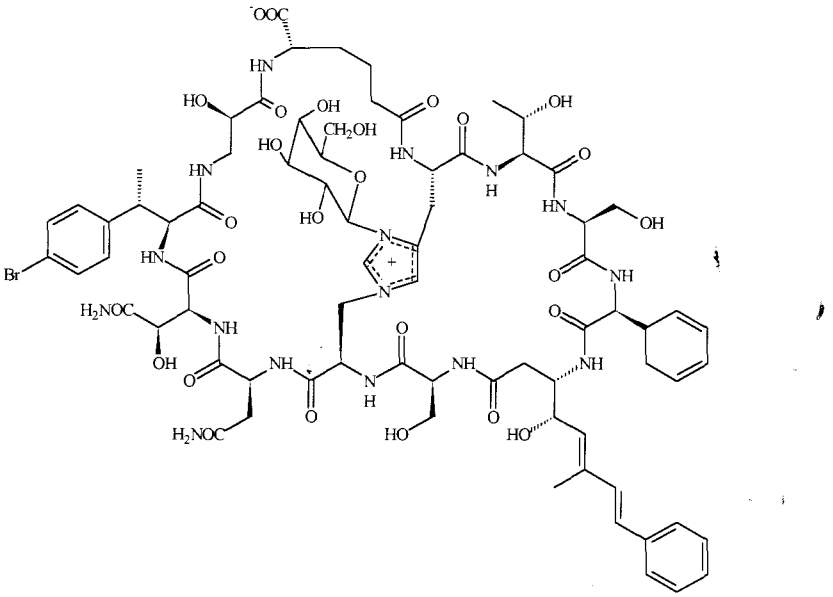
60

The known cytotoxin swinholide A **(61)** and the new bicyclic peptide theopalauamide **(62)**, were isolated concurrently from separate specimens of the lithistid sponge *Theonella swinhoei* collected in Mozambique and Palau.[50] The structure of theopalauamide was determined from spectroscopic data, and its stereochemistry was determined by chemical degradation and analysis of the products by chiral GC-MS. In a previous study on the cellular location of metabolites in *T. swinhoei*, Faulkner *et al.*[51] showed that **61** was associated with a mixture of unicellular bacteria and that **62** was located in filamentous

eubacteria that are found only in the interior of the sponge.[51] Swinholide A was previously obtained from a South African specimen of *Theonella conica.*[51]



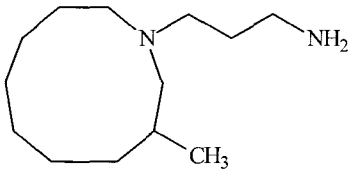
61



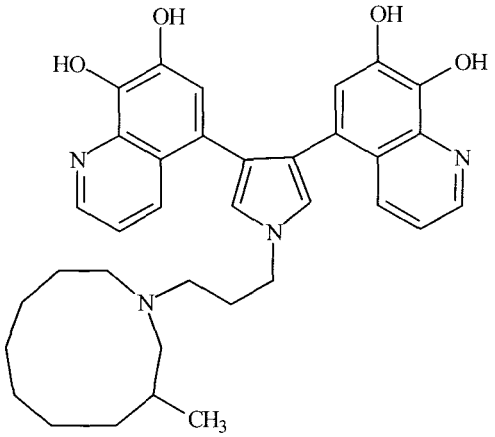
62

Cytotoxic extracts of the Indo-Pacific sponge *Haliclona tulearensis*, collected in Sodwana Bay, contained two alkaloids, halichlorensin **(63)**[52] and the bisquinolinylpyrrole, halitulin **(64)**.[53] The structure of halitulin was determined by spectroscopic methods and chemical degradation. Compound **64** is air and light sensitive and is cytotoxic to P-388 murine

leukemia (IC_{50} 0.025 μg mL^{-1}), A-549 human lung carcinoma (IC_{50} 0.0121 μg mL^{-1}), HT-29 human colon carcinoma (IC_{50} 0.012 μg mL^{-1}) and MEL-28 human melanoma (IC_{50} 0.025 μg mL^{-1}).

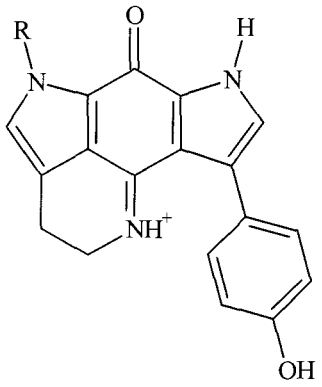


63



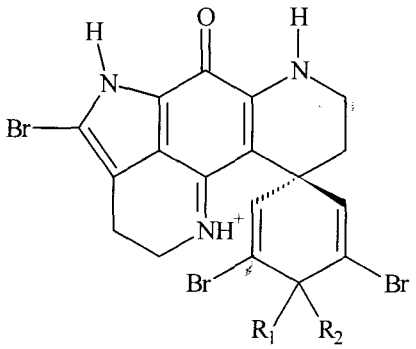
64

An undescribed latrunculid sponge collected in the Tsitsikamma Marine Reserve, off the south eastern coast of South Africa, was found to be a morphological intermediate between the genera *Latrunculia* and *Zyzzya*, both of which contain pyrroloiminoquinone-producing sponges.[54] Chemical investigation of this sponge resulted in the isolation of the antimicrobial bispyrroloiminoquinone alkaloids, the tsitsikammamines A **(65)** and B **(66)**, as well as two new brominated derivatives of discorhabdin C, compounds **(67)** and **(68)**.[54]



65 R = H

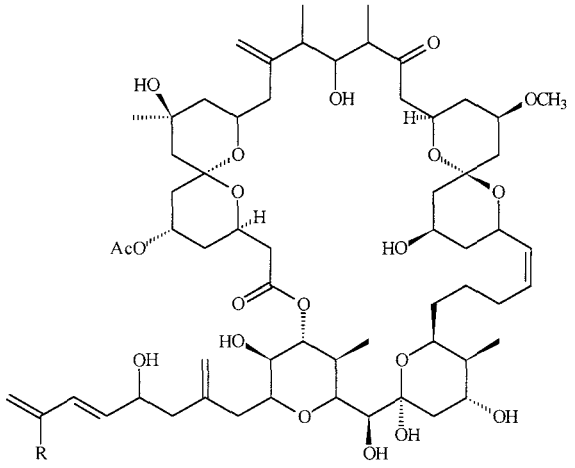
66 R = CH_3



67 $R_1 + R_2 = O$

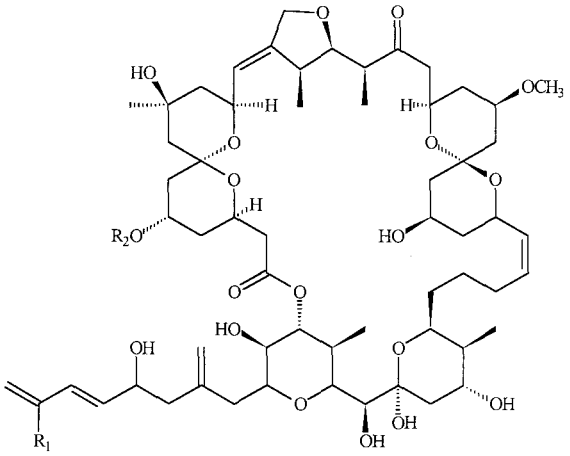
68 R_1 = H, R_2 = OH

An investigation of the antineoplastic constituents of the brightly coloured sponge, *Spirastrella spinispirulifera,* collected off the south coast of South Africa near Cape Town, provided the macrolides, spongiastatins-4 **(69)**, spongiastatin-5 **(70)**, spongiastatin-6 **(71)**, spongiastatin-7 **(72)**, spongiastatin-8 **(73)** and spongiastatin-9 **(74)**, in minute quantities.[55-57] The potent activity exhibited by these compounds in the NCI's panel of 60 human cell lines, led to increasingly larger and ecologically controversial collections (up to 2409 kg) of *S. spinispirulifera* to provide sufficient material for further testing.



69 R = Cl

71 R = H



70 R_1 = Cl, R_2 =H

72 R_1 = R_2 = H

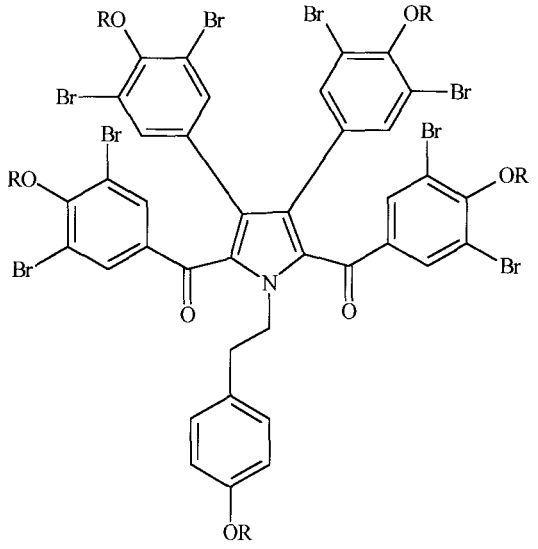
73 R_1 = H, R_2 = Ac

74 R_1 = Cl, R_2 = Ac

1.4.3 Ascidians

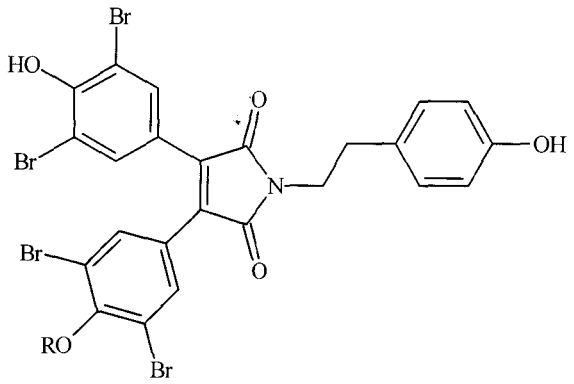
Ascidians, commonly called tunicates or sea squirts, belong to the phylum Chordata which encompasses all vertebrate animals. Adult ascidians are sessile filter feeders but they produce "tadpole-like" larvae with a primitive notochord. Although ascidians are very common along the South African coast, they have not been well studied.[14] In some of South Africa's benthic reef communities, the population of ascidians is disproportionately large relative to other filter feeders. For example, 36% of a recent random collection of invertebrates from Algoa Bay by the Coral Reef Research Foundation comprised these animals.[58] Ascidians are known to be a rich source of unique and biologically active secondary metabolites.[7,59]

An investigation of the Indo-Pacific ascidian *Polycitor* sp., collected in Sodwana Bay, yielded three novel alkaloids, polycitone A **(75)** and polycitrins A **(76)** and B **(77)**.[60] The structures of these compounds, which are related to the lamellarins,[61] were established from NMR spectroscopic data. Confirmation of the structure of **75** was provided by an X-ray analysis of the penta-*O*-methyl derivative **(78)**. Compound **76** was found to inhibit the growth of SV40 transformed fibroblast cells at a concentration of 10 μg mL^{-1}.



75 R = H

78 R = CH_3

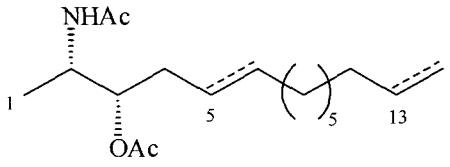


76 R = H

77 R = CH_3

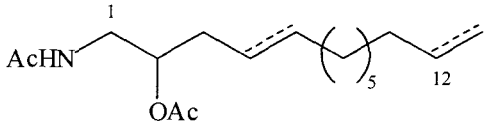
Four new antimicrobial, unsaturated amino alcohols were isolated as their acetate derivatives **(79-82)** from the ascidian *Pseudodistoma* sp., collected in the Tsitsikamma

Marine Reserve along the southern coast of South Africa.[62] Standard spectroscopic methods and chemical conversions were used to determine the structures of these compounds.



79 Δ^5, Δ^{13}

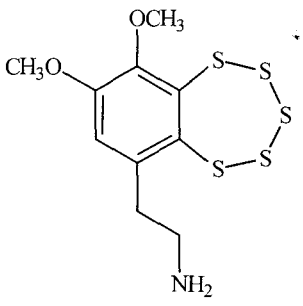
80 Δ^5



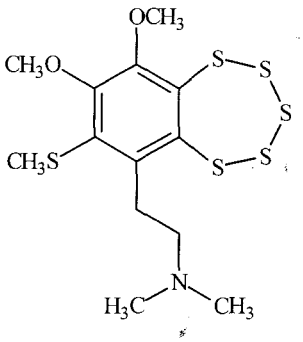
81 Δ^4,Δ^{12}

82 Δ^4

Bioassay-guided fractionation of the MeOH extract of the ascidian *Lissoclinum* sp., collected on Aliwal Shoal, off Umkomaas on the eastern coast of South Africa, led to the isolation of three known compounds; varacin **(83)**, *N,N*-dimethyl-5-(methylthio)varacin **(84)**, 3,4-dimethoxy-6-(2'-*N,N*-dimethylaminoethyl)-5-(methylthio)benzotrithiane **(85)**, and a new dimeric alkaloidal sulfoxide, lissoclin disulfoxide **(86)**.[63] Compound **86** inhibited the interleukin-8 receptors, IL-8 Rα and IL-8 Rβ with IC_{50} values of 0.6 and 0.82 μM, respectively, and also showed activity against phosphokinase C (IC_{50} 1.54 μM). Varacin exhibited potent antifungal and antimicrobial activity *in vitro* against *Candida albicans* and *Bacillus subtilus*.[64]



83



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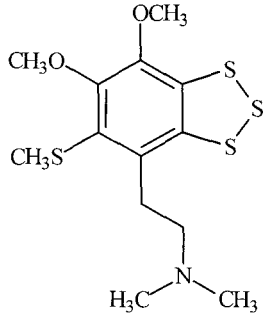

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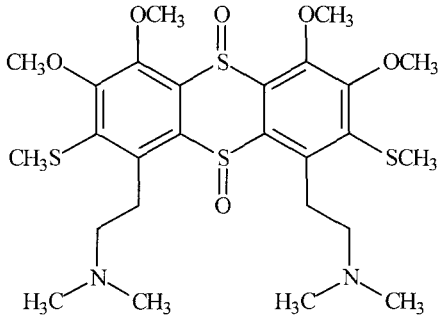
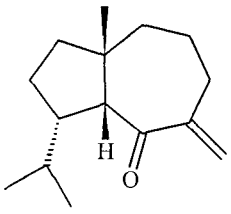


86

1.4.4 Molluscs

The phylum Mollusca is one of the largest marine invertebrate phyla, with more than 5000 species occurring in southern Africa.[14] These species are grouped into seven classes, of which the gastropods are the major producers of secondary metabolites. Class Gastropoda is divided into a further three subclasses: the Prosobranchia (true limpets), Opisthobranchia (nudibranchs and sea hares) and Pulmonata (false limpets). Nudibranchs constitute the overwhelming majority (*ca* 75%) of the nearly 300 species of opisthobranch gastropods found in South Africa.[17] Most gastropods are shelled, but nearly all molluscs of the subclass Opisthobranchia lack a protective shell and often sequester toxic secondary metabolites from their diet. These "defense chemicals" may then be exuded from glands in the skin when the animal is molested.[12,65,66]

Extracts of the endemic South African nudibranch *Leminda millecra*, collected at Coffee Bay on the Transkei coast, were found to contain the new sesquiterpenes, millecrones A **(87)** and B **(88)** and millecrols A **(89)** and B **(90)**.[67] These compounds are typical of the metabolites found in the soft corals on which the organism feeds. The structures of compounds **87-90** were established from spectroscopic data.[67]



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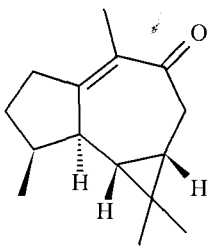

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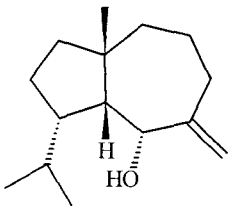

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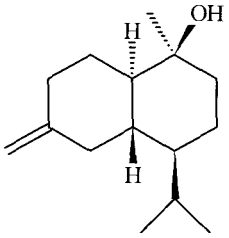
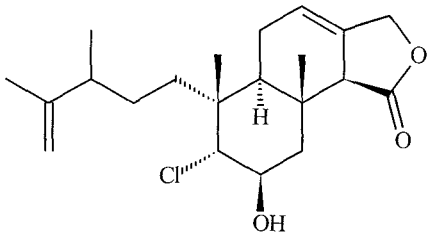


90

Specimens of the nudibranch *Chromodoris hamiltoni*, collected on Aliwal Shoal, contained four unusual chlorinated homo-diterpenes, hamiltonins A-D **(91-94)**, and a new sesterterpene, hamiltonin E **(95)**, in addition to the known compounds latrunculins A **(96)** and B **(97)**.[68] A more recent chemical investigation of *C. hamiltoni* collected from Malangaan reef off southern Mozambique, revealed the presence of latrunculin B and two new spongiane diterpene lactones **(98)** and **(99)**.[69]



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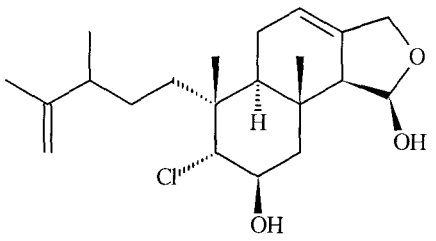

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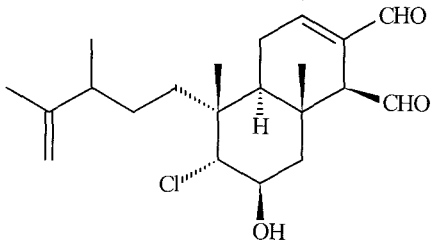

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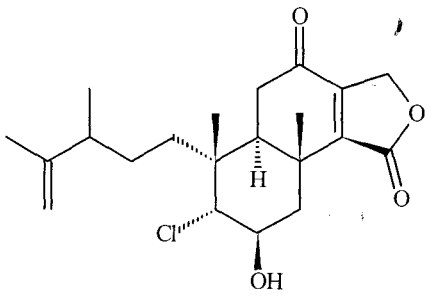

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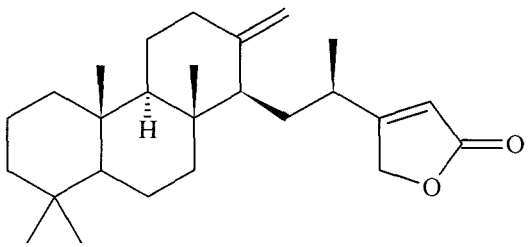

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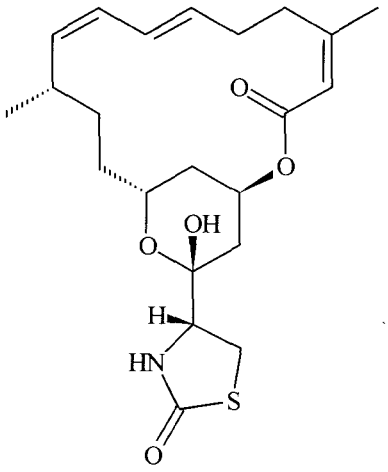

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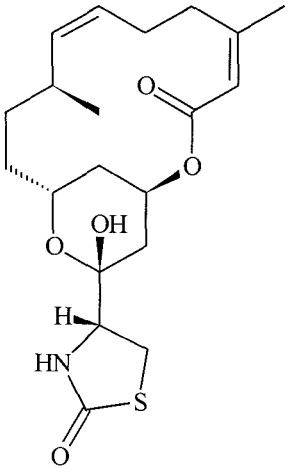

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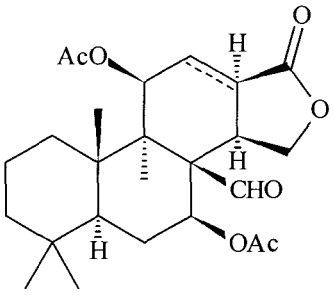
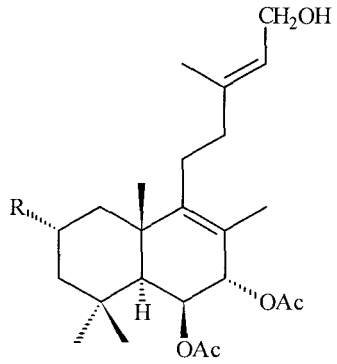


98

99 Δ^{12}

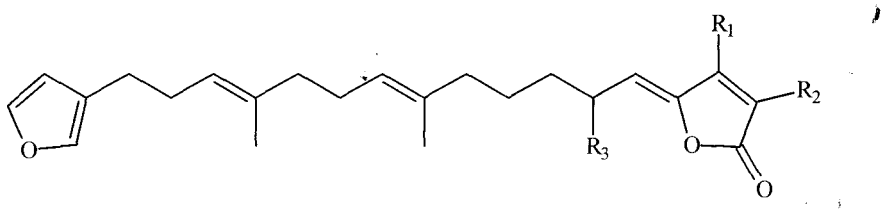
The endemic intertidal pulmonate mollusc *Trimusculus costatus* collected from the south-east coast of South Africa, contained the known labdane diterpene **(100)** and the new acetylated analogue **(101)**.[70] Both compounds **100** and **101** showed fish-feeding inhibitory activity. *T. costatus* is the only member of the genus *Trimusculus* ocurring in southern Africa.[70]



100 R = H

101 R = OAc

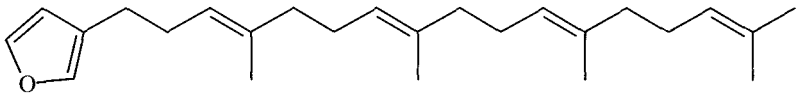
The endemic South African nudibranch *Hypselodoris capensis* was collected in the Tsitsikamma Marine Reserve together with the sponge *Fasciospongia* sp. that it was observed to feed upon. The acetone extract of *H. capensis* contained the known compounds variabilin **(102)**, 22-deoxy-variabilin **(103)**, nakafuran-8 **(104)**, nakafuran-9 **(105)** and the new compound 22-deoxy-23-hydroxymethylvariabilin **(106)**.[71] The sponge *Fasciospongia* sp. yielded compounds **102**, **103**, **106** and furospinosulin-1 **(107)**, clearly confirming that the sponge formed part of the nudibranch's diet.[71] Variabilin was also isolated from an *Ircina* sponge collected in the Tsitsikamma Marine Reserve.[19]



102 R_1 = OH, R_2 = CH_3, R_3 = α-CH_3

103 R_1 = H, R_2 = R_3 = CH_3

106 R_1 = H, R_2 = CH_2OH, R_3 = CH_3



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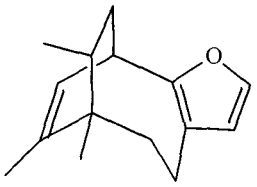

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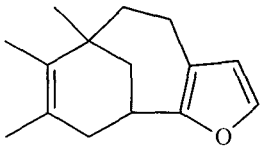
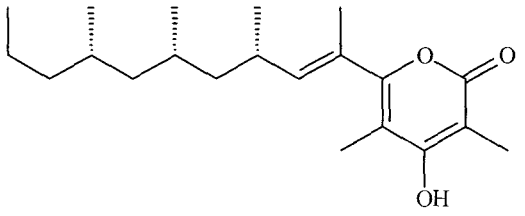


105

Intertidal pulmonate molluscs of the genus *Siphonaria* frequently contain polypropionate metabolites.[72] Nine *Siphonaria* species are known to occur along the South African coast.[73] Chemical investigation of *S. concinna* resulted in the isolation of the known polypropionate, pectinatone **(108)**,[19] while *S. serrata* yielded the new polypropionate, siserrone **(109)**.[74] It has been suggested that compound **109** may be a rearrangement product of dihydrosiphonarin A **(110)** which also occurs in *S. serrata*.[74]



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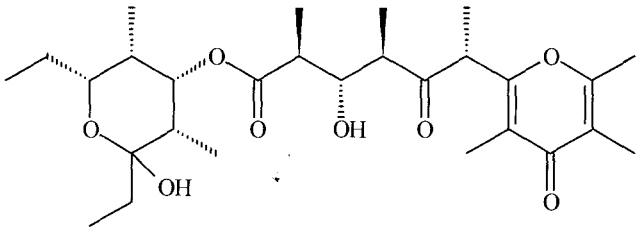

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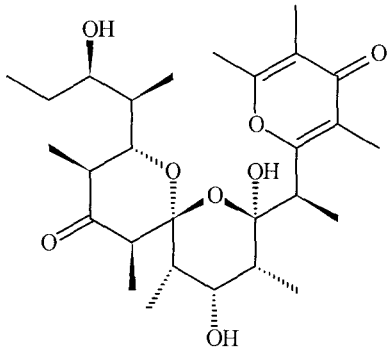
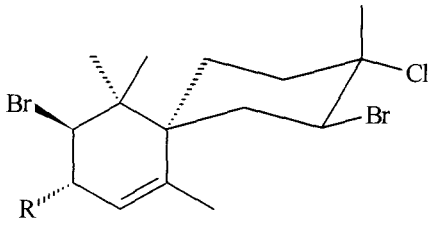


110

1.4.5 Marine Algae

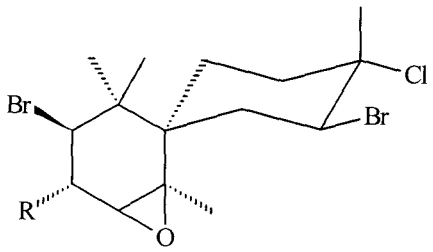
Marine algae are divided into 3 classes; Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae). Due to their abundance in shallow waters and ease of collection, seaweeds were one of the first groups of marine organisms to have their natural products chemistry studied.[4] The West Coast of South Africa is well known for its diverse algal populations, but very little work has been published on the natural product chemistry of South African algae.

Only one publication, describing the isolation of four halogenated chamigranes **(111-114)** from the South African red algae *Laurencia glomerata* is evident in the marine natural products literature.[75]



111 R = H

113 R = OH



112 R = H

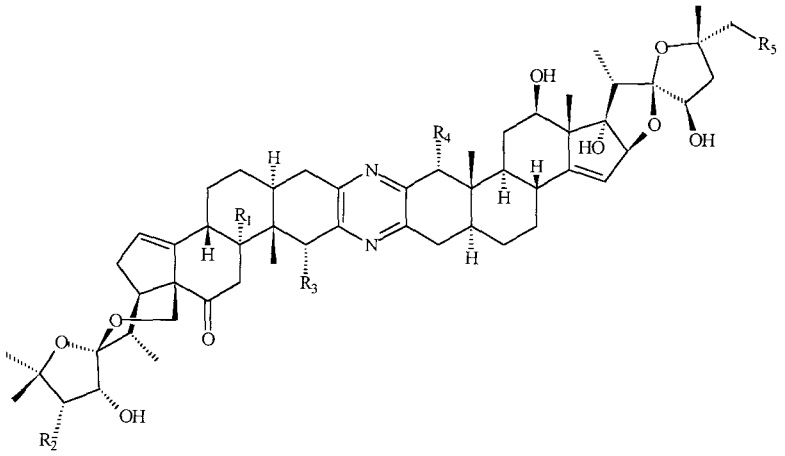
114 R = OH

Recently, Vlachos *et al.*[76] reported a study in which the crude extracts of 56 southern African seaweeds were screened for antimicrobial activity. No fractionation or characterization of the compounds responsible for the activity was reported.

1.4.6 Miscellaneous Marine Organisms

Pettit *et al.* have studied the metabolites produced by the South African marine worm, *Cephalodiscus gilchristi* over a period of 21 years. This prolonged study yielded the cephalostatins 1-17 **(132-148)** which are potent human cancer growth inhibitors.[77-84] In order to obtain sufficient quantities of these compounds for preclinical development, increasingly larger and again controversial collections (up to 450 kg) of these marine

worms were carried out. The most potent human cancer cell growth inhibitors in this series of seventeen compounds are cephalostatins-1 **(132)**, -7 **(138)**, -16 **(147)** and -17 **(148)** (ED_{50} 10^{-7}-10^{-9} μg mL^{-1}). The structures of these complex compounds were determined either by X-ray diffraction studies or by NMR methods and comparison of spectral data with those of other compounds in this series. Cephalostatins-7 **(138)** and -12 **(143)** have been synthesised *via* a biomimetic synthetic strategy.[85] This will, hopefully, prevent any further large-scale collections of the tube worm.



cephalostatin-1 **(132)** $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = OH$

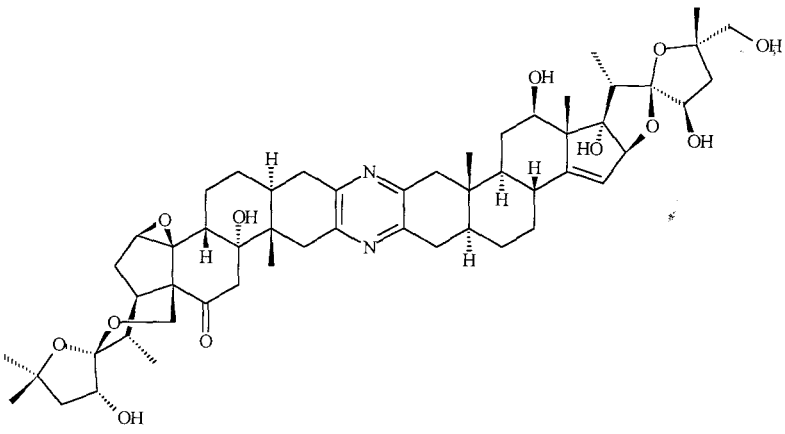
cephalostatin-2 **(133)** $R_1 = R_5 = OH$, $R_2 = R_3 = R_4 = H$

cephalostatin-3 **(134)** $R_1 = R_5 = OH$, $R_2 = CH_3$, $R_3 = R_4 = H$

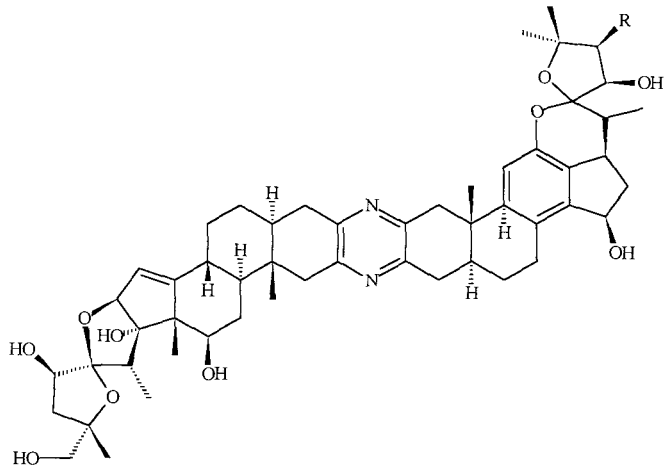
cephalostatin-10 **(141)** $R_1 = R_5 = OH$, $R_2 = R_3 = H$, $R_4 = OCH_3$

cephalostatin-11 **(142)** $R_1 = R_5 = OH$, $R_2 = R_4 = H$, $R_3 = OCH_3$

cephalostatin-17 **(148)** $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$

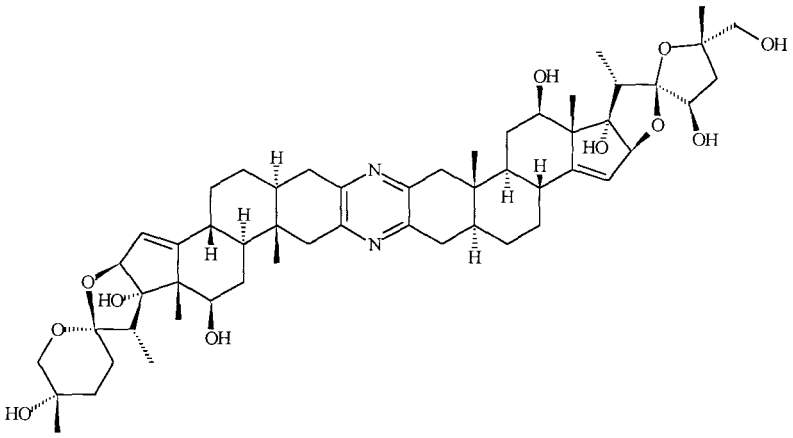


cephalostatin-4 **(135)**

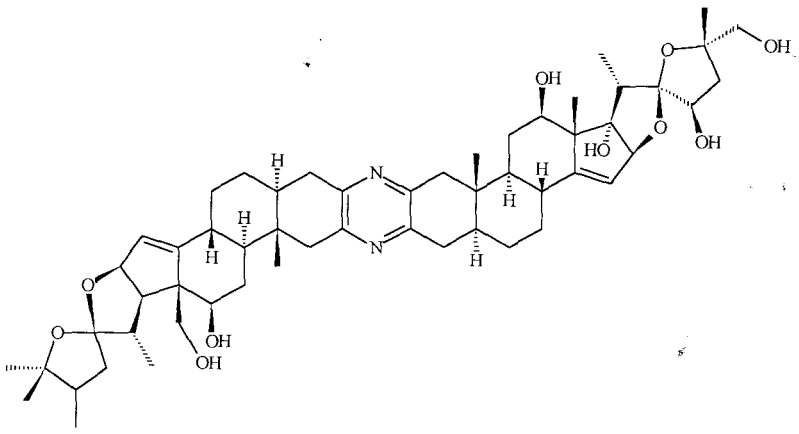


cephalostatin-5 **(136)** R = CH_3

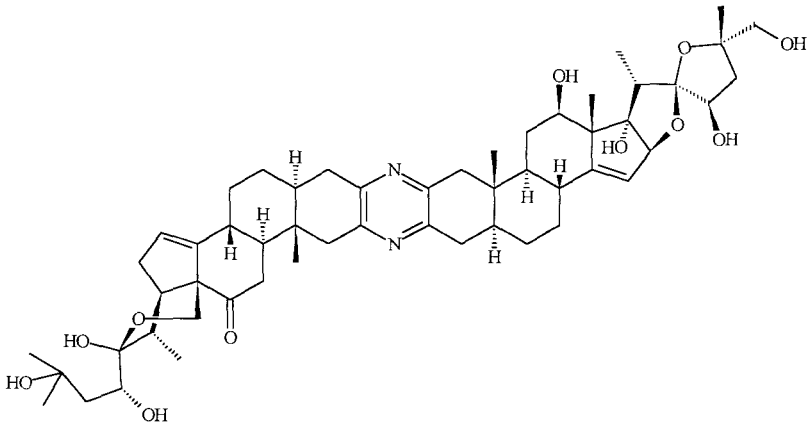
cephalostatin-6 **(137)** R = H



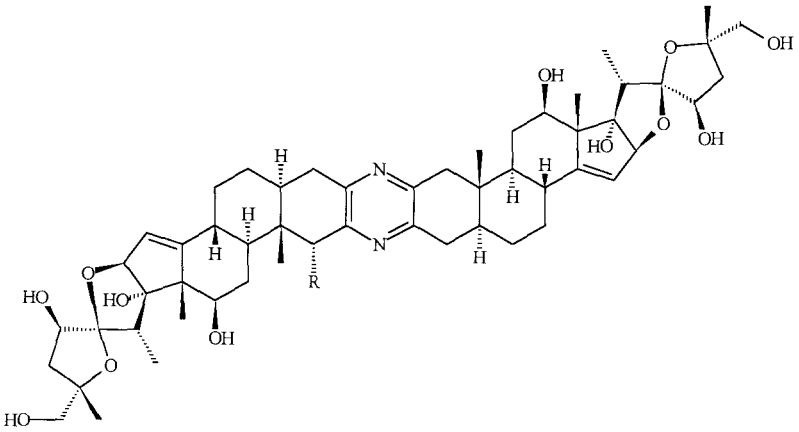
cephalostatin-7 **(138)**



cephalostatin-8 **(139)**

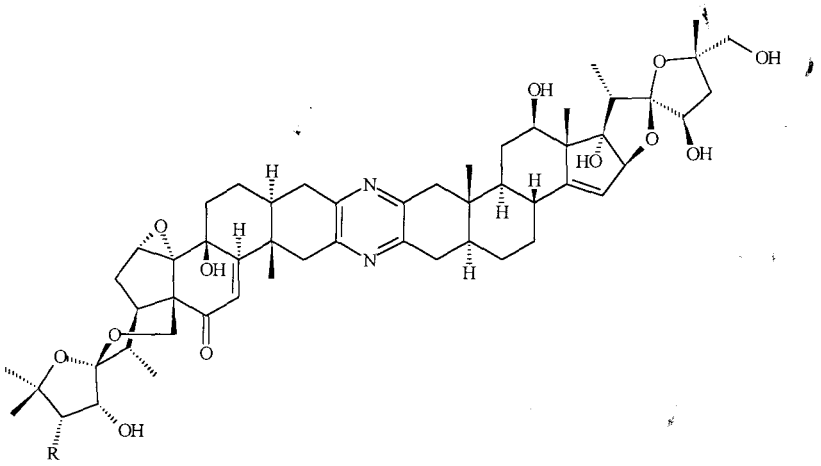


cephalostatin-9 **(140)**



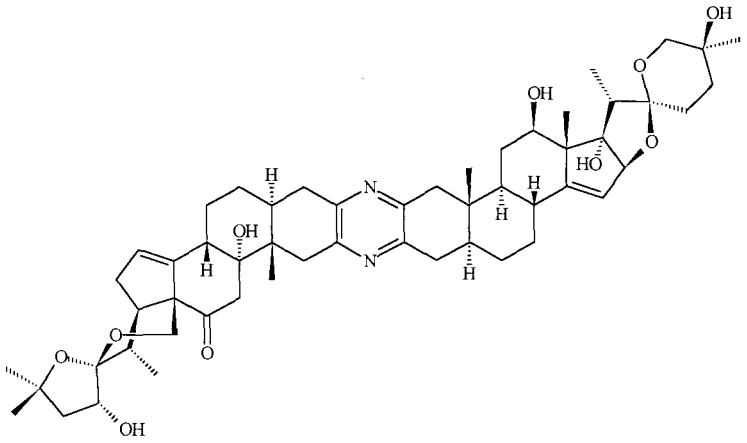
cephalostatin-12 **(143)** R = H

cephalostatin-13 **(144)** R = OH



cephalostatin-14 **(145)** R = H

cephalostatin-15 **(146)** R = CH_3



cephalostatin-16 **(147)**

1.5 THESIS OBJECTIVE

It is quite clear from the above review that South Africa has a relatively unexplored marine *fauna* and *flora*. Thus, the objective of this thesis was to study the natural product chemistry associated with selected South African marine organisms. New chemistry is often associated with endemic species and the high level of endemism encountered among Southern African marine organisms makes species from this region attractive targets for marine natural products investigations. Therefore, the marine invertebrates studied in this thesis were either endemic to the South African coast or had been selected, after screening of the Rhodes University invertebrate collection acquired as part of a collaboration with SmithKline Beecham. Crude methanol extracts of invertebrates collected in the Tsitsikamma Marine Reserve (101 organisms) and off Hout Bay, near Cape Town (133 organisms), were screened by ^{1}H NMR spectroscopy and for biological activity using simple antimicrobial assays or the brine-shrimp lethality assay. Extracts showing "interesting" ^{1}H NMR spectra and or potent biological activity were selected for further study.

Marine organisms generally produce very small amounts of bioactive natural products and often isolating sufficient quantities of these compounds for more comprehensive biological testing can be problematic. In Southern Africa this problem is exacerbated by the relative inaccessibility of many of the dive sites for large scale recollections, pressure from environmentalists, and the scarcity of taxonomic expertise to provide conclusive

field identifications of invertebrates such as sponges and ascidians. Therefore, in some instances, the synthesis of simpler bioactive marine natural products may be a viable alternative solution to the problem of supply. Accordingly, as part of this thesis, the synthesis of a group of indole alkaloids, isolated in trace amounts from a mixed collection of Cape sponges, was attempted.

Chapter Two

NEW POLYPROPIONATES FROM THE SOUTH AFRICAN FALSE LIMPET *SIPHONARIA CAPENSIS*

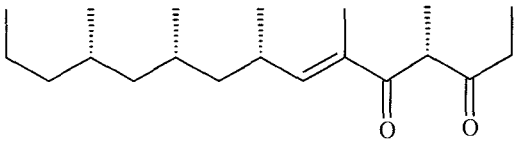
2.1 INTRODUCTION

Pulmonate limpets of the genus *Siphonaria* (Class: Gastropoda, Subclass: Pulmonata), also called false limpets, are air breathing molluscs commonly found on exposed rocks in the intertidal zone. Unlike true limpets (Subclass: Prosobranchia, Families: Patellidae and Acmaedae), siphonariids have lost all signs of their original gills, evolving a primitive lung in addition to a secondary gill which allows them to breathe air and also respire underwater.[86] During high tide, siphonariids remain firmly fixed to the rock surface leaving their "homescars" to feed on algae and micro-organisms as the tide recedes. When disturbed by potential predators, the mollusc exudes a toxic polypropionate-containing mucous from lateral pedal glands.[86-87]

2.1.1 Secondary metabolites from *Siphonaria* species

The chemistry of siphonariid limpets is dominated by polypropionates which are compounds derived from multiple condensation of propionate units.[7] The production of polypropionates is not restricted to *Siphonaria* species as they have also been isolated from micro-organisms, sponges, fungi and echinoderms.[73,88] Marine polypropionates are structurally similar to the actinomycete antibiotics, such as erythromycin, and this may have encouraged marine natural products chemists to study the polypropionate secondary metabolites of marine organisms in the hope of finding new molecules with similar biological activities. A large number of unusual, acyclic (*e.g* siphonariendione **(148)**),[89] and cyclic polypropionates containing furanone (*e.g.* siphonarienfuranone **(149)**),[90] 2-pyrone (*e.g.* pectinatone **108**),[91] 4-pyrone (*e.g.* vallartanone A **(150)** and B **(151)**)[92] or hemiacetal rings (*e.g.* denticulatins A **(152)** and B **(153)**)[93] have been isolated from siphonariids and these, together with other marine polypropionates, have been reviewed recently by Davies-Coleman and Garson.[73] It should be noted that the following polypropionates were omitted from this review:

norsiphonarienfuranone **(154)** from *S. lessoni*,[94] 2-acetylnorsiphonarienfuranone **(155)** from a Chilean species of *S. lessoni*,[95] 2-deoxysiphonarienfuranone **(156)** from *S. pectinata*,[96] and the unusual sulphated polypropionates 2,6-dimethylnonane-1-sodium sulphate **(157)** and 2,4,6-trimethylnonane-1-sodium sulphate **(158)** from the sea cucumber *Cucumaria frondosa*.[88]



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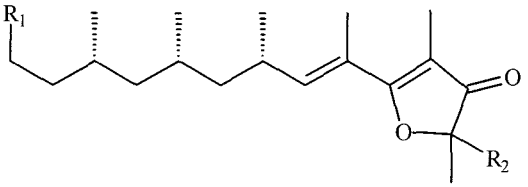
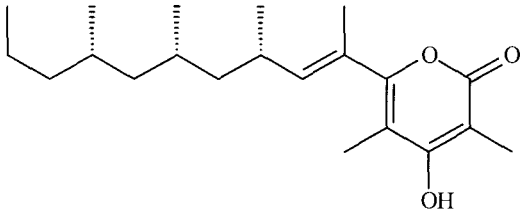


149 $R_1 = CH_3$, $R_2 = OH$

154 $R_1 = H$, $R_2 = OH$

155 $R_1 = H$, $R_2 = OAc$

156 $R_1 = CH_3$, R2 = H



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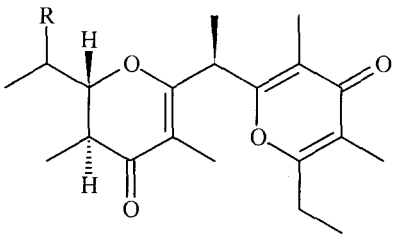
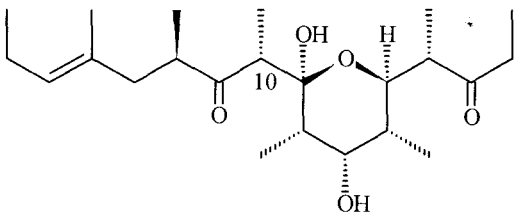


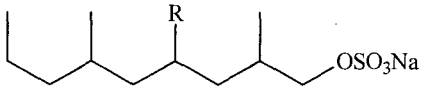
150 $R = CH_3$

151 R = H



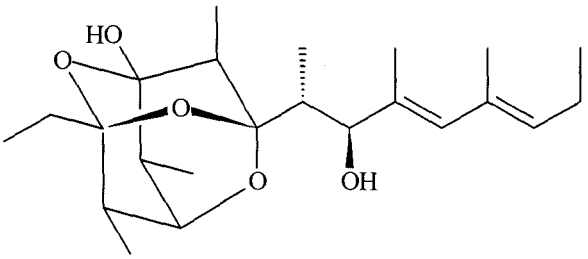
152

153 epimeric at C_{10}



157 R = H

158 $R = CH_3$



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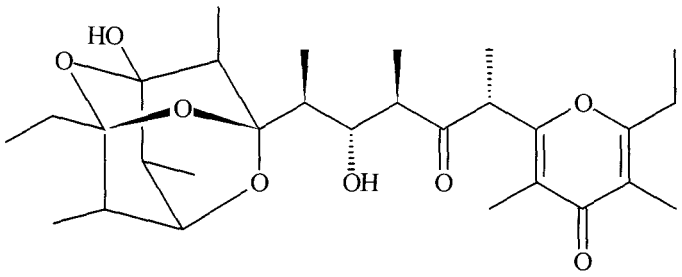


160

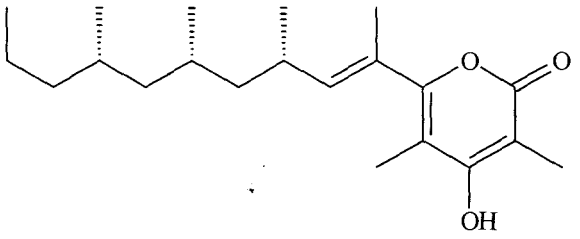
While the siphonariid polypropionates are structurally and biosynthetically related to the macrolide and polyether antibiotics, they show negligible antimicrobial activity and their biological and ecological function is uncertain.[73] The presence of polypropionates in the supposedly toxic mucous secretions of siphonariids suggests their involvement in chemical deterrence.[87,93,97] Unfortunately, few ecological studies have been carried out with pure polypropionates.[12,67] This may reflect the small quantities isolated and the instability of these compounds. Vallartanone B, when applied to krill at a concentration of 100 μg mg^{-1}, was found to deter the predatory fish *Thalassoma lunare*, while vallartanone A induced larval settlement in the tubeworm *Phragmatopoma californica*.[98] Denticulatins A and B, isolated from *S. denticulata* also showed ichthyotoxic activity toward goldfish.[99] Paradoxically, both *S. maura* and *S. denticulata* are commonly eaten by predators.[92,98] Although the role of polypropionates in chemical defence requires further investigation, the fact that these compounds may also have other ecological functions cannot be ignored.

Another possible reason for the puzzling lack of comprehensive evidence in support of polypropionate production as a chemical defence mechanism in *Siphonaria* may be the actual "natural product" status of many polypropionate metabolites. Synthetic studies have shown that a large number of cyclic polypropionates may be the result of non-enzymatic,

thermodynamically favoured cyclisations of acyclic precursors.[73] For example, the hemiacetal ring in denticulatin A and B, the trioaxaadamantane ring found in maumvatin **(159)** and caloundrin B **(160)** and the 4-pyrone ring found in vallartanone A and B may all arise "spontaneously" from acyclic precursors, with cyclisation taking place during the isolation process.[73,100-103] The above observations suggest that many reported siphonariid polypropionates are not genuine natural products and this may therefore account for their lack of biological activity. The actual natural products may be very unstable acyclic compounds which rearrange on isolation.[73] Therefore a challenge for future research in this field may be the isolation and identification of these "true" acyclic secondary metabolites of *Siphonaria* species with a concomitant investigation of their ecological role.

2.1.2 *Siphonaria capensis*

Of the nine recognised *Siphonaria* species[74] occurring in southern Africa, only two have been subjected to chemical investigation. *S. concinna* contained predominantly the ubiquitous polypropionate pectinatone **108**,[19] while *S. serrata* contained a new polypropionate, siserrone **109**.[75]



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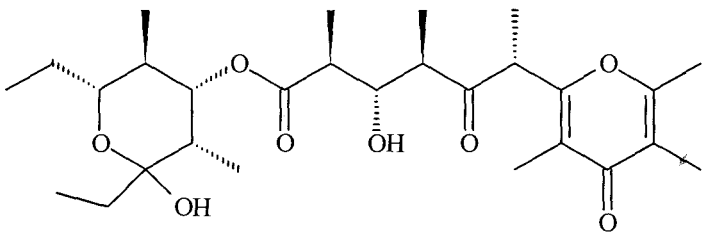


109

S. capensis is one of the most common limpets found in the mid to high shore areas around the coast of South Africa.[74,87] Some individuals remain submerged in intertidal pools, while others live on intertidal rocks that are exposed at low tide. Both groups feed only at low tide and return to a fixed scar after feeding.[87] The feeding behaviour of *S. capensis* therefore makes this mollusc susceptible to predation by both aquatic and terrestrial predators. The mantle and foot of *S. capensis* is richly supplied with complex mucous glands which produce copious amounts of a white mucous when directly stimulated or irritated. This mucous was observed to deter predatory whelks in field studies by Branch and Cherry,[87] who also studied the responses of a large number of other intertidal predators (both aquatic and terrestrial) to *S. capensis*. These included the giant clingfish (*Chorismodoris dentex*, an intertidal limpet specialist), a bird, the African black oyster catcher (*Haematopus moquini*) and the seastar *Marthasterias glacialis*. None of these predators were observed to feed on *S. capensis* even though they fed on the co-occurring patellid limpet, *Patella granularis*. An acetone extract of *S. capensis* showed marginal antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*.[104]

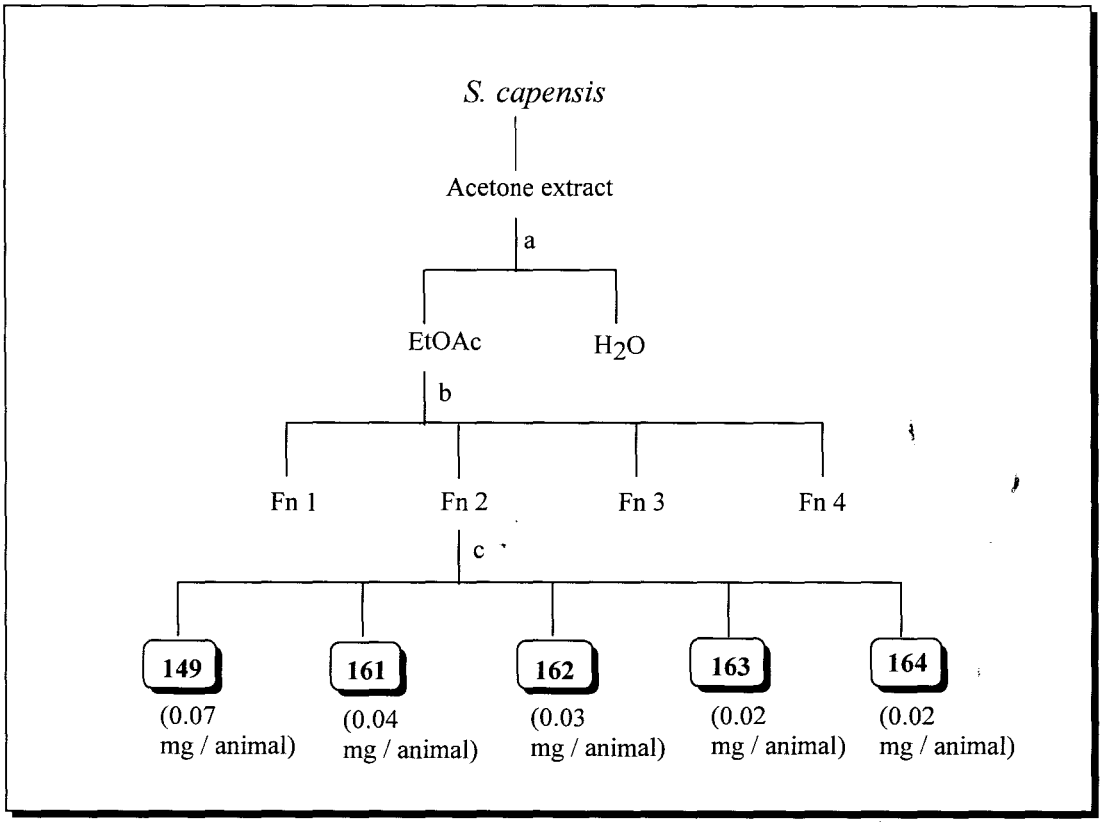
Figure 2.1 *S. capensis* from Bushman's River Mouth

In the following sections of this chapter, the isolation and characterisation of the polypropionate metabolites from *S. capensis* is described.

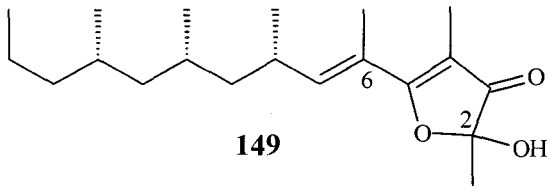
2.2 RESULTS AND DISCUSSION

2.2.1 Collection, extraction and isolation of polypropionates

Specimens of *S. capensis* were collected at the Bushman's River Mouth, South Africa, and exhaustively extracted in acetone. The combined acetone extracts were concentrated and fractionated according to Scheme 2.1. Solvent partitioning (EtOAc-water) followed by silica gel flash chromatography and normal phase HPLC, yielded C-2 epimeric mixtures of *E* and *Z* siphonarienfuranone, **149** and **(161)**, capensinone **(162)**, capensifuranone **(163)**, and (2*E*, 4*S*, 6*S*, 8*S*)-2,4,6,8-tetramethyl-2-undecenoic acid **(164)**.



Scheme 2.1 Extraction and isolation procedure for *S. capensis*: (a) Solvent partitioning (EtOAc-H_2O); (b) silica gel flash chromatography (EtOAc-hexane, 1:2); (c) Normal phase HPLC (EtOAc-Hexane, 1:4)



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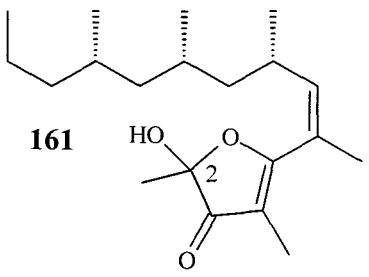

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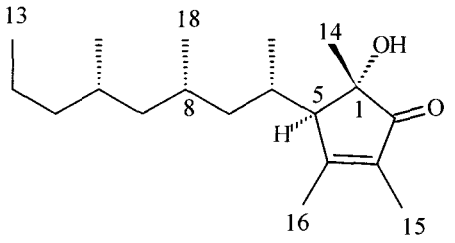

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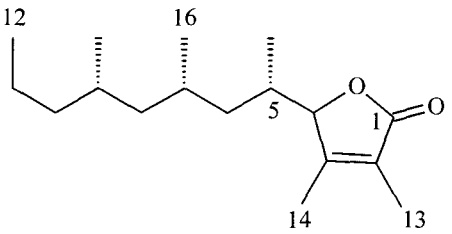

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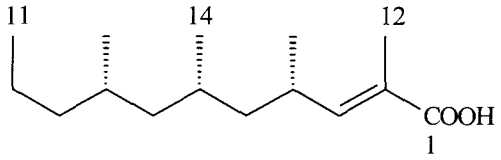

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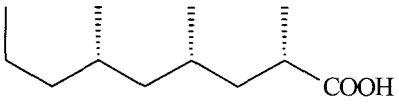
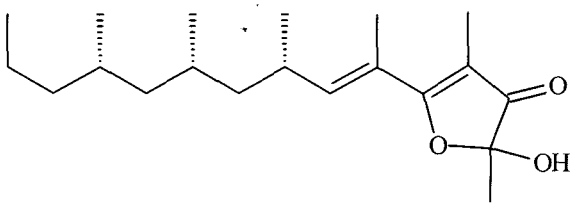


165

2.2.2 Structure elucidation of polypropionates

2.2.2.1 *Siphonarienfuranone*



149

The two geometric isomers, *E* and *Z* siphonarienfuranone, were the major metabolites present in the acetone extract of *S. capensis*. These compounds have been isolated previously, also as inseparable C-2 epimeric mixtures, from *S. grisea*[90] and *S. pectinata*.[96] The NMR data for the epimeric mixtures of **149** and **161**, isolated from *S. capensis*, including the characteristic duplication of the C-2 and C-5 ^{13}C NMR signals (δ 101.0/

101.1 and 181.8/ 182.0 respectively) were consistent with published values (Figure 2.2).[90] A significant difference between the optical rotation of the epimeric mixture of **149** ($[\alpha]_D$ + 54°, *c* 0.18, $CHCl_3$) from *S. capensis* and that reported by Norte *et al.*[90] for the same mixture from *S. grisea* ($[\alpha]_D$ +102°, *c* 0.14, $CHCl_3$) was observed. This prompted us to investigate the stereochemistry of the side chain in **149** and **161**. Oxidative cleavage of the double bond in **149** was achieved by treatment of **149** with $HIO_4/RuCl_3$.[105] The main product isolated was (2*S*, 4*S*, 6*S*)-2,4,6 trimethylnonanoic acid **165** ($[\alpha]_D$ +15°, *c* 0.50, $CHCl_3$; lit. +15°, *c* 0.60, $CHCl_3$) confirming the expected absolute stereochemistry of the three chiral centres in the side chain of **149** and **161**. Ruthenium tetroxide oxidation of pectinatone **108** ($[\alpha]_D$ +66°, *c* 0.17, $CHCl_3$; lit[90] +64°, *c* 0.11, $CHCl_3$), available from a previous investigation of the polypropionate constituents of the South African siphonariid *S. concinna*,[19] also gave 2*S*, 4*S*, 6*S*-trimethylnonanoic acid ($[\alpha]_D$ +14°, *c* 0.54, $CHCl_3$). This not only corroborated the consistency of the oxidative method employed, but also provided a suitable NMR standard for comparison with the oxidative degradation products of **149** and **164**. A possible explanation for the difference in optical rotation is the isomerisation of **149** to **161** (in addition to the C-2 epimerisation of each compound), also reported by Norte *et al.*,[90] and is a result of the different concentrations of each isomer at the time when the rotation was recorded. The isomerisation was confirmed when a pure sample of **149** was re-analysed by ^1H NMR after a few days, showing almost complete isomerisation to **161**.

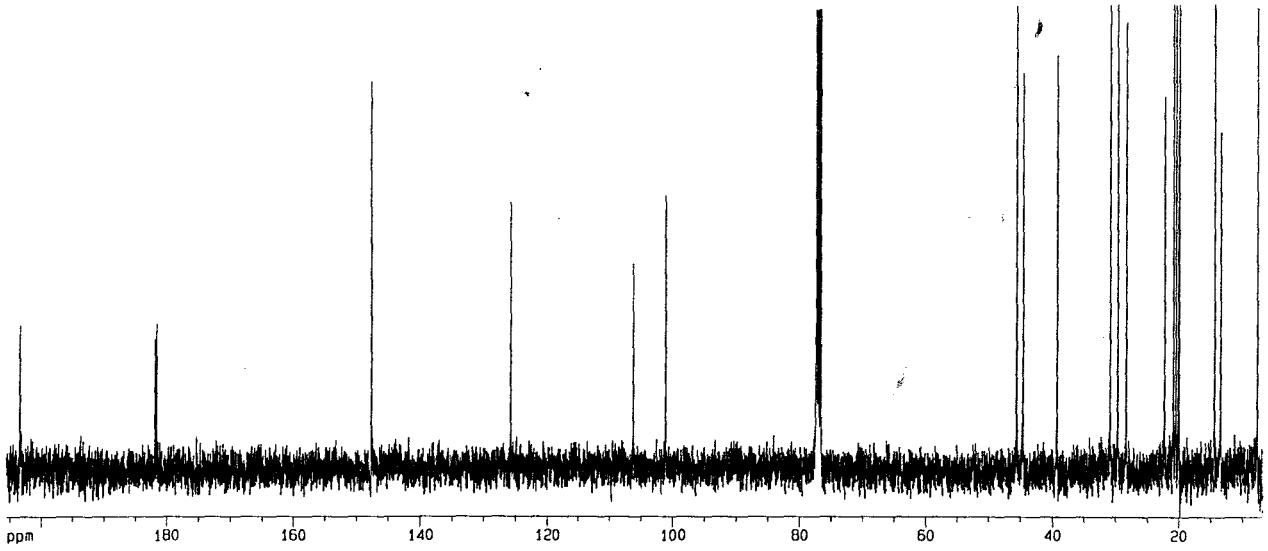
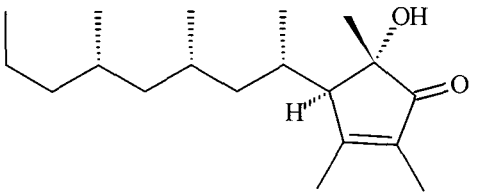


Figure 2.2 ^{13}C NMR spectrum ($CDCl_3$, 100 MHz) of epimeric (*E*)-siphonarienfuranone showing the duplication of signals.

2.2.2.2 *Capensinone*



162

Capensinone **162**, a minor polypropionate metabolite in the *S. capensis* extract, was isolated as a yellow oil ($[\alpha]_D$ -65°, *c* 0.42, $CHCl_3$). A molecular formula of $C_{19}H_{34}O_2$ was deduced from the NMR data and confirmed by HREIMS (*m/z* 294.2546, Δmmu -1.3). Two of the three double bond equivalents required by the molecular formula could be assigned to an αβ-unsaturated ketone (δ_C 210, 133, 171 and IR ν_{max} 1700, 1630 cm^{-1}),[106] suggesting that the structure of **162** contained a single ring to account for the third double bond equivalent. A broad IR absorption band at 3450 cm^{-1} and a quaternary signal at δ 77.6 in the ^{13}C NMR spectrum indicated the presence of a tertiary alcohol functionality. Significant overlap of signals in the methylene envelope region of the ^1H NMR spectrum complicated the assignment of resonances. However, changing the NMR solvent from $CDCl_3$ to C_6D_6 significantly improved the separation of overlapping signals (Figure 2.3). The ^1H NMR spectrum (C_6D_6) of **162** clearly showed the presence of seven methyl proton signals: three doublets (δ 0.75, 0.87, and 0.96), a triplet (δ 0.93) and three singlets (δ 1.25, 1.60 and 1.47), immediately suggesting a polypropionate skeleton for this compound. The signals at δ 0.75 (3H, d, J = 7.3 Hz), 0.87 (3H, d, J = 6.5 Hz), 0.93 (3H, t) and 0.96 (3H, d, J = 6.6 Hz) were assigned to four methyl groups on a 1,3,5-trimethyloctyl side chain. This assignment was confirmed by a combination of one- and two-dimensional NMR experiments. The ^1H and ^{13}C chemical shifts of **162** are presented in Table 2.1. The two deshielded resonances at δ 1.75 and 2.04 were suggestive of olefinic methyl groups. Definitive two- and three-bond HMBC correlations from H-5 (δ 2.70) to signals at δ 168.6 (C-4), 133.2 (C-3) and 77.6 (C-1) placed H-5 in the allylic position and also adjacent to the carbinol carbon. Further HMBC correlations from CH_3-14 (δ 1.25) to the carbinol carbon (δ 77.6) and to the carbonyl (δ 208.7) and from CH_3-15 to the carbonyl (δ 208.7), unequivocally established the substitution pattern in the cyclopentenone ring (Figure 2.4). A key HMBC correlation from H-5 (δ 2.70) to the methyl carbon signal at δ 17.9 (C-17) allowed attachment of the 1,3,5-trimethyloctyl side chain to the cyclopentenone ring, thus completing the structural assignment of **162**.

The relative stereochemistry at C-1 and C-5 was inferred from a series of 1D-NOE

difference experiments. Irradiation of H-5 did not produce enhancement of the CH_3-14 methyl protons and *vice versa*, suggesting that they are on opposite sides of the ring. It is appreciated that the absence of an NOE is not a strong criterion for assigning relative stereochemistry, and this assignment must thus be treated as tenuous. The all (*S*)-absolute configuration for the three chiral centres (C-6, C-8 and C-10) in the side chain is suggested on the assumption that **162** arose through a common biosynthetic pathway to **149** and **161** and that it would thus be unlikely that the stereochemistry in the side chain is different. This hypothesis is supported by a study conducted by Garson *et al.*[107] of the structural and stereochemical trends occurring among siphonariid metabolites. These researchers showed that polypropionates containing an alkenyl chain (*e.g.* pectinatone **108**, siphonarienfuranone **149** and siphonariendione **148**) all possess (*S*)-stereochemistry at the contiguous methyl-substituted chiral centres.

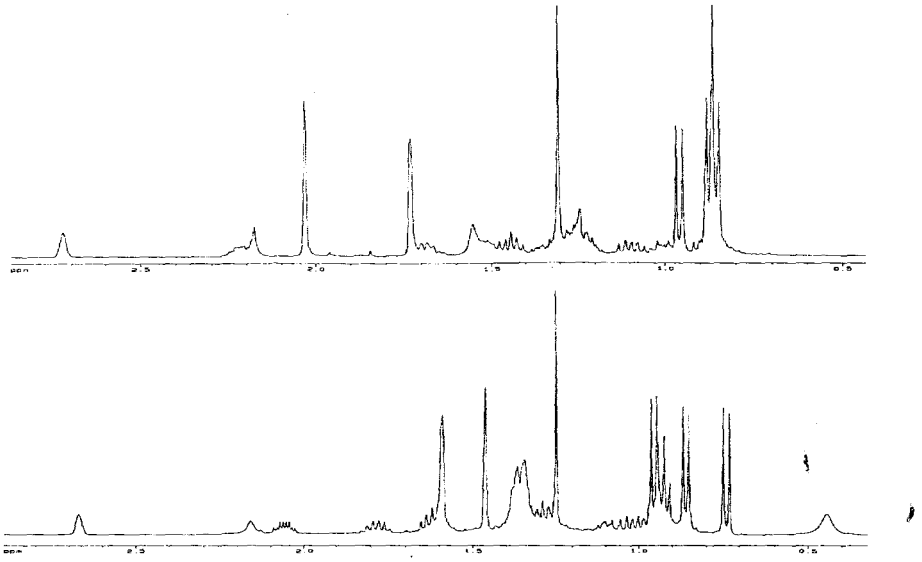


Figure 2.3 ^{1}H NMR spectra (400 MHz) for **162** in (a) $CDCl_3$ and (b) C_6D_6.

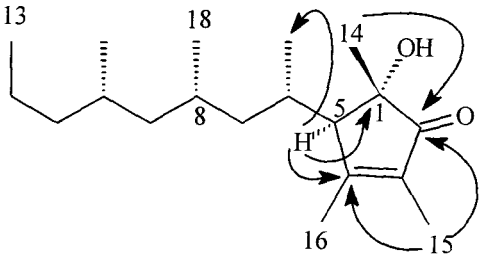
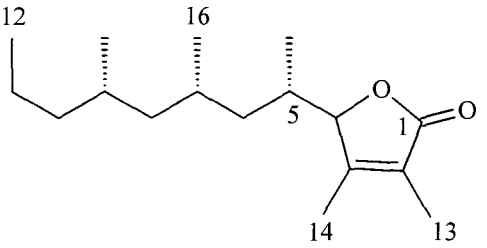


Figure 2.4 Key HMBC correlations for capensinone

Table 2.1 ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for capensinone and capensifuranone.

	Capensinone				Capensifuranone	
	δ_C $CDCl_3$	δ_C C_6D_6	δ_H, $CDCl_3$ m, *J* (Hz)	δ_H, C_6D_6, m, *J* (Hz)	δ_C $CDCl_3$	δ_H, $CDCl_3$, m, *J* (Hz)
1	77.3 s	77.6	-	-	174.8 s	-
2	209.7 s	208.7	-	-	124.6 s	-
3	133.4 s	133.2	-	-	158.0 s	-
4	170.8 s	168.6	-	-	87.8 d	4.68, br s
5	58.0 d	56.8	2.73, br s	2.70, br s	32.1 d	2.03, m
6	29.8 d	29.8	2.22, br m	1.60, m	36.6 t	0.90, m
7a	41.9 t	41.9	1.45, m	1.63, m	29.8 d	1.46, m
7b			1.10, m	1.02, m		
8	28.1 d	27.9	1.70, m	1.80, m	44.5 t	1.16, m
						0.78, m
9a	44.9 t	45.4	1.25, m	1.30, m	27.7 d	1.47, m
9b			0.90, m	0.95, m		
10	29.7	29.5	1.52, m	2.06, m	38.4 t	1.28, m
						0.96, m
11a	38.7 t	39.0	1.30, m	1.37, m	19.8 t	1.33, m
11b			1.02, m	1.18, m		1.22, m
12a	19.9 t	20.0	1.35, m	1.38, m	14.4 q	0.87, m
12b			1.25, m	1.27, m		
13	14.4 q	14.4	0.90, m	0.93, m	8.4 q	1.82, s
14	22.2 q	22.4	1.32, s	1.25, s	12.4 q	1.93, s
15	8.2 q	7.9	1.75, s	1.60, s	17.5 q	1.10, d, 7
16	16.0 q	14.8	2.04, s	1.47, s	21.2 q	0.83, d, 7
17	18.2 q	17.9	0.97, d, 7	0.75, d, 7.3	20.7 q	0.83, d, 7
18	20.6 q	20.4	0.95, d, 7	0.87, d, 6.5	-	-
19	20.8 q	20.5	0.87, d, 7	0.96, d, 6.6	-	-

2.2.2.3 *Capensifuranone*



163

The second minor metabolite, capensifuranone **163** was obtained as a colourless oil ($[\alpha]_D$ -15°, *c* 0.29, $CHCl_3$) which showed a molecular ion at *m/z* 266.2236 (Δmmu-10), consistent with a molecular formula of $C_{17}H_{30}O_2$, in its HREI mass spectrum. The ^1H NMR spectrum of **163** showed a deshielded methine resonance at δ 4.68 and two deshielded olefinic methyl singlets at δ 1.93 and 1.82. An αβ-unsaturated ester functionality was deduced from resonances at δ 174.8 and 158.0 in the ^{13}C NMR as well as a carbonyl absorption band at 1720 cm^{-1} in the infrared spectrum. This accounted for two of the three degrees of unsaturation implied by the molecular formula, suggesting the presence of a single ring. The absence of an hydroxyl absorption band in the infrared spectrum together with an oxymethine signal at δ 4.68, which was correlated to a signal at δ 87.8 in the HMQC spectrum, suggested the presence of a lactone ring. Definitive HMBC correlations from the CH_3-14 protons (δ 1.93) to the oxymethine resonance at δ 87.8 and the quaternary olefinic carbons at δ 158.0 and 124.6, as well as from CH_3-13 (δ 1.82) to the signals at δ_C 174.8 (carbonyl), 158.0 and 124.6, established the substitution pattern around the ring (Figure 2.5). Finally, HMQC and DEPT NMR spectra indicated the presence of a further four methyl, four methylene and three methine carbon signals which were immediately assigned to a 1,3,5-trimethyloctyl side chain by comparison of the NMR data of **163** with those of **162**. An HMBC correlation from CH_3-15 (δ 1.10) to the oxymethine carbon at δ 87.8 linked the side chain to the lactone ring. The (4*S*, 7*S*, 9*S*)-stereochemistry depicted in **163** is again proposed using a similar rationale to that followed for **162**. Unfortunately the configuration at C-4 could not be assigned due to the instability of **162**. In retrospect, it may be possible to assign the absolute chemistry at C-4 by interpretation of the $\pi \rightarrow \pi^*$ electronic transitions of the αβ-unsaturated lactone in the circular dichroic spectra of

162.[108] Table 2.1 lists the ^{1}H and ^{13}C NMR data for **163**.

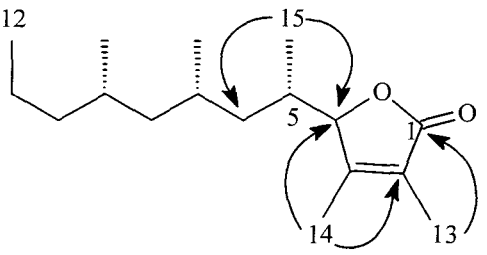
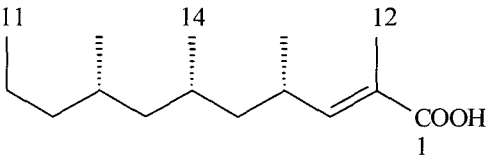


Figure 2.5 Key HMBC correlations for capensifuranone.

2.2.2.4 *Tetramethyl undecenoic acid*



164

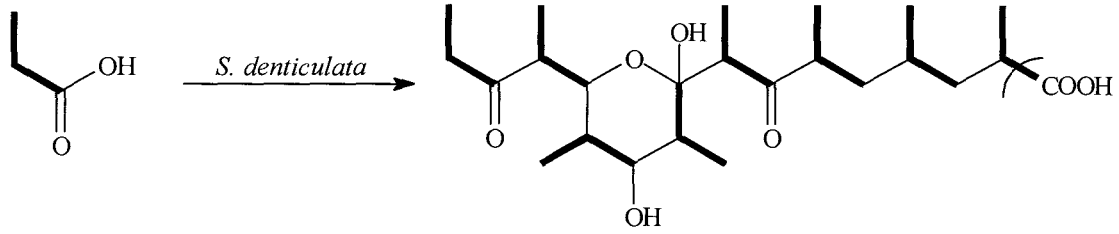
A molecular formula of $C_{15}H_{28}O_2$ was deduced for **164** from NMR data and confirmed by HREIMS (*m/z* 240.2092). Three deshielded resonances at δ 173.3, 151.0 and 125.4 in the ^{13}C NMR spectrum of **164**, together with infrared absorptions band at 3000 cm^{-1} and 1780 cm^{-1}, accounted for the two degrees of unsaturation implied by the molecular formula suggesting the presence of an αβ-unsaturated carboxylic acid. The analysis of the ^{1}H and ^{13}C NMR spectra indicated that **164** possessed the same 1,3,5,7-tetramethyldec-1-enyl moiety as siphonarienfuranone **149**. The olefinic methyl signal at δ 1.85 was correlated to a carbonyl signal at δ 173.3 and to the two olefinic carbons at δ 125.4 and 151.0 from HMBC data. The latter resonance showed an HMBC correlation to a methyl signal at δ 1.00 (3H-13) which was also correlated to a methine carbon at δ 31.1 (C-4) and a methylene carbon at δ 44.3 (C-5). A W-coupling (J = 1 Hz) from H-3 (δ 6.65) to CH_3-12 (δ 1.85) was also observed in the ^{1}H NMR spectrum of **164**. These connectivities thus defined the structure of the αβ-unsaturated moiety of **164**.

The *E*-configuration of the double bond followed from the ^{13}C chemical shift of the olefinic methyl (δ 12.1; a *Z*-configuration requires an olefinic methyl chemical shift of δ_C 20.9).[109] Oxidative degradation of **164** to 2*S*, 4*S*, 6*S*- trimethylnonanoic acid ($[\alpha]_D$ +19°, *c* 0.25, $CHCl_3$)[90] confirmed the stereochemistry of this compound. The all (*S*)-configuration in **164** further supports the tentative assignment of an (*S*)-absolute stereochemistry to each

of the three acyclic chiral centres in **162** and **163**.

2.2.3 Biosynthesis

Polypropionate metabolites may arise through either a linear combination of propionate units or a polyacetate pathway followed by methylation via a C_1-tetrahydrofolate metabolism. Manker *et al.*[98] showed that while [1-^{14}C]-propionate was incorporated into denticulatin A and B radiolabelled acetate was not (Scheme 2.2), confirming that an acetate-methionine pathway does not operate in *S. denticulata*. Further evidence in support of a propionate pathway in *Siphonaria* was obtained by Garson *et al.*[110] who showed that *S. zelandica* incorporated [1-^{14}C]-propionate into siphonarin A and B, while acetate was incorporated as a chain starter in the biosynthesis of siphonarin A (Figure 2.6). Other siphonariid polypropionates may arise in a similar manner. For example, pectinatone **108** can be considered to derive from a linear combination of seven propionate units and siphonarienfuranone **149** from six propionate units and an acetate chain starter.



Scheme 2.2 Proposed biosynthesis of the denticulatins

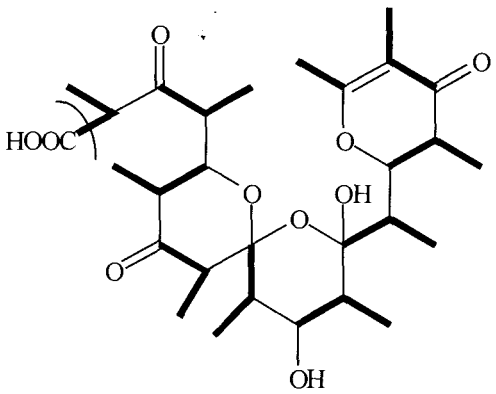
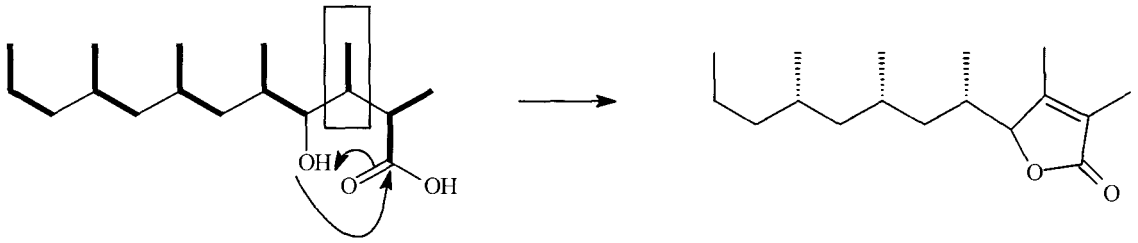


Figure 2.6 Proposed biosynthesis of siphonarin A

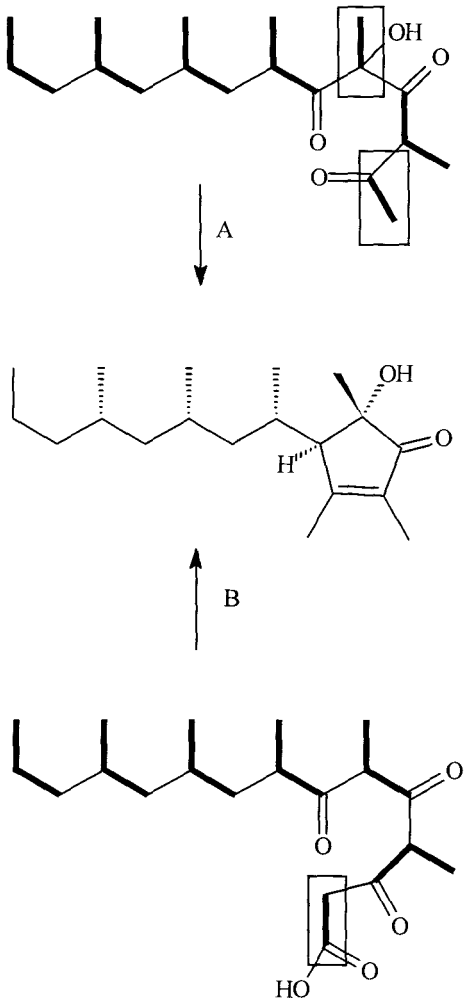
The biosynthesis of **164** from five propionate units is unambiguous, while the biosynthetic origin of capensinone and capensifuranone is uncertain and it would appear that acetate

needs to be incorporated in addition to propionate. A ^{14}C-labelling study may clear up some of these questions. Following discussions with Professor Garson (University of Queensland) a number of possible biosynthetic routes to capensinone and capensifuranone are considered here. Capensifuranone may arise from a linear combination of five propionates and an acetate unit (Scheme 2.3). The use of acetate in a chain building position, as opposed to a chain starter, has precedence in the biosynthesis of the cyercenes.[111]



Scheme 2.3 Proposed biosynthesis of capensifuranone

The biosynthesis of capensinone is more difficult to speculate upon. Firstly, a combination of five propionates and two acetates could be considered (Pathway A, Scheme 2.4), or alternatively, a combination of six propionates and an acetate followed by decarboxylation (Pathway B, Scheme 2.4). In both these examples, however, closure of the linear chain to give the cyclopentanone ring is problematic and has not been encountered in the biosynthesis of other marine polypropionates. The above discussion highlights the unusual structural features of the metabolites from *S. capensis*. The unusual, non-contiguous nature of the polypropionate chain in **149**, **162**, and **163** make these three compounds worthy of a biosynthetic study.



Scheme 2.4 Proposed biosynthesis of capensinone

2.4 SUMMARY AND CONCLUSION

Three new polypropionates have been isolated from the endemic South African false limpet *Siphonaria capensis* and their structures determined. Polypropionate metabolites with five-membered rings are rare in the marine environment and have been limited to furanones.[73] Capensinone is the first example of a marine polypropionate containing a cyclopentenone moiety. The vicinal arrangement of the two olefinic methyl groups in both **162** and **163** is most unusual for polypropionate-derived metabolites. The origin of the unusual structures of **162** and **163** would make an intriguing biosynthetic study. In view of the contradicting evidence concerning the ecological role of siphonariid polypropionates, combined with the ecological observations on *S. capensis* by Branch *et al.*,[87] a chemical ecological study of *S. capensis* would be useful.

Chapter Three

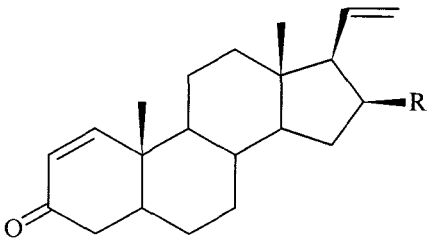
NEW PREGNADIENE STEROIDS FROM THE ENDEMIC SOUTH AFRICAN SOFT CORAL *PIETERFAUREA UNILOBATA*

3.1 INTRODUCTION

Marine invertebrates are prolific producers of novel steroids that have received considerable attention because of the frequent occurrence of unusual structural features when compared with steroids of terrestrial origin.[7,112-115] Pregnane-derived steroids, however, are relatively rare in the marine environment and have only been isolated from echinoderms,[114,116-119] octocorals,[31,32,121-131] and sponges.[113,131-139]

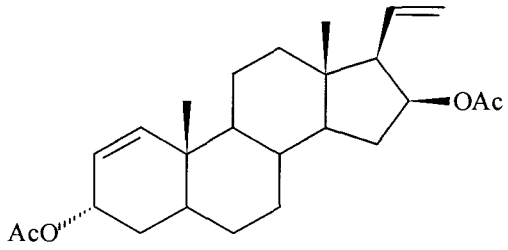
A limited number of pregnadiene metabolites have been isolated from octocorals (soft corals and gorgonians). Higgs and Faulkner[31] isolated 5α-pregna-1,20-dien-3-one **22**, pregna-1,4,20-trien-3-one **(166)**, 5α-pregn-20-ene-3α-ol 3-acetate **(167)** and 5α-pregn-20-ene-1α,3α-diol-3-acetate **(168)** from an unidentified soft coral from Canton Island. The structures of **22** and **166-168** were proposed from spectroscopic data and chemical conversion to known compounds. Compound **22** was also isolated from two colour variants of the South African soft coral *Capnella thyrsoida* together with two new 16-acetoxypregnene steroids **23** and **24**.[32]

Acetone extracts of *Gersemia rubiformis* contained **166** and pregna-5,21-diene-3β-ol **(169)**.[121,122] The structures of **167** and **169** were determined by extensive mass spectroscopic analysis and verified by synthesis from progesterone. Pregna-5,21-diene-3β-ol was also obtained by chemical degradation of the following glycosides: the muricins **(170-173)** isolated from *Muricea fruticosa*,[124] **(174)**, obtained from *Pseudoplexaura wagenaari*,[125] and **(175)** from the gorgonian *Eunicea* sp..[126] Compounds **170-173** inhibited growth of the pennate diatom *Phaeodactylum tricornutum* and may play an important role in reducing fouling on *M. fruticosa*.[125]



22 R = H

23 R = OAc



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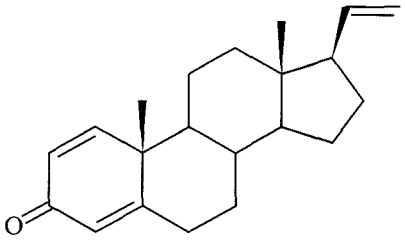
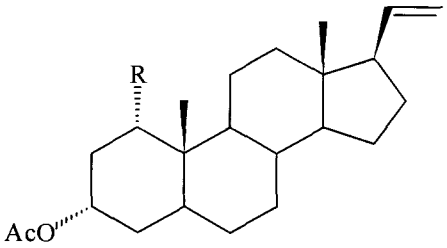
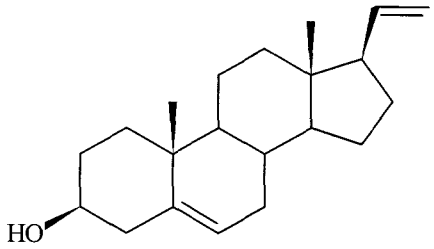


166

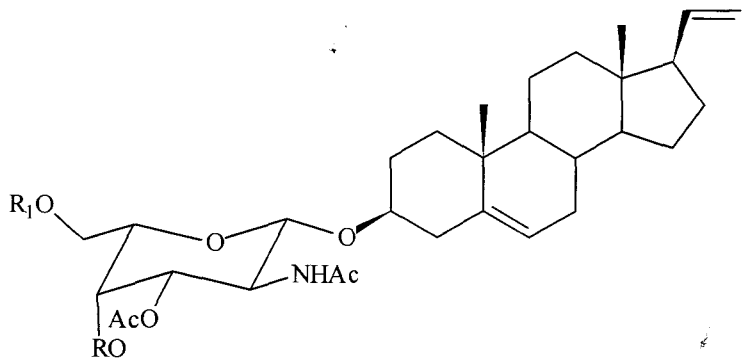


167 R = OH

168 R = H



169

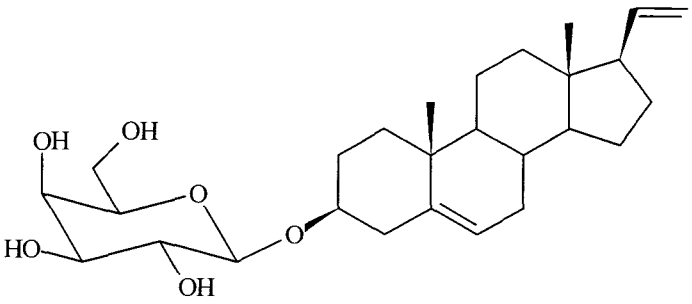


170 $R_1 = R_2 = Ac$

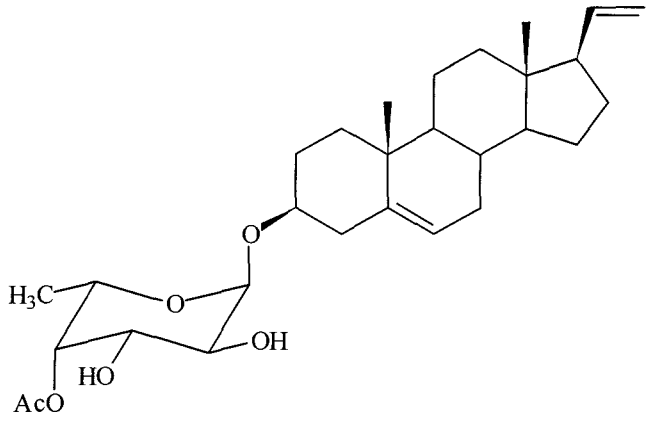
171 $R_1 = CO.CH_2CH_2CH_3$, $R_2 = Ac$

172 $R_1 = Ac$, $R_2 = CO.CH_2CH_2CH_3$

173 $R_1 = R_2 = CO.CH_2CH_2CH_3$

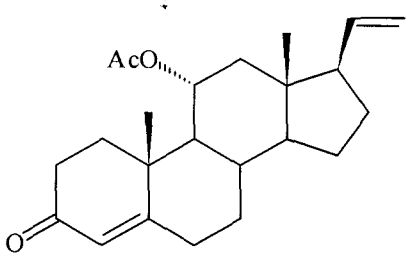


174



175

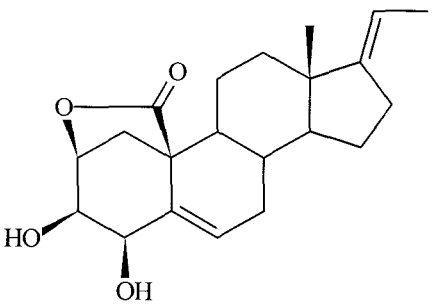
The 11-acetoxy pregnane **(176)** was isolated from the Mediterranean gorgonian *Eunicella cavolini* and its structure determined as pregna-4,20-dien-11α-ol-3-one 11-acetate from spectral data and partial synthesis.[123]



176

Extensive work has been carried out on the sterol composition of sponges and a large number of unusual steroids have been isolated.[113,131,132] The unusual pregnane-3β,4β-dihydroxy-10,2-carbolactones **(177-179)** were obtained from the Hawaiian sponge

Strongylophora sp.[137] Structures were assigned on the basis of extensive 2D-NMR techniques and confirmed by X-ray crystallography. These compounds are presumably derived from 2,3,4-trihydroxy-5-pregnenes. Pregnane **179** showed only marginal cytotoxicity.[137]



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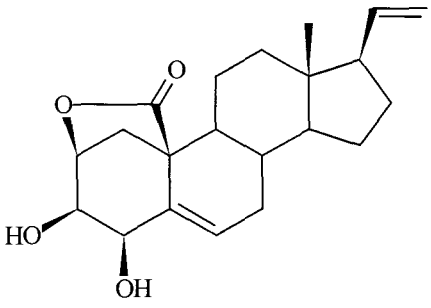

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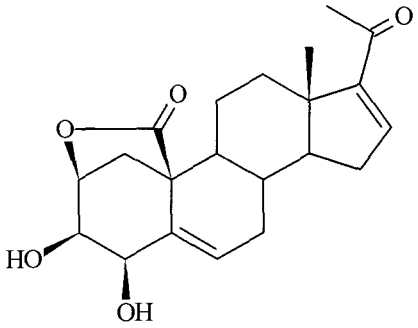


179

The high level of endemism characteristic of South African soft corals[16] make these invertebrates attractive targets for natural products investigation. Therefore, in continuation of our research group's systematic study of the chemistry of southern African soft corals[32,34] we investigated the endemic soft coral *Pieterfaurea unilobata* (Figure 3.1). Examination of the EtOAc extract of *P. unilobata* resulted in the isolation and identification of seven pregnadiene steroids, pregna-5,20-dien-3β-ol **169**, pregna-5,20-diene-3α,7α-diol 3α-acetate **(180)**, pregna-5,20-diene-3β,7α-diol 7α-acetate **(181)**, pregna-5,20-diene-3α,7α,11α-triol 3α-acetate **(182)**, pregna-5,20-diene-3α,7α,11α-triol 3α,7α-diacetate **(183)**, pregna-5,20-diene-3α,7α,19-triol 3α,19-diacetate **(184)**, pregna-5,20-diene-3α,7α,11α,19-tetrol 3α,7α,19-triacetate **(185)**. Pregnadienes **180-185** are the first 7-substituted pregnanes isolated from the marine environment.

3.2 RESULTS AND DISCUSSION

Two species of the genus *Pieterfaurea* (family Nidaliidae), *P. unilobata* and *P. khoisaniana* are known to occur along the coast of South Africa.[16] *P. unilobata* is endemic to the east coast of South Africa in the region stretching from East London to the Durban occurring at depths of 40-93 m depth.[16] Our collection of *P. unilobata* therefore extends the south-western range of this species to Port Alfred.

3.2.1 Collection, extraction and isolation

The purple coloured soft coral identified as *Pieterfaurea unilobata* (Figure 3.1) was collected by hand using SCUBA (-15 m) at Riet Point near Port Alfred in September 1994 and stored frozen. The frozen samples were diced, freeze-dried (143 g dry weight) and extracted with EtOAc (2 x 1.25 L). Removal of the solvent under reduced pressure gave a viscous, dark-orange oil (2.3 g) which was fractionated according to Scheme 3.1.

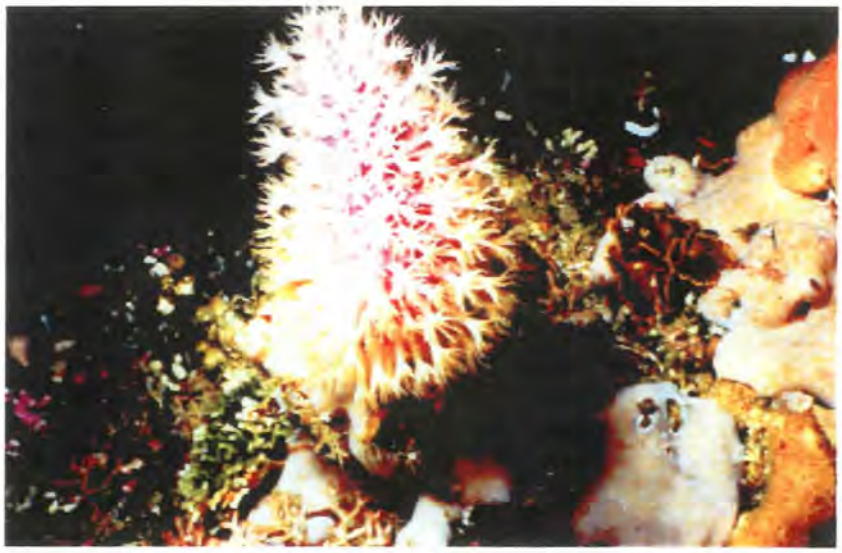
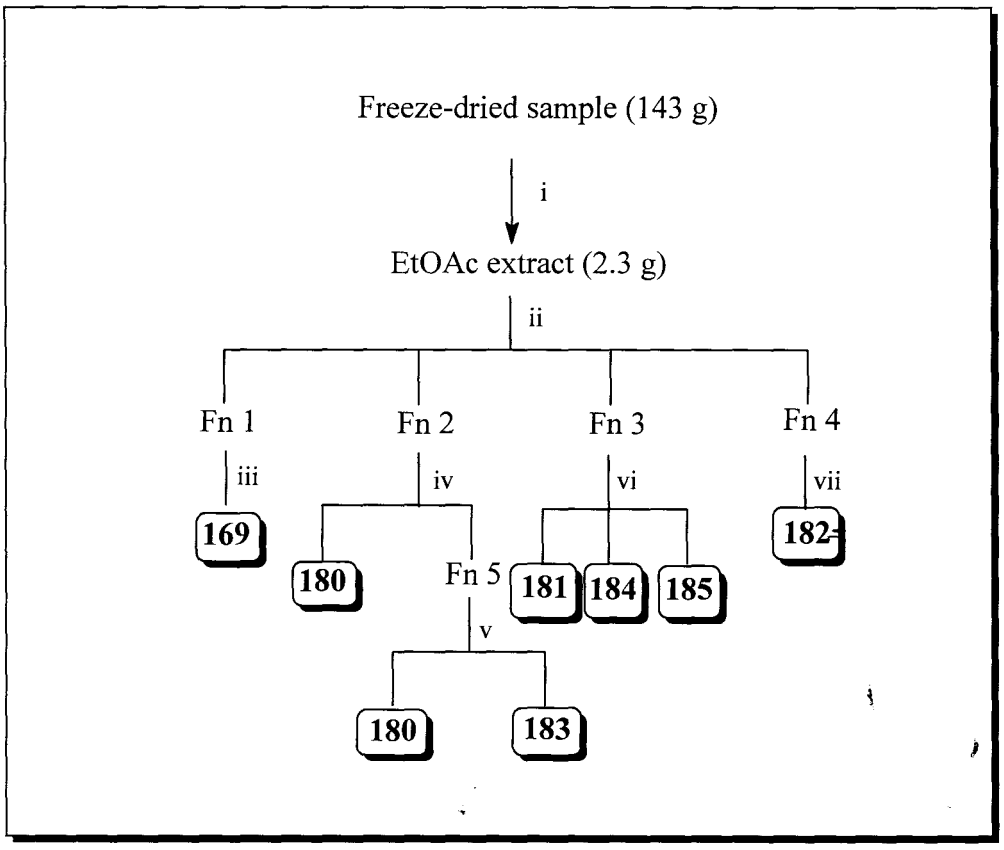


Figure 3.1 Underwater photograph of *Pieterfaurea unilobata.*

The ^{1}H NMR spectrum of the crude EtOAc extract indicated a number of interesting deshielded signals between δ 3.5 and 6.0 and a highly congested methylene envelope (δ 1.0 - 2.5), while thin layer chromatography (EtOAc-hexane, 1:1) revealed a number of brightly coloured spots when visualised with acid spray (10% H_2SO_4 in MeOH, heat).

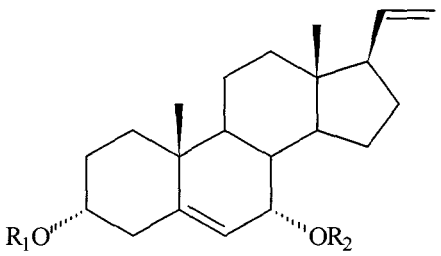
Flash chromatography of the crude extract on silica gel, using an EtOAc-hexane solvent gradient, gave several crude fractions which were analysed by TLC and ^{1}H NMR spectroscopy. Selected fractions were further purified by recrystallisation and normal phase HPLC to give **180** (128.5 mg, 0.09% dry wt), **183** (5 mg, 0.004% dry wt), **182** (3.7 mg, 0.0026% dry wt), **185** (11 mg, 0.008% dry wt), **184** (4.5 mg, 0.003% dry wt), **181** (19 mg, 0.013% dry wt) and **169** (5.2 mg, 0.003% dry wt).



Scheme 3.2 Extraction and isolation scheme for *P. unilobata*. i) Solvent extraction (2 x 1.25 L EtOAc; ii) Silica gel flash chromatography, solvent gradient, EtOAc-hexane (1:4, 1:3, 1:2, 1:1, 1:0); iii) Normal phase HPLC, EtOAc-Hexane (2:3); iv) Recrystallisation from EtOAc-hexane (3:14); v) Normal phase HPLC, EtOAc-hexane (2:7); vi) Normal phase HPLC, EtOAc-hexane (4:7); vii) Recrystallisation from EtOAc-hexane (3:7).

3.2.2 Structure determination

3.2.2.1 *Pregna-5,20-diene-3α,7α-diol 3α-acetate*



180 R_1 = Ac, R_2 = H

186 R_1 = R_2 = Ac

187 R_1 = R_2 = H

The major pregnadiene, **180**, crystallised from EtOAc-hexane (3:14) as well-formed plates (mp 156-159 °C). A molecular formula of $C_{23}H_{34}O_3$ was deduced from NMR data and confirmed by HREIMS (*m/z* 358.2522, Δmmu +1.4). Four olefinic carbon signals (δ 139.7, 144.3, 124.6, 114.6) and a carbonyl (δ 170.7) in the ^{13}C NMR spectrum of **180** accounted for three of the seven degrees of unsaturation required by the molecular formula, suggesting that **180** was tetracyclic. Readily recognisable signals at δ 0.60 (3H, s), 1.0 (3H, s), 5.0 (2H, m) and 5.75 (1H, m) in the ^1H NMR spectrum were in agreement with published values for a pregn-20-ene steroid skeleton.[32,139] An intense mass peak at *m/z* 298 (M^+ - CH_3COOH) in the LREI mass spectrum of **180** indicated the presence of an acetate group. This was confirmed by a characteristic sharp singlet at δ 2.0 (3H, s) in the ^1H NMR spectrum, ^{13}C NMR resonances at δ 170.7 and 21.4, as well as the presence of a strong IR absorption band at 1700 cm^{-1}. Further analysis of the IR, EIMS, ^1H and ^{13}C NMR data indicated the presence of a trisubstituted double bond (δ 144.3 and 124.6) and an oxymethine carbon (δ 64.4). Acetylation of **180** with acetic anhydride in pyridine at room temperature gave the diacetate **186**, which confirmed the presence of a secondary alcohol functionality. Having established the basic pregnene steroid skeleton for **180**, attention was then turned to positioning the acetate, hydroxyl and vinylic functional groups in the pregnene structure.

Structure elucidation of steroids is often hampered by the large number of overlapping signals in the ^{1}H NMR spectrum, especially in the region between 1 and 2 ppm.[139] Two-dimensional NMR techniques such as ^{1}H-^{1}H COSY and heteronuclear HMQC experiments are quite useful in assigning resonances in steroids. Strong COSY correlations were observed between the oxymethine resonance at δ 5.05 (H-3, C*H*OAc) and two methylene doublets at δ 2.55 (H-4β) and 2.25 (H-4α), as well as to a methylene multiplet at δ 1.8 (2H-2). Allylic coupling of the 2H-4 protons to the olefinic proton at δ 5.55 (H-6) and vicinal coupling of the latter proton to the second oxymethine proton at δ 3.84 suggested the partial structure as shown in Figure 3.2. Interestingly, an additional, and unusual, five bond homoallylic coupling from the oxymethine at δ 3.84 (H-7) to the signal at δ 2.55 (H-4β) was also observed (Figure 3.3). An HMBC NMR experiment provided further data to support this partial structure, revealing two and three bond correlations from the 2H-4 methylene resonances to the two olefinic carbons at δ 144.3 (C-5) and 124.6 (C-6) and also a two bond correlation to the oxymethine carbon at δ 70.3 (C-3).

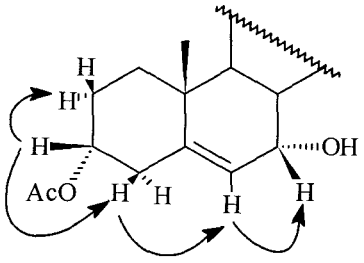


Figure 3.2 Selected COSY-90 correlations for compound **180** used to position the functional groups.

A number of procedures have been developed to differentiate between α and β orientated substituents in steroids. The most common method is by measuring the $W_{1/2}$ value (peakwidth at half height) of the relevant oxymethine resonance in the ^{1}H NMR spectrum.[140] Axial protons exhibit large vicinal, diaxial as well as smaller vicinal, axial-equatorial couplings, whereas equatorial protons show only small vicinal, axial-equatorial and vicinal, diequatorial couplings.[141-143] Normally, the number of closely spaced lines due to spin-spin coupling are not readily discernible and consequently a broad signal is usually

observed. Therefore, axial oxymethine protons typically show a $W_{1/2}$ of 15 - 30 Hz, while equatorial protons show a $W_{1/2}$ of 5 - 15 Hz.[140]

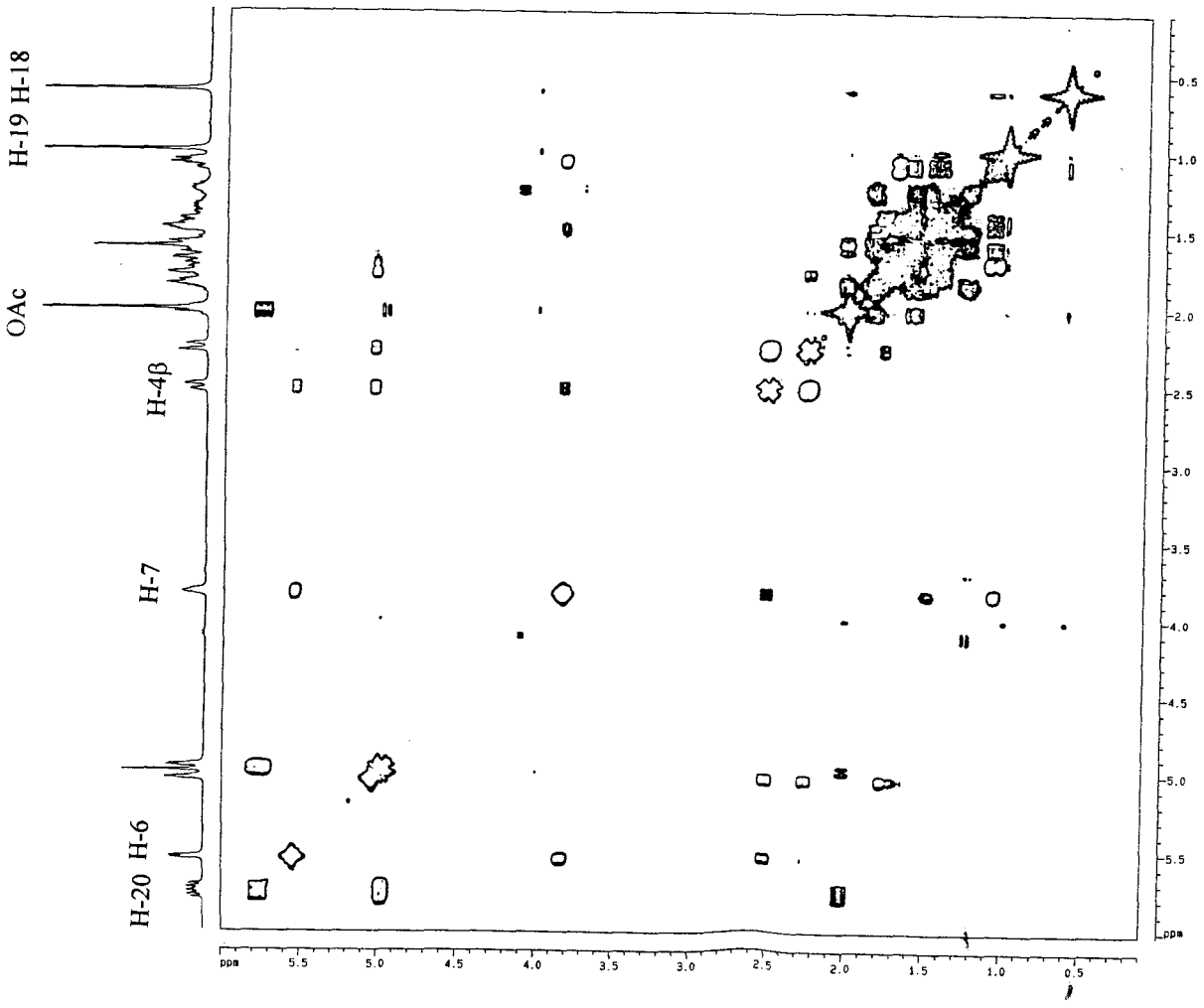


Figure 3.3 COSY-90 spectrum (400 MHz, CDCl3) of **180** showing the unusual 5-bond coupling from H-7 to H-4β.

Reduction of the ester group in **180** with LAH in ether gave the diol **(187)**. The ^{1}H NMR spectrum of **187** showed two oxymethine signals at δ 3.85 ($W_{1/2}$ = 14 Hz, H-7) and 4.10 ($W_{1/2}$ 10 Hz, H-3) indicating equatorial oxymethine protons and thus axial hydroxyl groups. Confirmation of this assignment was provided by NOE difference spectroscopy (NOEDS) which can be extremely useful in the determination of the relative stereochemistry of steroids.[144] Selected NOE enhancements which confirmed the assigned stereochemistry are illustrated in Figure 3.4. Irradiation of the H-4β proton (δ 2.55)

resulted in the enhancement of both the H-3 oxymethine and the H-19 methyl resonances, thus confirming the β-orientation of the H-3 proton.

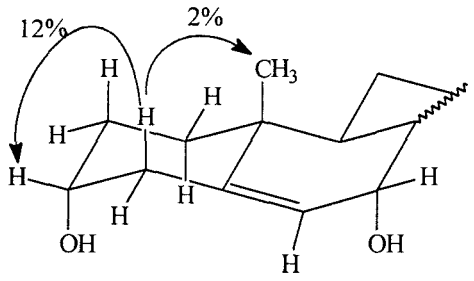


Figure 3.4 Selected NOE enhancements for **187**.

The structure and stereochemistry of **180** was unambiguously verified by single-crystal X-ray diffraction (Figure 3.5). The structure was solved by direct methods using the SHELXS program[145] and refined using the SHELXL-93 program.[146] The atomic coordinates are presented in Appendix 1. The C5-C6 bond length of 1.325(5) Å is consistent with a double bond at this position, as are the relevant bond angles. The unusually short exocyclic C20-C21 bond length (1.18 Å) and large C17-C20-C21 bond angle (133°) are attributable to positional disorder for C21. Attempts to refine a disorder model, however, were unsuccessful. The stereoscopic drawing of **180** is shown in Figure 3.5.

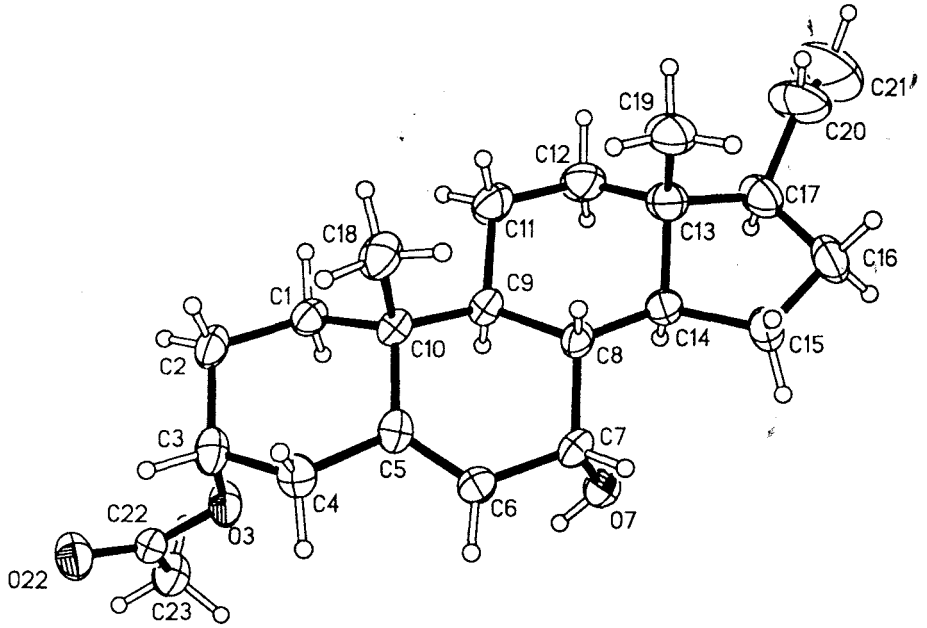


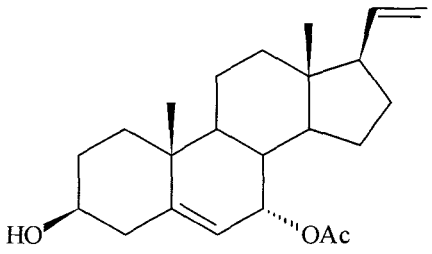
Figure 3.5 X-ray structure of compound **180**.

Comparison of the relevant spectroscopic data of sterols **169**, and **180-182** (Table 3.1) indicated that they differed only in the number and position of hydroxy and acetoxy groups.

Table 3.1 ^{13}C NMR Data (100 MHz, $CDCl_3$) for compounds **169, 180-187**.

	169	**180**	**181**	**182**	**183**	**184**	**185**	**186**	**187**
C-1	31.7	33.5	31.3	35.5	35.8	30.9	33.5	33.5	33.1
C-2	32.0	25.9	36.0	26.1	27.3	26.2	26.5	25.9	28.8
C-3	71.8	70.3	68.6	70.3	70.3	70.1	70.3	70.3	67.4
C-4	42.3	36.2	41.9	36.8	36.7	36.4	36.7	39.8	39.6
C-5	140.9	144.3	147.8	144.6	146.1	139.6	141.6	145.9	144.0
C-6	121.6	124.6	119.9	124.4	120.5	128.5	124.1	120.6	125.6
C-7	32.1	64.4	71.2	65.3	68.9	65.0	68.0	68.6	64.3
C-8	37.3	37.7	36.8	37.5	35.5	38.8	36.8	35.9	37.8
C-9	50.5	42.6	43.3	48.5	48.4	43.0	49.4	43.5	42.7
C-10	36.7	38.0	37.3	39.6	39.4	41.1	42.4	37.8	38.2
C-11	20.7	20.1	20.4	69.1	68.4	20.7	69.3	20.1	20.2
C-12	37.4	36.9	36.8	48.6	48.4	37.1	48.5	36.2	37.0
C-13	43.4	43.2	43.5	43.9	43.4	43.4	44.1	43.3	43.2
C-14	56.0	48.7	48.5	49.6	50.4	49.1	50.2	48.5	48.7
C-15	24.9	24.8	24.7	24.5	24.3	24.7	24.1	24.6	24.8
C-16	27.2	27.3	27.2	27.4	26.1	27.3	27.3	27.2	27.3
C-17	55.3	55.2	55.2	54.9	55.0	55.1	54.9	55.2	55.3
C-18	12.7	12.5	12.3	13.5	13.4	12.6	13.4	12.3	12.6
C-19	19.4	17.8	18.2	17.5	17.5	64.4	64.7	17.8	17.8
C-20	139.8	139.7	139.5	139.1	138.8	139.1	138.8	139.5	139.8
C-21	114.5	114.6	114.8	115.2	115.3	114.8	115.4	114.8	114.6
$CH_3\underline{CO}$		170.7	170.8	170.7	170.7	170.6	170.6	170.7	-
					170.7	170.5	170.5	170.5	
							170.2		
$\underline{CH_3}CO$		21.4	21.3	21.4	21.2	21.3	21.1	21.3	-
					21.2	21.0	21.0	21.2	
							20.9		

3.2.2.2 *Pregna-5,20-diene-3β,7α-diol 7α-acetate*



181

The HREIMS spectrum (*m/z* 358.2502, Δmmu -6, $C_{23}H_{34}O_3$) of **181** suggested that this compound was isomeric with **180**. ^{13}C, ^{1}H and IR data indicated the presence of acetate and hydroxyl substituents in addition to two double bonds. Significant differences in the ^{1}H NMR spectra of **181** and **180** included an upfield shift of one oxymethine proton from δ 3.84 ($W_{1/2}$ = 14 Hz) to δ 3.55 ($W_{1/2}$ = 21 Hz) and the collapse of the 2H-4 methylene protons from a double doublet to a multiplet centred at δ 2.3. Two dimensional NMR experiments were again very useful in assigning all the ^{1}H and ^{13}C NMR resonances of **181**. ^{13}C NMR shifts (Table 3.1), especially in the C and D rings were very similar to those of **180** suggesting a different substitution pattern in the A and B rings. Prominent COSY correlations were observed between the oxymethine at δ 3.55 (H-3) and two sets of methylene protons at δ 1.52 (2H-2) and δ 2.3 (2H-4). The latter signal showed long range coupling to the vinylic proton at δ 5.55 (H-6). The second oxymethine resonance at δ 5.0 (H-7) was coupled to a vinylic proton signal at δ 5.55 (H-6) and a methine resonance at δ 1.6 (H-8). All other spectral data were consistent with those established for a pregnadiene skeleton. The upfield shift of the H-3 proton from δ 4.1 in **187** to 3.55 in **181** together with a $W_{1/2}$ value of 21 Hz suggested an axial oxymethine proton thus indicating the a 3β-hydroxyl group.[147] The 7α configuration of the acetate moiety was suggested from the vicinal coupling constant ($J_{6,7}$ = 5.2 Hz) which was in accordance with that observed for **180** ($J_{6,7}$ = 5.5 Hz). The NMR data presented in Table 3.3 is consistent with the structure proposed for **181**.

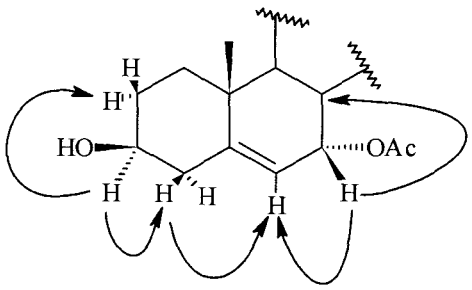
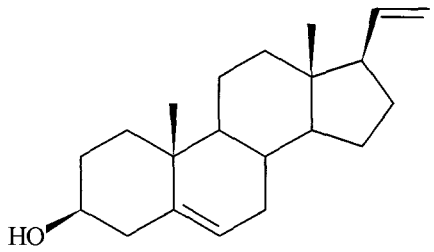


Figure 3.6 Selected COSY-90 correlations for compound **181** used to position the functional groups.

3.2.2.3 *Pregna-5,20-dien-3β-ol*

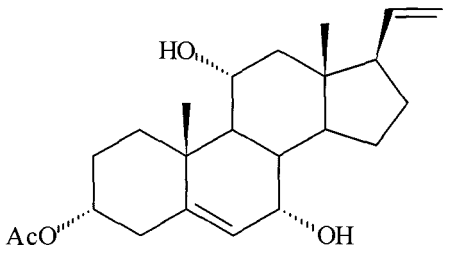


169

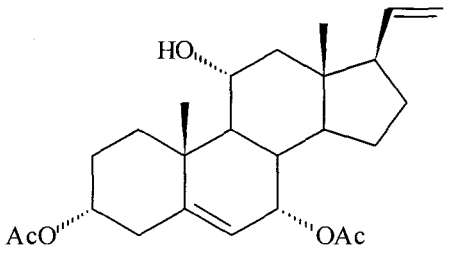
The spectroscopic data for **169** were very similar to those of **181**, but suggested the loss of the acetyloxy group at C-7 in **169**. The infrared spectrum indicated the presence of a hydroxyl group (3300 cm^{-1}) while the LREI mass spectrum showed a weak peak due to loss of the hydroxyl group at *m/z* 272 (M^{+}-H_2O). A molecular formula of $C_{21}H_{32}O$ (*m/z* 300.2470, Δmmu +17) was deduced from HREIMS data. An oxymethine proton resonance in the ^1H NMR spectrum (δ 3.55, $W_{1/2}$ = 22 Hz) suggested the presence of an equatorial hydroxyl group at position 3. The spectroscopic data for **169** was in accordance with pregna-5,20-dien-3β-ol, isolated previously from *Gersemia rubiformis*,[121,122] *Muricea fruticosa*,[124] *Eunicea* sp.[126] and *Pseudoplexaura wagenaari*.[125]

3.2.2.4 *Pregna-5,20-diene-3α,7α,11α-triol 3α-acetate* and *Pregna-5,20-diene-3α,7α,11α-triol 3α,7α-diacetate*

Pregnadiene **182** was found to be the most polar pregnane in this series and crystallised from EtOAc-Hexane (7:3) as fine needles (mp 226 - 229 °C, with decomposition).



182



183

The ^{1}H and ^{13}C NMR spectra of **182** were similar to those of **180**, but showed an additional oxymethine signal (δ 4.1, $W_{1/2}$ = 22.6 Hz) in the ^{1}H NMR spectrum and three carbinol carbons (δ 70.3, 68.9 and 68.4) in the ^{13}C NMR spectrum suggesting a trihydroxy pregnadiene. A molecular ion peak at *m/z* 374.2457 (Δmmu -6) in the HREIMS spectrum of **182** suggested a molecular formula of $C_{23}H_{34}O_4$. An IR absorption band at 1750 cm^{-1} and resonances at δ 2.0 (3H, s) and 170.7 in the ^{1}H and ^{13}C NMR spectra suggested the presence of a single acetate substituent. This was confirmed by a base peak at *m/z* 314 (M^+ - 60) corresponding to loss of acetic acid in the LREIMS spectrum. The positions of the acetate and hydroxyl groups were determined as before by interpretation of the COSY and HMBC NMR data. A $W_{1/2}$ value of 13.3 Hz and the $J_{6,7}$ coupling constant (5.5 Hz) were consistent with an α configuration of the C-7 hydroxyl group. The second hydroxyl moiety could be assigned to either the C or D rings. Comparison of ^{13}C NMR data of **182** with those of **180** suggested substitution on the C-ring. The position of the second hydroxyl substituent was deduced from COSY correlations between the oxymethine resonance at δ 4.1 (H-11) and resonances at δ 2.0 (H-8), 1.4 (H-9) and 1.10 (H-12b). Confirmation of this structural assignment was provided by a HOHAHA NMR experiment. The HOHAHA spectrum (Figure 3.7) revealed a contiguous spin system which included protons H-8 (δ 1.45), H-9 (δ 2.02), H-11 (δ 4.10) and 2H-12 (δ 1.57, 1.10). A series of NOE difference experiments (Figure 3.8) provided further support for the structure of **182** and established the β configuration of H-11. Irradiation of the H-11 proton resulted in 5% enhancement of the CH_3-18 protons and only slight enhancements of the CH_3-19 and H-8 protons. No enhancement of the H-9α methine proton was observed. A $W_{1/2}$ value of 22.6 Hz indicating an axial proton, confirmed the stereochemistry.

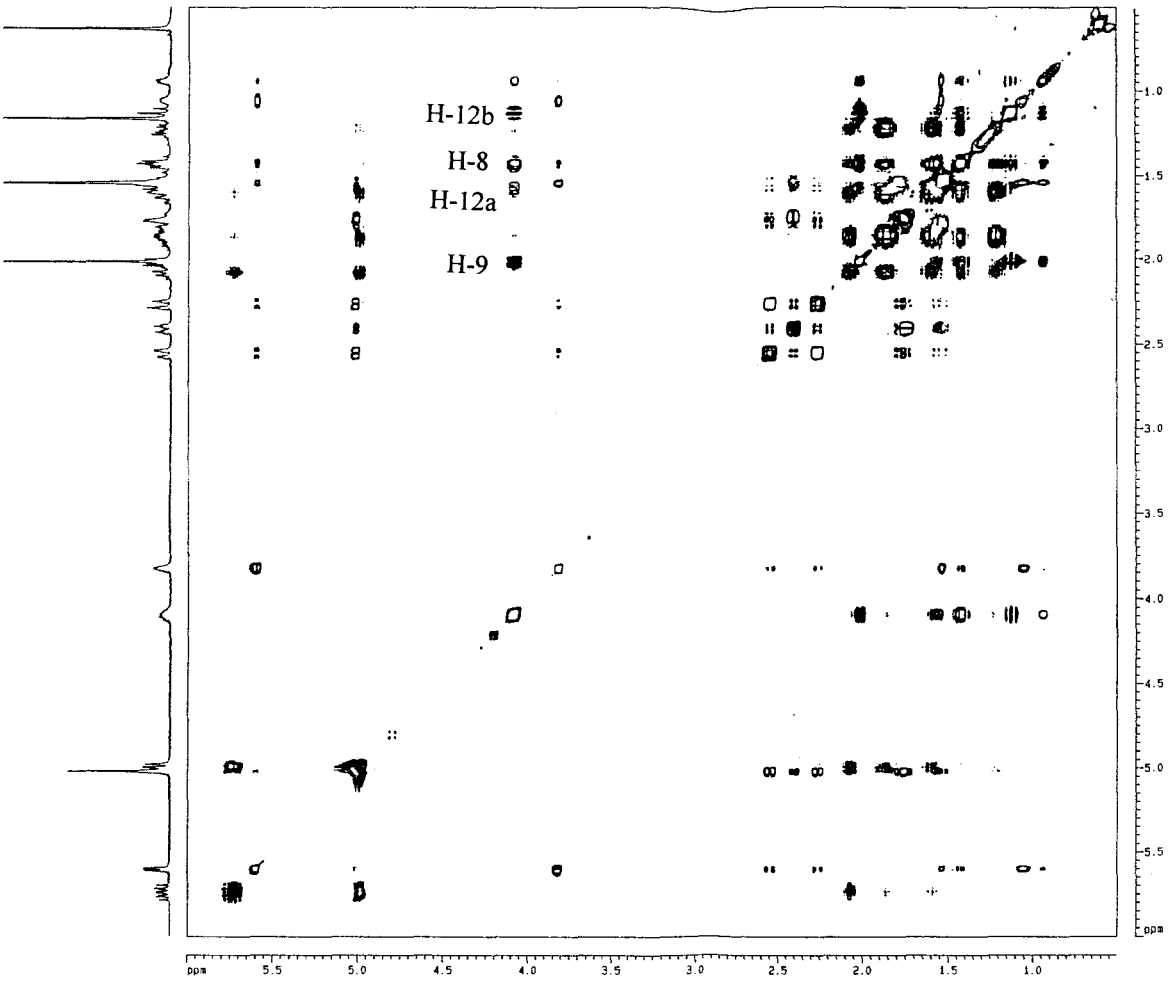


Figure 3.7 HOHAHA NMR spectrum of **182** (400 MHz, mixing time 200 ms, delay time 1s TD (F2) 4k, TD (F1) 256, SW8.01 ppm, $CDCl_3$)

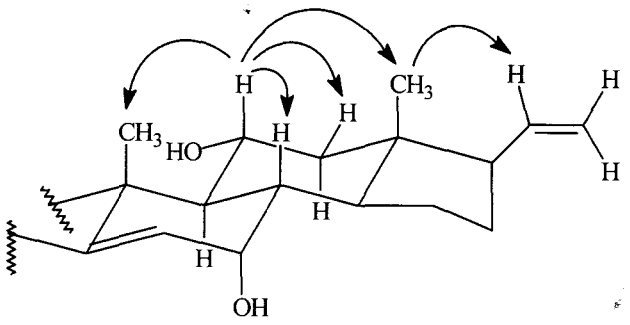
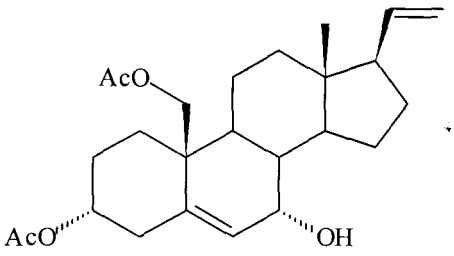


Figure 3.8 Selected NOE data for compound **182**

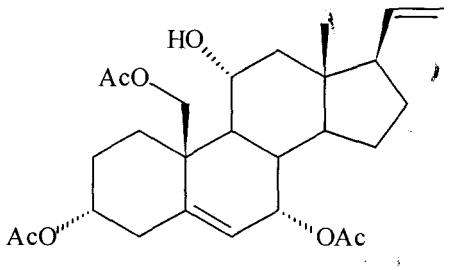
Pregnadiene **183** was isolated as an optically active oil ($[\alpha]_D$ -113°). The ^1H and ^{13}C NMR spectra of **183** were very similar to those of **182** but showed two acetate groups (δ 2.06 and 1.95) and the disappearance of the H-7 oxymethine proton resonance at δ 4.1, thus

suggesting that **183** was the diacetate derivative of **182**. This was confirmed by the presence of a molecular ion peak at *m/z* 416.2568 (Δmmu -7), in the HREIMS spectrum, which is consistent with a molecular formula of $C_{25}H_{36}O_5$. The one acetate group was placed at C-3 on the basis of COSY correlations from the oxymethine at δ 5.03 (H-3) to the two methylenes at 2H-2 (δ 1.75) and 2H-4 (δ 2.50 and 2.25). HMBC correlations from the vinylic methine at δ 5.6 (H-6) to C-4, C-7, C-8 and C-10 and from the oxymethine at δ 4.92 (H-7) to C-6, C-7 and C-8 positioned the second acetate at C-7. The position of the C-11 hydroxyl group and its stereochemistry was confirmed by the observation of COSY correlations from H-11 (δ 4.10, $W_{1/2}$ = 22 Hz) to H-8 (δ 1.65), H-9 (δ 2.00) and 2H-12 (δ 1.43 and 1.15). All other spectral data were consistent with the proposed structure of **183**. The 7α configuration follows from the $J_{6,7}$ (5.5 Hz) coupling constant. The identical chemical shifts (δ 4.1) and $W_{1/2}$ values (22 Hz) for H-11 in **182** and **183** thus suggested an 11α hydroxyl group in **183**. The 3α acetate group was assigned on the basis of the comparison of the chemical shifts and splitting patterns of the 2H-4 protons in this series of compounds (Section 3.2.3).

3.2.2.5 *Pregna-5,20-diene-3α,7α,19-triol 3α,19-diacetate* and *pregna-5-20-diene-3α,7α,11α,19-tetrol 3α,7α,19-triacetate*



184



185

An interesting feature of the ^{1}H NMR spectra (Figure 3.9a) of compounds **184** and **185** was the disappearance of the characteristic Me-19 signal (δ 1.0) and the conspicuous appearance of two deshielded oxymethylene doublets at δ 3.93 (J = 11.9 Hz) and 4.53 (J = 11.9 Hz). Compound **184** showed a molecular ion peak at *m/z* 416.2563 (Δmmu +5) in the HREIMS corresponding to a molecular formula of $C_{25}H_{36}O_5$. The LREIMS showed peaks at *m/z* 356 and 296 due to two successive losses of acetic acid. The ^{1}H NMR spectrum

showed a single peak at δ 2.10 corresponding to six protons (2 x OAc) and an oxymethine proton at δ 3.80 ($W_{1/2}$ = 12 Hz, H-7). The COSY NMR data were used to place the acetate at C-3 and the hydroxyl group at C-7 which is identical to coupling pattern observed in the COSY spectrum of **180** suggesting similar substitution in the A/B rings. The location of the acetyl group at C-19 was evidenced by strong geminal coupling (J = 11.9 Hz) between the two deshielded methylene protons at δ 4.55 and 3.95, the disappearance of the H-19 methyl signal and the HMBC correlations from the oxymethylene (H-19) to signals at δ30.9 (C-1), 43.0 (C-9) and 41.0 (C-10). The $W_{1/2}$ values for the H-3 (10.2 Hz) and H-7 (11.8 Hz) oxymethine protons defined the stereochemistry as 3α and 7α.

Compound **185** showed three acetate signals (δ 2.05, 2.02 and 1.95) in the ^{1}H NMR spectrum (Figure 3.9a) and three ester carbonyl resonances at δ 170.6, 170.5 and 170.2 in the ^{13}C NMR spectrum (Figure 3.9b) suggestive of a pregnadiene triacetate. The ^{13}C NMR spectrum showed a further four peaks at δ 70.3, 68.0, 69.3 and 64.7 corresponding to four carbinol carbons. A molecular formula of $C_{27}H_{38}O_7$ (*m/z* 474.2616, Δmmu -19) was determined from HREIMS data. Standard two dimensional NMR experiments and comparison of data with those of previous compounds in this series allowed the complete assignment of the structure of **185** as pregna-5,20-diene 3α,7α, 11α,19-tetrol 3α, 7α, 19-triacetate. An oxymethine proton at δ 4.13 (1H, br s, $W_{1/2}$ = 20.2 Hz) suggested an 11β proton.

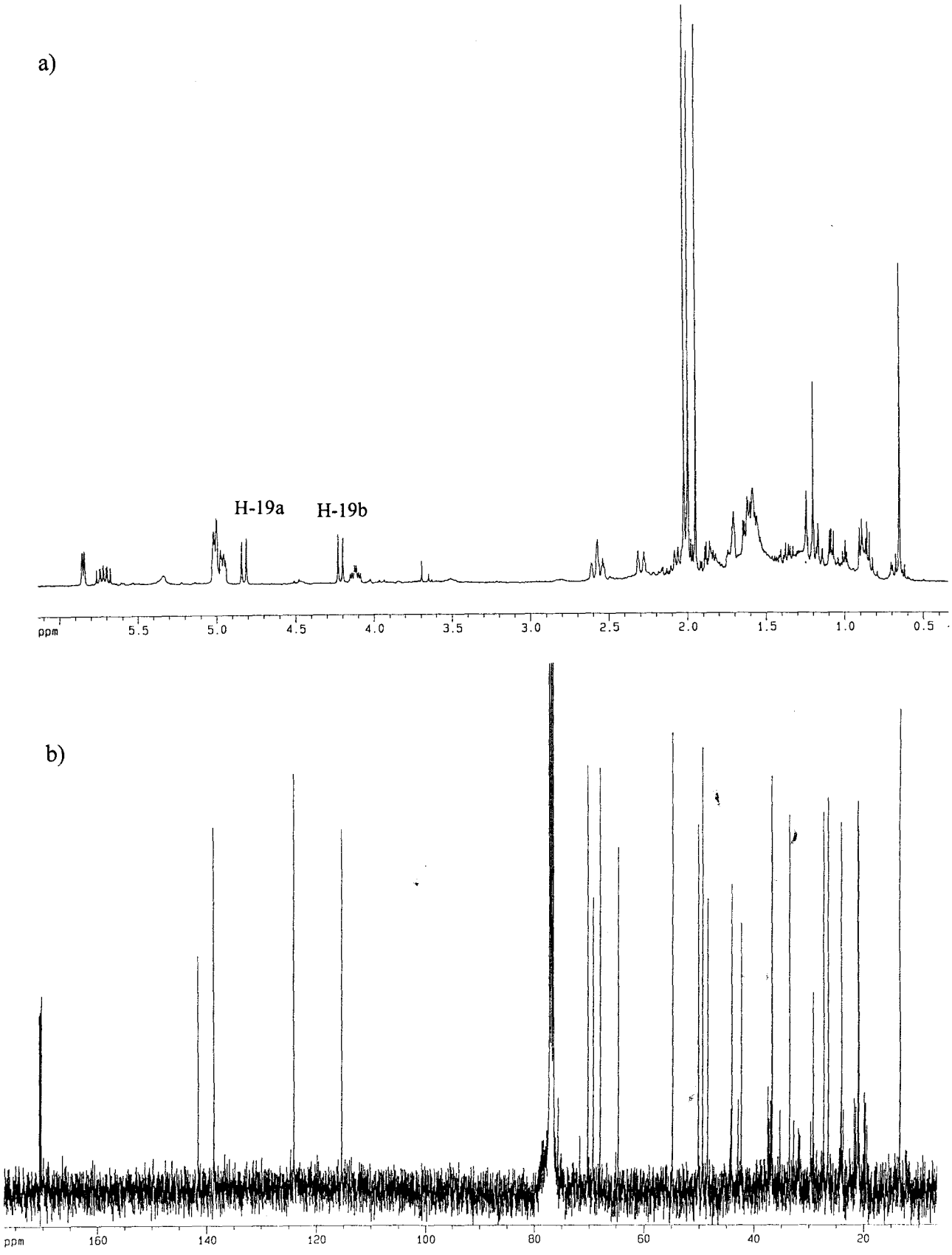


Figure 3.8 (a) ^{1}H (400 MHz, $CDCl_3$) and (b) ^{13}C NMR spectra of **185**

3.2.3 Determination of the relative conformation in 3-substituted pregnadienes

Measurement of $W_{1/2}$ values for pregnadiene acetates is often complicated by overlapping oxymethine and vinylic proton resonances. Reduction of the acetate to the secondary alcohol causes an upfield shift of the oxymethine proton into the unobscured region of the spectrum allowing easier measurement of $W_{1/2}$. Analysis of the ^{1}H NMR data for the compounds obtained in this study enabled us to propose an alternative procedure for the quick assignment of the C-3 stereochemistry in this series of pregnadienes, based on the chemical shift of the C-4 methylene protons. Electronegative substituents (X) affect the protons on the vicinal carbons in a predictable manner. In general, an equatorial X will shield the vicinal protons, while an axial X will deshield axial and shield equatorial protons in cyclohexane rings.[147] These changes in chemical shift were evident in the ^{1}H NMR spectra of pregnanes **169, 180-187**, which clearly showed two separated 2H-4 proton multiplets ($\Delta\delta \sim 0.3$ Hz) in compounds with a 3α-axial hydroxyl or acetate substituent (Figure 3.10), and a single complex multiplet centered at δ 2.3 for a 3β-equatorial substituent (Figure 3.11). The chemical shifts of the 4α- and 4β-protons in this series of pregnadienes are presented in Table 3.2.

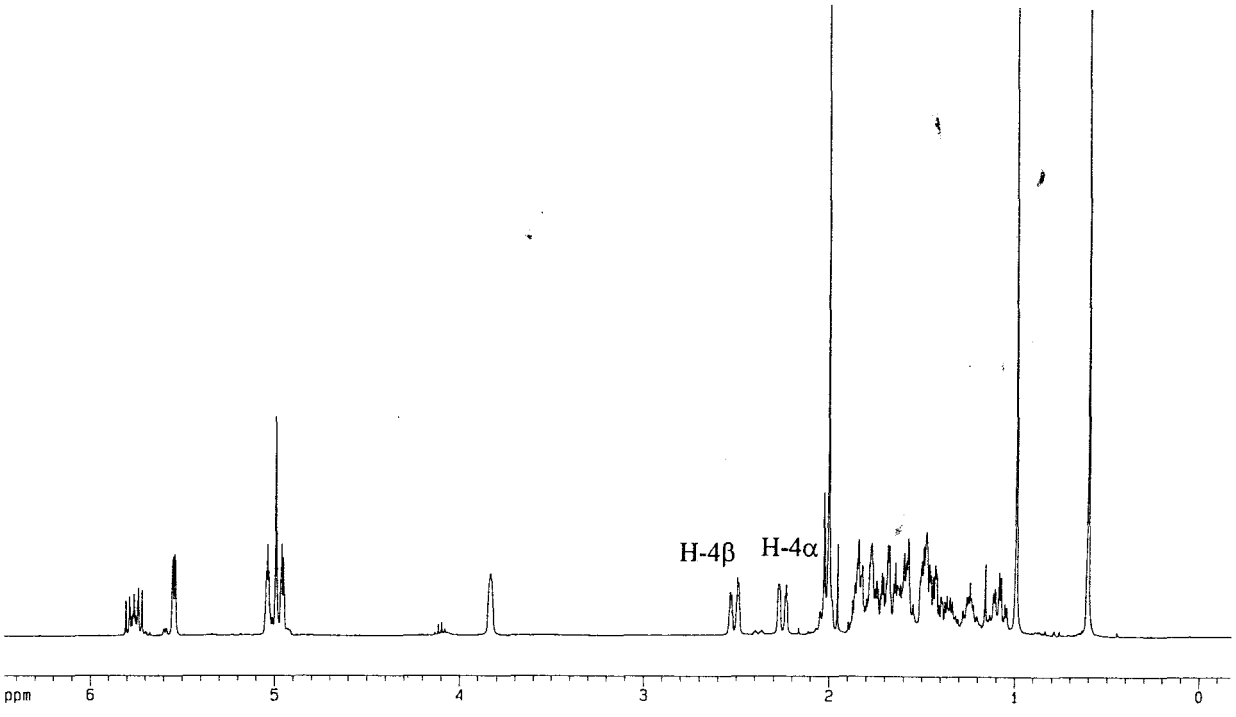


Figure 3.10 ^{1}H NMR spectrum of a pregnadiene with a 3α-acetate substituent.

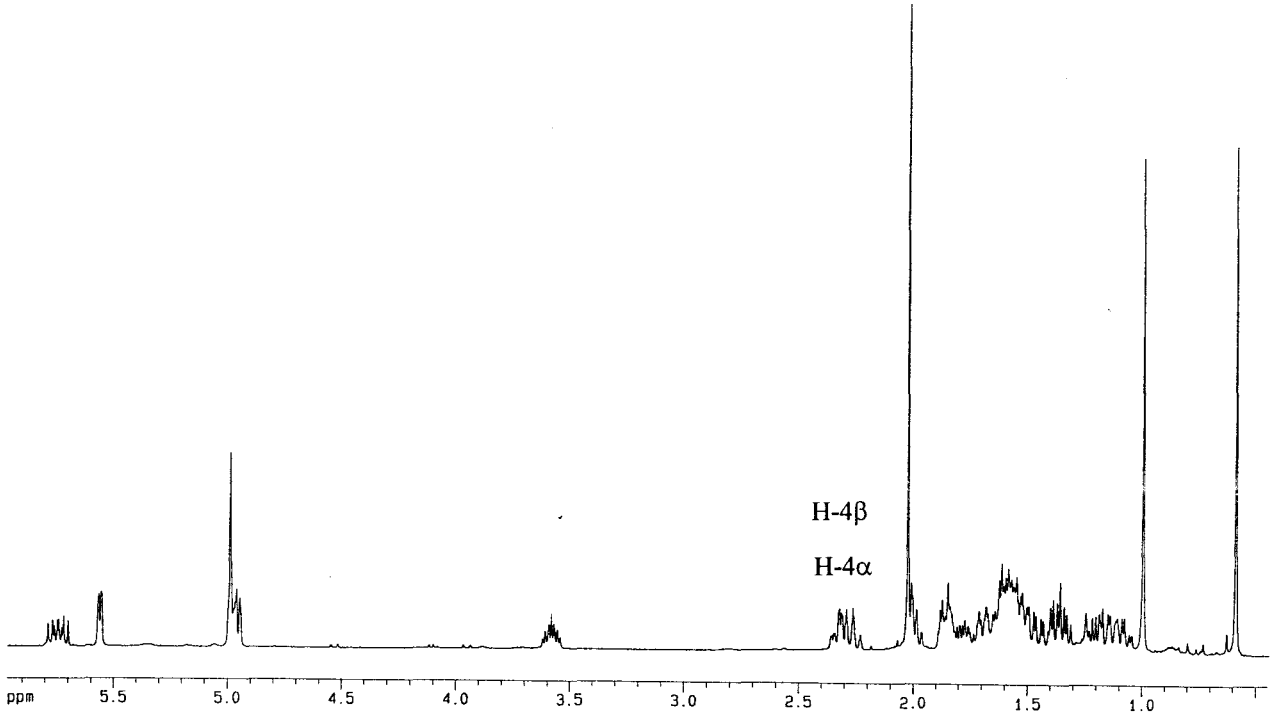


Figure 3.11 ^{1}H NMR spectrum of a pregnadiene with a 3β-hydroxyl substituent

Table 3.2 Comparison of the H-3 and 2H-4 ^{1}H chemical shifts for pregnadienes **169**, **180-187** (400 MHz, $CDCl_3$)

Cpd	δ_H (H-3)	$W_{1/2}$	H-4β (J, Hz)	H-4α (J, Hz)	Configuration at C-3	Δδ = (H-4β-H4α)
169	3.55	22.2 Hz	2.30, m	2.30, m	3β	0 Hz
180	5.05	14.0 Hz	2.55, dd, 15.3, 1.5	2.25, dd, 15.3, 1.5	3α	0.3 Hz
181	3.55	21.0 Hz	2.30, m	2.30, m	3β	0 Hz
182	5.05	a	2.57, dd, 15.1, 1.6	2.27, dd, 15.1, 1.6	3α	0.30 Hz
183	4.95	a	2.50, d, 15.0	2.25, d, 15.0	3α	0.25Hz
184	5.07	a	2.60, d, 15.2	2.30, d, 15.4	3α	0.30 Hz
185	5.03	a	2.60, d, 14.9	2.30, d, 15.0	3α	0.30 Hz
186	5.00	a	2.50, d, 15.2	2.25, d, 15.4	3α	0.25 Hz
187	4.10	10.0 Hz	2.60, d, 15.0	2.20, d, 15.0	3α	0.40 Hz

a) The $W_{1/2}$ for these resonances could not be measured because of overlap with the H-21 protons.

The origin of this characteristic change in chemical shift and splitting pattern can be ascribed to magnetic anisotropy, dipole and inductive effects, and van der Waals interaction associated with the C-3 substituent.[147]

3.4 SUMMARY AND CONCLUSION

Six new pregnadiene sterols were isolated from the soft coral *Pieterfaurea unilobata* together with the known compound **169**. Compounds **180-185** are the first pregnadienes obtained from the marine environment containing a C-7 substituent. Analysis of the ^{1}H NMR spectra of this group of sterols led to the proposal of an alternative procedure for the quick assignment of the absolute configuration at C-3 in this series of compounds.

Chapter Four

DISCORHABDIN ALKALOIDS FROM THREE SPONGES OF THE FAMILY LATRUNCULIIDAE

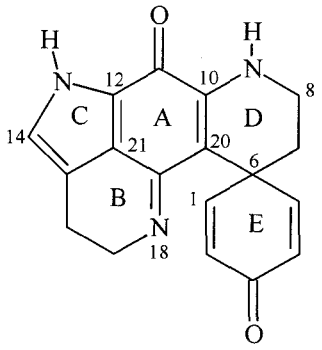
4.1 REVIEW OF MARINE PYRROLOIMINOQUINONE METABOLITES

4.1.1 Introduction

Alkaloids containing the pyrroloiminoquinone chromophore are generally associated with marine sponges of the genera *Latrunculia*, *Batzella*, *Damiria* and *Zyzzya*. The majority of these compounds exhibit potent *in vitro* cytotoxicity and a number of them have been screened as potential anticancer leads. Over forty of these biologically active compounds, which include the discorhabdins, makaluvamines, damirones, batzellines, isobatzellines, tsitsikammamines, epinardins and veiutamine, have been isolated. These alkaloids have been included in reviews of marine pyridoacridine alkaloids[148] but have not been reviewed *per se*. The chemistry, biological activity and biosynthesis of marine pyrroloiminoquinone metabolites will be discussed in this section.

4.1.2 Discorhabdins

The discorhabdins are the most complex of the pyrroloiminoquinone alkaloids, containing a pyrrolo-[1,7]-phenanthroline ring system and a spiro-cyclohexenone or spiro-cyclohexadienone moiety. Bromine is frequently incorporated into the skeleton with several metabolites also containing a tetrahydrothiophene ring. A few compounds containing a reduced C-3 carbonyl (*e.*g. the epinardins) or a bispyrroloiminoquinone (*e.*g. the tsitsikammamines) are also included here. A basic discorhabdin skeleton together with the atom numbering used by the original authors[149] is shown below **(188)**.



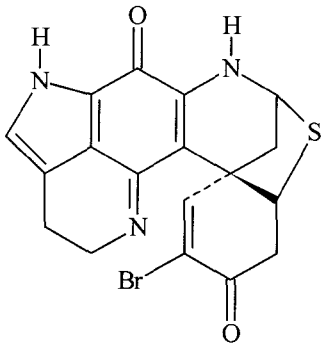
188

The isolation of the cytotoxic discorhabdin A **(189)**, also called prianosin A, was first reported from the Okinawan sponge *Prianos melanos*[150] and later recorded from the New Zealand sponge *Latrunculia brevis* (Ridley and Dendy)[151] and the Fijian sponge *Zyzzya marsailis*.[152] The structure of **189**, which features a tetrahydrothiophene ring in addition to the "usual" discorhabdin skeleton, was originally determined by single-crystal X-ray analysis.[150] Extensive analysis of COSY and HETCOR NMR data and comparison of these data with those of discorhabdin C **(190)** (the structure of discorhabdin C was reported before that of discorhabdin A), allowed the complete NMR assignment of discorhabdin A.[151] Discorhabdin A was found to be cytotoxic to murine leukaemia cells *in vitro* at IC_{50}'s of 37 and 14 ng mL^{-1} for L1210 and L5178Y murine leukeamia cells, respectively. Compound **189** also induced Ca^{2+} release from the sarcoplasmic reticulum.

The related alkaloid, discorhabdin B **(191)**, which also contains a tetrahydrothiophene ring, was obtained from an undescribed New Zealand latrunculid sponge (*Latrunculia* sp. B) and its structure determined by NMR methods.[151] Discorhabdin B showed *in vitro* cytotoxicity in the P-388 assay (ED_{50} 0.1 μg mL^{-1}) and also *in vivo* antitumour activity (T/C 117 % at a dose of 0.25 mg kg^{-1}).

Discorhabdin C **190** was discovered as a result of wide-scale screening of New Zealand's marine invertebrates for antiviral and antitumour activity.[149] This compound was isolated as the major component of the cytotoxic MeOH-toluene extract of a red-brown sponge identified as *Latrunculia* du Bocage. The structure of discorhabdin C was determined using a combination of spectroscopic techniques including X-ray analysis. The X-ray

structure showed that the pyrroloiminoquinone chromophore is approximately planar with the two six-membered heterocyclic rings in half-chair conformations with the apices of the half chairs, C-7 and C-17, residing above and below the plane, respectively. In addition, the planar cyclohexadienone ring is tilted at an angle of 76° relative to the plane of the chromophore. Perry *et al.*[151] subsequently assigned the complete NMR data for this compound which showed significant toxicity to L1210 and P-388 tumour cells (ED_{50}'s 100 ng mL^{-1} and 30 ng mL^{-1}, respectively).[151,153]



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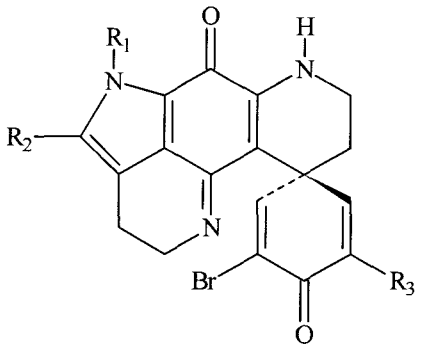
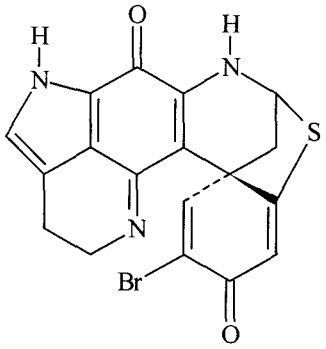


67 $R_1 = H$, $R_2 = R_3 = Br$

190 $R_1 = R_2 = H$, $R_3 = Br$

193 $R_1 = R_2 = R_3 = H$

202 $R_1 = CH_3$, $R_2 = R_3 = H$



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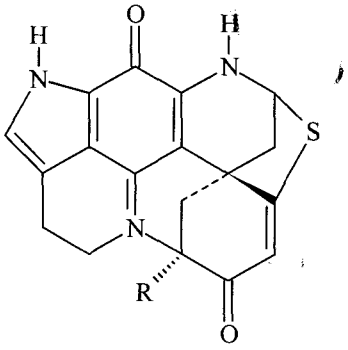


192 R = H

207 R = OH

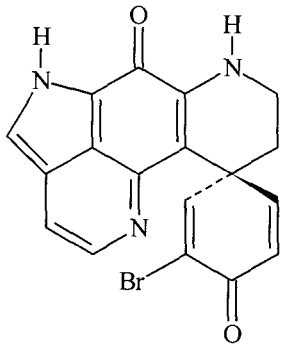
Discorhabdin D **(192)**, together with discorhabdin A, was isolated from the New Zealand sponge *Latrunculia brevis*.[154] The structure of **192**, which contains seven interlocking rings, was proposed from the comparison of the spectral data of **192** with those of known

discorhabdins. While discorhabdin D showed lower *in vitro* cytotoxicity when compared to discorhabdins A, B and C, this compound exhibited significant *in vivo* antitumour activity (T/C 132 % at 0.25 mg kg^{-1}).

Discorhabdin C and its 4-debromo-derivative, discorhabdin E **(193)**, was isolated during a second investigation of the New Zealand latrunculid sponge *Latrunculia* du Bocage (*L* cf *bocagei*).[155] The structure of discorhabdin E was determined mainly by comparing its spectroscopic data with those of **190**.

Blunt *et al.*,[156] in a review of New Zealand marine natural products, reported the isolation of discorhabdin F **(194)** from a New Zealand latrunculid sponge. Unfortunately, no spectroscopic data were reported for this compound.

Extracts of the Antarctic sponge *Latrunculia apicalis* (Ridley and Dendy) showed significant biological activity, affecting the feeding behaviour of the major Antarctic sponge predator, the sea star *Perknaster fucus*. Bioassay guided fractionation led to the isolation of discorhabdin G **(195)** and C.[157] The pure discorhabdin G was found to deter the predatory sea star, *Perknaster fucus* and showed inhibition of two common water column microorganisms isolated from seawater surrounding the sponge.



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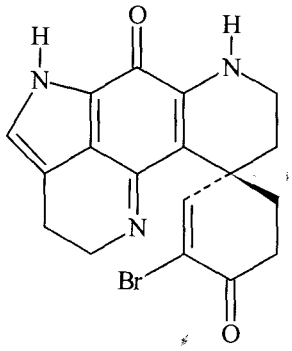
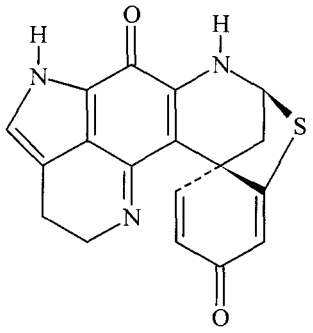


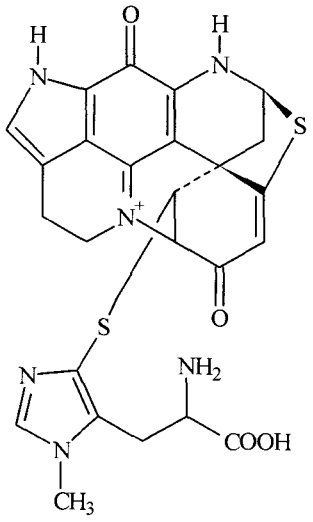
195

In a paper describing their biosynthetic studies on discorhabdin B, Munro *et al.*[158] reported that at least thirteen discorhabdins (A-M) had been isolated by their group. The structures of discorhabdins E-M, which were previously presented at the New Zealand Institute of

Chemistry Conference* (7-10 December 1993), were kindly provided to us by Professor Munro. It is unfortunate that the structures and spectral data for these compounds have not been published in the mainstream literature by Munro's group, as there is at least one example (discorhabdin G)[157] where a different structure has subsequently been assigned to one of their discorhabdins by an independent research group. The inclusion here of Munro's structures for discorhabdin G to M **(196*-201*)** is an attempt to clarify the complicated nomenclature dilemma which the inaccessibility of these structures has caused.



196*



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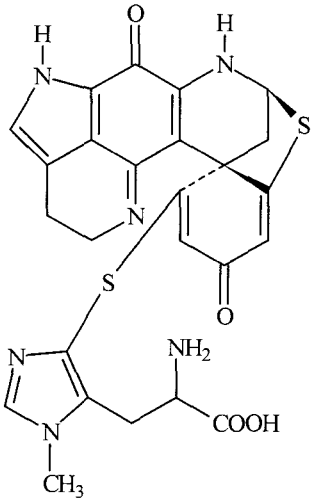

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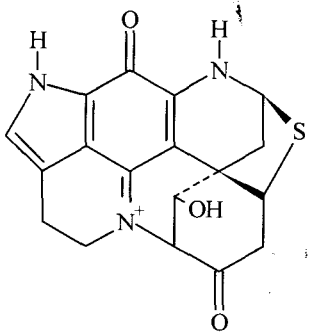
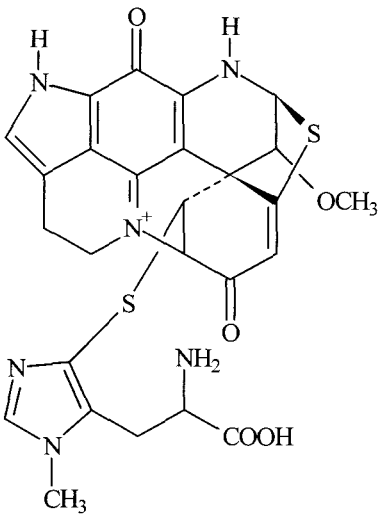
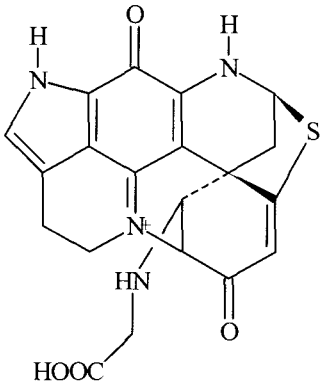


199*

* The structures of these compounds were also presented at The 37th Annual Meeting of the American Society of Pharmacognosy (1996).[159]



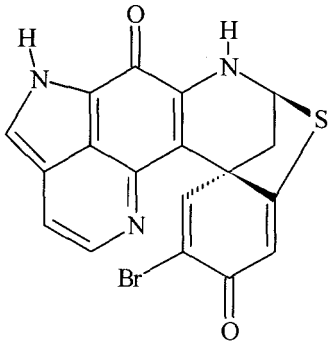
200*



201*

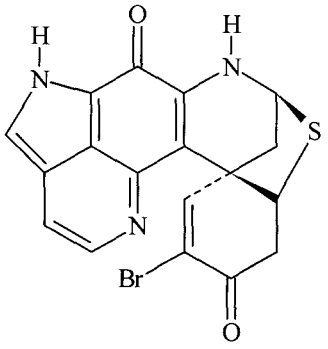
Bioassay-directed fractionation of an extract of the deep water Caribbean sponge of the genus *Batzella* led to the isolation of discorhabdin P **(202)** which is the N-13 methyl-derivative of discorhabdin C.[159] The structure of **202** was determined from a combination of NMR spectroscopy and single-crystal X-ray analysis. Discorhabdin P inhibited the phosphatase activity of calcineurin and the peptidase activity of CFP32 and also showed *in vitro* cytotoxicity to P-388 and A-549 tumour cell-lines. Calcineurin and the caspases (interleukin-2 converting enzymes) are implicated in the immune response. Interestingly, discorhabdin C, which only lacks the N-13 methyl group, showed no inhibition of calcineurin or CPP32 at the maximum tested concentration of 5 $\mu g\ mL^{-1}$.

Discorhabdin Q **(203)** was isolated from the cytotoxic extracts of four different sponges: *Latrunculia purpurea*, *Zyzzya massalis*, *Z. fuliginosa* and an undescribed *Zyzzya* species.[160] The structure of **203** was proposed as 16,17-dehydrodiscorhabdin B. Discorhabdin Q exhibited only moderate activity in the NCI 60 cell line antitumour screen. Dijoux *et al.*[160] suggested that the full aromatisation of the pyrroloiminoquinone system confers reduced cytotoxicity relative to the Δ^{16} saturated members of the family, but this hypothesis has yet to be confirmed.

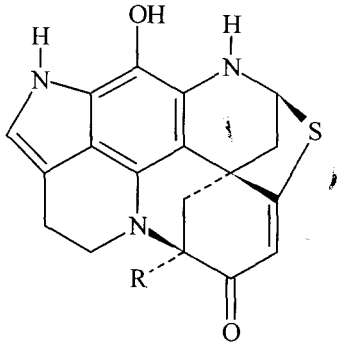


203

Prianosin B-D **(204-206)** were obtained from the Okinawan sponge *Prianos melanos*.[161] The structures of prianosin B, which is 16,17-dehydrodiscorhabdin A, and those of prianosin C and D were determined by standard spectroscopic techniques. The latter two compounds which contain aminophenol moieties were characterised as their acetate derivatives. Paradoxically, it was subsequently shown that prianosins C and D were artefacts of the acetylation procedure. The structures of **205** and **206** were thus revised to those of discorhabdin D and 2-hydroxydiscorhabdin D **(207)**.[156,162]



204

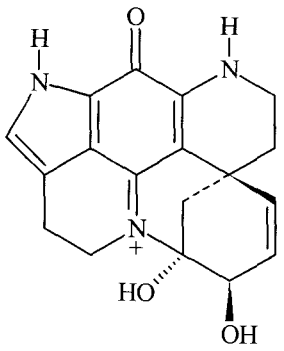


205 R = H

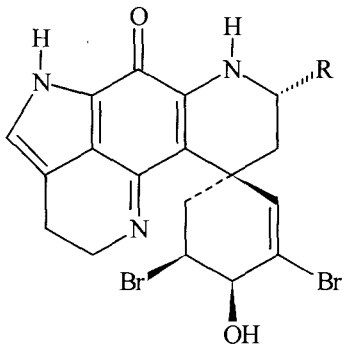
206 R = OH

An unidentified, spinach-green sponge from the South Indian ocean yielded a series of pyrroloiminoquine metabolites, the epinardins A-D **(208-211)**.[163] These compounds feature a cyclohexenol ring instead of the usual cyclohexenone ring characteristic of the other discorhabdins. The structures of the epinardins were determined by interpretation of NMR spectroscopic data. Epinardins A and C were cytotoxic to murine lymphocytic cells,

L1210 with IC_{50}'s of 1.2 and 0.32 μg mL^{-1} and to doxorubicin resistant L1210/DX cells at IC_{50}'s of 6.8 and 0.36 μg mL^{-1}, respectively.

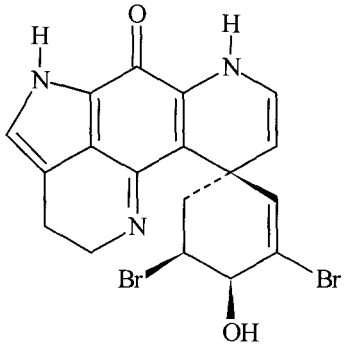


208



209 R = H

211 R = OCH_3

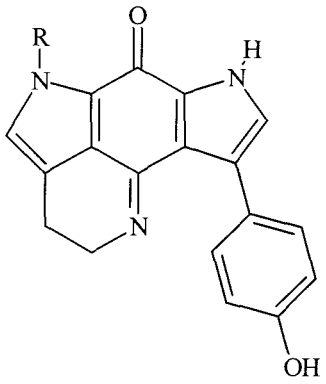


210

An investigation of an undescribed South African latrunculid sponge from a suspected new genus resulted in the isolation of 14-bromodiscorhabdin C **67**, 14-bromo-3-dihydrodiscorhabdin C **68**, and two new bis-pyrroloiminoquinone alkaloids, tsitsikammamines A **65** and B **66**.[54] The structures of these compounds, which showed antibacterial activity, were proposed from the interpretation of NMR data.

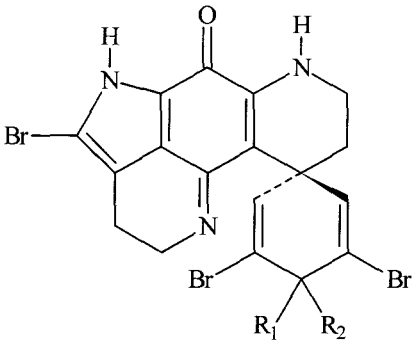
The related bis-pyrroloiminoquinone, wakayin **(212)**, was isolated from a Fijian ascidian *Clavelina* sp.[164] This is the only reported pyrroloiminoquinone metabolite from an ascidian which may suggest that these compounds are produced by symbiotic micro-organisms. The structure of wakayin followed from the interpretation of DQFCOSY, HMBC, and HMQC data. The biosynthesis of **212** differs from the other discorhabdins in that it must require

two molecules of tryptophan. Wakayin showed *in vitro* cytotoxicity against the human colon tumour cell line (HCT116, IC_{50} 0.5 μg mL^{-1}) and inhibition of the topoisomerase II enzyme (250 μM). Copp *et al.*[164] have suggested that **212** exhibits its cytotoxicity by interfering with, or damaging, DNA.



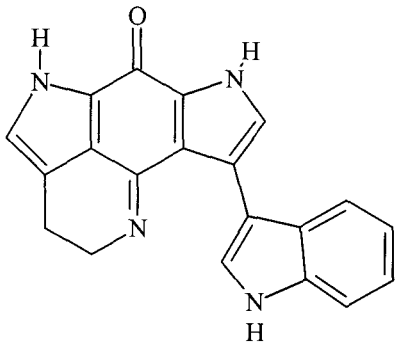
65 R = H

66 R = CH_3



67 $R_1 + R_2 = O$

68 R_1 = H, R_2 = OH



212

4.1.3 Makaluvamines

The makaluvamines are pyrroliminoquinone alkaloids that are considered to be biosynthetic precursors of the discorhabdins (Scheme 4.1) and have been isolated mainly from sponges of the genus *Zyzzya*. The basic makaluvamine skeleton may be derived from a single tryptophan amino acid.

A series of six pyrroloiminoquinones, makaluvamines A-F **(213-218)** together with discorhabdin A were obtained from the cytotoxic extracts of the Fijian sponge *Zyzzya* cf.

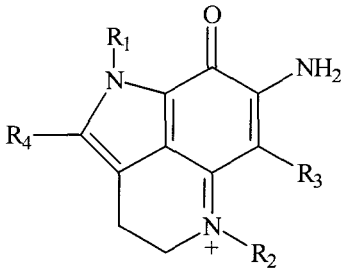
marsailis.[152] The structures of **213-218** were determined by standard two-dimensional NMR techniques. The makaluvamines exhibit potent *in vitro* cytotoxicity against the human colon tumour cell line (HCT-116) and also inhibit the topoisomerase II enzyme. In addition, makaluvamine A and C showed *in vivo* antitumour activity against the human ovarian carcinoma (Ovcar 3) implanted in mice. An increase in cytotoxicity with increasing structural complexity was observed for this series of compounds, *i.e.* discorhabdin A > makaluvamine F >> makaluvamine D. Cytotoxic activity against xrs-6, a Chinese hamster ovary (CHO) cell line sensitive to agents that cause double strand breaks in DNA, parallel the data obtained with HCT116. Makaluvamine A and C were only marginally active in the *in vivo* P388 murine leukaemia assay, but showed substantial reduction in tumour mass against the Ovcar 3 implanted in athymic mice.

The cytotoxic extract of the Indonesion sponge *Histodermella* sp (now *Zyzzya fuluginosus*)[165] contained makaluvamine G **(219)** as the major metabolite together with the minor compounds makaluvamines A and C.[166] The structure of **219** was determined by NMR methods. Makaluvamine G showed moderate cytotoxicity against murine leukaemia P-388 (IC_{50} 0.5 μg mL^{-1}), human non-small cell lung cancer (A-549, IC_{50} 0.50 μg mL^{-1}), human colon cancer (HT-29, IC_{50} 0.5 μg mL^{-1}), human breast cancer (MCF, IC_{50} 0.5 μg mL^{-1}) and against human oral epidermoid carcinoma KB (0.35 μg mL^{-1}). Compound **219** was a moderate inhibitor of topoisomerase I (IC_{50} 3.0 μM) and showed moderate inhibition of RNA (IC_{50} 15 μM), DNA (IC_{50} 15 μM) and protein (IC_{50} 21 μM) synthesis but did not significantly inhibit topoisomerase II.

Examination of the CH_2Cl_2-MeOH extract of the Pohnpeian sponge *Zyzzya fuliginosa* afforded the new compounds, makaluvamines H-M **(220-225)** and the known compounds makaluvamines C, D and G.[165] The structures of the six new compounds were elucidated by interpretation of spectral data. Makaluvamines D, G, H and J-M were inactive against topoisomerase I and II, but showed *in vitro* cytotoxicity against HCT-116 cells.

Makaluvamine N **(226)**, the only pyrroloiminoquinone metabolite containing a bromine substituent at position 6, was isolated as a minor metabolite from a Philippine specimen of the sponge *Zyzzya fuliginosa* together with makaluvamines A, C, D, E and I.[167] The

structure of **226** was determined spectroscopically. Makaluvamine N showed considerable *in vitro* cytotoxicity against human colon tumour cell line HCT-116 (LC_{50} 0.6 μg mL^{-1}) and exhibited 90% inhibition of the topoisomerase II unwinding of pBR322 at 5 μg mL^{-1}.



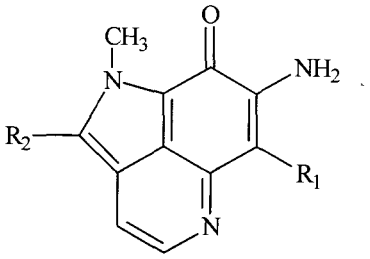
213 $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$

215 $R_1 = R_3 = R_4 = H$, $R_2 = CH_3$

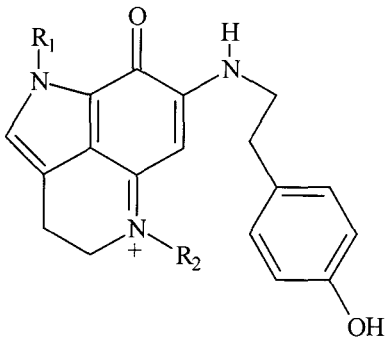
220 $R_1 = R_2 = CH_3$, $R_3 = R_4 = H$

221 $R_1 = R_2 = R_3 = R_4 = H$

226 $R_1 = R_2 = R_4 = H$, $R_3 = Br$



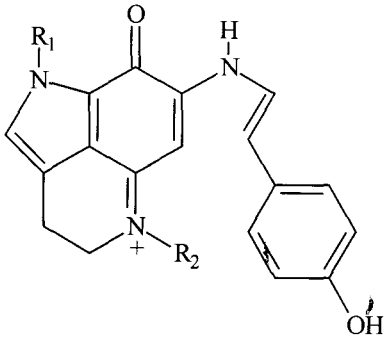
214 $R_1 = R_2 = H$



216 $R_1 = R_2 = H$

222 R_1 =H, $R_2 = CH_3$

223 $R_1 = CH_3$, $R_2 = H$

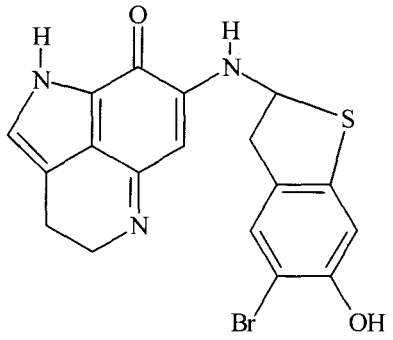


217 $R_1 = CH_3$, $R_2 = H$

219 $R_1 = R_2 = CH_3$

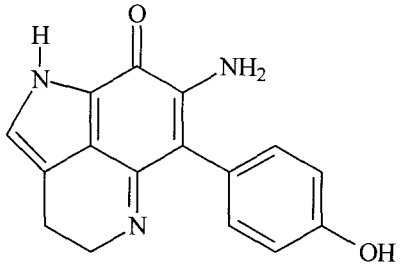
224 $R_1 = H$, $R_2 = CH_3$

225 $R_1 = R_2 = H$



218

Fijian specimens of the sponge *Zyzzya fuliginosa* contained the new alkaloid veiutamine **(227)** together with makaluvamines A-F.[168] Veiutamine, the first pyrroloiminoquinone alkaloid to be isolated bearing a C-6 para-phenolic substituent, showed potent *in vitro* cytotoxicity in a 25 panel cell line with a mean IC_{50} of 0.12 μg mL^{-1} and some selectivity against solid tumours.



227

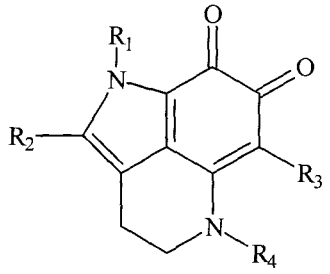
4.1.4 Batzellines, isobatzellines and damirones

The highly functionalised pyrroloquinoline metabolites, the batzellines A-C **(228-230)** are characterised by an *o*-quinone functionality and the rare appearance of chlorine in the condensed heterocyclic structure. Batzelline A-C were isolated from the deep water Bahamas sponge *Batzella* sp.[169] The structure of **228** was determined by X-ray analysis and those of **229** and **230**, by comparison of their spectral data with those of batzelline A.

A second examination of the deep water *Batzella* sponge resulted in the isolation of isobatzellines A-D **(231-234)**.[170] The structures of the isobatzellines, which contain an

aminoiminoquinone ring system, were based on structural comparisons with batzelline A. Isobatzelline A was converted to batzelline A by diazotisation-substitution in aqueous nitric acid. Compounds **231-234** show *in vitro* cytotoxicity against P-388 leukaemia cells and moderate antifungal activity against *Candida albicans*.

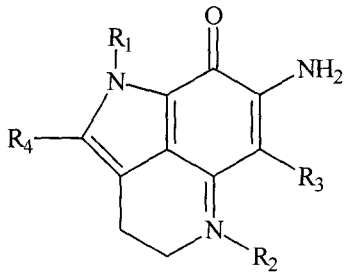
Damirone A **(235)** and B **(236)** are orthoquinone alkaloids resembling the batzellines and were first isolated from a Palauan sponge *Damiria* sp.[171] (now *Zyzzya fuliginosa*),[165] followed by their isolation from the Indonesian sponge *Histodermella* sp. (revised to *Zyzzya fuluginosus*).[165,166] The structures of **235** and **236** were determined by comparison of their spectral data with those of the batzellines. Damirone B was also isolated from Fijian specimens of the *Zyzzya fuliginosa*[168] and it co-occurred with makaluvone **(237)** in the Fijian sponge *Zyzzya* cf. *marsailis*.[152] Damirone C **(238)** was isolated from Pohnpeian specimens of *Zyzzya fuliginosa*.[165]



228 $R_1 = CH_3$, $R_2 = SCH_3$, $R_3 = Cl$, $R_4 = H$

229 $R_1 = R_4 = H$, $R_2 = SCH_3$, $R_3 = Cl$

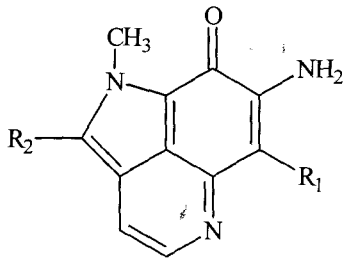
230 $R_1 = CH_3$, $R_2 = R_4 = H$, $R_3 = Cl$



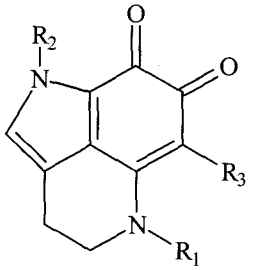
231 $R_1 = CH_3$, $R_2 = H$, $R_3 = Cl$, $R_4 = SCH_3$

232 $R_1 = CH_3$, $R_2 = R_3 = H$, $R_4 = SCH_3$

233 $R_1 = CH_3$, $R_2 = R_4 = H$, $R_3 = Cl$



234 $R_1 = Cl$, $R_2 = SCH_3$



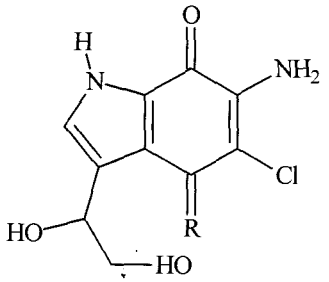
235 $R_1 = R_2 = CH_3$, $R_3 = H$

236 $R_1 = R_3 = H$, $R_2 = CH_3$

237 $R_1 = H$, $R_2 = CH_3$, $R_3 = Br$

238 $R_1 = R_2 = R_3 = H$

The secobatzellines A **(239)** B **(240)** are the most recent addition to the family of pyrroloiminoquinone metabolites. Compounds **239** and **240** were obtained from a deep-water Caribbean sponge of the genus *Batzella*.[172] Both compounds showed *in vitro* cytotoxicity against P-388 and A-549 cell lines. Secobatzelline A also inhibited the phosphatase activity of calcineurin and the peptidase activity of CPP32.



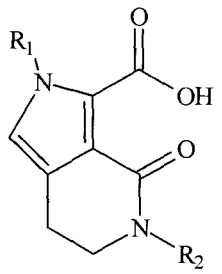
239 R = NH

240 R = O

4.1.5 Miscellaneous compounds

Makaluvic acids A **(241)** and B **(242)** are not classified as pyrroloiminoquinone metabolites, but have been included here because of their resemblance to, and co-occurrence with, the makaluvamines. The makaluvic acids are pyrrolecarboxylic acids and were isolated from the Micronesion sponge *Zyzzya fuliginosus* together with the previously reported makaluvamines A, E, and K.[173] The structures of **241** and **242** were determined

from spectroscopic data. Makaluvic acid A was inactive in an *in vitro* assay against murine leukaemia cell lines, which further supports the proposal that the pyrroloiminoquinone moiety is responsible for the cytotoxicity of these compounds. Fu *et al.*[173] suggested that oxidation of the batzellines, isobatzellines or makaluvamines may give rise to the makaluvic acids.



241 $R_1 = H, R_2 = CH_3$

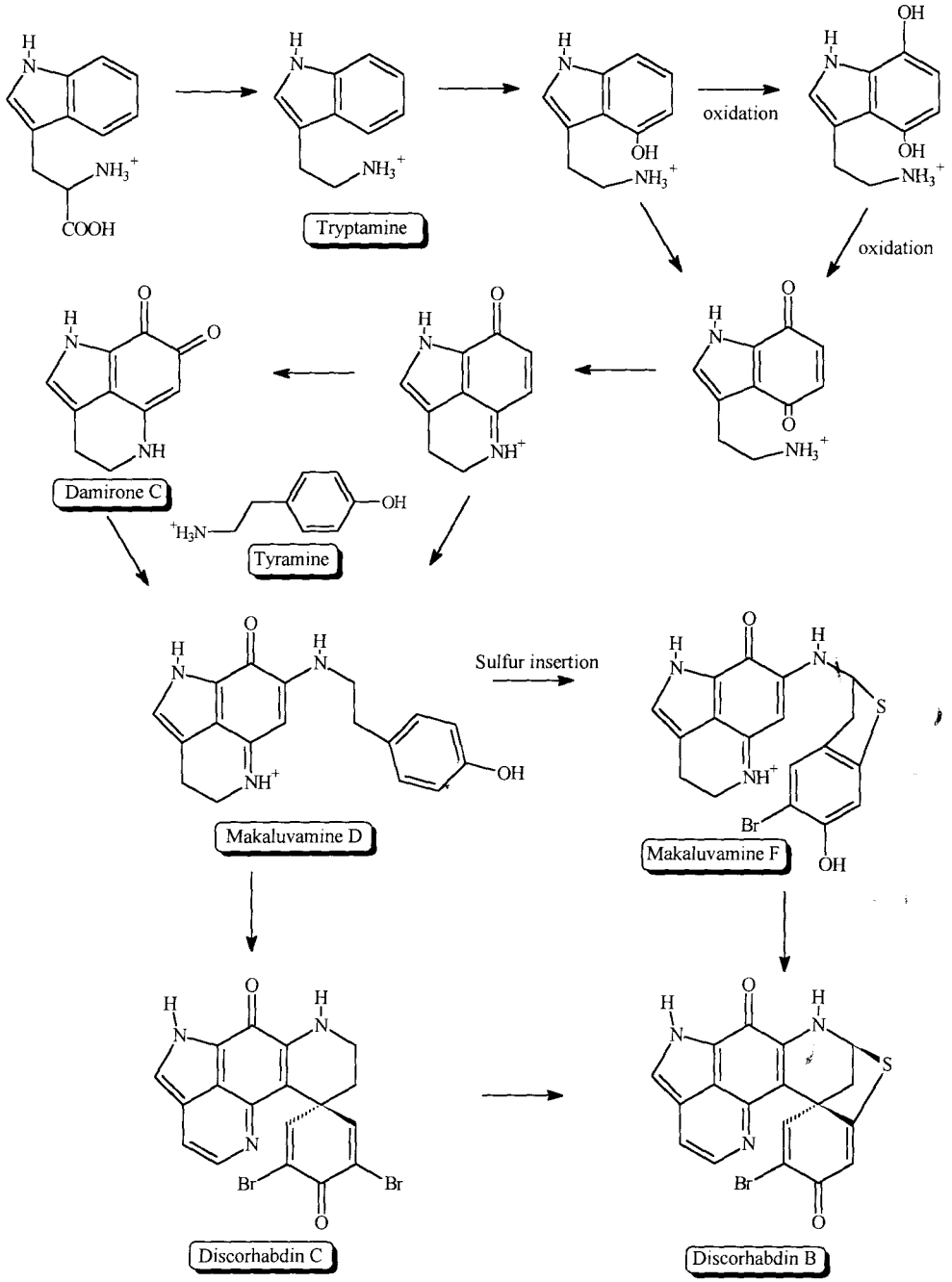
242 $R_1 = CH_3, R_2 = H$

4.1.6 Biosynthesis

The use of sponges in biosynthetic studies has been limited by their slow growth rates, their low incorporation rates of labelled precursors and the presence of symbiotic micro-organisms.[174] Pyrroloiminoquinone alkaloids are considered to be products of aromatic amino acid metabolism, but very little actual research has been done on the biogenesis of these compounds in sponges.

Munro *et al.*[158] suggested a biosynthetic pathway (Scheme 4.1) which interrelates the discorhabdins and other members of this class of natural products. The co-ocurrence of discorhabdin A, the makaluvamines, makaluvone and damirone B in the sponge *Zyzzya marailis*[152] is strong evidence in favour of this proposal. Tryptamine may be considered to be the direct precursor to the pyrroloiminoquinone backbone that characterise the damirones, batzellines, isobatzellines, makaluvone and the simpler makaluvamines. Further elaboration, by inclusion of the amino acid phenylalanine, may lead to the discorhabdins and the more complex makaluvamines, epinardins and the tsitsikammamines. Munro's hypothesis was tested in a study on the New Zealand sponge

Latrunculia sp. B.[158] The incubation of [U-^{14}C]-C-phenylalanine in tissue slices of *Latrunculia* sp. B produced discorhadin B with elevated ^{14}C incorporation. In addition, by performing the experiments in a solution containing a broad spectrum of antibiotics, some evidence was provided that the biosynthesis of discorhabdin B does not require the co-operation of symbiotic micro-organisms and that the discorhabdins are in fact true metabolites of the sponge. As mentioned previously wakayin differs from the other discorhabdins in that it seems to be biosynthesised from two molecules of tryptophan.



Scheme 4.1 Postulated biogenesis of the discorhabdins (Adapted from Munro *et al.*)[158]

4.1.7 Biological Activity

Most pyrroloiminoquinones show strong *in vitro* cytotoxicity against cultured tumour cells. In this section the general biological activities ascribed to the pyrroloiminoquinone metabolites are briefly discussed. Studies into the biological activity of these compounds suggest that the pyrroloiminoquinone moiety is essential for cytotoxicity. A trend evident in the literature that the orthoquinone alkaloids (makaluvone, damirones and batzellines) and the makaluvic acids are essentially non-cytotoxic.

Among the various discorhabdins tested by the NCI, the most active compound appeared to be discorhabdin C.[158] Copp *et al.*[155] explored the activity of various natural and synthetic derivatives of discorhabdin C. They suggested that the α-bromo-α,β-unsaturated ketone moiety, which is also present in a number of cytotoxic marine natural products, notably the tyrosine derivatives from the sponge families Aplysinidae and Aplysinellidae (order Verongida), may be partially responsible for the biological activity of the discorhabdins. This has been illustrated by comparing the cytotoxic activities of discorhabdin F (2-debromodiscorhabdin C) and 3-dihydrodiscorhabdin C to discorhabdin C. Interestingly, both derivatives were less cytotoxic than discorhabdin C.[153] Furthermore, discorhabdin D, which is less cytotoxic *in vitro* than discorhabdin A, B or C, showed significant *in vivo* antitumour activity suggesting a different mode of action. Discorhabdin A was found to induce Ca^{2+} release from the sarcoplasmic reticulum and was 10 times more potent than caffeine in this assay.[150]

Ireland *et al.*,[152] at the University of Utah, studied the mechanism of action of the makaluvamines and suggested that these compounds may act *via* a different mechanism to that of the discorhabdins. Their results indicated that makaluvamines A, C, D, E, and F may interfere with DNA replication by acting as topoisomerase II inhibitors. Topoisomerase II enzymes remove the entanglements of DNA strands during the replication process. Therefore, inhibition of this enzyme may lead to the discovery of new anticancer agents. Makaluvamine B, makaluvone, damirone B and discorhabdin A did not inhibit topoisomerase II, which again implicates the pyrroloiminoqionone moiety as the pharmacophore. Although the complete mechanism of cytotoxic activity of the

makaluvamines has not been completely elucidated, there is evidence that these compounds may also intercalate into DNA and can cause DNA single-strand breakage under reductive conditions. The planar-aromatic, fused-ring, positively charged molecular structure of the makaluvamines is consistent with compounds known to intercalate into DNA.[175] Wakayin, which also lacks the cyclohexadienone moiety, showed only marginal inhibitory activity toward topoisomerse II (250 μM), however, it showed significant differential cytotoxicity against mammalian cell lines which is indicative of DNA damage or interference with DNA processing.[164] Tsitskammamine B was also tested by the Utah group for topoisomerase I activity and exhibited activity in the same range as wakayin.[176]

A further observation concerning the cytotoxicity of the pyrroloiminoquinone metabolites was made by Dijoux *et al.*[160] who suggested that full aromatisation of the pyrroloiminoquinone system causes a reduction in the cytotoxicity relative to the 16,17-saturated members of this family of compounds, thus explaining the reduced activity of discorhabdin Q, prianosin B and makaluvamine B.

The Harbor Branch group showed that discorhabdin P and the secobatzellines A and B exhibited potent protein phosphatase inhibitory activity.[159,172] The enzyme calcineurin (CaN) is a protein phosphatase which is involved the regulation of the immune response. Immunosuppressants, such as cyclosporin A, exert their effect through the inhibition of CaN. The caspases (also known as interleukin-2 converting enzymes) are proteases which play a major role in the programmed cell death mechanism known as apoptosis and have been implicated in chronic neurodegenerative diseases such as Alzheimer's disease. Inhibitors of the caspases may serve to prevent the pathological damage induced by caspase-mediated apoptic events. Unfortunately, the majority of the pyrroloiminoquinone metabolites have not been screened for CaN or caspase activity.

4.2 RESULTS AND DISCUSSION

In our search for new biologically active compounds from South African marine invertebrates, we have screened a large number of extracts obtained from invertebrates collected around the coast of South Africa. Various assays, including antimicrobial,

isolated organ and the brine-shrimp assays were used to screen these extracts for activity. The heavily pigmented sponges SAF95-006, SAF95-058, UPES95-001 and SAF96-039 produced some of the most active extracts in the preliminary screening programme. In this section, the bioassay guided fractionation of the latter three extracts is described.

In addition to the work presented here, the chemistry of a further two latrunculid sponges are presently being studied in our laboratory. It is hoped that our ongoing work on the chemistry of South African latrunculid sponges will contribute to the chemotaxonomic database being complied for the family Latrunculiidae.[177]

4.2.1 The taxonomy of sponges UPES95-001, SAF95-058 and SAF96-039

The possible link between latrunculid sponge chemistry and taxonomy necessitates some understanding of the general morphological characteristics of the sponges studied. Generally, sponges of the genus *Latrunculia* are characterised by discorhabd microscleres which are scattered throughout the choanosome and ectosome (Figure 4.1). Discorhabds can be observed easily by a simple spicule preparation on a microscope slide.

Figure 4.1 Photomicrograph of the ectosome of a *Latrunculia* sponge showing a dense feltwork of megascleres with a layer of discorhabd microscleres lining the surface (T. Samaai).

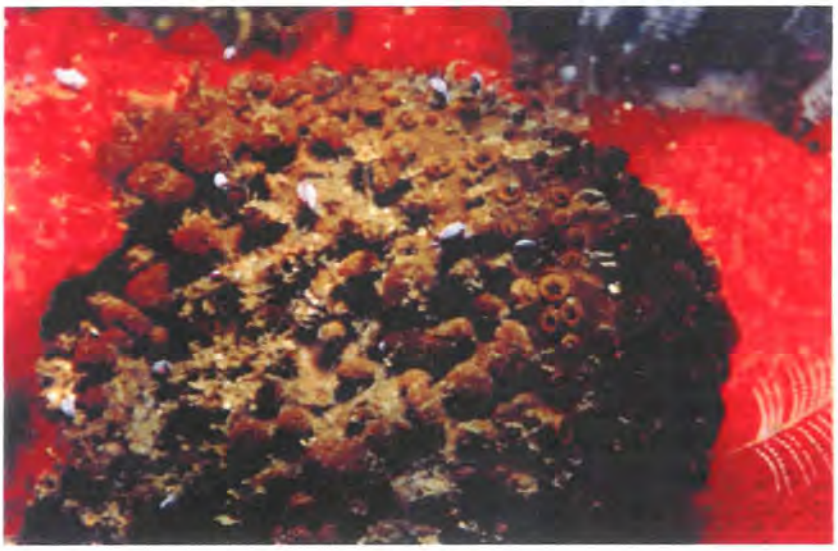


Figure 4.2. Underwater photograph of UPES96-001

A methanol extract of the sponge UPES95-001, collected as part of an initial survey of the marine invertebrates in Algoa Bay, Port Elizabeth, South Africa, showed potent antibiotic activity. The sponge was collected at a depth of 15 m in 1994 and identified by Dr Michelle Kelly-Borges as a *Strongylodesma* sp. as follows: "In life the sponge is spherical to semi-spherical, up to 6 cm high and 6 cm wide. The surface is smooth with volcano-shaped membranous oscules and smooth fungiform areolate structures. The texture is compressible and the colour is leather brown. The choanosomal skeleton consists of a sparse irregular square to polygonal-meshed reticulation of strongyles, with no distinction between primary and secondary tracts. The ectosome is made up of a shallow tangential layer of strongyles approximately 20 mm deep above which is a layer of perpendicular strongyles which pierce the surface. No microscleres are present. The sponge is most closely comparable to *Strongylodesma areolatea* Levi, 1969 (Family Latrunculiidae)."

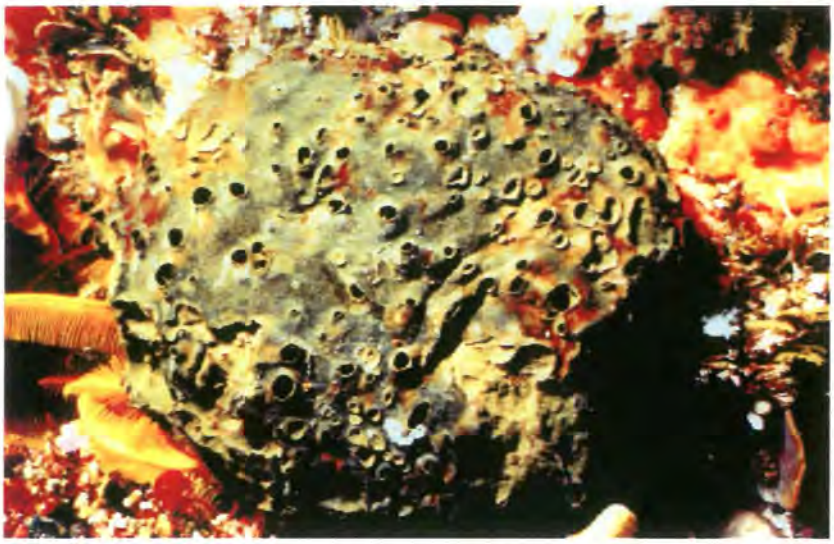


Figure 4.3. Underwater photograph of SAF95-058

SAF95-058 was collected in the Tsitsikamma Marine Reserve during a collaborative project with SmithKline Beecham set up to explore the biomedical potential of marine invertebrates from the Tsitsikamma Marine Reserve. A spicule preparation of the olive green sponge clearly showed the presence of the discorhabd microscleres characteristic of a latrunculid sponge. This was confirmed by Dr Michelle Kelly-Borges who identified it as an undescribed *Latrunculia* sp. as follows. "Sponge SAF95-058 was collected from a depth of 28 m in the Tsitsikamma National Park, South Africa in 1995. In life the sponge is semi-spherical, up to 5 cm high and 5 cm wide. The surface is smooth with regularly spaced raised oscules and crater-like areolate structures. The texture is compressible but leathery. The colour in life is olive green, mottled with brown patches. The choanosomal skeleton consists of a very irregular square to polygonal-meshed reticulation of anisostyles, with no distinction between primary and secondary tracts. The ectosome is made of a dense feltwork of anisostyles approximately 30 mm deep. A single layer of erect discorhabd lines the surface of the ectosome. The sponge is an undescribed species of *Latrunculia* (Family Latrunculiidae)."

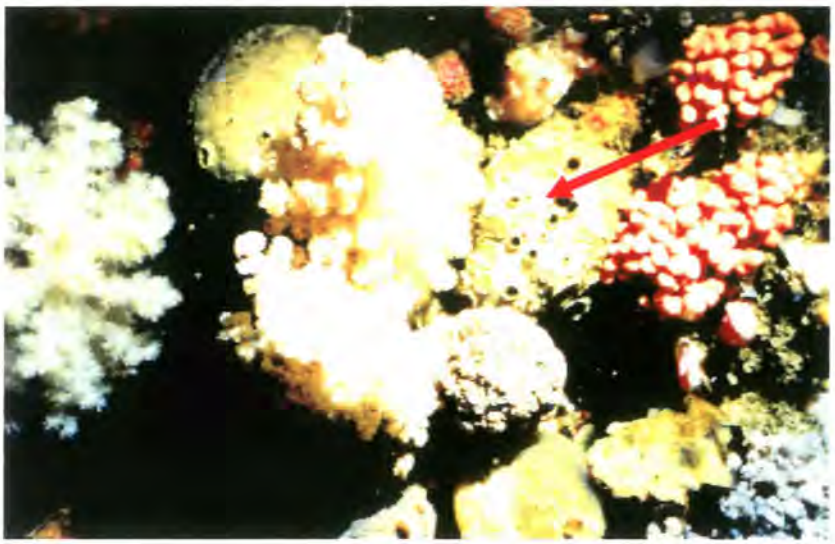


Figure 4.4. Underwater photograph of SAF96-039

In continuation of our program to explore the biomedical potential of South African marine invertebrates, we collected a number of specimens on the West Coast of South Africa near Cape Town. These invertebrates were investigated in collaboration with SmithKline Beecham and Prof D.J Faulkner at the Scripps Institute of Oceanography, University of California, San Diego. Ethanolic extracts of these organisms were screened by ^{1}H NMR spectroscopy and the brine shrimp lethality assay for general cytotoxicity. The green massive sponge (SAF96-039) showed significant activity in the brine shrimp assay and was selected for further investigation. A spicule preparation of this sponge showed the presence of the characteristic discorhabd microscleres indicating that it was a *Latrunculia* sp. The initial description of the sponge by Dr Kelly-Borges is as follows. "SAF96-039 was collected from a depth of 17-20 m at Oudekraal, Cape Town, South Africa in 1996. In life the sponge is semispherical, up to 4 cm high and 4 cm wide. The surface is smooth with low raised thick-lipped oscules, and crater-like pits which contain an inhalant sieve (areolate strcutures). These areolate structures have a distinct net-like cover. The texture is generally soft and leathery, but sandpapery to the touch. The colour in life is olive green, in alcohol the internal colour ranges from dark green to brownish green. The choanosomal skeleton consists of irregular polugonal-meshed reticulation of anisostyles as in SAF95-058, but the tracts is finer, giving the appearance of a more delicate reticulation. The megascleres are straight with polytylote expansions. Interstitial spicules are abundant and discorhabds are also scattered throughout the choanosome. The ectosome is made of a

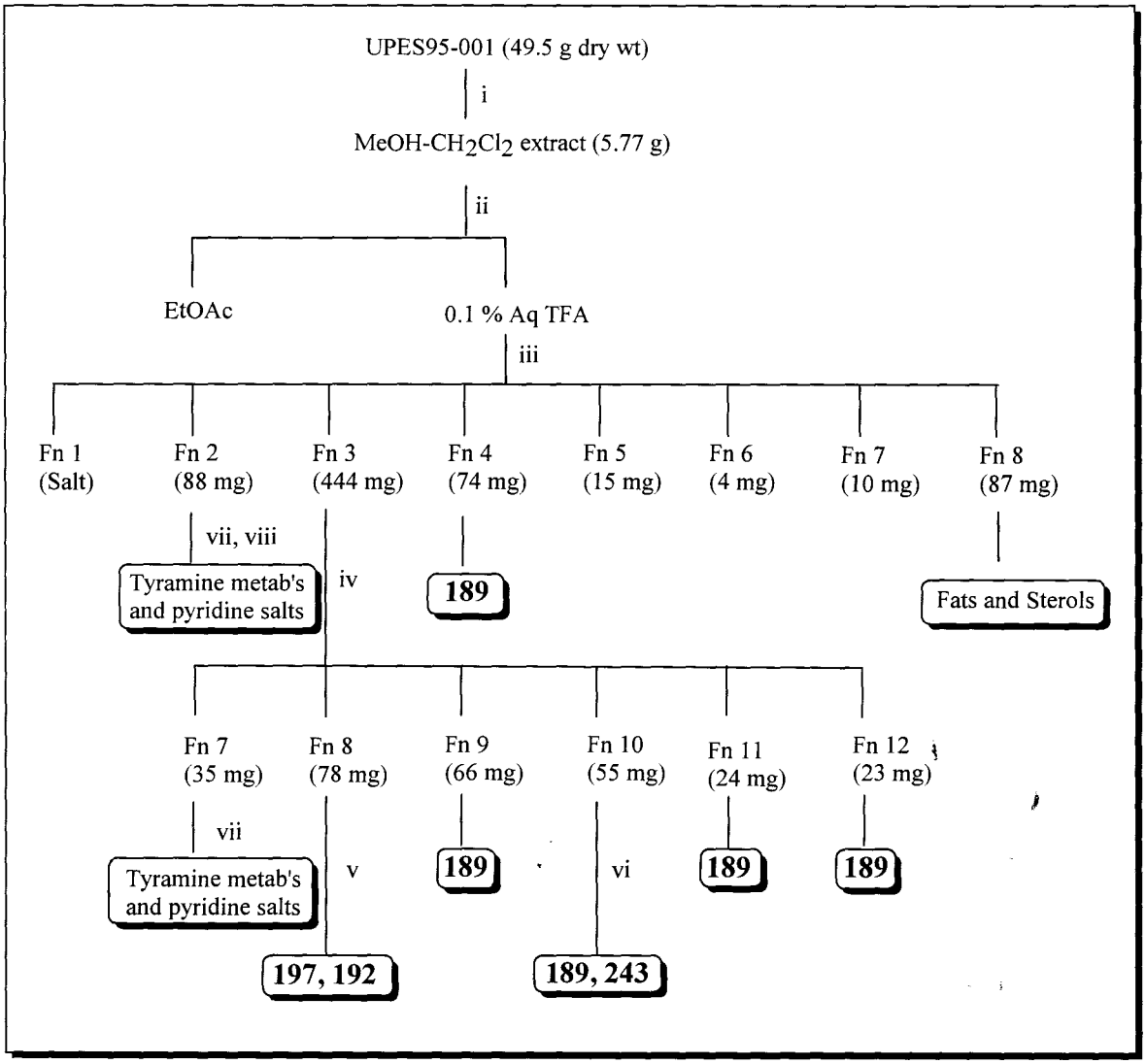
dense feltwork of anisostyles 30 mm deep. A very dense layer of interlocking discorhabds lines the surface of the ectosome, the discorhabds differ in that they have a greater degree of ornamentation. The sponge is a second undescribed species of *Latrunculia* (Family Latrunculiidae)."

4.2.2 Extraction and isolation of pyrroloiminoquinone metabolites from sponges UPES95-001, SAF95-058 and SAF96-039.

The discorhabdins, due to their polar nature, are extremely difficult to purify. A number of procedures have been developed by various researchers to overcome these difficulties. These include initial fractionation by C_{18} reverse phase flash chromatography[149] or by silica gel column chromatography[161] followed by reverse phase C_8 or C_{18} HPLC. We have investigated a number of procedures to isolate these compounds and found the most useful method to consist of a combination of LH-20 molecular exclusion chromatography, reverse phase Sep-Pak chromatography and reverse phase C_{18} HPLC. The following procedure was employed in the isolation of the discorhabdins from the sponge UPES95-001. A similar procedure was used for the work up of the other two sponges with only slight variations.

The freeze-dried sponge UPES95-001(49.5 g) was extracted with MeOH-CH_2Cl_2 (1:1) to give 5.77 g of a dark oil. The ^1H NMR spectrum of the crude extract showed only three conspicuous signals in the aromatic region. This extract, which contained a large amount of fats and sterols, was partitioned between H_2O (0.05% TFA) and EtOAc to give a dark coloured aqueous fraction that was concentrated and freeze-dried. A dark green "fluffy" powder (2.43 g) was obtained, still containing a large amount of inorganic salts. Desalting and further fractionation of a portion of this crude extract (2.0 g) was achieved on a C_{18} Sep-Pak cartridge using a solvent gradient from H_2O to MeOH (containing 0.05% TFA). Eight different coloured bands were collected (Fns 1-8, Scheme 4.2). The fraction eluting with 40 % MeOH (Fn 4) contained almost pure discorhabdin A. Fraction 3 which eluted with 30 % MeOH contained a number of interesting aromatic signals and was further purified on a C_{18} Sep-Pak cartridge followed by HPLC to give discorhabdin A **189** (131 mg, 0.32% dry wt), discorhabdin D **192** (5 mg, 0.01% dry wt), 3-dihydrodiscorhabdin C

(243) (10 mg, 0.02%, dry wt) and discorhabdin H **197** (11 mg, 0.03% dry wt). The most polar Sep-Pak fraction did not seem to contain any discorhabdins and was fractionated on a LH-20 gel column followed by reverse phase HPLC resulting in the isolation of a number of simple tyramine and pyridine derivatives that were not investigated further. The fractionation scheme for sponge UPES95-001 is summarised here in Scheme 4.2.



Scheme 4.2 Extraction and isolation scheme for UPES95-001: (i) Solvent extraction (MeOH-CH_2Cl_2, 1:1); (ii) solvent partitioning (0.1% aqueous TFA and EtOAc); (iii) C_{18} Sep-Pak fractionation using a solvent gradient from H_2O to MeOH; (iv) C_{18} Sep-Pak fractionation using a solvent gradient from H_2O to MeOH; (v) Reversed phase HPLC (MeOH-H_2O, 25:75, TFA pH 3); (vi) Reversed phase HPLC (MeOH-H_2O, 50:50, TFA pH 3); (vii) LH-20 column chromatography (MeOH-CH_2Cl_2, 70:30).

The EtOAc and methanol extracts for sponge SAF95-058 were obtained from SmithKline Beecham and showed potent antibacterial activity. The ^{1}H NMR spectrum of the crude EtOAc extract showed signals suggestive of fats and sterols whereas the methanol extract (Figure 4.5b) was very similar to that of UPES95-001 (Figure 4.5a) with the exception that the signal at δ 6.4 was absent. It was clear from the ^{1}H NMR spectra that the extracts of the two sponges contained similar metabolites and, given our chemotaxonomic interest in latrunculid sponges, a more detailed examination of the metabolites of SAF95-058 was pursued. A portion of the MeOH extract obtained from SmithKline Beecham was fractionated according to Schemes 4.3 to give discorhabdins A (180 mg) and H (4 mg).

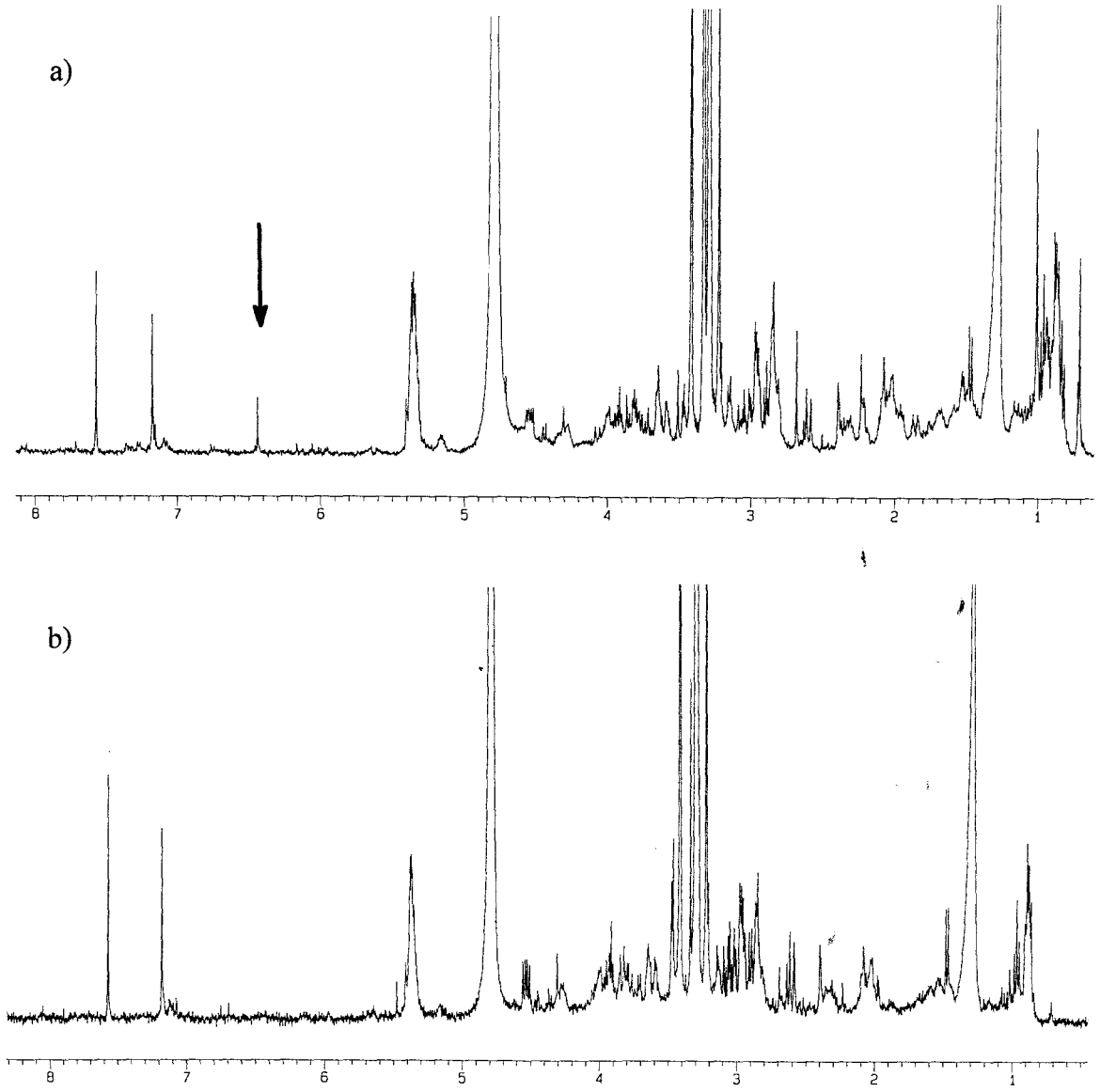
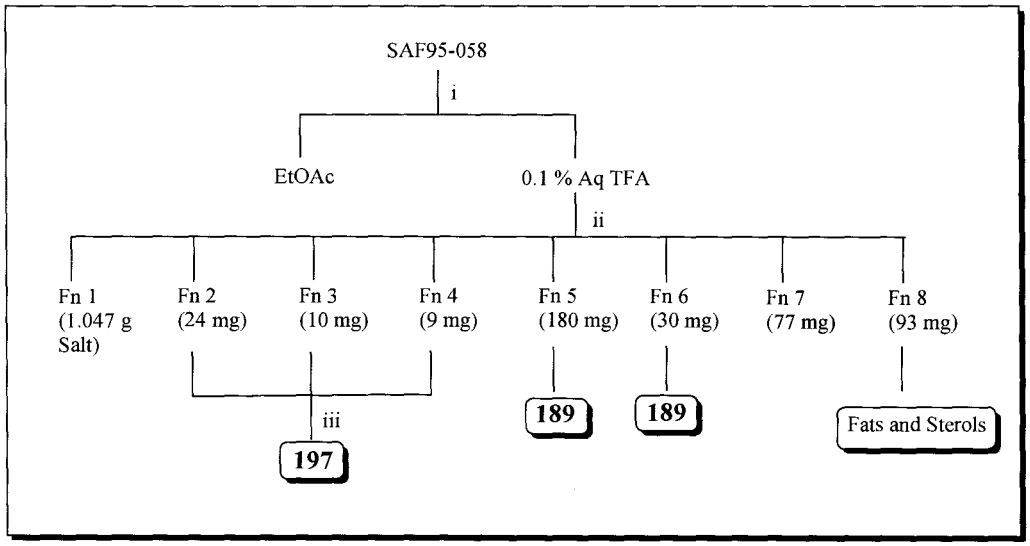
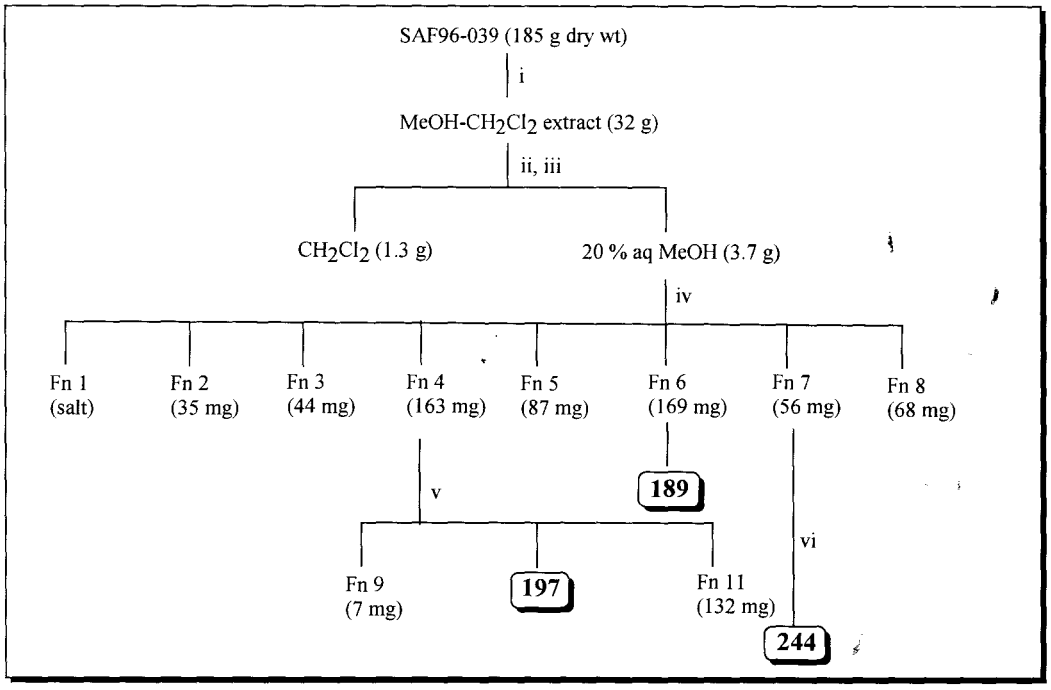


Figure 4.5 ^{1}H NMR spectra (400 MHz, CD_3OD) of (a) UPES95-001 and (b) SAF95-058.



Scheme 4.3 Extraction and isolation scheme for SAF95-058: (i) Solvent partitioning of MeOH extract (0.1% aq. TFA and EtOAc), (ii) C_{18} Sep-Pak fractionation (solvent gradient from H_2O to MeOH, (iii) Fns 2-4 were combined and fractionated by reversed phase HPLC (MeOH-H_2O, 25:75, TFA pH 3).

Fractionation of the MeOH-CH_2Cl_2 extract of SAF96-039 (Scheme 4.4) gave discorhabdins A (256 mg, 0.14% dry wt), H (201 mg, 0.11% dry wt) and 3-dihydrodiscorhabdin B (3.7 mg, 0.002% dry wt).

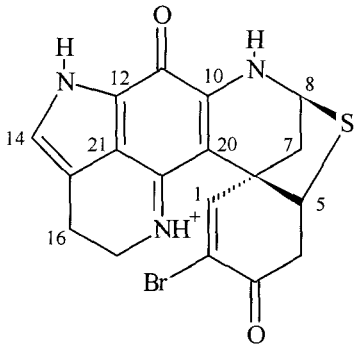


Scheme 4.4 Extraction and isolation scheme for SAF95-039: (i) Solvent extraction (MeOH-CH_2Cl_2, 1:1); (ii) Trituration with MeOH to remove inorganic salts; (iii) solvent partitioning (0.1% aqueous TFA and CH_2Cl_2), (iv) C_{18} Sep-Pak fractionation using a solvent gradient from H_2O to MeOH; (v) LH-20 column chromatography (MeOH-CH_2Cl_2, 1:1); (vi) Reversed phase HPLC (MeOH-H_2O, 40:60, TFA pH 3).

4.2.3 Structure elucidation of pyrroloiminoquinone metabolites

The discorhabdins show unfavourable solubility properties in most organic solvents. While CD_3OD was a useful solvent for the routine acquisition of the ^1H NMR spectra of crude fractions, the greater solubility of the pure pyrroloiminoquinones in DMSO-d_6 made the latter solvent the solvent of choice for the acquisition of two-dimensional, ^{13}C and DEPT spectra. Unfortunately, this resulted in the loss of resolution (*i.e.* loss of fine peak-splitting) when changing to the more viscous solvent (DMSO-d_6). Because of the abundance of quaternary carbons and non-protonated heteroatoms in these alkaloids, extensive ^1H-^{13}C long range correlations (HMBC)[178] were essential for the assignment of the ^1H and ^{13}C chemical shifts for each compound.

4.2.3.1 *Discorhabdin A*



189

Discorhabdin A was the major metabolite in all three sponges and was characterised as its green trifluoracetate (TFA) salt. This compound, present at approximately 1% dry wt, is probably responsible for the olive green colour of the sponges. A molecular formula of $C_{18}H_{15}N_3O_2BrS$ was deduced for the protonated compound from a 1:1 doublet at 416.0068/418.0049 in the high resolution FABMS spectrum. The ^1H NMR spectrum of **187** in CD_3OD (Figure 4.6) was relatively simple showing two aromatic methine signals (δ 7.57 and 7.18) and another two deshielded methine resonances at δ 5.37 (d, J = 3.1 Hz, H-8) and 4.52 (dd, J = 6.5 Hz, 12.2 Hz, H-5). The ^1H NMR spectrum in DMSO-d_6 showed three exchangeable protons at δ 13.23 (NH-13, br s), 10.36 (NH-9, br s) and 9.46 (NH-18, br s). A more complex situation was evident from the ^{13}C (Figure 4.7, Table 4.1) and

DEPT-135 NMR spectra which revealed eighteen well resolved signals including four methylene carbons, four methine carbons and ten quaternary carbons. An α,β-unsaturated ketone was evident from the carbon signal at δ 186.7 and an iminoquinone moiety from the signals at δ 165.8 and 153.6. The spectroscopic data were in agreement with those reported by Perry *et al.* for discorhabdin A.[151]

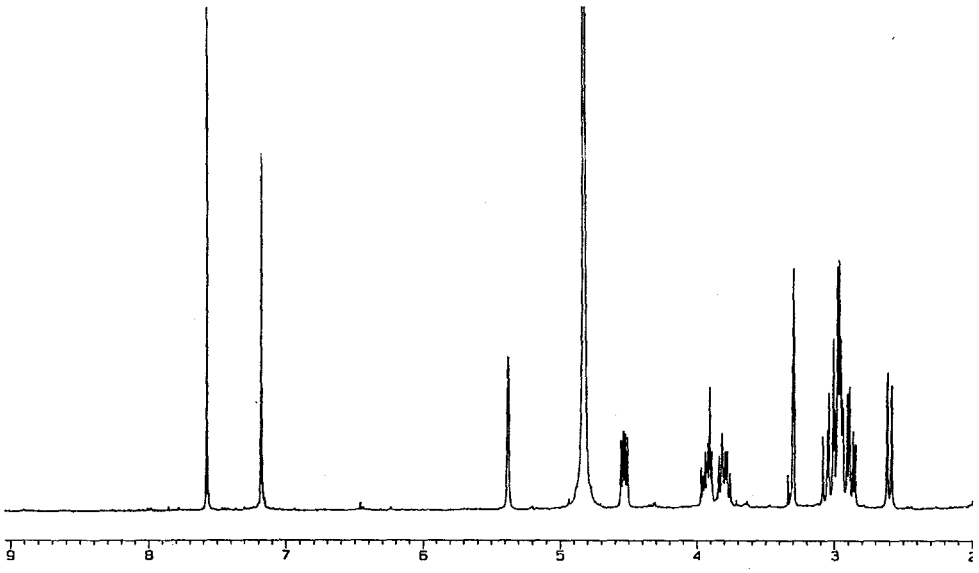


Figure 4.6. ^{1}H NMR spectrum of discorhabdin A (CD_3OD, 400 MHz)

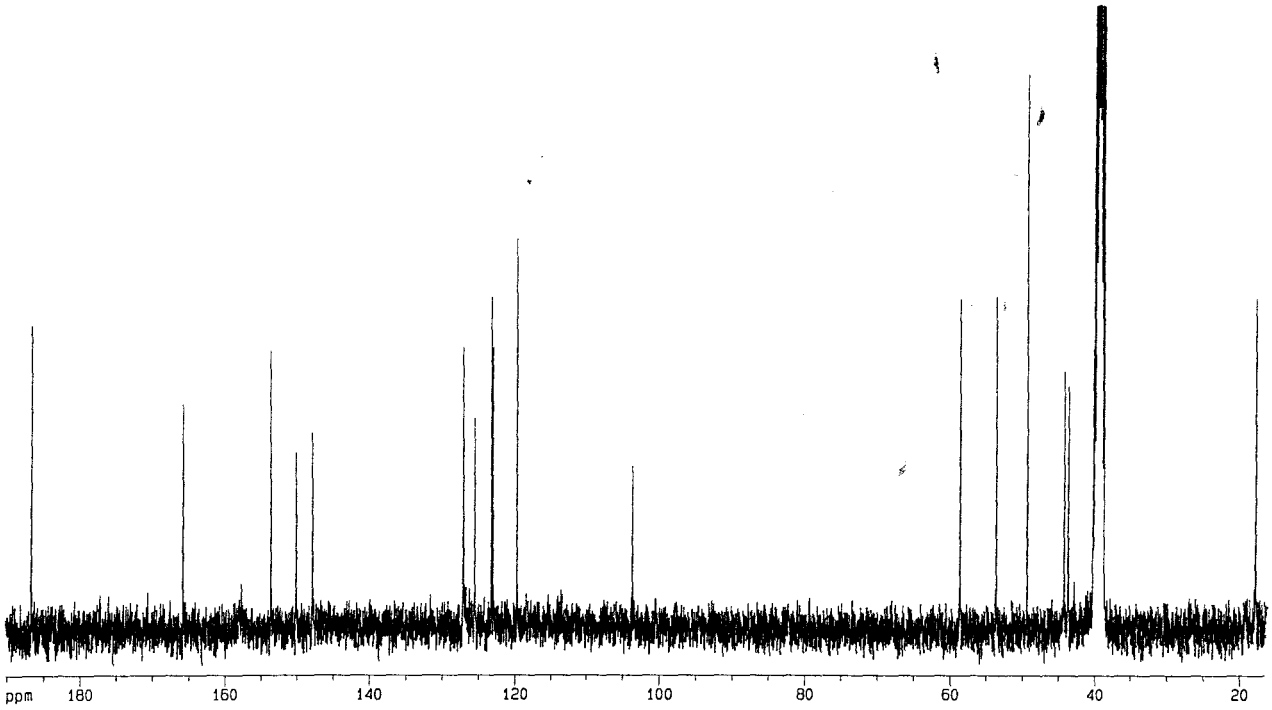


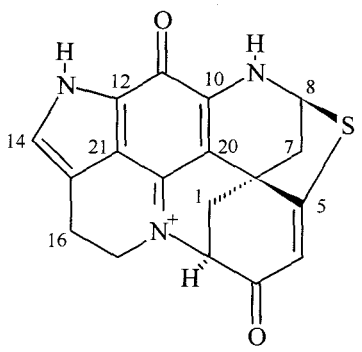
Figure 4.7. ^{13}C NMR spectrum of discorhabdin A (DMSO-*d*6, 100 MHz)

Table 4.1 ^{13}C NMR data (DMSO-d_6, 100 MHz) for discorhabdin A **189**, discorhabdin D **192**, 3-dihydrodiscorhabdin C **243**, discorhabdin C **190**, discorhabdin H **197**, 3-dihydrodiscorhabdin B **244**

Atom no	**189**	**190**[149]	**192**	**197**	**243**	**244**
1	147.9	151.4	30.1	51.0	133.8	127.8
2	125.5	122.8	62.1	64.9	122.2	116.7
3	186.7	171.5	182.9	181.4	69.5	62.4
4	44.2	122.8	112.3	113.2	122.2	111.3
5	53.6	151.4	172.9	169.0	133.8	140.3
6	49.4	44.9	41.0	45.2	42.0	48.1
7	40.2	33.8	38.6	37.6	33.7	30.8
8	58.6	38.5	62.6	62.5	37.5	63.9
10	150.1	152.0	147.7[a]	146.6	152.3	140.7
11	165.8	165.5	166.4	166.0	165.4	167.4
12	123.2	123.4	123.7[b]	123.6	123.3[c]	123.9
14	127.1	127.8	126.8	127.0	127.7	126.2
15	119.7	120.0	117.6	117.7	119.5	117.8
16	17.8	18.2	19.3	19.2	17.9	19.6
17	43.6	43.9	51.1	51.0	42.9	52.0
19	153.6	153.4	145.8[a]	147.6	153.1	147.3
20	103.6	91.9	99.5	99.6	92.8	99.6
21	123.0	123.7	121.4[b]	121.2	123.2[c]	121.0
2'				139.3		
4'				129.7		
5'				127.0		
6'				24.5		
7'				48.9		
8'				169.6		
9'				32.0		

[a,b,c] Chemical shift values with the same superscript may be interchanged

4.2.3.2 *Discorhabdin D*



192

The most polar compound, discorhabdin D, a green solid, was isolated as one of the minor metabolites of UPES95-001. The ^{13}C NMR and DEPT-135 spectra revealed the presence of the usual eighteen carbon resonances, including four methylene carbons, four methine carbons and ten quaternary carbons. An α,β-unsaturated carbon was evident from the carbon resonance at δ 182.9. The ^{1}H NMR spectrum (Figure 4.8), which was significantly different to that of discorhabdin A, lacked the signal at δ 7.57 (H-1) and, instead, contained a deshielded methine resonance at δ 6.06 (H-4) and a deshielded methylene at δ 4.35 (t, J = 2.7 Hz, H-2). The proton signals between δ 2.5 and 3.2 were also better resolved than those in the spectrum of discorhabdin A (Figure 4.6). The disappearance of the NH-18 signal at δ 9.5 in the ^{1}H NMR spectrum (DMSO-d_6) suggested an additional substituent at this position. All the spectroscopic data were consistent with those of discorhabdin D published by Perry *et al.*[154]

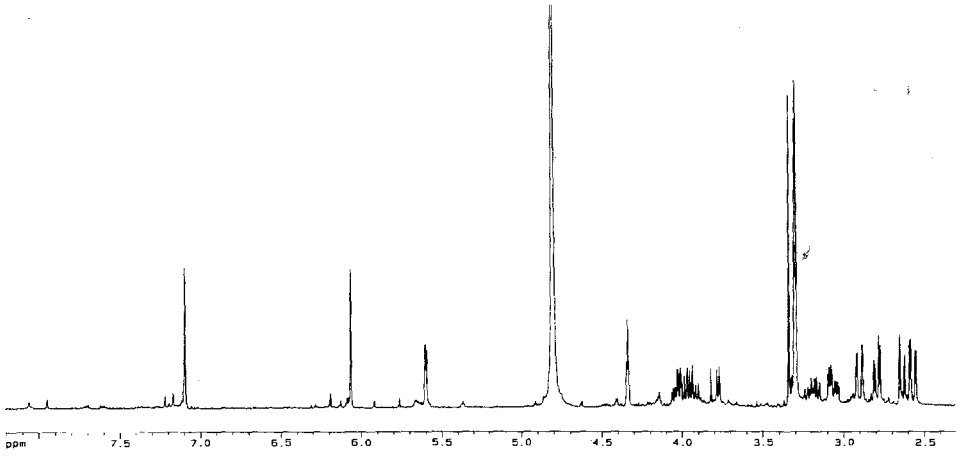
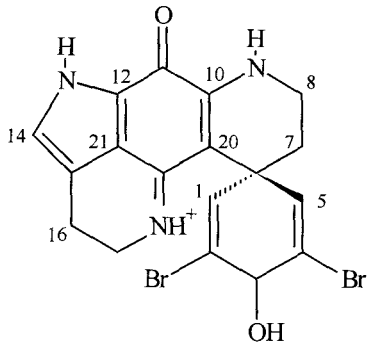


Figure 4.8. ^{1}H NMR spectrum of discorhabdin D (CD_3OD, 400 MHz)

6.2.3.3 *Dihydrodiscorhabdin C*



243

This compound was the second major metabolite obtained from UPES95-001 and was isolated as a bright red solid suggesting a reduction in the conjugation of the molecule when compared to the dark green compounds discorhabdin A and D. The ^{1}H NMR spectrum (Figure 4.9) of the pure compound looked deceptively simple with seven well-resolved signals. These included the indolic proton at δ 7.16, a sharp two proton singlet at δ 6.44, a deshielded methine at δ 4.71 and four methylene triplets suggesting two isolated pairs of mutually coupled methylene moieties. The H-8 methine signal which resonates at δ 5.37 and 5.60 in discorhabdin A and D, respectively, was conspicuously absent in **243**, suggesting a compound lacking a thio-ether functionality. This was confirmed by HRFABMS which gave a $[MH]^{+}$ peak at *m/z* 463.9609 corresponding to a molecular formula of $C_{18}H_{15}N_3O_2Br_2$. The ^{13}C NMR spectrum showed only sixteen of the expected eighteen signals indicating a plane of symmetry in the molecule. Two of the aromatic carbon signals (δ 133.8 and 122.4) were approximately twice the height of the neighbouring signals suggesting the presence of overlapping signals. The pyrroloiminoquinone structure followed from characteristic carbon signals at δ 166, 146, 99. All the protonated carbon signals were assigned from HMQC data. Analysis of the COSY-90 spectrum indicated that the four methylene groups constituted two isolated spin systems, identical to those reported for discorhabdin C,[149] and provided the initial evidence for the structural similarities between **190** and **243**. Further support for the discorhabdin C basic skeleton for **243** was established from exhaustive analysis of the HMBC data (Table 4.2) and comparison of the ^{1}H and ^{13}C NMR resonances (Table 4.1) of **243** with those of **190**. The absence of the α,β-unsaturated carbonyl signal (δ ≈ 182) in the ^{13}C spectrum,

combined with a methine resonance at δ 4.71 which was correlated to an oxymethine carbon at δ 69.5 in the HMQC spectrum, suggested a reduced carbonyl at C-3. The presence of a cyclohexadienol moiety was confirmed by HMBC correlations (Table 4.2) from the two proton singlet at δ 6.48 (H-1 and H-5) to carbons at δ_C 42.0 (C-6), 69.5 (C-3) and 122.4 (C-2 and C-4), thus completing the structural assignment of **243**. The stereochemistry at C-3 remains unassigned. 3-Dihydrodiscorhabdin C is related to the epinardins[163] and was previously synthesised from discorhabdin C by Blunt *et al.*[156] This is the first report of the occurrence of **243** in Nature.

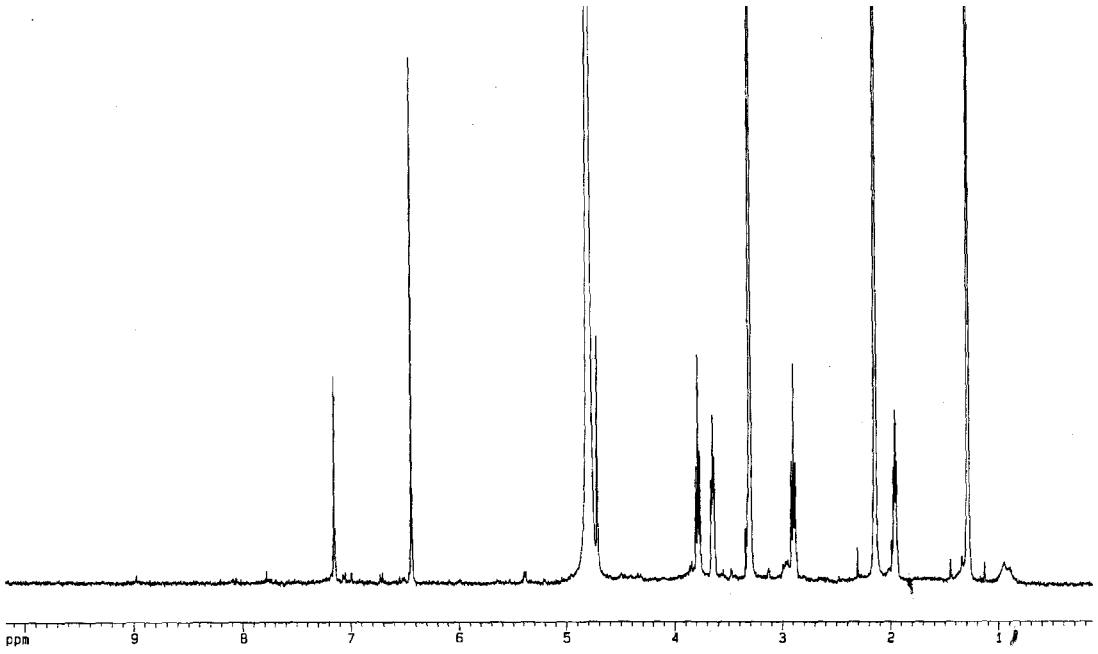
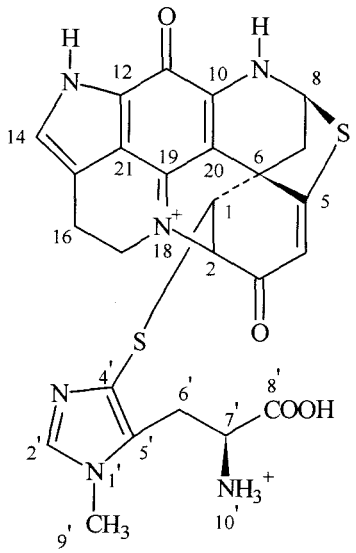


Figure 4.9. ^{1}H NMR spectrum of 3-dihydrodiscorhabdin C (400 MHz, CD_3OD)

Table 4.2 Spectral data for 3-dihydrodiscorhabdin C **243** and discorhabdin C **190**[149]

		243			**190**[149]
Atom no.	δ_C	δ_H (mult, *J*, Hz) DMSO-d_6	COSY	HMBC	δ_H (mult, *J*, Hz) CD_3OD
1	133.8, d	6.48, s	-	C-2, C-3, C-6, C-7, C-20	7.73, s
2	122.2, s	-	-	-	-
3	69.5	4.60, s	-	C-2 and/or C-4	-
4	122.2, s	-	-	-	-
5	133.8, d	6.48, s	-	C-2, C-3, C-6, C-7, C-20	7.73, s
6	42.0, s	-	-	-	-
7	33.7, t	1.86, t, 5.0	H-8	-	2.12, t, 6
8	37.5, d	3.59, t, 4.8	H-7	-	3.73, t, 6
NH-9	-	10.14, br s	H-8	-	10.3
10	152.3, s	-	-	-	-
11	165.4, s	-	-	-	-
12	123.3, s	-	-	-	-
NH-13	-	13.15, br s	H-14	-	13.4, s
14	127.7	7.37, s	H-16	C-15, C12/21	7.22, s
15	119.5, s	-	-	-	-
16	17.9	2.83, t, 7.4	H-17, NH18	C-15, C-17	2.90, t, 7
17	42.9	3.72, t, 7.4	H-16	C-15	3.79, t, 7
NH-18	-	7.80, br s	H-16	-	8.3, s
19	153.1, s	-	-	-	
20	92.8, s	-	-	-	
21	123.2, s	-	-	-	
OH	-	7.52	H-3	-	

4.2.3.4. *Discorhabdin H*



197

Discorhabdin H **197** was also isolated as a dark-green trifluoroacetate salt from all three sponges. The molecular formula was established by high resolution FABMS as $C_{25}H_{23}N_6S_2$ (*m/z* 535.1222) implying eighteen degrees of unsaturation. The ^1H NMR spectrum of **197** (Figure 4.10) revealed the presence of methine signals at δ 7.29 (H-14), 6.29 (H-4), 5.75 (H-8), δ 7.84 (H-2') and an N-methyl peak at δ 3.64 (H-9'). The ^{13}C NMR and DEPT-135 spectra indicated twenty five carbons, as expected from the molecular formula; including four methylene, one methyl and seven methine carbons and thus thirteen quaternary carbons. Further analysis of the ^{13}C spectrum showed carbon resonances at δ 166.0 (C-11), 147.6 (C-19), 99.6 (C-20) which were consistent with the presence of a pyrroloiminoquinone moiety. The carbon signal at δ 181.4 (H-3) suggested the presence of an α,β-unsaturated carbonyl. The large number of overlapping signals in the ^1H NMR spectrum, especially between δ 2.5 and 4.5, complicated the assignment of these signals. Nevertheless, careful interpretation of the HMQC and DEPT data allowed the assignment of all protonated carbons. The structure of **197** was not immediately apparent from direct comparison of the ^1H and ^{13}C NMR data of this compound with those of **189**, **192** and **243**. A detailed analysis of HMBC, HMQC and COSY-90 data was thus necessary. Two separate spin systems can be assembled from the two-dimensional NMR data of **197** (Figure 4.11). The left hand hemisphere of the discorhabdin carbon framework

was deduced from an analysis of COSY-90 and HMBC data of **197** as follows. The NH-13 proton at δ 13.20 showed vicinal coupling to H-14 (δ 7.29), while HMBC correlations from the latter signal to carbon resonances at δ 117.7 (C-15), 123,6 (C-12) and 121.2 (C-21) were observed. The 2H-16 methylene multiplet (δ 3.10) showed correlations to the carbon signal at δ 117.7 (C-15) and also vicinal coupling to 2H-17 (δ 3.80 and 4.0). The ^{13}C NMR chemical shift of C-17 (δ 51.0) suggested that this carbon was attached to a nitrogen atom.[106] The above correlations are illustrated as continuous bold bond on the left hand side of Figure 4.11.

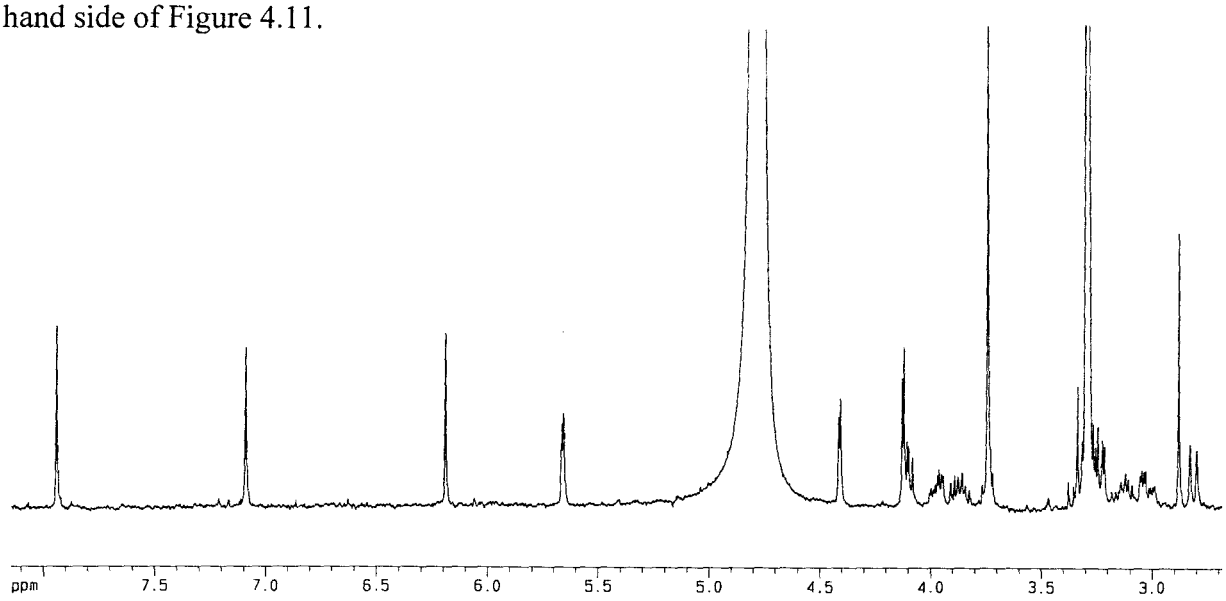


Figure 4.10. ^{1}H NMR spectrum of discorhabdin H (CD_3OD, 400 MHz).

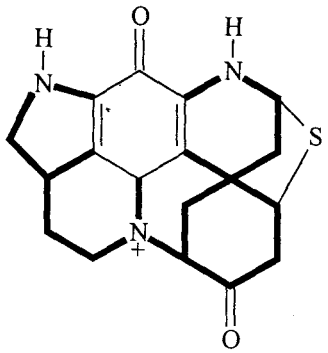


Figure 4.11 Contiguous spin systems deduced from COSY-90 and HMBC data for **197**.

The assignment of the right hand hemisphere of the discorhabdin skeleton was completed as follows. The deshielded methine at δ 5.75 (H-8) showed vicinal coupling to 2H-7 (δ 3.05 and 2.71) and also strong HMBC correlations to carbons at δ 37.6 (C-7), 45.2 (C-6), 146.6 (C-10) and 169.0 (C-5). Further important HMBC correlations from 2H-7 to C-5 (δ

169.0) and to C-20 (δ 99.6) were also observed. Finally, H-2 (δ 4.42) showed vicinal coupling to the methine at δ 4.02 (H-1) and HMBC coupling to C-19 (δ 147.6) C-3 (δ 180.5) and C-6 (δ 45.2), thus connecting the two hemispheres (Figure 4.11). Comparison of the above data with those of discorhabdin D confirmed that their structures were very similar.

It was quite clear that a substituent at C-1 would account for the remaining atoms required by the molecular formula. Again, COSY and HMBC spectra were useful in assigning the histidine structure of this substituent. The H-2' proton (δ 7.84) showed long range coupling in the COSY spectrum to the methyl signal at δ 3.64 (3H-9'). The chemical shift of this methyl group is consistent with an N-methyl moiety. HMBC correlations from 3H-9' to C-4' (δ 129.7) and C-2' (δ 139.4) supported an imidazole ring. The methylene protons at δ 3.25 (2H-6') showed vicinal coupling to H-7' and also HMBC correlations to C-4'(δ 129.7), C-5' (δ 127.0), and to a carbonyl at δ 169.6 (C-8'). Comparison of the ^{13}C data of a standard sample of histidine with those of the histidine residue from **197** confirmed the structural assignment. The additional sulfur atom required by the molecular formula could only be accommodated by a thio-ether link connecting the discorhabdin skeleton to the histidine residue. Evidence in support of this was provided by the chemical shift of C-1 (δ 51.0) which is consistent with the chemical shift value of a carbon bonded to a sulfur atom.[106]

The proposed structure of discorhabdin H contained five chiral centres. The relative configurations at C-1, C-2, C-6 and C-8 of discorhabdin H were determined by analysis of the ROESY NMR and molecular modelling data (Figure 4.12). The configuration at C-1 was arbitrarily chosen as *R*. The H-1 methine showed ROESY correlations to H-7b and H-2. The latter proton showed a further correlation to H-17b, suggesting that these protons reside on the same side of the ring. These data are in agreement with those reported for discorhabdin D.[154]

Although the structure of discorhabdin H was reported by Munro[158] the spectral data is not accessible. The stereochemistry of the histidine residue in **197** has not been previously assigned and was accordingly investigated as follows.

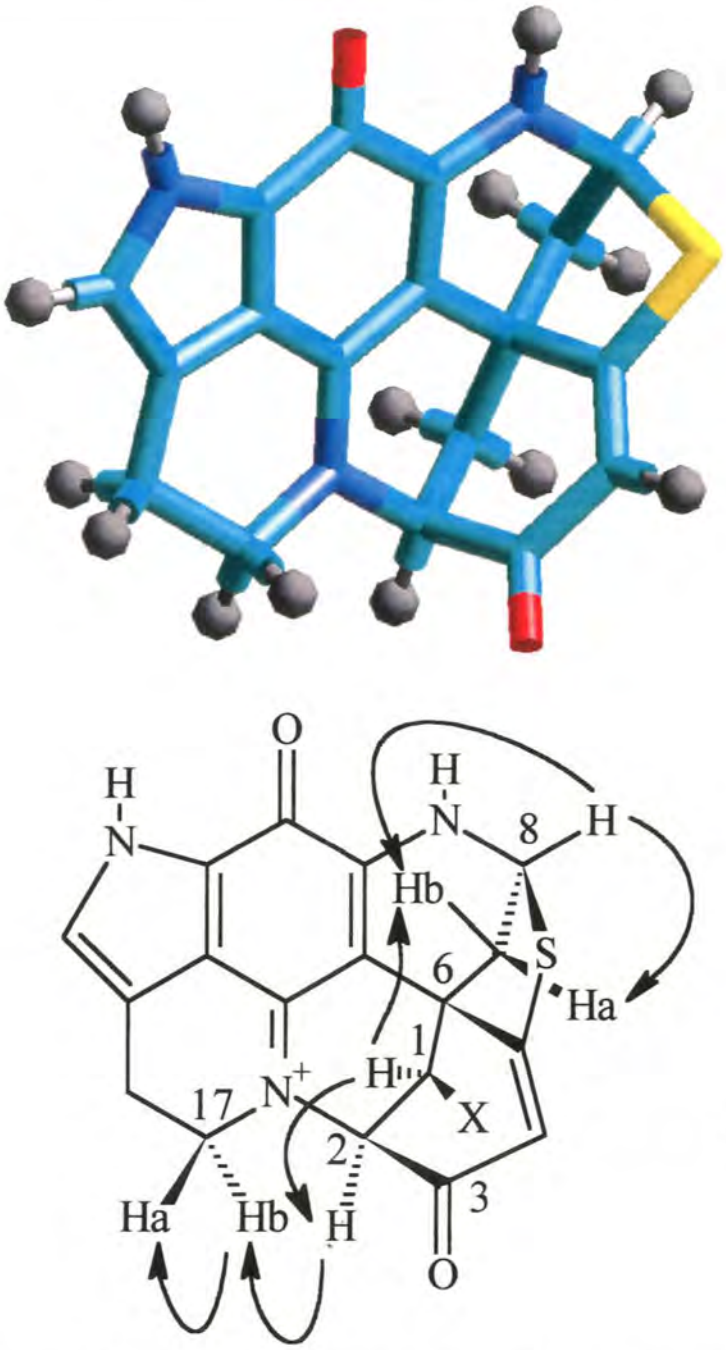
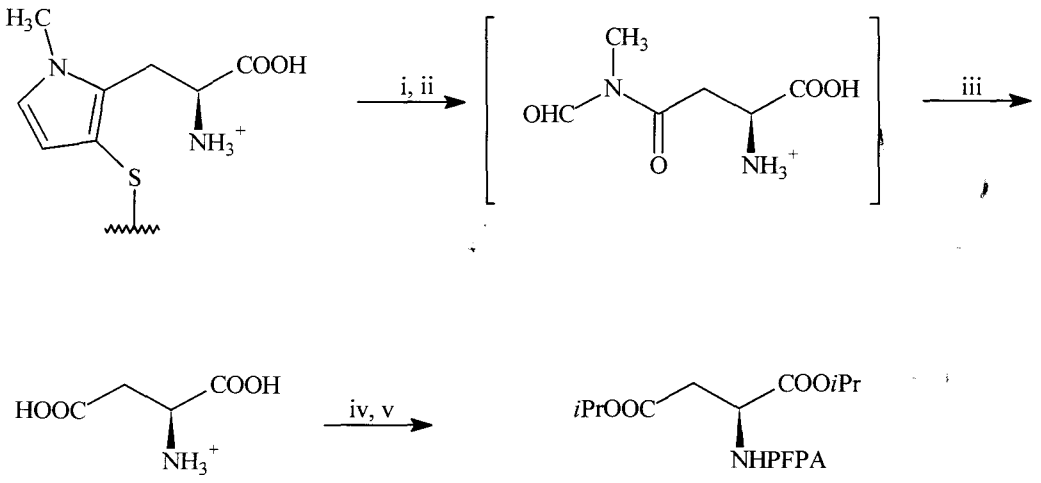


Figure 4.12 Key ROESY correlations (relaxation delay = 1 s, mixing time = 300 msec) for discorhabdin H together with its lowest energy conformation determined by molecular mechanics calculations (Cerius^2 version 3.5 molecular modelling program). Note: To simplify the diagram the histidine residue (X) is not shown.

A common procedure for the determination of the absolute configuration of amino acids is by GC-MS using a chiral GC column. The assignment of the absolute configuration of histidine or tyrosine residues in peptides is often conveniently achieved by ozonolysis of

these amino acids to give aspartic acid which is then analysed by GC-MS and compared to an authentic standard. The latter procedure was used to determine the stereochemistry of the histidine residue in discorhabdin H. Ozonolysis of discorhabdin H (400 μg), followed by an oxidative work up with hydrogen peroxide, gave a residue which contained the amino acid of interest. The latter residue was esterified with isopropanol followed by acylation with pentafluoropropionic anhydride to obtain derivatives suitable for GC-MS (Scheme 4.5) which were then analysed using a chiral column. The GC chromatogram (Figure 4.13) of these derivatives showed one main peak with a retention time of 25.66 minutes. The mass spectrum of this compound exhibited a "fingerprint" mass spectrum containing five prominent peaks at *m/z* 276, 262, 216 and 189 (Figure 4.13). Standard samples of *D*- and *L*-aspartic acid were derivatised in the same manner and were analysed under the same conditions as the sample. The retention times for the *D* and *L* derivatives were 25.57 and 25.69 minutes, respectively with both giving identical mass spectra to that of the sample derived from **197**. Co-injection of the standard *L*-aspartic acid with the sample derived from **197** gave a single peak confirming the *L*-configuration of the aspartic acid residue and thus the histidine residue obtained from discorhabdin H.



Scheme 4.5 Ozonolysis and derivatisation of discorhabdin H

Reagents and conditions: (i) O_3, -70°C, MeOH; (ii) H_2O_2; (iii) H_3O^+, 100°C, 16 h; (iv) *i*PrOH, CH_3COCl, 100°C, 1h; (v) PFPA, CH_2Cl_2, 100°C, 15 min

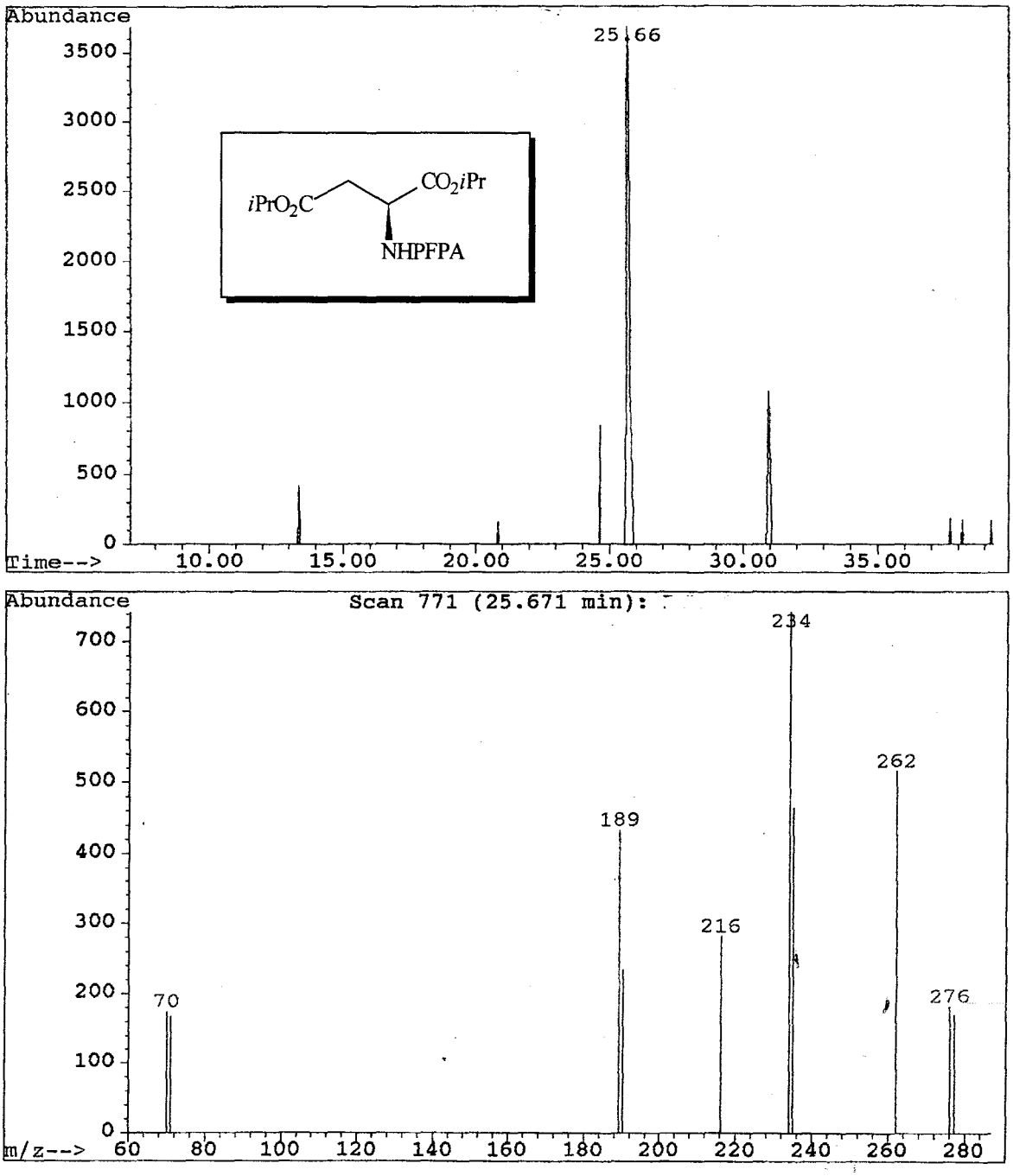
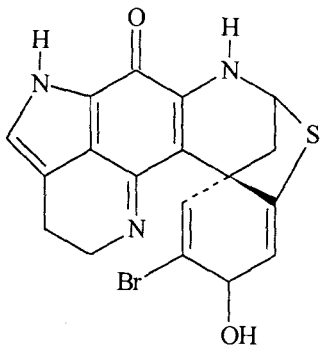


Figure 4.13 GC and MS spectrum of ozonolysis product from discorhabdin H

4.2.3.5 *Dihydrodiscorhabdin B*



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3-Dihydrodiscorhabdin B was isolated as a dark-green solid which exhibited a molecular ion peak at *m/z* 416.9968 in the HRFABMS spectrum, corresponding to a molecular formula of $C_{18}H_{15}N_3BrO_2S$. The ^1H NMR spectrum in DMSO-d_6 (Figure 4.14, Table 4.3) showed six methine signals and the usual two NH-13 (δ 10.08) and NH-10 (δ 10.96) signals. Eighteen well resolved signals in the ^{13}C NMR spectrum (Table 4.1) suggested the usual discorhabdin skeleton.

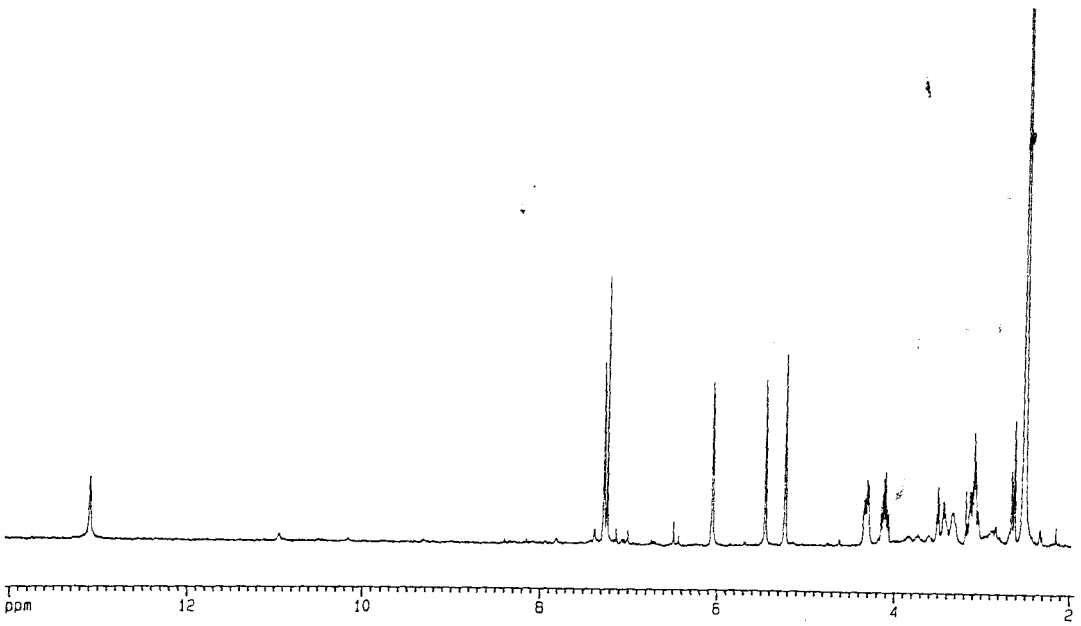


Figure 4.14 ^1H NMR spectrum of 3-dihydrodiscorhabdin B (400 MHz, DMSO-d_6)

Table 4.3 ^{1}H (400 MHz, DMSO-d_6) and ^{13}C (100MHz, DMSO-d_6) NMR data for 3-dihydrodiscorhabdin B

Atom no.	δ_C	δ_H (mult, *J*,Hz)	COSY	HMBC
1	127.8	7.22, s	-	C-2, C-3, C-6
2	116.7	-	-	-
3	62.4	5.21, s	H-7	C-6, C-2
4	111.3	5.43, d, 3.1	H-7, H-8	C-6
5*	140.3	-	-	-
6	48.1	-	-	-
7	30.8	2.63, dd, 12.9, 2.9	H-3, H-4, H-8	C-20, C-6
		2.51, m		
8	63.9	6.03, d, 2.9	NH-9, H-4, H-7	C-10 /C-5
NH-9	-	10.96, br s	H-8	-
10*	140.7	-	-	-
11	167.4	-	-	-
12	123.9	-	-	-
NH-13	-	13.08, br s	H-14	-
14	126.2	7.25, d, 2.0	NH-13	C-12, C-21, C-15
15	117.8	-	-	-
16	19.6	3.10, m	H-17	-
17	52.0	4.11, m	H-16	-
		4.29, m	H-16	
19	147.3	-	-	-
20	99.6	-	-	-
21	121.0	-	-	-

* chemical shift values may be interchanged

An iminoquinone chromophore was deduced from ^{13}C NMR signals at δ 167.4 and 147.3. The αβ-unsaturated carbonyl signal at about δ 180, characteristic of the C-3 carbonyl in discorhabdin A and C was conspicuously absent. Instead an additional oxymethine carbon at δ 62.4 appeared in the carbon spectrum suggesting an alcohol functionality at this position. A further ten aromatic carbon signals suggested the presence of five double bonds. The carbon skeleton for this compound was constructed by interpretation of 2D NMR spectra as for the other compounds. Two and three bond HMBC correlations from the methine at δ 7.25 (H-13) to carbon signals at δ 123.9 (C-12 or C-21), 121.0 (C-21 or C-12) and 117.8 (C-15) confirmed the presence of the pyrroloiminoquinone moiety. The

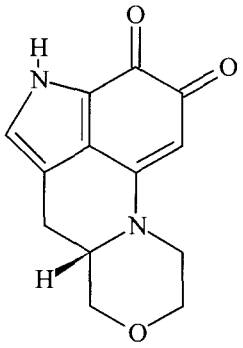
aromatic methine at δ 7.22 showed HMBC correlations to carbons at δ 116.7 (C-2), 62.4 (C-3) and 48.1 (C-6). All the spectroscopic data were consistent with the proposed structure of **244** which has also not been reported before.

4.2.4 Phyletic distribution and chemotaxonomy

Pyrroloiminoquinone metabolites have mainly been isolated from sponges (Phylum Porifera), with only a single report of their occurrence in a tunicate (Phylum Urochordata). Chemotaxonomic and morphological studies show general trends with respect to the occurrence of the various pyrroloiminoquinone alkaloids in the different sponge genera. The discorhabdins are found mainly in sponges of the genus *Latrunculia* (Family, Latrunculiidae, order Hadromerida), while the makaluvamines, which are considered to be intermediates in the biosynthesis of the discorhabdins, are generally found in sponges of the genus *Zyzzya*. Furthermore, the batzellines and isobatzellines have been reported only from the deep-water sponge *Batzella* sp. Exceptions include the occurrence of discorhabdin A in the sponge *Zyzzya fuliginosa* and discorhabdin P in the deep water Caribbean sponge of the genus *Batzella* (Class Demospongia, order Poecilosclerida, family Desmacididae).[159] Recently, discorhabdin Q was isolated from sponges belonging to the genera *Latrunculia* and *Zyzzya*. The following taxonomic revisions[165] have been suggested: The Fijian sponge *Zyzzya* cf *marsailis* and the Palauan sponge *Damiria* sp. have been reassigned to *Zyzzya fuliginosa*. The Indonesion *Histodermella* sp. is now also considered to be a species of *Zyzzya*.[165] Therefore, all the makaluvamines and damirones described to date have been isolated from sponges of the genus *Zyzzya*. An interesting case is the isolation of the tsitsikammamines from an undescribed South African latrunculid. The sponge shows morphological characteristics of the genera *Latrunculia* and *Zyzzya* as well as natural products which could be tentatively classified as intermediates between discorhabdins and makaluvamines.

Although there seem to be definite trends in the distribution of pyrroloiminoquinones in the Phylum Porifera, caution is necessary in the interpretation of these observations. The possibility that these compounds may be produced by symbiotic microorganisms cannot be excluded and thus additional research is required to address the problem. The isolation of

the pyrroloquinoline derivative, haematopodin **(245)**, a mushroom pigment from the fungus *Mycena haematopus*, further illustrates the fairly wide distribution of pyrroloiminoquinone metabolites.[179]



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One of the objectives of our study of South African latrunculid sponge chemistry was to explore the potential use of discorhabdin alkaloids as chemotaxonomic markers in the family Latrunculiidae.[177] Our investigations may be summarised as follows (Table 4.4): Discorhabdin A and H are produced by three morphologically different South African sponges which belong to the family Latrunculiidae suggesting that these sponges are genetically related. Only one sponge studied thus far does not produce these metabolites.[54] In addition to discorhabdins A and H these sponges also produce different minor metabolites. It is also interesting to note that reduction of the C-3 carbonyl moiety is also a common feature of some of the minor metabolites.

Table 4.4. Discorhabdins isolated from South African latrunculid sponges

	SAF95-058	UPES95-001	CT96-039	SAF95-006
Discorhabdin A	X	X	X	
Discorhabdin D		X		
Discorhabdin H	X	X	X	
Dihydrodiscorhabdin C		X		
Dihydrodiscorhabdin B			X	
Tsitsikammamine A				X
Tsitsikammamine B				X
14-bromodiscohabdin C				X
14-bromo-3-dihydrodiscorhabdin C				X

During our investigations we became aware of a number of minor metabolites that were present, in very small quantities, in the sponge extracts. The paucity of material hampered their isolation and structure elucidation, thus compelling us to seek an alternative method to investigate these compounds and complete our study. A preliminary study was initiated in order to explore the potential of HPLC-MS in as a sensitive tool to identify minute amounts of pyrroloiminoquinone metabolites.

Electrospray mass spectrometry (ESMS) hyphenated to HPLC is now widely used in the pharmaceutical industry for the detection and determination of drugs and their metabolites.[180] This is because many drugs contain basic nitrogen atoms that can be protonated in the electrospray process and hence are detectable by ESMS. The technique is extremely sensitive, providing detailed information regarding molecular mass and structure from very small quantities of material. ESMS relies on the production of multiply charged ions whose *m/z* values can be analysed by virtually all types of mass analysers. The "soft" ionization inherent in the ES technique means that not only can the molecular ion or its modifications (*e.g.* $[M+H]^+$, $[M-H]^-$) be monitored to reveal the molecular mass of a compound, but also non-covalent interactions between molecules in solution can be preserved in the gas phase.[180] In addition detailed structural information can also be obtained by resorting to MS-MS techniques.

Standard ESMS spectra were obtained for discorhabdin A, H, dihydrodiscorhbdin C and tsitsikammamine B. This was followed by the analysis of a crude discorhabdin mixture (from UPES95-001) by the positive ion HPLC-ESMS procedure using MeOH-H_2O (6:4) as eluant. The total ion chromatogram is shown in Figure 4.15. The mass spectra for each of the main peaks are shown in Figure 4.16 and the results tabulated in Table 4.4.

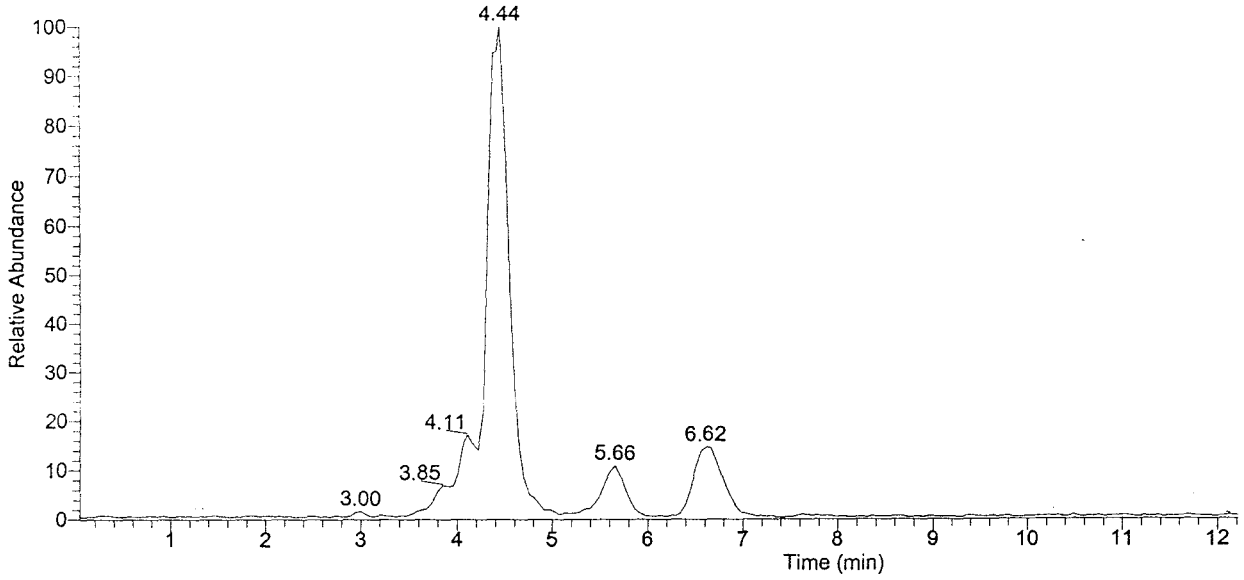


Figure 4.15 Total ion chromatogram for a crude discorhabdin mixture from UPES95-001.
Conditions: Column μ-Bondapak C_{18} (0.46 x 25 cm), Mobile phase MeOH-H_2O, (6:4, pH 3 TFA), Flow rate 1 mL min^{-1}

The above results show quite clearly the potential of this technique in screening crude extracts for discorhabdin alkaloids. The mass spectrum of the compound eluting at 4.11 minutes is consistent with discorhabdin D, but this will have to be confirmed by co-injection of a suitable standard. Unfortunately our sample of discorhabdin D, from UPES95-001, had degraded prior to the LC-MS analyses. Further work is required in order to elucidate the structures of the unknown compounds eluting at Rt 5.66 and 6.62 minutes.

We have established that this technique is suitable for the screening of crude extracts for discorhabdin-type alkaloids. Some preliminary purification of extracts may still be required, for example, desalting and crude fractionation on a Sep-Pak column. The use of gradient elution HPLC may simplify the work up procedure even further. Work is in progress to screen all the available latrunculid sponges in our collection by LC-MS.

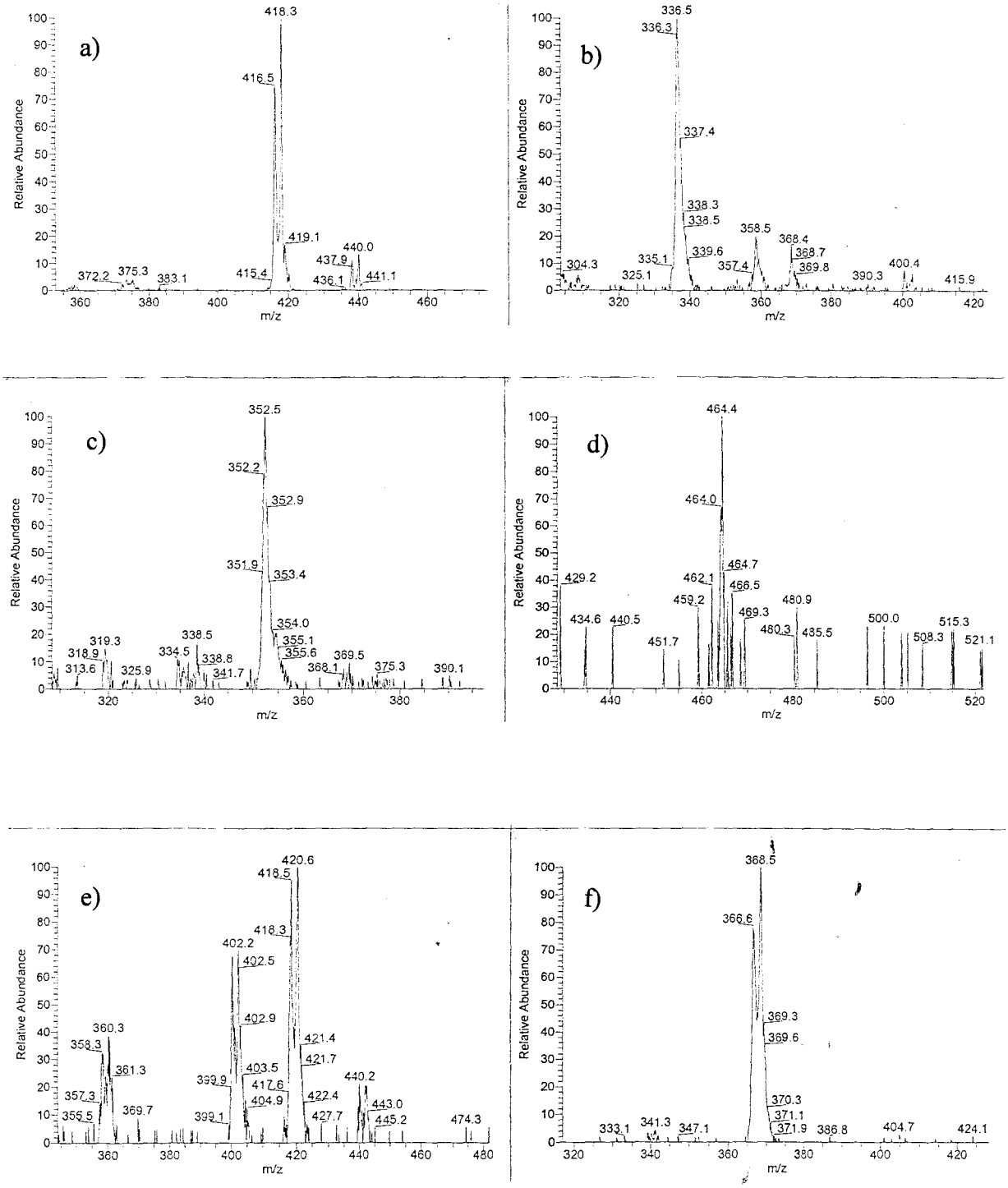


Figure 4.16 Positive ion ES mass spectra of the major peaks obtained from HPLC-ESMS. (a) Rt 4.44 min, (b) 4.11 min, (c) Rt 3.85 min, (d) Rt 4.87 min (e) Rt 5.66 min, (f) Rt 6.62 min

Table 4.5 Summary of data obtained from HPLC-ESMS

Peak	Retention time (min)	Molecular ion peaks (m/z)	Comment
1	3.85	352.5	unknown
2	4.11	336.6	discorhabdin D
3	4.44	416.5/418.3	discorhabdin A
4	5.66	400.2/402.2, 418.5/420.6	2 brominated unknowns
5	6.62	366.6/368.5	brominated alkaloid

4.3 SUMMARY AND CONCLUSION

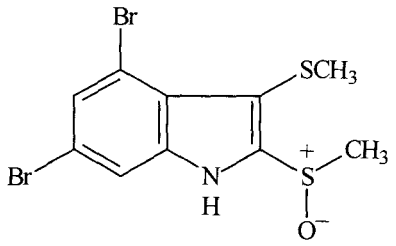
Sponges belonging to the family Latrunculiidae are producers of a growing number of pyrroloiminoquinone metabolites. The potential of South Africa latrunculid sponges in this regard has not been completely explored and the work presented here and elsewhere[54] support this impression. The three sponges studied here all produce discorhabdin A as the major metabolite as well as discorhabdin H as one of the minor compounds. Other minor metabolites isolated from these three sponges include discorhabdin D, 3-dihydrodiscorhabdin C and 3-dihydrodiscorhabdin B. We have also shown that LC-MS has great potential in studying the minor pyrroloiminoquinone metabolites present in these sponges.

Chapter Five

DILEMMAONES A-C, UNUSUAL INDOLE ALKALOIDS FROM A MIXED COLLECTION OF SOUTH AFRICAN SPONGES

5.1 INTRODUCTION

Indole alkaloids are well represented in several marine phyla with a variety of novel and biologically active metabolites having been identified.[7,181] Representative examples of marine indoles, illustrating their structural diversity, include the simple halogenated itomanindole **(245)** from the red alga, *Laurencia brongniartii*,[182] the cytotoxic and anti-migraine compound, aplysinopsin **(246)** from the sponge *Thorecta* sp.[183] the antimicrobial bisindole, fascaplysin **(247)** from the sponge *Fascaplysinopsis* sp.[184] the anti-tumour α-carboline alkaloid, grossularine 1 **(248)** from the tunicate *Dendrodoa grossularia* [185] the antiviral β-carboline alkaloid eudistomin A **(249)** from the ascidian *Eudistoma olivaceum*;[186] the carbazole alkaloid, hyellazole **(250)** from the cyanobacterium, *Hyella caespitosa*;[187] and the physostigmine alkaloid flustramine A **(251)** from the bryozoan *Flustra foliacea*.[188]



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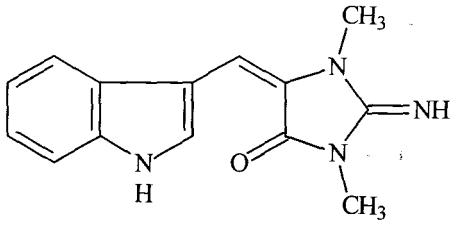

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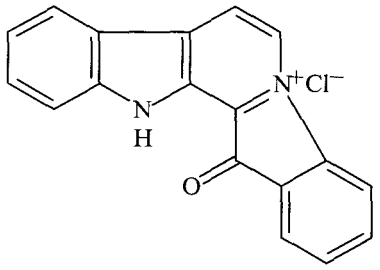

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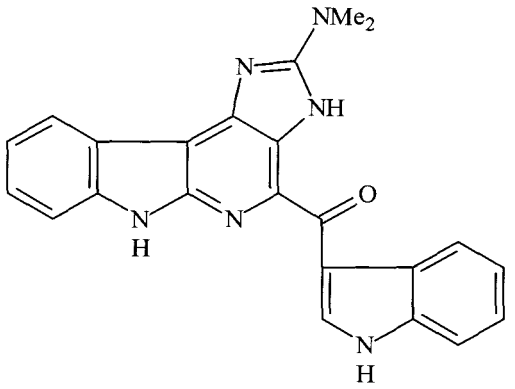

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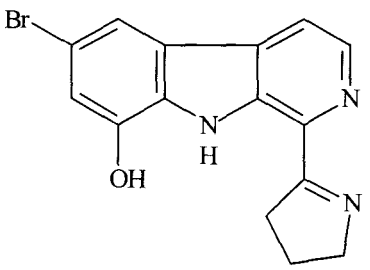

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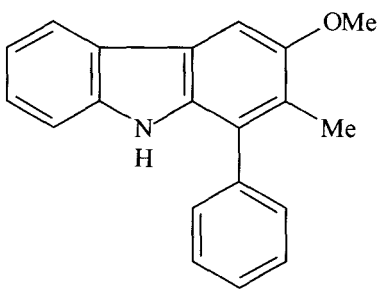

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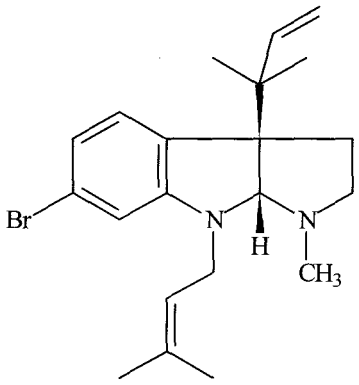

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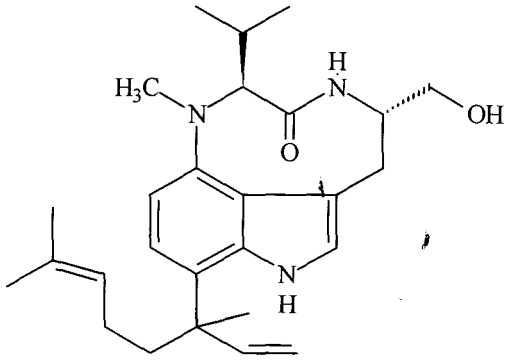
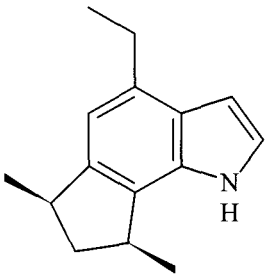


252

A common feature of many marine indoles is the incorporation of bromine into the indole skeleton, while only a few of the indole alkaloids show alkylation of the benzene ring. One of the few exceptions is lyngbyatoxin A **(252)** which contains a linalyl group attached at C-7.[189] Lyngbyatoxin, the causitive agent in "swimmer's itch", is a metabolite of the blue-green alga, *Lyngbya majuscula*. The majority of indole alkaloids, including marine indoles, are biosynthesised from the amino acid tryptophan.

The trikentrins **(253-257)** from the Australian sponge *Trikentrion flabelliforme*[190] and the herbindoles A-C **(258-260)** from an *Axinella* sp.,[191] are characterised by a cyclopentano-indole skeleton. These alkaloids, which lack a C-3 substituent, are apparently not derived from tryptophan.[191] The herbindoles showed significant feeding deterrence against generalist fishes and also cytotoxic activity against KB cells at MIC 5μg mL^{-1} for **258**, > 10μg mL^{-1} for **259** and 10 μg mL^{-1} for **260**.



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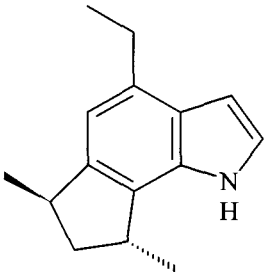

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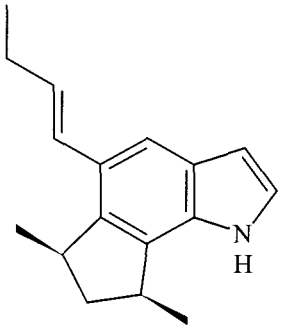

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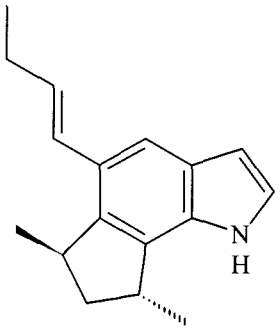

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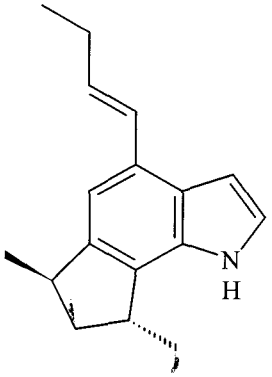

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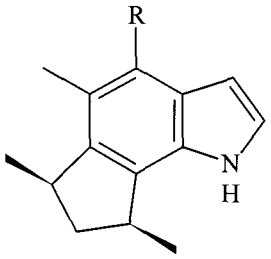
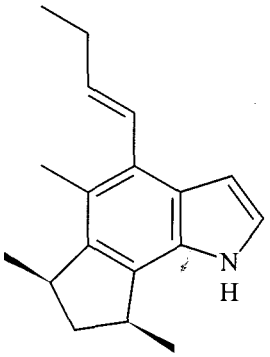


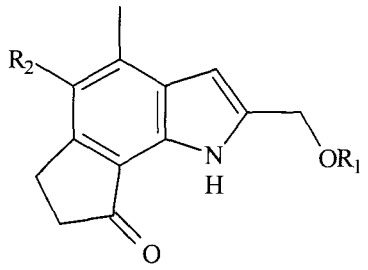
258 R = CH_3

259 R = CH_2CH_3



260

In continuation of our search for biologically active compounds from South African marine invertebrates, over one hundred and thirty specimens were collected on the South West coast of South Africa, near Cape Town. The crude voucher extracts were screened by ^{1}H NMR spectroscopy and for general cytotoxicity. From the results of this preliminary screening, sample SAF96-015 was selected for further investigation. Bioassay-guided fractionation of the crude extract led to the isolation of a cytotoxic glycosphingolipid as one of the active metabolites which was not investigated further. However, examination of the less active fractions by ^{1}H NMR spectroscopy revealed the presence of some interesting aromatic compounds which were subsequently isolated as minor metabolites. These compounds were identified as the new cyclopentanone indoles dilemmaones A-C **(261-263)**.



261 $R_1 = CH_3$, $R_2 = H$

262 $R_1 = R_2 = H$

263 $R_1 = CH_3$, $R_2 = OH$

The following sections describe the isolation and structure elucidation of dilemmaones A-C. The dilemma surrounding the sponge source of these compounds is also discussed.

5.2 RESULTS AND DISCUSSION

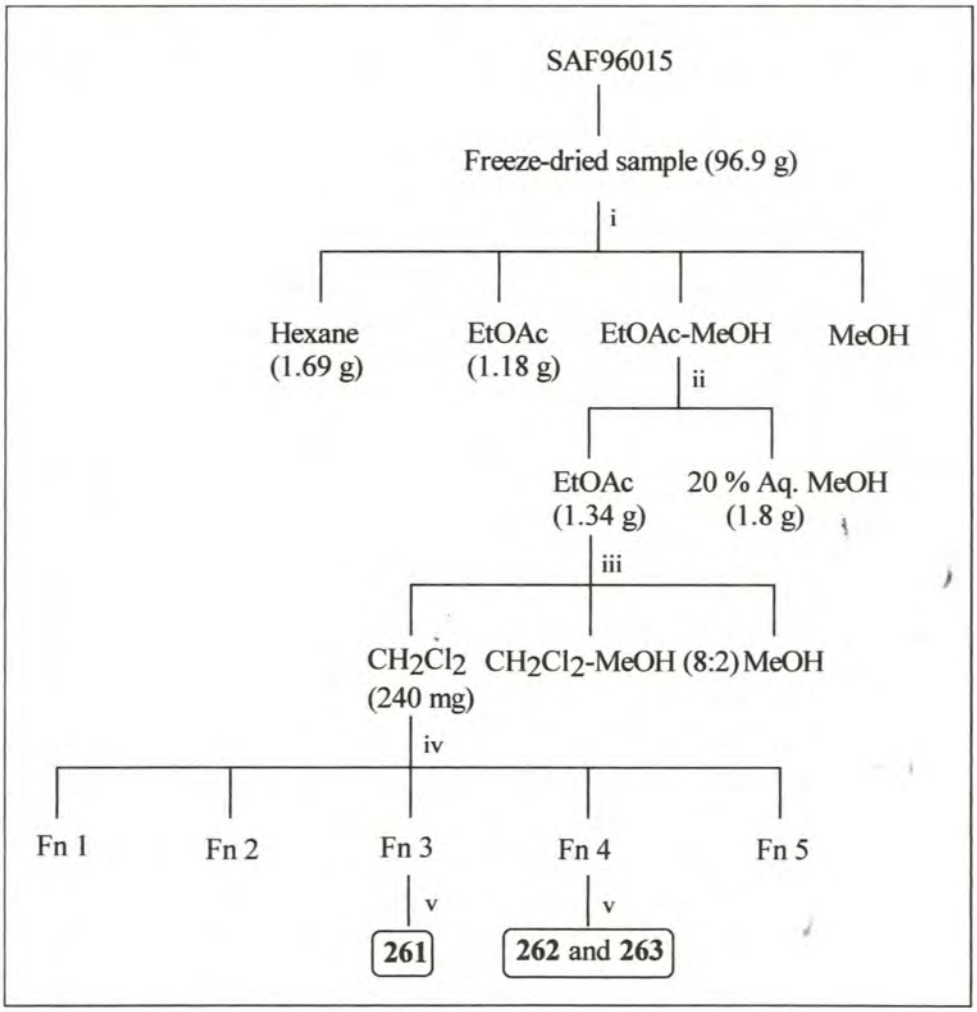
5.2.1 Collection and extraction of sponge SAF96-015 and isolation of the dilemmaones.

Specimens of the orange sponge (SAF96-015, Figure 5.1) were collected on two separate occasions by hand using SCUBA near Cape Town, South Africa, at a depth of 15 m. The

two samples were kept separate and were immediately frozen while a voucher specimen was stored in MeOH.

In the preliminary screening procedure, the MeOH extract of the voucher specimen was decanted, concentrated and partitioned between EtOAc and H_2O. Both the organic and aqueous fractions were dried and screened for cytotoxicity using the brine shrimp lethality assay. The EtOAc partition showed moderate activity (70% mortality at a concentration of 50 $\mu g\ mL^{-1}$), while the ^1H NMR spectrum showed a number of interesting aromatic signals. At this point the bulk sample was freeze-dried (96.9 g) and extracted sequentially with Hexane, EtOAc, EtOAc-MeOH (1:1) and MeOH. Interestingly, none of the crude extracts showed the prominent aromatic signals of the voucher extract and bioassay guided fractionation was used to isolate the active components. The brine shrimp assay indicated that the activity was present mainly in the EtOAc-MeOH (1:1) extract and this extract (3.14 g) was accordingly partitioned between EtOAc and H_2O. The organic fraction (1.35 g) obtained from the solvent partition was fractionated on a diol Sep-Pak cartridge (12 g, 35 mL) using a gradient of CH_2Cl_2, CH_2Cl_2-MeOH (8:2) and MeOH. Most of the activity resided in the MeOH fraction which consisted mainly of a single glycosphingolipid. The CH_2Cl_2 fraction (240 mg) exhibited interesting aromatic ^1H NMR signals (similar to those observed in the original voucher extract) and was further fractionated on silica gel using a gradient from hexane to EtOAc. Final purification was achieved by normal phase HPLC using EtOAc-Hexane (7:3) to give dilemmaones A (4.9 mg, 0.005 % dry wt), B (1.2 mg, 0.0012 % dry wt) and C (3.4 mg, 0.0035 % dry wt). The extraction and isolation procedure is outlined in Scheme 5.1.

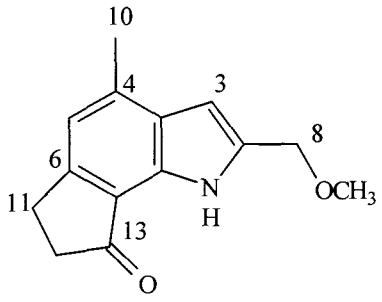
Figure 5.1 Red and orange sponges predominate on the Western Cape reefs.



Scheme 5.1 Isolation procedure for dilemmaones A-C. i) Solvent extraction; ii) Solvent partitioning; iii) Diol Sep-Pak; iv) Silica gel chromatography (Hexane-EtOAc gradient); v) Normal phase HPLC (EtOAc-Hexane, 7:3).

5.2.2 Structure elucidation of dilemmaones A-C

5.2.2.1 *Dilemmaone A*



261

Dilemmaone A, the most abundant of the minor metabolites, was obtained as an optically inactive solid (mp 146-148°C). A molecular formula of $C_{14}H_{15}NO_2$ was established for **261** from HRCIMS data. The ^{13}C NMR spectrum (Table 5.1) confirmed the presence of fourteen carbons, of which eight were aromatic. A signal at δ 206.5 in the ^{13}C NMR spectrum and an IR absorption band at 1655 cm^{-1} were consistent with a ketone moiety. The remaining three degrees of unsaturation implied by the molecular formula, could be accounted for by a tricyclic structure. An NH absorption peak at 3340 cm^{-1} in the infra red spectrum combined with a broad, exchangeable resonance at δ 9.53 in the ^1H NMR spectrum suggested the presence of a substituted indole. The ^1H NMR spectrum (Figure 5.2) also showed signals due to an aromatic methyl group (δ 2.61, 3H, s), a methoxyl group (δ 3.43, 3H, s), a downfield methylene (δ 4.60, 2H, s), two aromatic protons (δ 6.51, 1H, s and 6.98, 1H, s), and an isolated $-CH_2CH_2-$ fragment (δ 3.22, t, J = 5.5 Hz and 2.73, t, J = 5.5 Hz). A combination of HMQC, HMBC and NOESY NMR experiments were used to connect these fragments. HMBC correlations (Figure 5.3) from the methylene at δ 3.22 (H-11) to carbon signals at δ 152.9 (C-6), 120.0 (C-7), 36.7 (C-12) and 206.5 (C-13) and from the second methylene at δ 2.73 (H-12) to carbon signals at δ 152.9 (C-6), 29.5 (C-11) and 206.5 (C-13), indicated that the third ring was a cyclopentanone ring fused to the indole ring system (Figure 5.3a). The positions of the aromatic methyl, methoxymethyl and cyclopentanone ring were assigned as follows. HMBC correlations from the methylene signals at δ 4.60 (H-8) to carbons at δ 135.4 (C-2) and 100.8 (C-3) and a NOESY correlation from H-8 (δ 4.60) to the NH (δ 9.53), unambiguously assigned the

methoxymethylene group to position 2 on the indole ring (Figure 5.3b). The aromatic methyl (δ 2.61) was assigned to position 4 on the basis of HMBC correlations to carbon signals at δ 127.6 (C-3a) and 118.2 (C-5). NOESY correlations between CH_3-10 (δ 2.61) and the aromatic protons at δ 6.51 (H-3) and 6.98 (H-5) supported this assignment (Figure 5.3c). The positioning of the cyclopentanone ring followed from the presence of a NOESY crosspeak between H-11 (δ 3.22) and H-5 (δ 6.98) (Table 5.2). All the NMR data for dilemmaone A were consistent with the proposed structure.

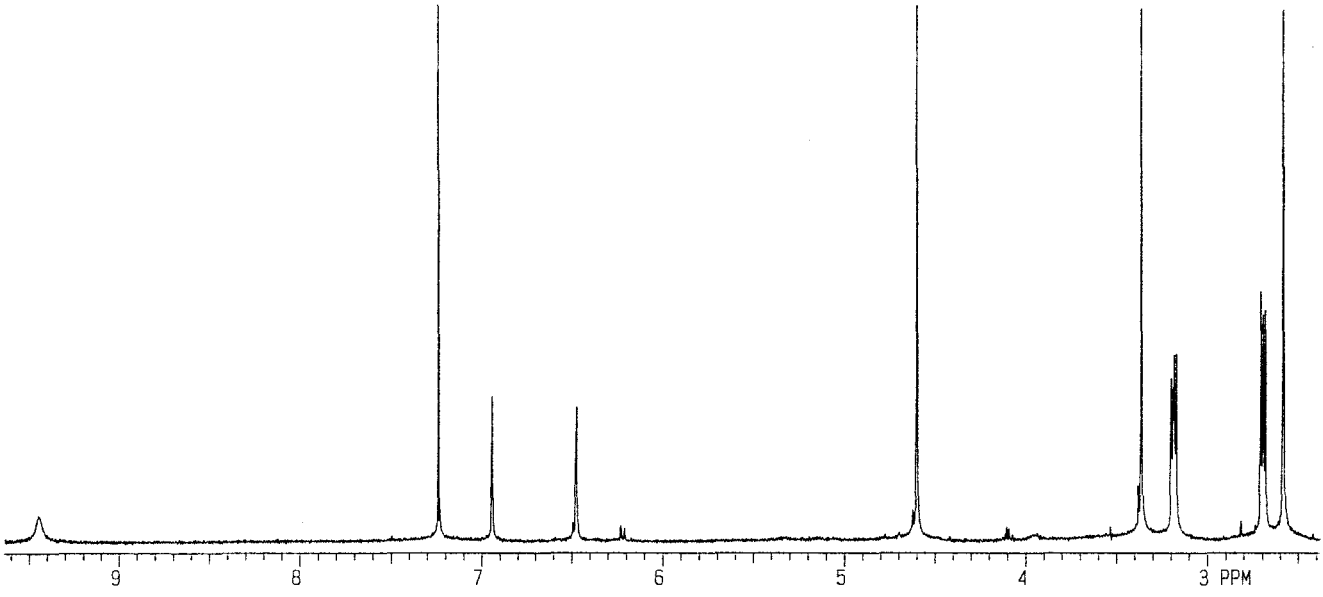


Figure 5.2 ^{1}H NMR spectrum of dilemmaone A (300 MHz, $CDCl_3$)

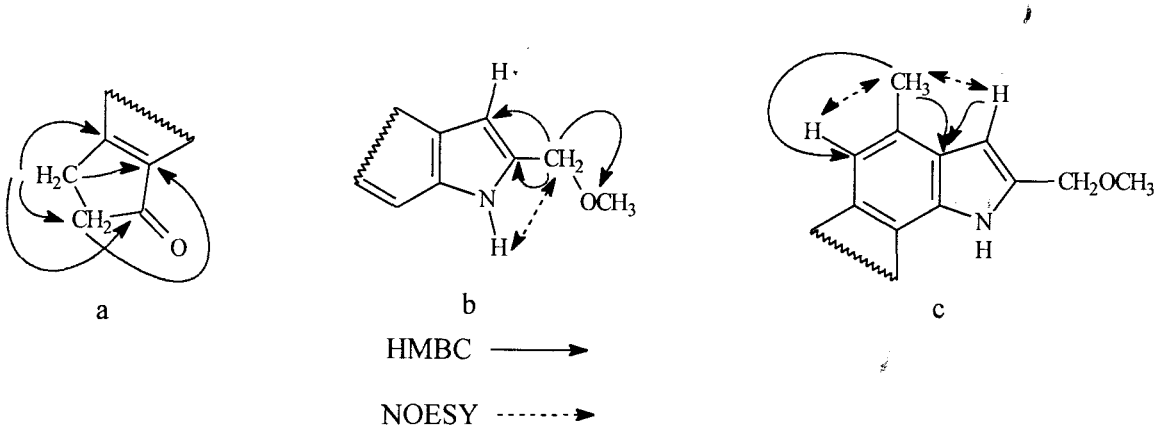


Figure 5.3 Key HMBC and NOESY correlations for dilemmaone A

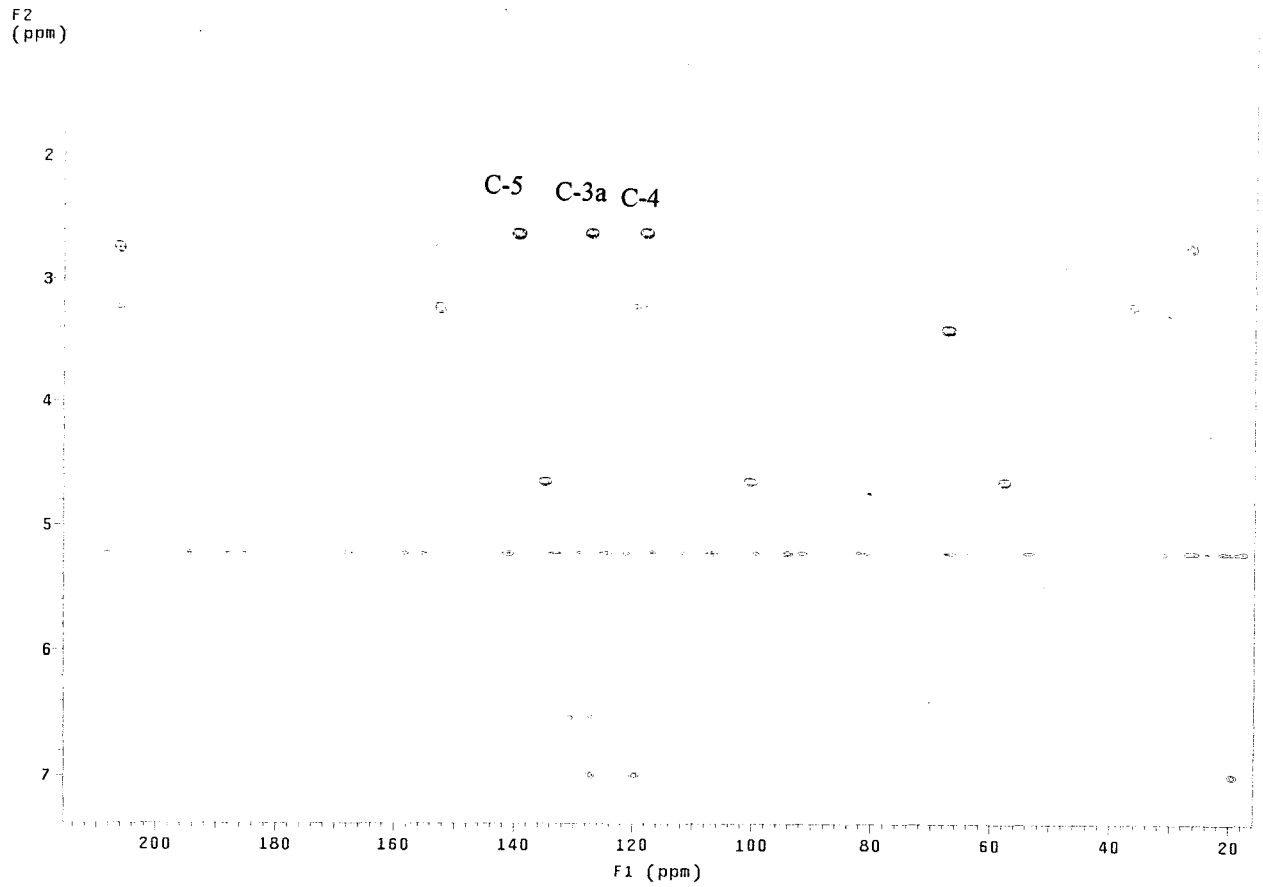


Figure 5.4 HMBC spectrum of dilemmaone A (300 MHz, CDCl3)

An alternative structure **264** (Figure 5.5), which seemed biosynthetically more plausible and which also fitted the NOESY data, was ruled out based on careful interpretation of the HMBC correlations.

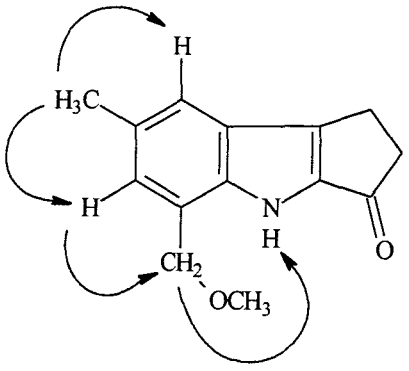


Figure 5.5 An alternative structure for dilemmaone A showing the NOESY correlations.

Table 5.1 ^{13}C NMR data for dilemmaones A-C (75 MHz).

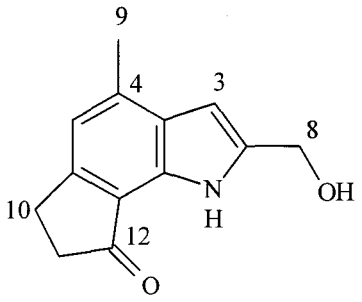
C no.	**261**	**262**	**263**
2	135.4	a	136.0
3	100.8	99.3	100.3
3a	127.6	a	126.6
4	139.7	a	139.3
5	118.2	118.3	147.8
6	152.9	a	144.1
7	120.0	a	120.0
7a	131.1	a	123.1
8	67.7	58.5	67.6
9	58.2	a	58.0
10	19.9	19.9	12.6
11	29.5	26.9	23.4
12	36.7	36.7	36.4
13	206.5	a	206.1

[a] Resonances for the quaternary carbons were not obtained due to the small sample size and long relaxation times.

Table 5.2 ^{1}H and NOESY NMR data for dilemmaone A ($CDCl_3$, 300 MHz).

C no.	δ_H, mult, J (Hz)	NOESY
3	6.51, s, 1H	H-8, H-10
5	6.98, s, 1H	H-11, H-10
8	4.60, s, 2H	H-9, H-3, NH
9	3.43, s, 3H	H-8
10	2.61, s, 3H	H-3, H-5
11	3.22, t, 5.6, 2H	H-12, H-5
12	2.73, t, 5.5, 2H	H-11
NH	9.53, br s	H-8

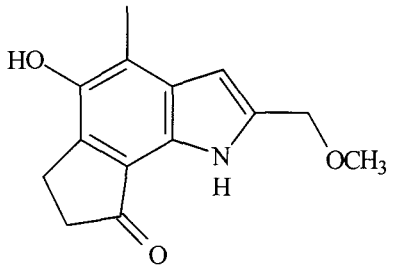
5.2.2.2 *Dilemmaone B*



262

Dilemmaone B was isolated as an unstable buff-solid (mp 168 - 170°C) and showed a $[M+H]^+$ peak at *m/z* 216.1026 in the HRCIMS spectrum corresponding to a molecular formula of $C_{13}H_{13}NO_2$. The ^1H NMR spectrum of **262** was very similar to that of **261**, but lacked the methoxyl signal suggesting that **262** was the free alcohol analogue of **261**. The downfield shift of the hydroxymethylene group from δ 4.60 to δ 4.88 in the ^1H NMR spectrum of **262** is consistent with the loss of a methyl group.[106] Because of the small amount of material isolated and its instability, complete NMR data could not be obtained for dilemmaone B. Relaxation times for the quaternary carbons were of the order of 5 seconds which made the acquisition times for ^{13}C NMR spectra on such a small amount of material prohibitively long. All the spectral data were consistent with the proposed structure for **262**.

5.2.2.3 *Dilemmaone C*

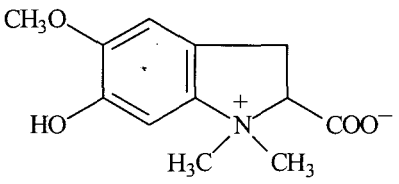


263

Dilemmaone C was isolated as a pale yellow solid (mp 187- 190°C) and showed a molecular ion peak at *m/z* 246.1130 in the HRCIMS spectrum which was consistent with a

molecular formula of $C_{14}H_{15}NO_3$. Again, the ^1H NMR spectrum of **263** was similar to that of **261** except that it lacked one aromatic proton resonance at δ 6.98 (H-5) suggesting an additional aromatic substituent. This, together with a signal at 3340 cm^{-1} in the infra red spectrum of **263**, suggested a hydroxyl substituent at position 5. The downfield shift of the C-5 and the upfield shift of the C-6 and C-4 carbon resonances (Table 5.1) are consistent with a hydroxyl substituent at position 5 when compared to **261**.[106] The remaining spectral data were fully consistent with the proposed structure for dilemmaone C.

The dilemmaones are related to the trikentrins and herbindoles, but are quite unusual with respect to the substitution of the aromatic ring as well as the hydroxymethylene and methoxymethylene groups situated at C-2. The methoxyl group in dilemmaone A and C may have arisen from the use of methanol in the extraction procedure. The biosynthesis of these compounds is uncertain because, like the trikentrins and herbindoles, they do not seem to be derived from tryptophan. Most marine indoles may be divided into simple and tryptophan-derived indoles, the biogenesis of which may involve either phenylalanine or tryptophan precursors. It is possible that the dilemmaones may arise from a phenylalanine precursor which gives rise to the methoxmethylene group at C-2. A simple indole **265**, apparently derived from phenyl alanine and bearing a C-2 carboxyl group, has been isolated from the Caribbean sponge *Pseudoceratina crassa*.[192]



265

5.2.3 Sponge dilemma

In order to isolate more material for further characterisation and biomedical screening, the second collection of SAF96-015 was extracted. It was immediately apparent that the ^1H NMR spectrum of the crude extract of the second collection was different to the ^1H NMR spectrum of the first collection. Careful chromatography using a similar procedure to that

used for the first collection did not afford any dilemmaones, introducing uncertainty as to the sponge origin of these compounds. With the help of sponge taxonomists, Dr Kelly-Borges and Ms Harper, the two collections were examined and the following concluded:

1. The first collection (SAF96-015-1), which yielded the dilemmaones, consisted of a mixture of three similar sponges. These were identified as (A) *Ectyonanchora flabellata* Levi (47% dry wt), (B) *Crambe chelastra* Levi (41% dry wt) and (C) an undescribed *Antho* sp (12% dry wt).
2. The second collection only contained the *Antho* sp. (subgenus Dirrhopalum, order Poecilosclerida, family Microcionidae)
3. The voucher specimen contained the *Antho* sp. only.

The dilemmaones do not resemble the known metabolites from *Crambe* species[193] and a comparison of the ^{1}H NMR spectra of the crude voucher extract and the pure dilemmaones (Figure 5.6) suggested that these compounds may be produced by the undescribed *Antho* sp. This obviously needs to be confirmed because the second collection did not contain any dilemmaones. A possible explanation is that the two collections were made on different days at different sites. The possibility that the dilemmaones may be produced by a contaminating microorganism cannot be ruled out at this stage and recollection of the sponges should be made in order to address the problem of the sponge source. This dilemma highlights the importance of careful sorting of sponge specimens in the field and the value, if possible, of having qualified taxonomists in the field when marine invertebrate samples are collected.

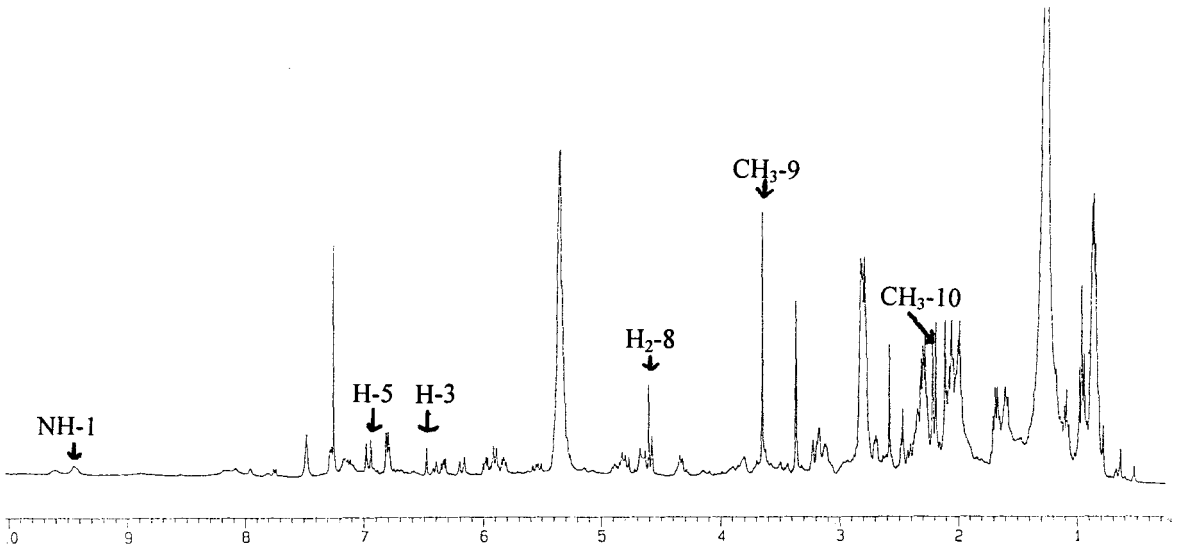


Figure 5.6 ^{1}H NMR spectrum of the crude voucher extract of SAF95-015 (400 MHz, $CDCl_3$).

5.3 SUMMARY AND CONCLUSION

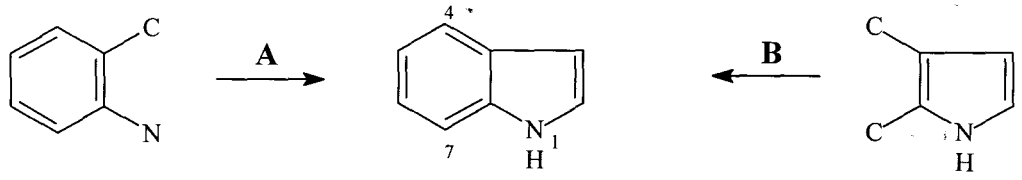
Dilemmaones A-C **(261-263)** were isolated in trace amounts by bioassay guided fractionation of an extract obtained from a mixed collection of sponges. This is the first report of cyclopentanone-indole alkaloids from the marine environment. Further work is required in order to determine the sponge source of these compounds.

Chapter Six

STUDIES TOWARDS THE TOTAL SYNTHESIS OF THE DILEMMAONES

6.1 INTRODUCTION

Many indolic secondary metabolites exhibit potent physiological effects, for example, LSD, reserpine, tryptophan, serotonin, indole acetic acid. Accordingly, the synthesis of indole alkaloids has long been a topic of interest to organic and medicinal chemists. The indole ring system is usually synthesised by an intramolecular ring-closure of either a mono-substituted or *ortho*-substituted benzene precursor.[194] Many classical organic syntheses of indoles such as the Fischer, Bischler, Madelung and the more recent Hemetsberger procedures fall into this category.[194-196] Using these procedures functional groups at the 2- and 3-positions are readily introduced on cyclisation; however, any substituents at positions 4 to 7 must be established in the arene precursor (Scheme 6.1 A). Less common are approaches to indoles that originate with a pyrrole and build up to the carbocycle (Scheme 6.1 B). This procedure offers versatility in the substitution of the carbocycle but suffers from difficulties associated with oxidation of the tetrahydroindole and the tendency of vinylpyrroles to polymerise.

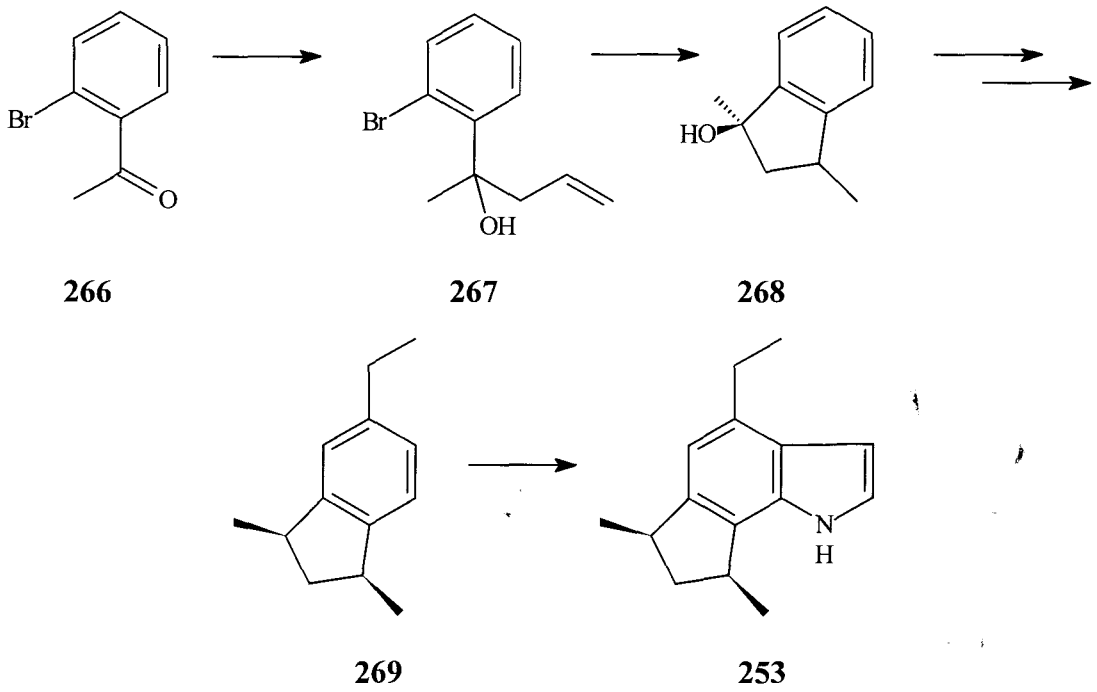


Scheme 6.1 Two general approaches to the synthesis of indoles.

The trikentrins, herbindoles and dilemmaones are characterised by a fused cyclopentane ring at C-6 and C-7 and a lack of substitution at C-3 as well as varying alkyl substitution at C-4 and C-5. A major challenge in the synthesis of these compounds is the regioselective introduction of the functionalities to obtain the highly alkyl-substituted aromatic system. Several syntheses have been reported of the trikentrins and herbindoles and are briefly

outlined below with emphasis given to the introduction of the alkyl substituents on the benzene rings of these two groups of compounds.

The first synthesis of the trikentrin skeleton was reported by Macleod *et al.*[197-199] who synthesised (±)-*cis*-trikentrin A **253** from bromoacetaphenone **(266)**. A key feature of their synthesis is the use of an aryl radical cyclisation of intermediate **(267)** to form the substituted indane system **(268)**. The C-4 substituent was introduced *via* Friedel-Crafts acylation of the aromatic ring followed by reduction and dehydroxylation to give **(269)**. The indole ring was formed by formylation of the benzene ring in **269** followed by Hemetsberger's indole synthesis. Key features of this synthesis are presented in Scheme 6.2.



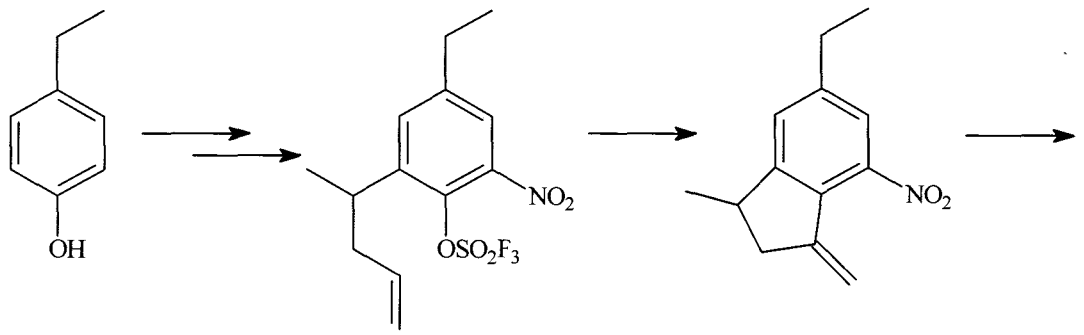
266 **267** **268**

269 **253**

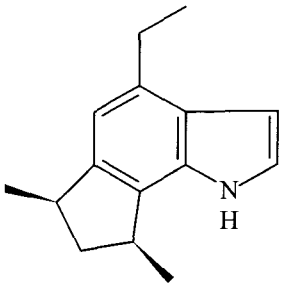
Scheme 6.2 Macleod's synthesis of (±)-*cis*-trikentrin A employing an aryl radical cyclisation reaction.[197-199]

Wiedenau[200] used an intramolecular Heck coupling of the triflate **(270)** to form the five membered ring of the indane system in *cis*-trikentrin A. This strategy meant that the C-4 substituent was already present in the starting material and would not have to be added at a later stage. Compound **270** was formed by acetylation of 4-ethyl phenol **(271)**, followed by

Fries rearrangement and condensation with allylmagnesium chloride and subsequent dehydroxylation. The indane ring system followed from an intramolecular Heck reaction catalysed by palladium(0). Formation of the indole ring in intermediate **(272)** was achieved *via* the Bartoli procedure (Scheme 6.3).



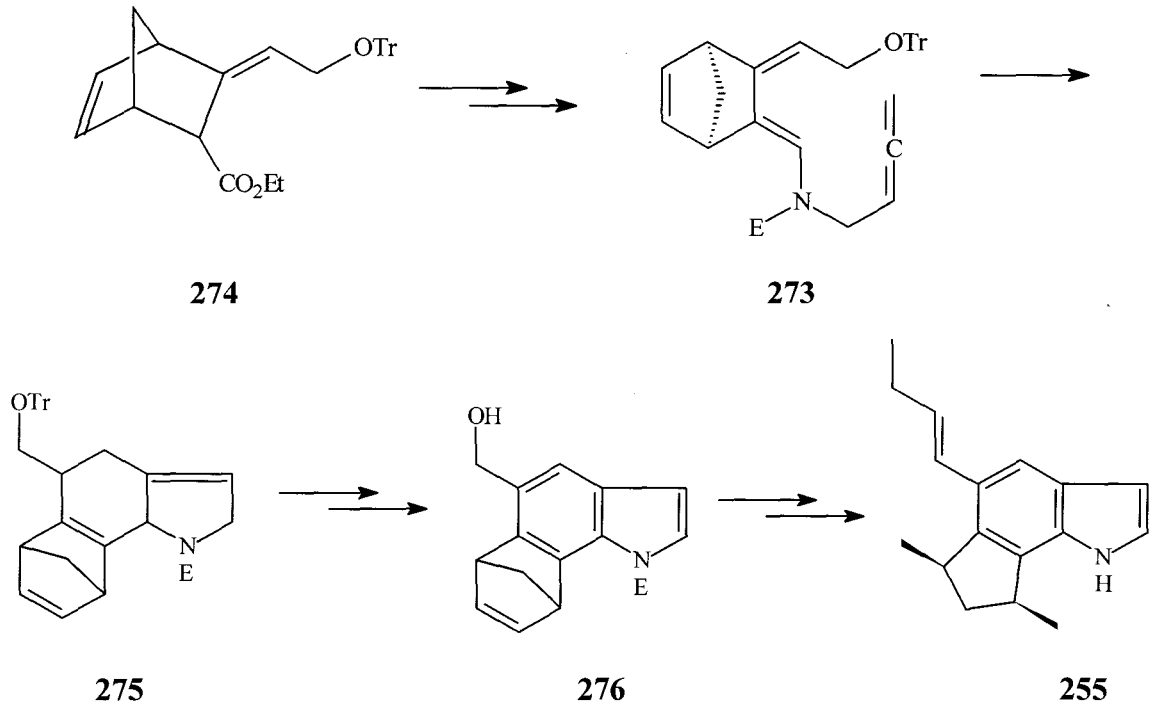
271 **270** **272**



253

Scheme 6.3 Wiedenau's synthesis of (±)-*cis*-trikentrin A *via* a intramolecular Heck coupling of a triflate.[200]

cis-Trikentrin B was synthesised using an intramolecular Diels-Alder reaction of 1,2,3-trisubstituted allenic dienamide **(273)**.[201,202] The C-6 substituent was already in place in the starting material **(274)** and the cyclopentane ring was masked in the bicyclic ring system of **273**. The dienamide **273** was subjected to an intramolecular Diels-Alder reaction followed by deprotection and aromatisation of the intermediate **(275)** to give compound **(276)**. Oxidative cleavage of the non-conjugated double bond in **276** and subsequent Wittig olefination of the oxidised side chain gave racemic *cis*-trikentrin B (Scheme 6.4).



274 **273**

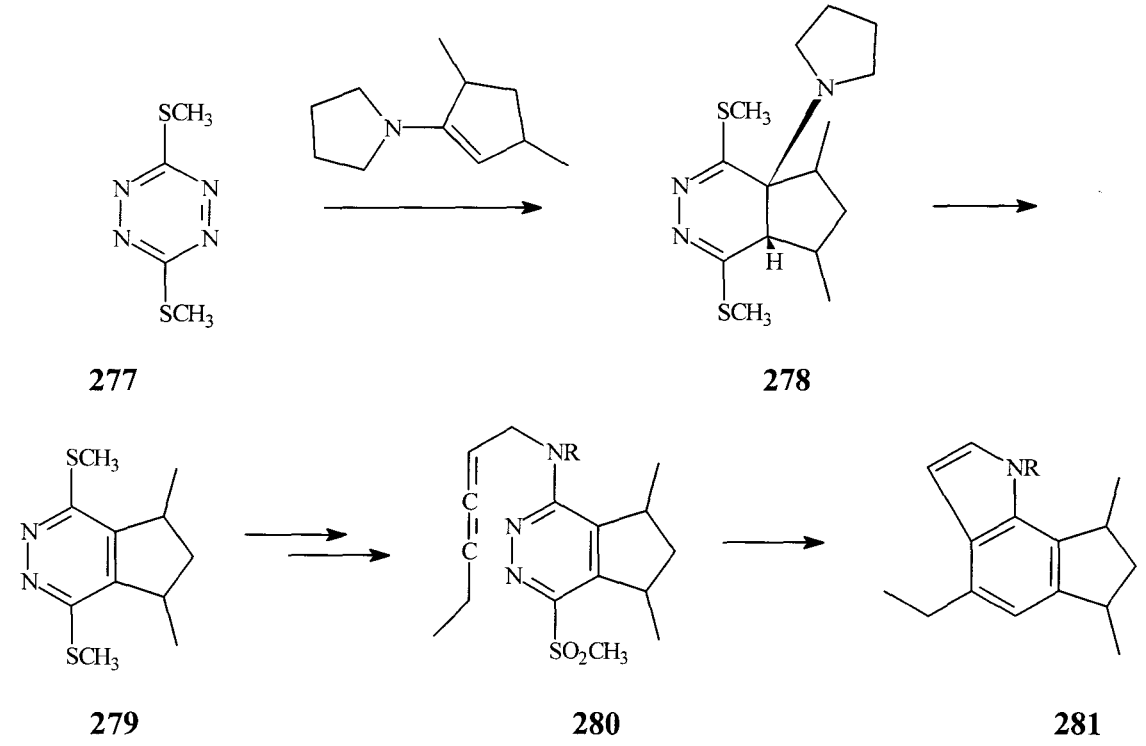
275 **276** **255**

Scheme 6.4 Synthesis of (±)-*cis*-trikentrin B *via* an allene intramolecular cycloaddition.

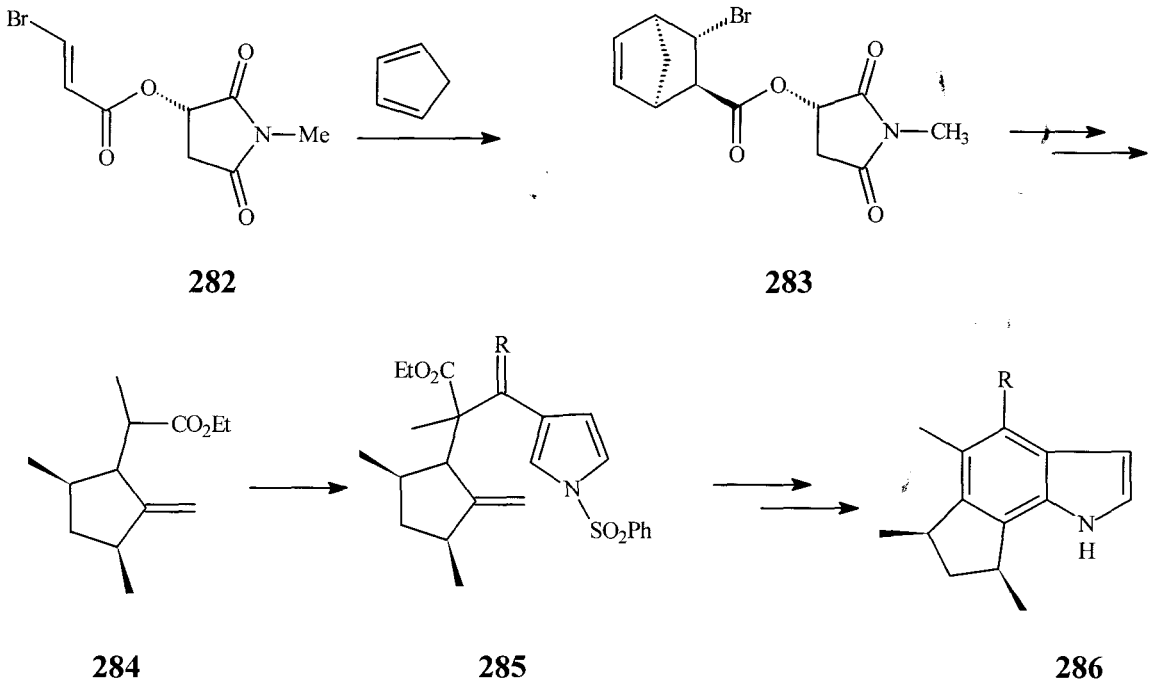
Boger *et al.*[203] reported a five step total synthesis (Scheme 6.5) of racemic *cis*- and *trans*-trikentrin A. Their approach featured two sequential heteroaromatic azadiene Diels-Alder reactions. The second Diels-Alder reaction is analogous to that used by Yasukouchi[201] and Lee[202] in their syntheses of *cis*-trikentrin A. The sequence starts with an intermolecular heteroaromatic Diels-Alder reaction of the tetrazine **(277)** and the pyrrolidine enamine of 2,4-dimethylcyclopentanone to give compound **(278)**. Loss of the pyrrolidine gave intermediate **(279)** and was followed by selective displacement of a single sulphone by 1-aminohexa-2,3-diene to give the advanced intermediate **(280)** which cyclised *via* an intramolecular Diels-Alder reaction to give **(281)**.

Muratake *et al.*[204-210] published a number of reports in which they describe an acid-induced indole cyclisation procedure of 2- and 3-substituted pyrrole derivatives in their syntheses of the trikentrins and the herbindoles. Diels-Alder coupling of compound **(282)** with cyclopentadiene gave the [2.2.1]-bicycloheptene system **(283)**. The *cis*-dimethylcyclopentenyl unit **(284)** was synthesised from **283** in several steps and condensed with 3-formyl-1-(phenylsulfonyl)-pyrrole to give the 3-substituted pyrrole derivative **(285)**. Acid-catalysed cyclisation of **285** gave the tricyclic system **(286)**. The use of the

phenylsulfonylmethyl group as the C-4 substituent facilitated the introduction of various substituents at this position.

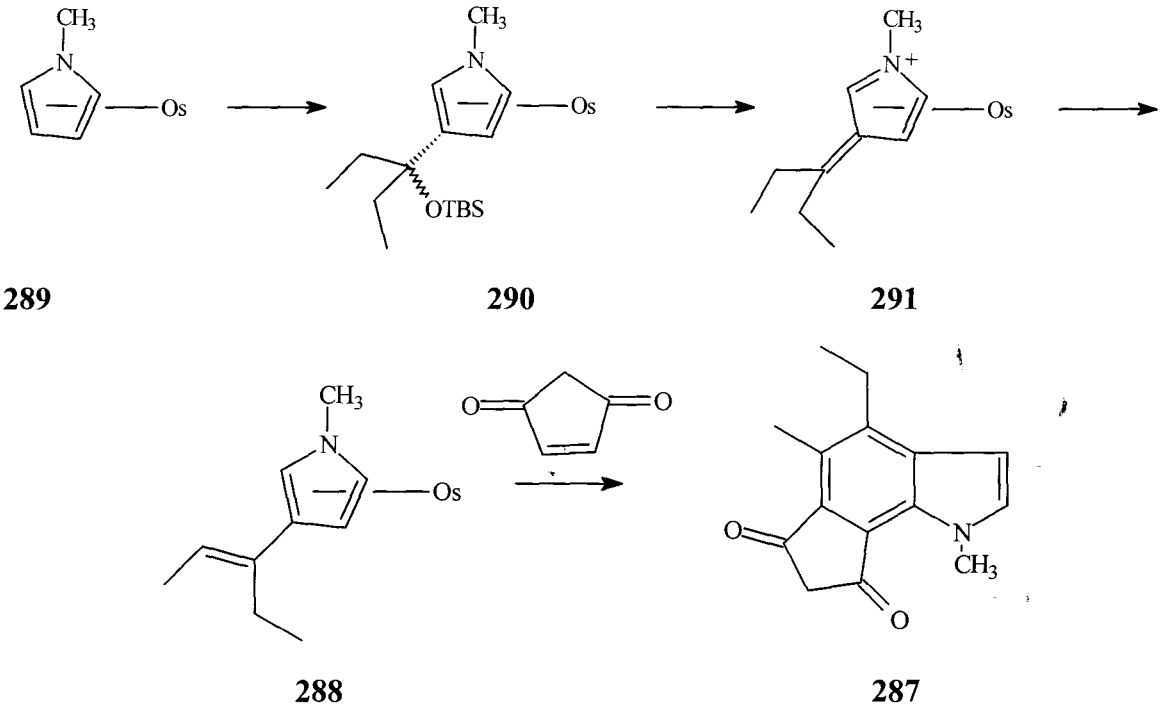


Scheme 6.5 Synthesis of (±)-*cis*- and (±)-*trans*-trikentrin A *via* a heteroaromatic azadiene Diels-Alder reaction.



Scheme 6.6 Synthesis of alkyl-substituted indoles by acid catalysed cyclisation of 1-phenyl-sulfonyl-2-pyrrolyl derivatives.

A further procedure that may be useful in the synthesis of highly substituted indoles has been reported by Hodges *et al.*[21] The indole **(287)** was synthesised in low yield (14%) *via* a Diels-Alder reaction of the β-vinylpyrrole complex **(288)** with 4-cyclopentene-1,3-dione (Scheme 6.7). Compound **288** was constructed from the N-methylpyrrole/osmium complex **(289)** *via* intermediates **(290)** and **(291)**. The pentaamineosmium(II) moiety dearomatised and activated the pyrrole ring toward electrophilic addition at the β-carbon. The β-vinyl complexes showed significantly greater reactivity toward dienophiles than their uncomplexed conterparts. Substitution at C-4 and C-5 was established by the appropriate choice of ketone, alkyne, acetal, or anhydride which led to the corresponding vinylpyrrole. This procedure is limited in that indoles, without substitution on the nitrogen, have only been synthesised in low yield. A further disadvantage is that electron-withdrawing groups deactivate the vinylpyrrole toward Diels-Alder reactions.



Scheme 6.7 Conversion of vinylpyrrole complexes to highly substituted indoles.[211]

The dilemmaones A-C **261-263** were isolated in trace amounts from a mixed collection of sponges, and in order to explore the biological activities of these unique compounds additional quantities were required. The source sponge for compounds **261-263** has not been unambiguously established and therefore recollection of the sponge(s) and isolation

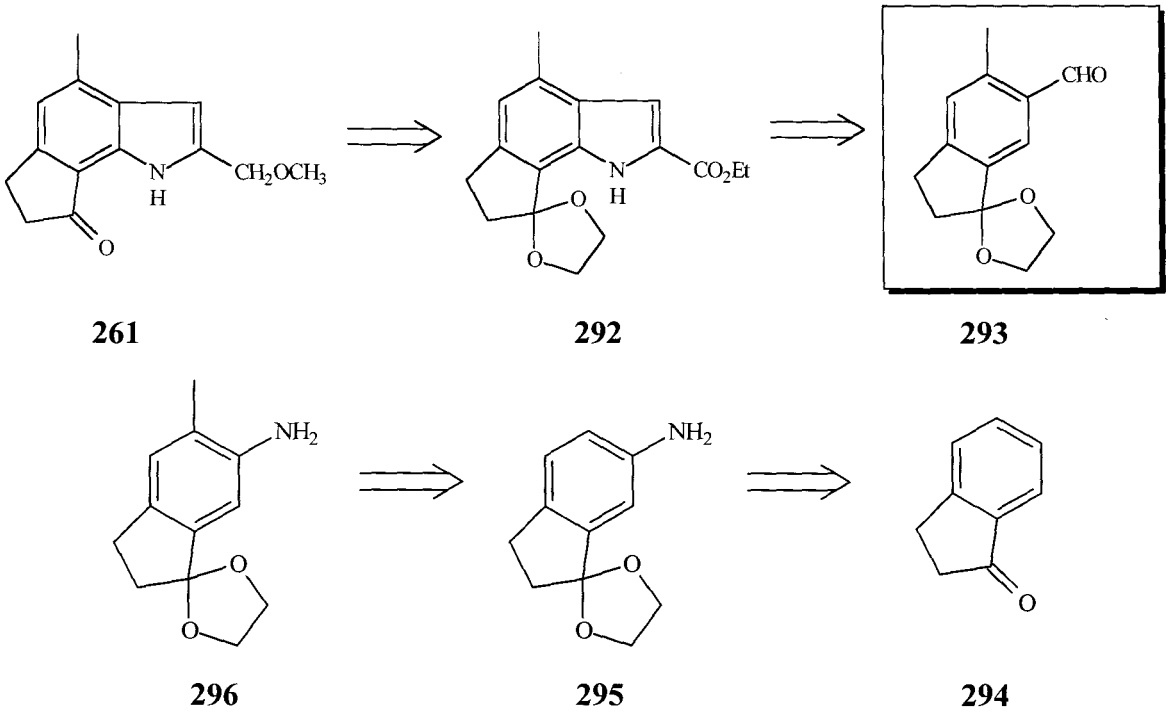
of sufficient quantities of the dilemmaones was problematic. This encouraged us to explore the feasibility of a total synthesis of these compounds. The following sections describe our synthetic approaches to the synthesis of the dilemmaones.

6.2 RESULTS AND DISCUSSION

6.2.1 Retrosynthetic analysis of dilemmaone A

In section 6.1 the syntheses of the trikentrins and herbindoles, which are structurally related to the dilemmaones were reviewed. Given the oxygenation at C-13 and substitution at C-2 and C-4 in the dilemmaones, these syntheses could not be adapted to the synthesis of **261-263** and another strategy was required. While a number of approaches to the dilemmaones may be possible the disconnection outlined in Scheme 6.8 provided us with, what seemed to be, the most feasible route to **261-263**.

LAH reduction of the precursor **(292)** followed by deprotection and methylation would yield **261**. The 2-substituted pyrrole ring in **292** may be constructed *via* application of Hemetsberger's indolisation procedure[212-214] to aldehyde **(293)** thus simplifying the synthesis of **261** to the preparation of this key intermediate (Scheme 6.8). A reasonable starting material for the synthesis of **293** is indan-1-one **(294)**, which on nitration, carbonyl protection and reduction of the nitro-group should furnish the amine **(295)**. A key step in our synthetic plan was the regioselective *ortho*-alkylation of amine **295** to the intermediate **(296)**. We envisaged achieving this by employing a novel and a relatively under-utilised procedure developed by Gassman *et al.*[215] for the *ortho*-alkylation of substituted anilines. Standard functional group conversion of the amino group in **296** to an aldehyde should then give the key intermediate **293**.



261 **292** **293**

296 **295** **294**

Scheme 6.8 Retrosynthetic analysis of dilemmaone A.

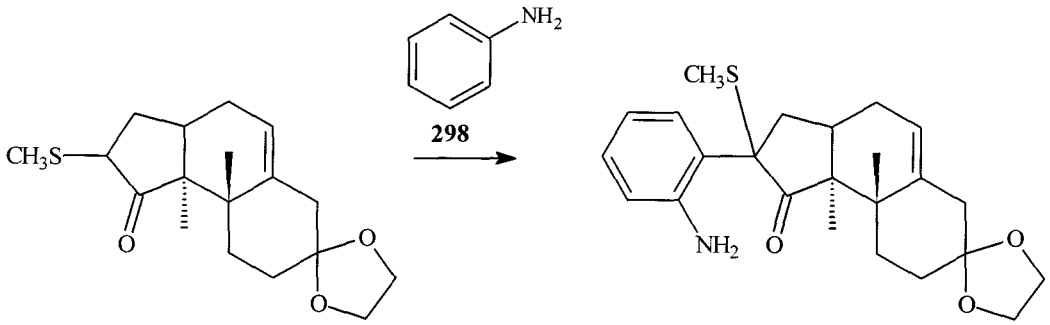
6.2.2 Gassman's alkylation procedure

Early in our synthesis we recognised the key importance of the Gassman's alkylation procedure and a brief discussion of this procedure is presented here.

The potential utility of Gassman's alkylation procedure in the alkylation of the indanone ring system attracted our attention. Surprisingly, this reaction has not been widely applied to natural product synthesis with only a handful of examples reported in the literature.[216-217] These examples include the crucial coupling of **(297)** with aniline **(298)** to give intermediate **(299)** (Scheme 6.9) in the total synthesis of the alkaloids paspalicine and pasplainine.[216]

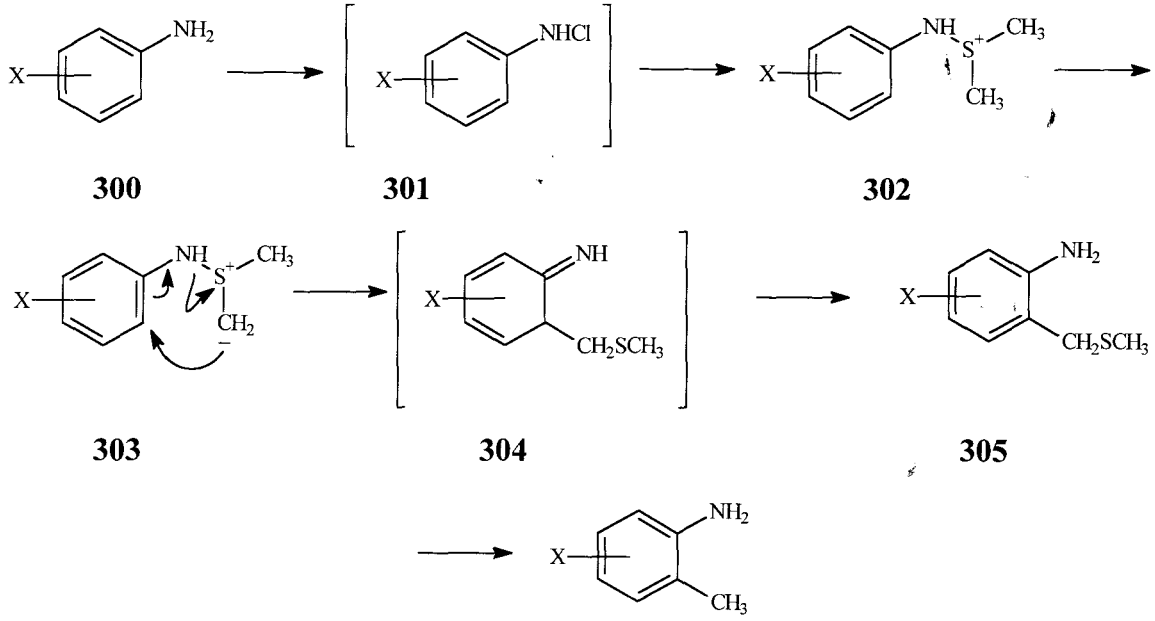
Gassman *et al.* published a series of reports in which they described a procedure for the *ortho*-alkylation of anilines and phenols and subsequently extended this reaction to the preparation of indoles.[215,218-224] This reaction is based on a Sommelet-Hauser type 2,3-sigmatropic rearrangement of an azasulfonium salt. The general procedure (Scheme 6.10) involves: (a) mono-*N*-chlorination of a substituted aniline **(300)** with a suitable

halogenating agent (*eg tert*-butylhypochlorite), (b) conversion of the *N*-chloroaniline **(301)** into an azasulfonium salt **(302)** through reaction with a dialkyl sulfide, (c) treatment of the azasulfonium salt with base to yield an azasulfonium ylide **(303)**, (d) Sommelet-Hauser type rearrangement of the ylide **303** to produce a substituted dienone imine **(304)**, and (e) hydrogen transfer and accompanying rearomatisation of the dienone imine to give the *ortho*-alkyl-α-thioalkoxy substituted aniline **(305)**. An advantage of Gassman's *ortho*-alkylation is that it is a one pot procedure which does not require isolation of the intermediate azasulfonium. Finally, Raney-nickel desulfurization would then produce the *ortho*-alkylated product **(306)**.



297 **299**

Scheme 6.9 Coupling of **297** with aniline *via* Gassman's procedure.



300 **301** **302**

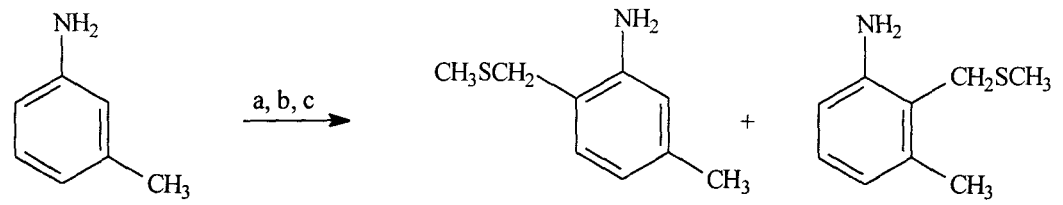
303 **304** **305**

306

Scheme 6.10 Mechanism of Gassman's *ortho*-alkylation procedure.

6.2.3 Model studies

In order to optimise the reaction conditions, Gassman's procedure was first explored using the simple model compound, *meta*-toluidine **(307)**. Treatment of a mixture of **307** and dimethyl sulfide (3 eq) in methylene chloride at -65°C with *tert*-butyl hypochlorite (1.2 eq) followed by sodium methoxide (1.2 eq) afforded a 1:1 mixture of *ortho*-isomers **(308)** and **(309)** (Scheme 6.11). The ^{1}H NMR spectrum of the mixture (Figure 6.1) clearly showed the Ar$\underline{CH_2}$S- and $\underline{CH_3}$S- signals.



307 R = CH_3 **308** **309**

Scheme 6.11 Model study: Gassman's alkylation of *meta*-toluidine

Reagents and conditions: (a) Bu^{t}OCl / CH_2Cl_2, (b) CH_3SCH_3, (c) NaOMe / MeOH

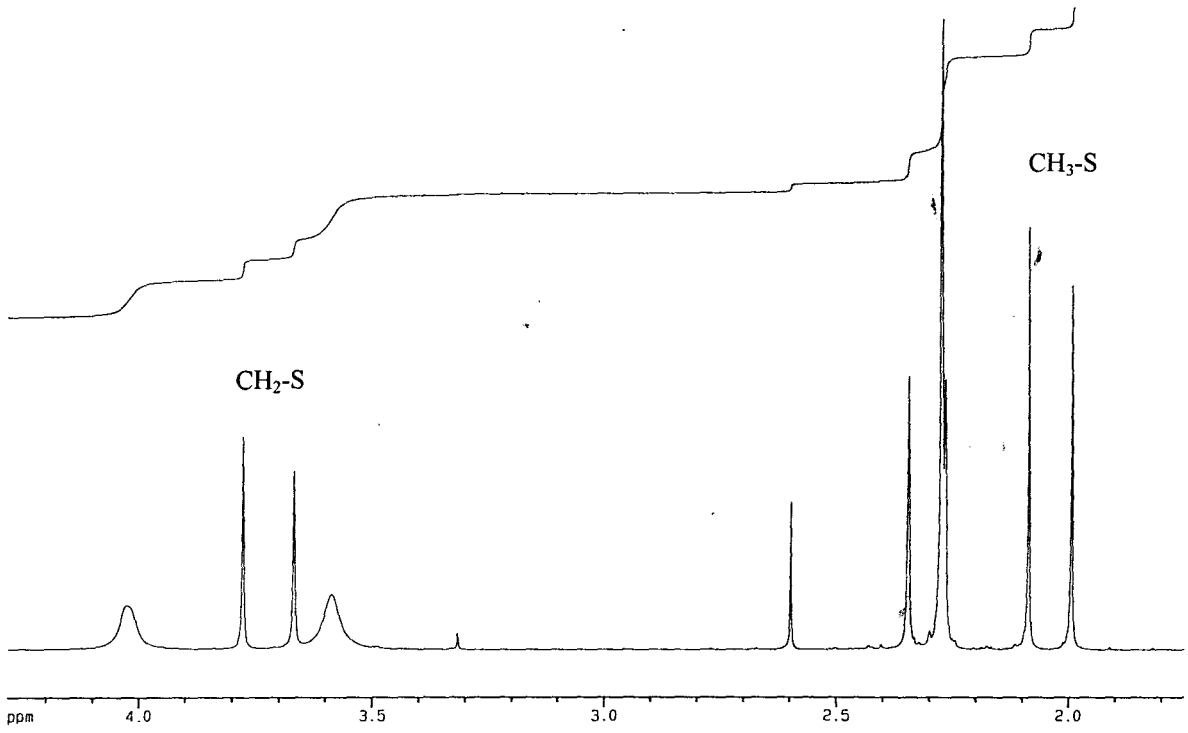


Figure 6.1 Upfield region of the ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the crude product mixture of **308** and **309**.

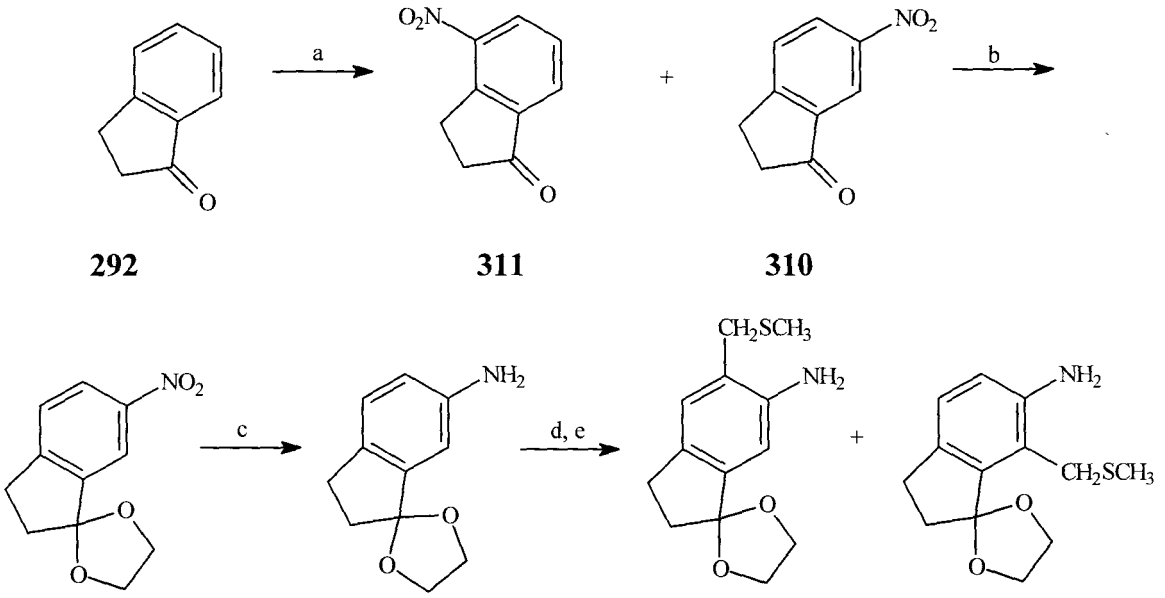
Having established the experimental protocol for Gassman's reaction in our laboratory we were ready to attempt the synthesis of the dilemmaones. The following section outlines an approach to dilemmaone A based on the retrosynthesis outlined in Scheme 6.8.

6.2.4 Attempted synthesis of dilemmaone A from indanone

The simplest procedure for the preparation of 6-aminoindanone derivatives was anticipated to be *via* a nitration/reduction route. The synthesis of the 6-aminoindan-1-one derivative **314** is illustrated in Scheme 6.12. Unfortunately, the nitration of indanone is complicated by the fact that complex mixtures are often produced in the presence of concentrated acids, while conversely, nitration does not take place very readily when dilute acids are used.[225-227] In our hands 6-nitro-indanone **(310)** was efficiently prepared by nitration of indanone with fuming nitric acid in concentrated sulfuric acid at 0°C. Maintaining the reaction temperature below 15°C was critical since higher temperatures led to the formation of an intractable gum. This reaction was conveniently performed on a 1-2 g scale as attempts at scaling the reaction up to 10 g again yielded an intractable gum. The 4- and 6-nitro-indanone derivatives **(311)** and **310** were formed in a 1:4 ratio and were easily separated by silica gel flash chromatography. Protection of the carbonyl group as the ketal was achieved by refluxing **310** with ethylene glycol and *para*-toluene sulfonic acid (PTSA) in dry benzene to provide **(312)** in 75% yield after purification. Hydrogenation of **312** over PtO_2 gave the amine **295** in quantitative yield.

Once we had the 6-aminoindanone derivative **295** in hand we were in a position to attempt the Gassman's procedure. Compound **295** was dissolved in dry dichloromethane, cooled to -65°C and treated with *tert*-butylhypochlorite and dimethylsulfide followed by reaction with a sodium methoxide solution. A mixture of rearranged products, **(313)** and **(314)**, was obtained in 25% yield (not optimised) after purification (Scheme 6.12). The ^{1}H NMR spectrum of the crude mixture clearly showed the two isomers, which were recognised by the splitting pattern in the aromatic region (a pair of singlets versus a pair of doublets), in a 27:73 ratio for the 5- and 7-substituted isomers, **313** and **314**, respectively. These two compounds were purified by normal phase HPLC and their ^{1}H NMR spectra are presented

in Figure 6.2. Unfortunately, the required regioisomer **314** was the minor product which necessitated a review of this approach to the dilemmaones.



292 **311** **310**

312 **295** **313** **314**

Scheme 6.12 Synthesis of aminoketal **295** and its alkylation by Gassman's procedure.

Reagents and conditions: (a) HNO_3/H_2SO_4, 86 %; (b) $HOCH_2CH_2OH$, TsOH, PhH, 76 %; (c) PtO_2/H_2, EtOH, 100 %; (d) Bu^tOCl/CH_2Cl_2, CH_3SCH_3; (e) NaOMe/MeOH

It is intriguing that the more hindered site is preferred for substitution. Gassman *et al.*[220,221] reported that even though no charge is built up on the ring, electronic and steric effects may play a role in the reaction of *meta*-substituted anilines. Electron-withdrawing groups appear to favour substitution *ortho* to the *meta*-substituent, while electron-donating groups in the *meta* position direct cyclisation to the position *para* to the substituent. This was illustrated in the literature by the application of Gassman's procedure to the synthesis of the 2-substituted indole **(315)** from meta-nitroaniline **(316)** *via* intermediate **(317)**[220] (Scheme 6.13). In this case substitution only occurred *ortho* to the nitro group.

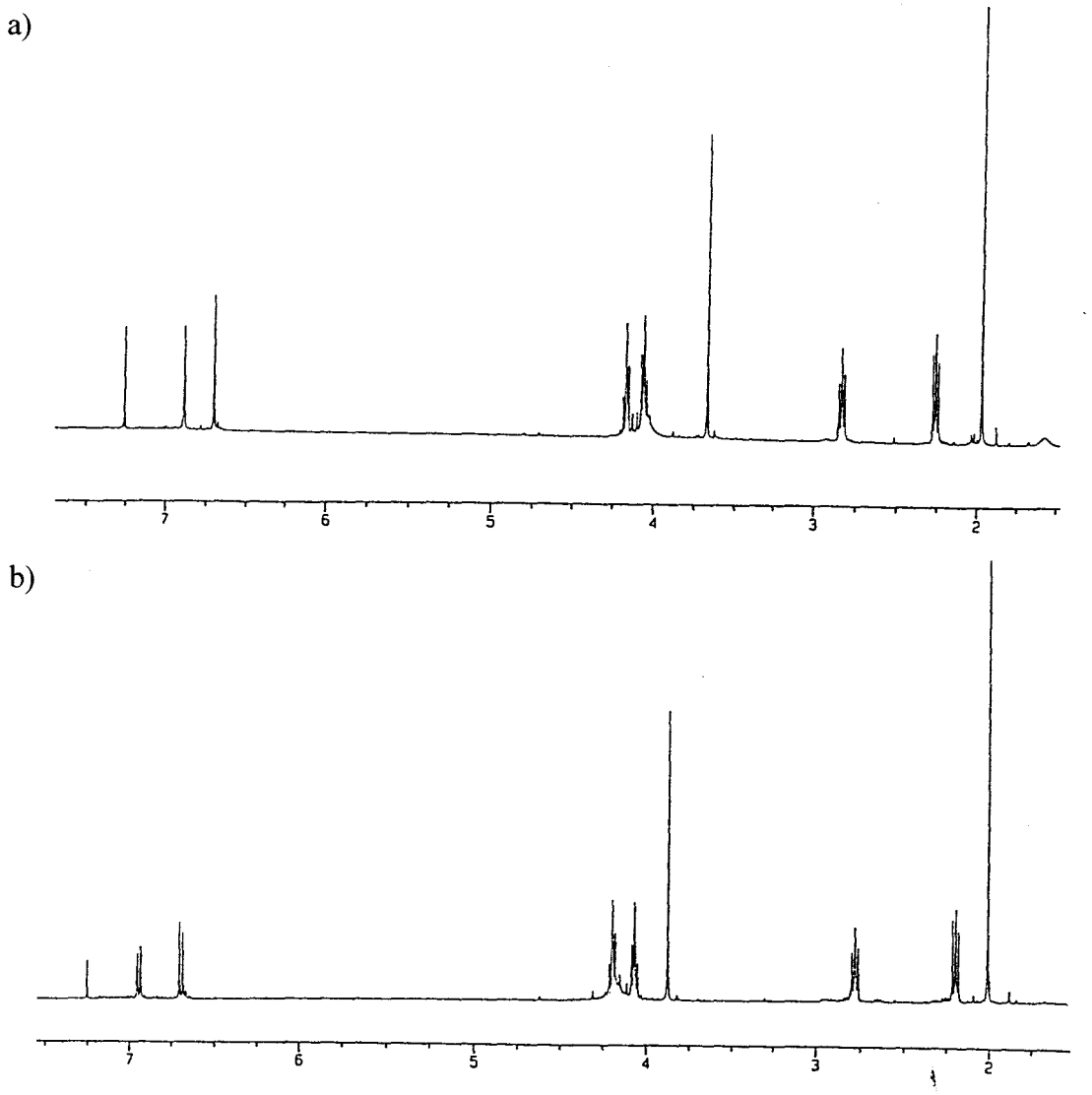
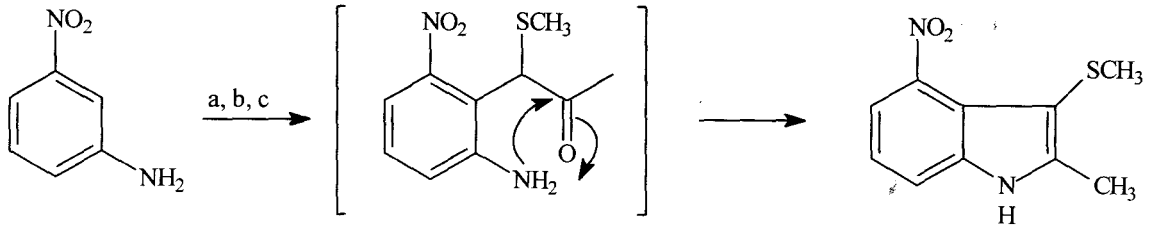


Figure 6.2 ^{1}H NMR (400 MHz, $CDCl_3$) spectra of (a) 5-methoxymethyl-6-aminoindanone derivative **313** and (b) 7-methoxymethyl-6-aminoindanone derivative **314**.

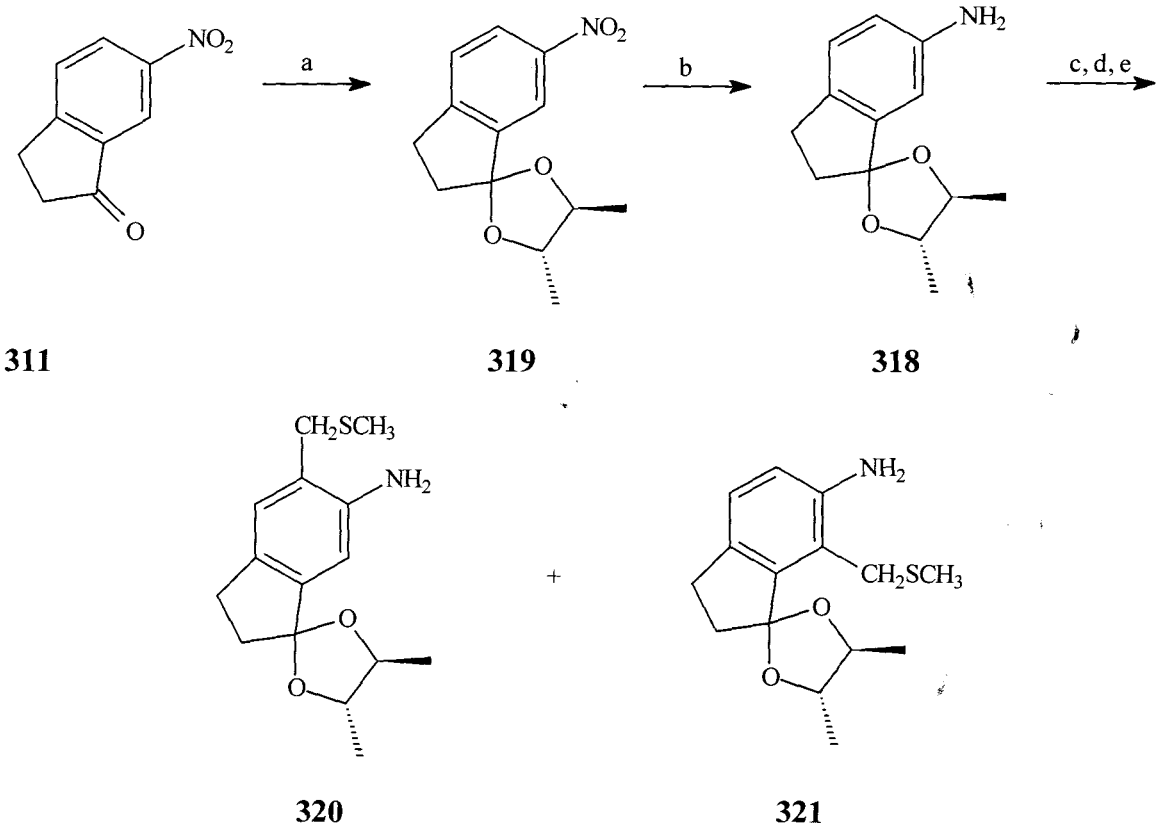


316 **317** **315**

Scheme 6.13 Gassman's alkylation of *meta*-nitroaniline.

Reagents and conditions: (a) Bu^tOCl, CH_2Cl_2, -65 °C, (b) $CH_3SCH_2C(O)CH_3$; (c) $(C_2H_5)_3N$

It is clear that the dioxolanyl protecting group is not bulky enough to hinder attack at C-7. Therefore, in an attempt to increase the steric bulk of the carbonyl protecting group aminoketal **(318)** was prepared from **311**. It was anticipated that the bulky 2,3-butanediol protecting group would be more effective in "blocking" the C-7 position during the Gassman's reaction. Consequently, compound **311** was reacted with 2*R*,3*R*-2,3-butanediol and PTSA in dry benzene to give the nitroketal **(319)** (Scheme 6.14) in only 22% yield. Hydrogenation of the nitro group under standard conditions afforded the aminoketal **318** which was characterised by standard spectroscopic techniques. The ^{1}H and ^{13}C NMR spectra of **318** are presented in Figure 6.3. Alkylation of **318** by Gassman's procedure gave the 5- and 7-substituted products **(320)** and **(321)** in a 24:76 ratio (Scheme 6.14). The ^{1}H and ^{13}C NMR spectra of **320** after purification by y HPLC are illustrated in Figure 6.4. Clearly, the expected steric hindrance provided by the bulky protecting group did not effectively shield the C-7 position during the Gassman's procedure.



Scheme 6.14 Preparation of aminoketal **318** and its alkylation by Gassman's procedure.

Reagents and conditions: (a) 2*R*,3*R*-2,3-butanediol, TsOH, benzene, reflux; (b) PtO_2 / H_2, EtOH; (c), Bu^tOCl, CH_2Cl_2, -65°C; (d) $(CH_3)2_S$; (e) NaOMe / MeOH

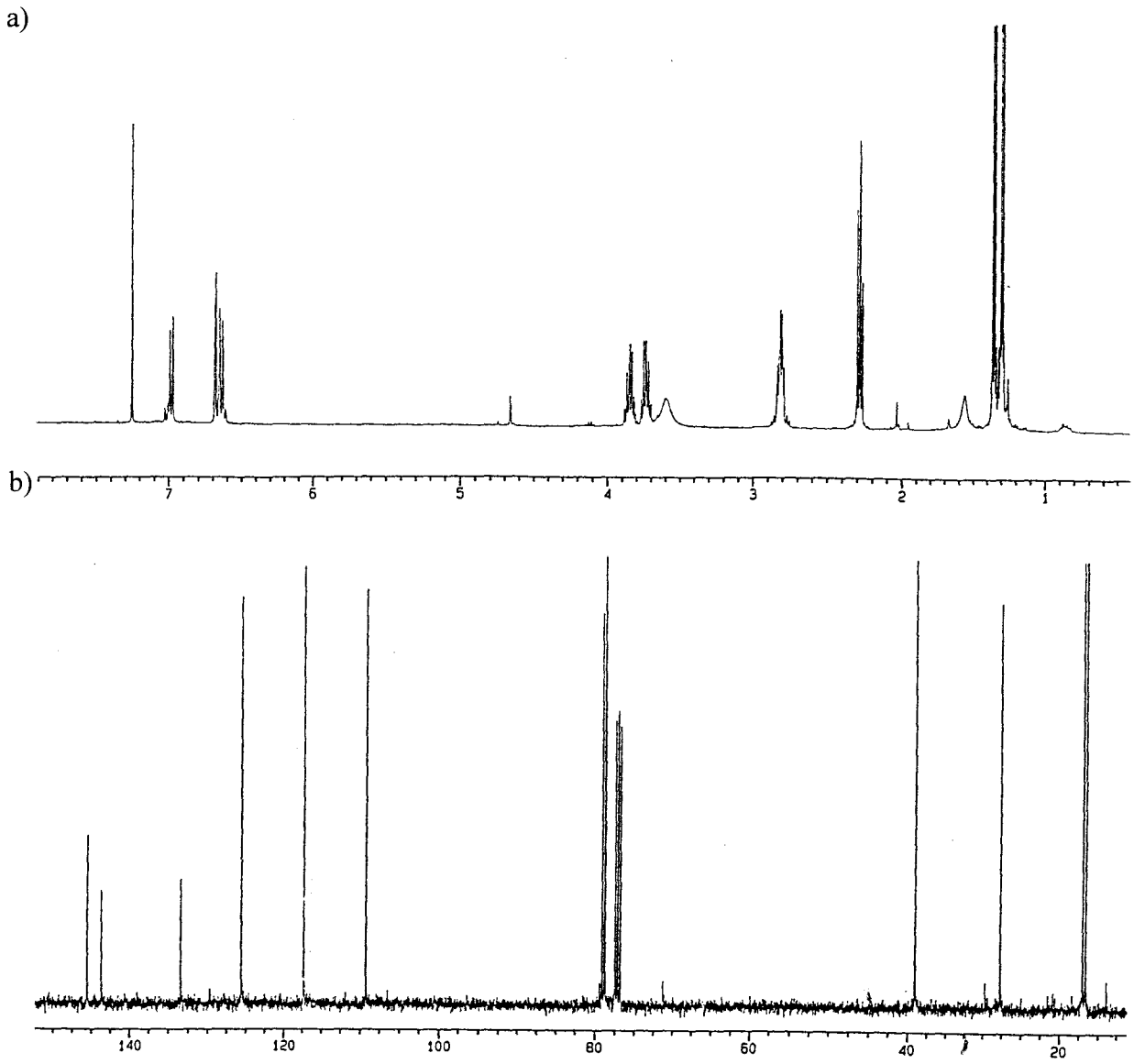


Figure 6.3 (a) ^{1}H (400 MHz, $CDCl_3$) and (b) ^{13}C (100 MHz, $CDCl_3$) NMR spectra of **318**.

In an attempt to explain the unexpected results of the above reactions we modelled the azasulfonium salt of **318** using the Cerius[2] molecular modelling package. The energy minimised structure of azasulfonium salt of **318** is presented in Figure 6.5 and from this structure it was apparent that the butanediol group used was insufficiently bulky to hinder attack at C-7. Disappointingly we realised that this was not a viable route to the dilemmaones and alternative procedures needed to be developed for the preparation of the key intermediate **293**. A logical alternative *i.e.* the direct alkylation of the indanone ring at the 5-position *via* Friedel-Crafts alkylation or acylation was eliminated as a possible alternative because of the directing effects of the ring substituents. Faced with this

alternative because of the directing effects of the ring substituents. Faced with this temporary *impasse* we decided to focus our attention on the synthesis of dilemmaone C. The attempted synthesis of dilemmaone C is described in the following section.

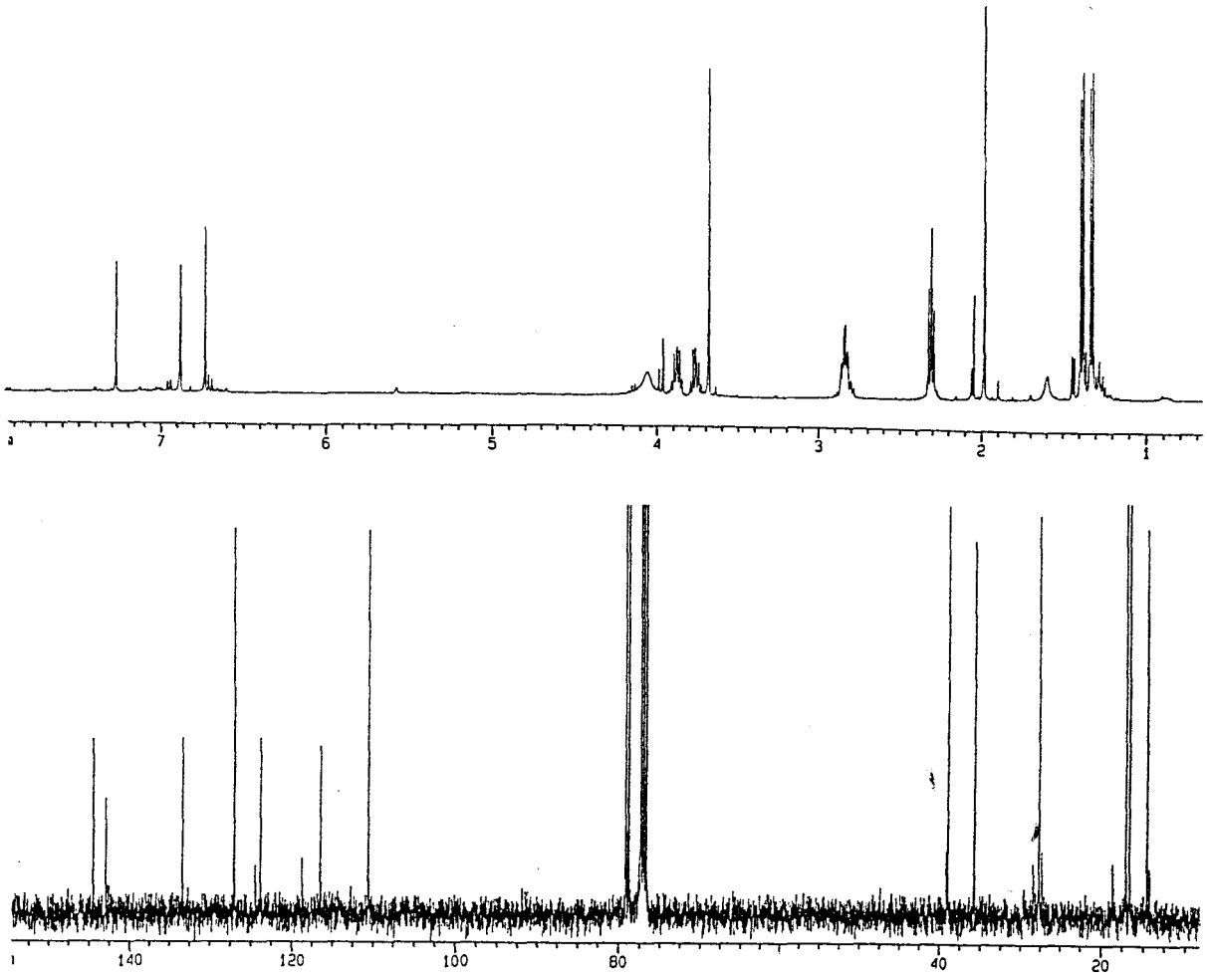


Figure 6.4 ^{1}H (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectra of 5-methoxymethyl-6-aminoindanone derivative **320**.

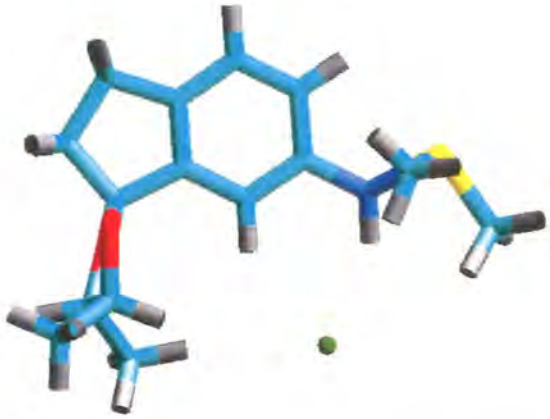
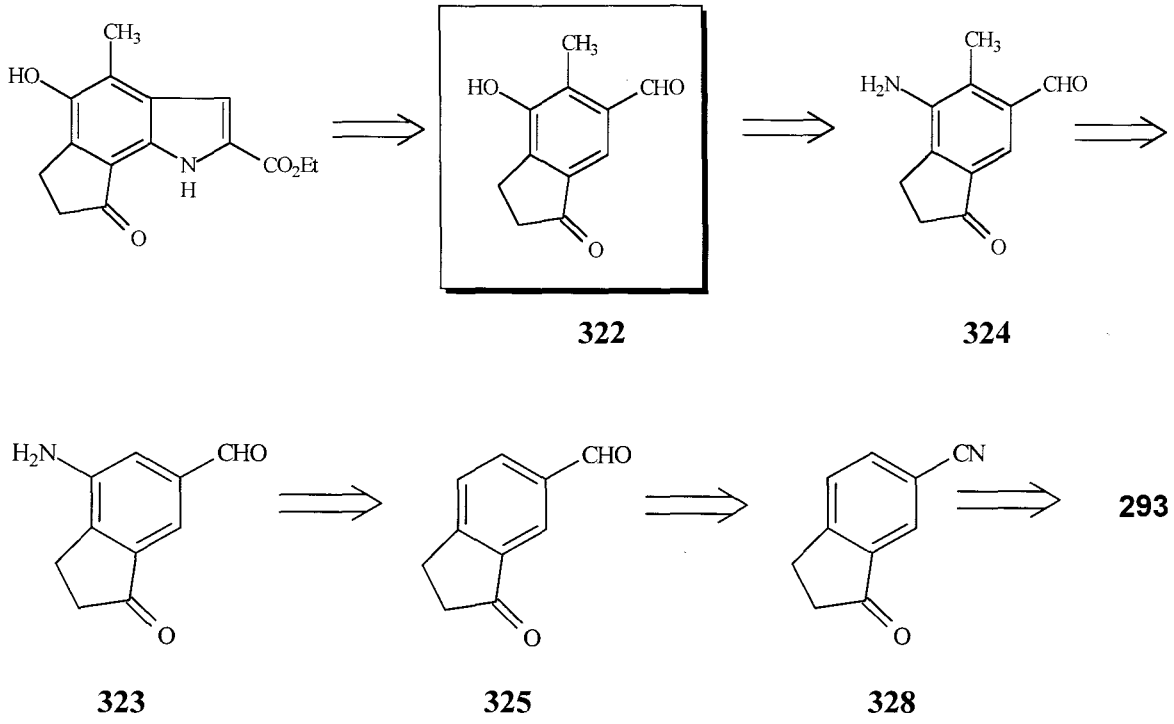


Figure 6.5 Energy minimised structure of the azasulfonium salt of **318**.

6.2.5 Attempted synthesis of dilemmaone C

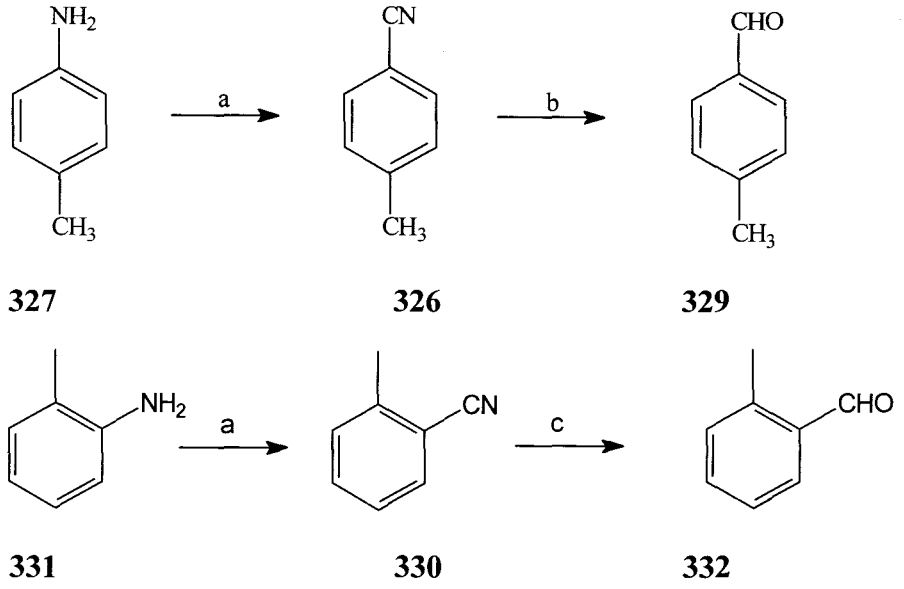
The retrosynthetic analysis of dilemmaone C (Scheme 6.15) indicated that the key intermediate in the synthesis was the aldehyde **(322)**. Aldehyde **322** may be prepared from Gassman's reaction of the 4-aminoindanone derivative **(323)** followed by conversion of the amino group in precursor **(324)** to an alcohol. The problems associated with the regioselectivity of Gassman's reaction would thus be avoided, since only one isomer can form.

The initial challenge was the synthesis of the 4-aminoindanone derivative **323** as it was anticipated that the direct formylation of indanone to give aldehyde **(325)** may be difficult because of the deactivated aromatic ring. An indirect procedure for formylation of **292** based on the conversion of an aromatic amine to an aldehyde was developed and initially explored using *p*-tolunitrile **(326)** as a model compound.



Scheme 6.15 Retrosynthetic analysis of dilemmaone C

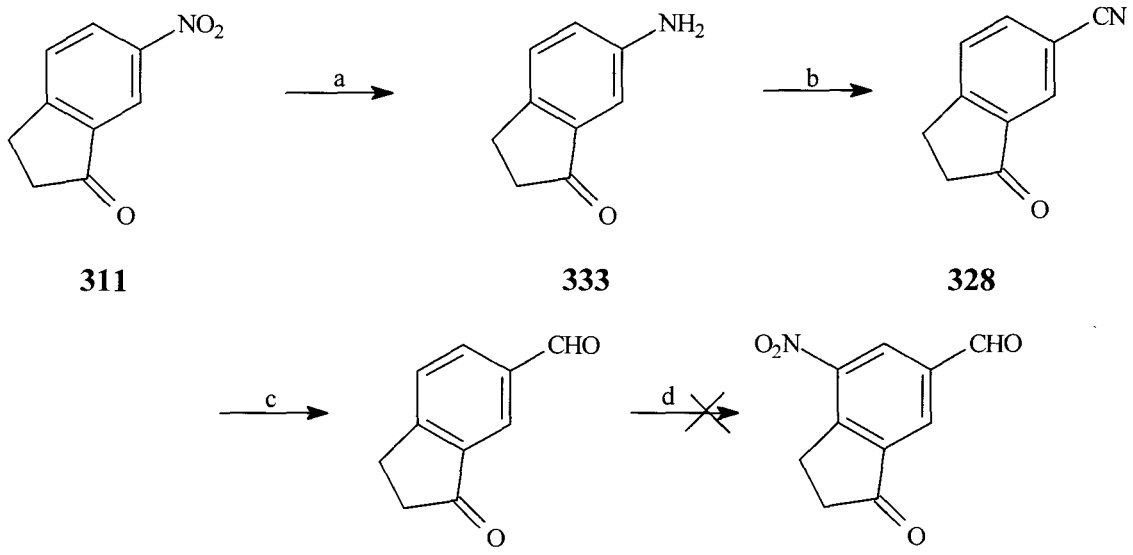
Para-tolunitrile was prepared from *p*-toluidine **(327)** *via* the Sandmeyer procedure[228] (Scheme 6.16). A number of procedures exist for the reduction of nitriles to aldehydes[229] of which the use of diisobutylaluminium hydride (DIBAH) is the most common. However, the use of DIBAH with **(328)** may lead to the reduction of both the nitrile and ketone and would thus require an additional protection step. On surveying the literature we discovered an under-exploited procedure for the reduction of aromatic nitriles to aromatic aldehydes. The procedure, developed by Staskun *et al.*,[230,231] employs nickel-aluminium alloy and formic acid and was reported to be compatible with a range of sensitive functional groups. We, again, explored this procedure with model compounds. *Para*-tolunitrile was refluxed with nickel-aluminium alloy and formic acid (75%) to give the aldehyde **(329)** in excellent yield (88%) yield after work up (Scheme 6.16). Interestingly, when this reaction was attempted using the more hindered *ortho*-tolunitrile **(330)**, prepared from *ortho*-toluidine **(331)**, only starting material was isolated. We found that the use of freshly prepared Raney nickel, in stead of nickel-aluminium alloy, and maintaining the temperature at 80-90°C allowed the yield of **(332)** to be improved to 89% for the more hindered *o*-tolunitrile (Scheme 6.16).



Scheme 6.16 Model reaction: Reduction of aromatic nitriles to aromatic aldehydes.

Reagents and conditions: (a) i) conc. HCl/H_2O, (ii) $NaNO_2/H_2O$, (iii) CuCN/KCN; (b) Ni-Al alloy, 75% HCOOH (reflux); (c) Raney nickel, 75% HCOOH (80-90°C).

The above sequence of reactions could now be applied to the synthesis of 6-formylindanone **(325)** (Scheme 6.17). Compound **328** was prepared by the direct hydrogenation of 6-nitroindanone **310** over PtO_2[227] followed by diazotisation of the resulting 6-aminoindanone **(333)** and reaction with cuprous cyanide under Sandmeyer conditions. Reduction of the nitrile **328** with formic acid and nickel-aluminium alloy gave **325** in good yield (77%). Unfortunately, all attempts at nitrating this very deactivated ring system, to introduce functionalisation at C-4, using nitric acid[232] or nitronium tetrafluoroborate,[233] were unsuccessful giving mainly complex mixtures of products or unreacted starting material (Scheme 6.17). Since this did not seem like a viable route we were obliged to refocus on the synthesis of dilemmaone A and devise an alternative strategy to prepare the indanone derivative **293**.



Scheme 6.17 Attempted preparation of 6-formyl-4-nitroindan-1-one.

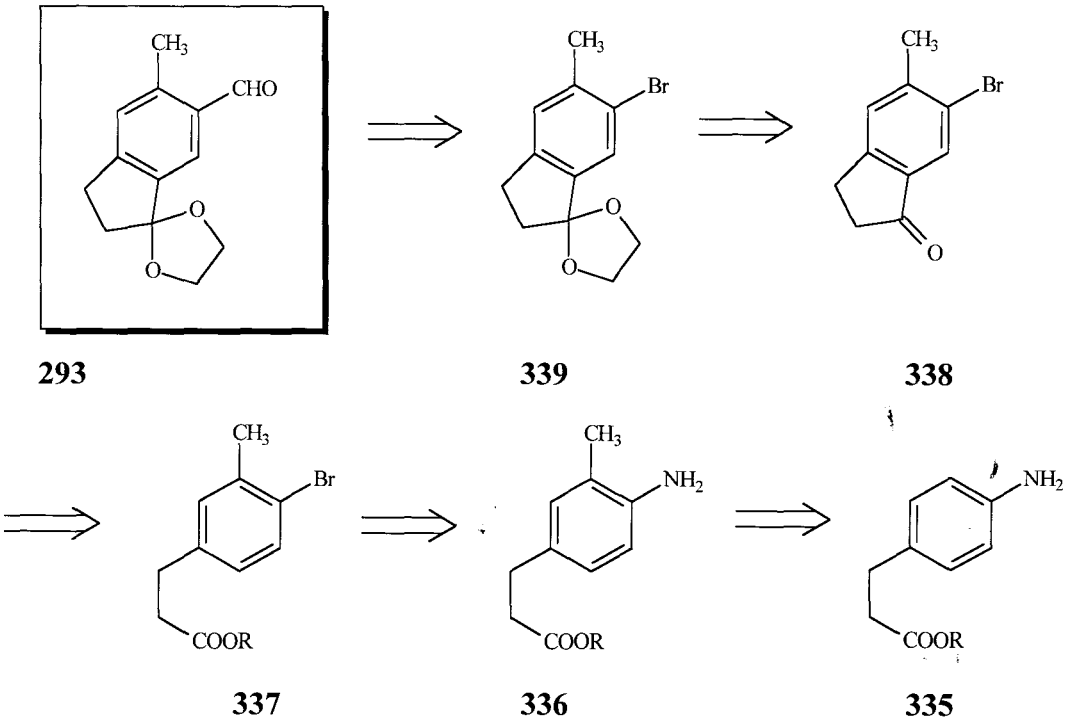
Reagents and conditions: (a) PtO_2, EtOH; (b) i) conc. HCl/H_2O, (ii) $NaNO_2/H_2O$, (iii) CuCN/KCN; (c) Nickel-aluminium alloy, 75% HCOOH; (d) HNO_3/H_2SO_4.

6.2.6 An alternative route to key intermediate 293

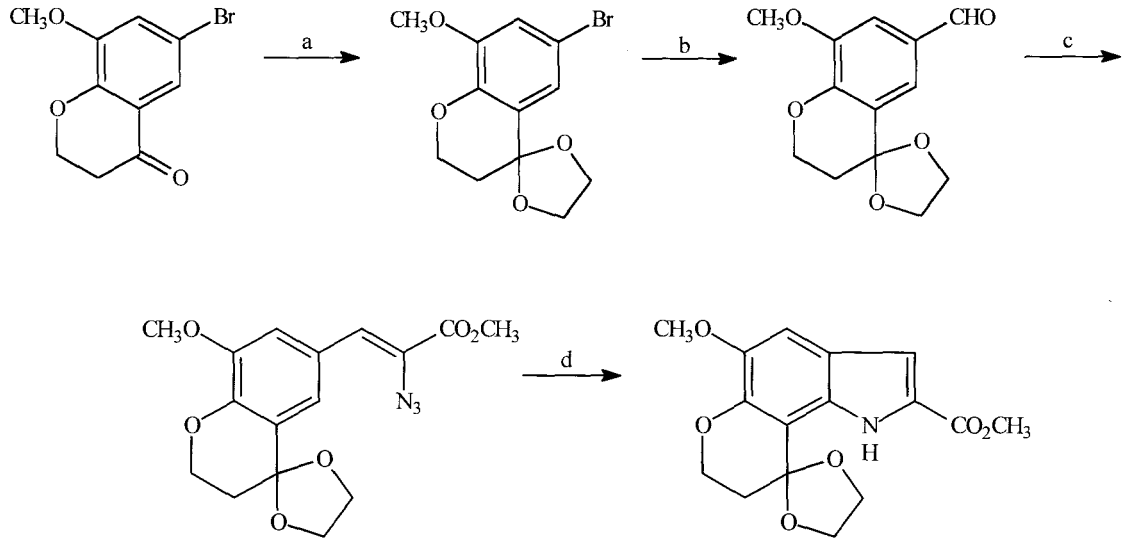
On reflection, we proposed that the problems associated with the regioselectivity of Gassman's procedure may be overcome by employing a symmetrical aniline in our synthetic sequence. Therefore, an alternative pathway, based on the formation of the indanone ring at a later stage in the synthesis was explored to prepare the key intermediate **293**. Many derivatives of phenylpropionic acid **(334)** such as the *meta*- and *para*-halogen isomers may be converted into the corresponding indanone derivatives by an intramolecular Friedel-Crafts acylation reaction.[225,234,235]

A retrosynthesis of intermediate **293** is presented in Scheme 6.18. The synthesis starts with amine **(335)** which may be synthesised from **334** by the usual nitration/reduction sequence. It was envisaged that *ortho*-alkylation by Gassman's procedure would give the *ortho*-substituted aniline **(336)**. Friedel-Crafts acylation of anilines do not take place readily because of complexation of the amino group with the Lewis acid catalyst. Furthermore, conversion of the amino group to a nitrile before cyclisation would deactivate the ring towards Friedel-Crafts cyclisation. We therefore elected to follow a procedure involving

the conversion of the amine **336** to the bromide **(337)** *via* the Sandmeyer[236] procedure followed by Friedel-Craft acylation as a feasible alternative. Cyclisation of **337** would give 6-bromo-5-methylindanone **(338)**. Protection of the carbonyl in **338** to give **(339)** and subsequent formylation *via* a halogen-lithium exchange protocol[237] should furnish the desired intermediate **293**. The protection and formylation steps in our planned synthesis were analogous to the procedure used by Kim *et al.*[238-239] in their synthesis of the oxo-pyranoindole **(340)** (Scheme 6.19). The protection of the ketone in **338** was deemed necessary for two reasons. Firstly, nucleophilic attack of *n*-butyl-lithium on the ketone would be a major problem and secondly, Kim *et al.*[238,239] reported that their attempted preparation of the azide **(341)** in the absence of a protected ketone led to nucleophilic attack of the methoxide anion on the ketone as the major reaction.



Scheme 6.18 Alternative retrosynthesis of intermediate **293**



341 **340**

Scheme 6.19 Synthesis of pyrannoindole **340** *via* Hemetsberger's protocol.[238,239]

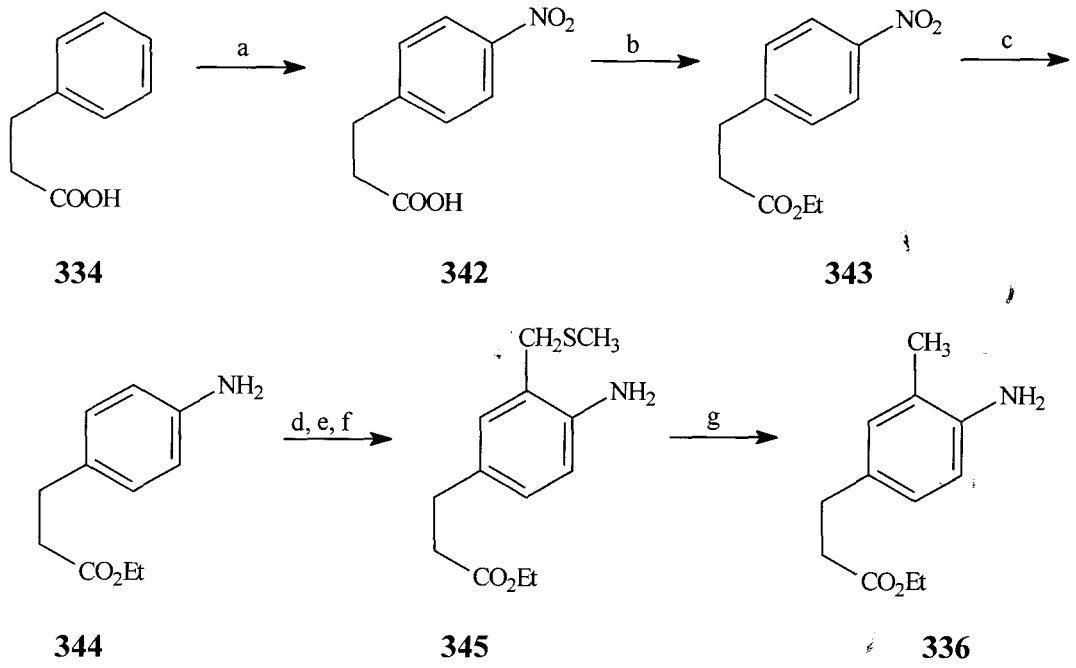
Reagents and conditions: (a) ethylene glycol, PTSA, toluene, 83%; (b) *n*-BuLi, DMF, 90%; (c) $N_3CH_2CO_2Et$, NaOMe; (d) xylene.

The first step in the synthesis of intermediate **293** was the nitration of phenyl propionic acid (Scheme 6.20). Initial attempts at nitration of phenyl propionic acid using a mixture of concentrated nitric and fuming sulfuric acids led to the formation of mainly the *ortho-para* dinitro-product. However, the required *para*-nitro product **(342)** was obtained almost exclusively, with a milder nitrating mixture consisting of nitric, sulfuric and glacial acetic acids. A small amount of the *ortho*-isomer (<10%) also formed in the reaction and was easily removed by silica gel flash chromatography. None of the dinitro product was evident. Protection of the carboxylic acid **342** as the ester **(343)** prevented interference of this group in the Gassman reaction and also aided in the purification of the subsequent reaction products. Thus, esterification of the carboxylic acid with EtOH / H_2SO_4 followed by reduction of the nitro group gave ethyl 3-(4-amino-phenyl)propanoate **344** in good yield (Scheme 6.20).

Treatment of **344** with *tert*-butyl hypochlorite at -65°C followed by addition of dimethyl sulfide gave the azasulfonium salt which was subjected to base-catalysed rearrangement by treatment with freshly prepared sodium ethoxide solution and warming to room temperature to give ethyl 3-(4-amino-3-[thiomethoxymethyl])propanoate **(345)** in 50%

isolated yield. The rearranged product was easily identified by its ^{1}H NMR spectrum (Figure 6.6) which contained three aromatic protons and characteristic signals for the $\underline{CH_2}$S-Ar and $\underline{CH_3}$S groups. The crude product contained a number of minor products that were not isolated. These were presumed to be products of aromatic chlorination[241,241] encountered elsewhere as by-products of the Gassman's alkylation.

A significant improvement in the reaction yield was obtained by mixing the aniline and the dimethyl sulfide **BEFORE** addition of the chlorinating reagent. This simple modification improved the yield from 50% to approximately 84%, based on isolated product. From our experience, an excess of base was also required which on aqueous work-up produced sodium hydroxide and frequently led to partial hydrolysis of the very susceptible ester. This was not a major obstacle since we could continue with either the ester or carboxylic acid. Desulfurization was achieved using freshly prepared Raney nickel to give ethyl 3-(4-amino-3-methyl-phenyl)propanoate **336** in yields of up to 93% (Scheme 6.20).



Scheme 6.20 Preparation of *ortho*-substituted amine **336**.

Reagents and conditions: (a) $HNO_3/H_2SO_4/CH_3COOH$; (b) $EtOH/H_2SO_4$; (c) PtO_2, EtOH; (d) Bu^tOCl, CH_2Cl_2, -65°C; (e) $(CH_3)_2S$; (f) NaOEt, EtOH; (g) Raney nickel, EtOH

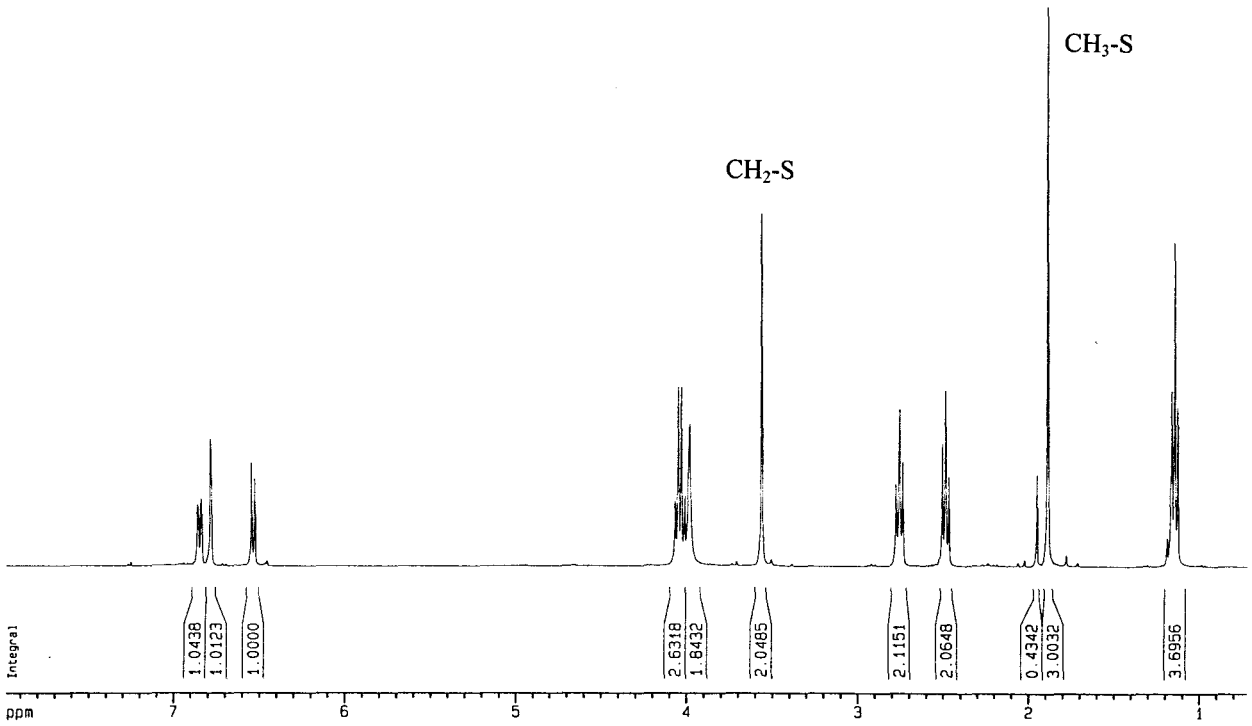
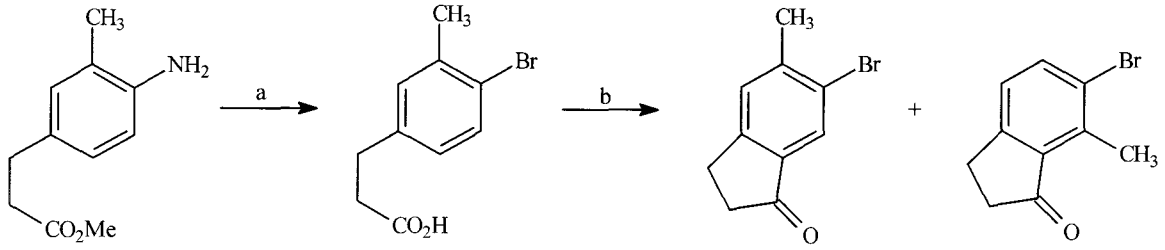


Figure 6.6 ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of ethyl 3-(4-amino-3-[thiomethoxymethyl])propanoate **345**

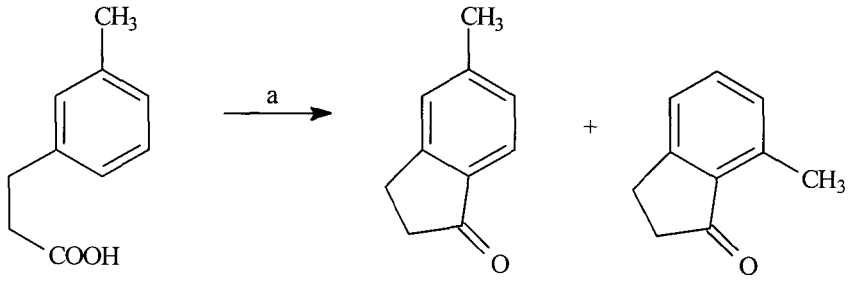
Having efficiently prepared the substituted aniline **336** we needed to synthesise the aryl bromide **337** (Scheme 6.21). Reaction of the diazonium salt of **337** with CuBr in hydrobromic acid at 0 °C was accompanied by facile ester hydrolysis to give the aryl bromide **337** in 52% yield. Intramolecular Friedel-Crafts acylation of the acid chloride of **337**,[225,226] using aluminium chloride as a catalyst gave a mixture of the two isomers **(338)** and **(346)** in 54% yield (Scheme 6.21). The ^{1}H NMR spectrum of the crude reaction mixture clearly showed a 2:3 ratio of isomeric products which were separated by HPLC and their structures verified by NMR and HREI mass spectrometry. It is interesting to note that the cyclisation preferentially occurred toward the more hindered position. This was not entirely unexpected since cyclisation of 3-(3-methyl-phenyl)-propionic acid **(347)** with methanesulfonic acid gave a 3:2 ratio of 2- and 6-acyl derivatives **(348)** and **(349)** whereas cyclisation with polyphosphoric acid (PPA)[242] gave a 1:1 ratio (Scheme 6.22). PPA was very effective in catalysing the cyclisation of phenylpropionic acid to indanone, but several attempts at cyclisation of **337** with PPA, under various conditions, were unsuccessful.



336 **337** **338** **346**

Scheme 6.21 Preparation of indanone derivative **338**

Reagents and conditions: (a) HBr, $NaNO_2$, 0°C; (b) i) $SOCl_2$, ii) $AlCl_3$, $(CH_2Cl)_2$



347 **348** **349**

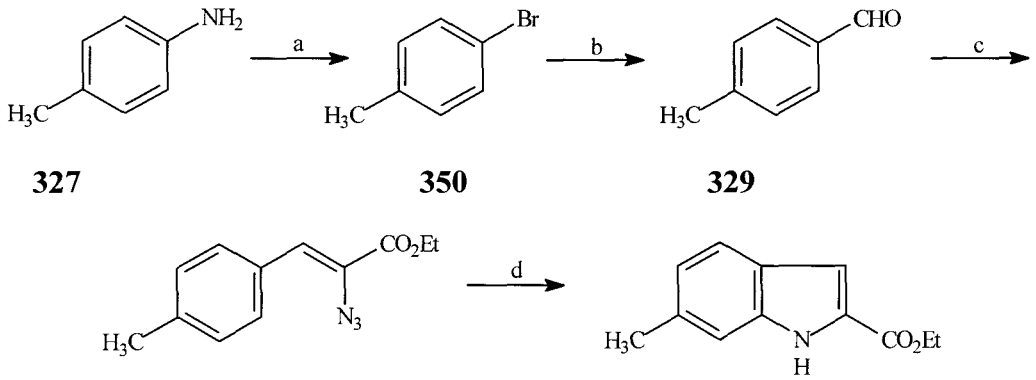
Scheme 6.22 Friedel-Crafts acylation of 3-(3-methylphenyl)-propionic acid.

Reagents and conditions: (a) PPA

Having synthesised the bromoindanone **338** in somewhat reduced yield we were now ready to attempt the formylation procedure followed by synthesis of the pyrrole ring. As mentioned previously, these reactions have been employed successfully by Kim *et al.*[238,239] in their synthesis of pyrannoindoles (Scheme 6.19). Since we had a limited amount of **338** available we explored the formylation procedure and the synthesis of the pyrrole ring with model compounds in order to optimise conditions.

The first step of the model reaction sequence was the preparation of the requisite *p*-bromotoluene **(350)** from *p*-toluidine **(327)** using the standard Sandmeyer reaction.[236] Treatment of **350** with *n*-butyllithium at -78°C followed by quenching of the organolithium intermediate with *N*-formylpiperidine gave the *para*-tolualdehyde **(329)** in 97% yield (Scheme 6.23). The next step in the model reaction sequence was to synthesise

the pyrrole ring using Hemetsberger's protocol. Accordingly, the aldehyde **329** was condensed with ethyl azidoacetate to give the reportedly unstable azidocinnamate **(351)**.[214] Surprisingly, **351** was stable enough to allow all the NMR data to be obtained. Some problems were encountered in carrying out this condensation, but it could be accomplished in reasonable yield by using a large excess of ethyl azidoacetate and maintaining anhydrous conditions. The cyclisation reaction of **351**, by thermolysis in refluxing toluene or xylene, furnished the indole **(352)** (Scheme 6.23). The ^{1}H NMR spectrum of **352** (Figure 6.7) clearly showed the indolic NH-1 proton at δ 9.0.



Scheme 6.23 Model reaction: Preparation of *para*-tolualdehyde from *para*-toluidine *via* a Sandmeyer bromination reaction and lithium-halogen exchange.

Reagents and conditions: (a) i) HBr, $NaNO_2$, ii) CuBr/HBr; (b) *n*-BuLi, formylpiperidine, Et_2O, -78°C, 97%; (c) $N_3CH_2CO_2Et$, NaOEt; (d) toluene, reflux.

With the successful synthesis of indole **352** we had hoped that the synthesis could be brought to a swift conclusion. However, all attempts at protecting the ketone functionality in **338** as the dioxolane were frustrated (Scheme 6.24). This was a crucial reaction since we could not continue with the lithiation reaction with an unprotected ketone. Unfortunately, due to time constraints and a paucity of the intermediate **338**, alternative procedures, such as the use of the trimethylsilyl derivative of ethylene glycol [$(TMSOCH_2)_2$] and trifluoromethane sulfonate (TMSOTf), to protect the ketone could not be explored.[243]

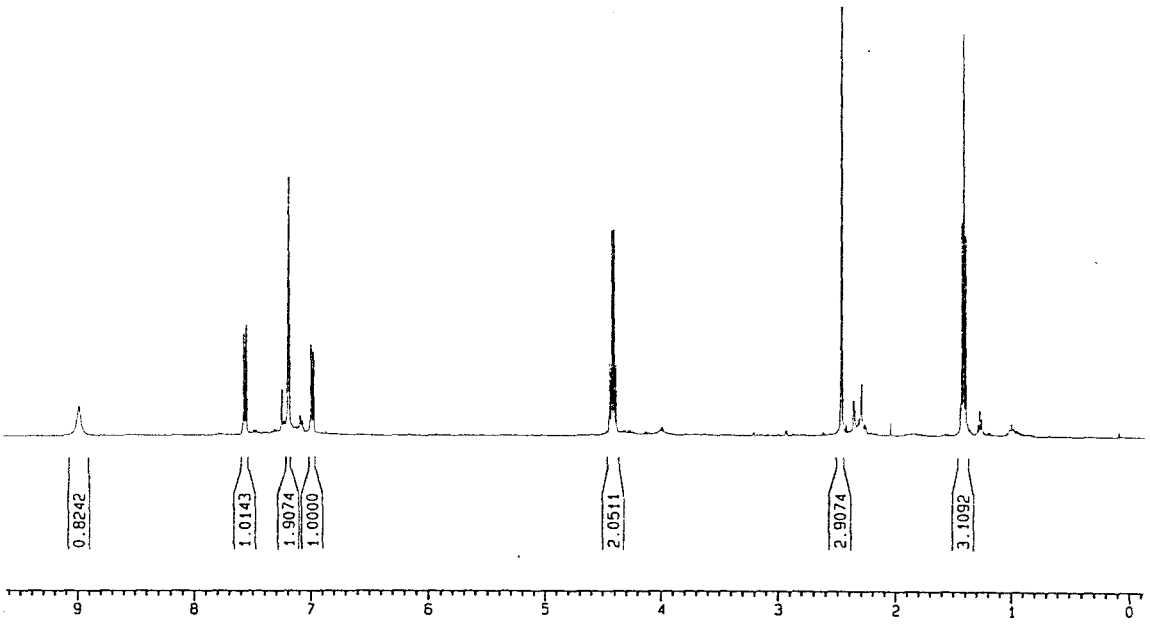
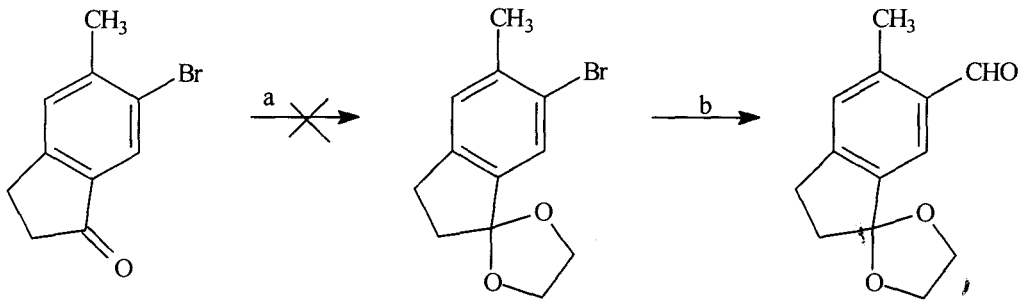


Figure 6.7 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of indole **352**.



338 **339** **338**

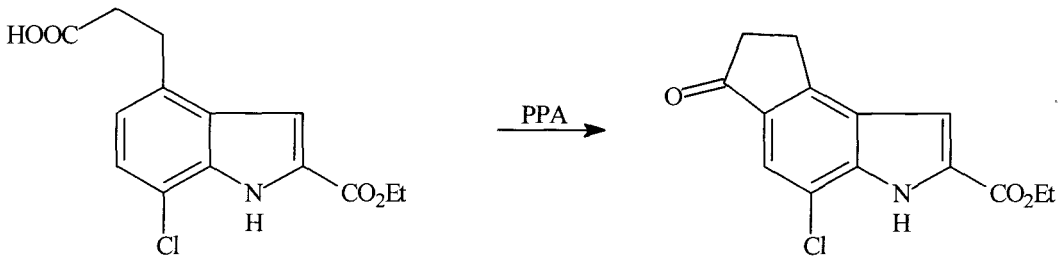
Scheme 6.24 Attempted protection of bromoketone **338**.

Reagents and conditions: (a) ethylene glycol, PTSA, C_6H_6; (b) i) *n*-BuLi, Et_2O, -78°C, ii) formylpiperidine.

6.2.7 Alternative strategies employed in the synthesis of dilemmaone A-C

Concurrently with our two main approaches to the synthesis of the dilemmaones discussed above we also explored other synthetic strategies. One of these strategies required the synthesis of the indole ring first followed by an intramolecular Friedel-Crafts reaction to form the pentanone ring. Nagasaka *et al.*[244] used the Friedel-Craft reaction to synthesise

the tricyclic ring system **(353)** from 3-(2-ethoxycarbonyl-7-chloroindole-4-yl)-propionic acid **(354)** (Scheme 6.25). This seemed like an obvious route to the dilemmaones and was investigated as follows.

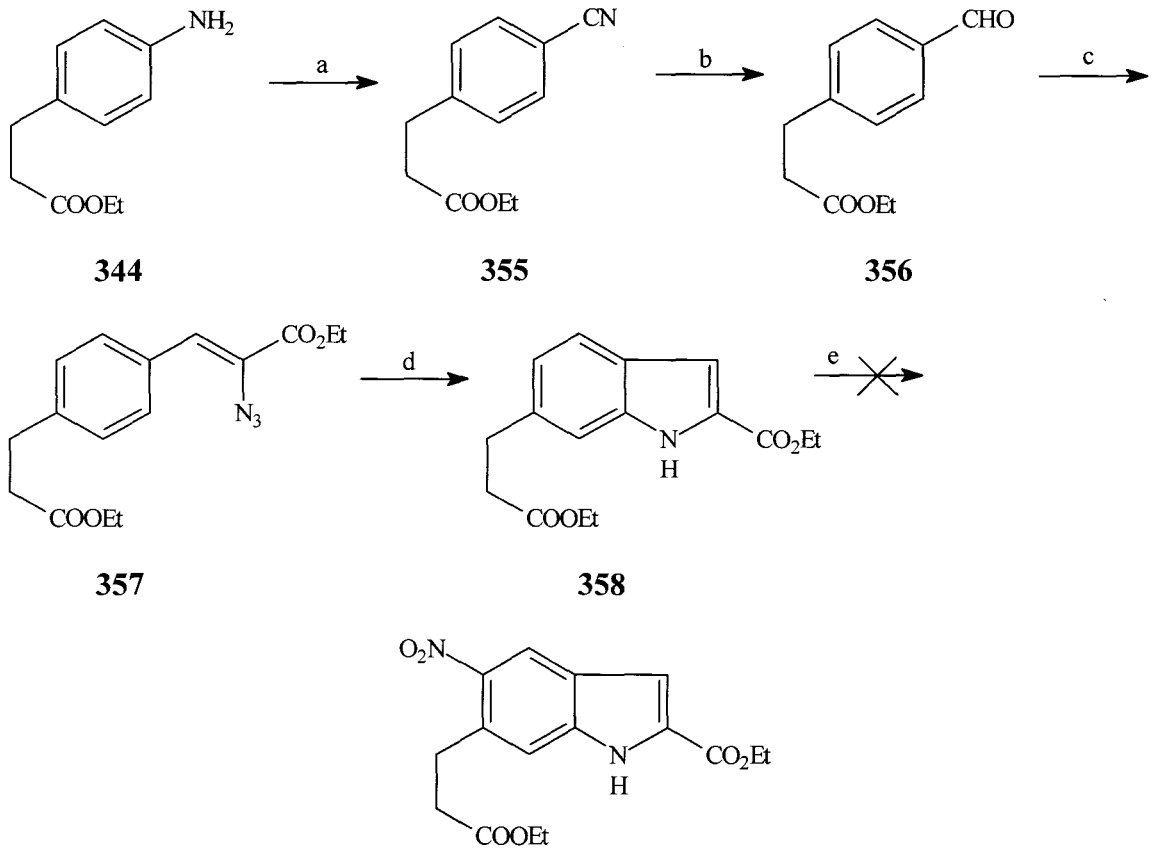


354 **353**

Scheme 6.25 Synthesis of indole **353**

Noland *et al.*[245] have shown that the indole ring may be nitrated under acidic conditions to give the 5-nitroindoles. This reaction would provide an alternative route to 5-aminoindoles which could then be alkylated and cyclised to provide the dilemmaone skeleton. An advantage of this procedure is that the alkyl group is introduced into the ring at a relatively late stage in the synthesis.

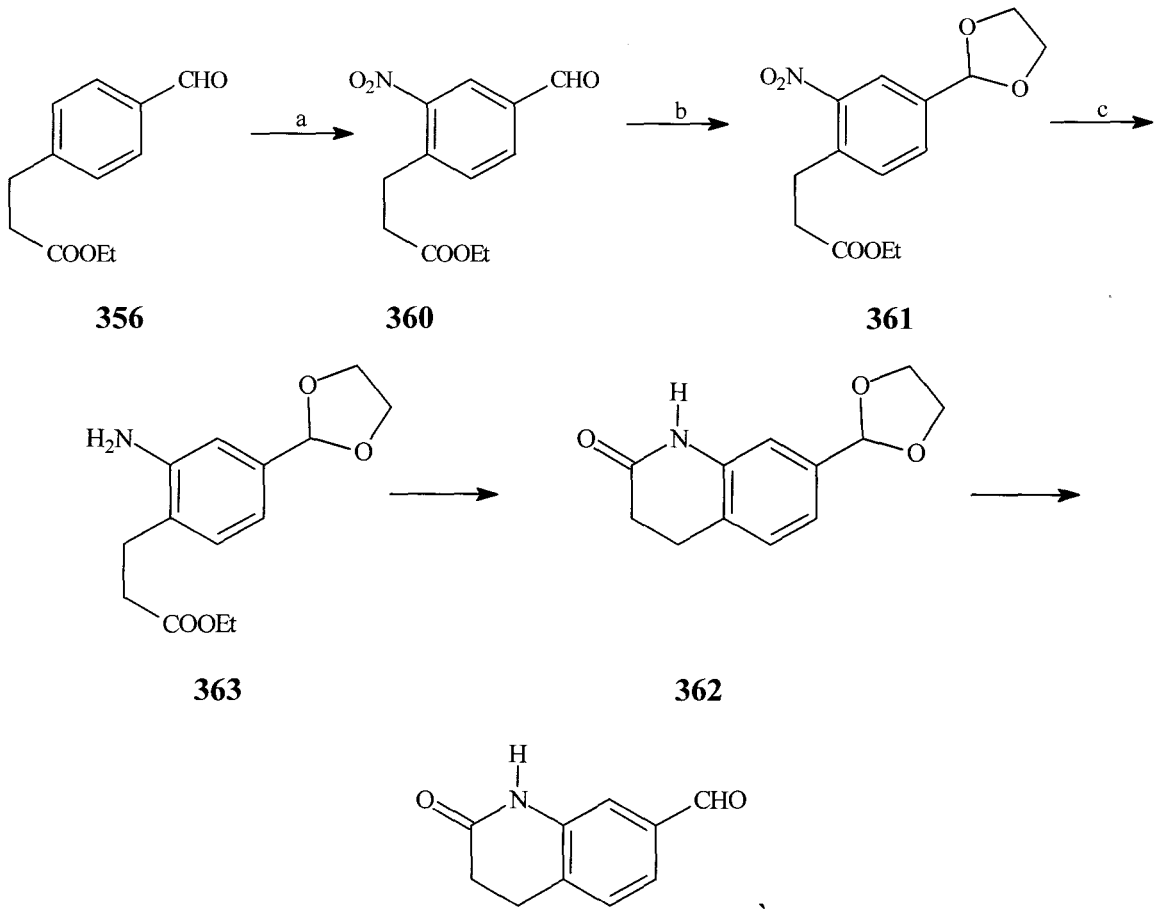
The synthesis was initiated by considering various formylating reactions of phenylpropionic acid. A number of procedures were attempted, including formylation with $Cl_2CHOCH_3/TiCl_4$,[246] and the Vilsmeier procedure ($POCl_3$/formylpiperidine)[247] without success. The indirect route, *via* the nitrile **(355)**, again provided easy access to the formyl derivative **(356)**. Aldehyde **356** was then subjected to two different synthetic sequences. Firstly, condensation of **356** with ethyl azidoacetate gave **(357)**. Thermolysis in refluxing toluene gave the indole **(358)** which was fully characterised by NMR and mass spectroscopy. Attempts at nitration of this indole gave a complex mixture of products with the required nitroindole **(359)** present in low yield (Scheme 6.26).



Scheme 6.26 An alternative procedure, leading to the synthesis of the indole ring

Reagents and conditions: (a) i) HCl/H_2O; ii) $NaNO_2/H_2O$, iii) CuCN/KCN; (b) Ni-Al alloy, HCOOH (75%); (c) $N_3CH_2CO_2Et$, NaOEt/EtOH; (d) toluene, reflux; (e) H_2SO_4/HNO_3.

Secondly, an alternative approach in which **356** was nitrated with concentrated nitric acid and fuming sulfuric acid gave the nitro derivative **(360)** which was subsequently protected as **(361)** to ensure the aldehyde would not be reduced in the next step of the reaction. Unfortunately, reduction of **361** gave a mixture of the lactam **(362)** and the amine **(363)**. Furthermore, **363** was converted to **362** on standing, even at 0°C which made this route unsuitable for the synthesis of the dilemmaones (Scheme 6.27). In addition the aldehyde protection group in **362** was fairly labile and this compound converted back to the free aldehyde **364** on standing.



356 **360** **361**

363 **362**

364

Scheme 6.27 An alternative procedure leading to the formation of the lactam **364**

Reagents and conditions: (a) H_2SO_4/HNO_3, (b) $(CH_2OH)_2$, PTSA, C_6H_6, (c)PtO_2/H_2, EtOH.

6.3 SUMMARY AND CONCLUSION

The objective of the research presented in this chapter was to find a convenient synthetic sequence to prepare the dilemmaones in order to secure an adequate supply of these compounds for biological testing. A number of synthetic strategies were designed and investigated. All the reactions were explored and optimised by using model compounds. A key feature of all these strategies was the use of Gassman's *ortho*-alkylation procedure in order to introduce the alkyl substituent at C-5 of the dilemmaone skeleton. Unfortunately, the most obvious approach to the dilemmaones from indanone was unsuccessful due to problems associated with the regiochemistry of Gassman's reaction. The use of bulky carbonyl protecting groups was not successful in overcoming these problems. The second

main approach was designed in order to overcome the problems associated with the Gassman's procedure by making use of a symmetrical aniline at the beginning of the synthesis. However, the regioselectivity of the intramolecular Friedel-Crafts acylation reaction adversely affected the yield of the substituted phenylpropionic acid derivatives. Further problems associated with the protection of the ketone and formylation of the indanone derivative still need to be addressed. Unfortunately, the paucity of material at the protection and formylation stage prevented further experimentation and because of the time constraints the sequence could not be repeated in order to provide more material.

Chapter Seven

EXPERIMENTAL SECTION

7.1 GENERAL EXPERIMENTAL

The following general procedures were followed unless otherwise stated. The ^{1}H (400MHz), ^{13}C (100MHz), DEPT-135, NOEDS, HMQC, HMBC, COSY-90, HOHAHA, NOESY and ROESY NMR spectra were recorded on a Bruker AMX400 spectrometer using standard pulse sequences. Chemical shifts are reported in ppm and referenced to residual undeuterated solvent resonances ($CHCl_3$ δ_H 7.25, δ_C 77.0; C_6H_6 δ_H 7.15, δ_C 128.02; CH_3OH δ_H 3.30, δ_C 49.05; DMSO δ_H 2.50, δ_C 39.43) and coupling constants are reported in Hz. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Infrared spectra were recorded on a Perkin-Elmer 180 Grating infrared spectrometer or a Perkin-Elmer Spectrum 2000 FT-IR spectrometer as films (neat) on NaCl or AgCl discs. Low resolution mass spectra were recorded on a Hewlett-Packard 5988A spectrometer at 70 eV by using the direct insertion probe and high resolution spectra were obtained by Dr P. Boshoff of the Mass Spectrometry Unit at the Cape Technikon, Cape Town. High-performance liquid chromatographic separations were performed on Whatman Magnum 10 Partisil and Phenomenex C-18 columns (9mm i.d., 50cm, flow rate 4ml.min^{-1}) using a Spectra-Physics liquid chromatography pump, Waters R401 differential refractometer and a rheodyne injector. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected.

All solvents were distilled before use. Normal phase TLC was performed on DC-Alufolien Kieselgel 60F_{254} plates and reverse phase TLC was performed on DC-Ferigplatten RP18F_{254}s plates. The plates were viewed under UV light (254 nm) and were developed by spraying with 10% H_2SO_4 in MeOH followed by heating. Flash column chromatography was performed using Kieselgel 60 (230-400 mesh) silica.

All reactions requiring anhydrous conditions were conducted in flame-dried apparatus. Where appropriate, solvents and reagents were dried by standard methods, *i.e.* by distillation from the usual drying reagent prior to use: tetrahydrofuran and diethyl ether

from sodium wire and benzophenone, dichloromethane from calcium hydride. Organic extracts were dried over sodium sulfate or magnesium sulfate. All reactions were magnetically stirred unless otherwise stated.

7.2 CHAPTER TWO: EXPERIMENTAL

General experimental procedures

Low resolution CIMS (CH_4) and EIMS spectra were obtained on a Hewlett Packard 5988a mass spectrometer.

Collection and Extraction of *Siphonaria capensis*:

558 specimens of *S. capensis* (identified by Dr Allan Hodgen of the Zoology Dept, Rhodes University) were collected from the intertidal zone at the Bushman's River mouth, South Africa, in the summer of 1992 and stored in acetone until work-up. The extract was decanted and the specimens re-extracted with acetone. The acetone extracts were pooled, concentrated and partitioned between EtOAc and water. The polypropionate compounds obtained from the concentrated EtOAc partition layer by silica chromatography (1:2 EtOAc-hexane), normal phase HPLC (1:4 EtOAc-hexane) and C-18 reverse phase HPLC (9:1 MeOH/H_2O) were C-2 epimeric mixtures of siphonarienfuranone **(149)** (0.07mg/animal) and the *Z*-isomer **(161)** of this compound (0.04 mg/animal), capensinone **(162)** (0.03 mg/animal), capensifuranone **(163)** (0.02 mg/animal) and (2*E*, 4*S*, 6*S*, 8*S*) tetramethyl-2-undecenoic acid **(164)** (0.02 mg/animal).

(*E*)-siphonarienfuranone (149):

Oil; $[\alpha]_D$ +53.7° (*c* 1.75, $CHCl_3$, lit[90] $[\alpha]_D$ +102°); IR (film) ν_{max} 3400, 2960, 2920, 2880, 1690, 1600, 1450, 1370, 1210, 1110, 1050, 930 cm^{-1}; ^1H NMR ($CDCl_3$, 400MHz) δ 6.13 (1/2 H, d, *J* = 10 Hz, ½ H-9), 6.10 (1/2 H, d, *J* = 10 Hz, ½ H-9), 2.70 (1H, br m, H-10), 1.95 (3H, d, *J* = 5.4 Hz, 3H-8), 1.80 (3H, d, *J* = 3.2 Hz, 3H-5), 1.53 (3H, d, *J* = 6.6 Hz, 3H-1), 1.47 (1H, m, H-13), 1.47 (1H, m, H-15a), 1.44 (1H, m, H-16), 1.38 (2H, m, H-12a), 1.30 (1H, m, H-19a), 1.23 (1H, m, H-18a), 1.23 (1H, m, H-19b), 1.10 (1H, m, H-15b), 1.02 (1H, m, H-18b), 1.02 (3H, d, *J* = 6.6 Hz, 3H-11), 0.94 (1H, m, H-12b), 0.86 (3H, m, 3H-20), 0.85 (3H, m, 3H-14), 0.80 (3H, d, *J* = 6.6 Hz, 3H-17); ^{13}C ($CDCl_3$, 100 MHz) δ 203.3/203.2 (C-3, s), 181.8/181.5 (C-6, s), 147.7/147.6 (C-9, d), 125.8/125.7 (C-7, s), 106.3/106.2 (C-4, s), 101.14/101.09 (C-2, s), 45.6 (C-12, t), 44.61/44.59 (C-15, t), 39.3/39.2 (C-18, t), 30.9 (C-10, d), 29.7 (C-13, d), 28.33/28.30 (C-16, d), 22.30/22.27

(C-1, q), 20.84 (C-11, q), 20.4 (C-14, q), 20.1/20.0 (C-17, q), 19.9 (C-19, t), 14.4 (C-20, q), 13.41/13.37 (C-8, q), 7.6 (C-5, q); CIMS (CH_4, 1.8 Torr, Quadrex column, 100926 @ 10°/min) *m/z* (int, %) 323 (24), 322.0 (100), 303.9 (18), 277.8 (13), 221.7 (31).

(*Z*)-siphonarienfuranone (161):

Oil; $[\alpha]_D$ +58.9° (c=5.93, $CHCl_3$); IR (film) ν_{max} 3340, 2950, 2910, 2880, 1685, 1590, 1450, 1370, 1200, 1130, 1050, 930 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.44 (1/2 H, m, H-9), 5.42 (1/2H, m, H-9), 2.34 (1H, br m, H-10), 1.95 (3H, s, 3H-8), 1.64 (3H, s, 3H-5), 1.55 (3H, s, 3H-1), 1.39 (1H, m, H-16), 1.30 (1H, m, H-19a), 1.25 (2H, m, H-15a), 1.25 (1H, m, H-13), 1.24 (1H, m, H-19b), 1.22 (1H, m, H-18a), 1.10 (1H, m, H-12), 1.02 (1H, m, H-18b), 1.00 (3H, m, 3H-11), 0.95 (1H, m, H-15b), 0.88 (1H, m, H-12), 0.85 (3H, m, 3H-20), 0.80 (3H, m, 3H-17), 0.73 (3H, m, 3H-14); ^{13}C ($CDCl_3$, 100MHz) δ 202.7 (s, C-3), 183.4 (s, C-6), 143.2/143.0 (d, C-9), 124.4 (s, C-7), 108.1/108.0 (s, C-4), 101.6/101.5 (s, C-2), 45.4 (t, C-12), 44.8/44.6 (t, C-15), 39.34/39.27 (t, C-18), 32.27/32.25 (d, C-10), 29.7 (d, C-13), 27.8/27.1 (d, C-16), 22.3/22.1 (q, C-1), 21.5/21.3 (q, C-11), 20.93 (q, C-8), 20.86 (q, C-14), 20.0 (q, C-19), 19.96 (t, C-19), 14.4 (q, C-20), 6.6/6.5 (q, C-5); CIMS: CH_4, 1.8Torr, Quadrex (Polar column), 100-260@10°/min *m/z* (int, %) 322.85 (24), 321.85 (100), 303.85 (19.0), 221.55 (23).

Capensinone (162):

Oil; $[\alpha]_D^{21}$ = -65° (*c* 0.42, $CHCl_3$); IR (film) ν_{max} 3450, 2960, 2920, 2840, 1700, 1630, 1450, 1370 cm^{-1}; ^1H and ^{13}C NMR data see Table 2.1; EIMS (70eV), *m/z* (int, %), 139 (100), 123 (10), 122 (31), 111 (15), 71 (8), 55 (13), 43 (34); CIMS (CH_4, 1.8 Torr) *m/z* (int, %) 295 (100), 294 (6), 277 (80); HREIMS obsd. *m/z* 294.2546, $C_{19}H_{34}O_2$ (M^+) requires 294.2559.

Capensifuranone 163: oil; $[\alpha]_D^{21}$ = -15.1° (*c* 0.29, $CHCl_3$); IR (film) ν_{max} 3440, 2960, 2920, 2840, 1720, 1450, 1370 1250 cm^{-1}; ^1H and ^{13}C NMR data see Table 2.1; EIMS (70eV), *m/z* (int, %), 139 (24), 113 (14), 112 (100), 111 (16), 99 (11), 85 (28), 71 (23), 57 (28); HREIMS obsd. *m/z* 266.2236, $C_{17}H_{30}O_2$ (M^+) requires 266.2246.

(2*E*, 4S, 6S, 8S)-2,4,6,8-Tetramethyl-2-undecenoic acid 164:

Oil; $[\alpha]_D^{21}$ = +32° (*c* 0.19, $CHCl_3$); IR (film) ν_{max} 3000, 2980, 2900, 1780, 1700, 1650, 1460, 1425, 1380, 1280 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.65 (1H, m, H-3), 2.63 (1H, m, H-4), 1.85 (3H, s, 3H-12), 1.48 (1H, m, H-8), 1.38 (1H, m, H-6), 1.36 (1H, m, H-5a), 1.32 (1H, m, H-10a), 1.24 (2H, m, H-9a, H-10b), 1.15 (1H, m, H-7a), 1.10 (1H, m, H-5b), 1.02 (1H, m, H-9b), 1.00 (3H, d, $J_{4,13}$ = 7 Hz, 3H-13), 0.90 (1H, m, H-7b), 0.88 (3H, m, 3H-11), 0.83 (3H, d, $J_{6,14}$ = 7 Hz, 3H-14), 0.82 (3H, d, $J_{8,15}$ = 7 Hz, 3H-15); ^{13}C NMR ($CDCl_3$) δ 173.3 (s, C-1), 151.0 (d, C-3), 125.4 (s, C-2), 45.6 (t, C-7), 44.3 (t, C-5), 39.3 (t, C-9), 31.1 (d, C-4), 29.6 (d, C-8), 28.2 (d, C-6), 20.5 (q, C-13), 20.4 (q, C-14), 20.0 (t, C-10), 19.9 (q, C-15), 14.4 (q, C-11), 12.1 (q, C-12); EIMS (70eV), *m/z* (int, %), 167.0 (40), 150.0 (12), 148.9 (100), 71.2 (14), 70.2 (11), 57.2 (19), 55 (9); CIMS (CH_4, 1.8 Torr) 269 (8), 241 (100), 223 (17); HREIMS obsd. *m/z* 240.2092, $C_{15}H_{28}O_2(M^+)$ requires 240.2089.

Oxidative degradation with ruthenium tetroxide:

The following method is representative. Compound **164** (6.7 mg, 0.028 mmol) was dissolved in CCl_4 (0.5 ml) and added to a solution of CH_3CN (0.5 ml), H_2O (0.75 ml), periodic acid (30 mg, 0.132 mmol) and a catalytic amount of $RuCl_3.3H_2O$. The reaction mixture was stirred vigorously (4.5 h) at room temperature, concentrated, and partitioned between H_2O (5 ml) and CH_2Cl_2 (3 x 5 ml). The combined CH_2Cl_2 partition fractions were dried, concentrated and subjected to normal phase HPLC (EtOAc/Hexane 3:7) to give (2*S*, 4*S*, 6*S*)-2,4,6 trimethylnonanoic acid **(165)** as an oil; 2.5 mg (45% yield): $[\alpha]_D$ = +19° (*c* 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 2.58 (m, 1H), 1.74 (m, 1H), 1.53 (m, 2H), 1.18 (3H, d, *J* = 7 Hz), 0.88 (3H, m), 0.88 (3H, m), 0.82 (3H, d, 3H, *J* = 7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.9 (s), 45.3 (t), 41.2 (t), 39.2 (t), 37.1 (d), 29.6 (d), 28.2 (d), 20.3 (q), 20.0 (q), 19.9 (t), 18.0 (q), 14.4 (q).

7.3 CHAPTER THREE: EXPERIMENTAL

Invertebrate material:

The soft coral was collected in September 1994 off Port Alfred, South Africa, by SCUBA (-15m) and identified as *Pieterfaurea unilobata* (class Anthozoa, order Alcyonacea Lamaouroux, 1816, family Nidaliidae) by Dr. Gary Williams (California Academy of Sciences). *P. unilobata* is characterised by the possession of conspicuous permanent conical protuberances formed by a palisade-like arrangement of large spindles. Large tuberculate and clubbed spindles are present throughout the surface as well as the interior of the colony, while anthocodial sclerites are absent.[16] Voucher specimens were deposited at the California Academy of Sciences and Rhodes University Marine Invertebrate Collection (PA94-003).

Extraction and Isolation:

The frozen sample was diced and freeze-dried to give 143.5 g dry material which was extracted with EtOAc (2x 1.25l). Removal of the solvent under reduced pressure left a viscous, dark-orange oil (2.3g) which was subjected to flash chromatography on a silica gel column using a solvent combination of increasing polarity (n-hexane : EtOAc, 4:1 , 3:1, 2:1, 1:1, 0:1). Fractions of 10 ml each were collected and combined based on TLC analysis to give four main fractions Fn 1-4.

Sterols **180** (128.5mg, 0.09% dry wt) and **183** (10.3mg, 0.0072% dry wt) were obtained from Fn 2 (313.4mg) by recrystallization (n-hexane:EtOAc, 7:1.5) followed by semi-prep HPLC (n-hexane-EtOAc, 7:2). Semi-prep HPLC (n-hexane-EtOAc, 7:1) of Fn 3 (193mg) gave sterols **185** (18.8mg, 0.013% dry wt), **184** (4.5mg, 0.0031% dry wt) and **181** (10.8mg, 0.0075%dry wt). Sterol **169** (5.2mg, 0.0036% dry wt) was purified from Fn 1 (320.7mg, mainly cholesterol) by normal phase HPLC (n-hexane:EtOAc, 3:2). Sterol **182** (3.7mg. 0.0026% dry wt) was recrystallised from Fn 4 (99mg, n-hexane:EtOAc, 7:3).

Pregna-5,20-diene-3α,7α-diol 3α-acetate (180):

Colourless crystalline plates (n-hexane-EtOAc), mp 156-159°C; $[\alpha]^{25}_{D}$ -84° (*c* 0.02, $CHCl_3$); IR (KBr disk) ν_{max} 3490 (OH), 2940, 1700 (OAc), 1380, 1285, 1030, 1010, 890

cm^{-1}; ^1H NMR ($CDCl_3$) 5.77 (1H, m, H-20), 5.55 (1H, dd, J = 5.5, 1.8, H-6), 5.05 (1H, m, H-3), 5.00 (2H, m, H-21), 3.84 (1H, br s, $W_{1/2}$ = 14 Hz, H-7), 2.25 (1H, dd, J = 15.3, 1.5 Hz, H-4), 2.55 (1H, dd, J = 15.3, 1.5 Hz), 2.03 (1H, m, H-17), 2.00 (3H, s, OAc), 1.85 (1H, m, H-15), 1.85 (1H, m, H-16a), 1.80 (2H, m, H-2), 1.72 (1H, m, H-12a), 1.68 (1H, m, H-1b), 1.60 (1H, m, H-16b), 1.60 (1H, m, H-11a), 1.50 (1H, m, H-8) 1.50 (1H, m, H-14), 1.45 (1H, m, H-11b), 1.45 (1H, m, H-1b), 1.35 (1H, m, H-9), 1.25 (1H, m, H-15b) 1.10 (1H, m, H-12b) 1.00 (3H, s, H-19), 0.60 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) see Table 3.1; LREIMS (70 eV) *m/z* (int, %) 298 (100), 280 (19), 265 (15), 105 (16), 93 (17), 91 (25); HREIMS *m/z* 358.2522 ($C_{23}H_{34}O_3$ requires 358.2508)

Pregna-5,20-diene-3β,7α-diol 7α-acetate (181):

Yellow oil: $[\alpha]^{25}_D$ -198° (c 0.17, $CHCl_3$); IR (dry film) ν_{max} 3400 (OH), 2940, 1710 (OAc), 1360, 1240, 1200, 1040, 100, 880 cm^{-1}; LREIMS (eV) *m/z* 298 (94), 265 (100), 211 (61), 143 (55), 105 (54), 91 (81); HREIMS *m/z* 358.2502 ($C_{23}H_{34}O_3$ requires 358.2508); ^1H NMR ($CDCl_3$) δ 5.75 (1H, m, H-20), 5.55 (1H, dd, J = 5.2, 1.6 Hz, H-6), 5.00 (2H, m, H-21), 5.00 (1H, m, H-7), 3.55 (1H, br m, $W_{1/2}$ = 21 Hz, H-3), 2.30 (2H, m, H-4), 2.01 (1H, m, H-17), 2.00 (3H, s, OAc), 1.85 (1H, m, H-2a), 1.85 (1H, m, H-12a), 1.80 (1H, m, H-1a), 1.80 (1H, m, H-16a), 1.70 (1H, m, H-12b), 1.60 (1H, m, H11a), 1.60 (1H, m H-16b), 1.57 (1H, m, H1b), 1.57 (1H, m, H-15a), 1.52 (1H, m, H-2b), 1.45 (1H, m, H-11b), 1.37 (1H, m, H-9), 1.35 (1H, m, H-14), 1.20 (1H, m, H-15b), 1.13 (1H, m, H-8), 1.00 (3H, s, H-19), 0.60 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) see Table 3.2.

Pregna-5,20-dien-3β-ol (169):

Colourless oil; $[\alpha]^{25}_D$ -58° (c 0.05, $CHCl_3$); IR ν_{max} (film) 3020 (OH), 1210, 740 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.75 (1H, m, H-20), 5.35 (1H, m, H-6), 5.0 (2H, m, H-21), 3.55 (1H, br m, $W_{1/2}$ = 22.2, H-3), 2.30 (2H, m, H-4), 2.00 (1H, m, H-7a), 2.00 (1H, m, H-17), 1.85 (1H, m, H-1a), 1.85 (1H, m, H-2a), 1.73 (1H, m, H-12a), 1.73 (1H, m, H-15a), 1.55 (1H, m, H-7b), 1.55 (1H, m, H-11a), 1.55 (2H, m, H-16), 1.50 (1H, m, H1b), 1.50 (1H, m, H-2b), 1.45 (1H, m, H-11b), 1.23 (1H, m, H-15b), 1.10 (1H, m, H-8), 1.10 (1H, m, H-12b), 1.00 (1H, m, H-14), 1.00, (3H, s, H-19), 0.97 (1H, m, H-9), 0.6 (3H, s, H-18); ^{13}C NMR ($CDCl_3$) see Table 3.2.; LREIMS (70 eV) *m/z* (int, %) 272 (2), 267 (52), 213 (30), 145

(33), 107 (28), 105 (40), 91 (44), 85 (52), 83 (100); HREIMS *m/z* 300.2470 ($C_{21}H_{32}O$ requires 300.2453).

Pregna-5,20-diene-3α,7α,11α-triol 3α-acetate (182):

Fine needles; mp 226-229°C (with decomposition); $[\alpha]^{25}_D$ -65° (c 0.08, $CHCl_3$); IR (KBr disk) ν_{max} 3300 (OH), 2920, 1760, 1260 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 5.75 (1H, m, H-20), 5.60 (1H, m, H-6), 5.05 (1H, m, H-3), 5.00 (2H, m, H-21), 4.10 (1H, br s, $W_{1/2}$ = 22.6 Hz, H-11), 3.80 (1H, br s, $W_{1/2}$ = 13.3 Hz, H-7), 2.57 (1H, dd, *J* = 1.6, 15.1 Hz, H-4β), 2.42 (1H, m, H-1a), 2.27 (1H, dd, *J* = 1.6, 15.1 Hz, H-4α), 2.06 (1H, m, H-17), 2.02 (1H, m, H-9), 1.95 (3H, s, OAc), 1.90 (1H, m, H-16a), 1.85 (1H, m, H-15a), 1.80 (2H, m, H-2), 1.60 (1H, m, H-16b), 1.57 (1H, m, H-1b), 1.57 (1H, m, H-12a), 1.45 (1H, m, H-8), 1.45 (1H, m, H-14), 1.25 (3H, s, H-19), 1.23 (1H, m, H-15b), 1.10 (1H, m, H-12b), 0.65 (3H, s, H-18); ^{13}C NMR see Table 3.1; LREIMS (70 eV) *m/z* (int, %) 314 (100), 157 (10), 145 (16), 121 (11), 107 (17), 105 (17), 93 (13), 91 (22); HREIMS *m/z* 374.2450 ($C_{23}H_{34}O_4$ requires 374.2457)

Pregna-5,20-diene-3α,7α,11α-triol 3α,7α-diacetate (183):

Colourless oil; $[\alpha]^{25}_D$ -113 (c 0.09, $CHCl_3$); IR (film) ν_{max} 3450 (OH), 2940, 1750 OAc), 1450, 1420, 1370, 1240, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), 5.73 (1H, m, H-20), 5.60 (1H, m, H-6), 5.03 (1H, m, H-3), 5.00 (2H, m, H-21), 4.92 (1H, m, H7), 4.10 (1H, br s , H-11), 2.50 (1H, m, H-4β), 2.35 (1H, m, H-1a), 2.25 (1H, m, H-4α), 2.10 (1H, m, H-17), 2.06 (3H, s, OAc), 2.00 (1H, m, H-9), 1.95 (3H, s, OAc), 1.85 (1H, m, H-15a), 1.75 (2H, m, H-2), 1.65 (1H, m, H-8), 1.60 (1H, m, H-1b), 1.60 (1H, m, H-14), 1.60 (1H, m, H-15b), 1.60 (1H, m, H-16a) 1.43 (1H, m, H-12a), 1.20 (1H, m, H-16b), 1.17 (3H, 2, H-19), 1.15 (1H, m, H12b), 0.6 (3H, s, H-18); ^{13}C NMR see Table 3.1.; LREIMS (70 eV) *m/z* (int, %) 314 (100), 298 (29), 296 (22), 149 (42), 145 (30), 91 (33); HREIMS *m/z* 416.2563 ($C_{25}H_{36}O_5$ requires 416.2563).

Pregna-5,20-diene-3α,7α,19-triol 3α,19-diacetate (184):

Colourless oil; $[\alpha]^{25}_{D}$ -82° (c 0.04, $CHCl_3$); IR (film) ν_{max} 3500 (OH), 2930, 1730 (OAc), 1365, 1230, 1030, 1010, 950, 890 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.80 (1H, m, H-6), 5.75 (1H, m, H-20), 5.07 (1H, m, H-3), 5.00 (2H, m, H-21), 4.55 (1H, d, J = 11.9 Hz, H-19a), 3.95 (1H, d, J = 11.9 Hz, H-19b), 3.80 (1H, br s, $W_{1/2}$ = 11.8 Hz, H-7), 2.60 (1H, d, J = 15.2 Hz, H-4β), 2.30 (1H, d, J = 15.4 Hz, H-4α), 2.10 (3H, s, OAc), 2.10 (3H, s, OAc), 2.00 (1H, m, H-17), 1.90 (1H, m H-1a), 1.90 (1H, m, H-16a), 1.87 (1H, m, H-15a), 1.85 (1H, m, H-8), 1.80 (1H, m, H-2a), 1.75 (1H, m, H-12a), 1.70 (1H, m, H-2b), 1.67 (1H, m, H-11a), 1.60 (1H, m, H-16b), 1.50 (1H, m, H-1b), 1.50 (1H, m, H-11b), 1.45 (1H, m, H-14a), 1.42 (1H, m, H-9), 1.27 (1H, m, H-15b), 1.10 (1H, m, H-12b), 0.65 (3H, s, H-18); ^{13}C NMR see Table 3.1.; HREIMS *m/z* 416.2568 ($C_{25}H_{38}O_5$ requires 416.2563)

Pregna-5,20-diene-3α,7α,11α,19-tetrol 3α,7α,19-triacetate (185):

Yellow oil; $[\alpha]^{25}_{D}$ -114 (c 0.13, $CHCl_3$); IR (dry film) ν_{max} 3500 (OH), 2930, 1750 (OAc), 1365, 1240, 1010cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.85 (1H, d, J = 4.4 Hz, H-6), 5.73 (1H, m, H-20), 5.03 (1H, m, H-3), 5.00 (1H, m, H-21), 4.95 (1H, m, H-7), 4.95 (1H d, J = 11.9 Hz, H-19a), 4.22 (1H, d, J = 11.9 Hz, H-19b), 4.13 (1H, br s, $W_{1/2}$ = , H-11) 2.60 (1H, d, J = 14.9 Hz, H-4β), 2.56 (1H, m, H-1a), 2.30 (1H, d, J = 15.0 Hz, H-4α), 2.10 (1H, m, H-17), 2.05 (3H, s, OAc), 2.02 (3H, s, OAc), 2.00 (1H, m, H-12a), 1.95 (3H, s, OAc), 1.87 (1H, m, H-9), 1.85 (1H, m, H-16a), 1.80 (1H, m, H-8), 1.70 (2H, m, H-2), 1.63 (1H, m, H-14), 1.63 (1H, m, H-16b), 1.60 (1H, m, H-1b), 1.60 (1H, m, H-15a), 1.20 (1H, m, H-15b), 1.18 (1H, m, H-12b), 0.65 (3H, s, H-18); ^{13}C NMR see Table 3.2.; LREIMS (70 eV) *m/z* (int, %) 312 (75), 276 (67), 263 (86), 143 (76), 141 (86), 131 (78), 129 (63), 91 (100); HREIMS *m/z* 474.2616 ($C_{27}H_{28}O_7$ requires 474.2514, weak M^+, 414.2408 = M^+-CH_3OOH).

Acetylation of compound 180:

Compound **180** (11.1mg) was treated with Ac_2O (0.5 mL) and pyridine (0.5 mL) at room temperature overnight. The solvent was removed under reduced pressure and the white powder dissolved in EtOAc (1ml). Removal of the EtOAc *in vacuo* afforded the diacetate

as fine needles (12.2mg):

$[\alpha]^{25}_D$ -182° (*c* 0.01, $CHCl_3$); IR (KBr) ν_{max}, 2950, 1740 (OAc), 1375, 1245, 1020, 1000, 900 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.60 (3H, s, H-18), 1.00 (3H, s, H-19), 1.10 (1H, m, H-12b), 1.20 (1H, m, H-15b), 1.40 (1H, m, H-14), 1.45 (1H, m, H-11b), 1.50 (1H, m, H-1b), 1.55 (1H, m, H-9), 1.55 (1H, m, H-16b), 1.58 (1H, m, H-15a), 1.60 (1H, m, H-11a), 1.65 (1H, m, H-8), 1.68 (1H, m, H-1a), 1.73 (1H, m, H-12a), 1.80 (1H, m, H-16a), 1.80 (2H, m, H-2), 1.97 (3H, s, OAc), 2.00 (1H, m, H-17), 2.03 (3H, s, OAc), 2.25 (1H, m, H-4b), 2.50 (1H, m, H-4a), 4.93 (1H, m, H-21), 4.93 (1H, m, H-7), 5.00 (1H, m, H-3), 5.03 (1H, m, H-6), 5.75 (1H, m, H-20); ^{13}C NMR ($CDCl_3$) see Table 3.1.; LREIMS (70 eV) *m/z* (int, %) 298 (100), 280 (21), 265 (16), 211 (16), 159 (21), 143 (17), 105 (18), 91 (25)

Reduction of compound 180:

LAH (50mg) was added to a stirred solution of **180** (19.6 mg) in dry ether (6 mL). The solution was cooled in ice and 5% KOH (8 ml) added. The basic solution was extracted with ether, dried over anhydrous Na_2SO_4, filtered and concentrated to yield pregna-5,20-dien-3α,7α-diol as a clear oil (15.9mg):

$[\alpha]^{25}_D$ -122° (c 0.08, $CHCl_3$); IR (dry film) ν_{max} 3360 (OH), 2940, 1420, 1370, 1210, 1110, 1000, 985, 890, 745 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.60 (3H, s, H-18), 1.00 (3H, s, H-19), 1.10 (1H, m, H-12b), 1.25 (1H, m, H-15b), 1.25 (1H, m, H-2b), 1.42 (1H, m, H-8), 1.43 (1H, m, H-9), 1.45 (1H, m, H-11b), 1.50 (1H, m, H-14), 1.58 (1H, m, H-1b), 1.60 (1H, m, H-16b), 1.65 (1H, m, H-11a), 1.65 (1H, m, H-1a), 1.75 (1H, m, H-12a), 1.80 (1H, m, H-2a), 1.90 (1H, m, H-15a), 1.90 (1H, m, H-16a), 2.05 (1H, m, H-17), 2.20 (1H, m, H-4b), 2.60 (1H, m, H-4a), 3.85 (1H, br s, H-7), 4.10 (1H, br s, H-3), 4.98 (2H, m, H-21), 5.60 (1H, m, H-6), 5.75 (1H, m, H-20); ^{13}C NMR ($CDCl_3$) see Table 3.1.; LREIMS (70 eV) *m/z* 298 (100), 265 (33), 145 (230, 119 (23), 105 (26), 93 (22), 91 (35), 83 (40).

7.4 CHAPTER FOUR: EXPERIMENTAL

Invertebrate material:

UPES95-001 was collected in Algoa Bay, Port Elizabeth, South Africa. The sponge was collected at a depth of 15 m initially in 1994, recollected in 1995 and identified by Dr Michelle Kelly-Borges as a *Strongylodesma* sp. (Section 4.2.1).

SAF95-058 was collected from a depth of 28 m in the Tsitsikamma National Park, South Africa in 1995 and identified by Dr Michelle Kelly-Borges as an undescribed *Latrunculia* sp. (Section 4.2.1).

SAF96-039 was collected from a depth of 17-20 m at Oudekraal, Cape Town, South Africa, in 1996 and identified by Dr Michelle Kelly-Borges as an undescribed *Latrunculia* sp. (Section 4.2.1).

Extraction and isolation:

UPES95-001:

The freeze-dried sponge UPES95-001(49.5 g) was extracted with MeOH-CH_2Cl_2 (1:1) to give 5.77 g of a dark oil. This extract was fractionated according to Scheme 4.2. to give discorhabdin A (131 mg, 0.32% dry wt), discorhabdin D (5 mg, 0.01%), 3-dihydrodiscorhabdin C (10 mg, 0.02% dry wt), and discorhabdin H (11 mg, 0.03% dry wt).

SAF95-058:

Separate EtOAc (4.9 g) and MeOH (17.5 g) extracts were provided by Dr Brad Carte (SmithKline Beecham). A portion of the MeOH extract (1.59 g) was fractionated on a C-18 Sep-Pak cartridge, using a solvent gradient from H_2O to MeOH, containing 0.1% TFA. Fractions 5 and 6 (eluting with 30-40% aq. MeOH) contained pure discorhabdin A (180 mg). Fractions 2-4 (eluting with 20-30% aq. MeOH) were combined and further purified by C-18 reverse phase HPLC (MeOH-H_2O-TFA, 30:70:0.05) to give discorhabdin H (4 mg).

SAF96-039:

The frozen sponge was diced and freeze dried to give 185 g of dried material which was sequentially extracted with MeOH-CH_2Cl_2 (1:1) and MeOH. The MeOH-CH_2Cl_2 extract (32.2 g) was triturated with MeOH to give 5 g of MeOH soluble material which was partitioned further between 20% aq. MeOH and CH_2Cl_2. The aq. MeOH partition contained 3.7 g of a crude discorhabdin alkaloid. A portion of the crude alkaloid extract was fractionated on a C-18 Sep-Pak cartridge followed by reverse phase HPLC (Scheme 4.4) to give discorhabdin A (256 mg, 0.14%), discorhabdin H (201 mg, 0.11%) and 3-dihydrodiscorhabdin B (3.7 mg,0.002%).

Discorhabdin A (189), a dark-green solid, was characterized as its TFA salt, IR ν_{max} (KBr) 3440, 1740, 1650, 1620, 1520 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.57 (1H, s, H-1), 7.18 (1H, s, H-14), 5.37 (1H, d, J = 4 Hz, H-8), 4.53 (1H, dd, J = 6.5 Hz, H-5), 3.91 (1H, m, H-17a), 3.82 (1H, m, H-17b), 3.07 (1H, m, H-4a), 3.00 (1H, dd, J = 12.5, 4 Hz, H-7a), 2.97 (2H, m, H-16), 2.88 (1H, m, H-4b), 2.60 (1H, d, J = 12.5 Hz, H-7b); ^1H NMR (DMSO-d_6, 400 MHz) δ 13.18 (1H, br s, NH-13), 10.35 (1H, br s, NH-9), 9.43 (1H, br s, NH-18), 7.42 (1H, s, H-1), 7.38 (1H, s, H-14), 5.33 (1H, s, H-8), 4.58 (1H, dd, J = 6.4 Hz, H-5), 3.85 (1H, m H-17a), 3.71 (1H, m, H-17b), 2.7-3.1 (5H, m, H4, H-7a, H16), 2.48 (1H, d, J = 12 Hz, H-7b); ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 4.1, HRFABMS *m/z* 416.0072 $[M+H]^+$ (calcd for $C_{18}H_{15}{}^{79}Br\,N_3O_2S$)

Discorhabdin D (192), a dark-green solid, was characterised as its TFA salt; IR ν_{max} (KBr) 3400, 1650, 1620, 1550, 1520 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.09 (1H, s, H-14), 6.06 (1H, s, H-4), 5.60 (1H, d, J = 2.9 Hz, H-8), 4.34 (1H, t, J = 2.8 Hz, H-2), 3.98 (2H, m, H-17), 3.21 (1H, m, H-16a), 3.05 (1H, m, H-16b), 2.89 (1H, dd, J = 13.5, 2.5 Hz, H-1a), 2.79 (1H, dd, J = 12, 3.5 Hz, H-7a), 2.64 (1H, d, J = 12 Hz, H-7b), 2.57 (1H, dd. J = 13.4, 2.9 Hz, H-1b); ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 4.1.

3-Dihydrodiscorhabdin C (243) was characterised as its TFA salt, red solid; IR ν_{max} (film) 3300, 1690, 1670, 1690, 1530 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.16 (1H, s, H-14), 6.44 (1H, s, H-1 and H-4), 4.71 (1H, s, H-3), 3.64 (1H, m, H-8), 3.78 (2H, t, J = 7.4 Hz, H-17), 2.89 (2H, t, J = 7.4, H-16), 1.96 (2H, t, J = 5.0 Hz, H-7); ^1H NMR (DMSO-d_6,

400 MHz) see Table 4.2; ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 4.1, HRFABMS *m/z* 463.9609 $[M+H]^+$ (calcd for $C_{18}H_{16}{}^{79}Br_2N_3O_2$)

Discorhabdin H (197), a dark-green solid, was characterised as its TFA salt; IR ν_{max} (KBr) 3450, 1740, 1640, 1630, 1540 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 7.95 (1H, s, H-2'), 7.09 (1H, s, H-14), 6.19 (1H, s, H-4), 5.66 (1H, d, *J* = 3.6 Hz, H-8), 4.41 (1H, d, *J* = 3.2 Hz, H-2), 4.12 (1H, d, *J* = 3.2 Hz, H-1), 4.10 (1H, m, H-7'), 3.98 (1H, m, H-17a), 3.88 (1H, m, H-17b), 3.75 (3H, s, H-9'), 3.34 (1H, m H-6'), 3.25 (1H, m H-7a), 3.10 (2H, m, H-16), 2.80 (1H, d, *J* = 12.2 Hz, H-7b); ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 4.1, HRFABMS *m/z* 535.1226 $[M+H]^+$ (calcd for $C_{25}H_{23}N_6O_4S_2$).

3-Dihydrodiscorhabdin B (244), a red solid, was characterised as its TFA salt; IR ν_{max} (film) 3300, 1690, 1670, 1690, 1530 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) see Table 4.3; ^{13}C NMR (DMSO-d_6, 100 MHz) see Table 4.1, HRFABMS *m/z* 416.9968 $[M+H]^+$ (calcd for $C_{18}H_{15}{}^{79}BrN_3O_2S$).

Determination of Absolute configuration of histidine residue in discorhabdin H:

(a) Ozonolysis of discorhabdin H: Discorhabdin H (400 μg) in MeOH (400 μL) was cooled to -70 °C and a stream of ozone was bubbled into the cooled solution for 30 minutes (fading of colour). Hydrogen peroxide (200 μL, 50%) was added after allowing the solution to warm to room temperature. The solution was allowed to stand at room temperature for 60 min, after which excess reagent was removed under a stream of nitrogen. The reaction products were taken up in 5.0 N HCl (500 μL) and heated to 100 °C in a sealed reactivial for 16 h and cooled. The solvent was removed in a stream of dry nitrogen with heating, followed by drying under a high vacuum.

(b) Derivatisation and analysis by chiral GC-MS: To the hydrolysate obtained above was added 2-propanol (400 μL) and acetyl chloride (100 μL) in a 1 mL thick-walled reactivial which was securely capped. The solution was heated to 100 °C for 45 min, cooled and the solvent removed under dry nitrogen. Pentafluoropropionic anhydride (PFPA, 400 μL) in CH_2Cl_2 (400 μL) was added to the residue, the vial capped and the

solution heated at 100 °C for 15 min. The solvent was removed under a stream of dry N_2, the residue dissolved in CH_2Cl_2 (100 μL) and analysed by GC-MS using an Alltech Chirasil-Val capillary column (0.32 x 25 m). The oven temperature was ramped from 50 to 200 °C at 3 °C / min continuing to 210 °C at 5 °C / min, and a mass range of 50 to 600 Da was recorded every 1.96 s. The identity of the aspartic acid was confirmed by coinjection with a solution of authentic standard D and L aspartic acid which had been derivatised in the same manner.

Analysis of discorhabdin alkaloids by HPLC-ESMS

Mass spectral data were obtained with a Finnigan Matt LCQ Mass Spectrometer system (Finnigan Co., San Jose, CA) coupled to a Spectrasystem P2000 HPLC system using a μ-Bondapak C_{18} column (0.46 x 25 cm) with a mobile phase consisting of MeOH-H_2O, (6:4, pH 3 TFA), flow rate 1 mL min^{-1}. The mass spectrometer was operating in positive ion spray mode, set with a spray needle voltage of 4.45 kV, capillary temperature of 200°C, sheath gas flow of 30 U and auxiliary gas flow of 0 U. Sample concentration was 0.1 mg mL^{-1} and the injection volume was 20 μL.

7.5 CHAPTER FIVE: EXPERIMENTAL

General experimental procedures:

IR and UV spectra were recorded on a Perkin-Elmer 1600 and Lambda 3B instruments, respectively. ^{1}H, ^{13}C and DEPT NMR spectra were recorded on a Varian Gemini 400 spectrometer at 400 and 100 MHz. HMQC, HMBC and NOESY NMR experiments were recorded on a Varian Inova 300 spectrometer. Chemical shifts are reported in ppm and referenced to residual undeuterated solvent resonances ($CHCl_3$ at δ_H 7.26, $CDCl_3$ at δ_C 77.0) and coupling constants are reported in Hz. High resolution CI mass spectra using NH_3 were run on a VG 7070 mass spectrometer at the Regional Mass Spectrometry Facility at University of California, Riverside. Low resolution EI mass spectra (70 eV) were run on a Hewlett Packard 5988A mass spectrometer.

Collection, extraction and isolation:

Specimens of the orange coloured sponge were collected off Hout Bay, South Africa, by hand using SCUBA. The sponge was immediately frozen until it was freeze-dried and extracted sequentially in hexane, EtOAc, EtOAc-MeOH (1:1) and finally with MeOH. The EtOAc-MeOH extract (3.14g) was partitioned between H_2O and EtOAc and the organic partition was concentrated to give 1.34 g of crude material which was further fractionated on a diol Sep Pak (12 g, 35 mL) using CH_2Cl_2, MeOH-CH_2Cl_2 (20:80) and MeOH as eluants. The CH_2Cl_2 fraction (240 mg) was subjected to column chromatography on silica gel using a step gradient of hexane-EtOAc. Final purification was achieved by HPLC on a silica column using EtOAc-hexane (70:30) as a mobile phase to give dilemmaones A **(261)** (4.9 mg, 0.005% dry wt), **(262)** (1.2 mg, 0.0012% dry wt) and **(263)** (3.4 mg, 0.0035% dry wt).

Animal material:

In life, the sponge (*Antho* sp.) forms a relatively thick, elastic, encrustation that is bulbous or mammilate in places with the surface being finely dimpled. The exterior colour of the sponge is a bright reddish-orange and a brighter orange internally. The ectodermal skeleton consists of a thick crust of paratangential auxiliary subtylostyles with microspined heads. The choanosomal skeleton consists of a basal isotropic renieroid

reticulation of acanthostrongyles, with a primary dentritic plumose skeleton of smooth or slightly spined substylostyles arising from the sponge base. Hastate acanthostyles echinate the primary tracts. Microscleres are palmate isochelae accompanied by at least three sizes of toxas. The sponge is an undescribed species of the genus *Antho*, subgenus *Dirrhopalum* (order Poecilosclerida, family Microcionidae).

Dilemmaone A (261):

An off-white solid; mp 146-148 °C; IR (film, AgCl) ν_{max} 3342, 1654, 1131 cm^{-1}; UV ($CHCl_3$) λ_{max} 310 (ε 4100), 250 (ε 8060); ^1H NMR (300 MHz, $CDCl_3$) see Table 5.2; ^{13}C NMR (100 MHz, $CDCl_3$) see Table 5.1; LRCIMS *m/z* (rel. int) 230 $[M+H]^+$ (100), 200 (8), 198 (11); HRCIMS *m/z* 230.1177 $[M+H]^+$ (calcd for $C_{14}H_{16}NO_2$, 230.1181); LREIMS *m/z* (rel. int) 229 (97), 198 (100), 170 (42).

Dilemmaone B (262):

Buff solid; mp 168-170 °C; IR (AgCl) ν_{max} 3383, 1646, 1221, 1021, 772 cm^{-1}; UV ($CHCl_3$) λ_{max} 310 (ε 4230), 250 (ε 8420); ^1H NMR (300 MHz, $CDCl_3$) δ 9.68 (1H, br s, N-H), 6.98 (1H, s), 6.50 (1H, s), 4.88 (2H, s), 3.22 (2H, t, J = 5.4 Hz), 2.74 (2H, t, J = 5.4 Hz), 2.61(3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) see Table 5.1; LRCIMS *m/z* (rel. int) 216 $[M+H]^+$ (100), 200 (22), 198 (8); HRCIMS *m/z* 216.1026 $[M+H]^+$ (calcd for $C_{13}H_{14}NO_2$, 216.1024).

Dilemmaone C (263):

Pale yellow solid; mp 187-190 °C; IR (AgCl) ν_{max} 3337, 1671, 1143 cm^{-1}; UV ($CHCl_3$) λ_{max} 310 (ε 12330), 250 (ε 7950); ^1H NMR (300 MHz, $CDCl_3$) δ 9.37 (1H, br s, N-H), 6.44 (1H, s), 4.62 (2H, s), 3.46 (3H, s), 2.77 (2H, t, J = 5.4 Hz), 3.15 (2H, t, J = 5.4 Hz), 2.51 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) see Table 5.1; LRCIMS *m/z* (rel. int) 246 $[M+H]^+$ (100), 245 (15), 216 (17), 215 (9), 214 (23); HRCIMS *m/z* 246.1140 $[M+H]^+$ (calcd for $C_{14}H_{16}NO_3$, 246.1130); LREIMS *m/z* (rel. int) 245 (52), 214 (100).

7.6 CHAPTER SIX: EXPERIMENTAL

Preparation of *tert*-butyl hypochlorite:[248]

Regular Jik (500 mL), containing 3.3% w/v of NaOCl was placed in a 1 L flask equipped with a stirrer and the solution cooled to below 10 °C in diffuse light. A mixture of Bu^tOH (23 mL) and glacial acetic acid (19 mL) was added in a single portion and the mixture stirred for 5 min. The resulting mixture was transferred to a separator and the bottom aqueous layer was separated. The organic layer was washed with 10% aqueous Na_2CO_3 solution (50 mL) followed by H_2O (50 mL). The Bu^tOCl was dried over $CaCl_2$ and filtered to give 14.63 g (56%) of material which was stored over $CaCl_2$ under a N_2 atmosphere at 5°C.

Note: Bu^tOCl decomposes upon exposure to bright light, $CHCl_3$ and acetone with considerable evolution of heat; reacts violently when exposed to rubber, strong light or overheating.

Preparation of 3-methyl-2-(thiomethoxymethyl)aniline 309 and 5-methyl-2-thio-methoxymethyl)aniline (308)[222]

A vigorously stirred solution of *meta*-toluidine (0.44 g, 4.1 mmol) and dimethyl sulfide (0.76 g, 12.3 mmol) in 20 mL of dry CH_2Cl_2 was cooled to -65 °C under nitrogen. To this was added a solution of *tert*-butyl hypochlorite (0.44 g, 4.1 mmol) in dry CH_2Cl_2 (10 mL) over a 10 min period. The stirring was continued for an additional 2 h followed by warming to -30 °C over a 1 h period. Sodium methoxide (0.27 g, 4.92 mmol) in methanol (50 mL) was added over a 5 min period to the reaction mixture. This solution was allowed to warm to room temperature overnight. TLC analysis (SiO_2, EtOAc:hexane. 4:6) showed no starting material and indicated the presence of two new spots. The reaction mixture was quenched with water (20 mL) after which it was concentrated. The aqueous solution was extracted with CH_2Cl_2 (2 x 50 mL) and the combined organic extracts were dried over anhydrous sodium sulfate, filtered, and the solvent removed *in vacuo* to leave a dark oil (0.53 g) which was not purified any further. The ^1H NMR spectrum (400 MHz, $CDCl_3$) of the crude product mixture clearly showed paired signals at δ 1.99/2.09 (2 x $ArCH_2S\underline{C}H_3$), 2.27/2.35 (2 x $ArCH_3$), 3.67/3.78 (2 x $Ar\underline{C}H_2SCH_3$) due to the two isomers.

Preparation of 6- and 4-nitro-indan-1-one (310) and (311)[225,227]

Indan-1-one (4 g, 30.3 mmol) was dissolved in concentrated sulfuric acid (20 mL) and cooled to 0 °C. Fuming nitric acid (2 mL, 45.0 mmol) was added dropwise to the vigorously stirred mixture while maintaining the temperature below 10 °C. After all the nitric acid had been added the mixture was poured onto crushed ice (200 mL) and the aqueous solution was extracted with EtOAc (3 x 50 mL). The combined organic extracts were washed with saturated sodium bicarbonate solution, dried (Na_2SO_4) and concentrated to give a waxy solid. The two isomers were separated by chromatography on silica gel (EtOAc-Hexane, 1:1) to give the 6-and 4-nitro-indanones in a 22:78 ratio (61.6% total yield).

4-Nitroindan-1-one (311):

A yellow oil; IR ν_{max} (film) 1710, 1611, 1516 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) 8.45 (1H, dd, J = 0.6, 7.9), 8.44 (1H, dd, J = 0.7, 7.5), 7.60 (1H, t, J = 7.8), 3.64 (2H, t, J = 6.0), 2.79 (2H, t, J = 6.1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.4 (s), 150.0 (s), 146.4 (s), 140.2 (s), 129.9 (d), 129.6 (d), 128.7 (d), 35.8 (t), 26.8 (t); EIMS (70eV), *m/z* (int, %) 177 (560, 160 (48), 103 (93), 102 (100), 77 (64).

6-Nitroindan-1-one (310):

Yellow oil; IR ν_{max} (film) 1702, 1600 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.49 (1H, d, J = 1.7), 8.41 (1H, dd, J = 2.1, 8.4), 7.65 (1H, d, J = 8.4), 3.27 (2H, t, J = 6.0), 2.82 (2H, t, J = 6.1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.3 (s), 160.7 (s), 147.9 (s), 138.1 (s), 129.6 (d), 128.7 (d), 119.0 (d), 36.6 (t), 26.1 (t); EIMS (70eV), *m/z* (int, %) 177 (100), 103 (66), 91 (33), 77 (66).

Preparation of 6-nitro-1-(1,3-dioxolan-2-yl)indane (312):

The ketone **310** (0.99 g, 5.6 mmol), ethylene glycol (0.42 g, 6.72 mmol) and PTSA (20 mg) were heated under reflux in dry benzene (50 mL) with a Dean and Stark trap overnight. The mixture was washed with brine (3 x 25 mL), dried (Na_2SO_4), filtered and concentrated to give 1.14 g of crude material which was chromatographed on silica gel (EtOAc-Hexane, 35:65) to give 0.82 g (67 %) pure ketal **312** as a clear oil.

IR ν_{max} (film) 1611, 1516 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.12 (2H, m), 7.32 (1H, d, J = 8.6 Hz), 4.19 (2H, br s), 4.07 (2H, br s), 2.98 (2H, t, J = 8.6 Hz), 2.33 (2H, t, J = 7.0

Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.7 (s), 147.8 (s), 144.7 (s), 125.8 (d), 124.6 (d), 119.1 (d), 115.2 (s), 65.2 (t), 65.2 (t), 37.8 (t), 35.5 (t).

Preparation of 6-amino-1-(1,3-dioxolan-2-yl)indane (295):

The nitro compound **312** (575 mg, 2.60 mmol) was dissolved in EtOH (100 mL, 95%) and hydrogenated over PtO_2 (28 mg) until no more H_2 was absorbed. The catalyst was filtered off (celite) and the filtrate concentrated to give 444 mg (90 %) of pure amine **295**.

IR ν_{max} (film) 3412, 2923 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, d, J = 8.2 Hz), 6.65 (2H, m), 4.15 (2H, br s), 4.05 (2H, br s), 2.82 (2H, t, J = 6.7 Hz), 2.25 (2H, t, J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.5 (s), 143.6 (s), 133.2 (s), 125.6 (d), 117.5 (d), 115.0 (s), 110.5 (d), 65.1 (t), 65.1 (t), 37.3 (t), 35.5 (t); EIMS (70eV), *m/z* (int, %) 219 (100), 175 (28), 160 (72), 146 (36).

Preparation of 6-amino-1-(1,3-dioxolan-2-yl)-5-(thiomethoxymethyl)indane (313) and 6-amino-1-(1,3-dioxolan-2-yl)-7-(thiomethoxymethyl)indane (314):

A mixture of the amine **295** (300 mg, 1.57 mmol) and dimethyl sulfide (384 μL, 5.25 mmol) was taken up in dry CH_2Cl_2 (10 mL). The flask was covered with aluminium foil (to keep out light) and cooled to -60 °C under a dry N_2 atmosphere. *Tert*-butyl hypochlorite (300 μL, 2.52 mmol) in CH_2Cl_2 (250 μL) was added dropwise over 1 h at this temperature and the mixture was stirred for an additional 1 h. A freshly prepared solution of sodium methoxide (2.52 mmol) in MeOH (2 mL) was added and the reaction allowed to warm to room temperature overnight. The mixture was concentrated and passed through a silica gel plug to give 303 mg of crude material. Normal phase HPLC using EtOAc-Hexane (1:1) of a portion of this extract (89 mg) gave the 7-and 5-substituted isomers **(314, 313)** in a ratio of 77:23 (33% overall yield).

6-Amino-1-(1,3-dioxolan-2-yl)-5-(thiomethoxymethyl)indane (313):

IR ν_{max} (film) 3420, 2956 cm^{-1};^1H NMR ($CDCl_3$, 400 MHz) δ 6.88 (1H, s), 6.69 (1H, s), 4.16 (2H, m), 4.05 (2H, m), 3.66 (2H, s), 2.82 (2H, t, J = 6.8 Hz), 2.25 (2H, t, J = 6.8 Hz), 1.97 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.5 (s), 142.1 (s), 133.5 (s), 127.1 (d), 124.0 (s), 117.3 (s), 110.5 (d), 65.1 (t), 65.1 (t), 37.4 (t), 35.7 (t), 27.6 (t), 14.6 (q); EIMS (70eV), *m/z* (int, %) 251 (33, M^+), 204 (100), 160 (22); HREIMS *m/z* 251.0969 (calcd for $C_{13}H_{17}NO_2S$, 251.0979).

6-Amino-1-(1,3-dioxolan-2-yl)-7-(thiomethoxymethyl)indane (314):

IR ν_{max} (film) 3435, 2957 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, d, J = 8.1 Hz), 6.69 (1H, d, J = 8.0 Hz), 4.20 (2H, m), 4.06 (2H, m), 3.87 (2H, s), 2.78 (2H, t, J = 7.0 Hz), 2.20 (2H, t, J = 7.0 Hz), 2.01 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.3 (s), 138.6 (s), 134.9 (s), 124.5 (d), 119.0 (s), 118.9 (d), 118.8 (s), 64.3 (t), 64.3 (t), 37.2 (t), 28.5 (t), 27.1 (t), 14.3 (q); EIMS (70eV), *m/z* (int, %) 251 (67), 240 (38), 160 (100), 146 (30); HREIMS *m/z* 251.0979 (calcd for $C_{13}H_{17}NO_2S$, 251.0979).

Preparation of 6-nitro-1-(4'R, 5'R-4',5'-dimethyl-spiro-1',3'-dioxolan-2-yl)indane (319):

A solution of **311** (0.71 g, 4.0 mmol), (-)-(*R,R*)-2,3-butanediol (0.41g, 4.5 mmol), PTSA (36 mg) and dry benzene (60 mL) in a flask equipped with a Dean and Stark trap was heated at reflux for 11 h. After cooling to room temperature, the benzene was evaporated and the crude oil chromatographed on silica gel using EtOAc-Hexane (1:1) as eluant to give 0.22 g of the pure ketal **319** (22 %) as a clear oil:

IR ν_{max} (film) 3010, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.18 (1H, s), 8.16 (1H, m), 7.33 (1H, d, J = 8.0 Hz), 3.93 (1H, m), 3.78 (1H, m), 3.00 (2H, dt, J = 2.5, 6.7 Hz), 2.39 (2H, t, J = 6.8 Hz), 1.38 (3H, d, J = 6.0 Hz), 1.32 (3H, d, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 150.9 (s), 147.6 (s), 144.8 (s), 125.8 (d), 124.7 (d), 119.0 (d), 115.0 (s), 79.5 (d), 79.2 (d), 38.8 (t), 28.7 (t), 16.8 (q), 16.4 (q).

Preparation of 6-amino-1-(4'R, 5'R-4',5'-dimethyl-spiro-1',3'-dioxolan-2-yl)indane (318):

The nitro ketal **319** (170 mg, 0.68 mmol) was hydrogenated over PtO_2 (20 mg) in EtOH (50 mL) until no more H_2 was consumed. The catalyst was filtered off and the filtrate concentrated to give 90 mg (60%) of pure amine **318**.

IR ν_{max} (film) 3457, 3010 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, d, J = 8.1 Hz), 6.68 (1H, d, J = 2.1 Hz), 6.64 (1H, dd, J = 2.1, 8.0 Hz), 3.84 (2H, m), 3.74 (2H, m), 3.60 (2H, br s), 2.81 (2H, m), 2.25 (2H, t, J = 6.9 Hz), 1.35 (3H, d, J = 5.9 Hz), 1.29 (3H, d, J = 5.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 145.5 (s), 143.7 (s), 133.4 (s), 125.6 (d), 117.3 (d), 109.3 (d), 79.1 (d), 78.7 (d), 39.0 (t), 39.0 (t), 27.6 (t), 17.0 (q), 16.6 (q); EIMS

(70eV), *m/z* (int, %) 219 (100), 175 (28), 160 (72)146 (36); HREIMS *m/z* 219.1266 (calcd for $C_{13}H_{17}NO_2$, 219.1258)

Preparation of 6-amino-5-(thiomethoxymethyl)-1-(4'R, 5'R-4',5'-dimethyl-spiro-1',3'-dioxolan-2-yl)indane (320) and 6-amino-7-(thiomethoxymethyl)-1-(4'R, 5'R-4',5'-dimethyl-spiro-1',3'-dioxolan-2-yl)indane (321):

A mixture of the amine **318** (80 mg, 0.37 mmol) and dimethyl sulfide (68 μL, 0.93 mmol) was taken up in dry CH_2Cl_2 (5 mL). The flask was covered with aluminium foil and cooled to -65 °C under a dry N_2 atmosphere. *Tert*-butyl hypochlorite (53 μL, 0.44 mmol) in dry CH_2Cl_2(250 μL) was added dropwise over 1 h at this temperature and the mixture stirred for an additional 1 h. A freshly prepared solution of sodium methoxide (24 mg, 0.44 mmol) in MeOH (2 mL) was added and the mixture allowed to warm to room temperature overnight. The mixture was concentrated and passed through a silica plug (EtOAc) to give 302 mg of crude material. Normal phase HPLC using EtOAc-Hexane (35:65) of the crude extract (82 mg) gave **320** and **321** in a ratio of 24:76 (30% overall yield).

6-Amino-5-(thiomethoxymethyl)-1-(4'R, 5'R-4',5'-dimethyl-spiro-1',3'-dioxolan-2-yl)indane (320):

IR ν_{max} (film) 3398 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.85 (1H, s), 6.70 (1H, s), 4.03 (2H, br s), 3.85 (1H, m), 3.75 (1H, m) 3.66 (2H, s), 2.81 (2H, m), 2.28 (2H, t, J = 6.8 Hz), 1.95 (3H, s), 1.35 (3H, d, J = 6.0 Hz), 1.29 (3H, d, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 144.6 (s), 143.0 (s), 133.4 (s), 127.1 (d), 123.9 (s), 116.5 (s), 110.6 (d), 79.1 (d), 78.8 (d), 39.0 (t), 35.7 (t), 27.7 (t), 18.6 (t), 17.0 (q), 16.6 (q), 14.6 (q); EIMS (70eV), *m/z* (int, %) 279 (37), 232 (100), 188 (18); HREIMS *m/z* 279.1292 (calcd for $C_{15}H_{21}NO_2S$, 279.1292)

6-Amino-7-(thiomethoxymethyl)-1-(4'R, 5'R-4',5'-dimethyl-spiro-1',3'-dioxolan-2-yl)indane (321):

IR ν_{max} (film) 3456 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, d, J = 8.0 Hz), 6.68 (1H, d, J = 8.1 Hz), 4.05 (2H, br s), 3.85 (1H, m), 3.75 (1H, m), 3.65 (2H, s), 2.79 (2H, m), 2.28 (2H, t, J = 6.6 Hz), 1.98 (3H, s), 1.35 (3H, d, J = 6.0 Hz), 1.30 (3H, d, J = 6.0 Hz); ^{13}C, NMR ($CDCl_3$, 100 MHz) δ 145.3 (s), 138.7 (s), 134.6 (s), 124.5 (d), 119.0 (s),

118.8 (s), 116.5 (s), 79.2 (d), 78.8 (d), 39.0 (t), 35.7 (t), 27.6 (t), 16.8 (q), 16.5 (q), 14.6 (q); EIMS (70eV), *m/z* (int, %) 279 (100), 206 (72), 160 (73), 120 (24); HREIMS *m/z* 279.1285 (calcd for $C_{15}H_{21}NO_2S$, 279.1292).

Preparation of *para*-tolunitrile (326):[228]

Para-toluidine **327** (1 g, 9.35 mmol) was suspended in a mixture of concentrated hydrochloric acid (2.4 mL) and water (2.4 mL) and cooled to 0 °C with vigorous stirring. A solution of sodium nitrite (0.67 g, 9.71 mmol) in water (2 mL) was added dropwise, while maintaining the temperature below 5 °C. The mixture was stirred for an additional 15 min after which it was neutralised by the addition of solid sodium carbonate (0.56 g). The copper reagent was prepared by dissolving copper(I)cyanide (0.94 g, 10.5 mmol) in an aqueous solution of potassium cyanide (1.44 g, 22.1 mmol in 5 mL water) and heating it to 60 °C. The cold, neutralised, diazonium salt solution was added slowly to the vigorously stirred copper reagent at 60 °C. Heating was continued for another 20 min after which the mixture was allowed to cool to room temperature. The aqueous suspension was extracted with EtOAc (4 x 50 mL) and the combined organic extracts washed with brine and dried (Na_2SO_4). The extract was concentrated under reduced pressure to give 0.74 g of crude oil. Chromatography on silica gel (EtOAc-hexane, 1:4) gave pure **326** (57%).

IR ν_{max} (film) 2228, 1607, 1509, 1451; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.52 (2H, d, J = 8.2 Hz), 7.25 (2H, d, J = 8.0 Hz), 2.41 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 143.6 (s), 132.0 (d), 132.0 (d), 129.8 (d), 129.8 (d), 119.1 s), 109.3 (s), 21.8 (q).

Preparation of *para*-tolualdehyde (329) *via* nickel-aluminium / formic acid reduction of nitrile (326):[230, 231]

Para-tolunitrile **326** (100 mg, 0.86 mmol) was refluxed with formic acid (3 mL, 75%) and nickel-aluminium alloy (120 mg) for 1 h. The catalyst was filtered off and the filtrate diluted with water (20 mL). The aqueous solution was extracted with ether (3 x 20 mL) and the combined organic extracts were washed with saturated sodium bicarbonate solution, dried (Na_2SO_4) and concentrated to give 100 mg (88%) of pure aldehyde **329**.

IR ν_{max} (film) 2924, 1704, 1605, 1577; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.94 (1H, s), 7.75 (2H, d, J = 8.0 Hz), 7.43 (2H, d, J = 8.0 Hz), 2.39 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.8 (s), 145.5 (s), 134.1 (s), 129.9 (d), 129.8 (d), 129.6 (d), 129.4 (d), 21.8 (q).

Preparation of *ortho*-tolunitrile:[228]

o-Toluidine (36 g, 0.33 mol) was dissolved in a mixture of HCl (85 mL, conc.) and water (85 mL). The clear light brown solution was cooled to 0 °C in an ice-salt bath with vigorous stirring. The hydrochloride salt of *o*-toluidine precipitated out as a fine suspension. A solution of sodium nitrite (24 g, 0.35 mmol) in water (50 mL) was added slowly to the cold mixture while maintaining the temperature below 10°C. (30 min). The diazotised solution was neutralised with sodium carbonate (~20 g) maintaining the temperature below 10°C.

Copper(I)cyanide (25.8 g, 0.4 mol) was dissolved in a solution of potassium cyanide (52 g) and water (125 mL) and heated to 60 °C. The cold, neutral, diazotised solution was added slowly to the copper reagent at 60°C (frothing). The mixture was refluxed for 15 min and then steam-distilled . The distillate was collected (300 mL) and extracted with ether (3 x 100 mL). The combined ether layers were dried (Na2SO4), filtered and concentrated to give 19.37 g (50%) of pure *o*-tolunitrile.

IR ν_{max} (film) 2225, 1601, 1487, 1458, 761; ^{1}H NMR ($CDCl_3$, 400 MHz) 7.57 (1H, d, J = 7.7), 7.46 (1H, t, J = 7.2 Hz), 7.30 (1H, d, J = 7.8 Hz), 7.25 (1H, t, J = 7.6 Hz), 2.53 (3H, s).

Attempted preparation of *ortho*-tolualdehyde *via* nickel-aluminium / formic acid reduction of *o*-tolunitrile:[230]

o-Tolunitrile (1 g, 8.55 mmol) was refluxed with formic acid (3 mL, 75%) and nickel-aluminium alloy (1 g) for 1 h. The catalyst was filtered off and the filtrate diluted with water (20 mL). The aqueous solution was extracted with ether (3 x 20 mL) and the combined organic extracts were washed with saturated sodium bicarbonate solution, dried (Na_2SO_4) and concentrated to give 0.9 g of crude starting material.

Preparation of *ortho*-tolualdehyde *via* Raney nickel / formic acid reaction of *ortho*-tolunitrile: [231]

o-Tolunitrile (1 g, 8.55 mmol) was taken up in formic acid (25 mL, 75%) and approximately 3 g of Raney nickel was added. The reaction mixture was refluxed for 1.5 h, cooled and filtered through a celite pad. The filtrate was diluted with water (50 mL) and extracted with EtOAc (2 x 50 mL). The combined EtOAc layers were washed with water (2 x 50 mL), saturated sodium bicarbonate solution (2 x 50 mL) and dried (Na_2SO_4) to give 0.84 g (82%) of pure *o*-tolualdehyde.

IR ν_{max} (film) 2860, 2737, 1727, 1694, 1666, 1601, 754; ^{1}H NMR ($CDCl_3$, 400 MHz) 10.48 (1H, s); 7.79 (1H, d, J = 7.6 Hz), 7.47 (1H, t, J = 7.4 Hz), 7.35 (1H, t, J = 7.5 Hz), 7.27 (1H, d, J = 5.6 Hz), 2.67 (3H, s).

Preparation of 6-aminoindan-1-one (333):[227]

The 6-nitroindanone **311** (1.6 g, 9.0 mmol) was taken up in ethanol (100 mL, 95%) and was hydrogenated over PtO_2 (140 mg) until no more H_2 was consumed. The catalyst was filtered off and the filtrate concentrated to give 1.15 g (86 %) of pure amine.

IR ν_{max} (film) 3469, 1710 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.22 (1H, d, J = 8.1 Hz), 6.97 (1H, d, J = 2.2 Hz), 6.93 (1H, dd, J = 8.1, 2.2 Hz), 2.99 (2H, t, J = 5.6 Hz), 2.63 (2H, t, J = 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.8 (s), 145.9 (s). 145.8 (s), 138.0 (s), 127.1 (d), 123.0 (d), 107.8 (d), 36.8 (t), 25.0 (t);

Preparation of 6-cyano-indan-1-one (328):

The amine **333** (1.1 g, 7.48 mmol) was dissolved in a mixture of concentrated hydrochloric acid (10 mL) and water (10 mL) and the mixture heated to 60 °C for a 5 min until a clear solution was obtained. The amine hydrochloride solution was cooled on a salt-ice bath to -5 °C and a sodium nitrite (0.57 g, 8.23 mmol) solution in water (5 mL) was added drop wise. The reaction was stirred for another 20 min at -5 °C followed by the addition of sodium bicarbonate to neutralise the solution. Copper cyanide (0.67 g, 7.48 mmol) was dissolved in an aqueous solution of potassium cyanide (1.22 g, 18.7 mmol, in 20 mL water) with the aid of heat and cooled to 20 °C. The neutralised diazonium salt solution was added to the copper cyanide reagent and a thick, rust coloured crust formed which was difficult to stir. Benzene (50 mL) was added to improve the stirring and the

mixture was stirred overnight after which it was heated to 60 °C (30 min). The benzene layer was run off and the aqueous solution extracted with EtOAc (2 x 30 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated to give 0.92 g (78%) of crude material. Chromatography on silica gel (EtOAc-Hexane, 1:1) afforded the pure nitrile **328** in 39% yield (0.46 g).

IR ν_{max} (film) 2922, 2225, 1715, 1613 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.97 (1H, s), 7.79 (1H, d, J = 8.0 Hz), 7.59 (1H, d, J = 7.9 Hz), 3.21 (2H, t, J = 6.0 Hz), 2.73 (2H, t, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 204.5 (s), 159.0 (s), 137.6 (s), 137.0 (d), 127.9 (d), 127.9 (d), 117.9 (s), 111.6 (s), 36.0 (t), 26.2 (t); EIMS (70eV), *m/z* (int, %) 129.1 (72), 128.1 (45), 69.0 (100); HREIMS *m/z* 157.0524 (calcd for $C_{10}H_7NO$, 157.0528)

Preparation of indan-1-one-6-carbaldehyde (325):

The nitrile **328** (168.4 mg, 1.1 mmol) was heated at 90°C in formic acid (75 %, 10 mL) with nickel-aluminium alloy (160 mg) for 1 hr. The reaction mixture was cooled and filtered through a celite pad which was washed with chloroform (20 mL). Water (20 mL) was added and the lower chloroform layer was separated. The aqueous layer was extracted with a further 2 portions of chloroform. The chloroform extracts were combined and washed with sodium bicarbonate solution, dried (Na_2SO_4) and concentrated to give 136 mg (77 %) of pure aldehyde **325**.

IR ν_{max} (film) 2987, 1725, 1695 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.97 (1H, s), 8.10 (1H, s), 8.03 (1H, d, J = 7.9 Hz), 7.57 (1H, d, J = 7.9 Hz), 3.17 (2H, t, J = 6.0 Hz), 2.69 (2H, t, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.5 (s), 191.0 (s), 161.0 (s), 137.6 (s), 135.7 (s), 133.9 (d), 127.2 (d), 125.9 (d), 36.2 (t), 26.1 (t); EIMS (70eV), *m/z* (int, %) 148.1 (39), 131.1 (55), 120.1 (29); HREIMS *m/z* 160.0524 (calcd for $C_{10}H_8O_2$, 160.0524).

Attempted nitration of indan-1-one 6-carbaldehyde (325) with HNO_3 / H_2SO_4:

The aldehyde **325** (55.5 mg, 0.35 mmol) was dissolved in concentrated sulfuric acid (0.5 mL) and cooled on a salt-ice bath. Potassium nitrate (40 mg, 0.4 mmol) was added in a single portion and the mixture stirred at 5 °C for 10 min. The ice bath was removed and the solution stirred at room temperature for 1 h. The mixture was quenched with ice water (5 mL) and extracted with EtOAc (3 x 5 mL). The EtOAc extracts were combined,

washed with brine, dried (Na_2SO_4) and concentrated. The ^1H NMR spectrum of the crude showed a complex mixture of products.

Attempted nitration of indan-1-one 6-carbaldehyde (325) with nitronium tetrafluoroborate:[233]

To a stirred mixture of nitronium tetrafluoroborate (20 mg, 0.15 mmol) and acetonitrile (2 mL) at 5°C, was added a solution of indan-1-one 6-carbaldehyde (24.2 mg, 0.15 mmol) in acetonitrile (0.5 mL). The mixture was allowed to warm to room temperature (20 min) and was then heated to 60°C and maintained at this temperature for 30 min. The mixture was allowed to cool and was then quenched with ice-water (5 mL). The acetonitrile was evaporated off and the aqueous mixture extracted with CH_2Cl_2 (3 x 10 mL). The combined CH_2Cl_2 layers were dried (Na_2SO_4), filtered and concentrated. The ^1H NMR spectrum of this crude mixture showed mainly unreacted starting material.

Dinitration of phenyl propionic acid (334):[249]

Powdered phenyl propionic acid (10 g, 66.7 mmol) was added with, vigorous stirring, to concentrated sulfuric acid (25 mL, 98%) at 0°C. To this mixture was added fuming nitric acid (20 mL, 93%) while maintaining the temperature below 15°C followed by stirring at this temperature for another 2 h. The acid mixture was poured onto 500 mL of ice and the off-white precipitate was filtered off and dried to give 12.6 g (80%) of the dinitro-product. ^1H NMR ($CDCl_3$, 400 MHz) 8.63 (1H, d, J = 2.1 Hz), 8.25 (1H, dd, J = 8.1, 2.2 Hz), 7.58 (1H, d, J = 8.1 Hz), 3.17 (2H, t, J = 7.4 Hz), 2.60 (2H, t, J = 7.4 Hz).

Preparation of 3-(4-nitro-phenyl)-propionic acid (342):[250]

Powdered phenylpropionic acid (21.51 g, 0.143 mol) was dissolved in a mixture of glacial acetic acid (50 mL) and concentrated sulfuric acid (50 mL) and the yellow coloured solution cooled to 0 °C on an ice-salt bath. A mixture of concentrated sulfuric acid (50 mL) and nitric acid (50%, 20ml) was added drop wise to the vigorously stirred mixture while maintaining the temperature below 10 °C. The resulting thick suspension was poured onto 500 mL of crushed ice and the white precipitate filtered off and dried to give 24.69g (88%) of 4-nitrophenylpropionic acid **342**. This product contained a negligible amount of the ortho isomer and was used without further purification.

IR ν_{max} (film) 1725, 1600, 1525, 1350 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.97 (2H, d, *J* = 8.5 Hz), 7.24 (2H, d, *J* = 8.4 Hz), 2.89 (2H, t, *J* = 7.5 Hz), 2.49 (2H, t, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) 174.9 (s), 148.3 (s), 146.7 (s), 129.2 (d), 129.2 (d), 123.7 (s), 123.7 (s), 34.6 (t), 28.1 (t).

Preparation of ethyl 3-(4-nitro-phenyl)-propanoate (343):[250]

4-Nitrophenylpropionic acid **342** (13.02, 66.8mmol) was refluxed with absolute ethanol (15 mL) and concentrated sulfuric acid (0.5 mL) for 3 h. The ethanol was evaporated and the oil dissolved in EtOAc (50 mL) and washed with water (2 x 50 mL) and sodium bicarbonate solution (2 x 50 mL). The EtOAc extract was dried (Na_2SO_4) filtered and concentrated to give 13.73g (98%) of ethyl ester **343**.

IR ν_{max} (film) 3336, 1725, 1600, 1525, 1350 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$) δ 8.11 (2H, d, *J* = 8.0 Hz), 7.25 (2H, d, *J* = 8.6 Hz), 4.10 (2H, q, *J* = 8.0 Hz), 3.03 (2H, t, *J* = 7.6 Hz), 2.66 (2H, t, *J* = 7.6 Hz), 1.20 (3H, t, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.1 (s), 150.1 (s), 148.1 (s), 130.6 (d), 130.6 (d), 124.6 (d), 124.6 (d), 60.2 (t), 36.2 (t), 30.2 (t), 14.2 (q).

Preparation of ethyl 3-(4-amino-phenyl)-propanoate (344):

The nitroester **343** (10.16g, 49mmol) was dissolved in ethanol (100 mL, 95%) and hydrogenated over PtO_2 (100mg) until no more H_2 was consumed (10 h). The catalyst was filtered off and the filtrate concentrated to give 8.74g (100%) of aminoester **344**.

IR ν_{max} (film) 3431, 3340, 1730, 1614, 1519, 1435, 1273, 834 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$) δ 6.97 (2H, d, *J* = 8.2 Hz), 6.60 (2H, d, *J* = 8.2 Hz), 4.11 (2H, q, *J* = 7.2 Hz), 3.53 (2H, br s, NH_2), 2.83, (2H, t, *J* = 7.8 Hz), 2.55 (2H, t, *J* = 7.8 Hz), 1.22 (3H, t, *J* = 7.2 Hz); ^{13}C (100MHz, $CDCl_3$) δ 173.1 (s), 144.6 (s), 130.5 (s), 129.1 (d), 129.1 (d), 115.2 (d), 115.2 (d), 60.2 (t), 36.3 (t), 30.2 (t), 14.2 (q).

Preparation of ethyl 3-(4-amino-3-[thiomethoxymethyl]phenyl)propanoate (345):

The aminoester **344** (5 g, 26.0 mmol) and dimethyl sulfide (4.6 mL, 65.0 mmol) were taken up in dry dichloromethane (30 mL), the flask was covered with aluminium foil and cooled to –65 °C. *Tert*-butyl hypochlorite (3.7 mL, 31.0 mmol) in dry dichloromethane (10 mL) was added dropwise over a 45 min period. The reaction was followed by TLC

which showed only a small amount of starting material present after stirring at –65 °C for 2 h. Clean sodium (1.2 g, 52.0 mmol) was dissolved in anhydrous ethanol (15 mL) under a N_2 atmosphere. This sodium ethoxide solution was added slowly to the azasulfonium salt solution maintained at –63 °C and the reaction stirred at this temperature for another 1 h. The cooling bath was removed and the reaction stirred at room temperature overnight. A thick gel formed which was dissolved in dichloromethane (50 mL) and partitioned with water (50 mL) to give 3.54 g of organic extract. Flash chromatography of this extract on silica gel gave 1.66 g (25%) of pure **345**. The aqueous partition was carefully neutralised to pH 6 (dilute HCl) and extracted with EtOAc (3 x 30 mL) to give 2.37 g of pure carboxylic acid (45%).

IR ν_{max} (film) 3370, 1734, 1508, 1437, 824 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$) δ 6.91 (1H, d, J = 8.0 Hz), 6.83 (1H, s), 6.60 (1H, d, J = 8.0 Hz), 4.10 (2H, q, J = 7.2 Hz), 3.65 (2H, s), 2.81 (2H, t, J = 7.8 Hz), 2.53 (2H, t, J = 7.8 Hz), 1.96 (3H, s), 1.22 (3H, t, J = 7.2 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ 173.0 (s), 143.4 (s), 130.6 (d), 129.0 (d), 128.3 (d), 116.6, s), 115.2 (s), 60.3 (t), 36.3 (t), 35.5 (t), 30.1 (t), 14.6 (q), 14.2 (q); EIMS (70eV), *m/z* (int, %) 253 (60), 206 (100), 120 (62); HREIMS *m/z* 253.1143 (calcd for $C_{13}H_{19}NO_2S$, 253.1137).

Preparation of ethyl 3-(4-amino-3 methyl-phenyl)-propanoate (336):

Raney nickel was prepared, fresh before use. Nickel-aluminium alloy (6 g) was added slowly to a solution of NaOH (7 g) in H_2O (50 mL) at room temperature. When all the alloy had been added the mixture was stirred until H_2 evolution slowed down. The mixture was then heated on a boiling water until H_2 evolution slowed down again (2 h). The mixture was cooled and the alkaline solution decanted. The precipitated Raney nickel was washed with water (3 x 50 mL) and EtOH (3 x 50 mL, 95%).

The activated catalyst was transferred to a flask containing compound **345** (1.659 g, 6.56 mmol) and EtOH (50 mL) and mixture heated under reflux for 3 h. The catalyst was filtered off and the filter cake washed well with EtOH. The filtrate was concentrated to give 1.34 g (87%) of **336**.

IR ν_{max} (film) 3452, 3375, 1732, 1626, 1509, 756 cm^{-1}; ^1H NMR (400MHz, $CDCl_3$) δ 6.88 (1H, s), 6.85 (1H, d, J = 8.0 Hz), 6.59 (1H, d, J = 7.9 Hz), 4.11 (2H, q, J = 7.2 Hz), 3.50 (2H, br s, NH2), 2.81 (2H, t, J = 8.0 Hz), 2.55 (2H, t, J = 7.9 Hz), 2.13 (3H, s), 1.22

(3H, t, J = 7.1 Hz); ^{13}C NMR (100MHz, $CDCl_3$) δ 173.2 (s), 142.7 (s), 130.7 (s), 130.4 (d), 126.6 (d), 122.5 (s), 115.1 (d), 60.3 (t), 36.5 (t), 30.2 (t), 17.3 (q), 14.2 (q); EIMS (70eV), *m/z* (int, %) 207 (60), 120 (100); HREIMS *m/z* 207.1241 (calcd for $C_{12}H_{17}NO_2$, 207.1260).

Preparation of 3-(4-bromo-3-methyl phenyl)-propionic acid (337):

The aniline **336** (713 mg, 3.44 mmol) was taken up in a mixture of H_2O (4 mL) and HBr (4 mL, 48%). A pink solid formed immediately which dissolved on heating to give a clear light brown solution. The amine hydrochloride solution was cooled to 0 °C and a solution of $NaNO_2$ (280 mg, 4.06 mmol) in H_2O (2 mL) was added dropwise. The copper reagent was prepared by mixing cuprous sulfate pentahydrate (1.2 g, 4.8 mmol) and sodium bromide (0.76 g, 7.4 mmol) with H_2O (5 mL). A clear light blue solution formed which was heated on a water bath for 5 min. The copper bromide precipitated as a fine powder on the addition of a solution of sodium metabisulfite (1.05 g, 5.54 mmol) in water (3 mL). The mixture was cooled to room temperature and the aqueous solution decanted. The CuBr precipitate was washed with H_2O (3 x 5 mL) and finally dissolved in HBr (4 mL, 48%). The CuBr reagent was cooled in a salt-ice bath to 0 °C and the cold diazonium salt solution added drop wise. A clear dark solution formed which was stirred on the ice bath for another 1 h and stood overnight (no stirring). A thick crust formed after a few hours which gradually disappeared. The aqueous solution was extracted with $CHCl_3$ (3 x 15 mL). The combined $CHCl_3$ extracts were washed with brine, dried ($MgSO_4$) and concentrated to give 0.52 g of crude material. Column chromatography (SiO_2, $CHCl_3$) gave **337** in 52% yield.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.41 (1H, d, J = 8.1 Hz), 7.06 (1H, d, J = 1.7 Hz), 6.88 (1H, dd, J = 2.0, 8.1 Hz), 2.87 (2H, t, J = 7.7 Hz), 2.63 (2H, t, J = 7.7 Hz), 2.36 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz) 178.3 (s), 139.4 (s), 137.8 (s), 132.3 (d), 130.8 (d), 127.2 (d), 122.6 (s), 35.3 (t), 29.8 (t), 22.8 (q).

Preparation of 6-bromo-5-methyl indan-1-one (338) and 6-bromo-7-methyl indan-1-one (346):

The carboxylic acid **337** (0.45 g, 1.86 mmol) was placed in a dry 50 mL RB flask equipped with a magnetic stirrer, condenser and $CaCl_2$ drying tube. Thionyl chloride (0.2 mL, 2.8 mmol) was added *via* a syringe and the reaction stirred gently at room temperature for 30 min, followed by heating at 70 °C for 10 min. Excess $SOCl_2$ was removed under reduced pressure (water aspirator) gently heating the mixture to 70 °C (10 min). The light yellow oil was dissolved in dry dichloroethane (5 mL) and powdered $AlCl_3$ (320 mg, 2.4 mmol) was added. A reflux condenser and drying tube was attached and the mixture stirred at room temperature for 1h. The reaction mixture rapidly turned a dark brown to violet colour with concomitant evolution of gas. After a few minutes the gas evolution slowed down and finally stopped completely. The solution was cooled to room temperature and quenched with ice (50 mL). A few drops of concentrated hydrochloric acid was added to dissolve precipitated $Al(OH)_2$ and the mixture transferred to a separatory funnel and extracted with $CHCl_3$ (3 x 5 mL). The combined $CHCl_3$ extracts were washed with saturated sodium bicarbonate solution, dried (Na_2SO_4) and concentrated to give 226 mg of crude material. Column chromatography (SiO_2, CH_2Cl_2) of the crude material followed by normal phase HPLC (EtOAc-hexane, 3:4) gave **(338)** and **(346)** in a 2:3 ratio (36% overall yield).

6-Bromo-5-methyl indan-1-one (338):

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.91 (1H, s), 7.35 (1H, s), 3.04 (2H, t, J = 6.4 Hz), 2.69 (2H, t, J = 6.0 Hz), 1.53 (3H, s); ^{13}C NMR δ 205.1 (s), 154.0 (s), 144.9 (s), 136.8 (s), 128.6 (d), 127.5 (d), 124.4 (s), 36.6 (t), 29.7 (t), 23.9 (q); EIMS (70eV), *m/z* (int, %) 198.0 (14), 196.0 (25), 117.1 (100); HREIMS *m/z* 223.9849 (cald for $C_{10}H_9O^{79}Br$, 223.9837).

6-Bromo-7-methyl indan-1-one (346):

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.68 (1H, d, J = 8.1 Hz), 7.15 (1H, d, J = 8.2 Hz), 3.00 (2H, t, J = 6.1 Hz), 2.72 (3H, s), 2.69 (2H, t, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 206.7 (s), 155.0 (s), 138.7 (s), 137.6 (d), 135.8 (s), 125.2 (d), 125.0 (s), 37.1 (t), 24.7 (t), 17.0 (q); EIMS (70eV), *m/z* (int, %) 181.0 (19), 169.0 (17); HREIMS *m/z* 223.9845 (calcd for $C_{10}H_9O^{79}Br$, 223.9837).

Model reactions: Intramolecular cyclisation of phenyl propionic acid with polyphosphoric acid as catalyst:

Powdered phenyl propionic acid (0.5 g, 3.33 mmol) was added to polyphosphoric acid (3 g) at 90 °C (water bath). The mixture was stirred with a glass rod at this temperature until all the phenyl propionic acid had dissolved (5 min). The clear yellow coloured mixture was heated until a dark-red mixture was obtained (1.5 h). Excess PPA was hydrolysed by the addition of crushed ice and allowing the mixture to stand at room temperature for 1 h. Off-white crystals separated out which were collected by vacuum filtration to give 360 mg (82%) of pure indanone.

^{1}H NMR ($CDCl_3$, 400 MHz) δ7.74 (1H, d, J = 7.6 Hz), 7.60 (1H, t, J = 7.4 Hz), 7.46 (1H, d, J = 7.6 Hz), 7.35 (1H, t, J = 7.4 Hz), 3.13 (2H, t, J = 5.5 Hz), 2.67 (2H, t, J = 5.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.1 (s), 155.1 (s), 137.0 (s), 134.6 (d), 127.2 (d), 126.5 (d), 123.7 (d), 36.2 (t), 25.8 (t).

Attempted cyclisation of 3-(4-bromo-3-methylphenyl)propionic acid (337):

3-(4-Bromo-3-methylphenyl)propionic acid (100 mg, 0.41 mmol) was added to polyphosphoric acid (1 g) at 90 °C (water bath). The mixture was stirred with a glass rod at this temperature for 1.5 h. Excess PPA was hydrolysed by the addition of crushed ice and allowing the mixture to stand at room temperature for 1 h. The aqueous mixture was extracted with CH_2Cl_2 (3 x 15 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated. ^{1}H NMR of this crude showed only unreacted starting material.

Preparation of *para*-bromotoluene (350):[236]

Para-toluidine **(327)** (5.4 g, 50 mmol) was taken up in a mixture of H_2O (15 mL) and HBr (15 mL, 48%). The amine hydrochloride solution was cooled to 0 °C on an ice-salt bath and a solution of $NaNO_2$ (3.5 g, 50 mmol) in H_2O (10 mL) was added dropwise. The copper reagent was prepared by mixing cuprous sulfate pentahydrate (12 g, 48 mmol) and sodium bromide (1.5 g) with H_2O (10 mL). A clear light blue solution formed which was heated on a water bath for 5 min. The copper bromide precipitated as a fine powder on the addition of a solution of sodium metabisulfite (11 g, 55.4 mmol) in water (10 mL). The mixture was cooled to room temperature and the aqueous solution decanted. The CuBr precipitate was washed with H_2O (3 x 5 mL) and finally dissolved in HBr (15 mL, 48%). The CuBr reagent was cooled in a salt-ice bath to 0 °C and the cold diazonium salt

solution added drop wise. A clear dark solution formed which was stirred on the ice bath for another 1 h and stood overnight (no stirring). A thick crust formed after a few hours which gradually disappeared. The aqueous solution was extracted with $CHCl_3$ (3 x 25 mL). The combined $CHCl_3$ extracts were washed with brine, dried ($MgSO_4$) and concentrated to give 6.2 g (72%) of **350**.

^1H NMR ($CDCl_3$, 400 MHz) 7.32 (2H, d, J = 8.1 Hz), 6.94 (2H, d, J = 8.1 Hz), 2.25 (3H, s).

Preparation of *para*-tolualdehyde (329) *via* a lithium-halogen exchange procedure:[237]

p-Bromotoluidine (1.0 g, 5.85 mmol) was taken up in dry THF (5 mL) and cooled to -50°C under a N_2 atmosphere. *n*-Butyllithium (3.7 mL, 5.85 mmol, 1.6 M in hexane) was added over a 10 min period. After stirring for 1 hr at -50 °C, formylpiperidine (0.65 mL, 5.85 mmol) was added and the reaction allowed to warm to room temperature. The mixture was stirred under N_2 overnight (8 h). Concentrated hydrochloric acid (5 mL) was added and the mixture stirred for 30 min. The THF was evaporated under reduced pressure and the aqueous solution was extracted with $CHCl_3$ (3 x 20 mL). The combined $CHCl_3$ extracts were washed with a saturated sodium bicarbonate solution, dried and concentrated to give 0.69 g (97%) of *p*-tolualdehyde **329**.

Preparation of ethyl azidoacetate:

Preparation of ethyl chloroacetate:[251]

A mixture of monochloroacetic acid (65g). absolute ethanol (95g) and concentrated H_2SO_4 (11g) was refluxed for 36 h, then poured into excess of water, and the lower layer separated. The organic layer was washed with $NaHCO_3$ solution, dried (Na_2SO_4), and distilled through an efficient fractionating column to give 65g of pure ethyl chloroacetate, bp. 142°C/751mm.

Preparation of ethyl azidoacetate:[252]

Ethyl chloroacetate (23.1 g, 0.19 mol) and absolute ethanol (40 g) were heated under reflux during 3 h with sodium azide (13.6 g, 0.21 mol), and sufficient water (40 mL) too maintain the salt in solution. The mixture was transferred to a separator and water (70 mL) added. The lower organic layer was washed with water (2 x 20 mL), dried over $CaCl_2$, and redistilled under reduced pressure to give 10.6 g of ethyl azidoacetate.

Preparation of ethyl 2-azido-3-(4-methylphenyl)propenoate (351):[214]

Sodium (0.758 g, 33 mmol) was dissolved in anhydrous ethanol (15 mL) at room temperature, under a N_2 atmosphere. The resulting sodium ethoxide solution was cooled to -10 °C and a mixture of the aldehyde **329** (1.03 g, 8.58 mmol), ethyl azidoacetate (3.89 g, 33.8 mmol) and anhydrous ethanol (2 mL) was added slowly during 1 h. The mixture was stirred for another 1 h at -10 °C after which it was allowed to warm to room temperature and was then poured into water (20 mL). The aqueous mixture was extracted with EtOAc (3 x 30 mL). The combined EtOAc phases were washed with aqueous ammonium chloride solution (10%, 30 mL), dried (Na_2SO_4) and concentrated to give 82% of the unstable azide **351** which was used without further purification.

IR ν_{max} (film) 2982, 2121, 1713, 1617, 764; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.71 (2H, d, J = 8.1 H), 7.18 (2H, d, J = 8.1 Hz), 6.89 (1H, s), 4.36 (2H, q, J = 7.2 Hz), 2.37 (3H, s), 1.39 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 163.6 (s), 139.7 (s), 130.5 (d), 130.5 (d), 130.4 (s), 129.1 (d), 129.1 (d), 125.5 (s)124.6 (s), 62.1 (t), 21.4 (q), 14.1 (q).

Preparation of ethyl 6-methyl indole-2-carboxylate (352):[214]

The azidocinnamate **351** (500 mg, 2.17 mmol) was heated in refluxing toluene (10 mL) for 3 h. The toluene was removed under *in vacuo* to give a yellow powder. The crude product was chromatographed on silica gel (EtOAc-Hexane, 2:8) to give the indole carboxylate **352** (23%).

^{1}H NMR ($CDCl_3$ (400 MHz) δ 8.99 (1H, br s), 7.56 (1H, d, J = 8.2 Hz), 7.19 (2H, s), 6.98 (1H, d, J = 8.3 Hz), 4.41 (2H, q, J = 7.1 Hz), 2.47 (3H, s), 1.42 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 162.2 (s), 137.4 (s), 135.5 (s), 126.9 (s), 125.4 (s), 122.9 (d), 122.1 (d), 111.5 (d), 108.6 (d), 60.9 (t), 21.9 (q), 14.4 (q); EIMS (70eV), *m/z* (int, %) 203 (80), 175 (100), 157 (22), 129 (29), 102 (14).

Attempted preparation of 6-bromo-5-methyl-1-(1,3-dioxalan-2-yl)indane (358):

Bromoketone **357** (50 mg, 0.22 mmol), ethylene glycol (100 mg, 1.6 mmol) and PTSA (5 mg) was refluxed in dry benzene (25 mL) for 24 h. The solution was cooled and the benzene evaporated *in vacuo* to mainly unreacted starting material.

Attempted formylation of phenyl propionic acid with dichloromethyl methyl ether and titanium tetrachloride.

Phenyl propionic acid (250 mg, 1.67 mmol) was dissolved in dry CH_2Cl_2 (10 mL), under a N_2 atmosphere and the solution cooled to 0°C. Titanium tetrachloride (0.31 mL, 2.8 mmol) was added dropwise followed by the addition of dichloromethyl methyl ether (0.12 mL, 1.39 mmol). The reaction mixture was stirred at 0°C for 5 min and allowed to warm to room temperature (30 min) after which it was heated at 35°C for 15 min. The reaction was quenched by the addition of ice-water (10 mL). The organic fraction was separated, and the aqueous fraction was then extracted with CH_2Cl_2 (3 x 25 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated to give 233 mg of crude material which was shown by TLC and ^{1}H NMR to consist mainly of mainly starting material.

Attempted formylation of phenyl propionic acid by the Vilsmeier procedure (formylpiperidine and phosphorus oxychloride):

N-formyl piperidine (190 μL, 1.7 mmol), phosphorus oxychloride (160 μL, 1.7 mmol) and CH_2Cl_2 (250 μL) were mixed under a dry N_2 atmosphere and stood for 15 min. Phenyl propionic acid (250 mg, 1.67 mmol) in CH_2Cl_2 (0.5 mL) was added dropwise over 5 min and the mixture was stirred at room temperature for 1 h.The reaction was quenched with water and extracted with CH_2Cl_2 (2 x 10 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated to give 216 mg of crude material which contained mainly unreacted starting material as determined by TLC and ^{1}H NMR.

Preparation of ethyl 3-(4-cyano-phenyl)-propanoate (355):

The amine **344** (6.1 g, 31.6 mmol) was dissolved in a mixture of concentrated hydrochloric acid (15 mL) and water (15 mL) and cooled to 0 °C. A fine white precipitate formed to which was added a solution of sodium nitrite (2.4 g, 34.8 mmol) in water (15

mL). The mixture was stirred for 1 h at 0 °C. The copper cyanide reagent was prepared by dissolving copper cyanide (3.2 g, 34.8 mmol) in a solution of potassium cyanide (5.16 g, 79.0 mmol) and water (40 mL) and heating the mixture on a water bath until a clear light brown solution was formed. The cold diazotised solution was neutralised by the addition of sodium bicarbonate until it was slightly basic to litmus paper. This solution was added slowly to the copper reagent at room temperature with vigorous stirring. A rusty brown precipitate formed that was stirred overnight. The addition of 50 mL of benzene improve the stirring. The mixture was heated to 60 °C for 30 min and cooled. The benzene layer was run off and the aqueous partition extracted with dichloromethane (3 x 30 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to give 5.673 g, (88 %) of crude material. Chromatography of the crude on silica gel (EtOAc-Hexane, 35:65) gave 3.81 g (59%) of pure nitrile **355**.

IR ν_{max} (film) 2228, 1734, 1608 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.59 (2H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.1 Hz), 4.11 (2H, q, J = 7.1 Hz), 2.99 (2H, t, J = 7.6 Hz), 2.62 (2H, t, J = 7.6 Hz), 1.21 (3H, t, J = 7.1); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1 (s), 146.2 (s), 132.2 (d), 132.2 (d), 129.2 (d), 129.2 (d), 118.9 (s), 110.3 (s), 60.6 (t), 35.1 (t), 30.9 (t), 14.1 (q); EIMS (70eV), *m/z* (int, %) 203 (26), 149 (29), 129 (100); HREIMS *m/z* 203.0943 (cald for $C_{12}H_{13}NO_2$, 203.0946);

Preparation of ethyl 3-(4-phenylcarbaldehyde)propanoate (356):

The nitrile **355** (1.5 g, 7.39 mmol) was heated gently (80-90 °C) with nickel-aluminium alloy (1.46 g) in formic acid (50 mL, 75%) for 1 h. The mixture was cooled, filtered through celite and transferred to a separator. Dichloromethane (30 mL) was added and sufficient water to allow 2 phases to form. The combined CH_2Cl_2 layers were washed with brine (3 x 30 mL), dried (Na_2SO_4) and concentrated to give 1.3709 g (90%) of crude material that gave a positive DNP test. The crude material was chromatographed on silica gel (EtOAc-Hexane, 3:7) to give 1.12 g (62%) of a clear yellow oil.

IR ν_{max} (film) 2982, 1737, 1702, 1607, 1576; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.94 (1H, s), 7.77 (2H, d, J = 8.1 Hz), 7.34 (2H, d, J = 8.0 Hz), 4.09 (2H, q, J = 7.2 Hz), 3.00 (2H, t, J = 7.6 Hz), 2.63 (2H, t, J = 7.6 Hz), 1.19 (2H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.9 (s), 172.4 (s), 147.9 (s), 134.8 (s), 130.0 (s), 130.0 (d), 130.0 (d), 129.0 (d), 60.6

(t), 35.2 (t), 31.0 (t), 14.1 (q); EIMS (70eV), *m/z* (int, %) 135.0 (44), 132.1 (100), 105.1 (27); HREIMS *m/z* 206.0935 (calcd for $C_{12}H_{14}O_3$, 206.0943)

Preparation of ethyl 2-azido-3-(ethyl 4-phenylpropanoate)propenoate (357):

Clean sodium (0.462 g, 20.1 mmol) was dissolved in anhydrous ethanol (15 mL) at room temperature under a N_2 atmosphere. The sodium ethoxide solution was cooled to -10 °C and a mixture of the aldehyde (1.0 g, 4.85 mmol) and ethyl azidoacetate (2.233 g, 19.4 mmol) and dry ethanol (2 mL) was added drop wise over 30 min at -10 °C. The mixture was stirred for an additional 30 min at -10 °C and allowed to warm to room temperature over 1 h. The mixture was diluted with EtOAc (30 mL) and transferred to a separator and washed with aqueous ammonium chloride solution (10%, 3 x 15 mL). The EtOAc partition was dried (Na_2SO_4) and concentrated to give 316 mg (82%) of crude azide **357** which was used without further purification.

IR ν_{max} (film) 2112, 1732, 1606 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.71 (2H, d, J = 8.1 Hz), 7.19 (2H, d, J = 8.0 Hz), 6.87 (1H, s), 4.33 (2H, q, J = 7.1 Hz), 4.23 (2H, q, J = 7.1 Hz), 2.94 (2H, t, J = 7.7 Hz), 2.60 (2H, t, J = 7.7 Hz), 1.35 (3H, t, J = 7.1 Hz), 1.20 (3H, t, J = 7.2 Hz); ^3C NMR ($CDCl_3$, 100 MHz) δ 172.3 (s), 163.5 (s), 142.2 (s), 131.3 (s), 130.7 (d), 130.7 (d), 128.5 (d), 128.5 (d), 125.1 (d), 125.1 (s), 62.1 (t), 60.4 (t), 35.5 (t), 30.8 (t), 14.1 (q), 14.1 (q)

Preparation of ethyl 6-(2-ethoxycarbonyl-ethyl)-indole-2-carboxylate (358):

The azidocinnamate **357** (500 mg, 1.58 mmol) was refluxed in toluene (25 mL) for 3 h. The toluene was evaporated and the residue chromatographed on silica gel (EtOAc-hexane, 2:8) to give 177 mg (39%) of pure indole **358**.

IR ν_{max} (film) 3330, 1711, 1525, 1247, 1197 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.25 (1H, br s), 7.50 (1H, d, J = 8.2 Hz), 7.23 (1H, s), 7.16 (1H, s), 6.98 (1H, d, J = 8.3 Hz), 4.39 (2H, q, J = 7.1 Hz), 6.57 (2H, q, J = 7.1 Hz), 3.03 (2H, t, J = 7.7 Hz), 2.64 (2H, t, J = 7.7 Hz), 1.37 (3H, t, J = 7.1 Hz), 1.19 (3H, t, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ EIMS (70eV), *m/z* (int, %) 215.1 (18), 202.1 (50), 169.1 (20), 69 (100); HREIMS *m/z* 289.1321 (calcd for $C_{16}H_{19}NO_4$, 289.1314).

Attempted preparation of ethyl 5-nitro-6-(2-ethoxycarbonyl-ethyl)-indole-2-carboxylate (359):

The indole diester **358** (100 mg, 0.34 mmol) was dissolved in concentrated sulfuric acid (0.5 mL) and the cherry-red mixture cooled to 0 °C on a salt-ice bath. A mixture of fuming nitric acid (60 mg, 0.9 mmol) and concentrated sulfuric acid (0.5 mL) was added drop wise to the cooled, well-stirred solution. The mixture was stirred for another 30 min after which it was poured onto crushed ice and extracted with EtOAc (3 x 15 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated to give 123 mg of crude material. ^{1}H NMR of the crude indicated a complex mixture with only a small amount of the nitroindole present in the mixture.

Preparation of ethyl 3-(2-nitro-4-phenylcarbaldehyde)propanoate (360):

The aldehyde **356** (1.3 g, 6.31 mmol) was dissolved in concentrated sulfuric acid (4 mL) and cooled on a salt-ice bath. A mixture of fuming nitric acid (0.5 g, 7.57 mmol) and concentrated sulfuric acid (4 mL) was added drop wise over 10 min with vigorous stirring. Stirring was continued for another 30 min at this temperature after which the mixture was poured onto crushed ice. The off-white precipitate that formed was extracted with EtOAc (2 x 20 mL) and the combined extracts washed with sodium bicarbonate solution, dried (Na_2SO_4) and concentrated to give 1.39 g of crude nitro compound. Chromatography of the crude oil on silica gel (EtOAc-hexane, 1:1) gave 1.02 g (65%) of the pure nitro compound **360**.

^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.03 (1H, s), 8.4 (1H, d, J = 1.3 Hz), 8.03 (1H, dd, J = 1.4, 7.8 Hz), 7.61 (1H, d, J = 7.9 Hz), 4.11 (2H, q, J = 7.1 H), 3.29 (2H, t, J = 7.1 Hz), 2.73 (2H, t, J = 7.4 Hz), 1.21 (3H, t, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 189.3 (s), 171.8 (s), 141.9 (s), 135.7 (s), 133.3 (d), 132.7 (d), 126.0 (d), 100.6 (s), 60.8 (t), 34.3 (t), 28.4 (t), 14.1 (q).

Preparation of ethyl 3-(4-[1,3-dioxolan-2-yl]-2-nitrophenyl)propanoate (361):

The aldehyde **360** (5.7 g, 22.7 mmol), ethylene glycol (1.549 g, 25 mmol) and PTSA (216 mg, 1.14 mmol) were heated under reflux with dry benzene (100 mL) on a Dean and Stark trap equipped with a calcium chloride drying tube and 4Å molecular sieves. After 4.5 h

the reaction mixture was cooled and washed with saturated sodium bicarbonate solution (2 x 30 mL), dried (Na_2SO_4) and concentrated to yield 6.86 g (100%) of the ketal **361**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (1H, d, *J* = 1.4 Hz), 7.60 (1H, dd, *J* = 1.4, 7.9 Hz), 7.40 (1H, d, *J* = 7.9 Hz), 5.81 (1H, s), 4.06, (6H, m), 3.20 (2H, t, *J* = 7.5 Hz), 2.67 (2H, t, *J* = 7.5 Hz), 1.21 (3H, t, *J* = 7.1 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.2 (s), 149.1 (s), 138.5 (s), 136.3 (s), 132.3 (d), 131.0 (d), 123.0 (d), 102.0 (d), 65.4 (t), 65.4 (t), 60.6 (t), 34.7 (t), 28.1 (t), 14.1 (q).

Attempted preparation of ethyl 3-(2-amino-4-phenylcarbaldehyde)propanoate (363): The nitro compound **361** (6.78 g, 22.98 mmol) was dissolved in ethanol (100 mL) and hydrogenated over PtO_2 (100 mg) until no more H_2 was absorbed. The catalyst was filtered off and the filtrate concentrated. Chromatography of this crude (5.79 g) on silica gel (EtOAc-Hexane, 1:1) eluant gave 3.45 g (57%) of pure lactam **362**. A small amount of the very unstable aminoester **363** was also produced which cyclised to the lactam on standing.

Ethyl 3-(2-amino-4-[1,3-dioxolan-2-yl]phenyl)propanoate (363):
^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.99 (1H, d, *J* = 7.7 Hz), 6.78 (1H, d, *J* = 8.1 Hz), 6.74 (1H, s), 5.66 (1H, s), 4.09 (2H, q, *J* = 7.1 Hz), 4.04 (2H, m), 4.00 (2H, m), 2.77 (2H, t, *J* = 7.5 Hz), 2.57 (2H, t, *J* = 7.5 Hz), 1.20 (3H, t, *J* = 7.1 Hz); ^{13}C NMR ($CDCl_3$ (100 MHz) δ 173.1 (s), 144.3 (s), 136.9 (s), 129.2 (d), 125.6 (d), 116.4 (d), 113.3 (d), 103.4 (d), 64.9 (t), 64.9 (t), 60.3 (t), 33.3 (t), 25.7 (t), 13.9 (q).

Lactam 362: IR ν_{max} (film) 3200, 3099, 2894, 16821593, 1385, 1081 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.29 (1H, br s, NH), 7.11 (1H, d, *J* = 7.7 Hz), 7.05 (1H, dd, *J* = 1.3, 7.7 Hz), 6.92 (1H, d, *J* = 1.1 Hz), 5.71 (1H, s), 4.06 (2H, m), 3.97 (2H, m), 2.91 (2H, t, *J* = 7.5), 2.58 (2H, t, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.0 (s), 137.5 (s), 137.4 (s), 128.1 (d), 124.5 (s), 120.9 (d), 113.5 (d), 103.0 (d), 65.1 (t), 65.1 (t), 30.5 (t), 25.0 (t); EIMS (70eV), *m/z* (int, %) 219 (92), 218 (100), 174 (77), 147 (94), 119 (35); HREIMS (for deprotected aldehyde, **364**) *m/z* 175.0636 (calcd for $C_{10}H_9NO_2$, 175.0633)

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APPENDIX 1 Table Atomic coordinates [x 10^4] and equivalent isotropic displacement parameters [Å x 10^3] for **180** (esd's in parentheses).

atom	*x/a*	*y/b*	*z/a*	*U (eq)*[a]
O3	6096(1)	4376(5)	364(1)	36(1)
O7	3278(1)	4659(5)	1733(1)	35(1)
O22	6999(2)	4987(6)	-383(1)	41(1)
C1	6477(2)	3452(7)	1690(2)	31(1)
C2	7110(2)	4561(8)	1280(2)	35(1)
C3	6600(2)	5885(7)	779(2)	33(1)
C4	5947(2)	7433(7)	1044(2)	31(1)
C5	5390(2)	6470(6)	1525(2)	27(1)
C6	4526(2)	6936(7)	1516(2)	30(1)
C7	3917(2)	6222(7)	1986(2)	30(1)
C8	4446(2)	5271(7)	2559(2)	26(1)
C9	5191(2)	3765(7)	2363(2)	27(1)
C10	5879(2)	5039(7)	2019(2)	27(1)
C11	3636(2)	2409(8)	2908(2)	41(1)
C12	4962(3)	1377(9)	3302(2)	44(1)
C13	4341(2)	3054(8)	3538(2)	37(1)
C14	3844(2)	4117(7)	2973(2)	30(1)
C15	3091(2)	5390(8)	3235(2)	38(1)
C16	2844(3)	4024(10)	3790(2)	45(1)
C17	3536(3)	2168(9)	3859(2)	43(1)
C18	6483(2)	6479(8)	2463(2)	38(1)
C19	4869(3)	4682(10)	3968(2)	48(1)
C20	3723(3)	1369(13)	4515(2)	75(2)
C21	3516(5)	-210(22)	4766(4)	129(4)
C22	6408(2)	3976(7)	-179(2)	30(1)
C23	5947(2)	2105(8)	-503(2)	37(1)

[a] *U*(eq) is defined as one third of the trace of the orthogonalized U_{ij} tensor